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Animal Science

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volume 16

supplement 1

2017

ASPA 22nd CONGRESS

Perugia, June 13–16, 2017

Book of Abstracts

Guest Editors: Massimo Trabalza-Marinucci (Coordinator),
Cesare Castellini, Emiliano Lasagna, Stefano Capomaccio,
Katia Cappelli, Simone Ceccobelli, Andrea Giontella

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Italian Journal of Animal Science

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Italian Journal of Animal Science

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- Animal derived food quality and safety
- Animal genetics and breeding
- Aquaculture, poultry, companion and wildlife
- Livestock systems, management and environment
- Non-ruminants nutrition and feeding
- Production physiology and biology
- Ruminants nutrition and feeding

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Italian Journal of Animal Science

volume 16, supplement 1, 2017

ASPA 22nd Congress

Perugia, June 13-16, 2017

Guest Editors

Massimo Trabalza-Marinucci (Coordinator), Cesare Castellini,
Emiliano Lasagna, Stefano Capomaccio, Katia Cappelli,
Simone Ceccobelli, Andrea Giontella

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Perugia, June 13-16, 2017

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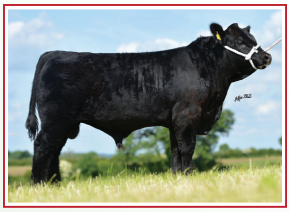

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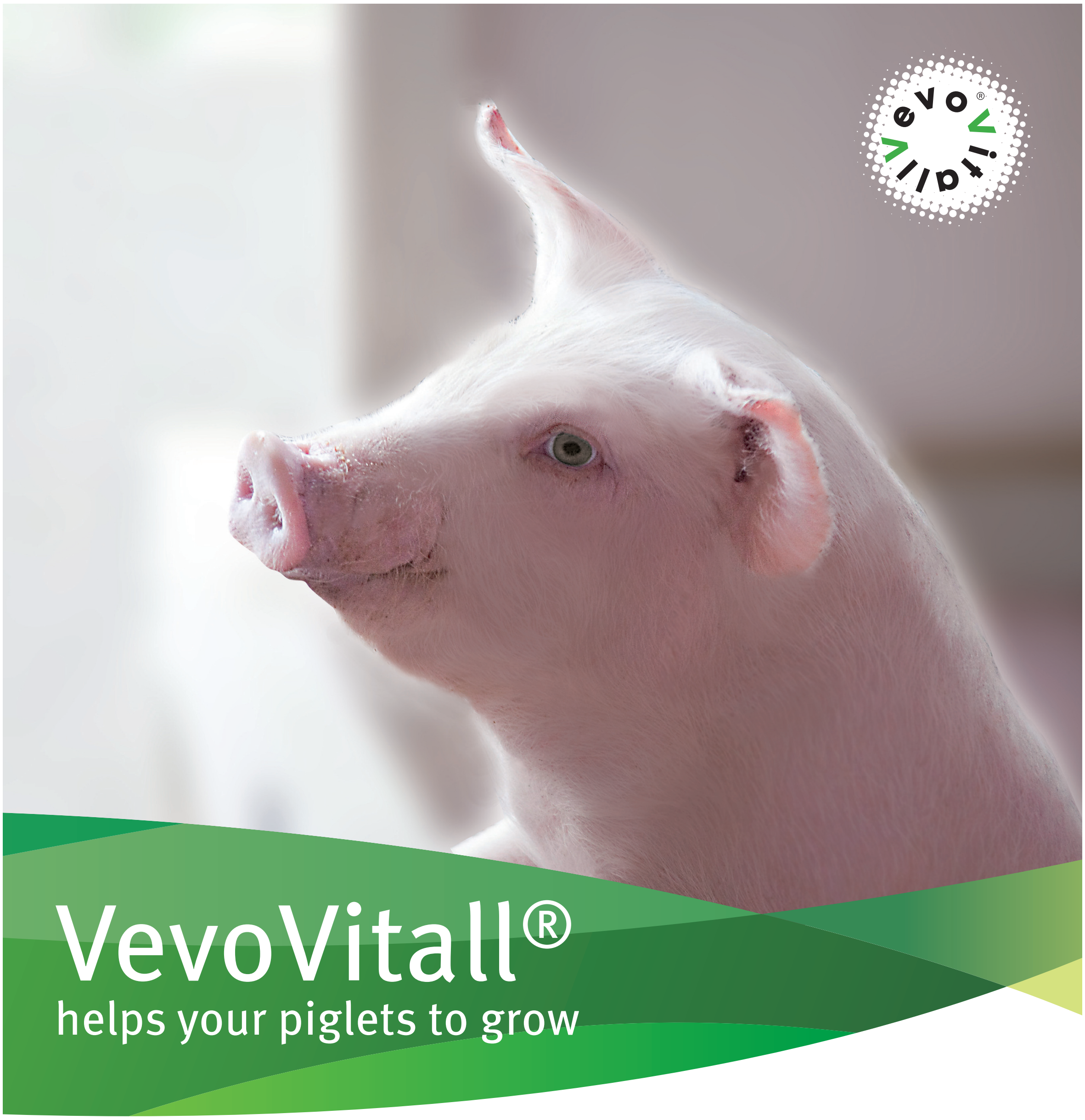

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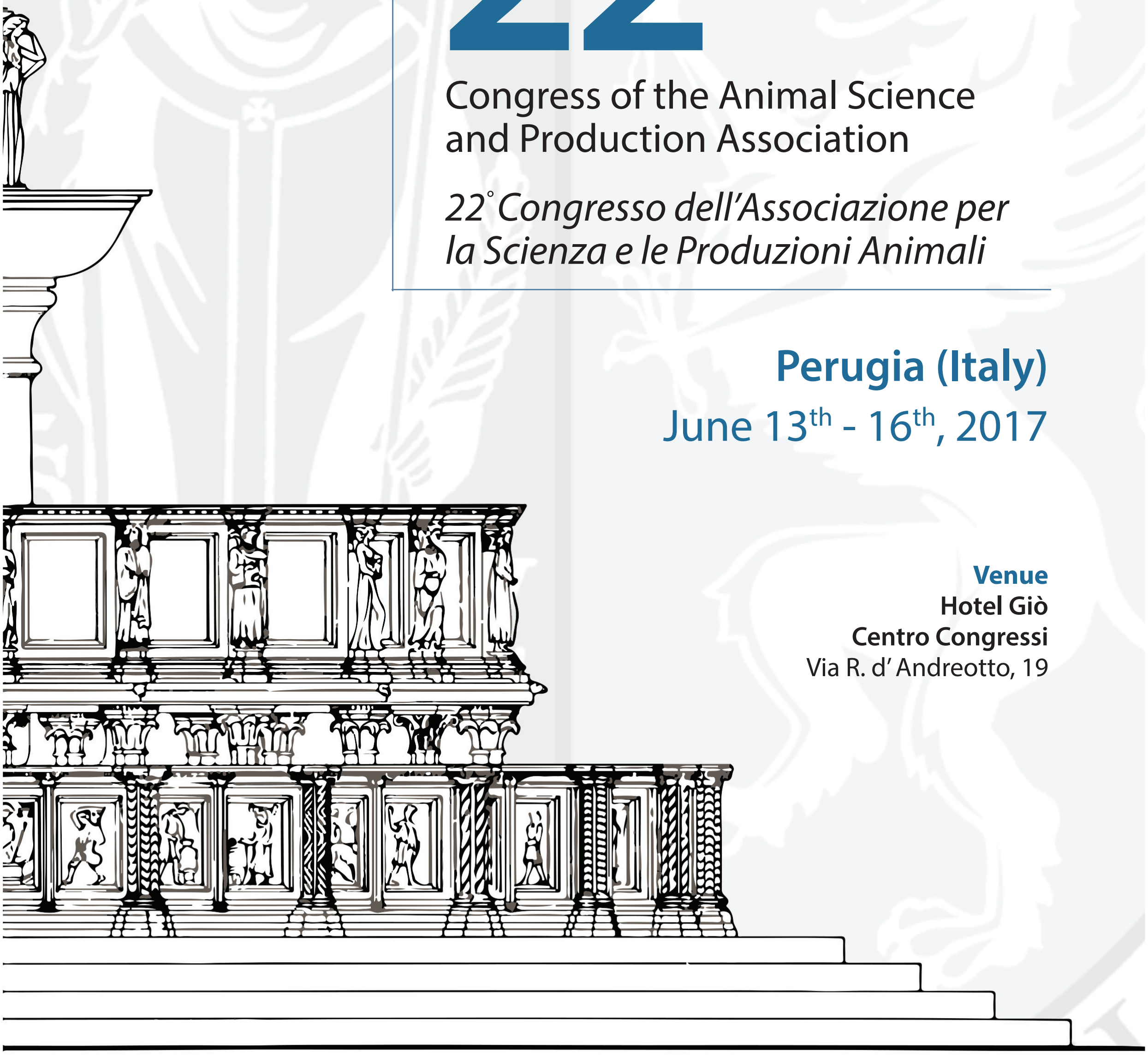
22nd

Congress of the Animal Science and Production Association

22° Congresso dell'Associazione per la Scienza e le Produzioni Animali



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June 13th - 16th, 2017

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ASPA 22nd Congress

Perugia, June 13-16, 2017

22nd ASPA CONGRESS PROGRAMME AT A GLANCE

#ASPA2017
June 13-16, 2017 - Perugia

Tuesday, June 13th					
09.30 12.30	*IJAS EDITORIAL BOARD MEETING*				
13.00 15.00	*ARRIVAL - REGISTRATION*				
	ROOM AU		ROOM T1		ROOM T2
15.00 16.15	ORAL PRESENTATIONS **AB** **"Horse genetics"** (5)	15.00 16.30	ORAL PRESENTATIONS **NF** **"Ruminal metabolism"** (6)	15.00 16.00	ORAL PRESENTATIONS **FQ** **"Meat quality"** (4)
17.30 19.30	**OPENING CEREMONY** ***(Plenary Room - Dept. of Agricultural, Food and Environmental Sciences, University of Perugia)*** **INVITED LECTURE** Livestock welfare issues in Europe or what is needed to maintain livestock production in the future? ***Matthias Gauly, EAAP, Italy***				
19.30 20.00	*Concert of "Perugia University Choir" and Welcome Cocktail*				

LEGEND

AB: Animal Breeding and Genetics
AE: Animal Husbandry and Environmental Impact
APR: Aquaculture, Poultry and Rabbit Production
AW: Animal Welfare, Health and Behaviour
NF: Nutrition and Feeding
FQ: Animal Food Quality and Safety

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Wednesday, June 14th							
	ROOM AU		ROOM T1		ROOM T2		ROOM H
8.45 9.45	**MAIN LECTURE - AB** Genetic adaptation of livestock to the challenges of climatic changes **Olivier Hanotte, UK**	8.45 9.45	ORAL PRESENTATIONS **NF** **"Monogastric nutrition"** (4)	8.45 10.15	ORAL PRESENTATIONS **APR** **"Poultry and rabbit nutrition"** (6)	8.45 10.30	ORAL PRESENTATIONS **AW** **"Husbandry, welfare and behaviour"** (7)
09.45 10.45	ORAL PRESENTATIONS **AB** **"Adaptation strategies"** (4)	9.45 10.30	**INVITED LECTURE - NF** Precision feeding ***Luigi Calamari, Italy***				
10.45 11.15	*Coffee break and POSTER SESSION*						
	ROOM AU						
11.15 13.00	**PLENARY SESSION WORKSHOP** Foods of animal origin and consumer attitudes **Chairman:** ***Luca Buttazzoni, Italy*** Politics of risk: questioning the naturalization of danger in meat production and consumption. ***Cristina Papa, Italy*** Food fact versus food fiction - The never-ending struggle between unbiased information and preconceived ideologies. ***Luigi Scordamaglia, Italy*** Foods of animal origin – how do we help the consumer understand sustainability implications of diet choice? ***Judith L. Capper, UK***						
13.00 14.00	*Lunch and POSTER SESSION*						
	ROOM AU		ROOM T1		ROOM T2		ROOM H
14.00 15.00	**MAIN LECTURE - NF** Gastrointestinal tract functionality and its relationships with eubiotics ***Pietro Celi, USA***	14.00 16.00	ORAL PRESENTATIONS **AW** **"Cattle health and metabolism"** (8)	14.00 16.15	ORAL PRESENTATIONS **AB** **"Beef cattle genetics"** (9)	14.00 16.30	**ROUND TABLE** Adaptation to hot climate and strategies to alleviate heat stress in dairy cows **ASPA Commission** **"Adattabilità dei sistemi zootecnici ai cambiamenti climatici"**
15.00 16.00	ORAL PRESENTATIONS **NF** **"Monogastric nutrition"** (4)	16.00 16.45	**INVITED LECTURE – AW** Nutritional impact on lameness in dairy cows ***Hugh Galbraith, UK***				
16.45 17.15	*Coffee break and POSTER SESSION*						
17.15 19.30	**AGROTEAM ROUND TABLE (ROOM AU)** *Innovative strategies to improve livestock production efficiency*						
20.00	*Dinner at Convention Center*						

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Thursday, June 15th

	ROOM AU		ROOM T1		ROOM T2		ROOM H
8.30 9.15	**INVITED LECTURE - AB** A brief history of statistical developments in animal breeding ***Daniel Gianola, USA***	8.30 10.30	ORAL PRESENTATIONS **NF** **"Feed evaluation"** (8)	8.30 9.30	ORAL PRESENTATIONS **APR** **"Husbandry, health and welfare"** (4)	08.30 10.15	ORAL PRESENTATIONS **FQ** **"Milk and cheese quality"** (7)
9.15 10.30	ORAL PRESENTATIONS **AB** **"Genome-wide studies"** (5)			9.30 10.30	**MAIN LECTURE - APR** Insects as sustainable feed ingredients ***Laura Gasco, Italy***		
10.30 11.00	*Coffee break and* ***POSTER SESSION***						
	ROOM AU		ROOM T1		ROOM T2		ROOM H
11.00 12.00	**MAIN LECTURE - FQ** Feeding strategies to reduce the use of antibiotics in pig production ***Joris Michiels, Belgium***	11.00 12.45	ORAL PRESENTATIONS **Joint Session NF+FQ+APR** **"Insects in animal nutrition"** (7)	11.00 12.45	ORAL PRESENTATIONS **AB** **"Dairy cattle genetics"** (7)	11.00 12.15	ORAL PRESENTATIONS **APR** **"Husbandry, health and welfare"** (5)
12.00 13.00	ORAL PRESENTATIONS **FQ** **"Meat quality"** (4)					12.15 13.00	**INVITED LECTURE – APR** Welfare in poultry ***Martina Gerken, Germany***
13.00 14.00	*Lunch and* ***POSTER SESSION***						
	ROOM AU		ROOM T1		ROOM T2		ROOM H
14.00 15.00	**MAIN LECTURE - AW** The role of mother-offspring behaviour in lamb survival ***Cathy Dwyer, UK***	14.00 15.30	ORAL PRESENTATIONS **Joint Session NF+FQ** **"Dietary strategies and quality of foods"** (6)	14.00 15.30	ORAL PRESENTATIONS **AB** **"Small ruminant genetics"** (6)	14.00 15.30	ORAL PRESENTATIONS **AE** **"Husbandry systems and socio-economic analysis"** (6)
15.00 15.45	**INVITED LECTURE – AW** Animal welfare and food security: role and tasks of the Italian farmers ***Franco Valfré, Italy***						
	ROOM AU		ROOM T1		ROOM T2		ROOM H
15.45 16.30	**INVITED LECTURE – AE** Constrains and solutions to reduce environmental impact for developed livestock farming **Andrea Rosati, EAAP, Italy**	15.45 16.45	ORAL PRESENTATIONS **AW** **"Pig health and behaviour"** (4)	15.45 16.45	ORAL PRESENTATIONS **AB** **"Dairy cattle genetics"** (4)	15.45 16.45	ORAL PRESENTATIONS **APR** **"Genetics"** (4)
16.45 17.00	***Coffee break and POSTER SESSION***						
17.00 18.30	MEETING OF ASPA MEMBERS (ROOM AU)						
20.00	***Social Dinner - Palazzo Bernabei, University of Perugia (Assisi)***						

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22nd ASPA CONGRESS PROGRAMME AT A GLANCE

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Friday, June 16th

	ROOM AU		ROOM T1		ROOM T2		ROOM H		
9.00 10.00	**MAIN LECTURE - AE** How to solve the (un)sustainability dilemma ***Simone Bastianoni, Italy*** Environmental impact of animal production ***Matteo Crovetto, Italy***	9.00 10.00	ORAL PRESENTATIONS **FQ** **"Food safety"** (4)	9.00 10.45	ORAL PRESENTATIONS **NF** **"Ruminant nutrition"** (7)	9.00 11.00	ORAL PRESENTATIONS **APR** **"Aquaculture"** (8)		
		10.00 10.45	**INVITED LECTURE - FQ** Foodborne pathogen: the case of *Campylobacter* in poultry ***Lisa K. Williams, UK***						
11.00 11.30	***Coffee break and POSTER SESSION***								
	ROOM AU		ROOM T1		ROOM T2		ROOM H		ROOM C
11.30 13.30	**SIB ROUND TABLE** Dairy cow welfare and ethology	11.30 13.00	ORAL PRESENTATIONS **Joint Session NF+FQ** **"Dietary strategies and quality of foods"** (6)	11.30 13.15	ORAL PRESENTATIONS **AB** **"Other species"** (7)	11.30 13.00	ORAL PRESENTATIONS **AW** **"Small ruminant health and behaviour"** (6)	11.30 13.30	ORAL PRESENTATIONS **AE** **"Husbandry systems and environmental impact"** (8)

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Agenda Invited Speakers

June 13th

Plenary Room - Dept. of Agricultural, Food and Environmental Sciences, University of Perugia

June 14th

Room AU - Animal Breeding and Genetics - Adaptation strategies

Chairmen: Paolo Ajmone-Marsan, Stefano Capomaccio

Room T1 - Nutrition and Feeding

Chairmen: Antonella Baldi, Luigi Gallo

Room AU - PLENARY SESSION WORKSHOP

Foods of animal origin and consumers attitudes

Chairman: Luca Buttazzoni

Room AU - Nutrition and Feeding

Chairmen: Paolo Bosi, Domenico P. Lo Fiego

Room T1 - Animal Welfare, Health and Behaviour - Cattle health and metabolism

Chairmen: Cinzia Marchitelli, Erminio Trevisi

June 15th

Room AU - Animal Breeding and Genetics

Chairmen: Nicolò P. P. Macciotta, Alfredo Pauciullo
08.30-09.15 Daniel Gianola
A brief history of statistical developments in animal breeding IS009

Room T2 - Aquaculture, Poultry and Rabbit Production

Chairmen: Federico Sirri, Alessandro Dal Bosco
09.30-10.30 Laura Gasco
Insects as sustainable feed ingredients IS010

Room AU - Animal Food Quality and Safety

Chairmen: Andrea Serra, Paolo Polidori
11.00-12.00 Joris Michiels
Feeding strategies to reduce the use of antibiotics in pig production IS011

Room H - Aquaculture, Poultry and Rabbit Production - Husbandry and welfare

Chairmen: Cecilia Mugnai, Massimiliano Petracci
12.15-13.00 Martina Gerken
Welfare in Poultry IS012

Room AU - Animal Welfare, Health and Behaviour

Chairmen: Silvana Mattiello, Massimo Trabalza-Marinucci
14.00-15.00 Cathy M. Dwyer
The role of mother-offspring behaviour in lamb survival IS013
15.00-15.45 Franco Valfré
Animal welfare and food security: role and tasks of the Italian farmers IS014

Room AU - Animal Husbandry and Environmental Impact

Chairmen: Luca M. Battaglini, Maurizio Silvestrelli
15.00-15.45 Andrea Rosati
Constrains and solutions to reduce environmental impact for developed livestock farming IS015

June 16th

Animal Husbandry and Environmental Impact

Chairmen: Bruno Stefanon, Mariano Pauselli
09.00-10.00 Simone Bastianoni
How to solve the (un)sustainability dilemma IS016
Gian Matteo Crovetto
Environmental impact of animal production IS017

Animal Food Quality and Safety - Food safety

Chairmen: Luisa A. Volpelli, Vincenzo Chiofalo
10.00-10.45 Lisa K. Williams
Foodborne pathogen: the case of Campylobacter in poultry IS018

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Agenda for oral presentations

Tuesday June 13th - room AU

Animal breeding and genetics - Horse genetics

Chairmen: Donata Marletta, Roberto Mantovani

15.00-15.15 Andrea Giontella, Francesca M. Sarti, Giovanni P. Biggio, Andrea Taras, Raffaele Cherchi, Maurizio Silvestrelli, Camillo Pieramati
Sardinian Anglo-Arab horses: genetic parameters of conformation and performances O001

15.15-15.30 Stefano Capomaccio, Andrea Giontella, Ted Kalbfleisch, Scotty DePriest, Andrea Verini-Supplizi, Silvia Sorbolini, Raffaele Cherchi, Maurizio Silvestrelli, Katia Cappelli
Transcriptome analysis of racehorses PBMCs: insights on transcribed introns and repeats O002

15.30-15.45 Andrea Verini-Supplizi, Stefano Capomaccio, Luisa Pascucci, Cinzia Bazzucchi, Marco Pepe, Mauro Coletti, Rodolfo Gialletti, Stefano Petrini, Fabrizio Passamonti, Franco Moriconi, Maurizio Silvestrelli, Katia Cappelli
RNA-cargo characterization of equine Ad-MSCs vesicles: first step towards cell-free therapeutic procedures O003

15.45-16.00 Katia Cappelli, Elisabetta Chiaradia, Andrea Viglino, Maurizio Silvestrelli, Francesca Beccati, Livia Moscati, Stefano Capomaccio
Circulating miRNAs as biomarkers of stress and training in endurance horses O004

16.00-16.15 Andrea Giontella, Hovirag Lancioni, Irene Cardinali, Marco R. Capodiferro, Stefano Capomaccio, Katia Cappelli, Camillo Pieramati, Alessandro Achilli, Maurizio Silvestrelli
Mitochondrial DNA haplotypes unveil mistakes in Maremmano horse pedigree records O005

Tuesday June 13th - room T1

Nutrition and feeding - Ruminal metabolism

Chairmen: Lucia Bailoni, Andrea Formigoni

15.00-15.15 Giuseppe Conte, Corrado Dimauro, Alice Cappucci, Eleonora Bulleri, Francesca Ciucci, Andrea Serra, Marcello Mele
Relationship between ruminal biohydrogenation products and milk fat depression O006

15.15-15.30 Alberto Palmonari, Giorgia Canestrari, Elena Bonfante, Ludovica Mammi, Andrea Formigoni
Effect of replacing soybean meal with rapeseed meal on productive performance and ruminal conditions of lactating dairy cows O007

15.30-15.45 Marica Simoni, Federico Righi, Francesca Martuzzi, Massimo Malacarne, Marco Renzi, Afro Quarantelli
Study of the in vivo and in vitro variations of the enzymatic activity of bovine rumen fluid inocula O008

15.45-16.00 Fabio Correddu, Alberto S. Atzori, Gianni Battacone, Giuseppe Pulina, Anna Nudda
Effect of exhausted berries of myrtle on ruminal metabolism in dairy sheep O009

16.00-16.15 Fiorentina Palazzo, Filippo Biscarini, Federica Castellani, Andrea Vitali, Giulia Masetti, Lisa Grotta, Giuseppe Martino
Characterization of the rumen microbiota in dairy calves receiving copper or grape-pomace feed supplementation O010

16.15-16.30 Federica Mannelli, Mariano Pauselli, Francesco Pini, Alice Cappucci, Andrea Serra, Giuseppe Conte, Marcello Mele, Sara Minieri, Stefano Rapaccini, Luciana Giovannetti, Carlo Viti, Arianna Buccioni
Effect of olive oil pomace dietary supplementation on rumen microbial community profile: a metagenomics approach O011

Tuesday June 13th - room T2

Animal food quality and safety - Meat quality

Chairmen: Edi Piasentier, Luciano Morbidini

15.00-15.15 Francesco Sirtori, Riccardo Bozzi, Alessandro Crovetti, Chiara Aquilani, Antonio Pezzati, Antonio Bonelli, Carolina Pugliese
Effects of salting time on seasoned meat in two pigs genotypes O012

15.15-15.30 Michela Contò, Maria Miarelli, Sabrina Di Giovanni, Paola Lavermicocca, Francesca Valerio, Sebastiana Failla
Effect of the broking down of cold chain on beef meat oxidation O013

15.30-15.45 Amalia Simonetti, Roberto Giudice, Annamaria Perna, Emilio Gambacorta
Comparison of oxidative status and antioxidant compounds of pig meat from Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig before and after cooking O014

15.45-16.00 Alessandro Crovetti, Francesco Sirtori, Doria Benvenuti, Antonio Bonelli, Andrea Lombardo, Giovanni Brajon, Riccardo Bozzi
Wet aging effect on beef meat physical traits O015

Wednesday June 14th - Room AU

Animal breeding and genetics - Adaptation strategies

Chairmen: Paolo Ajmone-Marsan, Stefano Capomaccio

09.45-10.00 Miranda Bryan, Christian Maltecca, Kent Gray, Clint Schwab, Yijian Huang, Francesco Tiezzi
Genetic variance for sow tolerance to heat stress O016

10.00-10.15 Elia Vajana, Mario Barbato, Licia Colli, Marco Milanesi, Estelle Rochat, Enrico Fabrizi, Christopher Mukasa, Marcello Del Corvo, Charles Masembe, Vincent Muwanika, Fredrick Kabi, Riccardo Negrini, Stéphane Joost, Paolo Ajmone-Marsan, the NEXTGEN Consortium
Combining landscape genomics and ecological modelling to investigate local adaptation of indigenous Ugandan cattle to East Coast Fever O017

10.15-10.30 Tri Satya Mastuti Widi, Nuzul Widyas
Variation of Madura Cattle in Madura Island, Indonesia O018

10.30-10.45 Salvatore Mastrangelo, Paolo Ajmone-Marsan, Alessandro Bagnato, Luca M. Battaglini, Riccardo Bozzi, Antonello Carta, Gennaro Catillo, Martino Cassandro, Sara Casu, Roberta Ciampolini, Elena Ciani, Paola Crepaldi, Mariasilvia D'Andrea, Rosalia Di Gerlando, Luca Fontanesi, Maria Longeri, Nicolò P. P. Macciotta, Roberto Mantovani, Donata Marletta, Donato Matassino, Marcello Mele, Giulio Pagnacco, Camillo Pieramati, Baldassare Portolano, Francesca M. Sarti, Fabio Pilla
BOVITA: a first overview on genome-wide genetic diversity of Italian autochthonous cattle breeds O019

Wednesday June 14th - Room T2

Animal breeding and genetics - Beef cattle genetics

Chairmen: Liliana Di Stasio, Camillo Pieramati

14.00-14.15 Francesca M. Sarti, Simone Ceccobelli, Andrea Giontella, Piera Di Lorenzo, Emiliano Lasagna, Andrea Quaglia, Fiorella Sbarra, Roberta Guarcini, Camillo Pieramati, Francesco Panella
Effects of Single Nucleotide Polimorphisms (SNPs) in candidate genes for growth traits in Italian beef cattle breeds O020

14.15-14.30 Fiorella Sbarra, Andrea Quaglia, Giovanni Bittante, Roberto Mantovani
Heritability of reproduction traits and type latent factors and their genetic correlations in Marchigiana, Chianina and Romagnola beef breeds O021

14.30-14.45 Domenico Aiello, Laura Salvadori, Sara Chiappalupi, Simone Ceccobelli, Emidio Albertini, Guglielmo Sorci, Emiliano Lasagna
Histological muscle characterization in hypertrophied Marchigiana cattle breed O022

14.45-15.00 Simone Savoia, Alberto Brugiapaglia, Liliana Di Stasio, Alessandro Ferragina, Alessio Cecchinato, Giovanni Bittante, Andrea Albera
"QualiPiem" - Innovative tools for selection of meat quality in Piemontese breed O023

15.00-15.15 Cinzia Marchitelli, Bianca Moioli, Roberto Steri, Gennaro Catillo
Twinning project: increasing of twin births in Maremmana breed O024

15.15-15.30 Mauro Fioretti, Stefano Biffani, Riccardo Negrini, Riccardo Bozzi
Genetic improvement and population structure of Italian Limousine O025

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15.30-15.45 Hovirag Lancioni, Piera Di Lorenzo, Simone Ceccobelli, Licia Colli, Irene Cardinali, Taki Karsli, Marco R. Capodiferro, Luca Ferretti, Paolo Ajmone-Marsan, Francesca M. Sarti, Emiliano Lasagna, Alessandro Achilli
Ancient distinctive migration routes suggested by the current mitochondrial gene pool of Podolic cattle breeds in Italy O026

15.45-16.00 Filippo Biscarini, Salvatore Mastrangelo, Gennaro Catillo, Bianca Moioli, Roberta Ciampolini
Genome-wide homozygosity in Maremmana cattle O027

16.00-16.15 Rosalia Di Gerlando, Maria T. Sardina, Marco Tolone, Anna M. Sutera, Salvatore Mastrangelo, Baldassare Portolano
Genome wide Copy Number Variation (CNV) detection in Cinisara cattle breed O028

Wednesday June 14th - Room T2

Aquaculture, poultry and rabbit production - Poultry and rabbit nutrition

Chairmen: Antonella Dalle Zotte, Cesare Castellini

08.45-09.00 Nieves Núñez-Sánchez, Gabriele Acuti, Domenico Brusco, Simona Mattioli, Oliviero Olivieri, Massimo Trabalza-Marinucci
Prediction of nutrient digestibility and chemical composition of rabbit diets using faecal near infrared spectroscopy (FNIRS) O029

09.00-09.15 Marco Birolo, Angela Trocino, Francesco Gratta, Gerolamo Xiccato
Performance and feeding behaviour of group–housed rabbits with free or time–limited access to feed O030

09.15-09.30 Paula A. Toalombo-Vargas, Nelson A. Duchi-Duchi, Luis R. Fiallos-Ortega, José V. Trujillo-Villacís, Luis E. Hidalgo-Almeida, Rafael Buenaño-Nuñez
Effect of chamomile (Matricaria chamomilla) on health in chickens O031

09.30-09.45 Paula A. Toalombo-Vargas, Nelson A. Duchi-Duchi, Luis R. Fiallos-Ortega, Antonio J. Morales- delaNuez, Luis E. Hidalgo-Almeida, Matiza L. Vaca-Cárdenas, Noé F. Rodríguez-G
Effect of chamomile (Matricaria chamomilla) on productive performance in chickens O032

09.45-10.00 Angela Trocino, Francesco Gratta, Marco Birolo, Alessandra Piccirillo, Massimiliano Petracci, Luc Maertens, Gerolamo Xiccato
Effects of the feeding system on performance and myopathy occurrence in two broiler chicken genotypes O033

10.00-10.15 Siria Tavaniello, Rossella Mucci, Acaye Ongwech, Marek Bednarczyk, Giuseppe Maiorano
Probiotic and prebiotic supplementation in broiler chickens: growth performance, carcass traits and meat quality O034

Wednesday June 14th - Room H

Animal welfare, health and behaviour - Husbandry, welfare and behaviour

Chairmen: Flaviana Gottardo, Andrea Verini-Supplizi

08.45-09.00 Giulio Cozzi, Luisa Magrin, Isabella Lora, Thomas Beorchia, Barbara Contiero
Milk yield, culling rate and reasons of pure Holsteins and second generation crossbred cows from Montbéliard × (Swedish Red × Holstein) O035

09.00-09.15 Riccardo Moretti, Stefano Biffani, Stefania Chessa, Riccardo Bozzi
Predictive models for locomotion issues in Italian Holstein dairy cows O036

09.15-09.30 Vincenzo Lopreiato, Andrea Minuti, Elisabetta Fratto, Marcello Marotta, Giovanni Loprete, Domenico Britti, Erminio Trevisi, Valeria M. Morittu
Use of an electronic rumination-monitoring system in pre-weaned calves O037

09.30-09.45 Giorgio Marchesini, Davide Mottaran, Matteo Gazziero, Massimo Mirisola, Severino Segato, Igino Andrighetto
Effect of different ventilation systems on beef cattle during the early fattening period O038

09.45-10.00 Veronica Redaelli, Marta De Santis, Silvia Gozzo, Laura Contalbrigo, Annalisa Stefani, Marta Toson, Luca Farina, Marta Borgi, Francesca Cirulli, Emanuela Valle, Fabio Luzi
Use of Infrared Termography (IRT) in equine assisted interventions: physiological aspects O039

10.00-10.15 Aristide Maggiolino, Alessandra Tateo, Pasquale De Palo, Giovanna Calzaretti, Pasquale Centoducati
Evaluation of different habituation protocols for training dairy donkey jennies to the milking parlor: effect on milk yield, behavior and heart rate O040

10.15-10.30 Monica Battini, Sara Barbieri, Elisabetta Canali, Francesca Dai, Emanuela Dalla Costa, Valentina Ferrante, Lorenzo Ferrari, Silvana Mattiello, Michela Minero
Outcomes of a web-survey for collecting stakeholders' opinion on welfare requirements for sheep, goats, turkeys, donkeys, and horses O041

Wednesday June 14th - Room T1

Animal welfare, health and behaviour - Cattle health and metabolism

Chairmen: Cinzia Marchitelli, Erminio Trevisi

14.00-14.15 Andrea Minuti, Nusrat Jahan, Matteo Mezzetti, Fiorenzo Piccioli-Cappelli, Lorenzo Bomba, Stefano Capomaccio, Juan J. Loor, Paolo Ajmone-Marsan, Erminio Trevisi
Relationship between blood energetic markers and circulating leukocytes transcriptome in transition dairy cows O042

14.15-14.30 Erminio Trevisi, Andrea Minuti, Matteo Mezzetti, Annarita Ferrari, Fiorenzo Piccioli-Cappelli
Supplements of Aloe arborescens improve health and inflammo-metabolic status of transition dairy cows O043

14.30-14.45 Paola Cremonesi, Federica Riva, Maria F. Addis, Camilla Ceccarani, Marco Severgnini, Erminio Trevisi, Massimo Amadori, Claudia Pollera, Joel Filipe, Giulio Curone, Lauretta Turin, Vittorio Tedde, Alessandra Pagnoni, Daniele Vigo, Paolo Moroni, Andrea Minuti, Valerio Bronzo, Bianca Castiglioni
A multidisciplinary study investigating the bovine milk microbiome and its association with mastitis resistance traits in Holstein Friesian and Rendena dairy cows O044

14.45-15.00 Daniele Dipasquale, Loredana Basiricò, Patrizia Morera, Umberto Bernabucci
Anti-inflammatory effects of conjugated linoleic acid isomers and essential fatty acids in bovine mammary epithelial cells O045

15.00-15.15 Sandy Sgorlon, Danilo Licastro, Bruno Stefanon, Monica Colitti
Cows relocation affects exosomes and their cargos in bovine milk O046

15.15-15.30 Luisa Magrin, Marta Brscic, Leonardo Armato, Giulio Cozzi, Flaviana Gottardo
Lameness in finishing beef cattle: distribution of claw disorders in 2716 hind feet O047

15.30-15.45 Edyta Bauer, Justyna Żychlińska-Buczek
Using mineral-vitamin boluses to improve somatic cell count prevention value in Polish dairy farms O048

15.45-16.00 Juan J. García Bustos
Treatment of bovine diseases with agroforestry resources in farms of the Colombian Amazon piedmont O049

Wednesday June 14th - Room T1

Nutrition and feeding - Monogastric nutrition

Chairmen: Antonella Baldi, Luigi Gallo

08.45-09.00 Elena Mariani, Nicoletta Rizzi, Guido Invernizzi, Alessandro Agazzi, Adriana Maggi, Giovanni Savoini
Spatio-temporal analysis of the Nrf 2 activation in transgenic reporter mouse fed with high dosage of saturated or unsaturated fatty acids using in vivo bioluminescent imaging O050

09.00-09.15 Maria G. Cappai, Maurizio Picciau, Maria G. A. Lunesu, Corrado Dimauro, Raffaele Cherchi, Walter Pinna
Circulating levels of nutrient-related metabolites in healthy Anglo-Arabian foals from weaning to 18 months of age O051

09.15-09.30 Mirco Dalla Bona, Giuseppe Carcò, Enrico Fiore, Stefano Schiavon, Luca Carraro, Massimo Morgante, Luigi Gallo
Effects of dietary protein and lysine content on growth performances, carcass traits and estimated nitrogen input-output flow of growing pigs O052

09.30-09.45 Micol Bertocchi, Paolo Trevisi, Diana Luise, Vincenzo Motta, Chiara Salvarani, Anisa Ribani, Aude Simongiovanni, Etienne Corrent, Luca Fontanesi, Paolo Bosi
Dose-response for different levels of leucine to lysine ratio on the growth performance of weaning pigs with different genotypes at the α-aminoadipate d-semialdehyde synthase gene O053

Wednesday June 14th - Room AU

Nutrition and feeding - Monogastric nutrition

Chairmen: Paolo Bosi, Domenico P. Lo Fiego

15.00-15.15 Vincenzo Motta, Michele Pesciaroli, Paolo Trevisi, Diana Luise, Ludovica Curcio, Lucilla Cucco, Francesca R. Massacci, Chiara F. Magistrali
Dietary calcium-butyrate promotes a shift of gut microbiota of weaned pigs O054

15.15-15.30 Chiara Aquilani, Francesco Sirtori, Oreste Franci, Alessandro Crovetti, Carolina Pugliese
Effects of protein restricted diet on in vivo performances and slaughtering traits of Cinta Senese pigs O055

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15.30-15.45 Matteo Ottoboni, Carlotta Giromini, Marco Tretola, Davide Gottardo, Daniela Marchis, Valentina Caprarulo, Federica Cheli, Antonella Baldi, Luciano Pinotti
Nutrients content and in vitro digestibility of ex-food as feed ingredient for pig diets O056

15.45-16.00 Eleonora Seoni, Gianni Battacone, Paolo Silacci, Frigga Dohme-Meier, Giuseppe Bee
Impact of increasing levels of condensed tannins from sainfoin in grower-finisher diets of entire male pigs on growth performance and carcass characteristics O057

Thursday June 15th - Room AU

Animal breeding and genetics - Genome-wide studies

Chairmen: Nicolò P. P. Macciotta, Alfredo Pauciullo

09.15-09.30 Mariasilvia D'Andrea, Valentino Palombo, Marco Milanesi, Sandy Sgorlon, Stefano Capomaccio, Giuseppe Conte, Paolo Ajmone-Marsan
Genome-wide association study of milk fatty acid composition in Italian Simmental and Italian Holstein cows O058

09.30-09.45 Alessio Cecchinato, Sara Pegolo, Christos Dadousis, Núria Mach, Yuliaxis Ramayo-Caldas, Marcello Mele, Stefano Schiavon, Giovanni Bittante
Genome-wide association and pathway-based analysis for milk fatty acids profile in dairy cattle O059

09.45-10.00 Alberto Cesarani, Antonio Puledda, Maria G. Manca, Fabio Correddu, Jessica Serdino, Silvia Sorbolini, Nicolò P. P. Macciotta
Genome-wide association study of cheese-making properties in sheep milk O060

10.00-10.15 Anna M. Sutera, Salvatore Mastrangelo, Maria T. Sardina, Rosalia Di Gerlando, Marco Tolone, Baldassare Portolano
Genome-wide association study for milk production traits in Valle del Belice sheep O061

10.15-10.30 Salvatore Mastrangelo, Marco Tolone, Maria T. Sardina, Anna M. Sutera, Rosalia Di Gerlando, Baldassare Portolano
Genome-wide scan for runs of homozygosity in Valle del Belice sheep O062

Thursday June 15th - Room T2

Animal breeding and genetics - Dairy cattle genetics

Chairmen: Giulio Pagnacco, Mauro Penasa

11.00-11.15 Marco R. Capodiferro, Shaoqiang Wang, Ningbo Chen, Tao Zhang, Hovirag Lancioni, Irene Cardinali, Hucai Zhang, Yongwang Miao, Vongpasith Chanthakhoun, Metha Wanapat, Marnoch Yindee, Yi Zhang, Hongzhao Lu, Ruihua Dang, Yongzhen Huang, Xianyong Lan, Martin Plath, Hong Chen, Johannes A. Lenstra, Chuzhao Lei, Alessandro Achilli
Looking into the Swamp Buffalo genetic history through the whole mitogenome analysis O063

11.15-11.30 Chiara Roveglia, Giulio Visentin, Mauro Penasa, Raffaella Finocchiaro, Maurizio Marusi, Martino Cassandro
Genetics of alternative somatic cell count traits in Italian Holsteins O064

11.30-11.45 Hugo Toledo-Alvarado, Gustavo de los Campos, Ana Vazquez, Rob Tempelman, Giovanni Bittante, Alessio Cecchinato
Predictive ability of Fourier transform infrared spectroscopy and milk components to assess the pregnancy status of dairy cows O065

11.45-12.00 Cristina Sartori, Francesco Tiezzi, Nadia Guzzo, Roberto Mantovani
Causal relationships between milk yield, somatic cells, fertility, longevity and fighting ability in Aosta Chestnut-Black Pied Cattle O066

12.00-12.15 Corrado Dimauro, Elisabetta Manca, Alberto S. Atzori, Massimo Cellesi, Giustino Gaspa, Nicolò P. P. Macciotta
Use of discriminant analysis to early detect lactation persistency in dairy cows O067

12.15-12.30 Marco Milanesi, Mario Di Guardo, Stefano Capomaccio, Katia Cappelli, Valentino Palombo, Mariasilvia D'Andrea, Marcello Del Corvo, Sandy Sgorlon, Andrea Minuti, Yuri Tani Utsunomiya, José Fernando Garcia, Erminio Trevisi, Riccardo Negrini, Bruno Stefanon, Paolo Ajmone-Marsan
Identification of genomic variants associated with metabolic stress in Italian Simmental and Holstein cows O069

12.30-12.45 Elisabetta Viale, Francesco Tiezzi, Fabio Maretto, Massimo De Marchi, Mauro Penasa, Martino Cassandro
Candidate gene association analysis for milk yield and composition traits, coagulation properties and somatic cell count in Italian Holstein Friesian bulls O078

Thursday June 15th - Room T2

Animal breeding and genetics - Small ruminants genetics

Chairmen: Elena Ciani, Emiliano Lasagna

14.00-14.15 Beatriz López, Vincenzo Landi, Mayra M. Gómez Carpio, Beatriz Agudo, Amparo Martínez Martínez
Preliminary results on the genetic variation and the population structure within a Spanish Merino breed commercial population O070

14.15-14.30 Mokhethi M. Selepe, Simone Ceccobelli, Emiliano Lasagna, Nokuthula W. Kunene
An overview on the genetic diversity in South African Nguni (Zulu) sheep O071

14.30-14.45 Silvia Sorbolini, Giustino Gaspa, Donata Marletta, Paola Crepaldi, Bianca Moioli, Antonello Carta, Baldassare Portolano, Emiliano Lasagna, Elena Ciani, Mariasilvia D'Andrea, Fabio Pilla, Nicolò P. P. Macciotta, Italian Sheep Consortium (BIOVITA)
Detection of signatures of selection in Italian sheep breeds O072

14.45-15.00 Sara Casu, Tiziana Sechi, Mario G. Usai, Stefania Sechi, Mario Barbato, Paolo Ajmone-Marsan, Antonello Carta
Toward the identification of causal mutations and LD markers associated to disease resistance in sheep O073

15.00-15.15 Licia Colli, Marco Milanesi, Marcello Del Corvo, Andrea Talenti, Francesca Bertolini, Minhui Chen, Alessandra Crisà, Kevin Daly, Bernt Guldbrandtsen, Stéphane Joost, Johannes A. Lenstra, Ezequiel L. Nicolazzi, Estelle Rochat, Benjamin D. Rosen, Max F. Rothschild, Bertrand Servin, Tad S. Sonstegard, Roberto Steri, Elia Vajana, Curtis P. Van Tassell, Paolo Ajmone-Marsan, Paola Crepaldi, Alessandra Stella, the ADAPTmap Consortium
Drawing up worldwide goat diversity and post-domestication history: update from ADAPTmap project O074

15.15-15.30 Andrea Talenti, Stefano Frattini, Salvatore Mastrangelo, Baldassarre Portolano, Emiliano Lasagna, Francesca M. Sarti, Simone Ceccobelli, Marco Milanesi, Licia Colli, Elena Ciani, Dominga Soglia, Stefano Sartore, Roberta Ciampolini, Alessandra Crisà, Roberto Steri, Gennaro Catillo, Donata Marletta, Salvatore Bordonaro, Mariasilvia D'Andrea, Stefania Chessa, Bianca Castiglioni, Pasqualino Loi, Tiziana Sechi, Antonello Carta, Riccardo Negrini, Alessandra Stella, Alessio Valentini, Francesco Panella, Giulio Pagnacco, Fabio Pilla, Paolo Ajmone-Marsan, Paola Crepaldi, the Italian Goat Consortium
Italian Goat Consortium: a collaborative project to study the Italian caprine biodiversity O075

Thursday June 15th - Room T2

Animal breeding and genetics - Dairy cattle genetics

Chairmen: Paola Crepaldi, Giovanni Bittante

15.45-16.00 Nadia Guzzo, Cristina Sartori, Alessandro Ferragina, Giovanni Bittante, Roberto Mantovani
Genetic parameters of new FTIR milk phenotypes in Rendena breed O076

16.00-16.15 Valentino Palombo, Marco Milanesi, Stefano Capomaccio, Sandy Sgorlon, Mariasilvia D'Andrea
Pathways interaction analysis for identification of novel candidate genes O077

16.15-16.30 Marco Iannaccone, Marina Papaianni, Flora Ianniello, Andrea Fulgione, Daniela Gallo, Gianfranco Cosenza, Rosanna Capparelli
Identification of a novel polymorphism in the 3'untraslated region of the interferon gamma gene as potential marker associate with bovine tuberculosis in water buffalo (Bubalus bubalis). O079

16.30-16.45 Sara Pegolo, Christos Dadousis, Núria Mach, Yuliaxis Ramayo-Caldas, Marcello Mele, Stefano Schiavon, Giovanni Bittante, Alessio Cecchinato
SNP co-association and network analyses for bovine milk fatty acid profile O080

Thursday June 15th - Room T2

Aquaculture, poultry and rabbit production - Husbandry, health and welfare

Chairmen: Federico Sirri, Alessandro Dal Bosco

08.30-08.45 Cristina Zomeño, Marco Birolo, Francesco Gratta, Andrea Zuffellato, Gerolamo Xiccato, Angela Trocino
Influence of group housing, lactation system and floor type on performance and injuries in rabbit does O081

08.45-09.00 Marta Brscic, Michele Gazzo, Barbara Contiero, Flaviana Gottardo
Main welfare problems in intensive broiler chicken production systems: is there any way to differentiate farms? O082

09.00-09.15 Kunlayaphat Wuthijaree, Christian Lambertz, Matthias Gauly
Prevalence and burden of helminths in free-range laying hens in Northern Italy O083

09.15-09.30 Sabine Mueller, Lorena Taddei, Ruth E. Messikommer, Michael Kreuzer, Isabelle D. M. Gangnat
Growth and slaughter performance of two dual-purpose poultry types compared with extensive broiler and layer types fattened for 67 and 84 days O084

Thursday June 15th - Room H

Aquaculture, poultry and rabbit production - Husbandry and welfare

Thursday June 15th - Room H

Aquaculture, poultry and rabbit production - Genetics

Thursday June 15th - Room H

Animal husbandry and environmental impact - Husbandry systems and socio-economic analysis

Thursday June 15th - Room T1

Animal welfare, health and behaviour - Pig health and behaviour

Chairmen: Carolina Pugliese, Giovanna Martelli

15.45-16.00 Silvia Parrini, Anna Acciaioli, Valentina Becciolini, Gustavo Campodoni, Oreste Franci
Study on the behaviour of Cinta Senese and Large White x Cinta Senese pigs reared at pasture O100

16.00-16.15 Giuseppe Carcò, Mirco Dalla Bona, Luca Carraro, Luigi Gallo, Maria A. Latorre, Manuel Fondevila, Stefano Schiavon
The daily feeding behaviour of growing pigs subjected to a feed restriction and to a reduction of dietary indispensable amino acid contents O101

16.15-16.30 Eleonora Nannoni, Luca Sardi, Marika Vitali, Giovanna Martelli
Growth traits, carcass and meat quality of heavy pigs receiving different enrichment tools O102

16.30-16.45 Luciana Rossi, Serena Reggi, Giovanni L. Alborali, Paolo Trevisi, Diana Luise, Angela Lombardi, Eugenio De Martini, Antonella Baldi
Seed-based vaccine immunogens administered in feed for the control of verocytotoxic E.coli infection in pig livestock O103

Thursday June 15th - Room T1

Nutrition and feeding - Feed evaluation

Chairmen: Paolo Bani, Pier Paolo Danieli

08.30-08.45 Afro Quarantelli, Marco Renzi, Marica Simoni, Alessandro M. Rosato, Federico Righi
Use of a new instrument for the in vitro evaluation of ruminants feeds through gas production: preliminary study O104

08.45-09.00 Martina Novacco, Matteo Ottoboni, Eleonora Fusi, Silvia Gastaldello, Valentina Furlan, Claudio Maran, Vittorio Dell'Orto, Federica Cheli
Electronic nose for the detection of mycotoxin contaminations in maize kernels O105

09.00-09.15 Monika Hejna, Antonella Baldi, Elisabetta Onelli, Davide Gottardo, Salvatore R. Pilu, Vittorio Dell'Orto, Mauro Zaninelli, Luciana Rossi
Evaluation of heavy metals in intensive animal production systems O106

09.15-09.30 Silvia Parrini, Anna Acciaioli, Antonio Pezzati, Doria Benvenuti, Riccardo Bozzi
Application of FT- NIRS to estimate chemical components of freeze-dry herbages of Tuscany natural pasture O107

09.30-09.45 Marco Isidori, Fabrizio Rueca, Gabriele Mannucci, Massimo Trabalza-Marinucci
Palatability of extruded dog diets supplemented with Ascophyllum nodosum O108

09.45-10.00 Pier Paolo Danieli, Roberto Ruggeri, Riccardo Primi, Francesco Rossini, Emiliano Chiaretti, Catia Stamigna, Domenico Chiaretti, Vincenzo Natoli, Bruno Ronchi
A preliminary nutritional assessment of six genotypes of tef (Eragrostis tef) as potential forage sources O109

10.00-10.15 Marta Castrica, Carlotta Giromini, Raffaella Rebucci, Davide Gottardo, Valentino Bontempo, Antonella Baldi
Evaluation of polyphenolic content and antioxidant activity in agri-food wastes and by-products using chemical and physiological extraction O110

10.15-10.30 Andriyani Astuti, Cuk Tri Noviandi, Ali Agus, Subur Priyono Sasmito Budhi, Ristianto Utomo, Evi Septiani
The effect of duration of storage and storage method on chemical composition and in vitro digestibility of complete calf pellet O183

Thursday June 15th - Room H

Animal food quality and safety - Milk and cheese quality

Chairmen: Andrea Summer, Umberto Bernabucci

08.30-08.45 Sudeb Saha, Giovanni Bittante, Stefano Schiavon, Giacomo Cesaro, Matteo Gianesella, Leonardo Armato, Enrico Fiore, Luigi Gallo
Effects of rumen fluid pH on rumen activity and quality and technological properties of milk O111

08.45-09.00 Massimo Todaro, Raimondo Gaglio, Maria L. Scatassa, Giovanna Sala, Francesca Mazza, Isabella Mancuso, Luca Settanni
Effect of the wooden vats on traditional cheese characteristics O112

09.00-09.15 Federica Salari, Iolanda Altomonte, Rosario Licitra, Mina Martini
Vitamin D content in donkey milk: preliminary results O113

09.15-09.30 Giorgia Stocco, Andrea Summer, Massimo Malacarne, Alessio Cecchinato, Giovanni Bittante
Factors affecting the detailed mineral profile of bovine milk from multi-breed herds O114

09.30-09.45 Claudio Cipolat-Gotet, Alessio Cecchinato, MaryAnne Drake, Bruno Martin, Giovanni Bittante
Influence of dairy farming system and animal factors on sensory descriptors of model cheeses from individual cows O115

09.45-10.00 Matteo Bergamaschi, Alessio Cecchinato, Flavia Gasperi, Franco Biasioli, Giovanni Bittante
Discrimination of dairy systems using the volatile fingerprinting of ripened cheese detected by a fast and non-invasive spectrometric technique (PTR-ToF-MS) O116

10.00-10.15 Alessandro Dalla Riva, Jasmina Burek, Daesoo Kim, Greg Thoma, Martino Cassandro, Massimo De Marchi
Life cycle assessment of Italian mozzarella cheese manufacturing O117

Thursday June 15th - Room AU

Animal food quality and safety - Meat quality

Chairmen: Andrea Serra, Paolo Polidori

12.00-12.15 Andrea Serra, Giuseppe Conte, Elisabetta Giannessi, Laura Casarosa, Carla Lenzi, Alessandro Baglini, Francesca Ciucci, Alice Cappucci, Marcello Mele
Cholesterol content and fatty acid composition of phospholipids and triglycerides are affected by the histological characteristics of semimembranosus and triceps brachii muscle fibre of Maremmana and Limousine bovine breeds O118

12.15-12.30 Roberto Tocci, Eleonora Pippi, Matteo Campostrini, Andrea Martini, Clara Sargentini
Consumer acceptance of ewe meat deriving from traditional and halal Tuscan market O119

12.30-12.45 Alice Cartoni Mancinelli, Simona Mattioli, Alessandro Dal Bosco, David Ranucci, Raffaella Branciari, Cesare Castellini
Geese reared in organic vineyard: qualitative traits of breast meat O120

12.45-13.00 Simone Mancini, Giovanna Preziuso, Sara Minieri, Alessandro Dal Bosco, Valentina Roscini, Gisella Paci
Enhanced antioxidant capacity and reduced lipid oxidation in cooked rabbit burgers added with Zingiber officinale O121

Thursday June 15th - Room T1

Joint session (NF + FQ + APR) - Insects in animal nutrition

Chairmen: Marina Pasquini, Achille Schiavone

11.00-11.15 Maria F. Iulietto, Paola Sechi, Margherita Ceccarelli, Luca Grispoldi, Beniamino T. Cenci-Goga
Exploring consumer perception of entomophagy: data from an online survey O122

11.15-11.30 Andrea Dama, Marco Meneguz, Manuela Renna, Carola Lussiana, Achille Schiavone, Francesco Gai, Enrico Bressan, Laura Gasco
Innovative protein sources in animal diets: bioconversion of vegetable waste with black soldier fly (Hermetia illucens) O123

11.30-11.45 Simona Mattioli, Valentina Roscini, Giacomo Rossi, Gabriele Rondoni, Maurizio Servili, Alessandro Dal Bosco, Mariano Pauselli, Cesare Castellini, Eric Conti
Fatty acids profile of Tenebrio molitor larvae grown in different oil-enriched substrates O124

11.45-12.00 Andrea Osimani, Federica Cardinali, Lucia Aquilanti, Nino Loreto, Sara Ruschioni, Paola Riolo, Maria F. Trombetta, Marina Pasquini
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12.00-12.15 Sihem Dabbou, Marco Cullere, Michele De Marco, Ilaria Biasato, Elena Biasibetti, Maria T. Capucchio, Stefania Bergagna, Daniela Dezzutto, Francesco Gai, Laura Gasco, Achille Schiavone, Antonella Dalle Zotte
Effects of black soldier fly (Hermetia illucens L.) larvae fat inclusion on growth performance, blood traits, carcass characteristics morphometric and histological parameters of finisher broiler chickens O126

12.15-12.30 Marco Cullere, Michael Woods, Giulia Tasoniero, Stefaan De Smet, Eric Claeys, Simone Vincenzi, Elsje Pieterse, Louwrens C. Hoffman, Antonella Dalle Zotte
Apparent nutrient digestibility of broiler quail diets supplemented with black soldier fly larvae reared on two different substrates O127

12.30-12.45 Enrico Bressan, Francesco Gai, Sihem Dabbou, Achille Schiavone, Manuela Renna, Andrea Dama, Marco Meneguz, Giovanni Perona, Ivo Zoccarato, Laura Gasco
Hermetia illucens partially defatted meal in piglets nutrition: preliminary results O128

Thursday June 15th - Room T1

Joint session (NF+FQ) - Dietary strategies and quality of food

Chairmen: Maria F. Trombetta, Giuseppe Luciano

14.00-14.15 Giulia Rossi, Stefano Schiavon, Giovanni Bittante, Giovanna Lomolino, Alberto Simonetto, Claudio Cipolat-Gotet, Marta Cattin, Franco Tagliapietra
Garlic as supplement for lactating cows: consequences on milk and cheese products O129

14.15-14.30 Federica Castellani, Nadia Bignardi, Andrea Vitali, Elettra Marone, Fiorentina Palazzo, Lisa Grotta, Giuseppe Martino
Effect of cow feeding supplementation with olive pomace on the development of aromatic compounds in milk and dairy products O130

14.30-14.45 Mirco Corazzin, Alberto Romanzin, Edi Piasentier, Stefano Bovolenta
Milk fatty acid profile of Italian Simmental cows as affected by pasture type and supplement level O131

14.45-15.00 Lucia Bailoni, Nadia Guzzo, Gianfranco Gabai, Roberto Mantovani
Fatty acids profile in early and commercial milk of dairy cows supplemented with flaxseed during the dry period O132

15.00-15.15 Miriam Iacurto, Germana Capitani, Federico Vincenti, Umberto Bernabucci
Effects of glycerol esters (Butyrflex®) on productive performances and meat quality of Limousine young bulls O134

15.15-15.30 Bernardo Valenti, Valentina Roscini, Giuseppe Luciano, Umberto Rossetti, Alessandro Priolo, Marcella Avondo, Mariano Pauselli
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Friday June 16th - Room T2

Animal breeding and genetics - Other species

Chairmen: Roberta Ciampolini, Fabio Pilla

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11.45-12.00 Simone Ceccobelli, Luigi Orrù, Simone Trabalza, Piera Di Lorenzo, Antonella Lamontanara, Francesca M. Sarti, Alessandro Dal Bosco, Emiliano Lasagna
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12.00-12.15 Agata Szczerba, Takashi Kuwana, Aleksandra Dunislawska, Luiza Chojnacka-Puchta, Dorota Sawicka, Grazyna Plucienniczak, Marek Bednarczyk
Efficiency of in vitro and in vivo chicken primordial germ cells modification O138

12.15-12.30 Martina Zappaterra, Roger Ros-Freixedes, Martina Bordini, Joan Estany, Roberta Davoli
ELOVL6 gene and SNP effects on fatty acid composition of backfat in Italian Large White pigs O139

12.30-12.45 Samuele Bovo, Gianluca Mazzoni, Giuseppina Schiavo, Francesca Bertolini, Giuliano Galimberti, Antonia B. Samorè, Stefania Dall'Olio, Luca Fontanesi
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12.45-13.00 Elena Facchini, Rita Rizzi, Giulio Pagnacco, Giulietta Minozzi
Estimation of genetic parameters for honey production in the honeybee – Preliminary results O141

13.00-13.15 Andrea Talenti, Dayna L. Dreger, Stefano Frattini, Beatrice Coizet, Federica Danelli, Stefano P. Marelli, Amedeo Picchi, Jacopo Riva, Enrico Moretti, Raffaella Cocco, Daniele Bigi, Luigi Liotta, Michele Polli, Gustavo Gandini, Giulio Pagnacco, Elaine A. Ostrander, Paola Crepaldi
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Friday June 16th - Room H

Aquaculture, poultry and rabbit production - Aquaculture

Chairmen: Alessandra Roncarati, Emilio Tibaldi

09.00-09.15 Chiara Ceccotti, Simona Rimoldi, Chiara Ascione, Genciana Terova
Effects of butyrate and taurine supplementation in European sea bass diet based on soybean meal O143

09.15-09.30 Gloriana Cardinaletti, Paola Beraldo, Bianca M. Poli, Maria Messina, Emilio Tibaldi
Including marine microalgae in European seabass (Dicentrarchus labrax) diets: effects on digestive-absorptive functions O144

Friday June 16th - Room C

Animal husbandry and environmental impact - Husbandry systems and environmental impact

Chairmen: Marcello Mele, David Meo Zilio

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Animal welfare, health and behaviour - Small ruminant health and behaviour

Chairmen: Gianni Battacone, Gabriele Acuti

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Herbage intake of Sarda suckler cows characterized by two different calving periods in a mountain pasture O167

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10.30-10.45 Ramy Elgendy, Federica Castellani, Fiorentina Palazzo, Mery Giantin, Lisa Grotta, Mauro Dacasto, Giuseppe Martino
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09.00-09.15 Margherita Ceccarelli, Luca Grispoldi, Paola Sechi, Maria F. Iulietto, Filippo Bertero, Serena Franceschini, Marco Revoltella, Beniamino T. Cenci-Goga
Prevalence of Escherichia coli from lymph nodes of bovine carcasses and detection of stx1, stx2 and eaeA genes with multiplex polymerase chain reaction O172

09.15-09.30 Karama Musafiri, Luca Grispoldi, Isidro García-Meniño, Jesús Blanco, Margherita Ceccarelli, Paola Sechi, Maria F. Iulietto, Rosalia Materazzi, Jorge Blanco, Beniamino T. Cenci-Goga
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09.30-09.45 Paola Sechi, Maria F. Iulietto, Salvatore Barbera, Luca Grispoldi, Margherita Ceccarelli, Filippo Bertero, Serena Franceschini, Beniamino T. Cenci-Goga
Effect of lactic acid bacteria on the quality of beef hamburger under different storage conditions O174

09.45-10.00 Marco Tretola, Ambra Di Rosa, Matteo Ottoboni, Valentina Caprarulo, Carlotta Giromini, Francesco Leone, Vittorio Dell'Orto, Vincenzo Chiofalo, Luciano Pinotti
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Joint session (NF + FQ) - Dietary strategies and quality of food

Chairmen: Miriam Iacurto, Giovanni Savoini

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11.45-12.00 Claudio Forte, Raffaella Branciari, Massimo Trabalza-Marinucci, Gabriele Acuti, Dino Miraglia, David Ranucci, Rossana Roila, Roberta Ortenzi, Michela Codini, Andrea Valiani
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Perugia, June 13th–16th, 2017
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Agenda for poster presentations

ANIMAL BREEDING AND GENETICS

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ANIMAL HUSBANDRY AND ENVIRONMENTAL IMPACT

AQUACULTURE, POULTRY AND RABBIT PRODUCTION

ANIMAL WELFARE, HEALTH AND BEHAVIOUR

ANIMAL FOOD QUALITY AND SAFETY

NUTRITION AND FEEDING

Foreword

The 22nd Congress of the Animal Science and Production Association (ASPA) is hosted in Perugia, a wonderful university city with a long tradition in agriculture, veterinary and animal sciences. The Congress is the result of the precious collaboration among the Department of Agricultural, Food and Environmental Sciences and the Department of the Veterinary Medicine of the University of Perugia as well as other Italian scientific Institutions such as the Università Politecnica delle Marche (Ancona), University of Camerino, Università degli Studi della Tuscia (Viterbo) and the Council for Agricultural Research and Economics – CREA ZA (Monterotondo, Roma).

This edition of the ASPA Congress has received a total of 333 scientific contributions: 180 oral presentations and 153 posters have been selected. The Congress sessions with their relative number of papers are listed as follows: Animal breeding and genetics (97), Nutrition and feeding (70), Animal food quality and safety (53), Animal welfare, health and behaviour (43), Aquaculture, Poultry and Rabbit production (40), and Animal husbandry and environmental effect (30).

I would like to point out that 18 main lectures will be presented across all the scientific sessions.

I wish to thank first of all the Organizing Committee, Dr Emiliano Lasagna (Chairperson), Dr Gabriele Acuti (Secretary), Dr Stefano Capomaccio, Professor Alessandro Dal Bosco, Dr Miriam Iacurto (Secretary), Professor Paolo Polidori, Professor Maria Federica Trombetta. I also wish to thank all the members of the Scientific Committee, Professor Cesare Castellini (Chairperson), Professor Umberto Bernabucci, Dr Luca Buttazzoni, Dr Katia Cappelli, Dr Simone Ceccobelli, Dr Andrea Giontella, Professor Nicola Lacetera, Professor Massimo Trabalza-Marinucci (Secretary), Professor Luciano Morbidini, Professor Marina Pasquini, Professor Mariano Pauselli, Professor Alessandra Roncarati, Professor Francesca Maria Sarti, Professor Maurizio Silvestrelli, Dr Carmela Tripaldi.

Special thanks to Professor Oliviero Olivieri, President of the Congress, for the contribution of the University of Perugia in the organisation of the Congress. Additional thanks to the Technical organizing Secretariat, MV Congressi SpA, in particular to Mrs Roberta Pasquini. Indeed, I would like to congratulate and to thank all those persons who have organised the meeting and have collaborated in reviewing the summaries and thank them for what they have done for ASPA and for the Italian Animal Science. A special thank goes to Professor Rosanna Scipioni, Editor-in-Chief of the Italian Journal of Animal Science, for having contributed to the edition of these proceedings.

Finally, I acknowledge the University of Perugia and the institutional agencies, in particular the Ministry of Agricultural, Food and Forestry Policies (MIPAAF), the Region Umbria, the Municipality of Perugia and the main sponsors.

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IS001

Livestock welfare issues in Europe or what is needed to maintain livestock production in the future?

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More than any other sector, agriculture in many Western European countries is the focus of public attention. Examples are discussions on the killing of one-day old male laying chicken, amputations or year-round tie-stall housing of livestock. In particular, modern livestock production already lost against the background of this criticism a significant part of its reputation. Following, livestock farming not only in these countries is facing major challenges, also because a continually increasing alienation of the society from livestock farming is observed since years. “Industrial livestock production” or “factory farming” (in the US) has become a synonym for less animal-friendly husbandry systems. This is very frequently discussed in the media, while being strongly negative connotated (McCarty 2005). This is taking place, even though scientifically-based knowledge on the relationship between farm/herd sizes and animal welfare are very rare (Meyer-Hamme et al. 2016). In the whole society, even if on different levels, a negative attitude towards “industrial livestock production” and the associated production methods is observed (Busch et al. 2013). But, are the problems discussed actually existing or are they constructed and overestimated? The question arises where fields of problems and needs for actions in practical livestock farming in Europe are and which requirements have to be fulfilled for positive changes.

In the following, specific problem areas, challenges but also opportunities will be demonstrated based on examples from fattening pigs, dairy cattle as well as poultry production. Meyer-Hamme et al. (2016) examined conventional fattening pig farms in Germany using the Welfare Quality® protocol. The authors concluded, that in terms of animal welfare the overall situation on farms has to be considered as unsatisfactory. This mainly applies to factors such as behaviour and health, thus diseases, injuries and other harms to the animals, which are caused by housing conditions. Inter alia, overcrowding and insufficient water supply, and therefore welfare-relevant aspects were identified as major problems. Approximately 40% of the observed pens were over-crowded (in respect to legal regulations) and these pens were found in 92% of the farms.

In general, animal health has to be considered as critical and requires significant improvements for all livestock species (Gauly 2015). Two examples, which emphasize the close relationship of this problem to animal performance and breeding. According to Sandilands (2011), 53% of the laying hens suffer from at least one bone broken during the laying period, which most probably is caused by the extremely high laying performance of the genotypes. Studies on the relationship between yield levels and animal health also display considerable problems (Roffeis and Waurich 2013). Beside the association to performance, a low proportion of not veterinary treated, thus healthy, cows is noted. Its proportion only ranges around 10% of the animals. The culling age of Holstein-Friesian dairy cows in Germany averages 5.4 years, just about two lactations. This clearly has to be considered as too low (Römer 2011) and points towards problems in terms of animal health. Longevity (duration of the productive live in months from first calving to culling) shows a similar, unsatisfactory situation on the phenotypic side. There are two hypotheses on the explained development in all livestock species: on the one hand, the high genetically-based performance cannot or only hardly be handled by many farmers. On the other hand, it has to be noted that for certain developments (e.g. increases of yields) the current housing systems in practice are not feasible. Against this background, the new opportunities of genomic selection provide opportunities and risks at the same time.

The fact that dairy cattle, despite the identified problems, are positively perceived by consumers in terms of welfare when compared to other livestock production systems in many countries, is most probably due to the visibility of the animals on pasture and its perceived and partly real advantages in terms of sustainability and welfare. But, welfare and health of the animals also depend whether or not they are able to express their natural behaviour. This seems to be the case when dairy cows are on pasture (Armbrecht et al. 2015). Consequently, the development of year-round indoor husbandry systems, which is taking place in many European countries (Reijis et al. 2013), may lead to a considerable risk for the reputation of livestock farming in general.

The overall aim of all efforts should be to keep livestock farming in Europe! Various issues have to be modified to achieve this goal. In order to attain a wide acceptance of the public for a modern livestock production, identification and a clear and honest announcement of problems, compliance of legal standards, establishment of an on-farm self-evaluation, good expertise and training of farmers are necessary. Beside, the factor “emotions of animals” and its relevance for animal welfare and consumer perception is essential. The specific aim for animal science has to be the improvement of husbandry systems, production techniques and breeding products towards superior animal protection and environmental standards. Furthermore, it should contribute to a better elucidation and strengthen transparency.

Only with joint efforts the future of livestock farming can be ensured in many parts of Europe.

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IS002

Genetic adaptation of livestock to the challenges of climatic changes

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By 2100, it is predicted that the global average earth temperature will increase by 1.4 °C to 5.8 °C. Global average annual precipitation will also increase with significant variation between regions. Extreme climatic events will become more common (IPCC 2014). Overall, we will be living on a hotter but also a more humid planet. Moreover, human population will continue to increase with estimation around 7 to 15 billions people by 2100. Not only will our livestock and crops need to adapt but they will need to produce more and sustainably. Fortunately, livestock species, breeds or/and populations, throughout their genetic history have became adapted to nearly all agro-ecologies and environments (FAO 2015). Livestock genetic diversity is a treasure drove of adaptation to environmental challenges, which, when characterised will represent major entry points to mitigate the impact of climatic changes.

The field of livestock genetic diversity characterisation may be divided into two overlapping phases. In the 1990th and early 2000th years, the focus was the understanding of the origin and geography distribution of livestock diversity. Major progresses were achieved in resolving the center of origin and history of our main livestock species and while some unknowns remain, we do now understand the human evolutionary driven history of most of our domesticates (FAO 2015). The importance of these early genetic characterisation works should not be underestimated. Selection, genetic drift and admixture are the three main factors shaping the genetic diversity of a species or a breed. Unravelling these processes are providing baseline knowledge for the second phase, the identification of functional diversity and the causative mutations underlining it. This has been greatly facilitated through the availability of livestock genome of references and new genomic tools and information (e.g. genome wide high density Single Nucleotides Polymorphisms (SNPs) chips, full genome sequences).

Adaptation to climatic changes is an important component of this functional diversity. The genetic mechanisms of such adaptation are expected to be complex. They should not only be understood as adaptation to temperature and/or water availability, but also as adaptation to the consequence of these two, such as changes in infectious and parasitic diseases distribution, forage availability and changes in vegetation cover and/or plant species etc. Similarly, heat tolerance is a physiological trait dependent on the complex interactions between many factors, including properties of the skin and hair, sweating and respiration capacity, metabolic heat production, behaviours etc. Researchers have focused so far on the identification of adaptation to extreme climatic environments. For examples, comparative genomic analyses among sheep living in contrasting environments, including arid *versus* humid zone and desert *versus* temperate environment, have identified genes pathway associated with water reabsorption (Yang et al. 2016). In chicken, a species where the wild ancestor is naturally adapted to a diversity tropical environment, a north – south comparison of chicken breeds identified signatures of selection in northern European chicken linked to physiological processed related to energy homeostasis (Fleming et al. 2017). However, it is in cattle that the search and the identification of genetic adaptation to the challenges of climatic changes are probably the most advanced. An early study, which compared on station the heat tolerance of selected breeds and crossbreed of temperate *Bos taurus*, tropical *B. taurus*, and *B. indicus* cattle, showed that tropical *B. taurus* breeds, which include Senepol, had a level of heat tolerance similar to that of *B. indicus* (Hammond et al. 1996). In the Senopol it was shown that its heat tolerance is due to its short and sleek hair coat under control of a single dominant gene (SLICK). A recent study identified the prolactin receptor (PRLR) as the likely candidate gene for the slick phenotype (Littlejohn et al. 2014). More recently. Kim et al. (2017) compared indigenous African cattle with commercial breeds developed in temperate areas. They identified remarkable long-range haplotypes across tropical African cattle within a heat tolerance QTLs, in one of the cattle heat shock proteins, in a chromosomal region including SOD1 (a gene knows to bind to heat shock proteins), in physiological pathways involved in regulating skin blood flow as well as at the prolactin releasing hormone gene region. As in the Senopol the prolactin signalling pathway is likely playing here a major role in thermoregulation.

Application of these findings to breeding improvement programs may follow different routes. If the identification of the candidates causative mutation linked to the adaptive traits is opening the door to genome editing, the identification of a relevant chromosomal region may be sufficient for the design of a genetic introgression program (Dikmen et al. 2014). Also, taking into account the quantitative nature of many genetic adaptations, genomic selection approaches may be more appropriated (Hayes et al. 2009). These approaches all required a prior understanding at genome level of the nature of the adaptations. It is a matter of urgency before our livestock genetic diversity fade away.

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IS003

Precision feeding

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Precision feeding (PF) applies the research results of traditional nutrition as well as linked areas of animal nutrition in order to meet the specific nutritional requirements of a specific animal or group of animals kept under definite conditions with maximum accuracy. This means that the nutrient availability does not limit animal's performance and animal welfare, in the interest of a safe, high quality and efficient production, in an economically viable and environmentally sustainable way. For these purpose various principles have to be considered. The first is the use of a precise matrix for both nutrient requirement and nutrient content of the ingredients, as well as the use of adequate nutrition models to estimate nutrient availability and evaluating the real fulfilment of the requirements. In addition the proper use of modifiers (prebiotics, probiotics, etc.) and other feed additives should be included, as well as the reduction of toxicants and antinutritional factors. Furthermore, the use of improved feed and feedstuff processing techniques that will lead to better nutrient utilization should be considered. Finally, the monitoring of the animal response is crucial in a PF system. With the availability of many animal monitoring systems, we can get useful information to better understand the nutritional framework, optimize the diet, and offer daily a more consistent and balanced ration. The examples below refer particularly to the PF in dairy herds.

Variability of feedstuffs characteristics and then of diets, particularly in dairy cows, is receiving increased attention because of its effects on cow performance, farm profitability, and on the environment. Control of this variation can occur during diet formulation, diet preparation, or at the time feed ingredients are sourced. Forages are among the most variable feed ingredients. It is quite difficult to maintain the consistency of dietary composition with the traditional approach by sampling feeds for laboratory analysis. The progress in NIR technology and the availability of portable NIR at an affordable cost, allow to analyse on site the forages and grains. Today are also available systems based on NIR technologies applied directly on the TMR wagon. These systems are able to control on-line the changes of the main nutrients contents of the feedstuffs and perform automatically actions in order to keep the final TMR as consistent as possible during the time. The first experiences were carried out by managing the rations on dry matter basis instead of as fed basis, by using analysis of NIR scanner installed on the TMR wagon. This approach can result in a more constant amount of DM delivered every day, and more stability of chemical and nutritional characteristics of the TMR, particularly for diet based on feeds with high and variable humidity (i.e. silages). In a recent study on dairy farms, the adoption of this system allowed the improvement of feed efficiency, with an increase of income over feed cost of 0.025 € per kg of milk. The availability of automatic feeding system makes these systems of analysis in real time even more interesting, allowing a more complete automation.

The estimation of the nutrient requirements is the other critical point in balancing the diet to fulfil the real requirement of the animals. Traditionally these estimations are performed during diet formulation. The availability of systems that can measure continuously the animal performance may allow to obtain useful information to the proper definition of the specific requirements, allowing a continuous and constant readjustment of the diet to better fulfil the real needs of groups of animals or possibly of each animal. Precision feeding with individual daily-basis feeding compared with group-feeding in growing pigs can significantly reduce digestible lysine intake by 26% and nitrogen excretion by 30% and feeding costs by 10%, without compromising the performance of growing pigs. In dairy cows the introduction of TMR have simplified and automated the feeding of cows and have allowed for substantial increases in milk production. However, feeding TMR has some limitations. One of the main is that some cows in the group receive more and some others less nutrients than they actually need. Based on the availability of daily milk yield, milk fat and protein content, and live weight, a system was proposed and able to allow preparation and delivery of different feeds (in both quantity and composition) in milking parlour according to the individual requirements calculated by the system, for an individual dynamic feeding approach. This system allows feeding a basal TMR with a low nutrient density available to all cows, and then only those needing more nutrients receive the necessary supplements during milking.

The interaction between nutrition, endocrine and immune system, and effects on metabolism and their relationships with health are important tasks. The physiological regulation is the most critical factor in animal productive capacity, and the genetic capacity can only be utilized if the environmental conditions, including diet, meet the demands of the animal, and animal is in optimal health and welfare conditions. The technology development offers the possibility of animal monitoring, including some physiological measurements. Information of these physiological changes can be obtained in dairy cows by automated measurement of milk yield, of the main milk composition (fat, protein), and particularly through the measurements of other milk components and metabolites, offering information to optimize diet and nutrition in the short and or long term. Milk fat and protein are proposed in order to obtain information on ketosis and sub-acute rumen acidosis. The on line measurement of urea and β-OH-butyrate in milk was proposed in order to optimize nitrogen and energy nutrition. Recent results have shown that the mid-infrared analysis of milk may be a viable method of predicting individual cow energy status for a large scale of application. Other biosensors, beside the system online milk monitoring, are available. A body condition score recording system based on analysis of images collected by using 3D-camera was proposed as a valuable tool to support evaluations of feed efficiency and health. Recently boluses measuring pH continuously and using wireless telemetry have been used for research purposes. Ruminal pH is an important parameter for nutritional status. The benefits of rumen pH are reduction in feed costs and a reduction in risk by identifying the immediate effects of management and diet changes. It was also proposed an adjustment of the concentrate allocation rate in early lactation based on rumination time continuously and automatically recorded, obtaining a potential effect on energy corrected milk yield in primiparous cows. All this information obtained with these technologies have to be recorded in a single database and articulately processed. Some researches in dairy cows have suggested that better decision support for the dairy farmer on daily questions around feeding, is made possible using a variety of big data sources containing static and dynamic sensor data of individual cows. All this information in a PF system will allow to optimize the diet and better meet the individual nutritional requirements, improving health and welfare, resulting in a greater efficiency in a more economically and environmentally sustainable way.

IS004

Politics of risk: questioning the naturalization of danger in meat production and consumption

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From an anthropological perspective, food is considered more than a mere issue of nutrition. Food production and consumption has been recognized as a symbolic practice in different societies in order to set up, maintain or overcome boundaries, cultural obligations or social hierarchies. Therefore, food can be understood as a "total social fact" (Mauss 1925) that constitutes a prism to observe broader societal dynamics. In this perspective, we will explore a range of interconnected issues.

In our presentation, the relation between risk and meat production/consumption will be investigated by combining anthropological literature with empirical material from a qualitative research in Umbria (Central Italy). Cultural and political strategies to avoid the risk of contamination have been at the centre of many classical anthropological work, in particular in relation to why certain food is considered “taboo” and other is not. In her ground-breaking and sophisticated reading of lifestyle, danger and food consumption, Mary Douglas (1966) considers the establishment of food taboos such as pig meat that is reputed as impure in Jewish and Islamic cultures, as practices of social distinction rather than misplaced attempts of primitive health practices. Lévi-Strauss (1962, 1966) considers food taboos as forms of classifications that are regarding the modes in which different beings are either distanced from each other or related through specific regimes of exchange and reciprocity. Tambiah (1969) shows how different levels of social distance, marked by rules of marriage and sex regulation, and scale of spatial distance in domestic activity, are correlated with specific “edibility distance” between human and domestic and wild animals. In a more radical fashion, recently the so-called “ontological turn” in anthropology has questioned the “great divide” between the natural and the cultural sphere on which relies much of Western thinking about relations between human and non-human animals (Koensler and Papa 2013). “Who should eat whom, and who should cohabit with whom?”, asks Donna Haraway (2008) in her cyborg manifesto *When Species Meet*. In this text, the chapter, entitled “We have never been human”, alludes and plays with Bruno Latour’s (1993) work, inspired by the long-standing anthropological legacy to question our taken-for-granted assumptions about how we perceive ourselves within society.

The ambiguities and contradictions of strategies to avoid risk emerge also in our examples from empirical and narrative material based on a qualitative data deriving from focus groups realized in Umbria with organic poultry farmers, gym-goers, organic buying group organizers and researchers in animal disease control and prevention. The research highlights social and political practices that are employed either by individuals or institutions in order to avoid or mitigate risk in everyday consumption of poultry meat. On a deeper level, these practices are depending on what is precisely perceived as a risk and what not, what seems to present itself as “natural” and what not, questioning the way how danger is naturalized.

In this context, two salient tensions emerge in the politics of risk. First, one tension occurs out of contemporary disputes over the exploitation of animals and how far rights of “persons” should be extended, if non-humans that have specific sentiments, agency and moral capacities should be included or excluded in risk mitigating strategies. In other words, disputes over how to treat animals and about the legitimacy or not to eat meat are questions that employ scientific, economic or political arguments in order to interrogate and redefine the boundary between humans and non-humans. In particular, industrial livestock production has been transformed animals into consumer commodities, in opposition of an understanding of some non-human animals as persons (Milton 2009). Animals can be understood either as “things” in terms of their benefit to others or as “persons”: as moral beings for whom activists fight to extend rights and debate their status as individuals, as happens for example in animal rights movements (Manceron and Roué 2009). The research demonstrates that meat consumption implies numerous contradictions, based on an unusual mix of specialist knowledge and personal experiences.

Second, another tension in the politics of risk arises in relation to disputes over how to regulate meat processing in order to avoid health risks, as well as mitigating implications on animal welfare. Within the realm of increasingly complex global meat chains, public scandals like the “mad cow” have attracted much attention. Another example has been the so-called “horse meat scandal” in which horse meat was mixed into processed beef products during complex food supply chains involving sub-contractors and suppliers in Luxemburg, the Netherlands, Cyprus, Poland and Romania. Rather than being part of a “natural” risk associated with breeding, an anthropological perspective goes beyond a search for more technical solutions. The research confirms that many consumers prefer personal relations in order to establish trust in the traceability in the products they buy, including in collective buyer groups (Martino et al. 2016; Papa 2012).

Focusing on everyday practices of farmers and food producers, an anthropological perspective explores how existing norms and regulations have always been in different degrees silently challenged, eroded or subverted in everyday practices (Papa 2015). Emblematically, the rapidly growing movement “Genuinely Clandestine” in Italy unites small-scale independent growers who oppose the EU-driven regulations of food certification, considered to be driven by global agribusiness interests and promotes so-called “participatory self-certifications” that aim to be more inclusive and democratic for the production of “genuine” food products (Koensler 2015). In contrast to ethical consumerism like the “slow food” movement, these practices are provocatively situated in grey legal spaces and re-appropriates the right to certify the quality of food in more democratic and inclusive ways (Koensler 2016). These elements show how a growing attention to risks of food chains cannot be met only with more sophisticated forms of control of supplier chains or other forms of surveillance, but rather needs to be considered a prism to understand broader political and socio-cultural elements that can be changed, rather than practical problems that be solved through technical interventions.

In conclusion, we emphasize the importance of an anthropological perspective that allows to question how political interests are masqueraded and hidden behind the veil of practices and discourses of “naturalization”. In this way, in order to explore contradictions in contemporary politics of risk we

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remark the necessity to "question the unquestionable" (Butler, 1997), also with particular regard to what seem natural patterns of food consumption.

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IS005

Food fact versus food fiction - The never-ending struggle between unbiased information and preconceived ideologies

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No diet could be better - healthy, good and balanced, the Italian population's secret of long life. At least, that is what the rest of the world thinks about how we eat. Only last week, the *Bloomberg Global Health Index* placed Italy first out of 163 countries; and our diet, that comprehensive array of essential foods surely deserves much of the credit for this. Hardly cause for dispute. Not so here at home, it would seem. With our products in worldwide demand and exports soaring, we are forced to witness the staples of Italian food - fresh and cured meats, sugars and carbohydrates, even some of our olive oils! - on the receiving end of consistently bad press at the hands of minority groups as fierce and loud-voiced as they are small. And whose communication strategy proves enormously damaging while devoid of scientific grounds. Minor incidents or the criminal behaviour of a few are fed to the public through the mass media and made to appear as the norm, casting a denigrating and undeserved shadow over the entire food industry.

Vegetarians, vegans, animal-rights activists… perfectly respectable individual choices take centre-stage and are voiced through self-appointed, usually unqualified opinion makers leading one-sided TV and media discussions, passing judgement on this or that food or on the supposed impact of intensive livestock farming on the environment or on animal welfare. One wonders how many of these people have ever actually visited a livestock farm or can honestly say they are in a position to assess the implementation of European rules on animal welfare.

All too many journalists take the opportunity of privileged access to television and media exposure in order to spread their own biased opinions and convey a distorted picture of our livestock industry - which, indeed, they would abolish altogether. This is tantamount to misinformation and certainly does not match our idea of the role of the press and investigative journalism, by no means at issue here. If animals, as they would have it, have rights just like humans do, then why is no mention ever made of the 1965 Brambell Report? A document that guarantees the 5 freedoms and offers a guide to farmers engaged in biosecurity protocols and in the fight against anti-biological resilience, in line with the EU's One Health vision.

And speaking of information, what about those celebrity chefs, now all the rage on television, who are supposed to provide consumers with advice on nutrition and balanced diets and actually do little more than just entertain? Now, this is a crucial topic that requires handling with the utmost care - let us just consider the political decisions on school lunches and children's diet. Reports of children removed from vegan parents' care because of intellectual disability and serious malnutrition caused by animal protein deprivation from infancy alert us to the extreme care required when addressing the general public on these all-important issues. Granted, some people can manage without animal protein, but as a rule it is a fundamental factor in the development of human intelligence and it is irreplaceable in children, athletes and people undergoing heavy pharmacological therapy. In any case, excluding animal protein from the diet must be done under expert supervision, if serious health problems are to be avoided, such as deficiencies in vitamin B12, heme iron and other nutrients that only meat and animal products can provide.

As we said, veganism is an individual choice that we respect, but it goes against our omnivorous nature: our intestines and teeth are proof that we were intended to eat meat and not spend hours on end chewing like chimpanzees (which, by the way, occasionally do eat meat!).

So much for the more general misconceptions and confusion.

More specifically, increasing amounts of meat-sounding vegan foods with absolutely no animal content are now making their way onto supermarket shelves. What guarantees have we that consumers are not being misled? Prominent EP members Paolo De Castro and Giovanni La Via have recently raised this issue and, in response to a question from another influential member of the European Parliament, Renate Sommer, the EU Commission has already expressed its concern over information that can lead to "*misunderstanding regarding the characteristics of a food*" when "*a naturally present component or ingredient normally used in that food has been replaced by a different component or a different ingredient*". Aside from any legal aspects, the fact that consumers may be led to believe they are eating a perfect substitute for a steak or other meat-based food - when they are not - is cause for great concern.

Of course, vegetable foods are an essential part of the make-up of a well-balanced diet, yet at the same time there is no disputing that meat, milk and eggs contain particular nutrients that are not available elsewhere with the same bioavailability characteristics. Crackers, prepacked vegetarian steaks and the like, usually made from soya, wheat, oats or legumes, for instance, and which are commonly sold in supermarkets, are almost completely lacking in fundamental minerals such as iron and zinc.

Basically, all we are asking for is correct and unbiased information, which can only stem from an objective and professional approach to food and nutrition issues. We are ready to play our part in driving out those few who do not follow the rules, but we believe that we deserve respect, as our industry contributes to the social and economic development of this country with a turnover in excess of 30 billion euros and a workforce of more than 180,000.

IS006

Foods of animal origin – how do we help the consumer understand sustainability implications of diet choice?

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The world population currently stands at over seven billion people and by 2050, is predicted to surpass nine billion, requiring a 48% increase in food production (FAO 2017). This population growth will also confer increased competition for resources, therefore the need to "produce more, using less" will be increasingly important. Livestock provide a myriad of nutritional, economic and social benefits, yet foods of animal origin are often perceived as having undesirable environmental impacts. As many consumers have no connection to animal agriculture, overcoming food production myths propagated via traditional and social media sources is a significant challenge to the global livestock industry (Capper and Yancey 2015).

Sustainable intensification is key to reducing environmental impacts of livestock production while improving productivity and global food security (FAO 2006). In the USA and other regions, improved animal nutrition, management and genetics over the past century have considerably reduced the environmental impact per unit of animal-origin foods. For example, improving cattle growth rates and slaughter weights between 1977 and 2007 resulted in 19% less feed, 33% less land, 12% less water and a 16% reduction in GHG emissions per unit of beef produced in the USA (Capper 2011). Similarly, a four-fold increase in milk yield per dairy cow between 1944 and 2007 allowed for reductions in feed use (77%), land use (90%), water use (65%) and GHG emissions (63%) per kg of milk in modern U.S. systems (Capper et al. 2009).

The greatest environmental mitigation effects may be conferred by management practices that allow livestock to perform to their genetic potential, optimizing key performance indicators (KPI) such as growth, yield, fertility, nutrition, health and product quality (Capper 2013b). These KPI have the advantage of being irrespective of system, operation size or region. For example, if calving rate in beef cows improves from 60% (characteristic of Brazil, Argentina and South Africa) or 90% (the U.S. average) to the ideal (100%), by

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whatever practices, tools or technologies are appropriate to the system, environmental impacts and economic costs are reduced (Capper 2013a). Compared to an ideal (100%) calving rate, a 90% calving rate increases land use by 8,1% and water use by 5,2% per unit of beef, whereas land use is increased by 53,2% and water use by 34,1% at a 60% calving rate. As calving rate improves, GHG emissions decrease from 7.945 kg CO_2-eq/363 kg beef (60%) to 5.841 kg CO_2-eq/363 kg beef (90%) or 5.459 kg CO_2-eq/363 kg beef (100%) and with a 36,3% decrease in feed costs per unit of beef between a 60% and 100% calving rate.

Foods of animal origin are generally associated with greater GHG emissions per unit of food compared to plant-based proteins, yet the perception that low-carbon diets are inherently sustainable is not necessarily accurate, as foods with the lowest GHG emissions (sugars and syrups) are not analogous with healthy diets (Drewnowski et al. 2015). Campaigns such as “Meatless Mondays”, which advocate for reduced meat consumption on the grounds of cutting dietary GHG emissions are gaining in popularity, yet these schemes may have relatively little impact, even if widely adopted. For example, in the UK, milk and meat production contribute ~6.0% to national GHG emissions (Department of Energy and Climate Change 2016). To take the simplistic view that a 1 day per week withdrawal from meat consumption would therefore cut meat production by one-seventh, if every one of the UK’s 65 million inhabitants adopted this dietary change, the projected annual reduction in national GHG emissions would be equal to less than one percent.

Improved monogastric feed efficiency is often cited as evidence for reducing consumption of beef or dairy products, however, given current concerns regarding competition between livestock and humans for feed and food crops, assessing feed efficiency per unit of human-edible energy or protein input vs. output rather than the traditional mass-based approach may be a better mechanism for assessing food sustainability. Due to the quantities of human-inedible forage used within the diet, both dairy and forage-fed beef produce a greater amount of human-edible food than they consume, with 0.71 kg and 0.92 kg human-edible protein input per kg of human-edible protein output for dairy and forage-fed beef respectively (Wilkinson 2011). In addition, to the efficient use of human-inedible by-product feeds, livestock production also yields multifarious by-products, including hides, fertilizer, tallow and pharmaceuticals. It is not clear what the global environmental impacts would be from replacing manure with inorganic fertilizers or pharmaceutical ingredients with synthetic alternatives.

Improving sustainability in the face of a consumer market that is increasingly sceptical about food production transparency and management practices is a considerable challenge. A relatively small percentage of consumers believe that the agricultural industry is transparent, yet the majority trust food producers (Capper and Yancey 2015). Livestock producers therefore have significant opportunities to explain management systems and practices to the consumer, thus improving the social acceptability of foods of animal origin. Sustainability will continue to be an issue for livestock producers worldwide; however, there is no single solution or one-size-fits-all system. Instead, improving productivity and efficiency throughout the industry; allowing producers access to management practices that confer productivity gains; and communicating the environmental gains made by livestock industries are all important components of maintaining and improving consumer acceptability of foods of animal origin.

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IS007

Gastrointestinal tract functionality and its relationships with eubiotics

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Optimal gastrointestinal functionality is essential for sustainable animal production. Effective functionality (digestion and absorption of feed) of the gastrointestinal tract (GIT) and its health (effective immune status, normal and stable microbiota, absence of inflammatory state) are important factors in determining animal performance (growth, milk yield, meat and egg quality). It is clear that several, complex mechanisms are involved in GIT functionality and health, therefore it is crucial to deepen our knowledge of these interactions so that strategies for the modulation of GIT functionality and health, in context of improved animal performance, can be developed. Over the last few decades, the adoption of genetic selection for high growth and reproductive traits, the implementation of advanced husbandry techniques (hygiene, vaccination, housing, transport, etc.), clarity of understanding in digestive physiology and dietary requirements of farmed animals has led to significant improvements in productive performance. In this regard, a crucial question for animal scientists is: "has farm animal performance reached its genetic/physiological limits"? It is within this context that the concept of "gut health" has started to attract significant interest within the animal science community (Kogut and Arsenault 2016). However, while gut health is an increasingly important topic in animal nutrition, a clear scientific definition is still lacking although it has been used repeatedly in animal health (Kogut and Arsenault 2016). A clear definition of gastrointestinal health and functionality and how it can be measured is required to monitor animal health and to evaluate the effects of any nutritional intervention on animal performance. While in human medicine gut health is often associated with the "absence of clinical diseases", this definition cannot be applied to farm animals as it is well known that animal performance can be impaired without any clinical signs of disease.

As initially proposed by (Conway 1994) there are three major components of gut health namely the diet, the mucosa, and the commensal flora. The mucosa is composed of the digestive epithelium with its specific structure, the gut-associated lymphoid tissue (GALT) and the mucus overlying the epithelium. The GALT, microbiota, mucus layer and host epithelium interact, forming a complex and dynamic equilibrium within the GIT that ensures efficient functioning of the digestive system. Perhaps a more comprehensive definition of gut health would be "a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction". This definition combines the principal components of gut health, namely diet, effective structure and function of the GIT barrier (mucosa) and normal and stable microbiota, with effective digestion and absorption of feed and effective immune status. All these components play a critical role in GIT physiology, animal health, welfare (including animal behaviour) and performance. Clarity of understanding of gut health will require the characterization of the interactions between these components.

Modern animal production has consistently been intensified in order to improve productivity therefore optimal gastrointestinal health and functionality are essential for sustainable animal production. Reducing costs by increasing stocking density generates the risk that infectious diseases, being of viral, bacterial or parasitic origin, can rapidly spread through a flock or herd. In the past, such diseases were largely controlled by the prophylactic supplementation of farm animals with antibiotic growth promotors (AGPs). Even in the absence of clinical diseases, these compounds exerted a beneficial effect on performance of growing farm animals, since they were capable to modulate the gut microflora in a positive direction. Since the ban of feed additive antibiotics in Europe in 2006, the incidence of intestinal problems has steadily increased. In order to offer the farm animal industry non-therapeutic alternatives for optimizing their operations various concepts, based on probiotics, prebiotics, organic acids and essential oils have been proposed. Since these products are supposed to change the GIT microbiota composition and activity from a dysbiotic into a eubiotic status and the compounds are considered to work differently from classical antibiotics, this product group has been named Eubiotics. Eubiotic products are able to beneficially modulate the gut microflora and thereby to transform a dysbiosis situation in the GIT into an eubiosis. Reports from controlled and field studies show that these concepts work, but on their own might not be powerful enough to consistently and reliably prevent digestive disorders and impairment of performance in farm animals under the current intensive husbandry conditions. Moreover, nutraceutical strategies have been reported to support the immune system and to modulate the oxidant/antioxidant balance and the inflammatory response (Celi and Gabai 2015; Chauhan et al. 2014; Cottrell et al. 2015).

The intestinal microbiome has the potential for both beneficial and harmful effects on the host animal. The use of prophylactic antibiotics as growth promoters has, to some extent, removed the need for careful study of the microbiome of the intestine and how this could be used to enhance productivity. Opportunities to control the GIT microbiota are considerable and in time this may extend to management of a specific equilibrium to avoid bacterial blooms and to generate useful compounds such as vitamins, peptides, fatty acids and energy. Thus, as AGPs are phased out there is an exciting opportunity to align husbandry (including biosecurity) with nutrition to ensure that the intestinal microbiome is buffered against rapid flux and to deliver a net advantage to the animal. Exogenous enzymes will form part of the solution but will be most helpful when associated with knowledge on ingredient quality (chemical and hygienic), substrate concentrations, nutritional gaps and relative nutritional value of the target feed. In conclusion, management of a post-AGP animal production paradigm requires increased routine surveillance of animal health and nutrition. Landscape analysis of feed ingredient quality, toxins and antinutrients, substrates, drinking water and animal health metrics on a global basis would greatly enhance the ability of the industry to align appropriate interventions to maintain optimal performance, welfare and sustainability.

The translation of the current knowledge into practical applications for animal scientists and nutritionists needs to be made a priority by the scientific community. This would allow the achievement of a better insight in the evaluation and in the progression of several GIT conditions and associated nutritional interventions to obtain optimal GIT functionality (a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction).

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IS008

Nutritional impact on lameness in dairy cattle

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Lameness in dairy cattle is a prevalent, painful and internationally endemic production disease in contemporary dairy herds. (See https://dairy.ahdb.org.uk/technical-information/animal-health-welfare/lameness/#.WLVawPLCDhU). Its aetiology is complex. It has been associated with selection of cows for milk yield, intensification of nutritional supply and adverse housing environments. Recognised causes include infectious digital dermatitis and "aseptic" claw horn lesions. Major symptoms of claw horn lesions include bruising and haemorrhage brought about by compression of the pedal bone on underlying soft tissues. Causation may include inflammation of the vascular dermal tissue as in laminitis and/or breakdown of laminar dermal connective tissue, catalysed by matrix metalloproteinases, and occurring particularly around parturition in response to physiological signalling by systemic hormones.

Important nutritional aspects concern meeting requirements for production and maintenance of important anatomical structures, particularly the normal suspensory apparatus supporting body weight, and the systematic provision of substrate for production of horn. These are in addition to preventing digestive imbalances brought about by excessive fermentable carbohydrate, inadequate buffering for rumen pH, and diets and systems of feeding predisposing to subacute ruminal acidosis (SARA). A role in SARA has been proposed for endotoxins arising from breakdown of bacterial cell walls in the rumen and with absorption into systemic circulation further along the digestive tract.

Important considerations also include the development of claw tissues in utero, post-natally and in response to homeorhetic mechanisms regulating reproductive and lactational states and determining partition of absorbed nutrients.

The major tissue components of the claw are the external horn-forming epidermis and the underlying basement membrane, dermis and hypodermis with the maintenance of integrity of all components necessary for synthesis of good quality horn such as in wall, sole and heel.

Horn is produced from soft tissue basal cells which divide and differentiate in migrating to form hard tissue cornified horn. A range of keratin proteins and intermediate filament associated proteins with a high requirement for supply of the sulphur-containing amino acid cysteine are expressed, along with proteins important in cell-cell and cell-matrix adhesion to maintain structural integrity.

The trace mineral zinc which is essential for cell proliferation and development, and the B-vitamin biotin have been ascribed important roles in production of claw tissues. Biotin acts in carboxylation reactions for elongating fatty acids which contribute to synthesis of "intercellular cementing substance" which links epidermal cells of the horn, and to lipids of the digital cushion which act as shock absorbers in locomotion. Certain lipids may also have anti-inflammatory activity of interest in modulating laminitis. Other vitamins and minerals are also important.

There remains a need to optimise supply of nutrients in diets to improve resistance to challenges of contemporary husbandry environments and to reduce lameness in the dairy cow.

IS009

A brief history of statistical developments in animal breeding

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Statistical methodology has played a key role in scientific animal breeding. Approximately one hundred years of statistical developments in animal breeding are reviewed. Some of the scientific foundations of the field are discussed, and many milestones are examined from historical and critical perspectives.

Application of statistical ideas to animal breeding and genetics traces back Galton (1822–1911) and Pearson (1857–1936), both of whom worked before Mendel's laws were rediscovered. Galton found out that, on average, descendants from tall parents were smaller than their parents, whereas progeny from shorter parents were taller. This "regression to the mean" is intimately related to the concepts of heritability and expected response to selection.

Contrary to simple Mendelian traits, genes affecting a complex trait are possibly many, as stated earlier. Lush (1896–1982), arguably the father of scientific animal breeding, often expressed the view that possibly all genes affect all such traits. In spite of spectacular advances in genomics, we still do not know the number of genes, the form of gene action, and the allelic frequencies and effects on most complex traits.

At least two types of statistical problems are encountered in the process of learning genetic values. First is assessing whether traits have a genetic basis, known as estimation of genetic parameters. Second is developing reasonably accurate methods for inferring merit or genetic evaluation, a termed coined by Charles Henderson (1911–1989) A third type of problem, not dealt with here, is that of deciding what to do with animals that have the best evaluations; examples are inbreeding avoidance schemes, mate allocation, and exploitation of heterosis if there is non-additive genetic variation.

In dairy cattle, it is relevant to infer genetic merit of males accurately, because of the impact these have on gain from selective improvement. Cows are evaluated as well, but at lower levels of precision than sires. Due to refinements in artificial insemination techniques and of widespread availability of frozen semen and embryos, some dairy bulls can produce thousands of daughters in several countries, creating an opportunity for international sire evaluation via genetic connectedness.

The emergence of massive amounts of data as a result of genome projects catalyzed the development of a new field: bioinformatics. Data-mining algorithms interrogate and analyze large, complex data sets often generated outside of statistically specified protocols. Perhaps the training of animal breeders will place more focus on bioinformatics and artificial intelligence in the future, a direction that few groups in animal breeding have taken. Most of the ground covered in the last 100 years of research include:

- Use of Mathematical Models
- Epistasis
- Models for Other Genetic Effects
- Best linear unbiased prediction
- The Mixed Model Equations (MME) and Solving the MME
- Bayesian ideas in animal breeding
- Non-linear models, survival analysis and longitudinal data
- Categorical and Counted Response Variables
- Survival Analysis
- Linear and Nonlinear Models for Longitudinal Data
- Coping with biases from selection processes
- Genomic selection methodology

Animal breeders have taken up new statistical ideas rapidly and have also contributed to the field of biological statistics, significantly so in the cases of BLUP, REML, Bayesian methods, and whole-genome prediction. Soon, genome sequence information on individuals will be increasingly available (e.g., the 1,000 Bull Genomes Project), and the expectations are huge. Many authors argue that all causal mutations will be present in the sequence, and that this advantage will be exploited fruitfully. Such a view is based on a somewhat linear map of the genome, i.e., that a string of bases can produce an accurate genotype-phenotype mapping. The second one is that more information is better; for instance, instead of 800,000 markers, there will be 10 million. With respect to the first thought, the DNA-protein process is not linear because of, e.g., protein folding and pervasive interaction and feedbacks in metabolism and nonlinear enzyme kinetics. DNA and methylation information may be crucial for breeding value assessment, but appropriate environmental with supplementary omics-type information should also be considered for building more effective prediction machines. Concerning the second argument, one difficulty with an explosive increase in potential covariate number from sequence information is that the p/n ratio will increase markedly. For instance, if 1,000 bulls are sequenced, the p/n ratio will easily surpass 1,000–2,000. The implication is that all linear regression coefficients will become minute because of strong regularization. Perhaps a deluge of genomic and postgenomic data will further complicate separation of signal from noise because of the temptation to over-model.

Given the continued growth in computer power and algorithms, there is flexibility for fitting more realistic error distributions, such as a t-distribution. Analysis of cross-validation residuals is also an important diagnosis tool, and use of bootstrap methods will enable us to obtain realistic measures of candidate-specific cross-validation reliability.

Animal breeders should also be cautious about making overly strong assumptions concerning the dimension of a model. A multivariate analysis is not necessarily better unless some traits enter as part of the prediction machines as valuable covariates, and not through correlations. Selection and ascertainment bias should be an issue of concern in genome-enabled prediction, and sources of bias will need to be assessed more carefully.

The advent of genomic data will also enable us to study relationships between genomic regions using systems and causal perspectives, e.g., gene networks affecting pathways via graphical and structural equation models. Systems analysis, however, is not new in animal breeding, and it is not unfair to state that dividends from this approach have been scarce.

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IS010

Insects as sustainable feed ingredients

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The International Feed Industry Federation (IFIF 2017) reports a world feed production of about 1 billion ton in 2015. Proteins represent an important share in feeds, not only from a nutritional point of view, but also with regard to the impact on feed cost (approximately 60% of the cost). For a few years now, the problem of scarcity and sustainability of some conventional protein sources has emerged, and alternatives have been sought.

By 2050, the demand for animal products will double with a consequent increase in feeds. An essential point for the future development of the livestock is to find new sources of protein to be included in the animal feeds.

FAO (2013) indicates insect larvae meals (IM) as one of the promising innovative sources as they contain high levels of protein (40% to 70% of dry matter) with a profile in essential amino acids similar to fishmeal and better than soybean meal, but also valuable other nutrients as lipids, vitamins and minerals (Makkar et al. 2014). Moreover, insects are considered more sustainable than other conventional protein sources as they can be large scale produced on organic streams (Oonincx et al. 2010; Oonincx and de Boer 2012).

The current EU legislation allows the use of protein insect meals only in aquaculture feeds (from July 2017) but their authorization for terrestrial monogastrics (poultry and pigs) is expected by 2020. As far as lipids from insect are concerned, great interest is showed by the feed industry as these lipids have the potential to increase animal health by modulating intestinal bacteria communities, and stimulating the immune system (Henry et al. 2015). Moreover, no limitations are reported for the use of insect oil in animal feeds.

Among insect species, black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), and common housefly (*Musca domestica*) are the most evaluated and results on their inclusion on animal feed are promising (Barroso et al. 2014; Makkar et al. 2014; Belforti et al. 2015; Veldkamp and Bosch 2015; Biasato et al. 2016; Gasco et al. 2016; Schiavone et al. 2017; Piccolo et al. 2017; Renna et al. *submitted*). EU regulation authorises among other insect species, both *H. illucens* and *T. molitor*. A number of researches report that non-herbivorous monogastric farm animals (fish, poultry and pigs) can be fed diets including different levels of IM. These reports also present how results are influenced by the specie of insect used, the processing technology to dry and obtain meal (e.g. defatting procedures), the feed formulation (level of IM inclusion and conventional protein substitution), and, of course, the animal age and species.

Generally speaking, dietary inclusion up to 35% to 40% seems feasible in freshwater species, without worsening the performance parameters, while in marine species, 25% inclusion results to be the maximum acceptable amount especially in fingerlings (Gasco et al. 2016; Piccolo et al. 2017). As far as poultry is concerned, inclusion levels of up to 15% lead to promising results. Little research has been done so far to evaluate IM in pig production but the inclusion of up to 10% results feasible (Bressan et al. 2017).

The dietary inclusion of IM and insect oil influences the meat and flesh quality, mainly the fatty acid composition (Belforti et al. 2015; Gasco et al. 2016; Schiavone et al. 2017; Renna et al. *submitted*). IM and insect oil are rich in saturated and monounsaturated fatty acids and completely lack in *n-3* long chain polyunsaturated fatty acids (Makkar et al. 2014; Henry et al. 2015). This could lead to dramatic changes in nutritional and functional properties of products obtained with these innovative ingredients. Nevertheless, the modulation of the insect fatty acid profile through the insect breeding substrate has been demonstrated (Makkar et al. 2014; Nguyen et al. 2015). This opportunity opens the way for interesting new researches.

Very few studies investigated the influence of IM on animal organs histology, but results obtained so far are promising.

One issue highlighted by research is the chitin. Chitin is a component of the insect exoskeletons and is poorly digestible by fish and monogastric terrestrial livestock due to low chitinase activity. High levels of chitin decrease feed digestibility (Henry et al. 2015). Even if not yet fully applied, extraction process can decrease the insect meal chitin content or its digestibility can be increased using enzyme treatments (Belluco et al. 2013; Sánchez-Muros et al. 2014). Nevertheless, at low levels, chitin can also be a valuable compound due to its bacteriostatic, antimicrobial and antifungal properties, as well as immune stimulant properties. Moreover, antimicrobial peptides have also been found in insects and their possible use as natural antibiotic or antifungal in farm animals is of great interest (Yi et al. 2014).

The transfer of undesirable contaminants (e.g heavy metals, pesticides, veterinary drugs) from insect rearing substrate to IM is another major issue. In the EU, the feed legislation (EC Directive 2002/32 and amendments) (EU 2002) sets the maximum concentration of undesirable substances allowed in animal feeds thus preventing any major risk. Outside EU other regulations are in force and IM should be carefully monitored when imported.

Preliminary investigations on consumer acceptance towards products obtained using IM showed positive consumer attitudes (Verbeke et al. 2015; Mancuso et al. 2016). Similarly, no negative impacts of IM on sensory properties have been reported so far (Lock et al. 2016; Borgogno et al. 2017).

To date, the price of IM especially in EU is not yet competitive due to low produced quantities. Nevertheless, with the approval of their use in aquaculture feeds by the EU Commission, a huge development of this market and a consequent decrease in price is expected in the next years.

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IS011

Feeding strategies to reduce the use of antibiotics in pig production

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Pig production is highly efficient and economically driven throughout the world. As profit margins for producers are low, high performance is expected and the risks for disease that may decrease performance should be kept to a minimum. This has resulted in a high use of antimicrobial growth promoters and/or veterinary antimicrobial products, depending on area and time. The main purpose of veterinary antimicrobial treatments are the reduction of morbidity and mortality and to prevent a subsequent loss in production. In pig production, often groups of animals are treated, either via (routine) prophylactic therapy or metaphylactically. Examples are treatment at the moment of castration of male suckling piglets or antimicrobial therapy for prevention of diarrhea after weaning.

Feeding strategies need to be tailored to reduce the susceptibility of the pig for disease outbreak, i.e. risk factors that favor pathogen proliferation must be acknowledged and tackled. This is particularly true for the weaned piglet, as the abrupt shift from highly digestible maternal milk containing protective antibodies, to a basically vegetable diet deteriorates gastro-intestinal function and microbial balance. Frequently, post-weaning diarrhea (PWD) is encountered in weaners. Though a multifactorial disease and still unclear pathogenesis, PWD is typically associated with faecal shedding of enterotoxigenic *E. coli* (ETEC) serotypes. It occurs mainly in the first two weeks following weaning. In this regard, three nutritional approaches will be illustrated.

Firstly, next to the use of veterinary antibiotics, such as colistin, supra-nutritional doses of Cu (170 mg/kg) and ZnO (2000-2500 mg Zn/kg) are well known to prevent scouring in weaned piglets. Though its mode of action has not been fully understood, ZnO might act by improving performance, reducing incidence of diarrhea, reduction of inflammation, improved histo-morphology, improved tight junction integrity, reduced secretory responses of the gut mucosa and antimicrobial activity by free Zn^{2+} which acts as radical inside the bacterium. The effects on the microbiome were summarized as: reduction of *Lactobacillus* spp., more heterofermentative species, increase in clostridia, increased diversity of *Enterobacteriaceae*, reduction in *E. coli* (1st week post-weaning), and reduced adhesion of ETEC. Recent recommendations by EMA and EFSA to withdraw pharmacological ZnO, and to bring total Zn and Cu levels in feed closer to nutritional needs, respectively, has evoked a vivid debate between authorities and stakeholders in EU. However, new ZnO formulations are being marketed which might offer equal efficacy at fairly low inclusion levels due to coating, chelation, or alternative processing to increase porosity and specific surface area. In a recent study in our group we could demonstrate that the additive HiZox®, containing ZnO, at 220 mg Zn/kg could equally reduce *E. coli* numbers in the distal small intestine and improve even better mucosal integrity and alkaline phosphate gene expression as compared to conventional pharmacological ZnO. Repeatedly, it was demonstrated that some essentials oils and medium-chain fatty acids (MCFA) show interesting antimicrobial activity. However, their *in vivo* application as antimicrobial is complicated by their fatty nature, and hitherto fast absorption, and their volatility and corrosiveness. Next to coating and esterification, new techniques are required for predicted delivery at the site where pathogens proliferate. Alternatively, new sources of MCFA like black soldier fly prepupae meal (*Hermetia illucens*), deserves attention.

A second concept that was conceived many years before is the interference with the pathogenesis of ETEC or with the bacterial stimulation of inflammatory pathways. Though appealing, effects remain inconsistent. In this respect we found recently that a mycotoxin binder down-regulated the gene expression of TLR-4 indicating decreased stimulation by LPS, and in addition it upregulated mRNA levels of the tight junction protein zona occludens (ZO-1) in weaned piglets, irrespective of the DON-contamination level in the diet.

The third approach is where the expertise of the nutritionist fully comes into play. The left column of the table below shows various predisposing factors that can favor proliferation of pathogens or provoke microbial imbalances in the pig's gut, causing PWD amongst others. Corresponding nutritional measures aimed at alleviating these factors are addressed in the right column. The application will depend on the weaning age, health status of the farm, basal feed ingredients, vaccination programs etc.

Predisposing factor	Nutritional approach
Initial anorexia followed by over-eating	Feed form (liquid, pelleted) and palatability of feed ingredients, restricted feeding between d4-8 post-weaning
Reduced gastric acidity barrier	Reduce buffering capacity of feed and use acidifiers (including fermented liquid feed)Role of trace minerals (e.g. ZnO), inert fibre (e.g. wheat bran), and antioxidants and its precursors (e.g. vitamin E, S-AA)
Reduced small intestinal integrity and increased inflammation and oxidative stress	
Increased ileal protein flow	Use highly digestible protein sources, reduce total dietary CP level by including indispensable AA, reduce ANF (e.g. TIA)
Increased viscosity caused by NSP's	Use NSP degrading enzymes, adapt feed processing
Increased proteolytic fermentation	Provide fermentable (soluble) carbohydrates (e.g. sugar beet pulp, inulin)
Reduced absorptive function of large intestine	Provide fermentable (soluble) carbohydrates and probiotics to produce SCFA (butyrate)
vImbalance of microbiota composition	Potential role for probiotics (preventative)
Reduced microbial colonization resistance	Potential ways to increase microbial diversity and immune development in early life (e.g. oligosaccharides)

Along with the intensification of livestock in past decades came the increased use of antibiotics. Minimizing the use of antibiotics and heavy metal compounds is essential for the long-term sustainability of the pig industry. Certain feed additives, and other nutritional measures are seen as alternatives/replacements to antibiotics, and have evolved in some cases to have important roles in everyday commercial pig nutrition. Nevertheless, and in general, there remains inconsistency and variability in the efficacy of some of these. Hence, the importance of improved management practices (e.g. biosecurity, all-in-all-out) and vaccination programs cannot be underestimated, adding to the idea that a holistic approach to reduce antibiotic usage is warranted.

IS012

Welfare in Poultry

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From the 1950s the application of genetic theory led to a rapid increase in productivity and the development of specialized fowl lines for meat and egg production (Tixier-Boichard et al. 2012). Concomitantly, management conditions became more intensified. The public concern about poultry welfare, in particular the keeping of laying hens in conventional cages (EFSA 2005), finally led to the ban of conventional cages in the EU from 2012. In the last decades, also intensive meat type production has come under increased scrutiny (European Commission 2000).

Laying hens

The key features of concern about conventional cages were: the limited space for escape and expression of behavior and the lack of features to fulfill behavioural needs such as nest building, foraging and dust bathing (EFSA 2005). In experiments it was shown, that deprived birds exhibit signs of frustration (Wood-Gush and Gilbert 1969). Furnished cages offer an intermediate level of complexity in that they provide access to nest sites, dustbathing facilities and perches. However, some behaviours are still prevented or inhibited depending on the cage design and the number of hens in the cage. Bone strength may be impaired due to insufficient opportunity for exercise. In addition, there is an increased risk of social aggression in large groups (Lay et al. 2011).

Non cage systems (e.g., aviaries, free range) offer more space and a more complex environment allowing hens to make choices and control its environment. But there are other challenges such as higher risk of diseases, (e.g. endo and ecto-parasites), predation or climate. Complex systems expose hens to an increased incidence of bone fractures which are a serious welfare concern because animals suffer from pain. Adequate design of perches and distances between tiers are crucial (Lay et al. 2011; Sandilands and Hocking 2012).

Injurious pecking (FP) remains one of the most widespread and important welfare issues of poultry production. The routine alleviation of FP by beak trimming of laying hens has come under increased scrutiny because this mutilation impairs sensory input and may cause painful neuromas (Gentle 2011). Accordingly, there are initiatives of several European countries to ban beak trimming. The term FP covers a group of behaviours such as gentle and severe FP, vent pecking and cannibalistic pecking. FP is regarded as redirection of normal behavior towards conspecifics and is related to feeding and foraging behavior. FP can be socially transmitted so that the occurrence of FP is much more difficult to control in larger groups such as large furnished cages or non-cage systems (Zeltner et al. 2000). The development of FP is a multifactorial process influenced by many genetic, behavioural and management factors (Buitenhuis et al. 2003; Rodenburg et al. 2013). In contrast to humans, chicken are sensitive to UV radiation and they use reflected cues from the plumage to gain information. However, artificial light in commercial poultry houses is without UV radiation and of low intensity. Under these conditions, eye development may be impaired thus affecting bird recognition of conspecifics and possibly influence FP (Bright 2007).

Early access to litter for foraging and control of fear and stress levels have been shown as important factors in reducing of FP (Rodenburg et al. 2013). Proactive individuals show an increased psychomotor activation possibly mediated by serotonin neurotransmission (van Hierden et al. 2002). Thus, automatic registration of locomotor activity might be used to develop early warning systems on farms.

Meat type poultry

In meat type poultry the long-term selection for high growth intensity and improved feed conversion ratio is accompanied by a number of welfare problems (European Commission 2000; Bessei 2006; De Jong et al. 2012). Concurrently with changes in feeding and housing, growth rate of broilers has more than doubled over the last 50 years. Across different species (broilers, turkeys), comparable conditions occur including cardiovascular and musculoskeletal defects, metabolic disorders and skin lesions. In recent years, animal-based measures to assess welfare on farms have been developed (EFSA 2012).

In contrast to broilers and Pekin ducks, FP is a sincere welfare issue in turkeys and Muscovy ducks. As shown for laying hens, environmental enrichment may reduce injurious pecking of turkeys (Martrenchar et al. 2001). There are initiatives of several European countries to ban beak trimming in turkeys.

The main behavioural phenomena in meat type poultry are changes in feeding behaviour and locomotion. The higher rate of food consumption in broilers can be attributed to an increased appetite. Over the whole growing period broilers may spend more than 75% of the time sitting (Gerken et al. 2003), compared to less than 30% in laying strains. Bone mass and strength change according to physical activity and mechanical stress and the lack of exercise is considered a main cause of leg weakness. The sharp increase in time spent sitting with age may results in skin lesions and contact dermatitis on poor litter conditions. Skin diseases such as breast blisters, hock burns and footpad dermatitis have increased most over the last 4 decades (De Jong et al. 2012).

The accelerated skeletal growth is combined with an increased incidence of bone disorders, most resulting from growth plate pathologies, such as tibial dyschondroplasia (Rath et al. 2000). The emphasis on breeding for high amount of breast muscle has caused broiler's centre of gravity to move forward and breasts became broader. Similar observations are made for turkeys (Hocking 1993). Lame birds spend less time on locomotor activities and have reduced walking abilities (Weeks et al. 2000). Musculoskeletal disorders resulting in degeneration or inflammation are likely to be very painful and the administration of an analgesic drug improved the walking ability of broilers (Danbury et al. 2000). The intensive metabolic processes involved in rapid growth cause problems regarding heat dissipation and increase the oxygen demand of the broilers which intensifies the activity of the cardio-pulmonary system. A lack of oxygen is the major problem in the aetiology of ascites and sudden death syndrome (SDS) (Julian 1998; EFSA 2010). Problems in heat dissipation increase the risk of cardiac failure in particular under adverse environmental conditions such as high ambient temperature and crowding. Panting as one form of thermoregulation is easily seen in broilers (Gerken et al. 2006).

The antagonistic relationship between growth and reproduction has resulted in the application of severe feed restriction in broiler parents regarded as a major concern in animal welfare (EFSA 2010b).

Many of the welfare problems in broilers can be reduced by slowing growth rate and encouraging locomotion. The genetic approach involves the use of slow growing lines and the modulation of the growth curve (EFSA 2010a; Tixier-Boichard et al. 2012). Management improvements include nutrition, lower stocking density, litter quality, and environmental enrichment (De Jong et al. 2012). The recent development of dual purpose fowl strains may become a new approach to overcome the welfare problems outlined for broilers. Such lines could also render the present routine of killing of male day old chicks in layer strains redundant (Damme et al. 2015).

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IS013

The role of mother-offspring behaviour in lamb survival

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Neonatal lamb mortality is a significant welfare and productivity concern in sheep farming, with the loss of up to 25% of lambs occurring before weaning. Nearly half of all mortality occurs on the day of birth, and is associated with dystocia and stillbirth, functional disorders, and a weak mother-offspring bond leading to starvation and hypothermia. Other deaths can be attributed to infectious disease, congenital malformation, predation and accident. In our work we have studied the behavioural relationships between ewes and lambs at birth, and the development of the lamb before birth, as a means to understanding the causes of lamb mortality, and to develop strategies to improve survival.

At birth ewes, which have previously shown little or no interest in lambs, exhibit intense and focused maternal care: licking or grooming the lamb and emitting low-pitched lambing bleats. The onset of these behaviours is controlled by changes in the concentrations of oestradiol and progesterone in late pregnancy, the release of central oxytocin at birth, and the presence and smell of amniotic fluids on the coat of the lamb. In addition to inducing the expression of maternal care, these physiological changes provide the opportunity for the ewe to form an olfactory bond with her lamb. Ewes are 'selective' for their own offspring, and failure to form a bond with the lamb will result in lamb abandonment and death without human intervention, as no other ewe will care for the lamb. Thus the successful onset of these maternal behaviours is crucial for lamb survival. The expression of maternal care is affected by ewe breed, maternal inexperience, undernutrition in late gestation, maternal temperament and birth difficulty. These factors appear to have their effects as they alter te signalling pathways that underlie the regulation of maternal behaviour, and are risk factors for increased lamb mortality.

However, the behaviour of the newborn lamb also plays a crucial role in its own survival, which is independent of maternal care. Lambs that stand and suck quickly after birth have significantly better survival than lambs that are slow to stand and need human intervention to suck. Lamb neonatal behavioural progress is reduced in lambs of some breeds, in male lambs, lambs born in larger litters, low birth weight lambs and lambs born after a difficult delivery. Lambs that are slow to suck have an increased risk of experiencing starvation, a reduced ability to maintain body temperature, an increased risk of developing infectious disease and are less well attached to their mothers, which may increase the risk of predation. We have shown that lamb neonatal behaviours are moderately heritable, suggesting that some improvement in lamb survival can be achieved by genetic selection, although this may be challenging in extensive environments where accurate assessments of behavioural phenotypes may be difficult. Management strategies to improve lamb survival include ensuring adequate maternal nutrition, a low stress lambing environment, which allows the ewe adequate time and space to develop a strong relationship with her lamb, and good lambing hygiene.

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IS014

Animal welfare and food security: role and tasks of the Italian farmers

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While celebrating the 60th anniversary of the founding of the European Union it is important to remember that one of the first actions taken was the eradication of tuberculosis and brucellosis. Subsequently, the prevention of diseases in farm animals was the objective of a long series of decrees. Only healthy animals produce safe milk and meat with suitable hygienic characteristics. This axiom was later the basis for the whole Community legislation, laying the foundations of a health prevention policy. In view of all this, it is clear that food security is at the heart of EU policy. Food safety was also at the base of the evolution of the concept of product quality such as in the case of milk and meat. In recent years, the concept of product quality was implemented by the development of the protected denomination of origin (PDO) certification and the environmental and social sustainability of animal production became more and more important. Animal welfare is one of the most relevant aspects of sustainability. With the support and close cooperation of the Member States, the European Commission has been promoting animal welfare for over 40 years, gradually improving the lives of farm animals. The Council Directive 98/58/EC, which gave general rules for the protection of animals of all species kept for production of food, wool, skin or fur or for other farming purposes (including fish, reptiles or amphibians) was one of the first important steps taken in this direction. When the Lisbon Treaty came into force in 2009, it amended the 'Treaty on the Functioning of the European Union' (TFEU) and introduced the recognition that animals are sentient beings. Article 13 of Title II states that: "In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage". National governments may adopt more stringent rules, provided they are compatible with the provisions of the Treaty, but Community legislation concerning the welfare conditions of farm animals lays down minimum standards. In the present study the system developed by the DQA and AIA, for a precise and accurate animal welfare measurement in dairy herds, is illustrated. An assessment of the whole herd animal welfare requires distinct investigation in two different areas: a) evaluation of farming systems in which an animal can live in wellness; b) evaluation of animal based parameters expressing whether the animal has a condition of wellness. Many scientific papers have been published on both the influence of farming systems on animal welfare and the animal based evaluation of animal welfare. Many protocols have been already applied in order to give a support to animal welfare control. Nevertheless, whereas herd evaluation is easier as it is referred to farm based measurements, the latter needs data processing as it is based on animal measurements (often analytical individual parameters). However, final evaluation has to convey the wellness level of all animals of the same herd. Several animal welfare risk factors have been identified by studies based on performance recording data. The aim of the work is to present an algorithm which calculates a unique herd animal welfare index from individual performance recording measurements. A set of risk factors are chosen and data are transformed into standardized values. Afterward, one value for each risk factor is calculated as an index of the animal welfare of the whole herd. An overall index, including all risk factors, is finally obtained in order to give useful information of herd animal welfare level. This final index is an intuitive instrument able to identify animal welfare situations from critical to optimal. An example using Italian performance recording data is provided. The proposed global index of animal welfare is a summary value based only on animal-based measures collected by official performance recording. It is composed by 5 different indicators related to 4 welfare hazard areas: reproduction, longevity, udder health and metabolic diseases. Longevity is assumed to be a direct consequence of the individual response to risk factors in term of animal welfare. The importance of this approach is also connected to three main aspects related to performance recording: continues monitoring of herds, long-term evaluation, guarantee of objectiveness and homogeneity in data collection. In current animal welfare assessment systems, animal-based measures are acknowledged as the base for the evaluation of the welfare of dairy cattle on farm. Almost all animal welfare items can be evaluated by animal-based measures.

IS015

Constraints and solutions to reduce environmental impact for developed livestock farming

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The world gross economic product due to animal farming is around 40 to 50% of total agricultural economic product. This economic sector supports around 1,3 billion producers. Animal products are essential part of human diet, both in terms of calories and component to diet. These few figures prove the importance of animal farming. Still animal production is linked to large environmental impact and, considering the growing demand of meat, milk and eggs, we are obliged to maintain, or eventually increase, the production level of livestock farming while reducing the environmental impact. Although the mitigation potential of reductions in livestock farming is large (up to 50% of the technical mitigation potential of the agriculture, forestry and land-use sectors), it is almost completely unknown the economic and social problems created by different animal farms management options.

A common goal should be searched through a "food production policy revolution" that must be universally agreed. The solutions to be adopted must also have a dual purpose: meeting the rising human needs and contributing to resilience and sustainability of the environment. Planning a general plan is impossible in absence of shared goals and a global accepted government. It is obvious that the food production policy revolution aims at long-term global results but facing many local short-term constraints. The overall goal to produce more animal products with less environmental impact must remain firm. The market will request more animal products in the next decades due to the increased number of human beings but even more due to the increased economic conditions of billions of people living mainly in East and South Asia, Brazil, etc. The request of animal products will likely remain the same in the developed countries, because protein level in the diet is sufficient and for the partly negative image of animal products.

Once defined and shared the global objective, the immediate second step, more difficult, is the choice of the methods to adopt to reach the goal. There are many possible options offered by the researches developed in the last years. Clearly there one method does not fit all systems for different times. The main difficulty is in fact because the choice of the method should be dynamically changed because the environmental, economic and social contexts are rapidly changing. The changes are also different for every local cluster. And again, every farm is different even within the same cluster in the same period. We currently live in a really dynamic world, where fast changes happen at every level, mainly in the economic and technical contexts. Considering the overall goals to produce more animal products while reducing environmental impact, the global governance must support research to find possible solutions. Developed countries will be facilitated since most of the research is done and applied there. There must be found a set of many possible solutions to fit any local situation. The fast dynamism of economic, social and technical conditions must generate lively scientific activity. The general set of technical solutions must be therefore constantly updated. Another crucial step is the decision moment of the new technique to apply. Developed countries should support farmers in the decision moment through supporting industry activities, through creation and support of extension service and through possible economic tools, like ad hoc benefits and fiscal policy.

The awareness of environment is much more considered in the developed countries than in developing. Respecting environmental parameters creates constraints to animal farmers and will create even more in the future. Therefore livestock farmers in developed countries certainly will have to consider that animal farming will be further limited by the environmental impact. The only way to increase or keep the same level of production while maintaining the environmental condition is to change farming models and to apply new technological developments for reducing environmental impact. The punctual application of the technique set of solutions is the key. Development and diffusion of new technologies is quite fast in developed and often farmers can be receptive to new proposals. The size of farms is generally growing and consequently the management is skilled and keen to invest to apply the new technologies.

Animal feeding strategies, genetics and precision livestock farming are the main area of technological application to render the farms more efficient and environmental friendly. Mainly for intensive animal farming system, the feeding strategies can be changed or modified to diminish the environment. But can happen only in developed countries and for specific conditions.

Improved genotypes are designed in developed countries for animals reared in high productive farming systems. As for any genetic selection plan the starting point is the breeding goal. It has been discovered that selecting for feed efficiency will also diminish the environmental impact of the selected animals. If those researches will be confirmed then genetic improvement can create immediate outcome for farmers and diminishing the environmental impact.

Among the new technologies we must include punctual application of "Precision Livestock Farming" although those are methods quite expensive to apply and require the possibility to invest and a necessary planned income to have safe return on investment. A vital and positive market is required therefore to apply new technologies for Precision Livestock Farming. Furthermore the farms must be culturally and technically receptive of such improvements.

The civil society perception of animal farming also has changed dramatically. The society and its acceptance and need of animal products are parameters changing rapidly and locally. And the usable set of available techniques must also consider the societal acceptance of farming methods. For instance there is a strong scientific justification for a shift from our current paradigm for animal farming (and more general, agriculture) focusing on productivity first and sustainability as a question of reducing environmental impact to a paradigm where sustainability constitutes the core strategy for livestock systems. But the absence of an accepted animal farming revolution (as part of a more general food production revolution) will delay and render heterogeneous, in the best option, the obtained results. In the worst option instead, the results will not be reached if not in few situation and therefore rendering globally useless the efforts.

For the first time global sustainability is increasingly understood at all levels, from local farming communities to international industries and policy makers. The human pressure is causing global environmental risks and agriculture is at the heart of the problem. Because of the essential role of agriculture in human existence and because of the caused huge environmental problems, policy makers, scientists, civil societies should find a shared goal and shared set of methods to cope with this problem. The examples and the guidance should come from the developed countries that for many reasons can better understand the problems and better achieve the solutions.

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IS016

How to solve the (un)sustainability dilemma

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Sustainability is a systems property and the use of systems indicators is a starting point to try to overcome the sustainability dilemma. Sustainability is an ideal state, and therefore it is difficult to measure per se. What can be measured is the distance from the ideal point of sustainability, i.e. UNsustainability. A key to the quantification is offered by H. Daly's principles of sustainability: 1) resources should be used at a rate that allows their re-formation (sustainable yield); 2) wastes should be produced at a rate which allows the environment to absorb them. It is possible to quantify what is used and/or what is released too fast and too much into the environment. These principles also show that unsustainability is related to the total amount of available resources and of waste produced (and to the speed of their formation/production). But up to now efforts to find indicators able to describe the sustainability level of a system have partly failed. This is probably due to the complex nature of the problem: if we try to divide it but still cover all its aspects we will have an exorbitant number of indicators (e.g. one for the concentration of any possible pollutant).

Unsustainability is an extensive problem because it is linked to the total and limited availability of resources and to the possibility of a limited system (e.g., the Earth) to absorb waste and pollutants. The improvement of intensive parameters (e.g., energy efficiency, CO_2/person or CO_2/\$) is not enough to reduce the distance from the desired state of sustainability. Therefore, it is not possible to assess sustainability/unsustainability by means of intensive parameters, because the problem is strongly correlated with the size of the system.

In recent years, indicators and methodologies have been proposed to represent sustainability. However, it is important to point out that these methods do not lead to the same information because they have different goals and horizons of application. For this, integrating information generated by each indicator becomes essential to have an all-comprehensive global picture of the system under study.

If we want to analyse animal production from a sustainability viewpoint, we have to consider also the complexity given by the fact that very often these productions do not have only one output, but multiple ones. Life Cycle Assessment (LCA), for example, if carried out on outputs such as bovine meat and milk, or chicken meat and eggs, sheep meat, wool and milk, can give quite arbitrary results due to the use of different allocation criteria. Emergy tries to solve this problem by introducing the "co-products" category to which the total emergy needed for the process is completely assigned. But also this can provide equivocal results if blindly applied. Ecological footprint chooses to identify only one output from each of the land types in which it can be divided. We will give criteria for a sound use of sustainability indicators for animal production systems.

IS017

Environmental impact of animal production

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Nowadays, the development of a sustainable farming system (in terms of environmental and economic sustainability) is a priority to preserve the natural resources and the environment and to guarantee the production of animal food. Moreover, the estimated increase in world population and the increasing demand for food of animal origin require a long-term global strategy to develop more intensive and sustainable animal production systems. The mission of agriculture and animal husbandry is to supply food nutritionally adequate, safe and healthy, maintaining the natural resources needed to produce it. The environmental sustainability of food production (of plant or animal origin) depends also on the types of human diet: increasing incomes and urbanization are heavily changing human diets, with a shift from the traditional diets to diets richer in refined sugars and fats, oils and meat. By 2050 these trends, if not checked, will increase the greenhouse gases (GHG) emission from food production by 80% (Tilman and Clark 2014).

The global livestock sector is characterized by a dichotomy between developing and developed countries. Total meat production in the developing world tripled between 1980 and 2002. In developed countries, on the other hand, consumption of livestock products is growing slowly although at high levels. In this regard, an excessive consumption of red meat and processed meat (>50 g/d) has been associated to some health issues (World Cancer Research 2007). However, the higher rate of chronic diseases associated with Western diets is also due to an excessive consumption of refined cereals, fried foods, soft drinks, sweets and energy-dense, nutrient-poor food products; overall, an ideal diet should include plenty of fruit, vegetables, nuts, legumes, unrefined cereals, and moderate amounts of meat, fish and dairy products (World Health Organization 2003).

Among the different livestock species, ruminants have a major environmental impact, mainly for the methane emission and the low feed efficiency, although they may utilize fibrous feeds, differently from monogastrics and humans. Particularly, beef production has the highest impact with a wide variability depending on the breed and the farming system (e.g.: intensive *vs* extensive; conventional *vs* organic; suckler *vs* dairy calf; high or low forage/concentrate ratio in the diet). A review on the environmental impact of beef production systems (de Vries at al. 2015) showed, per functional unit, a lower global warming potential (GWP), energy use and land use for dairy-based compared with suckler-based systems and for concentrated diets compared with roughage based diets. In suckler based system, maintaining the mother cow is the major contributor to all impacts; in the intensive systems (high concentrate diets) the lower impact is attributable to the faster growth rate and the more favourable feed conversion ratio; no large differences in GWP were found between organic and conventional systems. On the other hand, in central Italy, Buratti et al. (2017) found a higher carbon footprint with organic than with conventional systems (24.6 *vs* 18.2 kg CO_2eq/kg LW, respectively) in agreement with Meier et al. (2015) who showed that organic systems not always allow lower impacts than conventional systems. The adoption of organic systems reduces the environmental impact per unit of area, but not necessarily per unit of product (Tuomisto et al. 2012).

Considering pork, the impact on the environment is lower than beef. The Italian pig sector is mainly focused on the production of heavy pigs, with higher environmental impacts than light pigs. Bava et al. (2017) reported a wide variability in the environmental impact categories considered in 5 commercial farms of Northern Italy; for instance, GWP ranged from 2.7 to 5.8 kg CO_2eq/kg LW of heavy pigs, as compared to an average of 3.1 kg CO_2eq/kg LW of light pigs calculated from 12 studies reported by the authors. In the heavy pig production, feeds, especially protein sources, have the highest impact and some feeding strategies, such as lowering the dietary crude protein according to the physiological phase and supplementing diets with amino acid, should be implemented (Galassi et al. 2010; Schiavon et al. 2015).

Concerning milk production, it has to be underlined that most of the Italian dairy farms of the lowland areas are characterized by high stocking rates and intensive forage systems; hence, high inputs are required, for example N fertilizers which increase GHG and lower biodiversity. A higher use, consistent with the stocking rate, of legume and grass forages and of permanent pastures in place of maize silage, can reduce the purchase of N fertilizers and protein supplements on one side, and increase the C sink in the soil on the other side. Properly managed grass systems preserve soil C; particularly, replanting grasses in lands previously sown with annual crops can result in a significant increase in soil C, and in some cases the soil C gain more than offset all the GHG emissions from the farming system (Guyader et al. 2016). In this regard, as reported by Battini et al. (2016) a higher use of maize silage does not necessarily reduce the GWP per kg milk, due to the higher soybean meal import and the consequent carbon footprint associated to the land use change. In their study the lowest GWP (1.6 kg CO_2eq/kg milk) was registered for a farm of the intensive production Parmesan cheese area characterized by a crop rotation based on lucerne and grass hays; by contrast, the highest GWP (2.0 kg CO_2eq/kg milk) was estimated for a less intensive Parmesan cheese farm located in the hill/mountain area. Bava et al. (2014) found an average value of GWP equal to 1.3 ± 0.2 kg CO_2eq/kg milk, and a negative correlation with milk yield, dairy efficiency (kg milk/kg DMI) and stocking density.

The application of extensive farming systems, the reduction of external nutrient inputs, and the efficient use of nutrients at farm or regional levels have been described as advisable strategies for environmentally sustainable farming activity. However, extensive farming and strategies to decrease nutrient inputs are difficult to develop in several areas of the country (for example in the Po plain) and it must be underlined that intensive systems are generally less environmentally impacting than extensive ones when considered per kg product (meat, milk, eggs) or protein. Moreover, improvements in management techniques related to animal fertility (i.e. lower culling, lower replacement rates) can also reduce GHG/kg product (Crosson et al. 2011).

In conclusion, differences among cattle and pigs in terms of environmental impact are basically related to three factors: enteric methane emission, feed efficiency, and reproduction performance. Within each of these categories, significant improvements can be achieved applying specific strategies (e.g. starch/NDF ratio, lipid supplementation; Pirondini et al. 2015). However, it must be underlined that although technical measures can significantly reduce the environmental impacts attributable to agricultural and animal farming practices, other issues related to citizen education in the developed countries should be pursued. Among these, the importance of healthy balanced diets, lower food and energy intake, and less food waste should be emphasized to attain a more significant reduction of the environmental impacts.

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IS018

Foodborne pathogen: the case of *Campylobacter* in poultry

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Campylobacter is the leading cause of foodborne illness in humans worldwide, with the majority of cases attributed to the consumption of poultry. There are other sources of *Campylobacter* including environmental, cattle, pigs, wild animals and companion animals in particular puppies and kittens. However, there can be no doubt that poultry is the major reservoir of *Campylobacter* and the two species commonly found in poultry, *C. jejuni* and *C. coli*, are also those most commonly identified in the human population. *Campylobacter* has a long standing association with poultry, it is well suited to 42 °C, the body temperature of chickens, and is commonly found in the gastrointestinal tract of these animals. *Campylobacter* spp. are isolated throughout poultry production, including rearing and at slaughter and their occurrence is well documented. There are two main routes of transmission from poultry to humans, firstly cross contamination during processing and secondly via spread of the bacterium from the intestines to other organs including the liver. The chicken gut is colonised with high numbers of *Campylobacter* spp. and during processing, which is highly automated, these bacteria cross-contaminate the external surface of the carcass. *Campylobacter* can also be aerosolised and contaminate the carcass that way. More recently it has been shown that *Campylobacter* has the ability to leave the gut and infect other organs, the liver being the predominant one. Extra intestinal spread is a major public health issue: there have been several outbreaks of human campylobacteriosis linked to the consumption of chicken livers indicating that perhaps extra intestinal spread is more common than previously thought. The ability of *Campylobacter* to leave the gut is poorly understood and requires further investigation. A recent study at Swansea University examined isolates obtained from the ileum, caecum and liver of the same broiler chickens and found that there were more similarities between the isolates in the ileum and liver compared to those in the caecum and liver, suggesting that *Campylobacter* are leaving the gut from the ileum. It has also been shown that *Campylobacter* interacts with other intestinal pathogens, and takes advantage of damage to the gut epithelial cells. The interaction of *Campylobacter* with *E. coli* is particularly interesting, as it has been shown that these two pathogens interact with each other to cause damage to the gut. As above, recent studies at Swansea have shown that *C. jejuni* invasion into avian epithelial cells increased in the presence of *E. coli* but interestingly and perhaps surprisingly we also found that *E. coli* invasion into the same cell line increased in the presence of *C. jejuni*, this finding needs further work.

For many years *Campylobacter* was considered to be a commensal of chickens, it was thought not to cause any health or welfare problems in the birds. However, the majority of studies did not measure this and reported problems such as diarrhoea were attributed to other poultry pathogens. *Campylobacter* is considered to be part of the caecal microbiome of broiler chickens, because it is found in nearly every flock reared in the UK. It is generally accepted that every broiler chicken will be colonised with or exposed to *Campylobacter* during their lifetime irrespective of breed and rearing system used. Birds usually become colonised by the time they are 3 weeks old and due to the rapid growth and short lifespan of the birds, the immune system is under developed which may make them more vulnerable to infection. However, chickens do mount innate and adaptive immune responses to infection with *C. jejuni* but this does not result in clearance and may have the objective of confining the bacteria to the gut. There have been changes in the performance and welfare of broiler chickens that are associated with other conditions and with carcass rejections at slaughter, which are examined thoroughly. For in excess of 30 years *Campylobacter* were considered to be part of the normal gut microbiota of a chicken despite early evidence indicating otherwise.

There have been studies based on laboratory and field data, going back to 1981, which have demonstrated that *Campylobacter* can and does have an effect on the health, welfare and performance of broiler chickens. It was only recently accepted that chickens mount an immune response to *Campylobacter*. Prior to this, chickens were thought not to do so in the same way as they would not for other commensal pathogens such as *Lactobacillus*. As we develop a deeper understanding of the chicken immune system we begin to realise that it is more complex than previously thought and that *Campylobacter* is dealt with by the chicken immune system in a similar way to other pathogens. Whilst this is not universal to all strains, within some bird types, and with some isolates, the bacterium behaves as we would expect a commensal to and the immune response is not as pronounced. *Campylobacter* can also have an effect on the health, welfare and performance of broiler chickens and there has been an association between certain welfare associated conditions such as hock marks and pododermatitis. These foot and leg lesions are associated with diarrhoea in the birds, were risk factors for *Campylobacter* infection in commercial flocks and in experimental flocks the incidence of these foot and leg conditions were increased when *Campylobacter* was present in the flock compared to uninfected controls. There have also been studies examining the economic effects which have shown that performance and growth rate is affected by the presence of *Campylobacter* within a flock. With increasing evidence of impacts to health, welfare and performance of broiler chickens *Campylobacter* cannot be considered to be a true commensal of poultry, as in the right conditions it behaves more like a pathogen and needs to be considered as such.

There are many controls in place within the poultry industry to control *Campylobacter* levels in flocks during rearing at the farm including biosecurity, use of pre and pro biotics and additives and during processing such as rapid chilling and surface washes but the effectiveness of these is limited. There is also currently no effective vaccine against *Campylobacter*. Despite existing control measures the number of cases of human campylobacteriosis increases each year, with the majority of chickens containing *Campylobacter*. A combination of measures will be needed to control *Campylobacter* in chickens and understanding how the bacterium behaves in the chicken and the host response to this is needed.

O001

Sardinian Anglo-Arab horses: genetic parameters of conformation and performances

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Sardinia Island is famous in Italy for horse breeding. In this region, horses are managed in small breeding units (2 or 3 mares with their foals) and the more representative breed is the Anglo-Arab (AA). This breed originates from crosses between old Arabian Sardinian lines and Thoroughbred, and the Italian Stud book of the AA was established in 1967.

The AA pedigree file, including 43,624 horses (27,052 females and 16,572 males), born from 1853 up to 2012, and two dataset (morphological measurements of 6033 horses and the result of 1794 horses in 2139 flat races) were provided by AGRIS (Agricultural Research Agency of Sardinia).

The overall means are 160.8 cm for Wither Height (WH), 67.6 cm for Shoulder Length (SL), 185.9 cm for Chest Girth (CG), 19.7 cm for Cannon Bone Circumference (CB), and 73.2 points out of 100 for the Overall Morphological Score (OS); as expected, all the values were significant higher in males. The inbreeding coefficient, that reached its maximum of 2.9% in the foals born in 2010, significantly depressed the WH; the Arabian blood percentage significantly reduces WH, SL, CG and OS, whilst Thoroughbred reduces CG and OS, but increases SL. The heritability estimates were 0.78 ± 0.02 (WH), 0.35 ± 0.02 (SL), 0.47 ± 0.02 (CG), 0.57 ± 0.02 (CB) and 0.23 ± 0.02 (OS); the genetic correlations were all positive and ranged between 0.86 ± 0.02 (WH-SL) and 0.23 ± 0.04 (WH-OS).

The flat races results were used to calculate 6 “economical” traits from transformed earnings, 4 “statistical” traits from the placements, and 2 “Elo” traits from rankings. Each group of traits was analysed by a MT-BLUP, in order to find which trait could maximize the overall response of its group. The selected traits were the square root of total earnings ($h^2 = 0.41 \pm 0.02$) for the “economical” traits, the total number of placings ($h^2 = 0.48 \pm 0.05$) for the “statistical” traits, and the final rating ($h^2 = 0.29 \pm 0.04$) for the “Elo” traits. These three traits were eventually used in another MT-BLUP, were they showed genetic correlation ranging between 0.65 ± 0.08 (total number of placings – final rating) and 0.90 ± 0.03 (total number of placings - square root of total earnings).

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O002

Transcriptome analysis of Anglo-Arabian racehorse PBMCs: insights on transcribed introns and repeats

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The horse is probably the best animal model for investigating genomic response to exercise-induced stress, due to its natural aptitude for athletic performance and the relative homogeneity of its genetic background. We applied RNA-seq to Peripheral Blood Mononuclear Cells of six 3 years old race horses sex matched collected at rest and after a 2000-meter competition. Standard bioinformatics pipeline from mapping to reads counts evaluation (STAR, featureCounts, edgeR) was applied to reveal modulation in expression. Besides a rich list of differentially expressed genes after the effort, the analysis showed a large number of transcripts, corresponding to intergenic and intronic regions associated with new transcriptional elements. This data might be correlated with transcriptional activity related to nascent transcription, co-transcriptional splicing events or transcription of long noncoding RNAs or enhancer RNAs that are know to contribute to changes in intronic read counts. Moreover we observed a post-race increase of reads mapping to repeats, especially to LINE1 in intergenic and intronic regions. Indeed, around the 20% of the mammalian genome belongs to remnants of ancient viral infections called Long Interspersed Nuclear Elements L1 (LINE-1) and their spreading into the genome through retrotransposition is mediated by an RNA intermediate product.

Our results reinforce the hypothesis that transposable elements (TE) and intronic sequences may serve as transcriptional units capable of enriching transcriptomes through exonization and intron retention under stress conditions. We also found that 9 full-length LINE1 elements are up-regulated after the race. This suggests that a great effort induced by exercise may - in principle - activate LINE1 retrotransposition, as already demonstrated in human and mouse tissues and in certain sporadic cancers. Once confirmed, this hypothesis would open new scenarios for the understanding of low performance syndromes etiopathology due to strenuous exercise.

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O003

RNA-cargo characterization of equine Ad-MSCs vesicles: first step towards cell-free therapeutic procedures

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Equine adipose-derived Mesenchymal Stromal Cells (E-Ad-MSCs) are extensively studied for their anti-inflammatory and pro-regenerative properties observed at the muscular-skeletal level. Their biological effects seem linked, at least in part, to the release of nanometric extracellular vesicles (EVs) containing trophic factors, cytokines and coding and regulatory RNAs. This study aimed at investigating RNA content within EVs produced by E-Ad-MSC to explore the possibility to use vesicles instead of cells for therapeutic purposes.

EVs were obtained from 4 horses via ultracentrifugation of the E-Ad-MSC conditioned medium. Resulting pellet was used for electron microscopy analysis (TEM and SEM) and small RNA sequencing via Illumina technology.

TEM an SEM analyses revealed a mixture of shedding vesicles and exosomes as demonstrated by CD90 and Flotillin-1 reactivity with size ranging from 30 to 300 nm.

Standard bioinformatics analyses (trimming with Trimmomatic, mapping with STAR and counting with featureCounts) were applied: uniquely aligned reads and the last Ensembl gene annotation (86) were used to characterize the exosome cargo.

The annotated fraction of the genome accounts for the 40% of the produced sequences while the great part is positioned in the intergenic compartment. Of this 40%, the great part of the sequence fall on rRNA, followed by small nucleolar RNAs (snoRNAs) whereas miRNAs were represented at lower level as well as protein coding genes.

snoRNAs are conserved group of small non-coding RNAs recently considered regulatory with miRNA-like functionality. Concerning the protein coding fraction, ninety protein coding genes expressed over 10 RPKM were found and investigated for functional enrichment using String: pathway analyses revealed that there is a strong enrichment ($p = 2.74e\text{-}107$) for the “Ribosome” pathway with 57 proteins out to 90 considered. GO analysis revealed enrichment for term “Extracellular exosome” ($p = 3.23e\text{-}08$) cellular component vocabulary, as well as “RNA binding” ($p = 3.15e\text{-}07$) is one of the most represented molecular function. These data represent a key step in the characterization of E-Ad-MSC secretome and shed light on the mechanisms of action cell-derived vesicles.

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O004

Circulating miRNAs as biomarkers of stress and training in endurance horses

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Endurance exercise induces metabolic adaptation involving musculoskeletal, cardiovascular, respiratory, endocrine, immune systems and where muscle remodelling, mitochondrial synthesis and angiogenesis occur. Physical exercise has been recently associated to the modulation of a peculiar class of small noncoding RNAs (18–22 nucleotides), micro RNAs (miRNA), that act as post-transcriptionally regulators of gene expression. Released also in the body fluids, therefore named circulating miRNAs (ci-miRNAs), they have been recognized

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as optimal and accurate biomarkers in respect to classical serum/plasma biomarker and highly stability with high resistance to variations in temperature, pH value and multiple thaw and freezing cycles making samples storage and handling an easier task.
The aim of this study was to capture the whole picture of plasma circulating miRNAs through massive parallel sequencing in response to prolonged endurance exercise in samples obtained by four (4) trained and performing arabian horses. Plasma ci-miRNAs were analyzed before (T0) and two hours after the end of competition (T1), when the majority of the significant changes in ci-miRNAs occur. NGS libraries were built from plasma derived RNA and sequenced producing 50 nucleotide Single-End reads. After cleaning procedures, reads were aligned to the reference genome (equcab 2.0). Differential gene expression analysis, assessed with a count based approach using edgeR package, was applied comparing T1 versus T0 samples. Protein-Protein Interaction (PPI) network and significant enriched pathways of target genes were explored with Cytoscape 3.4.0 suite creating cluster of related targets from which Gene Ontology (GO) enrichment was calculated. Our results indicated the modulation of large set of miRNAs (up regulation of miR-1, 133, 206, 208b, 499-5p, down regulation of miR-486) arising from tissues involved in exercise response such as muscle, heart, liver, and blood and activation of correlated processes like inflammatory response, immunity, angiogenesis and cell proliferation. Ci-miRNAs high throughput sequencing is a promising approach for sport medicine itself beside the value of this specific work in horse athletes. Discovery of putative biomarkers for prediction of disease risks related to prolonged activity (i.e. overtraining syndrome) and metabolic adaptations monitoring to ultimately establish efficient training programs, could be transferred to all "sport species", including humans.

O005

Mitochondrial DNA haplotypes unveil mistakes in Maremmano horse pedigree records

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Nowadays traditional phenotypic and pedigree-based inferences are sustained by molecular data. Mitochondrial DNA is extensively used to genetically distinguish dam lines and to track ancestral origins. In order to obtain a more comprehensive overview on the Maremmano horse, we combined the available pedigree data with mitochondrial haplotypes, assessing the breed genetic diversity and verifying the reliability of extant genealogies. This important autochthonous Italian riding horse descended from an ancient local population spread in Maremma (in Southern Tuscany and Northern Latium) during the Etruscan period, and suffered, over the centuries, the influence of different genetic types introduced by people that dominated the Tyrrhenian coast. The phylogenetic analysis was performed on 90 female founders belonging to 70 different maternal lines, by sequencing 610 base pairs (bps) of the mitochondrial control region, from nucleotide position (np) 15,491 to np 16,100 and comparing to the Horse Reference Sequence (NC_001640). We were able to identify 52 haplotypes (32 unique and 20 shared between samples), classified through their diagnostic mutational motifs in 16 haplogroups (out of the 18 horse mitochondrial haplogroups already known), confirming the high variability within this breed. Indeed, this genetic feature of the Maremmano horse reflects the geographic position of Italy, in the centre of the Mediterranean Sea, and its cultural/economic past as a crossroad of migratory waves from the Near Eastern coasts into Europe and vice versa. Furthermore, for the most maternal lines (66 out of 70), the molecular analysis confirmed the available pedigree data, whereas four of them showed different mitochondrial haplotypes within the same mare line. This finding suggests that the current pedigree records might be somehow incorrect and confirms the efficacy of mitochondrial DNA surveys for testing Maremmano pedigree authenticity. The same approach could be extended also to other local breeds and livestock populations.

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O006

Relationship between ruminal biohydrogenation products and milk fat depression

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Milk fat content is affected by physiological and environmental factors. Milk fat depression (MFD) is observed in ruminants fed diet rich in starch or unsaturated fat. Several works proposed that MFD was related to a modification of ruminal metabolism, favouring the increase of specific biohydrogenation (BH) intermediates with antilipogenic effects. This role was attributed to CLAt10c12 because it is the only intermediate that clearly inhibits milk fat synthesis. However, dose-response studies carried out in different situations suggested that other fatty acids (FA) might inhibit milk fat synthesis.
The aim of this work was to individuate FA able to explain MFD. At this aim, we proposed a multivariate approach to discriminate cows affected by MFD syndrome.
Individual milk FA profile of 40 cows in MFD (T) was compared with that of 45 control cows (C). The two groups were composed of Italian Holstein cows in mid-lactation. All animals were fed the same diet (21.4 Kg of DM: 5.4 Kg of alfalfa hay +6.3 Kg of corn silage +9.7 Kg of concentrate). The level of high moisture corn in the diet was higher than 20%. According to literature, cows were considered in MFD when milk fat content was lower than 2.4%. FA composition was determined by gas chromatograph, while CLA isomers profile was obtained by HPLC.
The stepwise discriminant analysis was first exploited to select FA that best discriminated the T and C status. Then, using the 8 selected FAs (CLAt10t12, CLAt9t11, CLAt7t9, CLAc11t13, C10:1c9, C18:0, C18:1t6-t8, C18:1c11), the discriminant analysis correctly assigned, after a bootstrap resampling procedure, 92% of to animals to one of the two status.
Results highlighted that MFD was related to a modification of BH pathway, which involved not only CLAt10c12, but also other CLA isomers. Moreover, further milk FA were able to discriminate MFD status like C18:0, C18:1t6-t8, C18:1c11 (involved in BH) and C10:1c9 (involved in mammary metabolism). To our knowledge, this is the first time that MFD is investigated by comparing cows fed the same diet. When diet conditions are similar between the groups, a multivariate approach is a useful tool to evaluate mutual relationship among different groups of FA. Interestingly, CLAt10c12 was not selected by discriminant analysis, although its role on MFD is well known. Probably, the high correlation with other FAs masked its effect, reducing its discriminant power. On the contrary, CLAtt discriminate more clearly the effect of MFD.

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O007

Effect of replacing soybean meal with rapeseed meal on productive performance and ruminal conditions of lactating dairy cows

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The objective of this study was to investigate the effects of different levels of rapeseed meal inclusion in high producing cows diets on feed intake, rumination, total tract digestibility, ruminal pH dynamics and productive performance. Eight multiparous Holstein cows were assigned to a 4 x 4 Latin square design, with 3 weeks adaptation and 1 week of collection. Diets differed on primary protein source, while other dietary components remained the same. Treatments were identified as: S (2.5% DM basis of soybean meal), CB (1.8% soybean and 1% rapeseed meal), CM (0.9% soybean and 2.25% rapeseed meal), and CA (3.5% rapeseed meal). Chemical composition of the diets was similar among treatments, and the CP amount was 14.6% on average. Dry matter intake, milk production and quality, and rumination time were measured daily. Ruminal pH was measured continuously at 10 min intervals. Fecal samples were collected at multiple time points and averaged daily. Diets and feces were analysed for aNDFom, ADF, ADL, undigestible NDF (uNDF$_{240}$) and potentially digestible NDF (pdNDF), in order to calculate total tract fiber digestibility. All data were statistically analyzed with a factorial arrangement of treatments using the PROC MIXED model in SAS.
Milk production was not different, while ECM resulted higher in CM treatment (34.63 kg/d in CM compared to 32.47, 33.73, and 33.63 kg/d in S, CB and CA respectively, $p<.05$). Dry matter intake was lower in S treatment compared to the others ($p<.05$), while rumination time was not different among treatments. Ruminal pH was higher in CM treatment compared to others (5.92 against 5.88, 5.84, and 5.71 in CM, S,

CB, and CA respectively, $p<.05$). pH dynamics were also different among treatment, since both the area at pH <5.8 and <5.5 were significantly lower ($p<.05$) in CM compared to other treatments. In particular, it resulted: 72.1, 107.2, 165.0, and 255.0 in CM, S, CB and CA, respectively, for pH <5.8; 6.0, 15.0, 23.4, and 97.1 in CM, S, CB, and CA treatment, respectively, for pH <5.5. Total tract pdNDF digestibility decreased as the rapeseed inclusion increased, resulting in higher values for S treatment (61.25, 58.94, 56.63, and 54.32 in S, CB, CM, and CA respectively, $p<.01$). In conclusion, replacing soybean with rapeseed meal in diet for high producing cows led to an improved DMI, an increased ECM and protein amount, and to a more stable ruminal pH, with less time spent below sub–acidosis threshold.

O008

Study of the *in vivo* and *in vitro* variations of the enzymatic activity of bovine rumen fluid inocula

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Aim of the present work was to quantify the enzymatic activities (EAs) of rumen fluids (RFs) collected as inoculum in “*in vitro*” digestibility tests, in the attempt to assess the possible variations of RFs degradative capacity. A further objective was to evaluate the effects of the incubation of RFs on a standard substrate on enzyme expression by rumen microbes. In a first trial the activities of cellulase (C), amilase (A) and xylanase (X) were tested on 7 rumen fluid pools collected from two cows fed a hay-forage based diet in the interval June-September 2015. The EAs were tested using the radial enzyme diffusion method (RED) using substrate concentrations of 0.5%, 0.5% and 0.1% for C, A and X respectively. Wide variations of rumen fluid EA were observed with values (area of the halo surface; mm^2) ranging from 295.75 to 621.70 for C, from 310.26 to 495.40 for A and from 95.00 to 723.17 for X. The differences observed between the rumen fluids examined were significant for all the enzymes ($p<.001$) The EAs decreased in August, probably in relationship to heat/dietary stress. In a second trial, 3 different pools of (RFs), collected from cows fed no (NC), medium (MC) or high concentrate (HC) diets, were incubated in triplicate “*in vitro*” on a standard substrate (lactating cows total mixed ration). The relative EA were tested at different intervals (h: 1, 2, 4, 8, 24 and 48) to evaluate the dynamic of C, A and X enzymes expression pattern by ruminal microbiota, and to individuate the interval at which the different inoculums showed similar and/or maximum EAs. Initial EA were NC = 226.12; MC = 198.49 and HC = 282.93 mm^2 for A activity; NC = 250.32, MC = 365.67 and HC = 578.79 mm^2 for C; NC = 227.83; MC = 95.00; HC = 124.10 mm^2 for X. An increase of EA was observed for A from 4 to 8 hours, with maximum values of 321.71 for NC, 270.99 for MC and 304.27 mm^2 for HC. EA peaks were observed for C at 2 and 8 h. X activity showed a maximum value at 8 h for HC (208.67 mm^2) and after 48 h for NC (343.5 mm^2) and MC (288.07 mm^2). Similar values of EA in the 3 pools (NC, MC and HC) were observed at 24 h for C and at 4 h for X. Concerning A, two (MC and HC) out of 3 pools showed similar EA values at 24 h. The RFs EA can vary widely across inoculums from the same animals. Incubations affects EA, generally inducing an increase in RFs coming from NC diet, and a decrease in RFs derived from HC diet, with similar values at variable intervals depending on the enzyme.

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O009

Effect of exhausted berries of myrtle on ruminal metabolism in dairy sheep

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Utilization of polyphenols-rich by-products has become very interesting for their potential use in animal nutrition as modulators of the end products of rumen fermentation. Exhausted myrtle berries (EMB) is a by-product of the maceration process of myrtle (*Myrtus communis* L.) berries to produce a commercial liqueur called *Mirto*. The objective of this study was to evaluate the effect of the dietary inclusion of EMB on ruminal metabolism in lactating ewes. Rumen liquor samples were collected at week 3, 7 and 11 of the trial from 18 Sarda dairy ewes randomly assigned to 3 dietary treatments; a control diet (CON), a diet supplemented with 50 g/d per head of EMB (EMB50), or a diet supplemented with 100 g/d per head of EMB (EMB100). Sampling was performed 2 hours after the morning feeding, using a stomach tube and an evacuation pump. The samples were analyzed for ammonia, volatile fatty acids (VFA), fatty acids (FA) profile and dimethylacetals (DMA). The methane production was estimated according to the equation of Moss et al (2000). Data were analyzed with the PROC MIXED procedure of SAS. The model included the

dietary treatment, sampling and their interaction as fixed effects, and the animal nested within the treatment as random effect. Results evidenced that the concentration of rumen ammonia decreased ($p<.05$) in sheep supplemented with EMB (mean 11.8 mg/dL for EMB50 and EMB100) compared with CON (14.3 mg/dL). The total VFA concentration decreased in rumen of EMB100 group, compared with CON and EMB50 ($p<.05$), whereas the proportion of individual VFA were not affected by the diet. The acetate to propionate ratio and estimated methane production tended to increase with the EMB inclusion in the diet ($p<.10$). The total concentrations of FAME, OBCFA and DMA were not affected by the inclusion of EMB. The total concentration of CLA increased ($p<.05$) in EMB50 compared with CON, with that of EMB100 being intermediate. The C13:0, C16:1 and C17:0 DMAs, were reduced ($p<.05$) by EMB inclusion. In conclusion, the addition of EMB by-product to the diet of dairy ewes might improve rumen nitrogen metabolism, even though further in-depth studies are needed to confirm this result and to clarify possible additional effects.

O010

Characterization of the rumen microbiota in dairy calves receiving copper or grape-pomace feed supplementation

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In this study, the rumen microbiota of Holstein-Friesian calves was characterized, and the effect of two feed supplementations on its composition was investigated. Fifteen male calves of an average age of 195 days were assigned to three groups: copper supplementation (cupric sulphate: 300 g/100L of drinking water), grape pomace supplementation (1 kg/head/day), and control (no supplementation). The dietary treatment had a duration of 75 days. Copper is an essential trace elements; grape pomace is a source of polyphenols and resveratrol which have antioxidant and protective properties. Rumen fluid was sampled after slaughter for microbial-16S metabarcoding-sequencing. Taxonomic counts were then used to characterize the rumen microbiota and assess the impact of dietary supplementation.

The average number of different microbial OTUs (operational taxonomic units) was highest in the grape-pomace group (1583) compared to the copper (1507) and control (1500) groups. The species richness indicators (Chao1, abundance-based coverage estimator-ACE) gave a similar picture. The Shannon and Simpson diversity indexes were very similar among groups. On the contrary, the Fisher's alpha index showed a tendency of being higher in grape pomace group (256.4) than in copper and control ones (235.1 and 218.6 respectively). Overall, the most abundant microbial species in the rumen were Prevotella stercorea, Bacteroides uniformis and Ruminococcus flavefaciens, which accounted for 79% of total rumen bacteria. The same three species were the most abundant in the grape-pomace group (86% of the total), in controls (76% of total) and in the copper group (70% of total). Microbial counts were also used to classify calves in the three experimental groups. Random Forest (RF) was used for classification. When all OTUs were fitted to the classification model, the OOB (out-of-bag) classification error was high (66%). However, if only the four most relevant OTUs (from RF variable importance ranking) were fitted to the RF model, the OOB error dropped to 26.7%.

The rumen microbiota in dairy calves seems to be dominated by few taxas. Feed supplementation with grape-pomace and copper did not have a profound impact on the overall composition of the rumen microbiota. However, specific microbial species and/or taxa were differentially abundant in the treatment groups, and allowed for a certain degree of discrimination between them.

Acknowledgements

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O011

Effect of olive oil pomace dietary supplementation on rumen microbial community profile: a metagenomics approach

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The aim of this study is to investigate the effect of 2 different olive oil pomace (OOP) produced by a bi-phasic (OOP2) or by a three-phasic (OOP3) process of extraction on rumen microbiota using a metagenomic approach.

Twenty-four multiparous Comisana ewes were allotted into 3 experimental groups in multiple pens homogeneous for number, body weight and milk yield. The experimental diets were composed of chopped alfalfa hay administered *ad libitum* and 800 g of a concentrate containing 10% of extruded linseed (Control: C) or 10% of extruded linseed and 13.5% of OOP2, or 10% of extruded linseed and 11.25% of OOP3. At 28th day, the rumen liquor was individually sampled, by oesophageal pump, for fatty acid profile (GC-FID) analysis and microbiome characterisation. Microbial community profile was determined sequencing the V3-V4 region of the 16S rDNA on Illumina Miseq with 300 bp paired end.

Data of fatty acid composition of rumen liquor were analyzed by the following general linear model: $y_i = \mu + diet_i + e_{ij}$, where: y is the fatty acids; μ is the overall mean; $diet_i$ is the fixed effect of diet ($i = 1$ to 3). Illumina data were analyzed with QIIME. Analysis of variance, at different taxonomic levels, was done with R. Multivariate analysis (nMDS, UPGMA) were performed with PAST.

Rumen liquor from ewes fed with OOP3 and OOP2 shows a higher content of *cis*9 *cis*12 *cis*15 18:3 (α-linoleic acid, LNA). A significant decrease of *Anaerovibrio spp.* was found in OOP2 and in OOP3 respect C. Within this genus *A. lypolitica*, a rumen lipase-producing bacterium, is responsible for diglyceride hydrolysis. Lipolysis is the rate-determining step for the biohydrogenation of polyunsaturated fatty acids (PUFA). In vegetable oils, LNA is naturally present in Sn2 position of triglycerides, which is the last to be hydrolysed by microorganisms. Hence, a reduction of *Anaerovibrio spp.* concentration could be related to LNA accumulation.

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O012

Effects of salting time on seasoned meat in two pig genotypes

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The aim of the study was the effect of salting time on the chemical-physical traits in "Cuore di Spalla" product resulting from seasoning boneless shoulder. Fourteen Duroc x Large White (DxLW) and 14 Cinta Senese x Large White (CSxLW) castrated male pigs, were used. The subjects were reared in outdoor fences with a commercial mixture (12% crude protein, 2% fat and 3% crude fibre). The animals were slaughtered at the same age (14 months) and at an average live weight of 143 ± 15 kg for DxLW and 122 ± 15 kg for CSxLW. The right shoulder of each pig was salted for 3 days (L) while the left for 5 days (H). At the end of the salting period, the shoulders were stuffed into a natural gut. At the end of seasoning time (6 months), on a whole slice of salami the CIE L*; a*; b* color parametres and sensorial profile were determined. Chemical analysis (moisture, crude protein, ether extract, ash) were also performed. Data were analyzed by the GLM procedure (SAS, 2007) using breed, level of salt and their interaction as fixed effects. The results showed a greater growing in DxLW than CSxLW: DxLW showed, in fresh product, higher weight (3.1 *vs* 2.5 kg) and lower moisture percentage (63.3 *vs* 66.2%). On cured products DxLW showed higher fat content (12.8 *vs* 9.5%) and lower percentage of salt than CSxLW (6.8 *vs* 9.8%). These last results, Considering the same salting time for both genotypes, were linked to what observed on fresh meat (higher weight and lower moisture in DxLW respect to CSxLW. As regard color parameters DxLW recorded higher values of L* (40.2 *vs* 36.2), a* (18.0 *vs* 16.1) and b* (6.7 *vs* 4.9) than CSxLW. Color result in CxLW is probably attributable to the different maturation degree of the two genotypes at slaughter (Cinta Senese is an unimproved breed with slow growing rate). In regard of salting time effect, only DxLW group showed significant differences in salt percentage (7.8 *vs* 5.8% respectively for H and L). This result may be due to the smaller size of the product and to lower fat content of the CSxLW group which caused a salt saturation, already after three days of salting.

As regards sensorial traits, genotype effect affected oiliness, lean color and hardness of product, DxLW showed the highest values, probably due to the highest weight of animals and to the more matured meat. The products subjected to longest salting time showed the highest saltiness.

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O013

Effect of the broking down of cold chain on beef meat oxidation

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Foods are exposed to reactive oxygen species (ROS) that produce oxidative stress on lipids and proteins, chemical deterioration, discoloration, off-flavor and formation of toxic and carcinogenic compounds as the 4-Hydroxy-2-nonenal (HNE) and Malondialdehyde (MDA). Moreover the oxidation of meat during storage is accelerated at warm temperatures, compromising its shelf life. This study examined the relationship between protein and lipid oxidation and polyunsaturated fatty acid amount, considering also the effect of the cold chain breaking during storage. Forty-two samples of *longissimus dorsi* (L) and 42 of lean ground beef (M), belonging to 6 different young bulls were analyzed at time 0, 24, 48 hours and 7 days. The samples, after removal the samples at time 0, were divided into two groups, one (U) maintained at constant temperature (2 °C) and the second (B) was been undergone in the first 24 hours to three broken of cold chain (at 15 °C for 3 hours). Water loss, pH, metmyoglobin, thiols, carbonyls, MDA, HNE and fatty acid were determined. Data were submitted to analysis of variance with type of meat, temperature treatment, storage time as main effects. Moreover, Pearson correlation analysis was used to investigate the relationship among fatty acid and oxidation trend. The L samples maintained at constant temperature (U) did not present significant oxidation processes, while the M meat showed a significantly increase of HNE value ($p<.05$) from 0 to 7 days, reaching 13.5 nmol/g of meat. The lipid oxidation was already evident at 48 hours of storage for M samples undergone to chilling interruption (B), obtaining at 7 days a significantly ($p<.01$) high value of TBARS (1.59 mg/kg) and HNE (27.8 nmol/g). While protein oxidation (+18% of carbonyls $p<.05$) linked to a greater water loss ($p<.001$) and an increase of pH ($p<.05$) was significant only between zero and 7 days. Thiols group did not show significant differences during the stored times, due to high variability of the data. Significant positive correlations ($p<.05$) were found between the content of arachidonic, linoleic acid and the accumulation of HNE in L and M meat for B group. Therefore, the meat exposed to breaking of cold chain indicate an acceleration of lipid and protein oxidation, resulting in the accumulation of toxic substances harmful for health. Higher lipid oxidation are associated with higher amount of polyunsaturated fatty acids, making void the qualitative improvements of the beef meat.

O014

Comparison of oxidative status and antioxidant compounds of pig meat from Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig before and after cooking

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The aim of this study was to evaluate oxidative status and antioxidative compounds of meat from Suino Nero Lucano (SNL) and a modern crossbred (CG) pig, before and after coking. This study was carried out on 30 castrated male pigs purebred SNL pigs and 30 CG pigs ((Landrace x Large White ♀) x Landrace ♂). At about 90 days of age, they were located in the same farm, raised under a semi-wild system and fed with the same diet until their slaughter. SNL were slaughtered at a live weight of about 140 kg and 540 days of age and CG pigs at 160 kg of live weight and 480 days of age. *longissimus dorsi* (LD) muscle, was removed from each carcass. From each of the LD muscle samples, two pieces (150 g) were cut from the central portion, and distributed in two groups (raw and cooked). Cooking process (75 ± 3 °C at the center of the muscles) was performed in a convection-steam oven. All raw and cooked meat samples were analyzed for chemical composition (AOAC, 1995), vitamin E, creatine, carnosine and creatinine content (HPLC method) phenols (Folin-Ciocalteau method), thiols content (Ellman's method), and superoxide dismutase activity (spectrophotometric assay). Data were analyzed according to GLM procedure (SAS Institute, 1996). SNL raw meat showed higher fat content and lower protein content (23.63 *vs* 22.53% DM and 72.58 *vs* 73.84% DM, respectively; $p<.001$), higher vitamin E (2.75 *vs* 2.57 ug/g, respectively; $p<.001$), bioactive peptides (406.73 *vs* 347.63, 433.67 *vs* 347.91, 8.44 *vs* 7.26 mg/100 g, for creatine, carnosine and creatinine, respectively; $p<.001$), total phenols (133.62 *vs* 122.39 mg gallic acid/100 g; $p<.001$) and thiols (88.24 *vs* 73.59 nmol SH groups/mg protein; $p<.001$) content than CG raw meat. The cooking process has lead to a loss of all considered parameters ($p<.05$), except for creatinine content due to the conversion of creatine into creatinine during cooking process. CG cooked meat presented a higher creatinine content (11.79 *vs* 14.30 mg/100 g, respectively; $p<.001$). SNL cooked meat showed a higher vitamin E (2.17 *vs* 2.01 ug/g, respectively; $p<.001$), creatine and carnosine (205.53 *vs* 180.51, 234.96 *vs* 182.86 mg/100g, respectively; $p<.001$), total phenols (79.51 *vs* 71.39 mg gallic acid/100g; $p<.001$), and thiols (51.45 *vs* 39.82 nmol SH groups/mg protein, respectively; $p<.001$) content, indicating a better oxidative stability and a higher antioxidant capacity.

O015

Wet aging effect on beef meat physical traits

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The aim of this work was to study the evolution of meat quality traits during wet aging. Two hundred and two samples of *longissimus lumborum* muscle belonging to four genotypes (Limousine, Charolaise, Romagnola and crossbreed) were randomly sampled at the same slaughterhouse from April 2015 to June 2016. Slaughter age ranged from 11 to 30 months, while carcass weight ranged from 147 to 482 kg. *longissimus lumborum* was collected from the right half-carcass the day after slaughtering and 4 steaks were obtained by hand cutting. One steak was analysed the same day of sampling (day 1), the remaining three steaks were vacuum packaged and stored at 4 °C to perform wet aging at three different aging period (4, 9 and 14 days). Determinations carried out at each time were: pH, moisture, colour parameters L*, a* and b*, with Minolta chromameter CR200, finally TPA analysis were performed using a Zwick/Roell Z2.5 instrument. Data were analysed using the 'lsmeans' package of R software. Statistical model included the fixed effects of breed (4 levels), day (4 levels) and the covariate effects of carcass weight and slaughter age. Due to the difference height of the steaks this measure was included as covariate for TPA traits.
Results show that pH did not change significantly during the aging period, on the contrary L* (from 41.86 to 43.13), a*(from 21.78 to 20.49) and b*(from 6.45 to 7.16) changed significantly. Moisture was also significantly affected by the aging period (from 73.71% to 72.99%) as well as the TPA

parameters: hardness (from 1.79 N to 0.99 N), gumminess (from 0.7 N to 0.43 N) and chewiness (from 5.06 J to 3.09 J) decreased significantly with the aging period, while cohesiveness and springiness remained constant. In conclusion, wet aging could be considered a good alternative to dry aging; changes in meat colour, although statistically significant, are negligible, and loss of water during aging is contained. Vacuum packaging does not influence negatively meat tenderization process as confirmed by the reduction of hardness, gumminess and chewiness.

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O016

Genetic variance for sow tolerance to heat stress

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Poor sow reproductive performance due to heat stress (HS) can be partially improved with the use of genetic (genomic) selection. The objective of this work is to investigate the steps needed for its implementation, including the identification of traits and environmental covariates (ENV) of interest and estimation of variance components for tolerance to HS.

Reproductive and pedigree data were obtained for 22,339 Landrace litters (11,163 sows) from nucleus farms from Smithfield Premium Genetics (SPG) as well as 26,273 Landrace litters (11,696 sows) from The Maschhoffs (MAS). Traits included number born alive (NBA), total number born (TNB) and birth weight (BW).

Climate records (average, minimum and maximum temperatures, daylight duration and relative humidity) from the US National Climatic Data Center were downloaded and processed creating litter-specific measures. In addition, temperature-humidity index was computed. Phenotypes were analyzed using a sliding window approach: conception period was divided into three 1-week periods, pregnancy was divided into sixteen ∼1-week periods and lactation was divided into three 1-week periods.

SS-GBLUP random-regression animal models were run for each trait by ENV by time-period combination, including 80k SNP-chip genotypes for sows and their sires. The models included fixed effects of parity (cross-classified, four levels) and ENV (first order covariate), random additive genetic effect of sow and ENV first-order covariate nested within sow. Variance components were estimated using Gibbs sampler. The models provided estimates of heritability that are comparable to the traditional one and an estimate for the sow tolerance/sensitivity to the ENV (h^2_{TOL}), which is proportional to the variability of each sows ENV regression coefficient.

For both the populations under study, the h^2_{TOL} showed different magnitude depending on the ENV and the time range of ENV recording. For TNB, h^2_{TOL} was larger under relative humidity when this was measured before conception (∼0.075) and under maximum daily temperature when this was measured in late gestation (∼0.06). For BW, h^2_{TOL} was larger when ENV was recorded between 21 and 35 days of gestation under relative humidity (∼0.05). An explanation to this is that control of heat stress in sow farms is based on air flow systems, which can control temperature but not humidity.

Current results provide evidence of the existence of a genetic determination of sow tolerance to HS.

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O017

Combining landscape genomics and ecological modelling to investigate local adaptation of indigenous Ugandan cattle to East Coast Fever

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East Coast Fever (ECF) is a major and often fatal sickness affecting cattle populations of Central and Eastern Africa. The disease is caused by the protozoan *Theileria parva parva*, and transmitted by the hard-bodied tick *Rhipicephalus appendiculatus*. Indigenous herds, however, show tolerance to infection in ECF-endemically stable areas. Here, we used molecular data and epidemiological information from 823 indigenous cattle from Uganda to investigate the genetic bases underlying local adaption to *T. parva parva* infection. *R. appendiculatus* potential distribution over the study area, along with *T. parva parva* infection risk, were estimated and tested by means of a genotype-environment association (GEA) analysis (Stucki et al. 2016, Mol Ecol Resour. doi:10.1111/1755-0998.12629). We identified 41 and seven candidate adaptive loci for tick burden and *T. parva parva* infection, respectively. By exploring a region of 25 kbp around the significant loci, two genes were identified as putatively involved into local adaptation for ECF: PRKG1 (Protein kinase, cGMP-dependent, type I) and SLA2 (Src-like-adaptor 2). PRKG1 is an inflammatory response-related gene already described as associated with tick resistance in indigenous South African cattle. SLA2 is involved with lymphocyte proliferation regulatory pathways, which are known to be modified by *T. parva parva* infection. Additionally, a preliminary ancestry analysis of the highlighted candidate regions showed a mixed sanga (African taurine *x* zebu) and zebuine origin for PRKG1 region, and a prevalent sanga origin for SLA2 region.

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*Co-senior authorship

O018

Variation of Madura cattle in Madura Island, Indonesia

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Madura cattle are between the most important local cattle in Indonesia: they are probably a crossing of Bali (a domesticated form of the Banteng, *Bos javanicus*), *Bos indicus* and *Bos taurus* breeds. The uniformity of this hybrid was achieved after hundreds years of selection in the island of Madura. Madura cattle are embedded in many cultural aspects of local people. Two famous traditional festivals involving Madura cattle are Karapan (a bull racing) and Sonok (a cow/heifer beauty contest); animals from different areas are bred specifically for each event, and also the management practices are differently focused: as a result, there are visible phenotypic differences between the Karapan and Sonok sub-populations.

The aim of this study was to prove scientifically the existing phenotypic variation between these two sub-populations. Phenotypic traits were collected from 38 and 86 cows used for Karapan and Sonok breeding respectively, obtained data were analyzed using "R" programme. T-test was used to analyze body measurements data and showed that Sonok is significantly larger than Karapan ($p<.05$) in all dimensions. Exterior traits like body color patterns, dewlap, hump or backline were analized using χ^2 which also showed significant differences ($p<.05$) between the two sub-populations. Principal Component Analysis was used to visualize the underlying pattern of body measurements data. We could see separate clusters between the Sonok and the Karapan where the first two PC explained 67% and 16% of the total variance. These results allow discriminating the Sonok from the Karapan by means of body measurements and exterior traits.

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O019

BOVITA: a first overview on genome-wide genetic diversity of Italian autochthonous cattle breeds

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Analysis of genomic data is increasingly becoming part of the livestock industry and is an invaluable resource for effective management of breeding programs in small populations. The recent availability of genome-wide SNP panels allows providing background information concerning genome structure in domestic animals, opening new perspectives to livestock genetics. BOVITA was established to join local efforts and resources for the genomic characterization of Italian local cattle breeds. Despite the growing diffusion of some cosmopolite specialized breeds, several autochthonous breeds are still bred in Italy. The main aim of the BOVITA is to investigate the genomic structure of Italian local cattle breeds, to provide information on their genetic status that will be useful for the management of the genetic variability, as a contribution to biodiversity conservation and prioritization actions.

A total of about 800 animals (20-32 per breed) belonging to thirty Italian cattle breeds (Agerolese, Barà-Pustertaler, Burlina, Cabannina, Calvana, Chianina, Cinisara, Garfagnina, Italian Brown, Italian Holstein, Italian Simmental, Marchigiana, Maremmana, Modenese, Modicana, Mucca Pisana, Pezzata Rossa d'Oropa, Piedmontese, Pinzgau, Podolica, Pontremolese, Pustertaler, Reggiana, Rendena, Romagnola, Rossa Siciliana, Sarda, Sardo-Bruna, Sardo-Modicana and Ottonese-Varzese) and two cosmopolitan breeds (Charolaise and Limousine) genotyped with the Illumina BovineSNP50 v2 BeadChip array were collected for the analysis. The genotypes of several breeds were detected in the frame of the project, whereas for some breeds these data are derived by previous studies. The dataset will be analyzed to: study several aspects of population genetic diversity, multidimensional scaling plot, population structure, linkage disequilibrium, and runs of homozygosity. In addition, comparative analysis of conserved haplotypes will be conducted to identify genomic segments under selection pressure. Such information also provides important insights into the mechanisms of evolution and is useful for the annotation of significant functional genomics regions. Data analysis will also be useful to select SNPs suitable for parentage test and breed genetic traceability. The analysis of the data will pinpoint the genetic distinctiveness of Italian breeds. Moreover, the obtained results contribute to a better characterization of history and genetic structure of Italian cattle breeds.

O020

Effects of Single Nucleotide Polimorphisms (SNPs) in candidate genes for growth traits in Italian beef cattle breeds

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Chianina (CH), Marchigiana (MA) and Romagnola (RO) meat is worldwide recognized as top quality one; molecular markers could be used in the selection programmes of these breeds. In this study the effect of several candidate genes polymorphisms on growth and muscle traits is analysed. On 1196 young bulls (CH: 473, MA: 419, RO: 304) the following measurements were observed at one year of age: withers height (WH), trunk length (TL), chest girth (CG), rump length (RL), rump width (RW), shoulder width (SW), back width (BW), loin width (LW), and loin length (LL); furthermore 9 weights were recorded every 21 days from 6 up to 12 months of age during the performance test. In addition 5 BLUP indexes: Muscle Index (MI), Total Index (TI), Muscle Morphological Score (MMS), Dimension Morphological Score (DMS), Total Morphological Score (TMS) were considered. Fifteen SNPs in the following 10 genes were selected: *GHR* (AF126288, AY643807), *GDF8* (AJ438578, AJ794986), *GHRL* (AY455980), *LEP* (AB070368, AY138588, AY11369), *MYF5* (M95684), *IGF2* (AY237543), *LEPR* (AJ580801), *UCP2* (AY14782), *UCP3* (AF127030, AF127030), and *MC4R* (AF265221). The MA young bulls were genotyped at the GDF8 gene in order to know if they were hypertrophied or not. The gene and genotypic frequencies and the effect of each SNPs on the indexes were estimated by SAS software (PROC FREQ and PROC STEPWISE; $p \leq .05$); the average gene substitution (AGS) was computed by MTDFREML. In many SNPs the frequencies of the three genotypes were similar in the three breeds; the homozygote AA in GHR (g.300, A > G) was the most abundant one (CH: 60.2%, MA: 47.4% and RO: 54.0%) and the homozygote GG the loss represented one (5.4, 10.2, 5.9). The highest differences were observed between RO and CH. In body traits the AGS absolute values were in general lower than 0.5 cm; the most interesting SNP was MC4R (g.1069, G > C) in MA that got significant values in CG, RL, RW, SW and LW. The most important SNPs for the weights were LEPT (g.528, C > T) and MC4R. A general view of the AGS drives to take into consideration LEP (g.528, C > T) in CH, and MA, MYF5 (g.1948, A > G) in MA, and MC4R in MA and RO. The effects of the SNPs on the BLUP-AM indexes were rather strong in MC4R in CH (MI, TI, MMS), in GHR (g.300, A > G) in MA (MI, TI and DMS) and RO (MMS and DMS). According to these results should be suitable to consider SNPs markers in the selection procedures of the Italian beef cattle breeds.

Acknowledgements

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O021

Heritability of reproduction traits and type latent factors and their genetic correlations in Marchigiana, Chianina and Romagnola beef breeds

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Genetic improvement in beef cattle has been focused mainly on productive traits; however, reproductive, functional traits and correctness have gained interest in recent years. The aim of this study was to analyse the heritability (h^2) and the genetic correlations (rg) between reproductive and type latent factor in Marchigiana (M), Chianina (C) and Romagnola (R) Italian beef cattle. The 25 linear type traits scored on heifers using a 1-5 scale have been factorized to 4 latent factors (F1 to F4; eigenvalues ≥ 1), that explained from 58 (for R) to 64% (for C) of the total variance. In each breed F1 included traits related to muscularity (MU), F2 to body size (BS), F3 to fineness (FI) and F4 to feet and legs (FL), respectively. The final factorial scores obtained were merged with the reproductive data subsequently available for M, C, and R cows, i.e. the age at first calving (AFC; d) and the mean calving interval (CI; d). The number of data available varied from a minimum of 4348 (average CI of R cows) to a maximum of 13,960 (AFC of C cows). Bi-trait analyses were carried out to estimate h^2 and rg under animal model by means of a Gibbs sampling algorithm. The h^2 of the CI varied 0.057 for M to 0.111 for R, while the AFC showed greater h^2, i.e., from 0.229 for R to 0.298 for M. The h^2 values were moderate (0.104 to 0.226) for factorial MU, BS and FI and low (0.058 to 0.078)

for FL. The Highest Posterior Density 95% of rg distributions included zero value with some exceptions. The rg between BS and AFC was negative (favourable) in all breeds (-0.175 to -0.393); rg between FI and AFC was negative and favourable in M cows (-0.144) only; rg between MU and CI was negative and favourable in C cows (-0.302) only; and rg between FL and CI was positive (i.e., favourable) only in R cows (0.373). In conclusion, selection for type in M, C and R breeds produce small favourable effects on the CI, whereas AFC is indirectly positively improved by the current selection on factorials.

O022

Histological muscle characterization in hypertrophied Marchigiana cattle breed

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Myostatin (MSTN), also known as GDF8 (Growth Differentiation Factor 8), is one of the major regulators of skeletal muscle development. Mutations on *MSTN* gene are responsible for double muscling phenotype in several livestock animals. This phenotype occurs at a high frequency in some cattle breeds such as Belgian Blue and Piedmontese, and it was also identified in Marchigiana cattle, one of the most important Italian beef cattle breeds. A transversion mutation at nucleotide 874 in exon 3 (g.874G > T) of MSTN gene was found in Marchigiana breed.

In this work a Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) test was used to determine the genotype at MSTN locus. Besides, histological analysis were carried out to investigate on differences in muscle morphology between the three genotypes. Furthermore, considering the important role played by myostatin during myogenesis, a satellite cell specific marker (PAX7) was considered to verify if a myostatin deficiency leads to an increase in these stem cells. Ten bullocks aged between 18 and 24 months (except one which was 13 months old) were sampled. Blood and muscles were collected at slaughtering. All animals were genotyped at the *MSTN* locus. Three different muscles were characterized: *semitendinosus*, *psoas major* and *longissimus dorsi*. Formalin-fixed paraffin-embedded sections of muscle tissues were stained with haematoxylin/eosin. Myofiber cross sectional area (CSA) was used for muscle comparison. Part of each muscle samples were lysed and analysed for the expression of PAX7 by Western blotting. Genotyping at *MSTN* locus showed that two bullocks were homozygous for the mutation (TT), five were heterozygous (GT) and three were normal (GG). The CSA values in homozygous (TT) and normal (GG) were quite similar but microscopy analysis revealed muscle hyperplasia in homozygous (TT) bullocks, in accordance with previous studies. Western blotting analysis showed different expression levels of PAX7 in the three genotypes, with an increase in the mutant homozygous one. These findings confirm that lack of myostatin influences the proliferation of muscle precursor cells during myogenesis. Further studies about the mechanisms by which myostatin inhibits muscle growth are needed to better understand the particular muscle development in the hypertrophied Marchigiana cattle breed.

O023

“QualiPiem” - Innovative tools for selection of meat quality in Piemontese breed

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Genetic improvement of meat quality is complicated by the large number of traits involved and is usually limited by difficulties and costs related to sample collection and analytical procedures, which involve time-consuming lab methods.

The “QualiPiem” project is aimed at exploring the possibility of selecting meat quality traits in Piemontese cattle, with a particular concern to application and to cognitive aspects. The study provides the development of innovative tools for phenotypes detection on large scale at operational level based on visual and near-infrared spectroscopy (Vis-NIRS), the understanding of the genetic basis of meat quality traits and the implementation of genomic tools for selection.

Samples collection started on April 2015 and was carried on until February 2017. Twenty-four hours after slaughter, individual samples of the *longissimus thoracis* muscle were collected between the fifth and sixth thoracic vertebrae from 1,234 Piemontese young bulls, which were progeny of 193 A.I. purebred sires and 1,212 dams, all registered in the Italian Piemontese Herd Book. Animals were fattened on 135 farms and slaughtered at the same commercial abattoir. The SEUROP conformation of carcasses was also recorded at slaughter. Vis-NIRS spectra were collected on all fresh samples over a spectral range of 350 to 1,830 nm, in reflectance mode. After 8 d of aging physical attributes of meat samples were assessed by measurement of lightness (L*), redness (a*), yellowness (b*), pH (pH8d), drip loss (DL, %), cooking loss (CL, %) and shear force (SF, kg). Data on farms management were recorded to investigate environmental effects on quality traits.

Average age at slaughter (± Standard Deviation) was 542 d (± 62 d), average carcass weight was 437 kg (± 46 kg) leading to 0.820 g (± 0.11 g) of average carcass daily gain. “S”, “E”, “U” carcass conformation classes accounted for 26%, 67% and 7% of animals respectively. Average values (± s.d.) of physical parameters were 40.03 (± 5.98), 18.22 (± 3.09), 15.65 (± 2.75) for L*, a*, b* respectively and 5.56 (± 0.06), 4.60 (± 1.33), 16.53 (± 3.43) and 4.25 (± 1.15) for pH8d, DL %, CL % and SF kg respectively.

Five main management systems were identified by cluster analysis.

Next steps will concern the development of calibration equations for traits prediction from Vis-NIRS spectral data, the investigation of phenotypic and genetic variability and the implementation of genomic analysis for the selection of beef quality traits.

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O024

Twinning project: increasing of twin births in Maremmana breed

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The vast majority of fattened calves in Italy are imported. Therefore it is desirable an increase in national production of weanlings. Producing an extra calf in twin calving would be an opportunity to enhance the production output since an additional calf would be available for slaughtering purposes. Twinning is associated with a number of negative effects, as abortions, retained placentas, dystocia, longer interval before return to oestrus and reduced calf survival. The aim of twinning research project was to investigate the feasibility of selection for twinning in the local Italian breed of Maremmana cattle. The Maremmana herd of CREA-PCM experimental farm was the case study. From 2012 the herd is reared in two separate groups, one purebred and another crossed with Piedmontese bulls, to produce calves with a better beef conformation. A database containing all genealogical and phenotypic historical data (vital events, live weight, health data, and culling reasons) was set up. Calving records, including type (single or twin) were available since 1983 and all twins were considered dyzigotic. For this study, data from 1,260 individuals born from 1963 to 2014, 527 males and 733 females, were used. Statistical analyses were performed by the package Multivariate Mixed Models DMU. A total of 2,130 calving were recorded from 1983 through 2015: 2009 single and 121 twin births. During the four year of the experiment from 2012 through 2015 the twinning rate grew from 5.63% to 12.77%. Breeding values for twinning were estimated using two different animal models. Heritability estimates of twinning were 0.014 and 0.062 for the linear and the threshold model respectively. Also we calculated (chi-square test) the relation between twinning and calf mortality, purebred vs Piedmontese crosses and overall. Considering together single and twin calving, mortality from birth to weaning was significantly higher in purebred (7.60%) than in crosses (1.26%; p=.01). Genome-wide association study was performed on the corrected phenotype of all calving during the lifespan of each cow, using the Illumina BovineSNP54 BeadChip and DNA from119 cows living on farm in 2012. Different chromosomal regions and some candidate genes associated with twinning were identified. The preliminary results of this twinning project suggest that increasing twinning rate could offer an opportunity to improve the productivity of the Maremmana and of similar breeds kept on range conditions.

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O025

Genetic improvement and population structure of Italian Limousine

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The Limousine breed was firstly introduced in Italy in 1985 and since then has been experiencing an increasing and steadily growth. The herd book was officially recognized from the Italian ministry of the agriculture in 1999. In 2016 there were 1464 herds, 26,399 cows, 7425 heifers, 20,625 young animals and 1.273 bulls. The breed is well distributed across Italy, but the large number of herds/animals are in Sicily, Sardinia, Tuscany and Lazio regions. The objective of the present paper was to infer the population structure of the Italian Limousine and to implement a first genetic evaluation for average daily gain. Population structure was performed using the Endog program. Pedigree data included 275,083 animals born from 1950 to 2016. Base populations included 52,177 individuals. The effective number of founders was 1500 and the genetic contribution of 10, 50 and 302 most influent ancestors explained 10.2, 25.1 and 50% of the genetic variability in the population, respectively. Inbreeding and average relatedness coefficients were 0.68 and 0.21%, respectively. The effective number of ancestors was 468 and the effective population size was 360. Pedigree known (%) was 83, 67, 58 and 42% in generation 1, 2, 3 and 4, respectively.

Genetic evaluation for average daily gain (ADG) was performed using a single-trait repeatability test-day model. A total of 271,330 weights from 116,299 animals were included in the analysis. The pedigrees of the animals were traced back to 1950, and consisted of 276,518 animals. Genetic groups ($n = 15$) were defined based on origin and year of birth. The fitted model included herd-test day, sex and twinning as fixed effects and age at weigh by sex and age of the dam as linear and quadratic covariates. A minimum number of 2 weights from 30 to 365 days were required for each individual. The software DMU was used to estimate variances and predict the breeding values. Heritability and repeatability were 0.30 and 0.26, respectively. The ADG EBV was obtained dividing liveweight EBV by 335 days. Breeding values were standardised with mean = 100 and standard deviation = 10. The genetic base was fixed as the average ADG EBV for weighted animals born in 2011. BLUE estimates for age at weight by sex covariate were 1079 and 0.965 kg/d for males and females, respectively. These results are the first step towards the development of a national genetic evaluation.

O026

Ancient distinctive migration routes suggested by the current mitochondrial gene pool of Podolic cattle breeds in Italy

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Podolic cattle are a very ancient group of European bovine breeds, considered to be directly descendant of the aurochs (*Bos primigenius*). The aim of the present study was to assess the diversity of Podolic cattle breeds in order to obtain additional information on their genetic relationships and ancestral origin. Mitochondrial DNA (mtDNA) control-region sequences of 18 Podolic and 9 non-Podolic breeds have been analyzed: Piemontese (PI), Romagnola (RO), Marchigiana (MR), Chianina (CH), Maremmana (MA), Podolica Italiana (IP), Mucca Pisana (MP), Calvana (CA), Bianca di Val Padana (BP), Hungarian Grey (HG), Bulgarian Grey (BG), Istrian cattle (IC), Katerini (KA), Romanian Grey (RG), Slavonian Syrmian Podolian (SS), Turkish Grey (TK), Ukrainian Grey (UK), Podolsko (PO) and Valdostana (VA), Bruna Italiana (IB), Grigio Alpina (GA), Pezzata Rossa Italiana (RP), Modicana (MO), Reggiana (RE), Agerolese (AG), Cinisara (CI), Cabannina (CB). All sequences were aligned to the Bovine Reference Sequence and classified in haplogroups and sub-haplogroups. The analysis clearly highlighted the expected lack of maternal relationship between Podolic and non-Podolic groups. Intriguingly, two groups of the Italian Podolic breeds were genetically distinguished: the first clusters with other European Podolic cattle, and a second one more closely related to the Turkish Grey. Besides the possible explanation of a selection-driven effect, we propose the hypothesis of a dual ancestral origin among Italian Podolic breeds: a first group deriving from the Eastern European cattle, in agreement with historical records; a second directly from the ancient Turkish cattle, which might have reached the Italian peninsula by another route (possibly by sea). This study increases the knowledge on the diversity of Podolic breeds, and sets the basis for the development of appropriate strategies for the conservation of these ancient and popular cattle.

O027

Genome-wide homozygosity in Maremmana cattle

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The current availability of large numbers of single nucleotide polymorphisms (SNPs) throughout the genome makes these markers particularly suitable for the detection of patterns of genetic diversity and of genome-wide homozygosity in animal populations. The aim of this work was to estimate genetic diversity and homozygosity in the Maremmana cattle breed. We used a sample of 149 animals (males and females) genotyped with the BovineSNP50 v2 (54K) Illumina BeadChip. After editing for call-rate >0.9 and removing SNP unassigned or on the sex chromosomes, 128 animals and 50,814 SNPs were left. We estimated the following genetic parameters: observed and expected heterozygosity (Ho and He), minor allele frequency (MAF), and the F_{IS} statistic. We also scanned the genome for runs of homozygosity (ROH). In the present study, ROH were detected based on 20-SNP-long sliding-windows, and allowing for a maximum of 1 missing and 1 heterozygote genotype, and a maximum gap between consecutive SNP of 10^5 bp. ROH contained minimum 10 SNPs, and had a minimum length of 1 Mb and a minimum density of 1 SNP every 50 kbps.

The average Ho and He were 0.374 ± 0.132 and 0.365 ± 0.120, respectively, and the average MAF was 0.274 ± 0.130. These values are consistent with the range observed in other cattle breeds. We obtained some negative values for F_{IS} (-0.162 to 0.180) which corresponded to animals with lower than average homozygosity. In total, 10,465 ROH were detected (81.75 per animal), with an average length of 2.69 Mb. Most ROH (74%) had length ≤ 2 Mb. ROH are contiguous lengths of homozygous genomic segments where the two inherited haplotypes are identical. ROH indicate genomic regions where a reduction in heterozygosity occurred, and offer new opportunities to estimate inbreeding (F). The inbreeding coefficient based on ROH (F_{ROH}) was estimated by the ratio between the total ROH length and the size of the genome in each animal. Average F_{ROH} was 0.0869 ± 0.032. Unlike inbreeding estimated based on Ho, F_{ROH} is not influenced by allele frequencies (sampling) and can distinguish recent from ancient inbreeding. However, F_{ROH} requires SNP positions to be known (unlike Ho). Two genomic regions with ROH in over 60% of the animals were found: one on BTA6 (38.6-39.7 Mbps), one on BTA13 (54.3-54.8 Mbps). These may highlight regions where selective pressures have shaped the genome of the Maremmana breed.

O028

Genome wide Copy Number Variation (CNV) detection in Cinisara cattle breed

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Copy Number Variations (CNVs) are classes of polymorphic genomic regions including deletions, duplications and insertions of DNA fragments from at least 0.5 kb up to several Mb. CNV represents an important source of genetic variability that provides genomics structural information complementary to the single nucleotide polymorphism (SNP) data. Some CNVs have been shown to be important in both normal phenotypic variability and disease susceptibility in livestock. Several approaches to identify CNVs including FISH, aCGH, SNP array or NGS, were proposed and among these SNP genotyping is relatively low cost, high-throughput and high coverage method. The aim of this study was to identify the CNVs in 71 animals of Cinisara breed using Illumina BovineSNP50 BeadChip v2. PennCNV software, which incorporates Log R ratio and B allele frequency at each SNP marker, was used to identify CNVs. Seven animals showed not shared CNVs, as well as autosomes 19, 21, 22. Chromosome 25 presented no CNVs at all. A final number of 322 CNVs were detected. The average number of CNVs was 4.5 per individual, with an average length and median size of 143.04 kb and 122.14 kb, respectively. All CNVs were grouped in CNV regions (CNVRs) and a total of 107 CNVRs, ranged from 50 to $\sim$500 kb, were detected, which covered 4.90 Mb of polymorphic sequence and corresponded to

0.18% of the total genome length. In particular, we found 81 CNVRs with only gain (duplication), 22 with only loss (deletion), and four CNVRs with both. Furthermore, 8 CNVRs with >1%, 77 with >2.5%, and 22 with >5% frequency, were found. CNVRs having the highest frequency were located on Chr3:120501439-120647330 and Chr23:34673581-35007295, whereas the greatest number of genes was mapped in only one CNVR located on Chr 17:74123863-74393620. A total of 241 genes were included in the identified CNVRs. According to KEGG and DAVID database, most of the genes were involved in multiple signaling and signal transduction pathways in a wide variety of cellular and biochemical processes, such as immune response, adaptability, and olfactory receptors pathway. Further studies, using different algorithms and validating the CNVs discovered, will be conducted to corroborate these preliminary results on the CNVRs detected. These results will be used for the investigation of genomic changes and features of interest in the Cinisara breed, such as for association with functional or production traits and for biodiversity studies.

O029

Prediction of nutrient digestibility and chemical composition of diets using near infrared spectroscopy (NIRS) of rabbit feces

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Near infrared spectroscopy (NIRS) has been reported to be a fast, reliable and economic tool to accurately predict different attributes of feeds. NIRS has been successfully used to monitor nutrition in ruminants through diet and/or feces, but few studies have tested this technology to predict rabbit diets digestibility. The aim of this study was to predict the nutrient digestibility of rabbit feeds and chemical composition of rabbit feeds and feces from NIRS analysis of feces. Sixty New Zealand White growing rabbits were randomly housed in single cages divided in five groups and fed different experimental diets. A total of 75 individual fresh feces samples from 15 rabbits (3 for each group) were collected every three days along the last fifteen days of the trial. The feces were dried (65 °C for 24 hours) and ground (1 mm screen diameter), then analyzed in reflectance mode in a scanning monochromator using a rectangular quartz cup. Spectral absorbance values were recorded as log1/R, where R is the sample reflectance, in the visible and near-infrared region (400-2500 nm), every 2 nm. Fecal spectral data were fitted to the digestibility coefficients and to the chemical composition of feces and fed diets. Modified Partial Least Squares equations were performed using five cross-validation groups to select the optimal number of factors and to avoid overfitting. Several derivative and scatter correction pre-treatments of spectral data were used. The standard error of cross-validation (SECV) and the coefficient of determination of cross validation (r^2CV) were used to select the better calibration equations. Good predictive performance was obtained for the prediction of CP, NFC and NDF digestibility (SECV = 0.007, 0.006, 0.020, respectively; r^2CV = 0.93, 0.75 and 0.74, respectively). The prediction of all the feed nutritional parameters (DM, M, CP, CF, EE, Ash, NFE, NDF, ADF, ADL, NFC) showed good predictive results (SECV from 0.009 for DM to 0.583 for NDF and r^2CV from 0.98 for DM to 0.83 for CP) and for most fecal components (OM, CP, Ash, NDF, ADF; SECV from 0.280 for DM to 0.883 for NDF; r^2CV from 0.95 for CP to 0.75 for NDF). This work supports the viability of NIRS analysis of feces as a fast and reliable analytical method which could allow important savings in rabbit digestibility trials testing the nutritional values of feeds. Future work should be done to definitely validate these models with external samples.

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O030

Performance and feeding behaviour of group–housed rabbits with free or time–limited access to feed

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The present study aimed at evaluating the effect of the feeding system (AL: *ad libitum vs.* R: time–based feed restriction) on performance, feeding behaviour and slaughter results in group–housed growing rabbits. A total of 288 crossbred rabbits of both sexes were housed in 18 pens (16 animals per pen), half fed *ad libitum* and half restricted during the first three weeks, and controlled from weaning to slaughter (33 to 75 d of age). At the beginning of the trial, R rabbits had access to feeder for 10 h a day; feeding time increased by 1 h every 3–4 d until 16 h/d in the first three weeks; then, feeding time increased by 1 h/d until 24–h access to feeders in the 4th week; thereafter, all animals were fed *ad libitum*. Rabbit feeding behaviour was controlled at 39, 46, 53 and 60 d of age by weighing the feeders every hour by an automatic weighing system. Individual data (live weight, carcass data) were analysed by PROC MIXED of SAS with the feeding system as fixed effect and the pen as a random effect; pen data (feed intake and conversion) were analysed by PROC GLM of SAS. During the whole trial, AL rabbits exhibited small and frequent meals during all the day with minimum intakes (about 2–3 g/h) in the morning (9:00 to 11:00 h) and maximum intakes (7–10 g/h) in the evening (19:00 to 21:00 h). R rabbits showed higher hourly feed consumption than AL rabbits, with peaks of 18, 15 and 14 g in the first hour after accessing feeder at 39, 46 and 53 d of age when the feeding time was 11, 13 and 16 h/d, respectively. Once fed freely, R rabbits took only two days to reach the same intake pattern of AL rabbits.

In the first three weeks, feed intake was lower (–5%, i.e. 128 *vs.* 122 g/d; $p<.01$) and feed conversion was better (–5%, i.e. 2.42 *vs.* 2.29; $p<.001$) in R rabbits compared to AL ones. In the following three weeks, R rabbits showed a worse feed conversion than AL rabbits (+ 5%, i.e. 2.70 *vs.* 2.83; $p<.001$). In the whole trial, the feeding system did not affect health status, growth performance, slaughter weight (on average 2860 g), dressing percentage (61.0%) and meat quality traits including pH, colour, thawing and coking losses and tenderness.

In conclusion, feeding behaviour was heavily affected by the restriction program, but rabbits returned quickly to a normal behaviour once fed freely. The time–restriction program in the first three weeks reduced feed intake level at 95% of *ad libitum* without compromising growth performance and slaughter results.

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O031

Effect of Chamomile (*Matricaria chamomilla*) on health in chickens

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The study was carried out in San Pedro, Cacha Parish, Riobamba Canton, Province of Chimborazo it is located at 3040 meters above sea level. The aim of this work was assess the effect of four levels of chamomile extract (LCE) in the health of Campero-INTA" line chickens, which is a chicken commercial line with 25% Cornish, 25% Red Rhode Island y 50% Ross. Chickens line under intensive production system; the chickens were allocated to four treatments (T1; T2; T3 and T4 (0, 2, 4 and 6% chamomile extract), in drinking water. These treatments had four repetitions under a completely randomized design for 55 days. In this case was use 60 animals to each treatments, a total of 240 chickens. The results were processed using SPSS (16.0.2) statistic software. By means of the separation of means through the Waller Duncan test at a significance level of $p<.05$ and $p<.01$. This study was conducted to determine the health status of the animals, used three microbiological analyzes of the feces of the poultry before, during and at the end of the investigation. To the start the analyzed the percentages of gram positive bacteria (GPB) were found same for T1 and T3 with 70% GPB, followed by T0 and T2 with 50% BGP; to final to the study was showed the highest value has T0 with 90% BGP, followed by T1 y T3 with 80 and 75% BGP. Regarding Gram Negative Bacteria (GNB) before applying the treatments was registered a value of 30% (GNB), during the investigation, the lowest values were the same for T0 and T2 with 50% BGN, as for the highest values reached for T1 and T3 with 70%, during the final phase of the investigation, they registered similar values for T2 and T3 with 10% BGN; followed by T1 with 20% and T0 with 25% BGN. The total coliforms found before application of the treatments was 400,000 CFU/g; once subjected to the extract of chamomile was observed for T0: 1060,000; T1: 770,000; T2: 440,000; And T3: 380,000 CFU/g; In the final cycle of the investigation lower values were found, registering for T0: 280,000; T1: 260,000; T2: 240,000: and T3: 240,000 UFC/ml. 2200 HPG (eggs per gram), followed by (T1) with 2850 HPG, then (T3) with 4400 HPG and a higher parasitic load with (T0) 17300 HPG were recorded in the coproparasite analysis. In conclusion the polyphenols influence the health of chikens, the treatment that had less CFU was T3, in terms of lower parasite load was obtained with T2 and T3 (240000 HPG).

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O032

Effect of chamomile (*Matricaria chamomilla*) on productive performance in chickens

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Chamomile extract (*Matricaria chamomilla)* has a high concentration of polyphenols. This plant improves the broilers productive performance and hens egg production. The present study was carried out in San Pedro Neighbourhood (3040 mamsl), Chimborazo Province, Ecuador. The aim of this work was to determine the effects of chamomile extract in the productive parameter of chickens. For this experiment were used 240 "Campero-INTA" line chickens, which is a chicken commercial line with 25% Cornish, 25% Red Rhode Island and 50% Ross. These animals were randomly divided in 4 treatments with different levels of chamomile extract in the water: 0, 2, 4, and 6% in T1, T2, T3 and T4, respectively. The chickens' productive cycle lasted 55 days, with starter (0-15 days of life), grower (16-40 days of life) and finisher (41-55 days of life) concentrated feeds used in the trial. The daily weight gain, final body weight, feed conversion efficiency and carcass yield were recorded or calculated. For statistical analyses, unifactorial ANOVA was used and the Waller Duncan test was used to separate the means (SPSS 16.0.2). Differences in carcass yield, due to the chamomile extract concentration, were observed. The T3 group showed the highest carcass yield (75.6%), followed by the T2 (72.9%), T4 (70.4%) and T1 groups (69.2%). As for the other productive parameters, no differences due to the chamomile extract concentration were found. In conclusion, the chamomile extract increased the chickens' carcass yield. Further research is needed to investigate the role of polyphenols in the birds' growth process and other productive and health parameter must be evaluated.

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O033

Effects of the feeding system on performance and myopathy occurrence in two broiler chicken genotypes

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To evaluate whether performance and myopathy occurrence differed according to genotype (Cobb 500 vs. Ross 308) and feeding system (AL: *ad libitum* vs. ER: early restricted from 13 to 23 d of age, vs. LR: late restricted from 27 to 37 d; restriction rate: 80% of *ad libitum*), 828 day-old male chicks were assigned to 6 groups (2 x 3 arrangement), housed in 36 pens, and controlled for: individual live weight (weekly) and pen feed intake (daily) until slaughter (48 d); white striping and wooden breast occurrence at slaughter. Individual data were analysed by PROC MIXED of SAS (fixed effects: feeding system, genotype, and interaction; random effect: pen); pen feed intake and myopathy occurrence were analysed by PROC GLM and CATMOD, respectively. The feeding system affected performance: at the end of the first period (1-22 d), ER chickens showed lower weight gain (40.5 g/d *vs* 47.8 g/d and 48.0 g/d), feed intake (50.8 g/d *vs* 61.0 g/d and 60.2 g/d) and live weight (903 g *vs* 1056 g and 1059 g) than AL and LR broilers ($p<.001$); at the end of the second period (23-48 d), ER chickens showed higher weight gain (98.3 g/d *vs* 93.6 g/d and 90.2 g/d) and feed intake (182 g/d *vs* 177 g/d and 171 g/d) compared to AL and LR chickens ($p<.001$). Final live weight was the highest in AL group, intermediate in ER group, and the lowest in the LR one (3482 g, 3454 g, and 3399 g; $p<.01$). Feed conversion in the whole period did not change with the feeding system. At gross examination, white striping occurrence changed from 77.8% to 67.1%, and 81.7% in AL, ER and LR broilers ($p=.10$). Differences between genotypes were evident from the first day and, at the end of the trial, weight gain (74.3 g/d *vs* 70.1 g/d), feed intake (126 g/d *vs* 114 g/d), feed conversion (1.69 *vs* 1.64), and live weight (3548 g *vs* 3342 g) were higher in the Ross than in the Cobb chickens ($p<.001$). At slaughter, the rate of white-striped breasts was similar (on average 75.5%), but the occurrence of severely white-striped breasts was higher in the Ross than in the Cobb chickens (25.9% *vs* 7.41%; $p<.001$). Wooden breast occurrence (on average 5.1%) did not change with the feeding system or the genotype. In conclusion, under our conditions, a late feed restriction did not permit to recover performance at the end of the trial nor to control white striping occurrence. Moreover, the genotype affected growth rate and white striping degree: the highest the growth rate, the highest the severity of white striping.

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O034

Probiotic and prebiotic supplementation in broiler chickens: growth performance, carcass traits and meat quality

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Manipulations of the intestinal microbiota composition through pre- and pro-biotics may improve health and performance of chickens. A study was carried out to evaluate effects of a probiotic preparation (Lavipan®) and a combination of Lavipan® with the prebiotic RFO (raffinose family oligosaccharides), supplemented in feed, on economic impact (European Broiler Index, EBI), performance, carcass traits and meat quality of broiler chickens. 360 one-day-old female chicks (Ross 308) were randomly allotted to 3 dietary treatments: basal diet (Control, C); basal diet with 1% of Lavipan® (L) consisting of *L. lactis* IBB500, *C. divergens* S1, *L. casei* ŁOCK 0915, *L. plantarum* ŁOCK 0862 and *S. cerevisiae* ŁOCK 0141; basal diet with a combination of Lavipan® (1%) with RFO (0.8%) (LR). Both formulations were supplemented for the first 7 days of chick's life. Chickens were reared in floor pens (10 replicate pens/treatment, 12 chicks/pen). To provide commercial conditions, the poultry house was filled with 9000 as-hatched chicks. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR), at 10, 21 and 40 day of age, were calculated on pen basis. At 41 d of age, 10 randomly chosen birds per treatment were weighed and slaughtered. The yields of carcass, breast and legs were calculated. Pectoral muscle (PM) pH, color and water holding capacity were measured at 24 hours *post-mortem*. Total lipid and fatty acid analyses on PM were carried out. Data were evaluated by ANOVA. EBI was better in both L (342.7) and LR (354.6) compared with C group (326.0). Both L and LR supplementation significantly improved BWG within the first 10 days of life as compared with C group ($p<.05$). No significant differences in the BWG were found for the rest of the rearing period. However, treatment with LR and L was associated with modest increases in total BWG (+1.3% and +3.1%, respectively) as compared with C group. FI was higher in L group within the first 10 days of life as compared with C and LR groups ($p<.05$). The FCR was not affected by the treatment. Final body weight, carcass weight and carcass traits of slaughtered chickens were similar among groups. Any significant effect on physico-chemical traits, total lipid and fatty acid composition of PM was found. In conclusion, although the effect of bioactives supplementation on growth performance seems negligible the economic impact could be relevant if we consider the high number of animals in commercial conditions.

O035

Milk yield, culling rate and reasons of pure Holsteins and second generation crossbred cows from Montbéliard × Swedish Red × Holstein

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Crossbreeding has been proposed as management tool to improved fertility of dairy herds. Aim of this study was to compare milk yield, longevity and culling reason of pure Holstein cows (HO; $n = 990$) with those of second generation crossbred cows from Montbéliarde × (Swedish Red × HO) ($n = 193$). The research was carried out monitoring cows from a single dairy herd reared in a farm located in the Po Valley. All cows were loose housed in a barn provided with cubicles and they were subjected to the same management and feeding program. The sample considered 373 primiparous, 306 secondiparous and 504 multiparous cows (≥3 lactations) that calved from 2008 to 2016. Milk yield was estimated on 1699 closed lactations of the considered sample and pure HO had a higher average milk yield (10403 kg) than crossbred cows (9142 kg) ($p < .001$). However, when milk yield considered the whole productive career of the animals, there was no breed effect due the greater longevity of crossbred compared to pure HO (2.64 *vs* 2.40 lactations; $p = 0.02$). A total of 367 cows were culled during the recording period with an average culling rate of 31.0%. In line with longevity, culling rate was significantly ($p < .001$) lower for crossbred cows (12.4%) than for pure HO (34.7%) and a significant breed effect ($p < .001$) was observed particularly for culling rate of primiparous cows (30.6%), with pure HO showing the highest value (33.5% *vs* 10.4%). Culling reasons were gathered from farm recording sheets and peripartum disorders, as aggregate of post-partum problems, abomasum displacement and uterine prolapse, resulted the most frequent cause (15.8% of total culled cows) followed by low fertility (12.3%) and intestinal clostridiosis (10.3%). Odds ratios (OR) and 95% confidence intervals (CI) were calculated for these three main culling reasons considering crossbreeding as preventive term. The exposure to peripartum disorders was reduced by 60% ($p < .05$) for crossbred compared to pure HO cows (OR = 0.44; 95% CI 0.20-1.00). Similarly, crossbreeding decreased by 90% the likelihood of being culled for fertility reasons (OR = 0.09; 95% CI 0.01-0.64). There was no preventive effect of crossbreeding on the occurrence of intestinal clostridiosis, (OR = 0.49; 95% CI 0.19-1.29). A relevant environmental cause was hypothesized for this culling reason due to the recycling as bedding material of the solid fraction of the manure, after an incomplete composting process.

O036

Predictive models for locomotion issues in Italian Holstein dairy cows

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Locomotion system issues are major issues in dairy herds, affecting both animal welfare and farm productivity. Early detection could improve the effectiveness of treatments and increase the chances to cure lame cows. Currently, locomotion issues detection requires direct observation of cows walking (locomotion score). However, this is a time-consuming task and is not always an available option in large dairy farms. Aim of this preliminary study was to build a predictive model for locomotion system issues in Italian Holstein dairy cows using some novel phenotypes from automatic recording systems (milking parallel parlour and SCR Heatime and DataFlow2 system) as predictors. Data was recorded from a commercial farm located in the province of Mantua (Lombardy, Northern Italy) for a total of 413 animals, daily monitored for two years (Sept. 2014 – Dec. 2016). The response variable was binary (0/1: healthy and diseased, respectively). The selected variables were daily rumination time, parity, DIM, daily milk production, daily activity and month of recording. Summary statistics (mean and SD) were calculated: rumination time, 563.28 ± 88.48 min/day; parity, 1.93 ± 1.28; DIM, 171.34 ± 124.14 days; milk production, 24.14 ± 13.74 kg/day; activity, 614.00 ± 134.64 min/day. DIM were classified in four classes to assess the potential effect of the lactation stage: dry, early, mid, and late lactation (no lactation, <120 days, 120-240 days, and >240 days, respectively). Three different datasets were prepared, where rumination, milk, and activity were averaged as means of 1, 3, and 5 days before the response variable record. On each dataset, two models were fitted: logistic regression and random forest. All the analyses were performed in R using the caret package. Data were divided into a training and a testing dataset (proportion 80/20). Training data was used to train two different algorithms which were used to predict the class variable. The two selected algorithms were: 1. a logistic regression 2. a random forest.

For all the datasets, logistic regression was not able to predict diseased individuals, assigning all to the 'healthy' class.

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Random forest performed better, although with a high-class error. The 5-day window had the lowest OOB error rate (0.24%) and the lowest class error (0.71). Further tuning of the selected models will be necessary to build a valuable tool to predict locomotion system issues.

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O037

Use of an electronic rumination-monitoring system in pre-weaned calves

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The onset of rumination in calves starts approximately at 1-2 wks of age and increases up to 5 h/d at 4-6 wks. Nevertheless, the rumination behavior during the overall pre-weaning period and the daily rumination pattern is difficult to be investigated. The recent introduction of the Hi-Tag rumination-monitoring system (SCR Engineers Ltd., Netanya, Israel) in adult cows allows the automatic quantification of rumination time (RT) and opens new perspectives in the study of other physiological periods. This study was intended to evaluate the Hi-Tag system for monitoring rumination (RT and daily pattern) in pre-weaned calves and to assess the relationship of RT with inflammo-metabolic conditions. Nine Simmental calves were kept in individual pens from birth to 6 wk of age. At d 2 ± 1, calves were fitted with Hi-Tag loggers, which record the sounds of regurgitation and rumination. RT data were calculated in 2-h intervals. Calves were fed whole milk twice daily and also calf starter was provided. Blood samples were collected at d 0, 1, and then weekly. Data were analyzed using PROC MIXED and PROC CORR of SAS (SAS Institute Inc., Cary, NC). The average of daily RT was 91, 210, 313, 362, 373 and 377 min/d for wk 1, 2, 3, 4, 5 and 6, respectively. The daily RT observed at wk 6 represents the 63% of the mean daily RT value recorded in adult cows in mid lactation. Moreover, the daily rumination pattern observed in calves over weeks is similar to that reported in adult cows. At wk 6, the Hi-Tag loggers recorded values of RT at nighttime greater than those registered at daytime (61% *vs* 39%; $p<.001$) as already observed in adult cows. In all calves, RT was not influenced by milk suckling. Indeed, the RT in the 2-h interval recorded at milk feeding was markedly lower (especially in the morning) compared to RT in the 2-h interval before. Relevant correlations between daily RT and a number of plasma parameters (albumin: $r=0.50$, $p=.001$; paraoxonase: $r=0.59$, $p<.001$; cholesterol: $r=0.36$, $p<.01$; GGT: $r=-0.55$, $p<.001$) were observed. These associations demonstrate that RT in pre-weaned calves is well related with markers of liver functionality. These results represent a first attempt to use an automatic system to obtain a reasonable quantification of RT in calves at the pre-weaning stage. Despite this technology remains not validated for calves, the outcome of the present study seems promising to identify animals with poor post-birth adaptation or poor health status.

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O038

Effect of different ventilation systems on beef cattle during the early fattening period

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The aim of this study was to assess the effect of the ventilation system on the environment and the performance of beef cattle during the early fattening period. It was conducted on one group (n = 106) of young Charolais bulls from the first of August to the 9th of October. Animals were raised in a roofed, loose housing facility with straw bedding. The day after their arrival bulls (429 ± 24 kg) were grouped according to weight in 10 pens. Five pens were equipped with 2 ceiling fans (CF), whereas the other 5 had 2 horizontal fans (HF). The pens with different ventilation were separated by an empty pen with a windbreak. The trial lasted 70 days and it was divided into 3 periods. In the first and in the third periods all the fans were in operation, whereas in the second period they were switched off to see the effect of the ventilation on the animals and on the environment. In order to measure the average daily weight gain (ADG) and the effect of ventilation on the cleanliness of animals, at the beginning and at the end of the trial the animals were weighed and evaluated for their body surface covered in manure: from 1 (<10%) to 5 (>75%). The temperature-humidity index (THI) was continuously measured in CF and HF pens and outside the facility. Each pen was periodically checked for the dry matter of the bedding (DMB). Data on ADG were subjected to ANOVA using the ventilation system as a fixed effect, whereas cleanliness data were subjected to the nonparametric Mann-Whitney U test. Data on THI and on DMB were subjected to ANOVA using the combined effect of period and ventilation system. Period significantly affected THI (74.4 *vs* 75.2 *vs* 65.5, $p<.001$) that was the lowest in period 3, whereas THI was not significantly different between CF and HF. CF maintained a higher DMB (30.6 *vs* 45.1% ww; $p<.001$ and 22.2 *vs* 31.3% ww; $p=.002$) in periods 1 and 3, respectively. Bulls raised with the CF ventilation system not only remained cleaner, increasing their degree of dirtiness of only 1.16 points compared to 3 points of the others ($p<.001$), but they showed also a significantly higher ADG (1.20 *vs* 1.36 kg/day; $p=.039$) compared to animals raised with HF. These results indicate that CF help to improve the environmental conditions and the animal performance in hot weather conditions compared to the horizontal ventilation systems, and that the THI is not always an appropriate index to predict the effect of temperature and humidity on animal heat stress.

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O039

Use of Infrared Termography (IRT) in equine assisted interventions: physiological aspects

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This work is part of the project “Horse welfare in therapeutic sessions for children with autism: monitoring and assessment” (Ricerca Corrente IZSVe 13/2013), coordinated by the National Reference Centre for Animal Assisted Interventions (CRN IAA) and funded by the Italian Ministry of Health. This study focuses on the welfare of horses involved in Equine Assisted Interventions (EAI) and is designed to evaluate the use of innovative and non-invasive methods (such as thermography and measurement of heart rate variability and saliva collection) to inform the professionals involved in EAI, limiting stressful situations for the animals.

In particular, in this paper physiological indicators of the welfare of horses involved in therapeutic sessions of children with Autism Spectrum Disorder (ASD) are taken into account: cortisol levels in saliva and blood samples, surface body temperature in the area of the lacrimal caruncle, and heart rate variability. All data were collected from horses following the same session protocol with a child with ASD and a typically developing child (control group). Moreover, each therapeutic session was standardized and divided into different phases (baseline, grooming, start, riding, alt, end, post).

From the analysis of thermographic results significant temperature increases were registered from the baseline phase to grooming activities, start and post work session, only in the group consisting of children with ASI and not in typically-developing children.

In particular, it was noted that the horses ridden by children with ASD showed almost at all stages greater periocular temperature than horses ridden by control children, although this difference was statistically significant only in the post-work phase.

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O040

Evaluation of different habituation protocols for training dairy donkey jennies to the milking parlor: effect on milk yield, animal behavior and heart rate

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Actually, an interest in donkey milk production for human consumption, in particular in Italy, France and Belgium, is gaining popularity and economic interest. In this field, mechanization of milking procedures represents an important innovation in donkey milk production. The aim of the work is to evaluate if a habituation protocol for dairy donkeys to the milking parlor procedures could be useful to reduce animal stress.

Sixty lactating dairy donkey jennies (97±45 days in milk) were divided in three groups of 20 heads for each, homogeneous for age, parity and days in milk. All the animals were never subjected to mechanical milking procedures and never entered the milking parlor. Groups A and B were subject to different habituation protocols that lasted 9 days. These two groups had, then, 6 days of mechanical milking. Group C (negative control) was directly mechanically milked for 6 days. For the first 3 days, both groups A and B passed through the switched-off milking parlor. In the next 6 days donkeys in the group A received a "more gentle treatment" passing for 2 days through the switched-on milking parlor and for 4 days stopping in the milking stall. Group B, instead, was stopped in the milking stall during all 6 days, and their udders were neared to the switched-on milking cluster. Behavioral patterns and heart rate were measured during each session. Data obtained were submitted to 2-way ANOVA using the general linear model. Groups were then analyzed separately considering as fixed effect the milking session and applying the Tukey's test for repeated measures. Significance was set as $p<.05$.

Donkeys that received the pre-milking habituation handling (groups A and B) showed less reactive behaviors (kicks and steps) and incoming stops when the milking procedures started if compared to the C group ($p<.01$). Differences in heart rate were observed during the milking procedures between C group and other ones ($p<.05$). Milk production was greatly affected by pre-milking treatment. Group B showed a greater milk production at the first milking session ($p<.01$). From the second milking session, group C, without any pre-milking habituation period, showed lower milk production ($p<.01$). This suggests that the pre-milking habituation protocol providing a rapid contact with the milking cluster can result in a better response of animals with lower reactions behaviors, lower heart rate variation and higher milk production.

O041

Outcomes of a web-survey for collecting stakeholders' opinion on welfare requirements for sheep, goats, turkeys, donkeys, and horses

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The Animal Welfare Indicators (AWIN) project aimed at developing animal-based welfare assessment protocols for sheep, goats, turkeys, donkeys, and horses. To assure a good acceptance of the protocols, during the early stages of the project stakeholders were invited to participate in a multi-language web-survey, made available online for 15 months on the websites of several academic and international organisations. Participants answered open questions about appropriate requirements to guarantee high levels of animal welfare on farm. A total of 271 surveys were properly filled out (123 for horses, 81 for sheep, 36 for goats, 18 for turkeys and only 13 for donkeys). Answers came from 32 countries distributed in Europe (70.4% of respondents, among which 44.5% from Italy), America (16.2%; e.g. from United States, Canada, Chile, Colombia), Oceania (9.23%; e.g. from Australia, New Zealand), Asia (2.6%; e.g. from India, Vietnam), and Africa (1.5%; e.g. from Ghana, Lesotho). Gender of participants was balanced in all species, except for horses (85% women). Most of the participants (77%) aged between 31 and 60 years. Questionnaires on sheep and turkeys were compiled mainly by veterinarians, whereas questionnaires on goats and donkeys by farmers, on horses by owners. Text analysis was used to count the occurrence of the words used by the stakeholders and to assess the most prevalent ones. The term feeding was the most frequently used to describe welfare requirements (86.49% of goats' responses, 76.92% of donkeys, 74.39% of sheep, 66.67% of turkeys and 60.2% of horses). Specific terms were used for each species: protection from predators for goats and sheep (8.11% and 12.20% of responses, respectively), genetic for turkeys (11.11%), regular exercise for horses (27.6%), foot care for donkeys (30.77%).

The interest raised by the survey was below expectations. One reason could be that the distribution was insufficient or inappropriate: stakeholders from countries involved in the AWIN project were more engaged compared to other countries. Another possible explanation could be internet availability, which may represent a further limit in some geographic areas (e.g. developing countries). Finally, the characteristics of human-animal relationship seem to have played a role in stakeholders' involvement: horses, characterized by a stronger relationship, received the highest number of answers, whereas turkeys (high number of animals/farm, less strict human-animal relationship) received less answers.

The results showed that stakeholders' involvement, while crucial, would be susceptible to certain biases that need to be taken into account when conceiving and distributing a web-survey.

Acknowledgements

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O042

Relationship between blood energetic markers and circulating leukocytes transcriptome in transition dairy cows.

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The negative energy balance and the reduction of immunocompetence are interconnected conditions often experienced by the cows during the transition period. With the aim to study their relationship, the transcriptome profile of circulating leucocytes and plasma energetic markers were evaluated in the transition period of 6 healthy dairy cows. Blood samples were collected at -20 ± 2 d, -3 ± 1 d, 3, and 7 days from parturition. At each sampling time the total RNA was isolated from whole blood and transcriptome was analyzed by deep sequencing technology (Hiseq1000 Illumina, USA). Plasma obtained from the same animals was analyzed for glucose, NEFA and β-hydroxybutyrate BHB (ILAB 600, Instrumentation Laboratory, USA). The partial correlations between read counts (standardized for each samples and expressed as read counts for 100k read counts) and plasma parameters were calculated using the GLM procedure of SAS® (SAS Institute Inc.). The lists of genes significantly correlated ($P < 0.01$) with the blood parameters were used to run an enrichment analysis through the Dynamic Impact Approach (DIA) with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Glucose resulted correlated with 227 genes (197 positively and 30 negatively), NEFA resulted correlated with 62 genes (43 positively and 19 negatively), BHB resulted correlated with 540 genes (70 positively and 470 negatively). The NEFA concentration has shown a low effect on activation of: folate biosynthesis, starch and sucrose metabolism and fatty acid metabolism. Impact of NEFA on the gene expression can be considered irrelevant. Also the impact of glucose on KEGG pathways was limited and pertained the activation of: circadian rhythm, ABC transporters, homologous recombination, alpha-Linolenic acid metabolism and mismatch repair. Conversely, the effect of BHB was relevant and act by inhibiting several KEGG pathways as: DNA replication, glycosaminoglycan biosynthesis, non-homologous end-joining, homologous recombination, base excision repair, vitamin B6 metabolism. Overall, the plasma markers of the energy metabolism during peripartum showed a relationship with the transcriptome of the circulating leucocytes. The mobilization of body reserves (NEFA) is not the main player affecting the immune cells functionality; whereas the ability of the liver to oxidize properly the fatty acids (revealed by BHB concentration) seems an important and severe inhibitor of the immune cells functionality immediately after parturition.

O043

Supplements of *Aloe arborescens* improve health and inflammo-metabolic status of transition dairy cows

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During the transition period (TP) dairy cows show inflammations, reduced immunocompetence, negative energy balance (NEB) and oxidative stress, which worsen performances and increase metabolic and infectious diseases risks. The use of phyto-nutraceuticals could attenuate these risky conditions. *Aloe spp*. has long been utilized in folk medicine and we previously demonstrated that some of the typical polysaccharides of the *Aloe arborescens* Mill. *(AA)* can be absorbed in the intestine. Thus, homogenized preparations of *AA* (Dester Garden, BS) has been administered on 30 multiparous dairy cows to investigate effects on health and inflammo-metabolic profile during the TP. Cows were allocated in 3 homogeneous groups: Ctrl, A1 and A2 that received a drench of 0, 100 and 200 g/d of *AA* homogenate respectively during morning feeding, between -14 and 14 days from calving (DFC). Besides health status, body condition score (BCS) and milk yield (MY), blood profile (not esterified fatty acid, NEFA; beta hydroxybutyrate, BHB; haptoglobin; ceruloplasmin, CuCp; cholesterol; retinol and bilirubin) was frequently monitored from -35 to 35 DFC. Data were evaluated with ANOVA, using the MIXED procedure (SAS Inst.). In comparison to Ctrl, although not significant, treated cows showed less pro capita cases of clinical diseases in TP (1.0; 0.8; 0.5 in Ctrl, A1, A2, respectively), a mild increase of MY (37.4 *vs* 38.7 kg/d, for Ctrl and *AA*, respectively) and a lower reduction of BCS. During the first week of lactation A2 group showed a lower increase of NEFA (0.78 *vs* 0.46 mmol/L for Ctrl and A2, respectively; $p<.1$) and BHB (1.69 *vs* 0.61 mmol/L for Ctrl and A2, respectively; $p<.1$), suggesting a lower lipomobilization and NEB. In comparison to Ctrl, *AA* groups showed a less marked inflammatory status after calving, as suggested by the faster decrease of haptoglobin and CuCp (especially in A2

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group), and better liver functions, as confirmed by the higher ($p<.05$) concentrations of cholesterol (after 21 DFC) and retinol (between 14 and 28 DFC; $p<.05$), and by the lower levels of bilirubin (10.2 *vs* 6.0 μmol/L for Ctrl and A2, respectively; $p<.05$). In the overall, treated groups showed a numerically higher liver functionality index (LFI; -0.4; 0.3; 1.2 for Ctrl, A1 and A2, respectively), indicating a lower NEB, best health status and liver metabolism. These results demonstrate that a proper use of AA could allow the cows to successfully overcome the TP and could reduce the antibiotic treatments.

Acknowledgements

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O044

A multidisciplinary study investigating the bovine milk microbiome and its association with mastitis resistance traits in Holstein Friesian and Rendena dairy cows

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Highly selected dairy breeds, such as Holstein Friesians, are exposed to a high risk for disease, especially mastitis, during the transition period. In contrast, autochthonous and lower-yielding dairy cattle breeds, such as Rendena, are known to possess higher resistance to disease and resilience to intensive farming conditions, especially concerning udder health and metabolic disorders during periparturient period. A more detailed knowledge of the healthy milk microbial communities and of their interactions under physiological conditions might provide suitable information about milk quality and udder health.

With the aim to characterize milk microbial diversity during the transition period and its potential associations with mastitis, we used Next Generation Sequencing (NGS) of the 16S rRNA gene to investigate the bovine milk microbiome in 6 Holstein Friesian and 4 Rendena cows reared in the same farm and under the same environmental and management conditions. Further, in this study we applied a multidisciplinary approach to compare innate immune response patterns, inflammometabolic parameters and milk protein profiles.

Quarter milk samples and blood plasma were collected from all cows at dry-off (T1), 1 day after calving (T2), 7-10 days and 30 days after calving (T3 and T4, respectively). Quarter milk samples were analyzed for somatic cell counting, bacteriological growth, characterization of the milk microbiota by NGS, milk protein profiling by electrophoresis and densitometry. Moreover, for each milk sample inflammation markers and assessment of different innate immune-related mediators such as cathelicidin, lysozyme, CD45, IL-1beta, TNF-alpha, PTX3, IL-1R8, were measured by lyso-plate assay, ELISA and qPCR. In parallel, blood plasma samples were assessed for the main inflammometabolic (glucose, urea, non-esterified fatty acids, beta-hydroxybutyrate, acute phase proteins) parameters.

Our results indicate that the milk microbiome of Rendena cows profoundly differs from that of Holstein Friesian cows, and harbors some diversity and taxonomic markers of mammary gland health allegedly specific to Rendena cows, only. In addition, in comparison with Rendena cows, Holstein Friesian cows showed a more severe fat mobilization and systemic inflammatory response at T2 and T3.

In conclusion, in spite of the same farming conditions, several differences were observed in the two breeds providing useful hints about disease resistance in autochthonous rustic breeds.

O045

Anti-inflammatory effects of conjugated linoleic acid isomers and essential fatty acids in bovine mammary epithelial cells

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Fatty acids are important modulators of inflammatory responses, in particular, omega-3 and omega-6 essential fatty acids (EFAs) and conjugated linoleic acid (CLA) have received particular attention for their ability to modulate inflammation. The objectives of this study were to compare the effects of CLA and EFAs on the expression of pro-inflammatory cytokines and their protective efficacy against inflammatory status in mammary gland by an *in vitro* model based on bovine mammary epithelial cells (BME-UV1). BME-UV1 cells were treated with complete medium containing 50 μM of cis-9,trans-11 CLA (c9,t11 CLA), trans-10,cis-12 CLA (t10,c12 CLA), (α)-linolenic acid (aLnA), (γ)-linolenic acid (gLnA) and linoleic acid (LA). After 48 h by fatty acids (FAs) administration the cells were treated for 3 h with 20 μM of lipopolysaccharide (LPS) to induce inflammatory stimulus. Reactive oxygen species (ROS) production after treatments was assessed by dichloro-dihydro-fluorescein diacetate assay to verify and to compare the potential protection of different FAs against LPS-induced oxidative stress. The mRNA abundance of bovine pro-inflammatory cytokines (*TNFα*, *IL1β* and *IL6*) and peroxisome proliferator receptor-α/γ (*PPARγ/α*) were determined in BME-UV1 by qRT-PCR. The results showed that cells treated with FAs and LPS increased ROS production compared with control cells. Among treatments, cells treated with c9,t11 CLA and t10,c12 CLA isomers revealed significant lower levels of ROS production compared with other FAs. All FAs reduced the gene expression of pro-inflammatory cytokines. Among FAs, t10,c12 CLA, LA and gLnA showed an homogeneous reduction of the three cytokines and this may correspond to more balanced and efficient physiological activity and may trigger a better protective effect. The *PPARγ* gene expression was significantly higher in cells treated with t10,c12 CLA, aLnA and LA, whereas the *PPARα* gene expression levels were lower in cells treated with different FAs compared to the control. These results suggest that FAs inhibited the transcription of pro-inflammatory cytokines by the up-regulation of *PPARγ* expression and probably by PPARγ activation.

O046

Cows relocation affects exosomes and their cargos in bovine milk

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Extracellular vesicles in milk contains several compounds, as lipids, proteins, noncoding RNAs, and mRNAs. Among the different types of extracellular vesicles, exosomes have attracted researchers since their sorting cargos are a regulated, non-random process, which is heavily involved in cell-to-cell communication.

The aim of the study was to analyse the variations of RNAs in milk exosomes in response to the relocation of dairy cows between production groups. The study was carried out on 76 lactating cows during the relocation from high yielding group to low yielding group. The extent of stress was evaluated by means of milk cortisol in samples collected in the afternoon from 2 days before to 3 days after the change of the group. Mean cortisol concentrations significantly increase on the day of relocation (day 3) and on the days 4 and 5. Six cows were selected according to cortisol variations after the relocation. The 3 cows with the highest change and the 3 cows with the lowest change of cortisol after relocation were selected. Pools of Milk samples of days 1 and 2 (Before) and days 4 and 5 (After) were prepared for exosomes isolation using a commercial kit (ExoEasy Maxi Kit, QIAGEN).

Exosomes were visualized with TEM after immune labeling followed by negative stain and electron microscopy was carried out targeting CD63 and HSC70 and analyzed on Philips CM10. Total extracted RNAs were quality checked and processed with Illumina® TruSeq® Small RNA Library Prep protocol. Libraries were sequenced to 50 bp (average of 64 millions of reads per sample). Sequences were mapped against Btau_5.0.1 and analysed for differentially expression using Bioconductor Rsubread and DESeq2 software. Overall, 9 miRNA and 7 lncRNA resulted significantly differentially expressed (*FDR*<.05), suggesting a potential role of exosome cargos as a proxy for animal resilience. According to functional analysis, 5 differentially expressed miRNA were reported to be involved in the inflammation process in human. Further studies will be required to fully understand the regulatory activity of these miRNA in bovine inflammation.

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O047

Lameness in finishing beef cattle: distribution of claw disorders in 2716 hind feet

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Lameness impairs health and welfare of beef cattle, both reared in feedlot and indoor systems. This condition leads to an economic loss by the farmer, derived from costs for treatment or early culling of the animals that could result in emergency euthanasia at the farm. Despite the high impact of claw disorders in beef cattle, incidence of lesions causing lameness in intensive systems is not enough documented. The aim of this study was to evaluate the feet condition of beef cattle reared in different conditions through a *post-mortem* inspection. The study was carried out at 2 abattoirs in Veneto (Italy) during 19 observation sessions, on 96 different batches of animals. A total of 2,716 feet were inspected, 1704 of bulls and 1012 of heifers. Right and left hind feet of the same animal were collected, tied in a support and trimmed with a grinder by a vet claw trimmer. After that, claw disorders were identified using the classification proposed by ICAR Claw Atlas. All disorders were registered as binary measure (yes/no) and classified for their site within foot (lateral/medial). Results showed that 1734 out of the total inspected feet were affected by at least one claw disorder (64%), although the total diagnoses recorded were 2122. The most common disorder was the sole haemorrhage, which occurred in 66.5% of all diagnoses, most frequently in the lateral (93.1%) than in the medial claw. Incidence distribution of other disorders showed that heel horn erosion and white line lesion accounted for 12.6 and 11.1% of all diagnoses, respectively. Incidence of digital dermatitis was also considerable, above 6%. Corkscrew, toe and sole ulcer, swelling of coronet and interdigital dermatitis were recorded with a low frequency of about 1%. Considering the site of disorders, 55.5% of all diagnoses occurred in the lateral and 24.6% in the medial claw, and over 80% of cases with lesions in the medial claw had also lesions in the lateral claw. Classifying claw disorders by aetiology, 81.2% of all diagnoses were most commonly due to non-infectious causes, such as feeding and/or flooring systems. This study shows the high prevalence of claw disorders in beef cattle reared indoors in intensive systems, confirming that lameness is an important concern from animal welfare and economic standpoints as well in dairy cattle. Further developments of this study will consider data collection at the origin farm level to associate the incidence of claw disorders and possible risk factors.

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O048

Using mineral-vitamin boluses to improve somatic cell count prevention value in polish dairy farms

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Diseases of the mammary gland for years represent a major reason for economic losses in dairy farming in Poland. Somatic cell count (SCC) has been extensively used as a tool for monitoring mastitis in dairy herds. It has been observed that the application of CellStop® bolus to cows displaying a high SCC value is able to strongly reduce this parameter. The aim of this study was to use CellStop® bolus in Polish dairy farms to contrast milk SCC problems under practical conditions. Data were collected from 08.2013 to 07.2015 in milk production farm ($n = 370$) on Southern Poland. The herd study was composed of 73 Polish Holstein-Friesian (HF) in lactation, kept in freestall barn system. Cows produced, on average, $\geq$8300 kg of milk in 305 d. Data on milk SCC were collected monthly. Among the herd, 42 cows of different age, lactation stage and SCC levels were selected. Those cows exhibiting high moderate or high SCC ($>$400,000/ml) were treated with a single or double dose of CellStop® formulation. After treatment, cows were subjected to the control general physiological parameters, as well as of udder condition and milk quality using Toxin Terrain Reaction Cell (TOR). The work was based on personal observations, as well as on results of the control milk utility of Polish Federation of Breeders and Milk Producers (PFHBiPM). The data were analyzed to estimate the correlation of repeated measures of SCC within cow after treatment. The boluses impact after treatment on SCC level was compute by STATISTICA12® program.The result of the study showed the administration of the product CellStop® brings the best results for younger cows, which are less probability of occurring chronic mastitis. However, bolus preparation should be used preventively for the cows whose number of SCC does not exceed 300,000/ml in milk. The cows age or no. of lactation had no significant impact for parameters from above mentioned. Parity and the occurrence of clinical mastitis during the previous lactation were also significant risk factors for the development of clinical mastitis in the first 120 DIM and should be considered when using SCC information to make individual cow management decisions. Further research to evaluate the effectiveness of interventions that aim to decrease the incidence of mastitis in this group of quarters is warranted.

Acknowledgements

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O049

Treatment of bovine diseases with agroforestry resources in farms of the Colombian Amazon piedmont

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In a region of Amazonian piedmont in Colombia, an ethnoveterinary research was carried out to know how peasant farmers treat and control bovine affections with agroforestry resources available in their farms. Nineteen farms were randomly selected from those located (n = 200 farms approx.), in the area of the denominate "dual-purpose bovine production systems (Milking of the cow in the presence of the calf), in warm-climate mountain landscapes -SP4-" in Santo Domingo District of Florencia municipality, according to the geo-referenced and biophysical characterization of the land under agrarian use in Caquetá state by García and Collaborators (2002). Data collection was achieved through direct observation, participatory observation, surveys, semi-structured interviews and in-depth interviews with key informants. The development of the study concluded that in peasant farms analysed (n = 19), there are a large stock of agroforestry resources to supply health requirements of the herds, In that way, a total of 27 agroforestry sources were described. It was found that the specie with the highest number of reports was *Carica papaya*, reported in 42% of the farms, followed by *Cordia alliodora* (identified as antidiarrheal, antipyretic and antidote against snakes bite), reported in 31.5% of the farms. *C. papaya* was noted for its associations with anti-inflammatory properties, especially in mastitis, antipyretic, immunostimulant and milk ejector. Specific therapeutic aspects about the resources were not described at this stage of the research. In general terms, when peasants detect symptoms, they supply the products as green fodder extracts or infusions. they also perform some spraying and plastering of these to the animals. The research concluded that the conservation of therapeutic agroforestry knowledge is connected with oral peasant family tradition and the conformation of knowledge networks between friends and neighbours. Additionally and although the present research allowed to unveil the use of agroforestry sources in the area and health management of the herds based on the economic capacity and resources available, it is recommended, the development of experimental assays, in which the pharmacological properties of the different species identified, can be revealed in methodological schemes.

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O050

Spatio-temporal analysis of the Nrf2 activation in transgenic reporter mouse fed with high dosage of saturated or unsaturated fatty acids using *in vivo* bioluminescent imaging

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Nuclear factor-E2 related factor 2 (Nrf2) induces the transcription of genes coding for antioxidant enzymes by binding to the Antioxidant Response Element (ARE) to contrast oxidative stress in organisms. The aim of the study was to evaluate the activation of Nrf2 in transgenic reporter mice fed saturated or polyunsaturated fatty acids and the anti-inflammatory effect of estrogens on organism. Forty-eight ARE CRE OMO reporter mice were divided into 3 groups, consisting of 16 animals, based on presence/absence of estrogens (ovariectomized female, OVX; sham female, SH; male, MA). Each group was further split in 4 subgroups of 4 animals each and fed different diets (7.5% lard, 7.5% tuna oil, 20.0% lard and 20.0% tuna oil). All diets were isonitrogenous; low fat diets were mutually isocaloric, as well as high fat diets. Twice weekly animals were injected i.p. with 100μL luciferin 15 min before the imaging session (5 min., Charge Couple Device Camera, CCD) and kept under anaesthesia while performing ventral acquisition. Photon emission in selected body areas was measured using the Living Image Software. On day 70, experimental mice were subjected to a challenge with Sodium Arsenite and subsequently sacrificed. Specific organs (i.e. brain, muscle, heart, abdominal adipose tissue, urinary bladder, stomach, liver, lung, kidney, intestine, seminal vesicles, testis, spleen, bone, ovary and uterus) were dissected and immediately subjected to *ex vivo* imaging session. Data were analysed by MIXED and GLM procedures of SAS software and significant differences were declared for $p<.05$. The results showed that dietary treatments did not affect body weight and feed intake as well as Nrf2 expression in both pre- and post-challenge phases, excepting for the abdominal region ($p=.031$ pre-challenge); in this area, during the pre-challenge phase, OVX showed lower Nrf2 activation ($p<.001$). Ex vivo results outlined a significant effect of the challenge on all the considered organs ($p<.001$), while OVX subjects had higher Nrf2 expression on urinary bladder and kidney ($p<.05$) and high fat diet increased Nrf2 in urinary bladder ($p<.05$). Based on our findings, there is not any difference between addition of saturated or n-3 polyunsaturated fatty acids on oxidative stress. This study confirmed the protective role of estrogens under physiological condition.

O051

Circulating levels of nutrient-related and intermediate metabolites in healthy Anglo-Arab foals from weaning to 18 months of age

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A comparative trial was developed to assess the metabolic profile over growth stages of 12 Anglo-Arab foals, from weaning up to the age of 18 months. Two colts and 10 fillies were weaned at the age of six months and stabled in individual boxes: all animals were fed for one month (T_1) with a conventional diet based on concentrate (commercial pellet and oat grains, 50:50) and good-quality hay (oat, rye-grass, clover). All foals were then given access to a ryegrass pasture during the day and stabled at night with feed supplementation as in T_1 (adjusted to daily DM intake capacity) for five months (T_2) until yearling. During summer (T_3) the same protocol was followed. Blood sampling was carried once at each phase, at 8:00 a.m. in the morning between hay and concentrate administration. Laboratory analyses were carried out to check blood serum concentration of nutrient related and intermediate metabolites, with the determination of circulating enzyme concentration for organ function assessment and electrolytes. All analyses were carried out with an automated biochemical analyzer (Alcyon, Mindray BS 200). Variations in metabolite concentrations across growth stages were observed, except for serum protein (7.30 ± 0.62 g/dl) and total serum triglycerides (28.7 ± 5.20 mg/dl). Calcium to phosphorus ratio turned out to be constant throughout the trial (2.38 ± 0.04) but lower ($p<.001$) than own reference values for mature Anglo-Arabians. Blood serum urea showed to be constantly close to the upper limit of the physiological range for the horse (36.8 ± 5.51 mg/dl) [1]. Total serum cholesterol showed peaks ($p<.001$) at weaning (137 ± 28.6 mg/dl), to decrease at 18 months of age (84.8 ± 8.96 mg/dl). A similar trend was observed as to alkaline phosphatase (ALP) blood serum concentration, which differed between weanlings and 18 month-old foals ($p<.001$). Non-esterified fatty acids at 12 months (0.86 ± 0.42 mmol/dL) differed ($p<.001$) from values recorded at 6 and 18 months (0.17 ± 0.12 and

0.24 ± 0.22 mmol/dl), respectively. In this study, a peculiar concentration of circulating intermediate metabolites over growth was pointed out, which appeared to differ from average reference values of same blood serum parameters of the mature horse. The assessment of the nutritional state of Anglo-Arab growing horses might take into account both manifest growth performance and individual metabolic profile, contributing to the interpretation of foal's nourishment.

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O052

Effects of dietary protein and lysine content on growth performances, carcass traits and estimated nitrogen input-output flow of growing pigs

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This study was aimed to evaluate the effects of crude protein (CP) and standardized ileal digestible (SID) lysine on the performance of growing pigs of two commercial crossbreds (A and B) from 30 to 145 kg BW. The pigs (30.4 ± 2.0 kg of initial BW) were allotted in 8 pens (6 A and 6 B per pen) and received until around 60 kg BW the same diet containing 164 g/kg CP and 11.0 g/kg SID lysine. Afterwards, pigs of 4 pens were fed diets containing 163 to 146 g/kg CP and 9.4 to 8.5 g/kg SID lysine (HP), whereas the others were fed diets containing 158 to 126 g/kg CP and 8.0 to 6.5 g/kg SID Lys (LP). Individual feed intake was recorded daily by automated feeding stations (Compident Pig - MLP, Shauer Agrotronic, Austria) and animals were weighed weekly from the start to the end of the trial (145 ± 6.2 kg BW). From 60 kg BW onwards, the P2 subcutaneous fat thickness (P2BF) of all pigs was measured by ultrasound scanner equipped with a 5.5–10.5 MHz linear probe (Mylab OneVET - ESAOTE S.p.A., Genova, Italy), with a 3-week interval. The nitrogen (N) input-output flow was computed as difference between N intake and N retention, estimated either assuming a retention of 24 g N/kg BW or using the relationships between BW, P2BF and the estimated body protein content. At slaughterhouse, carcasses and main cuts were weighed and individual samples of *longissimus lumborum* were collected for chemical and physical analysis. Data were analysed according to a two way factorial mixed model including the effects of diet, crossbreds, their interaction and the random effect of pen within diet. Average growth rate of pigs in the 30 to 145 kg BW interval exceeded 0.95 kg/d, with an average feed conversion ratio of 2.57. Pigs fed LP diet showed similar growing performance and carcass and meat quality traits when compared to HP fed pigs, but a significant ($p<.001$) lower N intake and a significant ($p<.001$) lower estimated N excretion, irrespective of the method used for N retention prediction. Even if crossbreds differentiated ($p<.05$) for growth rate, feed intake and feed efficiency, no interaction was detected between CP/SID lysine dietary content and pig genetic line. In conclusion, an average reduction of 10 to 20% of CP and SID lysine dietary content did not impair pig performance, but greatly reduced the N output and the inclusion of soybean meal in the diets. This response does not seem affected by the genetic type of pigs.

Acknowledgements

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O053

Dose-response for different levels of leucine to lysine ratio on the growth performance of weaning pigs with different genotypes at the α-aminoadipate d-semialdehyde synthase gene

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A correct dietary supply of each essential amino acid and a balance against lysine (Lys) are required to maximize pig performance. Data on leucine (Leu) requirement in weaned pigs of modern lean types are scarce and do not consider the pig genotype. The Leu requirement was assessed by a 3-weeks dose-response trial in weaned piglets (9 to 20 kg), in a 2 (Pig genotype) × 5 (Diets) factorial arrangement. One hundred pigs (10 pigs/group) were selected at weaning (24 d of age) on the basis of the genotype at the α-aminoadipate d-semialdehyde synthase (AASS) gene, coding for an enzyme involved in the Lys metabolism. Piglets were equally distributed inside each litter for the two homozygous genotypes at the AASS gene, and fed the experimental diet after 1 week of adaptation (starting from 31 d of age) and for 3 weeks (19.32 ± 4.19 kg final BW). The standardized ileal digestible (SID) Leu:Lys contents of diets were 70%, 85%, 100%, 115% and 130%. The level of Leu affected significantly the growth and the feed intake of the animals during each of the experimental weeks and also for the whole period ($p<.001$). Feed to gain (F:G) was also affected by the diet (period from d 7 to end; $p<.001$). The AASS genotype did not affect the growth performance. Dose-response models on individual data (curvilinear-plateau, quadratic and broken-line models) were tested for ADG, feed intake and F:G. For ADG and F:G the quadratic model gave the best prediction (corrected R2 0.334 and 0.401) and predicted the maximum point of ADG and F:G at 110.7 and 108.7% SID Leu:Lys, respectively. The dose-response curves obtained for each AASS genotype were overlaying. Feeding weaned pigs with diets containing less Leu than Lys reduced the feed intake and the growth of the pigs. In our trial, the Leu requirement for weaned pigs from 9 to 20 kg LW was estimated to be in the range of 110% SID Leu:Lys.

Acknowledgements

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O054

Dietary calcium butyrate promotes a shift of gut microbiota of weaned pigs

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Butyric acid is a short-chain fatty acid produced by bacterial fermentation of non-digestible carbohydrates in large intestine, is involved in intestinal homeostatic maintenance and in prevention/protection against infections and inflammatory processes.
The aim of the study is to investigate the effects of dietary calcium butyrate supplementation in the modulation of intestinal bacterial community in growing pigs.
Ten 4-weeks-old pigs were divided in 2 groups; non-treated group (A) (5 pigs) received a commercial diet for weaners and treated group (B) (5 pigs) were fed the same diet supplemented with 3.52 g/kg of feed of fat coated calcium butyrate from their arrival (day 0) throughout the duration of the experiment (end of experiment day 49). Fecal samples were collected at day 0, 7, 28, 42 and 49 after the arrival at the animal facilities. Bacterial DNA was isolated using Qiamp stool mini kit (Qiagen) and V3-V4 region of the 16S rRNA gene was sequenced on MiSeq Illumina platform. The resulting reads were processed using QIIME software v1.9.1 and taxonomically assigned using GreenGenes database v13.8. The statistical analysis on Alpha and Beta diversity and on taxonomic composition were performed using Vegan and MetagenomeSeq packages in R v3.3.2.
Significant differences between A and B groups for alpha, beta diversity and for OTUs differential abundances were observed at 28 days of treatment. We found that group B was enriched in two *Prevotella* and one *Megasphaera* OTUs. Conversely, the group A was enriched in one *Ruminococcaceae* and one Treponema OTUs. Recently, two enterotype-like clusters were described for young pigs. One enterotype-like cluster is distinguished by a richness in *Ruminococcaceae/Treponema*, whereas the other is characterized by high *Prevotella* level. Better post-weaning performances and higher concentration of secretory IgA were associated with the *Prevotella* oriented enterotype-like cluster.
The calcium butyrate treatment seems to have favored the shift of intestinal microbiota to the *Prevotella* oriented enterotype-like cluster. Therefore, our results suggest the use of calcium butyrate to shape the intestinal microbial population of pigs.

Acknowledgements

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O055

Effects of protein restricted diet on *in vivo* performances and slaughtering traits of Cinta Senese pigs

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The aim of the study was to investigate the protein restriction effect on Cinta Senese pigs reared outdoor. Twenty-Two Cinta Senese pigs (13 barrows and 9 females), weighting averagely 38 kg, were equally divided in 2 groups. One group (NP) was fed with a 12% of crude protein (CP) diet, while the second (PR) with a protein restricted diet (9% of CP). After 5 months, the 2 groups were fed with the same diet (10% of CP). Pigs were weighted every 3 weeks; the average daily gain (ADG) and the feed conversion index (FCI) were calculated. Six hours after slaughtering, carcass weight and percentage of the main cuts were detected on the right side. On a loin sample joint (LS) (2th - 5th lumbar vertebra) the percentages of intermuscular fat, bone, inner and outer subcutaneous fat, lean, *longissimus lumborum* and *psoas major* muscles were determined. Data were analyzed by the analysis of variance (SAS). PR pigs, once fed with the 10% CP diet, showed an ADG from 5 months to slaughter of 0.496, which was significantly higher respect of that showed by NP pigs (0.443). Moreover, PR pigs resulted in a higher slaughtering weight (161.9 kg) than NP pigs (151.9 kg). Regarding the main cuts, the principal differences were found in loin and ribs percentage (14,12% in PR group and 15.93% in NP group) and in lard percentage, which showed an opposite trend, being higher (14.52%) in PR pigs than in NP pigs (11.69%). Examining LS tissue composition, protein restriction affected inner subcutaneous backfat (39.16% in PR pigs *vs* 33.31% in NP pigs) and *psoas mayor* (5.30% in PR group *vs* 6.62% in NP pigs) percentages. In conclusion, PR pigs grew more in the second period of trial; this is can be related to the greater lipid deposition pointed out by the higher lard and inner subcutaneous backfat percentages of PR pigs. However, further analysis should be carried out to determine the intramuscular fat deposition, which is expected to be the main parameter affected by protein restriction.

O056

Nutrients content and *in vitro* digestibility of ex-food as feed ingredient for pig diets

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Ex-food or Former Food Products (FFPs) represent a way by which losses from the food industry are converted into ingredients for the feed industry, thereby keeping food losses in the food chain. FFPs have been proposed as promising alternative feed ingredients. However, FFPs nutritional potential is not yet fully exploited. The aim of the present study was to perform a nutritional evaluation of selected FFPs.

Six samples of mixed FFPs, all based on bakery products, were analysed for Dry matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fibre (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), starch and ash. Nitrogen-Free Extractives (NFE) and Non-Structural Carbohydrate (NSC) were also calculated. Based on FFPs proximate analysis, Digestible Energy (DE) and Metabolizable Energy (ME) values for pigs were calculated. *In vitro* digestibility (IVD) of FFPs were evaluated using a multi-step enzymatic technique to predict the apparent total tract digestibility in pig. A wheat sample was included as control feed ingredient in the study. Data were analysed using IBM SPSS Statistics version 21 software (SPSS Inc.). *In vitro* digestibility values for FFPs samples were analysed using one-way analysis of variance in order to compare means.

FFPs have shown a nutrient composition comparable to that of cereal grains. In the tested FFPs dry matter concentrations ranged from 912.8 g kg^{-1} to 937.6 g kg^{-1}. The overall mean of CP content was 100 g kg^{-1} DM. Compared to wheat, FFPs were characterised by a relative high fat content (average EE 101.2 g kg^{-1} DM). The average starch content was 523.6 g kg^{-1} DM. Nitrogen-free extractives ranged from 611.7 g kg^{-1} DM to 746.8 g kg^{-1} DM, whereas NSC ranged from 585.4 g kg^{-1} DM to 792.7 g kg^{-1} DM. The relatively high NFE, NSC, starch and fat concentrations designated FFPs as valuable energy sources for pig. FFPs tested were characterized by valuable DE (17.2 MJ/kg) and ME (16.9 MJ/kg) values for pigs. However, DE and ME systems used may under/overestimate energy values due to the high lipid and starch content of FFPs. The average IVD value of FFPs samples (88.1% ± 5.77) was comparable to IVD of wheat (90.6% ± 1.62).

In conclusion, FFPs can be considered a fat-fortified version of common cereals grains. The high-energy content and digestibility values elect FFPs as promising non-traditional ingredients for target animals as pig.

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O057

Impact of increasing levels of condensed tannins from sainfoin in grower-finisher diets of entire male pigs on growth performance and carcass characteristics

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Diets containing condensed tannins (CT) have been reported to have anti-nutritional effects in monogastric animals as they reduce feed intake, growth rate and feed efficiency. However, recent findings suggested that hydrolysable tannins can impair development of accessory sex glands and by that influence boar taint in entire male pigs (EM). Unknown is whether CT have a similar effects as hydrolysable tannins. Thus, the goal of the study was to investigate the impact of increasing levels of CT from sainfoin on growth performance, carcass characteristics and morphometric organ data of EM. For the experiment, 48 Swiss Large White EM were assigned within litter to 1 of 4 grower (25-60 kg BW) and finisher (60-105 kg BW) diets supplemented with 0 (T0), 5 (T5), 10 (T10) and 15% (T15) sainfoin, respectively. Pigs were reared in one pen equipped with 4 automatic feeders, which allowed to monitor individual daily feed intake. All pigs were weighed weekly. They had *ad libitum* access to feed and water. At 170 d of age, pigs were slaughtered and carcass quality traits were evaluated. The inclusion of CT had no ($p>.05$ effect on growth performance in the grower period. Although feed intake tended ($p=.07$) to be 10.2% greater in T10 than T5, growth rate in the finisher period and feed efficiency in the grower and finisher period were similar among groups. Slaughter weight and hot carcass weight were not affected by the CT supplementation although T0 pigs had a 2.7% greater ($p<.001$) carcass yield than T15 pigs. Due to 6.4% heavier ($p<.05$) ham weights in T5 compared to T10 pigs, total lean cut percentage was 4.4% greater ($p<.05$) in T5 than T10 pigs, with intermediate values for T0 and T15 pigs. Expressed as percentage of hot carcass weight, relative liver weight was greater (2.05 *vs* 1.81%; $p<.05$) in T15 than T0 pigs whereas the relative salivary weight tended ($p=.08$) to be lower (0.09 *vs* 0.12%) in T10 than T5 pigs. Intermediate values were observed in T5 and T10 pigs for relative liver weight and for T0 and T15 pigs for relative salivary weight. No effects of sainfoin supplementation was observed on the weight of testis and bulbourethral gland. In conclusion, these results show that increasing dietary CT levels had no systematic negative effect on the traits under investigation in EM.

O058

Genome-wide association study of milk fatty acid composition in Italian Simmental and Italian Holstein cows

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Bovine milk is important for human nutrition. Among its components, fat is the main source of energy and influences the taste of milk and dairy products. Nonetheless milk fat has a high concentration of saturated (SFA) and a low concentration of monounsaturated (MUFA) and poly- unsaturated (PUFA) fatty acids beneficial to human health.

Fatty acid profile may change if a targeted breeding program could be applied. In this work the Italian Simmental (IS) breed, a dual-purpose cattle type (milk and meat production), was compared to the Italian Holstein (IH) breed, a milk production cow selected for high yielding, with the aim to highlight the genetic differences or similarities in fatty acid (FA) genetic architecture, using a genome-wide association (GWAS) approach.

Peripheral blood and milk samples were collected in 6 commercial farms of IS and 4 commercial farms of IH from about 1000 animals for DNA isolation and FA profiling respectively. Animals were sampled in the same physiological state (mid lactation), to minimize differences due to the stage of lactation. Each milk sample was split in two aliquots, one used for the determination of protein, fat, lactose and for somatic cell count (SCC) and the second frozen at -20 °C within 2 hours, for fatty acid analyses by gas chromatography technology.

For each breed, a single SNP genome-wide association analysis was carried out, using as fixed effects milk yield, fat and protein percentage content, SCC, parity, days in milk and farm. Thereafter, MUGBAS was used as gene-centred post-GWAS method for the identification of candidate genes. Significant candidate genes identified by MUGBAS were further included in a pathways interaction analysis.

A number of candidate genes were identified in both breeds. Some, as *DGAT1*, *SCD*, *FASN* known to be involved in fatty acid metabolism and found associated to FA composition in other studies. Others identified here for the first time, e.g.: *GHITM* on BTA 28 associated with PUFA/SFA and *ERLIN1* on BTA 26 associated with DI 14-1/(14 + 14-1) respectively in the IS and IH.

Although further investigations are required, different candidate genes in the two breeds, were found, which could explain the differences in FA profiles and might also be useful in future breeding programs.

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O059

Genome-wide association and pathway-based analysis for milk fatty acids profile in dairy cattle

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Fatty acids (FA) profile has a great impact on the nutritional and technological quality of milk and dairy products. Genome wide associations (GWAS) and gene-set enrichment analyses were conducted for 65 milk FA. Milk samples from 1152 Italian Brown Swiss cows reared in 85 herds were used. Animals were genotyped with the Illumina BovineSNP50 Bead Chip v.2. A single marker regression model was applied for GWAS using the GenABEL R package and the GRAMMAR-GC approach. A gene-set enrichment analysis was run on GWAS results, using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases, to reveal ontologies/pathways associated with the phenotypes of interest. In total 175 SNPs were significant ($p < 5\text{x}10\text{-}5$) spanning all *Bos taurus* autosomes (BTAs). The highest signals were detected on BTA26 (∼22.98 Mbp) and on BTA8 (∼3.66 Mbp). Four sub-regions were detected on BTA26 associated with 8 traits. The region 26a corresponded to only 1 SNP (∼9.87 Mbp) which was associated to both C10:1 and C14:1 indices. The regions 26b, 26c and 26d contained multiple SNPs. In particular, the region 26b included 4 SNPs (∼14.66-16.71 Mbp) associated to C14:1 index and C14:1 *cis*-9. In the region 26c (∼18.17-22.98 Mbp), signals were detected for C14:1 index, C10:1 index, C14:1 *cis*-9, monounsaturated FA, C18:1 *cis*-9, C10:0, C12:0 and C14:0. Finally, 6 SNP in the region 26d ($\sim$25.09-31.58 Mbp) were associated to C14:1 index and C14:1 *cis*-9. The high signal on BTA8 (Hapmap40047-BTA-119117) was significantly associated to C18:1 *trans*-16. Gene-set enrichment analyses showed significant results (Fisher's exact test, false discovery rate $<.05$) for 20 FA traits. In particular, C12:0 was associated with the highest number of overrepresented categories/pathways, including several connected to the regulation of mitogen-activated protein kinase (MAPK) activity and protein phosphorylation. The considerably large number of enriched GO and KEGG terms for C12:0 suggested that such phenotype might have a relevant biological role in the regulation of processes affecting milk fat composition. These findings might be helpful for follow-up studies designed to shed more light into the molecular mechanisms underlying FA synthesis and metabolism in the bovine mammary gland. The new insights could contribute to set up selective breeding programs aiming to obtain a healthier milk FA composition.

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O060

Genome-wide association study of cheese-making properties in sheep milk

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Sheep milk is almost all processed into cheese. For these reasons cheese making properties represent a key aspect of sheep milk quality. In this study, genomic regions associated with cheese making properties of sheep milk were investigated. Individual milk samples of 825 Sarda breed ewes, farmed in 47 flocks, were analyzed, by Formagraph, to determine milk coagulation properties (MCP): curd firmness (a_{30}), rennet coagulation time (RCT) and curd firming time (k_{20}). Individual cheese yield (ILCY) was determined through laboratory cheese micro-manufacturing method. Animals were genotyped with the Infinium Ovine SNP50 v1 BeadChip (54,241 SNPs). Marker map was based on the Oar3.1 genome assembly. Quality control was performed on animals (call rate >0.95) and SNP (markers unmapped, with minor allele frequency $<1\%$, call rate <0.975, or deviating from the Hardy-Weinberg equilibrium ($p<.01$) were discharged) by PLINK. Finally, 45,905 SNPs were retained for the analysis. The genome-wide study (GWAS) was performed using the GenAbel R-package, accounting for the genetic substructure of the population. In a first step data were corrected for the fixed effects of lambing month, parity, days in lactation and flock-test date. The residuals were analyzed with a linear model including SNP genotype as covariate and statistical significance of the SNP effects were adjusted on effective number of independent tests estimated using the number of blocks for RCT.
Considering a significance threshold of 0.05, for RCT three markers were found, two between 83.81 and 83.87 Mb on OAR2 and one on OAR15. In OAR2, *Basonuclin2* (*BNC2*), *Small Nuclear RNA Activating Complex Polypeptide 3* (*SNAPC3*) and *Coiled-Coil Domain Containing 171* (*CCDC171*) were annotated. In the region on OAR15, no genes were annotated. For a_{30} two markers have passed the threshold (one on OAR15 and one on OAR24). On OAR24 between 16.80 and 17.10 Mb, *Coenzyme Q7, Hydroxylase* (*COQ7*) and *Glycerophosphodiester Phosphodiesterase 1* (*GDE1*), were found. These two genes are involved with the metabolism of lipids and lipoproteins pathway.

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O061

Genome-wide association study for milk production traits in Valle del Belice sheep

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Over the last years, high-throughput technologies have provided the opportunity to explore the genomes of livestock species to identify regions influencing traits of economic interest. Genome wide association studies (GWAS) have been widely used to disentangle the genetic variation in complex phenotypes, such as milk production traits in sheep. The study presented here investigated the possible association of genome-wide SNPs and breeding values for milk production traits in the Valle del Belice sheep. The studied population consisted of a total of 481 ewes belonging to 9 half-sib families with available records for milk yield (MY), protein and fat percentage (P% and F%, respectively). All animals were genotyped for 54,241 SNPs, using the Illumina OvineSNP50K Genotyping BeadChip. During quality control, SNPs with minor allele frequency (MAF) $< 5\%$, call rate $<95\%$ and out of Hardy Weinberg Equilibrium (HWE) $p<.001$, were excluded from the analysis. In addition, sheep that missed more than 5% of genotypes were also removed. Finally, after quality control, 39,110 SNPs and 469 individuals were available for the GWAS. Univariate animal models were used to estimate variance components and breeding values for MY, P% and F%. The genome-wide association using mixed model and regression-genomic control approach as implemented in GenABEL was used. *P*-values of $\leq 5 \times 10^{-5}$ were considered as significant associations. Heritability estimates for MY, P% and F% was 0.21, 0.34, and 0.39 respectively. In total, nine significant SNPs on six different *Ovis aries* chromosomes (Oar) (Oar1: rs402356478; Oar2: rs406142196 and rs426814658; Oar3: rs399070200; Oar10: rs406329083 and rs405825255; Oar20: rs398454547; Oar24: rs405426757 and rs412849969) associated with MY, P% and F% were identified. All significant detected SNPs were within or close to known ovine genes. In particular, one SNP associated with P% was found within the alpha-lactalbumin (*LALBA*) gene on OAR3, which is a functional and positional candidate underlying this association, and one SNP associated with MY was found near the cyclic AMP-responsive element-binding protein (*CREB1*) on OAR2, a gene associated to genesis and secretion of milk, development of mammary glands and circadian rhythms. Genomic regions found to be significantly associated with milk production traits in this study will further contribute to the identification of the genes regulating those traits in dairy sheep.

O062

Genome-wide scan for runs of homozygosity in Valle del Belice sheep

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The current availability of very large numbers of single nucleotide polymorphisms (SNPs) throughout the genome makes these markers particularly suitable for the detection of genomic regions where a reduction in heterozygosity occurred and offers new opportunities to improve the accuracy of inbreeding (F) estimates. Runs of homozygosity (ROH) are contiguous lengths of homozygous segments of the genome where the two haplotypes inherited from the parents are identical. Here, we investigated the occurrence and the distribution of ROH in medium-density SNP genotypes ($\sim$50,000) in order to characterize autozygosity in 512 individuals of Valle del Belice sheep and identify the regions of the genome with high ROH frequencies. A total of 11,629 ROH were identified. All individuals displayed at least one ROH $>$1Mb. The mean value of FROH $>$1Mb was 0.084 ± 0.061. ROH that were shorter than 10 Mb predominated. The highest coverage of chromosome (OAR) by ROH was observed on OAR24, whereas the lowest one was observed on OAR1. A typical pattern was observed for the number of ROH per OAR with higher values in the first three chromosomes. There was a considerable difference among animals for the number of ROH segments and the length of the genome covered by ROH. The genomic regions most commonly associated with ROH were identified by selecting the top 1% of the SNPs most commonly observed in ROH within breed. A total of 239 SNPs were considered as candidate SNPs and we identified 107 potential candidate genes that may be under directional selection. Six genomic regions located on six chromosomes (OAR2, OAR3, OAR4, OAR10, OAR11 and OAR23), corresponding to ROH island, presented hotspot of autozygosity. According to KEGG database, a majority of the genes were involved in multiple signaling and signal transduction pathways in a wide variety of cellular and biochemical processes. The ROH islands spanned several candidate genes which influence traits that are associated with adaptability and with the regulation of immune responses (*NPAS2*, *PDCL3*, *SERPINF1* and *SERPINF2*) and we did not identified candidate genes with important influence on milk production traits in sheep. The Valle del Belice breed is subjected to limited breeding selection programs for milk production traits, but shows excellent adaptability to the local environments. Therefore, these results suggest at least a partial role of natural selection in shaping the genome of Valle del Belice sheep breed.

O063

Looking into the Swamp Buffalo genetic history through the whole mitogenome analysis

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Water Buffalo (*Bubalus bubalis*) is one of the most important livestock species in several Asian countries and is used for milk and meat production and draft power in rice cultivation. The domestic Water Buffalo in Asia is generally classified in two major subspecies, the dairy River Buffalo and the draft Swamp Buffalo, which differ in morphology, behavior and number of chromosomes. The swamp type is typical of Northeast India, China (southern regions and Yangtze Valley) and Southeast Asia. Based on mitochondrial DNA (mtDNA) analyses, the Swamp Buffalo was proposed to originate from the border region between south China and north Indochina. However, location, time, and mode of domestication are still unclear, because the Buffalo mtDNA sequences reported to date are fragmentary, mainly limited to the control region (only four complete mtDNA sequences deposited in GenBank). In this study, we report the complete mitogenome of 107 Swamp Buffalo from Southeast Asia to provide a comprehensive phylogeographic overview of the Swamp Buffalo matrilineal diversity and to allow quantitative inferences on origin and demographic history.

O064

Genetics of alternative somatic cell count traits in Italian Holsteins

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Mastitis is one of the most costly diseases in dairy herds. Alternative somatic cell count (SCC, cells mL^{-1}) traits have been proposed to improve udder health. The aim of this study was to estimate genetic parameters of alternative SCC traits in first-parity Italian Holstein cows. The original dataset was edited to include records between 5 and 305 days in milk (DIM). Contemporary groups were defined as cows calving in the same herd-year-season (HYS) and HYS with less than 5 animals were removed. After edits, 21,671 cows from 152 herds were available for statistical analysis. Alternative SCC traits were: mean and standard deviation of somatic cell score [(SCS, $3+\log_2(\text{SCC}/100{,}000)$] within lactation ($\text{SCS}_t$ and SD_SCS_t, respectively), between 5 and 150 DIM (SCS_{150} and SD_SCS_{150}, respectively), and between 151 and 305 DIM (SCS_{305} and SD_SCS_{305}, respectively); infection, a dichotomous trait indicating that at least one test-day record had SCC greater than 100,000 cells mL^{-1} within lactation; severity, the ratio between the number of test-days with SCC greater than 100,000 cells mL^{-1} and the total number of test-days; severity2, the ratio between the number of test-days with SCC greater than 400,000 cells mL^{-1} and the total number of test-days; and subclinical mastitis (SCM), identified as two consecutive test-days with SCC below 100,000 cells mL^{-1} followed by a test-day greater than 400,000 cells mL^{-1}. Means of SCS_t, SCS_{150}, SCS_{305}, infection, severity and severity2 were 3.48, 3.12, 3.44, 0.83, 0.40 and 0.14, respectively. Heritabilities of and genetic correlations between the aforementioned traits were estimated using univariate and bivariate animal models, respectively, considering HYS, age of the cow at calving, and number of lactation test-days as fixed effects, and additive genetic animal and residual as random factors. The pedigree included 73,009 animals (6 generations). Heritability estimates were 0.11 for severity, 0.08 for SCS_t, 0.06 for SCS_{150} and SCS_{305}, and 0.02 for SD_SCS_t, SD_SCS_{150}, SD_SCS_{305}, infection and SCM. The strongest genetic correlations (0.94 to 0.99) were between SCS traits (SCS_t, SCS_{150} and SCS_{305})

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and infection, severity and severity2. Although heritability estimates were generally low, exploitable genetic variation exists for SCC traits. Combination of such traits into an udder health index with appropriate emphasis may enhance genetic gain in resistance to mastitis of Italian Holstein population.

Acknowledgements

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O065

Predictive ability of Fourier transform infrared spectroscopy and milk components to assess the pregnancy status of dairy cows

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We used information from Holstein (16,956), Brown Swiss (31,528), Simmental (25,937) and Alpine Grey (12,572) cows reared in the north-Italy, to assess the predictive ability of milk components (fat, protein, lactose and casein) and the Fourier Transform Infrared (FTIR) spectral data to discriminate pregnant and empty cows. Pregnancy status (PS) was coded as a binary variable where the positive diagnose was indicated when a subsequent insemination was not registered within 90 days and it was confirmed by a subsequent calving. The milk samples were collected during the milk recording protocol and were analyzed using a MilkoScan FT+ 6000. The spectrum covers 1060 wavenumbers (wn) from 5010 to 925 cm^{-1}. The PS was predicted using generalized linear models (GLM) with fixed effects in the case of fat, protein, lactose, casein, and also for the association of PS with single FTIR spectral bands. We also fitted a GLM using all the available wavelengths (1060 wn) using a Bayesian model (a modified version of BayesB with a probit). This model was fitted using the BGLR R-package. The prediction accuracy was studied using a 10 fold cross-validation and calculating the area under a receiver operating characteristic curve (AUC) estimated between the predictions and phenotypes. Overall, the best results were obtained for the model that included the complete FTIR spectral data. We observed differences between breeds, the highest AUC value was for Alpine Grey cows with an AUC $= 0.644$, while Brown Swiss and Simmental had similar results (0.628 and 0.627 respectively) and the lowest value for FTIR-Spectra was for Holsteins with 0.606. In the single wave analysis important peaks were detected at: from wn 2973.2 to wn 2872.9 cm^{-1} where Fat-B is usually filtered; wn 1773.6 cm^{-1} where Fat-A is filtered; wn 1546.0 cm^{-1} where protein is filtered; wn 1468.9 cm^{-1} associated with urea and fat; wn 1399.4 cm^{-1} and wn 1245.1 cm^{-1} related to acetone; from wn 1025.2 cm^{-1} to wn $1013.7 \times \mathrm{cm}^{-1}$ where lactose is filtered. In conclusion, this research can provide new insights to conduct further research and implementation of pregnancy status screening on commercial herds.

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O066

Causal relationships between milk yield, somatic cells, fertility, longevity and fighting ability in Aosta Chestnut-Black Pied Cattle

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In animal breeding genetic relationships between traits are typically investigated through multi-trait mixed models (MTM), i.e., not considering relationships of causal dependence generally occurring in biological systems. Structural equation models (SEM) have been recently proposed to distinguish the genetic correlation between traits from the causal effect that phenotypes exert on each other. Focusing on the native Aosta Chestnut-Black Pied cattle, this study aimed to investigate the causal relationships involving traditional productive and functional traits and the attitude to dominance. The latter trait is routinely recorded as fighting ability (FIGH) score achieved during traditional cow contests (not cruel behaviours) and it is already considered as a breeding goal for this breed. Studies have shown that the ability to fight for dominance may be associated at phenotypic and genetic levels to a detriment in immune functions, health, and "feminine traits" as fertility and milk yield. Routinely collected test-day milk (TD-MY) and somatic cells score (SCS) records, whole lactation data (LACT) and lifetime information of calving intervals (CI), no. of parities and longevity (from birth on) were merged to FIGH scores achieved in 15 years of cow contests (~13,000 participants). Closeness in time for TD was ensured by editing productive information recorded later than 95 d from the fighting event. To robustly detect causal effects (λ) of a trait on another, the same trait pairs were analysed both as SEM and MTM running a Gibbs sampling algorithm. Negative genetic correlations with FIGH were found both for TD-MY recorded up to 30 days before (r ~-0.30), and LACT (r ~-0.60), but in both cases no casually dependencies of milk on FIGH were found (λ not different from zero). A minor causal effect of FIGH on SCS and CI was obtained (λ resp. ~0.05 and ~0.20 standardized units), suggesting a slight phenotypic loss of udder health and fertility due to FIGH. A positive causal effect (average $\lambda = 0.39$) of FIGH on longevity and no. parities was found. In MTM this effect was likely absorbed by genetic correlation (r ~0.40) since SEM revealed a negative genetic correlation occurring between FIGH and longevity (r ~-0.20). Concluding, SEM analysis allowed distinguishing phenotypic causation and common sources of variation (e.g. genetic) between attitude to dominance and secondary traits in Aosta Chestnut-Black Pied cattle baring out also cases of negative relationships.

O067

Use of discriminant analysis to early detect lactation persistency in dairy cows

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The traditional dairy cow length of lactation is around 305 days. The modern high-yielding cows are, however, capable to maintain an high persistency well beyond the traditional lactation standard length. The aim of the present research was to develop an algorithm able to early identify animals that, having a high milk production at 305 days, could be destined to have a long lactation. For those cows, the insemination could be delayed thus obtaining a number of health benefits as well as an improvement of fertility. Data consisted of 699,622 test day records for milk yield of 2305 lactations recorded in a farm placed in Italy (Arborea, Oristano) and in a farm located in Hungary (Tiszaalpar, Bács-kiskun). Animals were divided in two groups: primiparous (1025) and multiparous (1280). Then, they were grouped into two other classes: cows that at 305 days after parturition had a milk production lower than 20 kg and cows yielding more than 32 kg. Four different lactation curve models (Wood, Ali & Schaeffer, Legendre Polynomials and 4th Degree Polynomials) were fitted to individual lactations by using both the first 120 and 150 days in milk. The regression coefficients obtained in each model were used as variables in two multivariate discriminant techniques. The Canonical Discriminant Analysis (CDA) was used to test for possible differences, for each parity, between the two production classes. The Discriminant Analysis (DA) was than exploited to assign animals to the two production classes. To validate results, the complete dataset was randomly divided into training and validation datasets. This partition was iterated 5000 times by using a bootstrap procedure. The CDA significantly separated the two production classes for each parity. Among the different lactation models, the 4th degree polynomials were those that better assigned animals in the bootstrap procedure. In particular, by using the first 150 days of lactation, the error in assigning animals to the two production classes was 10% for primiparous and 13% for multiparous. Error slightly increased when 120 days of lactation were used: 12% and 17% for primiparous and multiparous, respectively.

O069

Identification of genomic variants associated with metabolic stress in Italian Simmental and Holstein cows

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The identification of genes and genomic variants associated with metabolic stress would allow the development of molecular tools for breeding in favor of animal welfare and production. In this study Ceruloplasmin (CuCp), Gamma-Glutamyl Transferase (GGT) and Paraoxonase (PON) blood levels were used as surrogate traits of metabolic stress in cows. Animals were sampled only once in the same physiological state (mid lactation), to minimize differences due to the stage of lactation. A genome wide association study (GWAS) was performed on 418 Italian Holstein and 412 Italian Simmental dairy cows, genotyped with the GeneSeek GGP Bovine 150k array. Marker-trait association was evaluated in each breed by single-marker regression using a mixed model that included farm, parity, days in milk, body condition score and milk yield as fixed effects (the last three fixed effect referred to the sampling day). The MUGBAS software (https://bitbucket.org/capemaster/mugbas) was used for post-GWAS analyses to pinpoint candidate genes. Interestingly, the main candidate genes identified were those coding for the proteins used as phenotype: ceruloplasmin-like (CP) gamma-glutamyltransferase 1 and 5 (GGT1 and GGT5) and paroxonase1 (PON1), likely indicating the existence of allele-specific patterns of gene expression at these loci. Except for CP, which was significant only in Holstein, the other three genes were significant in both breeds. In addition to the four stress-related proteins, other genes involved in inflammatory response and oxidative stress were found. Sequence data from the 1000 bulls project (http://www.1000bullgenomes.com) were explored and candidate causal mutations for the target genes identified. A validation step is planned to identify a suitable set of markers to be proposed for use in marker-assisted selection.

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O078

Candidate gene association analysis for milk yield and composition traits, coagulation properties and somatic cell count in Italian Holstein Friesian bulls

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Advances in DNA-based marker technology enabled the identification of genomic regions underlying complex phenotypic traits in livestock species. The incorporation of detected quantitative trait loci into genetic evaluation provides a great potential to enhance selection accuracies, hence expediting the genetic improvement of economically important traits. The objective of the present study was to evaluate the association of 96 individual single nucleotide polymorphisms (SNP), located in 53 candidate genes, with milk production (milk yield, MY; protein yield, PY; casein yield, CY; fat yield, FY) and composition (protein percentage, PP; fat percentage, FP; casein percentage, CP) traits, somatic cell score (SCS) and milk coagulation properties (MCP), i.e., rennet coagulation time (RCT) and curd firmness 30 min after rennet addition (a_{30}), in a population of Italian Holstein Friesian sires. Phenotypic records for 292,007 milk samples from 45,115 lactating cows reared in Veneto Region (northeast Italy) were collected and analyzed using mid-infrared spectroscopy. Through the use of de-regressed estimated breeding values (EBV), data from the semen sample of 423 Italian Holstein Friesian bulls were genotyped with the Illumina GoldenGate Assay (Illumina) to test the association of polymorphic SNP with the selected phenotypic traits. After data editing, of the 96-selected SNP, a total of 65 SNP in 43 candidate genes were successfully genotyped. Forty-five SNP in 32 genes were associated with at least one of the tested traits. In particular, most significant SNP-trait associations, having favorable effect on the milk traits, were observed for polymorphisms located in genes: *CCL3* and *AGPAT6* on FY; *DGKG* on MY; *PPARGC1A*, *AGPAT6* and *CSN1S1* on FP; *GHR* on PP and CP; *TLR4* on PP, CP and FP; *CSN2* and *POU1F1* on RCT; *GHR* on a_{30}; and *AGPAT6* on both RCT and a_{30}. *GHR* and *POU1F1* polymorphisms were associated with an important reduction of SCS. Genomic regions found to be significantly associated with milk technological traits will contribute to the identification of the genes regulating those traits in dairy cattle. Moreover, our results can be considered as preliminary foundation for further studies based on gene-assisted selection and genomic selection programs.

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O070

Preliminary results on the genetic variation and the population structure within a Spanish Merino breed commercial population

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The Merino sheep breed is undoubtedly the most emblematic in Spanish territory, not only because of its economic importance and the number of animals, but also because of its historical importance, being the sheep breed that has most participated in the formation of others ones throughout the world. After the fall of the importance of wool sector, in 50-60 years it was decided to convert this breed towards meat production, using the practice of crossing with Merino derived breeds, such as Berrichonne du Cher, Merino Precoz, among others. Parallel to this, the economic difficulties of the ovine sector and the large area in which the breed is raised, make the structure of the population very heterogeneous. The aim of this work was to study the diversity and genetic structure in a sample of 15 herds in the Merino breed, all assigned to the producers cooperative OVISO (Limited Cooperative Society of the Southwest Sheep), with the aim to determine their levels of breed purity, sources of introgression and genetics structure evolution in the cooperative context. We analysed 493 animals using 38 microsatellites markers. As comparison breeds we have included 30 animals belonging to other international breeds: Merino Precoz, Merino Landchaf, Merino Fleichaf, Berrichonne du Cher, Ile de France, as well as the Lacaune and Lojeña breed. The results show how genetic variability in the Merino breed is high compared with other Spanish breeds, with a heterozygosity of 0.68 and average number of alleles of 13. In addition, at intra-herd farms showed high levels of genetic diversity despite that allelic richness values showed heterogeneous values with a minimum of 1.71 up to a maximum of 3.65. The analysis of genetic distances, structure, as individual allocation, show that a high degree of admixture is not appreciated with the other breeds, although some isolated individuals may suggest the presence of crosses. Graphical representation of Structure software results shows, a high genetic diversity and distance between herds, that could suggest different geographical groups or genetic lines within the breed. These preliminary results provide the basis for a larger study that includes all herds in the breed in order to carry out complete and exhaustive picture of the genetic structure of Merino in the commercial context of OVISO cooperative enterprise, which count actually with a total number of 800,000 sheep in its breeding program.

O071

An overview on the genetic diversity in South African Nguni (Zulu) sheep

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The human history has been completely transformed by the domestication of animals and plants over the past 10,000 years. Survey based on Zulu sheep population size in a period of five years (2007 to 2011), reported that population size decreased due to crossbreeding. Data on the census of the Zulu sheep are unavailable. Use of only morphological features is insufficient and molecular markers are extremely useful for identifying and quantifying genetic diversity between populations. The aim of the study was to investigate the genetic diversity in Zulu sheep using 26 microsatellites loci. A total of 207 blood samples of Zulu sheep were randomly collected from the following areas: eShowe, Jozini, Makhathini research station, Mtubatuba, Nongoma, Ulundi, Nquthu and University of Zululand. In addition, 82 individuals of exotic breeds (Damara, Dorper and South African Merino) were included. The following parameters were analyzed: genetic variation, genetic differentiation, genetic distance, genetic structure and admixture. A total of 323 alleles were detected across the 26 microsatellites. The polymorphic information content revealed that all markers were informative (PIC = 0.59). Moreover, 26 loci showed a minimum number of alleles per locus (11.54). The mean number of observed alleles ranged from 3.84 (University of Zululand) to 6.64 (Nquthu). The highest expected heterozygosity (0.71) was observed in Nquthu, while the lowest (0.57) in eShowe. The inbreeding coefficient estimated ranged from -0.00642 in eShowe to 0.16465 in Jozini. The Reynolds weighted genetic distance revealed distinct clusters, first cluster including Makhathini research station and University of Zululand, second with Nquthu, Dorper, Ulundi, eShowe and Nongoma. The remaining populations could not be grouped in any

cluster. The structure and admixture analysis results ascertained that Makhathini research station and University of Zululand share common genetic structure, while four populations (Jozini, Nongoma, Ulundi and Nquthu) received genes from the Dorper breed. The values for observed and expected heterozygosity were above 0.50 in all populations indicating that the analyzed populations are characterized by a noticeable genetic variation. Nevertheless, Zulu sheep are threatened by inbreeding and crossbreeding (Dorper breed). Thus, there is a need for sustainable breeding and conservation programs to control the gene flow, in order to prevent the erosion of local sheep genetic resources.

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O072

Detection of signatures of selection in Italian sheep breeds

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Demographic events such as bottlenecks, genetic drifts, migrations, inbreeding and artificial selection change allele frequencies and combinations in a population. Also natural selection and local adaptation are decisive phenomena in the transmission of favorable genotypes leading to an increase of fitness. In livestock species, genetic diversity has often been assessed by comparing groups with different production aptitude. Contrariwise, the process of adaptive evolution has been frequently neglected because it is difficult to investigate. Sheep breeds, due to their pronounced ability to tolerate different climates and environments, to adapt to different production systems, and to the relative low level of artificial selection they have been subjected to, can represent a good animal model to investigate signatures of selection and adaptation, simultaneously. In this study, in order to detect genomic regions subject to selection a multiple-tests approach was adopted. To provide strong associations and improve power resolution, the canonical discriminant analysis, the fixation index for population differentiation and runs of homozygosity methodologies were implemented in twenty breeds representative of the Italian sheep population. The obtained results revealed the presence of known selection signals harbouring genes involved in milk, meat and wool production and morphological traits (*PRL, ABCG2, EDAR, NPR2, MC1R, RXFP2*) but also selective sweeps in loci related to sensory system (*TAS* family, *ORs* family), circadian rhythm (*CLOCK, PER3*), skull development (*GAB1, NPR3*), diseases resistance (*DEFB 134, IL6, CDH 26, PRP/PRND*) and xenobiotic metabolism (*PON1, DPH 6*) denoting a strong adaptation to environment. Moreover, new selection signatures were highlighted suggesting several new putative candidate genes for sheep breeds.

O073

Toward the identification of causal mutations and LD markers associated to disease resistance in sheep

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Several genomic regions significantly associated to disease resistance traits have been identified in a resource population of around 3000 Sarda ewes by using a LDLA approach based on Illumina Ovine50K BeadChip data. The investigated traits were mastitis through somatic cell count (SCC), nematodes resistance through fecal egg count (FEC) and paratuberculosis (PTB) assessed by the mean of the optical density values of the MAP antibodies from ELISA tests repeated along the

animals' life. A strategy to identify causal mutations and LD markers in the selected genomic regions has been set up for the most significant locations. For each trait, it consists in the whole genome re-sequencing of 2 animals with high probability to be homozygous at the favourable and unfavourable variants. A further animal with high probability to be heterozygous was re-sequenced as control. This approach was applied to investigate 4 regions associated with FEC, SCC and PTB. In this paper we present the strategy focusing on the position associated ($p<.10E-13$) with susceptibility to paratuberculosis on OAR 20 (24875590 bp). Genomic re-sequencing with 12X coverage was performed with an Illumina HiSeq sequencer. The quality controls were performed with fastQC, and sequences were mapped to the reference genome (Ovis aries v3.1, Archibald 2010), with BWA (Li, 2009). Variant call was performed with GATK (McKenna, 2010) with a base quality cut off >30 bases for calling. The segment to focus on was defined basing on IBD coefficients between the two analysed haplotypes (H+ and H-). The hypothesis was that causal mutations were alternatively homozygous in the two animals and included in the genome portion where the segment showing IBD = 1 between the 3 H+ overlapped with the segment showing IBD = 1 between the 3 H- ones. This explored region spanned from 24389525 to 25565801 bp, overlapping part of the MHC region. Identified polymorphisms were filtered for concordance with the expected genotypes (alternative homozygous). Functional annotation of filtered SNPs (n = 3535) and INDELs (n = 239) was performed by Variant Effect Predictor (McLaren et al. 2010) using the gene annotation database from Ensembl release 87 (2016). In the explored region, 1125 variants were identified on 21 annotated genes. Three non-synonymous SNP were identified in 2 protein coding regions (ENSOARG014759, and ENSOARG015866). A further Stop_gained variant was detected on ENSOARG015660.

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O074

Drawing up worldwide goat diversity and post-domestication history: update from ADAPTmap project

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The study of goat adaptation to different environments is a major aim of the international ADAPTmap project, which joins the genotyping and re-sequencing efforts of the International Goat Genome Consortium (IGGC), the African Goat Improvement Network (AGIN), Feed the Future program of United States Agency for International Development and NEXTGEN EU project.

Having a worldwide distribution, and thriving across a variety of contrasting habitats, goats offer an attractive opportunity to address the genetics of adaptation. This has to start with extensive analyses of the patterns of diversity, thus, a set of 144 breeds, representing 36 countries from 5 continents, has been genotyped with the Illumina GoatSNP50 BeadChip. Several analytical approaches have been adopted to describe the patterns of molecular variation across Africa, Europe and western Asia. The results obtained so far reveal a strong partitioning among continents. Three major gene pools correspond to goats from Europe, Africa and western Asia, while further sub-structuring reflects the main post-domestication migration routes. The reconstruction of past migration events highlighted several exchanges mainly between African populations, which often involve admixed and cosmopolitan breeds. In addition, extensive gene flow was revealed within specific areas (e.g., southern Europe, Morocco and Mali-Burkina Faso-Nigeria), while isolation due to geographical causes (e.g. insularity) or human management has brought a decrease in local gene flow. Taken together, these results confirm that after domestication in the Fertile Crescent in the early Neolithic era (approx. 15,000 BP), domestic goats spread to Europe, Africa and Asia through divergent migration routes, which determined the major genomic background of the continental populations. During the following centuries, due to geographical and reproductive isolation, further sub-structuring of diversity occurred at the local level. This has been accompanied by additional migrations and/or importations, the traces of which are still detectable, such as the clear African signatures in the goat populations of the Canary Islands and Southern America.

O075

Italian Goat Consortium: a collaborative project to study the Italian caprine biodiversity

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The Italian Goat Consortium (IGC), joined the effort of many Universities and Research Institutes, in a comprehensive study of the Italian goat population genetic makeup using a medium density (54K) SNPs chip. Currently IGC has genotyped more than 1,000 animals from more than 30 goat breeds and populations from all Italian geographical and agro-ecological areas of goat rearing.

The aim of this work is to obtain a clear picture of the Italian caprine biodiversity, to reconstruct the ancestry, to disentangle the genetic background and to assess the relationships among and within the investigated breeds. To date, the IGC dataset includes about 50 million genotypes. The data were quality checked by excluding markers and individuals on the basis of missing genotypes, minor allele frequency and close individual relatedness. Genetic relationships among and within breeds was investigated by Multi-Dimensional Scaling and Principal Component Analysis. Population structure, ancestry models and admixture were estimated by ADMIXTURE and fastSTRUCTURE software. Finally, phylogenic trees were reconstructed with PHYLIP software suite starting from shared-allele identity by state, and Reynolds distance matrices, while past migration events were modeled with TreeMix software.

The results confirmed high levels of genetic polymorphism and confirmed the North-South geographical pattern of diversity, previously reported on a smaller sample of Italian goat breeds. The analysis also revealed a pivotal role of Central Italy in connecting the genetic resources of the northern and southern areas of the country, and confirms the genetic isolation of insular breeds. Moreover, some breeds show clearly distinctive and homogeneous gene pools, whereas other breeds present complex and, in some cases, dishomogeneous genetic background.

Even if "*A breed is a group of domestic animals, termed such by common consent of the breeders*" (Lush J.L., 1994), genomic tools are useful in understanding the genetic background of populations and in defining their relationships or uniqueness. These tools can complement the traditional ones in providing farmers and their associations a powerful aid for a more conscious management of goat populations and their biodiversity.

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O076

Genetic parameters of new FTIR milk phenotypes in Rendena breed

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Heritability (h^2) and genetic correlations (rg) between traditional milk traits (i.e., milk yield, fat, protein and lactose %, and somatic cell score; SCS) and novel traits regarding milk coagulation properties (MCP), cheese yields (CY), curd nutrients and energy recoveries (REC) and daily cheese yields (dCY) were estimated in Rendena cattle breed. The new milk phenotypes accounted for 4 MCP traits, i.e., rennet coagulation time (RCT, min); time to a curd firmness of 20 mm (k20, min); and curd firmness at 30 and 45 min after rennet addition (a30 and a45, resp.; mm) and a group of cheese making traits accounting for 3 CY traits as % of milk processed weight (i.e., weight of fresh curd, $\%CY_{CURD}$; the curd dry matter, $\%CY_{SOLIDS}$; and curd moisture, $\%CY_{WATER}$); 4 REC traits (REC_{FAT}, $REC_{PROTEIN}$, REC_{SOLIDS}, and REC_{ENERGY}, %) calculated as ratio between single nutrient or energy in curd and in processed milk; and 3 dCY traits expressing the amount of cheese (dCY_{CURD}, kg/d), total solids (dCY_{SOLIDS}, kg/d) and water (dCY_{WATER}, kg/d) obtained from the milk yielded daily by a cow. Data were obtained from routinely milk recording (Trento province, 997 cows, 9,947 test-day records, 2010-2015). The new phenotypes were predicted with proper calibration equations from Fourier Transform Infrared (FTIR) spectral analysis of milk. Bi-trait analyses under a repeatability test-day model by means of a Gibbs sampling algorithm were carried out. The h^2 ranged from 0.10 to 0.39, except for SCS (0.01). Milk yield showed moderate negative rg with some MCP and REC traits (rg = -0.19 for RCT, a_{45}, REC_{SOLIDS} and REC_{ENERGY}) and CY traits (rg = -0.34) and high positive rg with dCY traits (0.88 on average). Negative, favourable, rg between fat and lactose (%) with RCT and k_{20} (rg = -0.24 and -0.23, resp.) and positive with a_{30}, a_{45}, CY and REC traits (average rg = 0.54 and 0.29, resp.) were estimated. Finally, protein (%) showed medium-positive rg with RCT, a_{45}, %CY, $REC_{PROTEIN}$, REC_{SOLIDS} and REC_{ENERGY} (average rg = 0.53) and negative rg with a_{30} and REC_{FAT} (average rg = 0.24). In conclusion, the relationships between traditional milk production and novel milk phenotypes in Rendena cattle resulted variable but some genetic correlations suggest a possible promising use of new traits for genetic improvement of technological properties and economic value of milk.

O077

Pathways interaction analysis for identification of novel candidate genes

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With rapid development of high-throughput sequencing technologies and resulting massive data production, researchers' concern is increasingly sliding over to ability to solve the challenge of data mining. For example, diffuse and strong associations with large chromosomal region are common when different genomic approaches are applied (e.g. selection signature, GWAS, ROH, etc.), making difficult to pinpoint true candidates among a huge list of significant genes. Filtering relying on positioning is commonly used, but the arduous and time-consuming traditional bibliographic research remains the last "validation" step.

Here we introduce a Pathways Interaction Analysis (PIA) to infer possible candidates among a list of genes. This approach has no species constraint and is based on gene relation investigations, taking into account both upstream and downstream pathways interaction. For this purpose, a genes list is ordered into respective KEGG metabolic pathways. Once pathways strongly related with the trait of interest are chosen (first degree interaction - FDI), a dependent interaction network is automatically created selecting the relative up/downstream pathways (second degree interaction - SDI). Ultimately, genes falling inside FDI and SDI are considered good candidates for the trait of interest having both positional and functional evidences at their sight.

First version of PIA is implemented in R environment. The script is programmed to download relevant files using KEGG REST API, and obtains annotations with KEGGREST and biomaRt Bioconductor R packages. Network visualization takes advantage of the DiagrammeR package.

The method was validated using GWAS outcomes on Italian Simmental and Italian Holstein fatty acids profile. Among the list of about 220 possible positional candidates, obtained with single-SNP and gene-based (MUGBAS - MUlti species Gene-Based Association Suite) pipelines, only 13 genes were selected with PIA approach, according to their function. The

bibliographic research confirmed their association with milk fatty acid traits.

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O079

Identification of a novel polymorphism in the 3'untraslated region of the interferon gamma gene in waterbuffalo (*Bubalus bubalis*)

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Interferon gamma (IFNG) is a pro-inflammatory cytokine produced by different cells of the innate and adaptive immune system. It plays a critical role orchestrating the immune response towards many pathogens including virus and intracellular bacteria. *Mycobacterium bovis*, the causing agent of bovine tuberculosis (bTB), is an intracellular bacteria that is phagocytized by alveolar macrophages in the lung following infection. Later, T-cell migrate to the lung and production and secretion of IFNG started. It has been demonstrated that polymorphisms in the *IFNG* gene affect the outcome of TB in human and KO ifng mice are susceptible to tuberculosis infection. For this reason, in this study we want to analyze the genetic variability in the *IFNG* gene and if the susceptibility to bTB in water buffalo (*Bubalus bubalis*) is influenced. To test our hypothesis, we collected blood samples from several buffaloes belonging to 5 herds of Campania (Southern Italy). Subjects were classified as cases (59 subjects), when they result positive to the delayed hypersensitivity test and microbiological isolation from lymphoid and controls (125 subjects) when they were negative to repeated intradermal test. Since we are firstly interested in polymorphism belongs to 3' untraslated region (3'UTR), we sequenced a fragment of 690 bp spanning the exon 4 of *IFNG* gene (NW_005783995.1) in 20 samples chosen randomly. A nucleotide transversion was detected at position g.4667G > A of the 3'UTR in 6 of 20 samples. TargetScan (http://www.targetscan.org/vert_71/) was utilized to forecast potential microRNA binding sites and verify if the g.4667G > A could have any potential regulatory effect on *IFNG* expression. MicroRNA (miR) are short non-coding RNA that influence the gene expression targeting a seed sequence which is located, in the majority of case, in the 3'UTR of a specific gene. Thus, we found that g.4667G > A polymorphism belong to 5' GGTTTTATCTCAGGGGCCAACTAGG 3' that includes the seed sequence of miR-125b (seed sequence is underlined and the polymorphism is highlighted in bold). This preliminary finding is a good starting point for extending the analysis of g.4667G > A to all samples and verifies whether the novel detected polymorphisms might be a potential marker of bTB.

O080

SNP co-association and network analyses for bovine milk fatty acid profile

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Nutritional and technological value of milk is largely influenced by milk fat quantity and fatty acid (FA) profile. However, the molecular mechanisms underlying the regulation of milk fat synthesis and secretion in the bovine mammary gland remain largely unknown. In the present study, we exploited an Association Weight Matrix (AWM) approach to build gene networks for bovine milk FA profile aiming to i) increase the knowledge about the functional relationships among set of genes affecting milk fat content and composition, ii) describe the biological functions regulating milk fat synthesis and secretion and iii) identify key transcription factors (TFs) controlling these mechanisms. The results of single-marker genome wide association studies (GWAS) across 65 FA and fat content in milk samples, as well as the genotypes for 37,568 single nucleotide polymorphisms (SNP) from a cohort of 1152 Italian Brown Swiss cows were used. An AWM-approach based on SNP co-associations allowed to predict a network of 791 genes related to milk FA profile. This network provided new insights into the crosstalk between distinct molecular pathways (e.g. mitogen-activated protein kinase (MAPK), lipid metabolism and hormone signaling) that were not detectable when analyzing individual SNP alone. In parallel, we focused on the TFs and their potential target genes within the AWM-derived network to identify the optimal subset of TFs spanning the majority of the network topology. Results highlighted BTB Domain and CNC Homolog 2 (BACH2), E2F Transcription Factor 3 (E2F3), and Lysine Demethylase 5A (KDM5A) as key regulators of milk FA metabolism. Functional analyses of the target genes by ClueGo confirmed that ontologies/pathways related to MAPK activity, cholesterol biosynthesis, hormonal signaling and reproduction were enriched (right-sided hypergeometric test, false discovery rate <0.05). In summary, our approach allowed to identify key regulators undetectable by the standard GWAS approach and provided novel insights into the physiological and cellular processes required for the synthesis and secretion of milk FA, improving the understanding of bovine mammary gland functionality. The new knowledge might help to develop selection strategies to improve milk quality for human consumption.

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O081

Influence of group housing, lactation system and floor type on performance and injuries in rabbit does

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To evaluate the effect of group housing, lactation system, and floor type, 40 crossbred multiparous pregnant rabbit does were transferred to individual pens (0.5 m x 1.0 m); 8 does were kept individually during all the trial (I; $n=8$); the other 32 does were kept in 8 collective pens (2.0 m x 1.0 m, by joining 4 individual pens) in stable groups of 4 individuals (G; $n=8$) from 8 d until 2 d before kindling and from 2 d until 33 d after kindling. Half pens had wire net floor covered with plastic mats (W; $n=8$) and the other half plastic slatted floor (P; $n=8$). Within G does, in half pens ($n=4$) each doe nursed its own litter (fixed lactation, F); in the remaining half, each doe randomly nursed the litters of the group (random lactation, R). Doe performance at kindling and during lactation, litter performance from standardization until weaning (33 d), and skin injuries on G does (5, 12, 19, 26 and 34 d after kindling) were monitored. The effect of the housing system on performance was tested by PROC GLM of SAS. Within G does, the effect of the lactation system and floor type was evaluated by PROC MIXED (pen as random effect); the injury frequency was analysed by PROC FREQ according to observation day, lactation system, and floor type. Housing system did not affect doe kindling or lactation performance; litter weight at standardization was higher for G than I does (680 *vs* 619 g, $p<.05$), but differences disappeared at weaning. The lactation system showed some residual effects when controlled lactation finished, nests were opened, and kits free of moving. In fact, from 19 to 33 d after kindling, does and litters had higher feed intake (679 g/d *vs* 602 g/d; $p<.05$) and, at 33 d, kits were heavier (775 *vs* 748 g, $p<.05$) in R than in F pens. Floor type (W *vs* P pens) affected milk production (244 *vs* 225 g/d; $p=.06$), and kit weight at the end of controlled lactation (301 *vs* 290 g; $p<.05$), without residual effects at weaning. The injury frequency changed after the group formation: 34% at 5 d, 47% at 12 d, 13% at 19 and 26 d, and 10% at 34 d of lactation ($p<.05$). In conclusion, under our conditions, group housing did not impair doe performance; lactation system and floor type had minor effects; aggressiveness among does was evident after group formation during early lactation but decreased in the late lactation. Further recordings on more reproductive cycles would be necessary to confirm the present results.

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O082

Main welfare problems in intensive broiler chicken production systems: is there any way to differentiate farms?

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In a scenario of intensive and standardized broiler chicken production system, the aim of this study was to assess the prevalence of main welfare problems using the Welfare Quality® assessment protocol and to differentiate farms. A shortened protocol (only on-farm) was applied in 13 farms in the Verona province from late-May to half-July 2016 with 35 housing facilities accessed in total. Animal-based measures were assessed: panting (indicator of heat stress); plumage cleanliness (indicator of litter quality); hock burn, food pad dermatitis and on farm mortality and culling rates (indicators of good health); and avoidance distance test (indicator of human-animal relationship quality). Information regarding the flock housed (breed, stocking density), environmental dust and litter quality were collected. The stocking density limit resulted in line with the regulation and there was a good litter management, proven also by the good level of plumage cleanliness (77.7 ± 24.5% observed birds clean). Prevalence of hock burn and foot pad dermatitis showed also positive results. Mortality and cullying rate were relatively high (3.5 ± 2.7% died/culled) and high numbers of birds were panting in an attempt to cope with heat stress in some farms (13.1 ± 22.6%). The avoidance distance test showed high fearfulness of animals towards humans in 2 farms with 0 animals touched. The application of a shortened protocol does not allow the calculation of an overall welfare score due to missing data and the differentiation of farms in this standardized production system could be done based on some negative results only contextually at measure level. Panting, and mortality and culling rate could point out farmers adopting efficient *vs* inefficient management of environmental temperatures and animals (0.0 *vs* 15.0% birds panting and 1.4 *vs* 4.4% of birds died/culled in first *vs* third quartile,

respectively). Low fearfulness levels of animals could reflect farmers having careful or potentially trained stockpeople in charge of the animals (11 *vs* 114 birds approached and 43 *vs* 149 birds touched in first *vs* third quartile, respectively). It could be concluded that, although not differentiating farms through a welfare score, assessing welfare of broiler chicken on-farm and gathering information on the management could point out potential critical factors. The overcoming of these and specific stockpeople training could improve animal welfare and production, particularly in the worst farms.

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O083

Prevalence and burden of helminths in free-range laying hens in Northern Italy

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Egg production is a relatively new farming sector in South Tyrol, an alpine region of Northern Italy. The vast majority of the hens are kept in free-range production systems. However, information on the infection status with gastrointestinal helminths, which are known as one of the major problems in these production systems, are not available, yet. Therefore, the present cross-sectional study aimed to estimate the prevalence (P) and burden of gastrointestinal helminths (WB). A total of 280 laying hens from 14 farms were randomly collected at the end of the laying period. After slaughter, the gastrointestinal tract was removed and examined for the presence of helminths. In addition, faecal samples were collected to estimate faecal egg counts (presented as eggs per gram of faeces (EPG)) and oocyst counts (oocyst per gram of faeces (OPG)), respectively. The P and mean WB of each helminth species and correlation between different worm species and total WB were calculated. Almost all hens (99.3%) were infected. The average WB was 171.1 (SD ± 261.2). The following nematodes were found: *Heterakis gallinarum* (P: 95.7%; WB: 111.6). *Capillaria* spp. (P: 66.8%; WB: 49.8) and *Ascaridia galli* (P: 63.6%; WB: 10.2). The overall prevalence of cestodes was 30.7%. The sex ratio (% of females) of *A. galli* and *H. gallinarum* were 56.0 and 53.8%, respectively. The percentage of EPG and OPG positive samples was 55.5% and 14.4%. The vast majority of the eggs quantified in the faecal samples were *A. galli* and *H. gallinarum*. Correlations between worm counts of different parasite species were highly positive ($p<.01$). A small proportion of the hens (13.6%) were infected with only one helminth species, while in 31.4% two and in 35.4% of the hens three species were found. Almost 20% of the hens were even infected with four species. In conclusion, free-range laying hens in the studied region were highly infected with gastrointestinal helminths at the end of the laying period. Generally, mixed infections were observed. Infections so far are rarely controlled with anthelminthics, but high infections indicate the necessity to develop efficient control strategies including management, the use of resistant genotypes and treatments.

O084

Growth and slaughter performance of two dual-purpose poultry types compared with extensive broiler and layer types fattened for 67 and 84 days

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Nowadays, newly hatched layer-type cockerels are culled. This could be avoided by using dual-purpose types, with females producing eggs and males fattened for meat. However, a lower performance is to be expected. Still they might be competitive in organic production where lower performing poultry types are employed. To test this, in an experiment growth and slaughter performance of dual-purpose types (Lohmann Dual, LD, and Novogen Dual, ND) were compared with an extensive broiler type approved for Swiss organic production (Hubbard S 757, HU) and a layer type (Lohmann Brown, LB), all slaughtered after either 67 or 84 days of fattening.

The 4 × 1350 birds were kept in 20 m^2 compartments of 270 birds each. All birds received an organic broiler diet (12.8 MJ metabolisable energy, 230 g crude protein/kg). Body weight (BW) and feed intake were determined weekly. At d 67 and 84, birds from 2 and 3 compartments per type were slaughtered. Carcasses, breast meat and legs from 24 birds per type and date were weighed. Statistical analysis considered effects of type, age at slaughter and the interaction. Feed intake during 67 d was around 68 g/d for LD, ND and HU and increased to 80 g/d during 84 d. In LB, feed intake and its increase were lower with 56 and 60 g/d during 67 and 84 d, respectively, compared to the other types. Feed conversion ratio

(FCR) was most unfavourable for LB (3.6) regardless of age at slaughter. Across 67 d, HU had the best FCR (2.6). There was no longer a difference to LD and ND across 84 d (range: 2.7 to 3.0). The average final BW of HU, LD and ND at d 67 and 84 were 1.7 and 2.4 kg, respectively, with LB being about 40% lighter. Similar differences were observed in carcass weights, with LD, ND and HU carcasses weighing about 1.1 kg at d 67 and 1.5 kg at d 84 d. Breast muscle proportions were greater for HU than LD and ND and did not change with age at slaughter (21, 17 and 18%, respectively). The breast meat proportion of LB was only 15%. Overall, leg proportion was greatest for LD (36.4%) followed by ND and HU (35.7 and 35.0%) and LB (32.8%). In conclusion, the dual-purpose types performed at a same level as the extensive broiler type except for breast meat, which is disadvantageous when breast meat is sold separately but less obvious when selling the entire carcasses. At d 84 compared to d 67, valuable cuts were heavier but similar in proportion of total carcass proportions. The layer cockerels were inferior in all important traits.

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O085

Effect of spaghetti-meat abnormality on quality and histological traits of broiler breast fillets

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During the last few years, the increasing growth rate and meat yield of modern broiler hybrids caused the appearance of several breast muscle abnormalities, such as white striping and wooden breast. Recently, a new myopathy termed spaghetti-meat (SM) characterized by extremely soft and friable *pectoralis major* when palpated after chilling has emerged. Since the occurrence of SM is often associated with white striping (WS), this study aimed at evaluating the effects of SM and/or WS conditions on meat quality and histological traits in broilers. For this purpose, 96 *pectoralis major* muscles were selected from homogeneous flocks (47 d of age and 2.8 kg of live weight at slaughter) into two independent trials and classified as follows: 24 Normal (N), 24 WS, 24 SM and 24 WS/SM. Each fillet was cut in order to separate the superficial layer from the deep one and both used to assess proximate composition, histological features, colour, pH and protein profile. Data were analyses by two-way ANOVA using abnormality and sampling position as the main variables. Proximate composition was found to be significantly modified according the type of abnormality, especially in the superficial layer of the fillets. The SM and WS/SM groups had a remarkable decrease in total protein content coupled with an increased moisture level ($p<.001$), while WS group was associated with higher lipid content. Histological evaluations of abnormal samples evidenced several degenerative aspects that almost completely concern the superficial layer of the fillets. The SM samples showed poor fibres uniformity and a progressive rarefaction of the endo- and peri-mysial connective tissue. The WS fillets exhibited necrosis and lysis of fibres, fibrosis, lipidosis, loss of cross striation and vacuolar degeneration, while WS/SM fillets exhibited intermediate histological features. No relevant effect on meat colour has been detected, however abnormal fillets showed increased yellowness (b*) and ultimate pH values ($p<.001$). Moreover, both WS and SM abnormalities were associated with a more intense proteolytic degradation of muscle tissue ($p<.001$), which led to the formation of high molecular-weight protein fragments. In conclusion, both muscle myopathies mainly affect the superficial layer of *pectoralis major* muscle, while deep section is poorly involved. Furthermore, if compared with WS, SM abnormality is associated with a more remarkable alteration of the proximate composition and quality traits of the meat.

O086

Changes in structural properties and desmin degradation of broiler wooden breast fillets during refrigerated storage

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A novel myopathy termed “Wooden breast” (WB) appeared worldwide about 5 years ago especially in fast-growing broilers slaughtered at heavier weights. Since severe WB affects meat appearance and texture, the poultry plants tend to downgrade WB and divert the meat to further processing where the sensory implications are less important. This study aimed at investigating the effect of a 7-days refrigerated storage on textural properties, particle size distribution and desmin degradation in broiler breast meat affected by WB. For this purpose, 45 Normal (NB) and 45 WB *pectoralis major* muscles were selected at 3 hours *post-mortem* from a single flock (Ross 508, males, 38-days old) and used to assess compression force measured at 40 and 80% of its initial height, particle size distribution by using a Malvern Mastersizer 3000 (Malvern Instruments Ldt, UK) and desmin degradation quantified by Western Blot. To evaluate the intra-fillet sampling position effect, both the superficial and the deep layer were considered and all measurements were carried out at 10, 24, 72, 120 and 168 h *post-mortem* after refrigerated storage (2-4 °C). Data were analysed by factorial ANOVA using abnormality, sampling position and storage time as the main variables. If compared to NB, superficial layer of WB exhibited higher 40 and 80% compression values measured at the earliest sampling time. In addition, the 80% compression values suggested a progressive softening process taking place within the superficial layer of the WB samples from 10 to 72 h *post-mortem* ($p\leq.001$). As for particle size distribution, the increased deposition of extracellular matrix and fibrosis in WB might contribute in explaining the different fragmentation patterns observed between the superficial and the deep layer in the WB samples, with the superficial part exhibiting a higher amount of larger particles and an increase in particles with larger size during storage, in comparison with NB. The results for Western Blot against desmin revealed that at 10 h *post-mortem*, the WB cases exhibited larger ($p\leq.05$) amounts of desmin. A sharp decrease of the intact desmin

band coupled with a progressive accumulation of its 39-kDa degradation fragment was observed without any significant difference among the groups. In conclusion, it was found that the harder consistency of the WB fillets is of structural origin and only partially counteracted by endogenous proteolytic processes taking place during the *post-mortem* period.

O087

Market chain of "Consortium for safeguard of Bianca e Bionda breeds" in Piedmont: 1. demography, housing and slaughtering conditions

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In Piedmont (North-West Italy), the poultry market chain safeguard of Bianca di Saluzzo (BS) and Bionda Piemontese (BP) by a local Consortium and the meat is a Slow Food Presidia. For this reason, a census was conducted to describe the alternative poultry chain of these two local breeds. Data were collected, in winter 2015, by visiting each farm included in the "Consortium for safeguard of Bianca di Saluzzo (BS) and Bionda Piemontese (BP) breeds" registering the farm location, the number of birds per farm, farm traits, number of hatched birds per year. Furthermore for each farm, information about birds live weight (LW), age, plumage condition, foot pad and breast blister at slaughter were recorded. Farms regional distribution varies according to the breed, being located for BS in Cuneo and Turin provinces (67% and 33%, respectively), whereas for the BP in the provinces of Asti (61%), Cuneo (28%) and Turin (11%). In total 41 farms rear these breeds, in particular: 6 farms rear BS birds and 35 farms the BP ones. The estimated annual production consists in 12,000 BS and 45,000 BP birds. Both breeds are reared, as imposed by their production disciplinary (that reports only other two rules, like the banning of OGM feed and allopathic treatments), in free-range systems, but with great density variability among farms for both breeds. In particular, indoor space availability range from 0.3 to 2.0 m^2/bird, whereas the outdoor space availability range from 1.4 to 30.0 m^2/bird. Moreover, all farmers reported that birds do not use indoor shelters, preferring to sleep in the trees. For both breeds, discarded females by the selection of hens for egg production are slaughtered. For BS were identified two commercial categories namely males and females, whereas for BP three commercial categories were found: males, females and capons. BS are slaughtered at 220 ± 54 days, when males reach 2.1 ± 0.2 kg LW and females 1.7 ± 0.2 kg LW; while BP are slaughtered at 184 ± 38, 226 ± 84 and 268 ± 8 days for males (LW = 2.3 ± 0.2 kg), females (LW = 2.0 ± 0.2 kg) and capons (LW = 2.9 ± 0.3 kg), respectively. Birds exterior characteristics were very good, showing undamaged plumage and none foot pads and breast blisters. Birds showed natural behaviour repertoire together with great exterior characteristics.

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O088

Market chain of "Consortium for safeguard of Bianca di Saluzzo e Bionda Piemontese breeds" in Piedmont: 2. Meat quality

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Consumer interest in unconventional poultry production systems, such as free-range and organic is growing in the view of improved animal welfare, sustainability and quality. The aim of the present study was to characterize meat quality of two types of local commercial products. These products from females of two local poultry breeds reared in free range, in very extensive housing conditions, are included in the list of the Slow Food Presidia and slaughtered for Piedmont's local market by the "Consortium for safeguard of Bianca di Saluzzo (BS) and Bionda Piemontese (BP) breeds. Females of these breeds, after a selection of hens for egg production, were discarded and slaughtered in winter 2015 (from January until March, in four successive slaughtering), for meat production (slaughter age: BS = 220 ± 54 days; BP = 226 ± 84 days). Ultimate pH (24 h) of breast meat was measured by

Crison 507 pH-meter fitted with a spear-type electrode. Colour measurements were performed, on the surface of breast, by a Minolta CM-600d spectrophotometer (8mm measuring area). Lightness, redness and yellowness (L^*, a^*, b^*) in the CIELAB colour space, were considered. To determine drip losses, 2cm thick breast samples were weight before and after storage at 4 °C for 24 h in a plastic container with a double bottom (Lundström and Malmfors, 1985). Cooking losses were measured by weighing 2cm thick breast samples before and after cooking. Samples were sealed in a polyethylene bag and cooked in a water bath. Shear force was measured on cooked meat ($1 \times 1 \times 2$ cm) by cutting them perpendicular to fiber direction, using an Instron 5543 equipped with a Warner–Bratzler shear device. Proximate analyses on breast meat, according to the AOAC (1990) methods, were performed. Breast meat of both breeds (N = 60 birds for each breed), did not show any difference in term of: pH, drip loss, L^*, a^*, b^*, dry matter, protein and ash contents. On the contrary BS birds showed, higher ($p<.05$) cooking loss and lower ($p<.05$) shear force. Concerning chemical characteristics of breast meat BP presented higher ($p<.05$) fat content. All the observed differences are probably due to variability of slaughter age and rearing conditions. Based on the obtained results, it appears obvious that big effortshave to be done to improve productive and qualitative homogeneity of products from Bianca di Saluzzo and Bionda Piemontese, to increase their market and thus to ensure their survival.

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O089

Captive morphological changes and growth parameters in Apennine rock partridges (*Alectoris graeca graeca*)

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The rock partridge (*Alectoris graeca*) is showing a continuing decline in the area of occupancy and its conservation status has been declared vulnerable at the national scale. Therefore, actions to reinforce the wild populations are required. In this view, the definition of captive breeding programs aimed to conserve the species *ex situ* and to produce birds suitable for restocking or reintroduction purposes are necessary. However, morphology responds and exhibits plasticity in adaptability to early environmental stimuli and captive morphological changes could be responsible of a loss of animal ability to survive in a natural environment. Such evidences exist on pheasants (*Phasianus colchicus*) but specific surveys were not performed on the rock partridge. With the aim to: i) highlight eventual morphological differences between rock partridges subjected to antipodal environemental stimuli, ii), identify at which time these changes occur, iii) estimate growth parameters and asymptotic weights, two groups of related rock partridge's offspring, respectively intensive reared and semi-natural reared, were measured and compared for six exterior morphological traits. Live weight, tarsal length, tarsal depth, tarsal width, head length and head width were recorded, starting from 14 days post hatching, up to 98 days post hatching. Significant differences in tarsal measurements and head length were detected between the two groups starting from 84 days post hatching. Larger and deeper tarsi and longer head were found in the group semi-natural reared, probably as the result of physical and mental exercise in a complex wild like environment. The integration constant (mean ± se = 1.1329 ± 0.0291) and the maturing index (mean ± se = 0.0222 ± 0.0004) estimated fitting observed data with Gompertz equation, suggest that the Apennine rock partridge grows up slower than Chukar partridges and other rock partridge's subspecies even if the asymptotic weight (mean ± se = 692.1013 ± 10.1554) seems in the range reported for the Alpine subspecies. In this study we present evidences that intensive rearing system could be successfully adopted providing that translocation to semi-natural acclimatization sites, occur before 70 days post hutching. In addition, within this time limit is also possible to translocate sex balanced groups.

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O090

Comparison of productive performance and intestinal transcriptomic profile of two fast-growing chicken hybrids

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Fast-growing chicken hybrids currently used in commercial conditions differ for growth rate, feed efficiency and reproductive traits. However, the reasons behind these differences are not clear. The aim of this study was to compare productive performance and intestinal transcriptomic profile of two fast-growing chicken hybrids (HA and HB). A total of 1170 one day-old female chicks from the same hatching session and breeder stock, were weighed, divided in 2 experimental groups of 9 replicates each and fed the same commercial diets. At the end of each feeding phase (9, 21, 34 and 43 d) productive performance were recorded. At slaughter (43 d), ileum mucosa was collected from one bird per replication and total RNA was extracted to perform microarray analysis for transcriptomic profile using Chicken Gene 1.1^{ST} Array Strip. Pathway analysis was performed through GSEA software considering enriched pathways with False Discovery Rate $<25\%$. At slaughter, HA reported lower body weight (BW) and daily weight gain (DWG) than HB (2607 *vs* 2734 g and 59.6 *vs* 62.6 g/bird/d, respectively; $p<.01$) whereas no significant difference was observed in feed conversion rate (FCR). Moreover, HA and HB birds showed different growth patterns throughout the study. Indeed, HA birds reported higher BW and better FCR from 0 to 9 d than HB (228 *vs* 217 g and 1.352 *vs* 1.419, respectively, $p<.05$) whereas HB chickens gained more weight than HA from 10 to 21 d (49.2 *vs* 45.3 g/bird/d, respectively; $p < 0.01$). From 35 to 43 d, HB birds showed higher DWG than HA (91.9 *vs* 83.3 g/bird/d, respectively; $p<.01$) but also a tremendous increase in FCR (1.769 *vs* 1.641, respectively for HB and HA; $p<.01$). Transcriptomic analysis revealed significantly enriched pathways for mitochondria and oxidative phosphorylation in HA birds and enriched pathways for immune system activation in HB ones. The activation of immune system in HB birds at 43 d of age may have had a negative impact on FCR increasing energy expenditure, even if they showed a better final growth than HA. On the other hand, the enrichment in pathways involved in mitochondria and oxidative phosphorylation in HA birds seems consistent with their phenotypic expression of feed efficiency showed from 35 to 43 d of trial. However, the ongoing metagenomic sequencing analysis of ileum microbiota could provide further insights to elucidate the determinants of the differences in the productive performance observed in the two chicken hybrids.

O091

Influence of a synthetic emulsifier supplementation on lipid metabolism and apolipoprotein gene expression in liver of female and male chicks

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Young chickens physiology is characterized by inefficient digestion and fat absorption due to a low level of natural endogenous lipase production. These evidences have increased the interest on the use of emulsifiers to improve fats utilization and the growth performance in young chickens. In our previous study we observed that supplementation with a synthetic emulsifier (AVI-MUL TOP/GP10, AMT) improves the growth performance of broiler chicks. The aim of this study was to evaluate the influence of AMT on plasma lipid profile, total lipid liver content and hepatic lipid metabolism-related genes of female and male chicks. A total of 1200 ROSS308 chicks (1-d-old) were allocated into 4 treatments with a 2 x 2 factorial design comparing gender (female or male) and different dietary treatments (basal diet supplemented without (CTR) or with emulsifier (AMT), 1 g/kg from d 0 to 12, 0.75 g/kg from d 12 to 22 and 0.5 g/kg from d 22 to 44, respectively). Each group consisted of 15 pens, 20 birds per pen, and one bird of each pen was randomly selected for the analysis. At the end of the trial, the plasma concentration of cholesterol, HDL, LDL, NEFA, triglyceride was evaluated. Liver samples were processed for total lipid content (Folch method) and the mRNA hepatic expression of apolipoprotein A-I (ApoA-I) and B (ApoB) was determined by real-time PCR. The data were analysed as a completely randomized design with a 2×2 factorial treatment arrangement by ANOVA (MIXED procedure). AMT supplementation increased the cholesterol, HDL, and LDL contents compared with the CTR group ($p<.01$; $p=.02$; $p<.01$ respectively). NEFA and triglyceride concentrations were not affected in AMT group. Moreover, it was observed a significant ($p<.05$) gender effect on plasma lipid parameters. No statistical difference was found in total hepatic lipid content between CTR and AMT groups. The AMT supplementation did not modify ApoA-I expression, while ApoB gene was significantly ($p=.005$) up-regulated in female.

ApoB is the main component of chicken plasma VLDL and determines the cellular uptake of lipoprotein in the liver. The obtained results indicate that AMT supplementation influenced the lipid metabolism and related gene expression in broiler chicks.

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O092

Parentage assignment for genetic improvement of farmed sea bream by new single nucleotide polymorphisms and single tandem repeats panels

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The number of selective breeding programs has increased over the years for all major European aquaculture fish species. This trend is supported by an increase in the number of selected traits and by the use of molecular tools in a larger number of selective programs. The management of modern selective breeding programs in aquaculture requires the use of pedigree information to carry out efficient genetic evaluations for multi-traits improvement, allowing a better control of inbreeding. Molecular markers represent a powerful tool for parentage inference of breeding candidates reared in communal tanks.

In this work, a large multiplex of 13 microsatellite markers (Single Tandem Repeats, STRs) and a panel of 59 Single Nucleotide Polymorphisms (SNPs) have been developed to infer complex pedigree structures in experimental batches of Gilthead sea bream (*Sparus aurata*).

STRs screening was carried out from a database of about 100 microsatellite (https://www.ncbi.nlm.nih.gov/nuccore/) and priority choice was given to the highly polymorphic microsatellite markers, with different sizes and known linkage on the sea bream linkage map. For the SNPs selection, a database of 49,000 ESTs (10,000 contigs) has been screened and processed for SNPs mining, annotation and mapping, and a final set of 128 SNPs markers was identified and spotted on array for OpenArray technology(Lifetechnologies).

SNPs and STRs panels have been validated on 4568 offspring assigned to 197 broodstock with PAF, a new parentage inference software.

The STRs multiplex allowed to unambiguously allocate 99.1% of the progeny to their parental pair, while 0.8% of the offspring remained unsolved.

The SNPs panel allowed to solve unambiguously 100% of the allocations, 99.1% of which in single match by deterministic approach, and 0.9% in multiple matches by stochastic approach. The Exclusion Probabilities (EP) given both parents were EP: 0.99999982 for STR markers and EP: 0.9999999 for SNP markers. The Identity Probability (IP) was IP: 2.370104E-24 for STR markers and IP: 1.060928E-30 for SNP markers.

The comparison between SNPs array and STRs multiplex opens the discussion on the limits and advantages of the two types of markers in parentage allocation analysis. The development of this efficient method of molecular fingerprinting, combined with specific broodstock handling and optimised breeding schemes, gives a great support for the implementation of breeding programs in sea bream.

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O093

Intestinal microbiota characterization by next generation sequencing in rainbow trout (*Oncorhynchus mykiss*) fed animal by-product meals as an alternative to fishmeal protein sources

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Bacteria associated with the epithelium of fish intestine play a critical role in host metabolism. Gut microbiota is involved in the anaerobic fermentation of complex dietary carbohydrates and oligosaccharides that are otherwise indigestible. Several genetic, nutritional, and environmental parameters influence the abundance and diversity of fish gut microbiota. Therefore, understanding the factors that regulate this community membership and structure is crucial for improving fish performance and could be highly relevant for aquaculture practice.

Animal by-product meals from the rendering industry could provide a sustainable and commercially viable alternative to fishmeal (FM) in aquaculture, as they are rich in most essential amino acids and contain important amounts of water-soluble proteins that improve feed digestibility and palatability. Among them, poultry by-product meal have given encouraging results in rainbow trout (*Onchorhynchus mykiss*). However, the introduction of new ingredients in the diet needs to be carefully evaluated since diet is one of the main factors affecting the microbial composition of fish intestine.

Accordingly, we investigated the effects of partial replacement of dietary FM with a mix of animal by-product meals and plant proteins on intestinal microbiota composition of trout. We used 1540 trout (initial mean body weight: 94.6 ± 14.2 g) that were distributed into 14 tanks of 3600 litres. Fish were fed for 12 weeks with seven different diets in duplicate (two tanks/diet). High-throughput 16S rDNA sequencing (MiSeq platform, Illumina) was used to study the gut microbial community profile, obtaining a mean of 384,730 ± 99,194 reads per sample. 4379 operational taxonomic units (OTUs) were identified, which predominantly mapped to the phyla of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*. Differences in gut microbiome composition were found between the dietary groups, whereas species richness was similar. The ratio vegetable: animal proteins proved to play a central role in determining microbiome profiles and *Firmicutes* and *Proteobacteria* phyla were particularly discriminatory for diet type in trout. Plant ingredients favoured a higher *Firmicutes*:*Proteobacteria* ratio than animal proteins. Acceptable abundance of *Firmicutes* was guaranteed by including at least 32% of vegetable proteins in the diet regardless of animal protein source and percentage. The results demonstrate that FM replacement with animal by-product meals did not induce significant changes in gut microbial richness and could be a suitable protein source for use in rainbow trout feed.

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O094

Potential of pure- and crossbred Les Bleues broiler for meat production for small-scale farmers

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In production systems for dual-purpose chicken both sexes might be raised together during the first weeks, before males are separated during their final fattening stage until slaughter, while females are raised for an entire laying period. Such a system seems to be suitable for small-scale mountain farmers in South Tyrol to satisfy the increasing demand for high quality and regionally produced products. However, systems (i.e., husbandry, feeding, slaughter weights) have to be adjusted to the specific conditions of the region. In this experiment, the performance of male broilers of two genotypes in such a system was compared. One day-old male purebred (P) Les Bleues ($n = 150$) and crossbred (C) New Hampshire x Les Bleues ($n = 150$) chickens were raised together with their female counterparts for 12 weeks in a floor husbandry system. Genotypes were kept in two separate compartments. Thereafter, males were moved to a mobile chicken house with free-range access, which was divided in two compartments, too. Until week 6, a starter (12.9 MJ ME, 22% XP, 8.5% EE) and until slaughter a broiler diet (12.3 MJ ME, 18% XP, 7.5% EE) was provided ad libitum. Animals were marked with wing tags in week 6 and slaughtered at weekly intervals from 12th to 23rd week of age. Individual live weight was measured weekly and carcass quality assessed. Feed consumption in the mobile house could only be recorded for both genotypes together due to technical equipment. Production costs were calculated. Results were analysed by one-way ANOVA using a t-test as post-hoc test. At 12 weeks of age, live weight of P (2075 g) was about 200 g higher than that of C (1865 g; $p<.05$). This equals to a daily weight gain of 24.1 g for P and 21.7 g for C. Until week 16, both genotypes reached an average live weight of more than 2500 g ($p>.05$). Dressing percentage was about 1% higher in C than in P when animals were slaughtered with 2500 to 3000 g live weight ($p>.05$). Proportion of legs, breast and wings was 34.3, 16.0 and 11.0% in P and 34.7, 15.5 and 12.1% in C ($p>.05$). Under the specific conditions of the region, production costs per kg of slaughter weight were 5.13 € for P and 7.14 € for C, mainly due to higher animal costs for C. Prices per kg of slaughter weight were 13 €. In conclusion, both genotypes showed an appropriate growth performance. However, production costs clearly demonstrated that production is only economically meaningful if animals are marketed as high-value premium products.

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O095

Ecosystem services and mountain livestock farming: a socio economic valuation

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The concept of 'Ecosystem Services' (ES) focuses on the linkages between ecosystems, including agroecosystems, and human wellbeing, referring to all the benefits, direct and indirect, that people obtain from ecosystems. Livestock systems can have multiple and significant ES and Ecosystem DisServices (EDS), depending on the intensity of the production systems and the use of resources.

This study aimed to identify the societal perception of a number of ES/EDS provided by dairy farming in alpine agroecosystems, and to analyze their socio-cultural and economic values. The study area was the Autonomous Province of Trento, a mountainous territory where dairy cattle systems are predominant. The experimental design was based on two steps. First, a survey examined the opinions of the population in the study area on the positive and negative effects of dairy cattle systems on environment (questionnaire based on a 5 points Likert scale). A total of 92 interviews were collected, 47 from dairy cattle farmers and 45 from non-farming stakeholders (extension service technicians, tourist operators and policy makers). The opinions on positive and negative aspects were compared using a Kruskall-Wallis test. Second, the most relevant ES identified with the first step were valued trough a choice model submitted to 504 respondents resident in the study area (102) and in the neighboring provinces (402). Data were analyzed with a mixed logit model to obtain the Willingness To Pay (WTP).

The first step indicated that for the local population the most relevant ES were water quality (that can be considered EDS depending on the level of provision), biodiversity, landscape, and high quality products. All the stakeholders, and farmers in particular, had a positive opinion on the outcomes of the dairy cattle systems and, in general, did not consider them negative for the environment. The choice model evidenced that the local and general population valued most the regulating ES water quality (49.8% of Total Economic Value – TEV). Provisioning ES (the offer of high quality cheeses) were considered already satisfactory (2.9% TEV), and neither improvement nor reduction were required. The WTP for the supply of non-provisioning ES exceeded the current level of public support, with a TEV of 159 € per person per year.

The results of this study evidenced that there is space to promote policy strategies aiming to improve the sustainability of mountain livestock systems.

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O096

How can working time analysis contribute to the production efficiency of mountain dairy farms?

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In mountainous regions, livestock farming has been the key for the maintenance of the landscape that contributes to the conservation of the local biodiversity and tourism. However, dairying is very labour-intensive under these conditions. The aim of the present study was to 1) estimate the labour input on dairy farms with loose and tie stall housing in South Tyrol and 2) propose strategies for maintaining the holdings and improving the economic situation of the farms. The study was performed on 102 dairy farms, of which half had tie and half loose housing systems. Daily and non-daily working activities and management practices and facilities used were surveyed with a standardized questionnaire. In addition, 9 tie stall and 10 loose housing farms were selected for on-site measurements to determine the exact working time of each single activity and validate questionnaire data. Average herd size of the tie stall (16.3 cows) and loose housing (23.2 cows) farms emphasized the representativeness of the sample for the region. Total working time requirement was higher for tie stall (177 man power hours (MPh) per cow and year) than for loose housing (113 MPh/cow/year) farms, while values varied from 63 to 440 MPh/cow/year and 40 to 270 MPh/cow/year for tie stall and loose housing accordingly. Milking estimated at 74 and 56 MPh/cow/year for tie and loose housing, respectively. Feeding followed with 34 and 27 MPh/cow/year. Labour costs per kg of milk estimated at 32.6 for tie stall and 16.9 Euro cents for loose housing, while production was determined at 41.4 and 79.7 kg/MPh, respectively. For both housing systems, farms with less than 10 cows had significantly higher working time requirement compared with larger herds. Results demonstrate that in both housing systems, working time is high and depends on herd size. Comparison between questionnaire and on-site measurements showed that farmers are able to estimate the overall working time, but have difficulties to separate it for single activities. Working time variation between farms was high and can be attributed to variations in management practices or to limited use of facilities that improve productivity and cost efficiency. Consequently, efficient organisation of working time and an increase in herd size might improve production efficiency. Accordingly, investment in farm facilities (i.e., milking system) and adoption of new management practices might further improve the economic situation of the farms.

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O097

Rural development and dialogue between the communities in Kosovo: an example of international cooperation

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The NGO Reggio Terzo Mondo (RTM) has been present in Kosovo since the early moments following the conflict of the '90s, with projects for supporting people's basic needs and rural development. The extreme importance and need to re-establish dialogue and coexistence between different ethnic groups emerged immediately.

A group of 12 women, Catholic Albanians and Orthodox Serbs, in the rural area of Klinë/Klina in western Kosovo, conscious of the need for their active role in the economic and social reconstruction of their Country, formed in 2013 the Cooperative EVA: the goal was to start an economic activity in the pigmeat sector, a traditional source of income for the Albanian Catholic community and the Serbian Orthodox.

With the collaboration of local and Italian institutions, RTM first conducted a feasibility study and a market survey. In December 2014 started, on EC funding, the project AWARE (Action of Women in Agribusiness for a new Regional Economy), combining the economic empowerment of women to dialogue between the Albanian and Serbian communities; the focus was the creation of a short chain of pork that guarantees quality products, recovery of local traditions and environmental sustainability of economic development.

UNIMORE has been involved in the organization of training modules direct both to breeders and to the local veterinary staff: the topics were the management, breeding facilities, nutrition, the use of local resources, slaughtering techniques, meat processing, and fight against diseases.

The breeding facilities owned by the members, in poor condition because of poverty and/or war, have been renovated or constructed *de novo* according to a standard model, adapted to the different situations. The main stage of the project was the design, construction and start-up of a modern slaughtering and meat processing plant. The project has also included exchanges with Italian companies active in the pigmeat sector. The small farms of members have a total of 24 sows and 220 growing pigs. The slaughterhouse works 10 pigs/week on average, producing fresh meat and processed products that come from a happy union between the Kosovar and the Emilian tradition.

This presentation reports the stages of the project: training, renovation and characteristics of the piggeries, construction and operation of the slaughterhouse, creation of a manual of correct farming practices and production rules.

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O098

Survey on dairy farmer use and interest for precision livestock farming tools

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The precision livestock farming (PLF) approach, the use of information and technologies to improve animal health, welfare, and farm efficiency, is a developing area of great scientific and commercial interest. To understand the current PLF tool adoption in Italian dairy farms and to analyse the perception of farmers of the importance of technologies, a survey was carried out in the Cremona province, for its economic importance in dairy industry. The technicians of Provincial Breeder Association (APA) of Cremona interviewed 490 farmers from July to September 2016 to collect information on: 1) farm extent, herd size (HS), average effective milk yield (EMY) and number of employed workers (EW); 2) presence of technologies for monitoring production, reproduction, health, and feeding parameters or interest for using them; 3) importance of criteria considered for evaluating possible investment in PLF tools (assigning a score from 1, not important, to 5, very important). Chi-squared analyses were performed using the FREQ procedure (SAS/STAT package) to compare differences in HS (<101, 101-200, >200 cows/herd: SHS, MHS and LHS), EMY (<9501, 9501-10500, >10500 kg/305 d: LMY, MMY and HMY), and cows to EW unit ratio (<33, 33-47, >47 cows/EW: LEW, MEW and HEW) categories (to obtain numerically comparable groups). About 93% of respondents were the owners of the farm; 23% of farmers were <40 year old and 41% were >51 year old. The most used and most interesting automatic systems were sensors to measure MY and cow activity (used by 41.2 and 48.6% of farmers, respectively) and to detect mastitis (used by 23.7% of farmers and interesting for 63.1%). The percentages of farms with automatic MY registration were 69.5% of LHS, 51.1% of HMY and 56.0% of HEW farms, while farmers that used sensors to detect oestrus were 73.8% of LHS, 64.2% of HMY and 61.7% of HEW. Sensors for automatic mastitis detection were present in 44.5% of LHS, 31.4% of HMY and 34.3% of HEW. Automated systems for monitoring rumination, milk composition and hoof health were poorly present, but highly interesting ($>50\%$). The most important factors for considering PLF investments were the benefit to cost ratio (score 4.74 ± 0.64) and the availability of a well-functioning local assistance (score 4.67 ± 0.63). This survey suggests most of the dairy farmers have positive aptitude for MY recording as well as for technologies that detect oestrus and mastitis, better if well supported by a good local service and support.

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O099

Milk quality and milking practices in dairy goat farms in Lombardy

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Aim of this work was to study the relationship between goat milk quality and milking management practices. The study was conducted on 148 dairy goat farms in Lombardy (Italy), with particular focus on milk somatic cell count (SCC) and milk fat/protein reversion syndrome. Direct interviews to the farmers were collected. Monthly bulk milk analyses in 1 year (fat, protein, casein and lactose percentages, somatic cell and standard plate count) were used for quality evaluation of goat milk.

Individual milk production was on average 1.26 ± 0.44 kg/d, collected during 1.94 ± 0.18 milkings a day. Average number of dairy goats was 74.3 ± 95.8; most of farms reared Alpine goats (38%), 36.5% Saanen, 16.3% Alpine and Saanen, and 9.2% local breeds. Milk quality was quite good (3.52 ± 0.73% fat, 3.40 ± 0.46% protein, 2.62 ± 0.40% casein). Milking was performed on bedded area in 13.8% of farms, inside the barn in 53.6% and in a separate milking room in 32.6%.

A multiple correspondence analysis was performed and high correlations were found (the first two dimensions explained 31.5% of variance) among farms characterized by local breeds, small size (<45 dairy goats), no official milk recording, milking on bedded area, no udder cleaning before milking, no forestripping, no teat post-dipping and no use of gloves by milkers. High correlation was found among farms with Saanen and Alpine goats, large size (>70 dairy goats), presence of official milk recording, milking inside the barn (but out of bedded area) or in a separate room, udder cleaning before milking, forestripping, teat post-dipping and use of gloves by milkers. A second multiple correspondence analysis was performed and high correlations were found among farms characterized by local breeds, low milk fat (<3.5%) and low protein content (<3.4%), milk for cheese processed by farmers, low content of SCC and standard count plate, probably due to a special care at milking for farm cheese making. On the other hand, high correlation was found among farms with Saanen and Alpine goats, large farm size (>70 dairy goats), high milk fat content, no milk fat/protein reversion syndrome and milk delivered to dairies, probably due to a special care in ration formulation and attention to milk quality. The study demonstrates the importance of milking and farming strategies to improve goat milk quality.

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O100

Study on the behaviour of Cinta Senese and Large White × Cinta Senese pigs reared at pasture

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In the last year, outdoor rearing system of pig has become more popular due to benefits in relation to animal welfare and a high acceptance by customers but, also, for economic management and higher quality of product. Understanding of the behaviour of traditional breeds on extensive environment is crucial both for knowledge of foraging strategies linked to a sustainable use of natural resources and for reduction of environmental risk linked to species-specific behaviour. In this research the native pure-breed Cinta Senese and its crossbreed with Large White farmed in a natural rearing system in Tuscany have been considered. The aim is the study of feeding and dynamic behaviour of pigs of the two genetic types during diurnal times through all the season of one year.

In situ direct observations were conducted on a total of 76 growing-fattening pigs during daylight hours and repeated for 3-5 consecutive days in every season. Animals behaviour was recorded by "scan sampling" at 20 minute intervals by a team of four observers, previously trained for observation under field conditions. Also environmental temperature was recorded at the same time interval.

The punctual observations were grouped into three daylight slots (morning, middle day and afternoon), to obtain the relative frequencies of the main activities within each diurnal time. Data were subjected to ANOVA with the proc. GLM of SAS using breed, daylight slot and season (and relative interactions) as discrete effects and temperature a continuous variable.

Both genotypes spent about 70% of the daylight hours in total feeding, but the growing temperature (from 7° to 29 °C) determined a linearly decrease of this activity.

Pigs showed greater interest on food in the afternoon, especially in grass feeding with significant differences respect to the morning when animals appeared less involved in active behaviours.

Animals were more interested in feeding in autumn and spring when they demonstrated, also, an intense rooting activity respect to other months. During summer, pigs showed greater propensity to rest respect to all other seasons ($p<.05$). Furthermore, Cinta Senese pigs in summer dedicated less time to the food research respect to the other genotype (44% *vs* 80%; $p=.001$). The results showed that pigs had the opportunity to express for many time feeding specific behaviours, but purebred Cinta Senese seems more affected by environment effect and by resources availability of extensive system.

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O101

The daily feeding behaviour of growing pigs subjected to a feed restriction and to a reduction of dietary indispensable amino acid contents

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This experiment aimed to evaluate the changes in the eating behaviour caused by a restriction of feed allowance and a reduction of the dietary indispensable amino acid content. Ninety-six Topig Talent × PIC barrows, allocated in 8 pens (12 pigs/pen), were individually fed according to an *ad libitum* (AL) or a restricted (R) feeding regime, from 47 to 145 kg body weight (BW). The feeders, one per pen, recorded the visits to the feeder, the time spent eating and the feed intake (FI) in each visit. The R pigs received amounts of feed close to their expected voluntary intake during 3 time intervals, 00:00 to 8:00, 8:01 to 16:00 and 16:01 to 23:59 h, when they were allowed to eat 33, 66 and 100% of the amount of feed planned for that day, respectively. The pigs of 4 pens received feeds with conventional standardized ileal digestible amino acids content (CAA), according to NRC (2012). The other pigs received feeds (LAA) that, in early (86-118 kg BW) and late finishing (118-145 kg BW), contained indispensable amino acid contents lowered by 0.09 and 0.18 with respect to the CAA feeds. Data, averaged by pig, were analysed as a 2 × 2 factorial design, with the following 4 levels: AL-CAA, AL-LAA, R-CAA, and R-LAA. The eating activity of pigs was largely diurnal. As overall mean, the pigs of this experiment consumed 2619 g/d in 57.7 min/d and made 8.8 eating visits, with a feeding rate of 47 g feed/min (Supplementary Table 1).

Feed restriction (R *vs* AL) reduced the daily FI (7%, $p<.001$) and the number of visits (27%, $p<.001$), but increased FI per visit (20%, $p=.001$) and the eating rate (10%, $p=.032$).

The dietary amino acid reduction applied in late finishing (LAA *vs* CAA) increased the daily FI (10.5%, p=.037), tended to increase the eating rate (19.6%, p=.06) with both feeding regimes, and tended to interact ($p<.10$) with the feeding regime for other eating traits. In fact, under AL conditions, dietary amino acid reduction increased the daily FI and the visit frequency, but reduced the FI per visit, where the opposite occurred under R conditions.

The coefficient of variation of all the behaviour traits (ranging 0.18 to 0.66) was always larger than that found for the daily FI (0.10). Strong relationships between FI per visit and the visit frequency ($R^2 = 0.93$), and between the eating rate and the time spent eating ($R^2 = 0.80$) were found. The behavioural flexibility of the pig in the attempt to achieve their desired feed intake was evidenced.

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O102

Growth traits, carcass and meat quality of heavy pigs receiving different enrichment tools

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Two separate and independent trials were carried out to investigate the effect of innovative enrichment devices on Italian heavy pigs' growth parameters, carcass traits, meat and ham quality. In Trial 1, 20 pigs received a hanging metal chain (C1) as environmental enrichment, and 20 received wood (poplar) logs (WL) placed inside a metal rack installed on one side of the pen. In trial 2, 20 pigs received a metal chain (C2), whereas 20 animals received a specifically formulated edible block (EB) placed inside the same metal rack described above. Enrichments were always available to pigs. Animal were kept on slatted floors and liquid-fed twice a day. Trials started when animals were approximately 80 days old (average Body Weight – BW = 28 kg) and ended at slaughtering (average BW = 158 kg, age: 285 days in Trial 1 and 280 days in Trial 2). Growth parameters, carcass traits and meat quality data were collected, (including hams weight during dry-curing) and submitted to analysis of variance. The pen (5 pigs) was taken as the experimental unit for growth parameters, the individual was used as the experimental unit for carcass and meat traits.

In both trials, no significant differences were observed as concerns growth parameters (final BW, average daily gain, feed consumption, feed conversion ratio). Only minor differences were detected in carcass traits, with WL carcasses having higher lean meat percentage (51.06 *vs* 49.86%, $p<.05$), lower backfat thickness (22.55 *vs* 25.15 mm, $p<.05$) but higher drip losses (1.23 *vs* 1.00%, $p<.05$) if compared to C1. EB carcasses had lower loin thickness if compared to C2 (59.20 *vs* 64.50 mm, $p<.05$). In both trials, these slight differences did not affect the overall yield in lean cuts, the overall meat water holding capacity or any other quality trait of meat (pH, colour, Warner-Bratzler shearing force). Ham weight losses during dry-curing were not affected by the experimental treatment ($p>.05$).

Our results show that providing heavy pigs with either poplar logs or an edible block did not affect their growth parameters, overall carcass traits, meat or ham quality; this observation supports the conclusion that such innovative and destructible/ingestible enrichment tools could be used in substitution of the metal chain. Further useful information will be available once the observation of the behavioural video-recordings will be completed, to get more insights on the type of interactions carried out by pigs on each enrichment device.

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O103

Seed-based vaccine immunogens administered in feed for the control of verocytotoxic *Escherichia coli* infection in pig livestock

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Porcine verotoxigenic *Escherichia coli* (VTEC), serotypes O138, O139 and O141 were associated with postweaning diarrhea, bloody stools, sudden death and oedema disease and were responsible of important economic losses. Verotoxins and F18 adhesive fimbriae are important virulence factors involved in the outbreak of the disease. The VTEC infection is characterized by absence of detectable neutralizing antibody in serum. This aspect suggests that local immunity plays a crucial role in the defense of the organism from the infection. The aim of this study was to evaluate the protective effect of edible vaccines represented by tobacco seeds expressing F18 adhesive fimbriae and the B subunit of VT2e toxin as alternative to antibiotic compounds. In particular 36 weaned piglets, after polymorphism analyses on FUT1,were randomly divided in two experimental groups (TG: n = 18 and CG: n:18) homogeneous per gender and age (50% male and 50% female, age 21+/-2 days) and allocated in individual pens in controlled environmental conditions. The treatment represented by 20 g (10g of F18 positive, 10 g of VT2eB positive) milled tobacco seeds mixed with 40 g of milk replacer on day 0, 1, 2, 7 and 14. The control group received the same amount of milled wild type tobacco seeds. Twelve animals per groups (respectively CTG and CCG) were challenged at day 20 with 10^{10} CFU of O138 VTEC strain by gavage. 6 piglets per group (UTG and UCG) were not challenged in order to observe the effects of the treatment without the interference of VTEC infection. Animals were evaluated for the zootechnical performance, general health status for 9 days after infection. Piglets were scored daily for main clinical signs (respiratory, palpebral oedema, epiphora, vitality, faecal consistency, and rectal temperature) with a point-score scale. The oral delivery strategy guaranteed the total consumption of the treatment. The fecal IgA content was evaluated by a sandwich ELISA assay using specific antibodies (anti-IgA; anti-F18; anti-VT2eB). The day before the challenge, the titer of fecal IgA resulted higher in TG then CG. CTG showed an average total score (from day 1 to day 9 post-challenge) significantly lower than infected controls for oedema, epiphora, vitality and depression. These results showed a significant reduction of the severity of clinical signs related to VTEC ($p<.01$). Edible vaccines could represent medical plants alternative to antibiotic in the control VTEC infection in pig livestock.

O104

Use of a new instrument for the *in vitro* evaluation of ruminants feeds through gas production: preliminary study

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The gas production technique has been applied, until now, only for scientific purposes and had relatively limited diffusion, since it was based mainly on the use of syringes and data collection was generally non-automated.

The present study was conducted to evaluate a new instrument to be used in the "*in vitro*" measurement of gas production as an alternative to the method by Menke and Steingass (1988) to evaluate dairy cattle feed nutritive value.

Starting from a patent of the University of Uppsala (Sweden), and with the Start up "Bioprocess Control", a new instrument called "Gas Endeavour" was enveloped and adapted to ruminal fermentation studies. This instruments allow to: a) operate with 500 ml batches b) simulate the physiological rumen movements by continuous or discontinuous stirring; c) operate using from 2 to 8 g of dry sample or about 20 grams of fresh forage, depending on the substrate; d) measure and register continuously the amount of gas produced on 15 batches.

Cumulative gas production profile of 24 hours interval were generated using the GAS ENDEAVOUR® at a temperature of 39 °C under discontinuous stirring (20 sec on and 40 sec off). Van Soest buffer, macro-mineral and micro-mineral solution were used to obtain the fermentation medium while the Van Soest method was the reference for the amount of rumen fluid inoculum, medium and sample substrate, that were proportioned to batches of 400 ml. The fermentation trials were made on four corn silage samples having different composition (starch content: 32.00, CSA; 18.31, CSB; 21.57, CSC and 27.74%, CSD) and on a sample of alfalfa hay on three different fermenters with of 5 g of pre-dried sample. In particular, CSA has been tested 11 times while measurements on CSB, CSC and CSD were repeated four times to assess the reproducibility of the fermentation process and the repeatability of the resulting amount of gas (CH4 + CO2) produced. The average amount of gas produced (ml/g DM), the relative standard deviation and the coefficient of variation (CV %) amounted to 199.94 ± 4.03 ml (CV 2.02%), 158.86 ± 4.69 ml (CV 2.95%), 176.09 ± 6.48 ml (CV 3.68%), 192.71 ± 5.88 ml (CV 3.05%). The alfalfa hay sample was repeated 5 times with a gas production (ml/g DM) equal to 141.09 ± 4.93 ml (CV 3.50%). Compared to bibliographic data, obtained with the use of syringes and other systems without automation, the GAS ENDEAVOUR equipment proved to be very reliable and performed highly repeatable measurement over time.

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O105

Electronic nose for the detection of mycotoxin contaminations in maize kernels

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The aim of this study was to evaluate the potential use of an electronic nose (e-nose) in cereal industry for rapid mycotoxin detection. Twenty-eight maize samples were collected from warehouses for stockpiling and analysed by commercial Lateral Flow Immuno Assays (LFIA) for the determination of total aflatoxins and fumonisins. Samples were stored at -18 °C in vacuum-sealed conditions prior to e-nose analysis. Two aliquots of each sample were processed and each analysis was run in triplicate. The headspace of each vial was analysed by the 10 MOS (Metal Oxide Semiconductor) sensors of a PEN3 e-nose. Ten different descriptors, representing each sensor of the e-nose, were used to detect aflatoxin and fumonisin contamination below (uncontaminated) or above (contaminated) the maximum acceptance limits for maize intended for feed. Data were analysed by Discriminant Function Analysis (DFA) procedures using IBM SPSS Statistics 22 (SPSS Inc.). Stepwise variable selection was done to select the e-nose sensors for classifying samples by DFA.

Discriminant function used to identify aflatoxin contaminated or uncontaminated samples included 3 e-nose sensors (W1C-aromatic, W3C-aromatic and W5C-arom-aliph). The overall leave-out-one cross-validated percentage of samples correctly

classified by the tri-variate DFA model for aflatoxins was 71.4%. In the case of uncontaminated samples, the percentage of samples correctly classified was 71.1%, while in the case of contaminated samples it was 75.0%.
Discriminant function used to identify fumonisin contaminated or uncontaminated samples included 6 e-nose sensors (W1C-aromatic, W3C-aromatic, W1S-broad-methane, W1W-sulphur-organic, W2W-sulph-chlor, W3S-methane-aliph). The overall leave-out-one cross-validated percentage of samples correctly classified by the six-variate DFA model for fumonisins was 81.7%. In the case of uncontaminated samples, the percentage of samples correctly classified was 87.9%, while, in the case of contaminated samples, it was 75.0%.
Even though a larger dataset is needed to perform an effective validation procedure, by using a dataset not included in the model e-nose seems to be a promising rapid/screening method to detect mycotoxin contamination in maize kernel stocks. From a technical perspective, contaminated samples misclassified as non-contaminated represent the worst outcome under in-field conditions in order to select samples that must undergo further accurate quantitative analysis.

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O106

Evaluation of heavy metals in intensive animal production systems

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Modern animal production systems produce large quantities of manure by-products that can be used as nutrient resource and soil conditioner in agriculture. Manure is also recognized as a significant source of contaminants of groundwater, surface water and soil with heavy metals (HM). Some HM are essential and are used as feed additives to enhance growth performance, improve meat quality and control diseases. The spread of high amounts of HM in the environment causes their accumulation in the food chain with negative effects on human health. For sustainable animal production and to develop effective approaches to preserve soil and water quality from the HM pollution, it is necessary to know the nutritional basis of the interaction between organisms and environment. The aim of this study was to evaluate the HM pollution in intensive animal production systems in order to establish which elements could represent critic aspects in sustainability and to set-up experimental conditions of phytoremediation strategy. Samples of feed, faeces and drink water were collected from ten intensive breeding farms (dairy cow and swine) in the North of Italy. Obtained samples were dried (on DM basis, AOAC), mineralized and analyzed using inductively coupled plasma mass spectrometry in order to detect the following elements: Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd and Pb. Considering feed samples of growing animals, principal component analysis allowed to separate cattle from pigs. Swine diets presented the highest concentration of minerals, depending on the herd ages. In fact, the highest amount was observed in the weaning phase (Zn: 884.22 ± 1201.59 mg/kg DM; Cu 176.27 ± 28.68 mg/kg DM; Mn: 147.42 ± 51.56 mg/kg DM; Se: 0.68 ± 0.51 mg/kg DM) indicating that these additives were widely applied in swine production and that farmers tend to use more additives to promote the growth of pigs. Co, Ni, As, Mo, Cd and Pb elements resulted under tolerable intake levels and did not represent an apparent risk. The general increase of HM content was registered in the livestock output, reflecting their content in feeds. The data showed that swine manure was an important source of Zn, Cu, Mn and Se to the environment. For the development of effective strategies of phytoremediation, integrated in the animal production systems, the attention should be focused on mineral supplementation that represents the major HM output of modern intensive farms.

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O107

Application of FT- NIRS to estimate chemical components of freeze-dry herbages of Tuscany natural pasture

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Near infrared spectroscopy has been successfully applied at analysis of animals feed, but not many authors have tested NIRs technologies on natural pasture and almost all the researches consider NIRS scansion of oven-dried samples. Nevertheless some parameters, such as vitamins or polyphenols, can be modified by the high temperature applied during the analysis. The object of this study is to investigate the FT-NIRs capacity to estimate chemical composition of natural pasture herbage scanned under freeze-dry condition.

Eighty herbage samples, collected in natural and naturalized pastures of Tuscany (Italy), were used. Each sample was freeze dried, grounded and analysed for: Dry Matter, Crude Protein, Ash, Ether Extract, Crude Fibre and fibrous fractions (Neutral Detergent Fibre, Acid Detergent Fibre, Lignin).

For each sample, three aliquots were scanned using FT-NIRs Antaris II (Thermo Scientific). Mathematical pre-treatments (Multiplicative Scatter Correction, Standard Normal Variate, 1st and 2nd derivate) were applied and outliers' spectra were identified and removed when necessary. Partial least square regression was used on the average spectrum and then the models were fully cross validated. Results are evaluated in terms of coefficient of regression and root mean square errors in calibration (R^2-RMSE) and in cross validation (r^2-RMSECV).

Calibration results, excluding EE and Ash, obtained R^2 higher than 0.87 for all parameters, with RMSE lower than 1.3 recorded for DM, CP and Lignin. Error values between 2.6 and 3 were achieved for CF, NDF and ADF, probably due to the broader range of variation in the primary analysis. In calibration, EE obtained a R^2 of 0.76 that need of further evaluation, while Ash showed R^2 values lower (>0.6), probably due to the absence of absorption in the near infrared region for the minerals. The cross-validation models achieved both lower coefficients of determination and higher RMSECV than the previous models; however error differences between calibration and validation steps were always smaller than the limit of 20% proposed by Moya (1993).

Hence, the FT- NIRS applied on freeze dried samples seems able to estimate the principal parameters of chemical components on samples of natural pasture herbages, characterized by wide variability. Further researches should aim to evaluate additional parameters and they can improve the accuracy of FT- NIRS on chemical constituents.

O108

Palatability of extruded dog diets supplemented with *Ascophyllum nodosum*

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Ascophyllum nodosum (AN) is a brown seaweed rich in minerals, vitamins and bioactive compounds, with prebiotic, antimicrobial and antioxidant properties. Although AN has been used in different pet animal feeds, studies aimed at evaluating the influence of this alga on foodstuff palatability are lacking.

A group of 28 medium-small sized naïve dogs was used. In all trials, the dogs were fed complete dry diets (21% crude protein) subjected to evaluation according to the "two pan-test". Animals were offered an amount of food based on their metabolic weight: two diets were offered in two separate bowls for five consecutive days, with the bowl position reversed daily to prevent 'left-right' bias. Food intake within the first 5 minutes and "first choice" eating behaviour were recorded.

An "obvious" palatability test with two dry foods, markedly different for composition and digestibility ("Premium" and "Economy"), was preliminary preformed. Palatability of AN was then evaluated in separate tests to make three pair-wise comparisons between a control extruded food (CTR) and two experimental foods, which were obtained by adding powdered AN at low (0.3%, AN-low) or high (1.0%, AN-high) concentration to the CTR food: 1) CTR *vs* AN-low; 2) CTR *vs* AN-high; 3) AN-low *vs* AN-high.

The "obvious" test confirmed that the food preference was driven by objective criteria: both first choice value and dry matter intake of the Premium food were higher ($p<.001$) than those observed for the Economy one (26.24 *vs* 2.38 g/kg BW^{75}, respectively).

Significant differences in feed intake were only recorded when the CTR and the AN-high foods were offered together (19.20 *vs* 7.22 g/kg BW^{75}, respectively; $p<.001$). In the remaining palatability tests, no differences were observed. As for the first choice behaviour, no differences were found in any of the three palatability test performed.

The results lead to the conclusion that, at the experimental conditions of the trial, AN shows an inhibiting and dose-depending effect on dogs' DM intake, which becomes significant only at the highest supplementation level (CTR *vs* AN-high). In addition, data obtained from the first choice behaviour evaluation induce to believe that the negative effects exerted by AN on palatability are predominantly related to gustatory factors rather than to olfactory stimuli.

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O109

A preliminary nutritional assessment of six genotypes of tef (*Eragrostis tef*) as potential forage sources

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Global climate changes are pushing the search for valuable, alternative crops highly resistant to drought and other stress conditions. In this context, some promising forage sources can be found within plant species native to the tropical areas. Tef (*Eragrostis tef* [Zucc.], *Poaceae*) is a warm-season C4 tropical grass, traditionally grown for grain in Ethiopia and is adapted to a wide range of climatic and geographic regions. The aim of the present study was to assess and compare the chemical composition and nutritive value for ruminants of six genotypes of tef, grown as warm-season fodder crops under the Central Italy climatic conditions. On the 1st of June 2016, six tef genotypes (namely tef06-08, tef11 and tef13-14) were sown at a density of about 420 plants m^{-2} in a meso-Mediterranean climatic area (Viterbo, Lazio, Italy) with a total water input of about 3,500 m^{-3} ha^{-1}, over the natural rainfall regime. Each genotype was seeded on 10 × 1.5 triplicate plots to be cut at two pre-heading stages (ct1, ct2) and at heading (ct3), starting from 21st of July till the 3rd of August. Proximate analysis was carried out on a total of 72 ground (1 mm) dry sub-samples. The CP contents of tef11 and tef14 (15.5 ± 0.55 and 15.1 ± 0.48% DM, respectively) were higher ($p<.01$) than those of the tef08 and tef13 genotypes (13.7 ± 0.32 and 13.3 ± 0.43% DM, respectively). The CP content was also affected by the cutting date and was higher ($p<.01$) at ct2, but not for all the tested genotypes due to a significant ($p<.05$) genotype × cutting date interaction. The NDF content (68.4% DM, overall mean) was not clearly affected by the genotype or cutting date. On the contrary, ADF was the lowest ($p<.01$) in ct1 plants (32.3 ± 0.63% DM) especially for the tef07-08 and tef13-14 genotypes. In comparison to ct1 and ct2 plants, lignin was higher ($p<.01$) at heading (ct3, 8.8 ± 0.5% DM). Cutting date also affected the ash and fat content for which the highest values were recorded in the ct2 (ash, 10.2 ± 0.2% DM) and ct3 (fat, 1.9 ± 0.1% DM) samples. The findings of this preliminary assessment suggest that tef can be regarded as a promising forage species also in meso-Mediterranean environments. Both the genotype selection and the agronomic practices (*i.e.*, time of harvest) may play a role in ensuring to achieve the best productive and nutritional performance of this grass species. The nutritive value for ruminants of the crude proteins of tef, deserves further investigations.

O110

Evaluation of polyphenolic content and antioxidant activity in agri-food wastes and by-products using chemical and physiological extraction

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EU-28 produces about 100 Mtonnes/year of food waste, of which 40% is generated from agri-food industries. Agri-food waste (AFW) and by-products may contain components that could be valorised for their bioactivity. However, the bioaccessibility of these products is highly variable and dependent on a wide range of factors, including food matrix characteristics. Thus, the aim of this study was to determine the total phenolic content and the antioxidant capacity of some by-products and AFW using two different extraction methods. Twelve samples of by products (grape marc, *Camelina sativa* cake, olive pomace and whey) and 12 samples of AFW (fruits and vegetables waste FVW, citrus pulp, strawberry and orange dried) were processed using: methanol extraction (chemical procedure) and *in vitro* physiological extraction simulating pig gastro-intestinal tract adapted from Regmi et al., 2009. Soy and wheat were included as controls. Afterwards, the polyphenolic content was assessed by Folin–Ciocalteu assay while antioxidant capacity was determined by 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) assay. Results obtained showed that the chemical extracts of by-products and AFW contain different amount of polyphenols; in particular, as expected, the grape marc showed the highest significant (4.5% w/w; $p<.05$) polyphenolic content compared to all samples considered. Of note, *Camilina sativa* cake, olive pomace, FVW, orange and strawberry dried showed a polyphenolic content of 1.3, 0.7, 1.3, 1.6 and 1.3% w/w, respectively. The antioxidant capacity of grape marc exhibited a higher ($p<.05$) value of 573.6 μmol Trolox equivalent (TE)/g compared to the other samples considered. *Camilina sativa* cake, olive pomace, FVW, orange and strawberry dried showed an antioxidant capacity of 29.2, 11.0, 28.4, 16.3 and 31.0μmol TE/g. The physiological extraction of grape marcs, *Camilina sativa* cake, olive pomace, FVW, orange, strawberry dried yielded a polyphenolic content of 3.6, 1.8, 3.2, 3.6 and 2.3% w/w, respectively. The antioxidant capacity showed a similar trend to polyphenolic content after physiological extraction. The results obtained in this study indicate that AFW and by-products could be considered a promising bioaccessible source of antioxidants and phenolic compounds with industrial applications for the production of ingredients to functionalize feedstuffs for monogastric animal.

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O183

The effect of duration of storage and storage method on chemical composition and *in vitro* digestibility of complete calf pellet

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An experiment was conducted to determine the effect of duration of storage and method of storage on the quality of complete calf starter pellet. Pelleting was done in order to facilitate the handling, storage, transport, and to improve the efficiency and palatability of feed. The calf starter ingredients were yellow corn (50%), soybean meal (24.5%), ricebran (25%), and premix (0.5%). The calf starter (65%) was them mixed with chopped corn straw (35%), and pelleted using expired milk powder as pellet binder to form a complete calf starter diet. Samples of the complete diet were stored in sealed polyethylene plastic bags, fibre sacks or plastic silos, and kept for eiher 0, 2 or 4 months before they were opened and analysed for proximate chemical composition, dry matter (DM), organic matter (OM), crude protein, ether extract, crude fibre and in vitro digestibility. The data were analyzed using a two-way ANOVA based on a 3 × 3 factorial design. Statistical analysis of the data showed significant differences only for duration of storage effect ($p < .05$). The DM of complete calf pellet on 0, 2, 4 months of storage were 87.94%, 89.20%, 89.36%. The DM and OM digestibilities of complete calf pellet at 0, 2, 4 months of storage were 68.50%, 66.31%, 66.94% and 69.09%, 66.67%, 66.39%, respectively. The proximate composition, DM and OM digestibility were not significantly different ($p > .05$) on main effect of storage method . It can be concluded that the chemical composition of complete calf pellet increased from 0 to 2 months of storage, while the digestibility of complete calf pellet decreased. The methods of storage (polyethylene plastic bag, fibre sacks, and plastic silo) had no effect on the quality of the complete calf pellet for at least 4 months storage.

O111

Effects of rumen fluid pH on rumen activity and quality and technological properties of milk

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Excess fermentation and accumulation of short chain fatty acids (SCFA) in the rumen is a frequent result of feeding dairy cows with high-grain diets. This allows to sustain increasing milk yield but has risen the risk of rumen acidosis. This study investigated the effects of rumen pH on milk composition traits, coagulation properties, cheese yield, and rumination activity (RA) of dairy cows. Rumen fluid samples were taken through rumenocentesis from 100 Holstein Friesian cows (5 to 75 days in milk - DIM) without clinical signs of disease kept in 2 herds, to determine pH and concentration of SCFA. Within the day of rumenocentesis individual milk samples were also taken from each cow. Further, RA was monitored using a microphone-based monitoring system (Ruminact, Milkline) fitted on cows, to record the time spent ruminating from 2 days before to 2 days after rumenocentesis. Data were analyzed using a linear mixed model including the fixed effects of parity and DIM class, the quartile of rumen fluid pH (QpH) and the random effect of herd-test date and also of cow when repeated observations per cow were analyzed. Rumen pH (5.98 ± 0.37) averaged 5.52 to 6.46 in the first to fourth quartile, respectively, with 32% of cows with rumen pH < 5.8. The concentration (mmol/l) of total ruminal SCFA and the proportion (%) of propionic acid on rumen fluid linearly increased ($p < .05$) as rumen pH declined, whereas that of acetic acid linearly decreased. Whereas the average time spent ruminating during the day was similar in cows of different QpH (on average 26%, but with a clear circadian variation), the circadian rumination pattern was influenced by rumen fluid pH, and the RA linearly increased ($p < .05$) in the daily interval 06:00 to 12:00 as rumen pH declined. Cows of different QpH did not differentiate for body condition score and test day milk yield (36.6 ± 8.0 kg/d). Likewise, QpH did not affect ($p < 0.05$) milk pH, somatic cell count (SCC), fat, protein, lactose and casein milk content.

Cows of different QpH provided milk with similar coagulation properties (rennet coagulation time, curd firming rate and curd firmness after 30 minutes) and cheese yield. Conversely, curd firmness and cheese yield linearly decreased as SCC content of milk ($\leq$100; 100 to 400; >400 x 10^3 cells/ml) increased. In conclusion, QpH altered SCFA content and proportion in rumen fluid and rumen activity, but not milk quality traits, even in cows with rumen pH <5.8.

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O112

Effect of the wooden vats on traditional cheese characteristics

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Wooden vats have been used for centuries to collect and transform milk by farmers and cheesemakers all over the world. Nowadays, the tree species mostly used to this purpose are Douglas fir and chestnut. The use of wooden vats is mandatory for the production of all PDO Sicilian cheeses, such as Ragusano, Pecorino Siciliano, Piacentinu Ennese and Vastedda della valle del Belìce. In the last years, the wooden vats have been deeply explored for the microbiological characteristics and they are covered by biofilms mainly represented by lactic acid bacteria (LAB) entrapped in a polysaccharide matrix. These microbial associations do not include pathogenic species. Up to date, there are no studies performed on the effect of wooden vats made with different tree species on the characteristics of the final cheeses. Thus, this aspect represents the main aim of the present study.

Eight wooden vats (20-L volume) were made with eight tree species (Calabrian Chestnut, Sicilian Chestnut, Cedar, Cherry, Ash, Wot, Pine and Poplar) and subjected to 15-d whey treatment for the activation of the LAB biofilms on the internal surfaces. All 8 wooden vats were then used to produce PDO Vastedda della valle del Belìce cheese, according to the EU Regulation. Each cheese making (carried out in duplicate at 7-d interval) was obtained from 14 l of raw ewes' milk. The cheeses were packaged under vacuum and, after 14 days of refrigerated storage, analysed for their physical (pH, a_w, CIELab parameters) and chemical composition (DM, fat, protein, N soluble, ash, salt) according to official methods. Moreover, peroxides, TBARs and polyphenols were also determined. Vastedda cheeses were subject to a sensory evaluation following the ISO indications. Several ANOVA models were employed to statistical analyses.

The results of the characterization of the cheeses revealed that the wooden vats did not influence their chemical composition, while Lightness (L^*) and hue angle were significantly modified by the tree species (table 1). In particular, the cheeses produced in vats made with Calabrian and Sicilian chestnut were characterized by a lower yellow intensity than the other tree species. Poplar vat produced Vastedda cheeses with significant lower peroxidase oxidation (1.68 mq/kg) and TBARs (0.0042 mg MDA/100 g of fat), probably due to the effect of natural antioxidant transferred from the wood to the milk. No particular differences among cheeses were appreciated by the panellists.

O113

Vitamin D content in donkey milk: preliminary results

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Vitamin D is an essential nutrient that plays a crucial role in the calcium homeostasis and bone metabolism and also acts as an hormone. Humans synthetize most of their vitamin D requirement from sunlight. However, an oral intake of vitamin D may be an important source in winter, when the UV-B-related synthesis is limited and for people who may not be exposed to sunlight. However, only a few foodstuffs, mainly of animal origin, provide a considerable source of vitamin D thus limiting the possibility of fulfilling the requirements of this vitamin. Milk contains above all vitamin D_3 and D_2. Although there are several studies on the content of vitamin D in bovine milk, there is little information regarding donkey milk. Therefore, the aims of this paper is to carry out a preliminary evaluation of the vitamin D content in donkey milk and to investigate its seasonal modifications. Raw bulk milk samples were collected for 10 months, every 15 days, from a dairy farm that produces donkey milk for human consumption. All the samples were analysed for chemical composition and vitamin D content by HPLC after extraction by the partially modified Silva et al (1992) method. Donkey milk showed a higher vitamin D content (2.31 μg/100 ml ± 0.83) compared to bovine and human milk. Vitamin D in donkey milk showed seasonal variations as reported in cows. In particular, vitamin D had a higher concentration in the summer than in winter, probably due to the different sun exposure of the animals. In conclusion, donkey milk shows a considerable vitamin D content, which could be useful to meet the deficiencies of this vitamin in humans. Further investigations are necessary to improve the vitamin D content in donkey milk through increasing its endogenous synthesis and transfer in milk and to clarify other variability factors.

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O114

Factors affecting the detailed mineral profile of bovine milk from multi-breed herds

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The aim of this study was to assess the independent variation of breed and herd in the content of 15 minerals in individual milk samples collected from cows raised in multi-breed herds. The 27 herds were classified into two categories according to average energy corrected milk productivity. Milk samples were collected from 238 cows of 6 breeds: 3 specialized dairy (Holstein Friesian, Brown Swiss, and Jersey) and 3 dual-purpose (Simmental, Rendena, and Alpine Grey), and were analyzed for macro- (Ca, P, Na, K, Mg, S), essential micro- (Cu, Fe, Mn, Se, Zn) and environmental micro- (B, Si, Sn, Sr) elements using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Data were analyzed using a linear mixed model that included fixed effects of DIM, parity, breed and herd productivity, and a random effect of herd-date within productivity level. An extended model was obtained from the previous one including linear covariate of milk yield, fat%, protein%, and SCS, to distinguish the direct effect of breed on minerals from the indirect effect due to breed differences in terms of milk yield and quality. Results showed that the effect of herd-date varied across minerals, but was large especially for environmental minerals (47% to 91% of total variance), while for macro- and essential micro-minerals the effect ranged from 11% to 61%. Milk samples collected from farms with high levels of productivity had a richer mineral profile than samples from low-productivity herds. Parity only influenced macro-minerals, with the exception of Ca and S, while DIM influenced almost all minerals, with a few exceptions among the environmental elements. Large differences were observed among breeds, both between the specialized and dual-purpose breeds, and within the two groups, and these differences remained even after adjusting for milk yield and quality. Milk samples from the Jersey and Brown Swiss cows had better mineral profiles than milk from Holstein Friesian cows; the other breeds of Alpine origin produced milk of intermediate quality. Our findings suggest that breed has a stronger effect on macro- and some of the essential micro-minerals than herd productivity, parity and DIM. Moreover, the variance of individual cows was greater than the variance of herds. We conclude that improvements in the macro- and micro-mineral contents in milk depend more on genetics (breed) rather than on environmental and management factors (productivity, herd, mineral supplementation).

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O115

Influence of dairy farming system and animal factors on sensory descriptors of model cheeses from individual cows

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The aim of this study was to investigate the effect of several sources of variation related to dairy farming system and cow characteristics on sensory profile of ripened model cheeses. Individual raw full-fat milk samples (1500 mL) of 1224 Brown Swiss cows were collected from 83 herds belonging to 4 dairy systems (ranging from traditional to modern ones, with different feeding and management systems) located in Trento Province (Italy). Milk samples were processed according to a lab cheese-making procedure to obtain model cheeses. Trained panelists assessed the sensory profile of all model

cheeses using a protocol scorecard composed by 7 main sensory descriptors related to smell and flavor intensity, taste (salt and sour), and texture (elasticity, firmness and moisture), and 40 sensory attributes describing in detail smell and flavor profile. Sensory traits were analyzed through mixed models which included the following fixed effects: dairy system, days in milk (DIM), parity, order of cheese presentation in the sensory session, the linear covariate of milk fat:protein ratio, the liner and quadratic covariates of cheese weight. Random effects included in the models were herd (within dairy system), animal (within DIM, parity and order of cheese presentation) and the effect of panelist. Overall, the effect of dairy system was negligible in explaining the variation of most of the traits, while the herd effect was much more relevant for many cheese sensory descriptors. Among the random sources of variation included in the statistical model, the effect of panelist explained a large part of the variation of many sensory traits. With respect to the cow characteristics, stage of lactation showed a significant effect for smell and flavor intensity as well as for salt sensory descriptor, while the effect of parity was almost trivial. Cheese smell intensity (and some related attributes) showed a quadratic trend over lactation with lower values in the middle, while flavor and salt descriptors resulted higher at the end. In conclusion, results confirmed that the use of lab standard cheese-making procedures to produce model cheeses from individual milk samples provided new knowledge on the influence of herd practices and of cow characteristics on the milk-to-cheese pathway.

O116

Discrimination of dairy systems using the volatile fingerprinting of ripened cheese detected by a fast and non-invasive spectrometric technique (PTR-ToF-MS)

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The volatile profile of ripened cheeses measured by proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS) was investigated to trace dairy systems (DSs). Moreover, the spectrometric peaks potentially useful for the DSs discrimination were tentatively identified. A total of 1075 individual model cheeses obtained using raw whole milk of individual Brown Swiss cows reared in 72 herds were analysed. Cows and herds were assigned to different groups according to: 3 -traditional (TR), modern (MO) with and no silage- and 5 -TR with and no automatic feeder, MO hay, MO total mixed ration (TMR) with and no silage- DSs using a linear discriminant analysis (LDA). Each group of cows and herds was divided randomly into 2 subsets: a calibration (Cow-Cal, Herd-Cal) set composed by 75% of the data, and a validation (Cow-Val, Herd-Val) set. The LDA correctly classified the 82.9% and 70.7% of the cows of the Cow-Cal and Cow-Val datasets, respectively when 3 DSs were assigned. The LDA successfully discriminated the 94.2% of the Herd-Cal dataset, while the discrimination was about 50.0% by the Herd-Val. The 75.0% and 52.1% of the cows were correctly classified in 5 DSs using Cow-Cal and Cow-Val datasets, respectively. The same differences was observed for the herds' dataset. A good discrimination was obtained when the linear functions obtained on Cow-Cal dataset were applied to Herd-Val dataset with a correct classification of 90.0% and 95.0% of the herds according to 3 and 5 DSs, respectively. Among the peaks that characterized the volatile profile of TR DSs (tied cows fed hay and some compound feed) we found m/z 61.028 (acetic acid), m/z 109.070 (pyrazine), and m/z 137.132 (terpene). The most important peaks related to MO DSs (loose cows, milking parlour, more concentrates) were m/z 71.086 (3-methyl-butan-1-ol, 3-methyl-3-buten-1-ol), m/z 101.097 (hexanal), m/z 123.117 (nonenal), and m/z 129.127 (octan-1-one). Within the MO DSs the main differences were attributable to the use or not of TMR and corn silage. Particularly, we found a positive association (higher loading) between m/z 77.060 (propanediol) and MO DSs without TMR. Similarly, the peak at m/z 101.060 (2,3-pentanediene) separates the MO DSs with TMR using corn silage from the other DSs. In conclusion, the volatile fingerprinting of ripened cheeses detected by a fast and non-invasive spectrometric technique could be used as potential method for discriminating different DSs and feeding strategy.

O117

Life cycle assessment of Italian Mozzarella cheese manufacturing

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Mozzarella is globally consumed, representing a strategic product in the future of dairy sector; Italy with 390 plants and 4300 operators produces 253,830 tonnes of cow's milk Mozzarella (66% of Bocconcino Mozzarella (BM) and 34% of

Pizza Mozzarella (PM)). BM is mainly produced using raw milk directly worked in Mozzarella plant, while PM is mainly produced using curd, coming from other dairy plants (mainly foreign plants). Mozzarella manufacturing requires several processes and resources, determining environmental impacts. Life Cycle Assessment (LCA) represents a methodology to estimate the environmental impacts of production process.

The environmental impacts of Mozzarella manufacturing were evaluated using a farm gate-to-plant gate LCA. System boundaries encompass milk transport from farm, Mozzarella-making and packaging. The third largest Mozzarella Italian plant was surveyed. Data collection regarded inputs (milk, energy, packaging, other materials, chemicals, water, land) and outputs (Mozzarella, co-products, waste). Ecoinvent® v3.2 database was used for background data. SimaPro© 8.1 was the modelling software. Environmental impacts on human health, ecosystem and resource use were assessed per kg of Mozzarella. Plant-specific information were used to attribute the amount of resources between Mozzarella and coproducts (whey and cream), otherwise the allocation approach was based on milk solids content of each product.

Most of the impacts (i.e. climate change, acidification, eutrophication) arose by milk transport, energy usage and packaging (cardboard boxes and plastic film). Pasteurization, Mozzarella-making and storage were the operations which required the largest amount of energy. Refrigerant gases caused ozone depletion, while process water and wastewater were main drivers of water depletion. Impact reduction can be achieved through implementation of energy usage efficiency (renewable energy input, energy control, heating/energy recovery), reduction of packaging and usage of more eco-sustainable packaging. Transport emissions can be reduced using control emission and a better logistic.

This analysis evidenced larger impacts for PM than BM; in fact PM production requires extra plant processes (curd production), and larger transport (transport of milk to curd plant and curd to PM plant). BM produced using Italian raw milk is preferable than producing PM using foreign curd, in order to have a better sustainable Italian Mozzarella-manufacturing.

O118

Cholesterol content and fatty acid composition of phospholipids and triglycerides are affected by the histological characteristics of *semimembranosus* and *triceps brachii* muscle fibre of Maremmana and Limousine bovine breeds

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Bovine breeds are characterised by a specific phenotypic conformation, which is largely due to the effects of extensive genetic improvement over the last 50 years. Genetic improvements have affected not only the animal productivity but also the meat quality. In fact, by selecting for the muscle growth of animals, a particular kind of muscle fibre has been selected, which, in turn, affect both the physical and nutritional quality of meat.

The aim of the present study was to investigate the histological characteristics of *semimembranosus* and *triceps brachii* muscle in two different bovine breeds: Maremmana (Ma) and Limousine (Lm). The former is an autochthonous beef cattle breed reared in Maremma, a rural area in the south of Tuscany (Italy), whereas the latter is a well-known beef cattle breed, genetically improved.

The animals were grazed in two adjoining pastures and received the same feed integration. The experimental period lasted nearly 12 months, from weaning (six months old and 210 ± 10 kg in weight) to slaughtering (about 19 months old). Muscle samples were collected at the animal slaughtering (Ma 18.9 ± 0.9 months, Lm 18.5 ± 1.0 months), before carcass cooling. Regarding the histological properties, the number of fibres (TNF), mean sarcolemma perimeter (MSP), cross section area (CSA), and total sarcolemma perimeter (TSP) were determined. Samples were also analysed for proximate composition, fatty acid composition of total lipid, phospholipids and triglycerides and for total cholesterol content.

Muscle fibres from Lm were smaller and more numerous than that from Ma. In fact MSP was 171.5 μm in Lm and 259.7 μm in Ma ($p<.05$) and CSA was 2356 μm^2 in Lm and 5472 μm^2 in Ma ($p<.01$). Consequently, TSP was higher in Limousine than in Maremmana (14.2 *vs* 12.2 mm, $p<.05$, respectively). This pattern dramatically affected the fatty acid composition of total lipids: Intramuscular fat from Lm was higher in PUFA (10.4 *vs* 5.8 g/100g of total lipids, $p<.01$) while that from Ma was higher in MUFA (34.8 *vs* 25.5 g/100g of total lipids, $p<.01$). Cholesterol resulted higher in intramuscular fat from Lm (3.0 *vs* 2.1 g/100 of total lipids). The results of this experiment put in evidence that changes in fibre traits resulted in significant changes in lipid composition of intramuscular fat.

Acknowledgements

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O119

Consumer acceptance of ewe meat deriving from traditional and halal Tuscan market

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Usually Tuscan people do not eat ewe meat and this product is appreciated only in some areas of the region. New sheep meat market, which consists of immigrated populations, requires this product. The ewe meat available on the market derives from at the end of career sheep or discarded reproducers.

Aim of the work was to appraise consumer's perception of ewe meat deriving from traditional and Halal Tuscan market using affective consumption test. One hundred thirty-three usual meat consumers (60% men, 40% women), but not usual ewe consumers, participated to the consumer test. The first question aimed at evaluating the "expectation" for the ewe meat. At the end of the test, consumers expressed the overall liking. The most represented age class was that ranging from 45 years old to 64 years old; 48% of consumers were graduated. Two identical salt and pepper steaks, one from the traditional market and one from the Halal market, identified with the letters A and B, respectively, were simultaneously tasted by the consumers. For every steak, the consumers were asked to express a vote for taste, odour, tenderness, and overall liking. The answers had a grading scale from 1 to 9 (1 = extremely negative; 9 = extremely positive).

Results were submitted to analysis of variance, with ewe meat market (traditional and Halal) and consumer test location (catering, conventional restaurant, ewe meat specialized restaurant) included as fixed factors in the model.

In comparison with men, women had lower expectations (6.8 *vs* 7.3; $p<.002$) but, at the end of the test, both sexes appreciated the ewe meat in a similar manner (average score: 7.5). As for the four considered parameters, men and women valuated in the same way the product. Consumers did not found differences between the traditional market meat and the Halal market meat. Considering the consumer test location, taste resulted better in both restaurants, while a higher score for tenderness was given in the conventional restaurant. The interaction between ewe market and consumer test location was significant in all considered parameters. The Halal steak cooked in both restaurants was the most appreciated. Excluding catering, the halal meat was always more tender than the traditional market meat. The ewe meat of the test was generally appreciated, reaching a mean value close to 7 for the overall liking.

In conclusion the ewe meat, even if not often appreciated in Tuscany, was estimated by the consumers both as traditional and as Halal meat. Considering these results, it is suggested that the Tuscan market may be a good opportunity for the ewe meat.

Acknowledgements

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O120

Geese reared in organic vineyard: qualitative traits of breast meat

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Agroforestry, by mean of suitable crops/animals interactions, could provide several benefits (economical, environmental, biodiversity, etc.) allowing the maintenance of agricultural landscape which is also useful for tourism activities. Considering the wine vocation of Italian territory, breeding geese in the vineyard could be considered a suitable agroforestry model. Thus, the aim of this study was to evaluate the quality and the oxidative status of geese meat reared in vineyard. One hundred and twenty geese were organically reared in 1 ha of vineyard (Experimental Group) in an organic wine farm (Perugia, Italy), whereas others 120 were intensively reared (Control Group) in the experimental farm of Perugia University. Birds were raised until the slaughter age (180 d) and, after slaughter, the *pectoralis major* muscle was dissected for analyses. The chemical composition, fatty acid profile and lipid oxidation of breast meat (TBARS, α- and ɣ-tocopherol, retinol) were determined. Data were analyzed by PROC ANOVA with rearing system as fixed effects. The breeding system significantly affected some traits of the breast meat; in particular the vineyard-reared geese showed a lower lipid amount (2.23% vs 4.32% respectively) and a higher content of antioxidants. Vineyard geese had also higher *n-3* long chain polyunsaturated fatty acid content (3.60 *vs* 2.14%), but unexpectedly, also a higher level of TBARS compared to the control one (0.18 *vs* 0.10 μg MDA/g). Furthermore, the geese reared in the vineyard showed meat darker and less tender. Such trend could be due to higher kinetic activity of such geese and also to the pro-oxidant activity of copper. Indeed, in organic vineyard copper treatments are allowed. Accordingly, the copper content of the geese liver was higher in geese reared in vineyard, although within the limits of EU regulations. Further researches are necessary to understand better the effect of this rearing system on *in vivo* oxidative traits and on other aspects of the production (economic and environmental impact, etc.).

O121

Enhanced antioxidant capacity and reduced lipid oxidation in cooked rabbit burgers added with *Zingiber officinale*

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Nowadays ready-to-cook products represent an important food productions in terms of market share. Rabbit meat could be a suitable matrix to obtain meat products with nutraceutical purpose for its high content of protein and amino acids of important biological values, and for its low fat content and the high percentage of unsaturated fatty acids. Natural antioxidant additives are extensively evaluated as alternatives to synthetic antioxidants due to their great acceptance by the consumers. *Zingiber officinale* Roscoe (ginger) is widely used as a spice and contains several compounds with biological activities such as gingerol, paradol and shogaols. In order to evaluate the effects of ginger powder on lipid oxidation (TBARS – thiobarbituric acid reactive substances) and fatty acids profile (SFA, MUFA, PUFA, PUFAω3 and PUFAω6) three formulations of rabbit burgers were compared (C, control; G1, ginger 1%; G2, ginger 2%). Burgers (100 grams) were cooked in a preheated oven at 163 °C to an internal temperature of 71 °C. Results of TBARS showed that burgers added with ginger were less susceptible to oxidation than control burger (0.19, 0.09 and 0.13 mg malondialdehyde/kg respectively for C, G1 and G2; $p<.05$). FA profile of ginger (high in unsaturated FA, mostly C18:2ω6) modified burgers' FA profile. PUFA and PUFAω6 were significant higher in burgers added with ginger than the C ones (PUFA: 27.46, 32.57 and 33.08, $p<.05$; PUFAω6: 23.11, 21.18 and 28.06, $p<.01$; respectively for C, G1 and G2). The low level of SFA in ginger powder affected the percentages of saturated FA of burgers; C burgers showed higher level of SFA than G1 and G2 (45.28, 40.62 and 41.25, respectively; $p<.05$). The addition of ginger to rabbit burgers decreased lipid oxidation and modified the FA profile increasing the PUFA content at the expense of the SFA content.

O122

Exploring consumer perception of entomophagy: data from an online survey

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The Food and Agriculture Organization of United Nations supports the production of edible insects as a promising and sustainable source of nutrients for the increasing demand of animal-derived products by the growing world population. Even if insects are part of the diet of more than two billion people worldwide, the practice of eating insects (entomophagy) raise challenging questions for the Western country where this practice is not a habit. Like any food, insects can be a vehicle of health hazards that must be identified and controlled for through appropriate procedures and consumers have to be aware of good handling procedures of these products (storage, cooking and eating).

This study investigates the worldwide consumer perception on including edible insects in the diet, through an online survey made of 31 closed questions, 1 open questions and free comments.

The multilingual survey (Italian, English and Spanish version) involved individuals ($n = 299$) from different backgrounds (29 countries, EU and non-EU) and different eating habits (about 8% were vegetarians), with different degrees of education and age (63.5% was between 20 and 30 years old).

The analysis showed that the majority of participants was already aware of the term *entomophagy* and 17% of participants have already eaten insects. Moreover, about half of the participants would be willing to pay the equivalent of a hamburger to buy 10 g of insects and 60% will consider eating insects in case of necessity.

On the contrary, 7% of participants would refuse to eat insects even in extreme conditions (hunger, natural disasters, wars) and the factors limiting entomophagy are mainly represented by fear of allergic reactions and microbiological hazards. Furthermore, most people would consider to find selective shelves in stores for insect-based products. In addition to this, most of the participants accept pet or farm animals to be fed by insect-based products. Finally, almost the totality of participants considered food safety of edible insects as a responsibility of the national competent authorities, as required for other foodstuff.

In conclusion, in an effort to explore the acceptability by consumers, the survey shows on one side, a predominant rejection of introducing entomophagy in Western diet and on the other side, a prevalent curiosity and awareness on environmental benefits of this source of nutrient and the potential use on animal feeding.

O123

Innovative protein sources in animal diets: bioconversion of vegetable waste with black soldier fly (*Hermetia illucens*)

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The aim of this study was to evaluate the effects of different vegetable substrates on growth and chemical composition of Black Soldier Fly larvae (BSFL). Two trials were performed using: 1) fruit (FRU) and vegetable waste (VEG) obtained from the large scale retail and 2) brewery (TRE) and grapes (VIN) by-products. The two trials were carried out in a climatic chamber with controlled temperature (T: 27 ± 0.5 °C) and relative humidity (RH: 70 ± 0.5%). Six replicates for each substrate were prepared containing one hundred 6-days old BSFL and 100 g of substrate. Every four days, 30 larvae were randomly sampled for three consecutive times from each container to observe the morphometric parameters. Larvae development (length and weight) was recorded. Data collection ended when the 30% of the larvae reached the prepupae stage. In order to obtain a sufficient quantity of larvae to perform chemical analyses at the end of the trial, 6 other replicates per substrate were prepared. The following parameters were analyzed: final larvae weight and length and chemical composition of the substrate and of the BSFL. Substrates had an influence of the growth performance and chemical composition. BSFL grown on TRE showed a faster growth and highest content in crude protein (CP: 52.6%) and those grown on VIN took longer time to reach 30% of pupae. BSFL grown on FRU reached the highest body weight (BW: 153 ± 39 mg) and showed the highest content of ether extract (EE: 40.70%). Considering results of this study TRE should be the most promising substrate in order to obtain BSFL to use in animal nutrition as feed rich in crude protein. The bioconversion with BSF larvae could be a new way to add value to wastes obtaining innovative raw material for feed and other purposes.

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O124

Fatty acids profile of *Tenebrio molitor* larvae grown in different oil-enriched substrates

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Several species of insects are currently considered a good and alternative source of proteins and lipids for animal and human. Producing edible insects on substrates containing bioactive compounds could improve insect growth and their nutritional characteristics. The main objective of this study was to evaluate the fatty acid (FA) composition in insect larvae fed different substrates, using *Tenebrio molitor* L. (Col. Tenebrionidae). A standard diet (51.8% wheat bran +40.7% soybean meal) was alternatively supplemented with the 7.5% of 3 different oils (sunflower, linseed or fish). The larvae were left to develop until the last molting (about 80 days from the beginning of the trial). Five replications of pooled samples for each dietary experimental group were carried out and a total of 20 FA were analyzed by gas chromatography (DL =0.6 μg/ml). Even the FA profile of dietary substrates was performed. Data were analyzed using GLM procedure of Stata software system. The results showed a direct correlation between substrate-enrichment and FA profile of the larvae. The dietary supplementation with sunflower oil determined a 1.3 fold increase of oleic acid (C18:1n-9; $p<.05$) with a contemporary reduction in linoleic acid (C18:2n-6, LA; 1.3 fold) content respect to the control. Linseed oil and fish oil supplementation determined a 12 fold and a 1.8 fold increase in α-linolenic acid (C18:3n-3, ALA; $p<.05$) content, respectively; conversely LA was reduced of 1.4 fold and 1.2 fold respect to the control ($p<.05$). Furthermore, the larvae supplemented with fish oil were characterised by the presence of eicosapentaenoic acid (C20:5n-3, EPA; 2.11 ± 0.53% total FA *vs* not detected) while docosahexaenoic (C22:6n-3, DHA) and arachidonic acid (C20:4n-6, AA), two long chain polyunsaturated fatty acids (LCP) of n-3 and n-6 series, respectively, resulting from the elongation and desaturation of ALA and LA were absent or in trace in all the samples. Such results suggest the deficiency of elongation/desaturation enzyme pattern in the *T. molitor*, considering that only when dietary LCP were administered they were found in the larvae. In conclusion, this preliminary study confirms that the lipid-enrichment of substrates could be an efficient way for improving the fatty acids profile of *T. molitor* and meanwhile opens new opportunities in the use of insects as LCP sources in animal nutrition. Therefore, the LCP metabolic pathway of *T. molitor* requires further investigation.

O125

The microbiota of fresh mealworms as revealed by PCR-DGGE

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The WHO reported that many human beings are actually suffering from some sort of malnutrition. In recent years, the idea of producing edible insects as food and feed has attracted the attention of media, researchers and food industry, due to their high nutritional value, fast reproduction, high feed conversion, limited rearing space, low emissions of greenhouse gases per kg of mass produced. Mealworm (*Tenebrio molitor* L.) larvae represent a very promising food source because they are very rich in protein and easy to rear. In the European Union there is still a lack of legislation regarding the production, processing and distribution of edible insects. Notwithstanding, a specific recommendation to include insects and their parts in novel foods is present in Regulation (EU) No 2015/2283. Actually, scientific studies on the microbiota in farmed insects are very scarce.

The aim of this investigation was to get an insight into the microbial population of laboratory-reared mealworms. To this end, three batches (100 g each) of fresh mealworm larvae, their feeding substrate (wheatmeal) and frass (excrement from what larvae had eaten mixed with substrate residues), were subjected to polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). The analysis of the DGGE profiles of microbial DNA extracted directly from the batches of fresh mealworms and their frass, showed the presence of *Enterobacteriaceae* (*Enterobacter* sp., *Escherichia* sp. *Klebsiella* sp.). *Lactococcus garviae* and *Enterococcus* species were also discovered. The presence of *Vibrio* sp. and *Spiroplasma velocicrescens* was also retrieved, the latter species with a sequence identity lower than 97%. The family *Enterobacteriaceae* dominated in frass where the genera *Xenorhabdus* sp., *Enterobacter* sp. and *Pantoea* sp. were found with sequence identities higher than 97%. *Vibrio* sp. together with *Brochothrix thermosphacta* and *Bacillus* sp., were also found. No closest relatives to bacterial species were discovered in wheatmeal used as insect feed. Although refereed to a limited number of samples, the high prevalence of *Enterobacteriaceae* suggests to deepen the safety issued posed by edible insects, as already highlighted by the European Food Safety Authority Scientific Committee Opinion on a risk profile related to production and consumption of insects as food and feed.

O126

Effects of black soldier fly (*Hermetia illucens* L.) larvae fat inclusion on growth performance, blood traits, carcass characteristics morphometric and histological parameters of finisher broiler chickens

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A trial was performed to evaluate the effects of a partial or a total replacement of soybean oil (SO) with *Hermetia illucens* larvae fat (HILF) in broilers chicken's finisher diets. At 21 days of age, a total of 120 male broiler chickens (Ross 308) were randomly allocated to three dietary treatments (5 replicates and 8 birds/pen). To a basal control diet (C), either the 50% or the 100% replacement of SO with HILF was applied (HI50 and HI100 group, respectively). Growth performance was evaluated throughout the trial. At day 48, 15 birds (3 birds/pen) per group were slaughtered at a commercial abattoir. Carcass yield and proportion of carcass cuts were recorded. Blood samples were taken from each slaughtered chicken for haematochemical indices. Morphometric analyses were performed on duodenum, jejunum and ileum. Samples of liver, spleen, thymus, bursa of Fabricius, kidney and heart were submitted to histological investigations. Data were analyzed by one-way ANOVA. Growth performance, carcass traits, blood parameters and gut morphometric indexes were not influenced by the dietary inclusion of HILF. HILF inclusion level did not significantly affect histopathological findings. Results suggest that the replacement of SO with HILF in broiler chickens diets have no adverse effects on growth performance and blood traits and do not significantly impair gut morphology and histological features.

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O127

Apparent nutrient digestibility of broiler quail diets supplemented with black soldier fly larvae reared on two different substrates

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In order to improve the sustainability of the livestock sector, the black soldier fly (BSF) is considered one of the most promising insect species to partly replace conventional feedstuffs. BSF requires lower land use than conventional crops and, in addition to its unquestioned nutrient content, it recycles by-products from food processing and organic waste generating BSF soil which is used as a soil enhancer. Recent trials on poultry species fed with BSF larvae meal (LM) showed encouraging results, even though the fatty acid profile of LM was rich in saturated and poor in n-3 fatty acids (FA), thus being sub-optimal in providing healthy meat for the modern consumer. Theoretically, being larvae monogastrics, they would deposit the FA that they are fed. To test this hypothesis, the present research tested the effect of a dietary inclusion with meal of larvae reared on two different substrates on the apparent nutrient digestibility of broiler quails. A total of 60 16-day-old quails were divided in three dietary groups: Control (commercial diet for growing quails, C), LM1 (C diet with 10% meal of larvae reared on 100% layer mash, low *n-3* FA) and LM2 (C diet with 10% meal of larvae reared

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on 50:50 layer mash and fish offal, high *n-3* FA). Each group (homogeneous for initial live weight and gender) had 5 replicates of 4 quails each. After 10 days of adaptation to the new diets, 4 days of excreta were collected. Feed and water intake were recorded daily. Excreta were weighed and packaged by cage, and stored at -40 °C for subsequent freeze-drying and the following chemical analysis: dry matter (DM), crude protein (CP), uric acid, ether extract (EE), gross energy, starch and chitin. Data were analysed by a one-way ANOVA with diet as fixed effect. The C quails produced more excreta than LM1 and LM2 birds ($p<.05$), and showed a lower apparent digestibility of DM ($p<.05$) and organic matter ($p<.05$) compared to LM2, with LM1 being intermediate. Starch was better digested in LM1 compared to C ($p<.01$), but did not differ from LM2, whereas C quails showed a higher EE apparent digestibility than LM2 ($p<.05$) but did not differ from LM1. Quails of group C displayed the lowest gross energy apparent digestibility ($p<.05$) with the same trend being observed for the metabolizable energy ($p<.001$). This study shows that a dietary inclusion of BSF larvae meal reared on a substrate rich in *n-3* FA did not negatively affect the nutrient apparent digestibility in broiler quails.

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O128

Hermetia illucens partially defatted meal in piglets' nutrition: preliminary results

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The aim of this trial was to investigate the effects of different inclusion level of *Hermetia illucens* (HI) defatted meal on the growth performance of piglets. Forty-eight newly weaned piglets were individually weighted (initial body weight: 6.09 ± 0.16 kg) and allocated in 12 different boxes to have a homogeneous initial live weight. Three different diets were formulated with increasing inclusion levels of HI (0%, 5% and 10%) in substitution of conventional protein sources. All diets were isonitrogenous and isoenergetic. Each diet was assigned to 4 replicates. Two phases feeding program (I from day 0 to day 23; II from day 24 to day 61) were studied. Piglets were individually weighted at the feed changing and at the end of the trial. Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) were calculated for each feeding phase and for the whole trial. The weight gain (WG) was calculated for the whole period. Data were analyzed by One-way ANOVA and differences of means by Duncan's test ($p\leq.05$).

No significant differences were observed on the individual final BW (kg) (31.88-HI0; 32.23-HI5; 33.06-HI10), on the WG (kg) (HI0: 25.78; HI5: 26.14; HI10: 26.96) and on the ADG (phase I, phase II and total). The ADG (kg) total ranged from 1.66 (HI10) to 1.72 (HI5). The FCR (phase I, phase II and total) and ADFI (phase I, phase II and total) showed no significant differences among the three treatments (FCR total: 1.86-HI0; 1.84-HI5; 1.92-HI10. ADFI total (kg): 3.14-HI0; 3.16-HI5; 3.17-HI10). These results are in line with other trial on piglets' nutrition. These results showed that the use of a partially defatted HI meal could replace the conventional protein sources up to 10% inclusion without adverse effect on growth performances.

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O129

Garlic as supplement for lactating cows: consequences on milk and cheese products

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It has been recently shown that garlic, or garlic components, used as feed additives could represent a good strategy to decrease the rumen CH_4 production of dairy cows. The present study assessed the effects of garlic as feed supplementation on dry matter intake (DMI), milk yield, feed conversion ratio (FCR), rumen fermentation parameters [(pH, volatile fatty acid profile (VFA), ammonia)], and milk and cheese organoleptic traits (smell and flavor intensity, saltiness, acidity and bitterness). The experiment was completed according to a 4 × 4 Latin Square with 4 Holstein-Friesian lactating cows fed in 4 consecutive periods with a total mixed ratio (TMR) not supplemented (CTR) or supplemented with garlic, as raw (G100: 100 g/d and G400: 400 g/d) or as allyl-sulfide, a pure derivative (SUL: 2 g/d). The cows were housed in single pens and fed using a TMR based on corn silage 29.1%, corn-barley concentrate 23.3%, sunflower-soy concentrate 17.3%, alfalfa hay 12.3%, grass hay 12%, sugar beet pulp 3.7%, other additives 2.3% (starch 25.3%, crude protein 13%, NDF 37.5%), on DM. Each experimental period lasted 7 d of transition and 14 d of treatment. At the 18th and 21th d of each period, rumen fluid and milk samples (10 L) were collected for chemical analysis and cheese-making. Raw milk and cheese (after 63 d of ripening) were evaluated for organoleptic properties by a trained sensory panel. The results showed that all the experimental treatments had no consequences on DMI, milk yield and FCR, urine pH, rumen fluid and feces, as well as the VFA production, and ammonia, compared to the CTR. The use of G100 and SUL did not affect the organoleptic properties of milk and cheese. In opposite the garlic treatment at the highest dosage (G400), had strong influence on the smell and flavor intensity of milk and cheese, as the saltiness, acidity and bitterness were higher compared to CTR. In conclusion, the use of garlic did not affect the production performances of lactating cows, but it influenced the organoleptic characteristics of both milk and cheese. In some market circumstances the use of garlic in the feeding of dairy cows can represent an opportunity.

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O130

Effect of cow feeding supplementation with olive pomace on the development of aromatic compounds in milk and dairy products

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Utilization of agro-industrial by-product in animal feeding represents an interesting and sustainable alternative to its disposal.

The aim of this study was to evaluate the effect of olive pomace (OP) as supplement feed in dairy cow on aromatic compounds in raw milk, spontaneous fermented milk and cheese. Twenty lactating cows were randomly divided in two homogenous groups and fed for 60 days with OP supplement (2 Kg DM/cow/die) in the experimental group (EG) and without in the control one (CG). Diets were isoenergetic and isoproteic.

Samples of bulk milk were collected at the end of the trial for both groups. Some of these were spontaneously fermented at 25 °C for 72h and thereafter analyzed. The rest of bulk milk was used to produce cheese that was sampled at 1, 7 and 30 days of ripening. A SPME-GC-MS analysis was performed to detect volatile compounds. Data were analyzed with GLM procedure and significance were set at $p<.05$.

In raw milk, besides free fatty acids, several secondary lipolysis catabolites (ethyl and methyl esters, lactones, aldehydes, methyl ketones) were detected and most of them (65%) were higher in EG ($p<.01$).

Higher lypolitic activity (48% of compounds) in fermented milk was recorded in EG confirming that already observed in raw milk ($p<.05$). Esters deriving from methionine catabolism were higher in EG fermented milk ($p<.05$). On the other hand, data generally showed an increase of first catabolites of branched amino acids in CG milk, while 45% of their related esters were most detected in the EG ($p<.05$).

Analysis of cheese highlighted differences ($p<.05$) between groups in some flavoring catabolites achieved at different times during ripening. Lypolitic catabolites were generally higher in the EG, especially at T7, with the exception for δ-nonalactone that was most observed in CG at every time ($p<.05$). First leucine catabolites (α-ketoisocaproic acid, α-hydroxyisocaproic derivative) were greater in EG cheese at every time ($p<.05$), while 3-methylbutanol were higher in EG at T7 ($p<.001$) and T30 ($p<.05$). Their related esters, isoamyl isopentanoate and isoamyl butyrate, increased in EG at T7 and T30 ($p<.01$) and at T30 ($p<.05$), respectively.

In conclusion, dietary supplement with olive pomace may affect lypolisis and proteolysis processes responsible of dairy products aroma. This pattern was already observed in raw milk and confirmed also in fermented milk and cheese, especially for compounds produced by esterase activity.

Acknowledgements

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O131

Milk fatty acid profile of Italian Simmental cows as affected by pasture type and supplement level

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The Alpine pastures are characterized by a spatial and temporal variability, and often they are not able to meet the nutritional requirements of lactating cows. The supplementation of the animals' diet with concentrate is a fairly widespread practice. Aim of the study was to assess the effect of pasture type and supplement level on fatty acid (FA) composition of milk. During summer grazing, 72 Italian Simmental cows were randomly assigned to 2 groups (36 animals each) balanced for milk yield and lactation stage in a 2×2 cross-over design. The groups received 3 (HS) or 1.5 (LS) kg/head/d of supplement. Cows grazed first a nutrient-rich pasture (RP, *Poion alpinae* alliance, 1500 m asl), and after a washout period of 10 d, they grazed a nutrient-poor pasture (PP, *Seslerion caeruleae* alliance, 1700 m asl). Pastures were grazed at the same phenological stage (flowering period of *Poaceae*) for 10 d. For 4 d at the end of the 2 grazing periods, the mixed milk of cows within group was sampled and analyzed for gross composition (FIL-IDF), and FA profile (GC/MS). Statistical analysis considered the fixed effect of supplement level, pasture type and group of cows. LS had greater fat (4.09 *vs* 3.92%, $p<.05$), odd-chain saturated FA (OCFA, 2.70 *vs* 2.55% of total identified FA, $p<.01$), branched-chain FA (BCFA, 3.95 *vs* 3.70%, $p<.01$), C18:1*t*11 (3.07 *vs* 2.96%, $p<.01$), conjugated linoleic acid (1.98 *vs* 1.79%, $p<.05$), C18:3n-3 (1.14 *vs* 1.03%, $p<.01$), polyunsaturated FA (PUFA, 6.59 *vs* 5.67%, $p<.01$), but lower level of C18:0 (10.66 *vs* 11.03%, $p<.01$), C18:1*c*9 (18.86 *vs* 19.72, $p<.01$) and monounsaturated FA (MUFA, 26.39 *vs* 27.64%, $p<.01$) than HS. Milk obtained from cows grazing on RP had lower fat (3.94 *vs* 4.07, $p<.05$), OCFA (2.50 *vs* 2.75, $p<.01$), and BCFA (3.64 *vs* 4.01, $p<.01$), but higher MUFA (27.17 *vs* 26.87, $p<.05$) level than PP. Phytanic SRR/RRR ratio was not affected neither by supplement level nor by pasture type (0.423 ± 0.017, $p>.05$). In conclusion, the supplement level showed a higher effect than pasture type on FA composition of milk of cows during summer grazing.

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O132

Fatty acids profile in early and commercial milk of dairy cows supplemented with flaxseed during the dry period

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The present study aimed to evaluate the effect of supplementing high yielding dairy cows with flacked flaxseed during the dry period on the fatty acids (FA) profile of early and commercial milk in the subsequent lactation. At the beginning of the dry period, 73 Holstein cows balanced for parity, days open, production level and with a close date of drying off were randomly assigned to a control (CTRL; $n=38$), or to an experimental flaxseed supplement dry-off diet (FLAX; $n=35$). The flaxseed used (200 g/head/day) contained about 44% of lipids on DM and 51.6% of n-3 FA on the total FA. During the dry period, cows were fed isoenergetic and isoproteic diets (12.3% CP, 48.8% NDF, and 0.78 milk feed units/kg on DM basis). After calving, the cows of both groups were fed ad libitum the same lactation diet containing 16.7% CP, 34.0% NDF, and 0.97 milk feed units/kg on DM. Early and commercial milk samples were collected at d 4, 15 and 30 after calving from the morning milking. Data were analysed using a hierarchical mixed model for repeated measures. As expected, sampling time affected significantly ($p<.001$) the early and commercial milk FA profile. In particular, SFA increased from d 4 to 15 to 30 after calving, MUFA decreased and PUFA resulted greatest at d 15 after calving (i.e., 62.8 *vs* 63.7 *vs* 65.7%; 32.9 *vs* 31.6 *vs* 29.8%; and 4.21 *vs* 4.63 *vs* 4.51% of total FA at d 4, 15 and 30, resp.). A significant ($p<.01$) enhancement of the total n-3 FA was observed in early and commercial milk of cows supplemented FLAX respect to CTRL. In particular, the content of alpha-linolenic acid and EPA resulted higher in FLAX than in CTRL group (0.35 *vs* 0.33%; 0.06 *vs* 0.05% of total FA; $p<.05$, resp.). No differences between the two diets were observed in total n-6 FA, although the diet affected significantly the content of dihommo-gamma-linolenic acid (i.e., C20:3n6; 0.11 vs 0.12% of total FA for FLAX and CTRL, resp., $p<.05$). The n6/n3 ratio resulted more favourable in early and commercial milk of FLAX cows than in CTRL (5.01 *vs* 5.35% of total FA resp., $p<.01$). In conclusion, flaxseed supplementation during dry period could have a positive effect on the FA profile of early and commercial milk collected within 30 days after calving.

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O134

Effects of glycerol esters (Butyrflex®) on productive performances and meat quality of Limousine young bulls

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One of the strategies to reduce antibiotics impact on farming is to use substances influencing the absorption of nutrients and/or strengthening the immune system. Glycerol esters supplementation showed positive effects on animal welfare and reduced antibiotic treatments in monogastrics. Because few information is available on ruminants, the aim of the present study was to analyse the effect of a feed additive containing glycerol esters on productive performances and meat quality in beef cattle. The study was carried out on one group ($n = 30$) of Limousine young bulls arrived from France: control group (C; $n = 15$) received no feed additive, and treated group (T; $n = 15$) received 40 g/d/head of Butyrflex®. Both groups were fed the same diet, based on corn silage (11.8 MJ/kg DM, 13% crude proteins and 36% starch on dry matter basis). Average body weight of the animals at the beginning of the study was 345 kg, and animals were slaughtered at commercial maturity. C group registered greater ($p<.0001$) weight (+47.2 kg) compared with T group at slaughter. The statistical model considered the glycerol esters supplementation as fixed effect and age at slaughtering as covariate and used PROC ANOVA of SAS Institute Inc. software. No significant differences on carcass weight (400 ± 33.6 kg), dressing percentage (63.3 ± 4.93%) and SEUROP value (E2) between groups were observed. These results were probably due to the different offal weight. Meat pH was greater in T group, both at slaughter (6.48 ± 0.10 *vs* 6.15 ± 0.09) ($p<.0001$) and after 48 h (5.86 ± 0.08 *vs* 5.63 ± 0.10) ($p<.02$), no significant differences were found after 4 and 6 days of ageing time. The cutting sample registered the same weight in both groups (6.06 kg) but the internal fat percentage was greater ($p<.04$) in C group (+1.18%). As regard to meat quality (drip and cooking loss; toughness and colour) T group showed lower ($p<.001$) a* index (10.27 ± 1.67 *vs* 13.14 ± 2.63), lower ($p<.003$) chroma (17.29 ± 1.33 *vs* 20.14 ± 3.14.) and greater ($p<.01$) hue angle (53.63 ± 4.35 *vs* 49.41 ± 3.85). The meat of T group appeared lighter than C group. In conclusion, the present study showed that feed additive containing glycerol esters influenced *in vivo*, slaughter performances and meat colour.

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O135

Dietary pomegranate pomace to improve milk fatty acid composition in grazing ewes

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Agro-industrial by-products may be rich in nutrients and bioactive compounds and could be usefully included in diets for ruminants to reduce feed cost and to improve product quality. In this sense, pomegranate pomace (PP) is of particular interest, as it is composed of pulp, seeds and peel which residue after the juice extraction and it is rich in bioactive polyphenols. Also, pomegranate seeds contain a peculiar fatty acid (punicic acid; *c-9 t*-11 *c*-13 C18:3) with beneficial effects on health.

The aim of this study was to evaluate the effect of dietary dried pomegranate pomace on the productive performance and the fatty acid (FA) composition of milk in grazing ewes. Twenty multiparous Comisana ewes at 140 ± 10 days in lactation equally divided into two groups, namely control (C) and pomegranate (P), balanced for live weight and milk yield, were gradually adapted to the experimental diet during 14 days. Over a 28-day experimental period, all the animals grazed on a sinantropic pasture for 6 h/d. In stall, each animal received an integration of alfalfa hay (500g) and, at milking, they also received barley (200 g) and one of two different pelletted concentrates (250 g): a conventional (C), or a concentrate containing 64% of dried PP.

Milk was collected weekly and all data were analysed with a repeated measures ANOVA to account for the effects of: diet, time of sampling and their interaction. Milk yield and composition (protein, fat, lactose and urea) were not affected by dietary treatment. Dietary PP significantly affected the FA composition of milk. In particular, percentage of *t*-9 18:1 (0.32 *vs* 0.25, $p<.001$), *t*-11 18:1 (1.45 *vs* 1.04, $p<.001$), *c-9 t*-11 18:2 (1.60 *vs* 0.69, $p<.001$), *c*-9 *c*-12 *c*-15 18:3 (1.60 *vs* 1.41, $p=.027$) and *c*-9 *c*-12 18:2 in tendency (2.70 *vs* 2.49 $p=.077$) were higher in the milk of P group in comparison with C ewes. All the mentioned FAs are involved in the ruminal metabolism of lipids, suggesting an effect of the bioactive compounds contained in PP on the FA biohydrogenation. In addition, *c-9 t*-11 *c*-13 C18:3, was detected only in the milk of P ewes, representing 0.19% of the total fatty acids.

In conclusion, the inclusion of PP in the diet of grazing ewes increased the proportion of healthy fatty acids arising from the biohydrogenation process with no negative effects on the milk yield and on the gross composition of milk.

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O136

Revealing avian oviduct cell proteome and secretome *in vitro* and its susceptibility to modification

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Avian oviduct tissue is a natural bioreactor which secretes large amount of proteins due to intensive production of eggs and as such is considered and important biotechnological tool to deliver human therapeutic proteins upon the genetic modification of oviduct cells. In order to characterize the *in vitro* model of oviduct cells, we investigated abundance of proteins in non-modified cultivated oviduct cells of hen and quail or in modified cells upon delivery of 2 exogenes to the cells: a reporter *GFP* and human interferon alpha 2a (*hIFNλ2a*) under ovalbumin promoter.

Quail (QOEC) and chicken oviduct epithelial cells (COEC) were isolated from laying quails ($n = 7$, age 12-20 wks) and hens ($n = 7$, age 30 wks). Non-liposomal X-tremeGENE 9 DNA Transfection Reagent (Roche) was used to introduce the plasmid pL-OG-OVAIFNEn-Egfp (IBA Warsaw) to the cells. Samples for proteomic analysis were prepared from pooled modified/non-modified QOEC and COEC, both from cells and culture medium. Peptides mixtures were analyzed by liquid chromatography coupled to tandem mass spectrometry and searched against *G. gallus* proteome. Proteins were identified by at least 2 peptides.

Fluorescent QOEC and COEC 24 h post transfection with elevated levels of *IFNλ2a* mRNA in the cells, were considered as transfected. Transfection efficiency was <10% which is typical for primary oviduct cells. As for the proteomic analyses, about 360 peptide families were identified in COEC and 265 in QOEC, both in modified and non modified cells. In culture medium, about 39 peptide families were found for COEC, and 20 for the QOEC cultures. Differences may be due to gaps in quail genome data. Our mass spec analysis did not detect presence of IFNλ2a exogenous protein, which may be due to a low expression efficiency. E.g., major proteins of egg white were detected in COEC cells and medium (Ovalbumin, Ovotransferrin, Ovomucoid), but in QOEC Ovalbumin was found only in cells and Ovotransferrin only in medium. The data will serve to further optimize analysis of oviduct secretome and to understand the impact of *in vitro* condition and genetic modification on the proteomic status of the oviduct cells.

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O137

RNA-Seq analysis reveals different mechanisms in fatty acids biosynthesis in two chicken strains

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Current human foods are considered unbalanced in term of n-6 and n-3 polyunsaturated fatty acids (PUFA) intake. Moreover, human has a poor conversion of these PUFA into long chain PUFA (LCP) due to the low efficiency of key enzymes of the elongation/desaturation pathway. Human diets must therefore contain proper level of LCP, mainly of *n-3* series, which is very low in developed society diets. Fish is the richest LCP food but it is not widely used in the human diet, while chicken meat is very popular and could represent an excellent and economic source of LCP.

In the present work RNA-seq technology was used to investigate differences in expression profiles between two poultry strains (Livorno and ROSS 308) characterized by slow- and fast-growing rate, respectively. For the experimental trial 10 chickens for each strain were reared under standard conditions. Birds of Livorno and ROSS 308 strains were slaughtered at 120 and 40 days of age respectively. Liver samples from three birds of each strain were collected and immediately frozen in liquid nitrogen and stored at -80 °C.

Total RNA samples were extracted using a commercial kit (Macherey Nagel). The RNA-seq analysis was carried out by an external service (Gene Wiz, USA) and all the library were sequenced at a depth of ~40 millions reads/library for each of the six samples. The reads used for the analysis showed an average quality score greater than Q30. Reads were aligned on the *Gallus gallus*.Galgal4.84 reference genome using TopHat. Aligned reads were counted using the featureCounts software and differential expression analysis was performed using the DESeq. The genes that significantly differed in gene expression were searched against the Kyoto Encyclopedia of Genes and Genomes (KEGG) to identify the pathways differentially regulated using DAVID Bioinformatics Resources.

In ROSS 308 compared to Livorno, a total of 2,416 genes were differently modulated (786 up-regulated and 1,630 down-regulated).

Analysis of distribution of the differentially expressed genes among the KEGG pathways allowed the identification of two pathways, involved in the LCP biosynthesis, in which the key genes resulted differentially regulated in the two poultry strains. The results highlighted that Livorno could carry allelic variants useful in the LCP biosynthesis; these alleles are often lost in selected genotypes. Accordingly, the Livorno chicken shows a more efficient LCP gene patterns than the commercial hybrid ROSS 308.

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O138

Efficiency of *in vitro* and *in vivo* chicken primordial germ cells modification

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In our laboratory the transgenic chicken generation has been attempted through chimeric intermediates produced by the transfer of donor genetically modified primordial germ cells (PGCs) into recipient embryo. However, the effectiveness of the transgenic method in birds has been still very low in many cases. The aim of this study was to compare the efficiency of primordial germ cells (PGCs) *in vitro* and *in vivo* modification using the Tol2 transposon system. We used X-tremeGENE 9 DNA Transfection Reagent complexed with pCMV-Tol2 with pL-miniTol2-OVA5IFN to stably transformd of PGCs. *In vivo* method relies on the direct injection of this complex through a window in the eggshell, into dorsal aorta of 14-17 HH recipient embryos. *In vitro* approach involved the isolation from blood of 2.5 -3 day embryos, *in vitro* culture, modification and reinjection of PGCs into dorsal aorta of recipient embryos. In both methods, after injection, the eggshell was sealed and the egg was returned to the incubator and incubated until hatching. The hatchability of manipulated embryos was calculated and compared to the control, windowed only, group. Gonads were collected from dead embryos during incubation. DNA was isolated by Sherlock AX isolation kit and real time PCR was performed for the presence of the miniTol2-OVA5IFN transgene. The hatchability of *in vivo*, *in vitro* and control embryos was: 31.4; 30.8 and 45.0%, respectively. A total of 52 gonads samples from manipulated embryos were analyzed and 23 (65.7%) and 11 (68.8) of *in vivo* and *in vitro* treated embryos, respectively, were confirmed as the transgene carriers. Irrespectively of the *in vivo* or *in vitro* method used, the percent of the hatched chicks as well as the percent of the modified gonads in the dead embryos was similar.

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O139

ELOVL6 gene and SNP effects on fatty acid composition of backfat in Italian Large White pigs

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Fatty acid (FA) profile has a relevant effect on the nutritional and technological qualities of meat products. The FA composition of backfat (BF) and pig meat is influenced by the genotype of pigs. Knowledge of genes influencing this trait is a target to perform a more precise selection for quality traits in pigs. In order to obtain genetic markers and genomic regions associated with FA composition of pig BF tissue, a Genome Wide Association (GWA) study was conducted on a population of about 800 Italian Large White (ILW) individuals, genotyped using Illumina PorcineSNP60 v2 BeadChip. After the quality control, the data were analysed using GenSel, a Bayesian statistics based software. GWA results indicated a strong association between the markers H3GA0025321, SIRI0000509, INRA0030422, and BF content of palmitic, palmitoleic, oleic, medium chain and long chain FAs (with H3GA0025321 showing Bayes Factors >1000 for BF content of medium and long chain FA). These three markers map in an intergenic region at 119-120 Mb on chromosome 8 (Sus scrofa build assembly 10.2), and were reported by Corominas et al. (2013) to be in linkage disequilibrium (LD) with SNPs in the promoter region of the nearby gene *ELOVL elongase 6* (*ELOVL6*), located on SSC8 at 120 Mb. In the analysed ILW population we confirmed the presence of LD between H3GA0025321, SIRI0000509 and INRA0030422 and *ELOVL6* promoter variants. These results seem to indicate the presence of a mutation influencing BF FA composition in the promoter sequence of *ELOVL6* gene. Furthermore, in addition to the GWA study evidencing the role of *ELOVL6* gene on BF FA quality, we performed for this gene an expression study in porcine BF tissue. Sixty samples chosen out of the 800 ILW for their extreme and divergent BF FA composition (high and low level of long chain FA) were analysed. qRT-PCR was performed using *YWHAZ* and *HPRT1* as normalising genes. *ELOVL6* showed different expression levels between samples divergent for the length of FA chain but no difference was found between different genotypes for the three considered markers. These findings suggest that *ELOVL6* has a strong role in determining BF FA profile and the identification of variants of this gene may provide markers for genetic selection addressed towards the improvement of pork FA composition.

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O140

Genome wide association studies for haematological and clinical-biochemical parameters in Italian Large White pigs

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Diseases are the most important causes of economic losses in all livestock species. Genetic improvement for disease resistance and resilience is becoming an essential issue in pig breeding programmes. Haematological and clinical-biochemical parameters reflect, at least in part, the immune capacity and the basic physiological states of the animals that can be considered as potential indicators of the capacity of the animals to cope with infections. We recently demonstrated that several of these parameters are highly heritable in pigs and that, for this reason, it could be possible to use them as proxies of disease resistance-related traits in breeding programmes. With the aim to identify genetic factors that might indirectly explain part of the genetic variability of these traits, we carried out genome wide association studies for 15 haematological and 15 clinical-biochemical parameters measured in about 900 performance tested Italian Large White pigs. All animals were genotyped with the Illumina PorcineSNP60 BeadChip genotyping tool. Association analysis was carried out using GEMMA. The most significant QTLs were identified for the number of basophils on porcine chromosome (SSC) 14, the number of eosinophils on SSC3, SSC7 and SSC10, the number of monocytes on SSC15, the levels of total cholesterol and LDL-cholesterol on SSC3, mean corpuscular hemoglobin level on SSC14, alanine aminotransferase activity on SSC7 and hemoglobin level and hematocrit on SSC18. These results will be useful for the identification of the causative mutations of these phenotypes that could be important to dissect disease resistance and resilience related traits in pigs.

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O141

Estimation of genetic parameters for honey production in the honeybee – Preliminary results

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The estimation of breeding values in genetic selection programs is a tool of paramount importance, as it allows to identify genetically superior individuals that can then become parents of the next generation, improving traits of economic interest. Honeybees represent an important productive livestock species due to hive products such as honey, bees wax, pollen and to its invaluable role as pollinators of agricultural crops and wild plant species. Genetic evaluation in the honeybee is considerably difficult due to their peculiar mode of reproduction. Phenotypes are measured on the colony, which consists of one queen and her daughters, *i.e.* thousands of

workers. The queen is the only fertile female of the colony that mates with 10 to 20 drones just once, and stores a life lasting reserve of sperm cells in her spermatheca. Drones are haploid and their gametes are clones of their genotype. Furthermore, drones die right after mating, which means they mate just once in their lifetime.

Restricted Maximum Likelihood (REML) and Best Linear Unbiased Prediction (BLUP) are important methodologies in livestock breeding for estimating variance components and predicting breeding values, respectively. These methods require information on the genetic relationship among tested individuals in order to estimate the individual additive genetic merit for a phenotypic trait. Ordinary rules for the estimation of genetic relationship are not applicable to honeybees due to queen's multiple mating and haploid males.

The aim of the work was to develop a numerator relationship matrix adapted to honeybee peculiarities, in order to estimate breeding value, variance components and genetic parameters for honey yield. Tested colonies ($n = 120$) derived from 8 parental lines have been distributed in 4 apiaries nearby Lodi (Lombardy). Honey was harvested twice during spring 2016. A modified R function was used to compute the inverse of numerator relationship matrix, which is required for the estimation of variance components and the prediction of breeding values. The fitted mixed model included fixed effects of apiary, strength of the colony and production, and both genetic additive and permanent environment random effects. Preliminary results show heritability and repeatability of honey production of 0.33 and 0.54, respectively. These results are in agreement with parameters estimated through different methods reported in literature.

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O142

Genomic landscape and biodiversity of Italian dogs

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The availability of genomic tools, such as SNP chip and whole genome sequencing technologies, have given researchers the opportunity to understand the relationships between, and genetic background of, dog populations. However, such international studies have included few Italian populations. This project aims to highlight the uniqueness of Italian dog populations and deepen our understanding of the genetic relationships that exists between them.

Almost 300 dogs from 18 important Italian breeds and ecotypes (17 ± 4 average number ± SD of animals per breed, breeds list: http://www.enci.it/libro-genealogico/razze-italiane) were considered for this study. Within each population, dogs were selected for analysis if they were unrelated at the second generation. Biological samples were collected according to the European regulations on animal welfare. All samples were genotyped with the Illumina 170K HD SNP chip. Data were checked for missing genotypes, minor allele frequencies, cryptic relatedness and duplicate individuals. Genotype phasing used individual pairwise identity by descend estimations. Distances were estimated as shared alleles identity by state between individuals, whereas populations distances were estimated as Reynolds distances. Both distances were then used to assemble a Neighbor-joining phylogenetic tree using the PHYLIP software. Finally, the genetic background of populations was defined using ADMIXTURE software. Those results provide the first deep insight into the genomic landscape of Italian dog breeds and ecotypes, highlighting their phylogenetic relationships. Our findings confirm most of the known history of the breed analyzed, grouping by their working ability and show an average inbreeding (± SD) of 0.15 (± 0.11).

Genomic data analysis has proven to be an important tool for revealing relationships within and across populations. These data can be used to define individual relationships, such as parentage and inbreeding, or those between populations, estimating genetic distances and phylogenetic relatedness. These studies will provide the Italian Kennel Club (ENCI) with new tools that, together with classic management instruments, can improve genealogical registration quality, selection strategies for breeding, as well as understanding of genetic makeup and breed composition, all leading to improved health and welfare for Italian breeds.

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O143

Effects of butyrate and taurine supplementation in European sea bass diet based on soybean meal

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With most wild fish capture fisheries at or above maximum sustainable yield, aquaculture cannot rely any more on oceanic resources for the manufacturing of aqua feeds. Substantial efforts have been made to reduce the proportion of fishmeal (FM) and fish oil in aquaculture feeds, by replacing forage fish with terrestrial plants. Of these, soybeans have been promoted as a viable substitute to FM because of their higher protein content, higher digestibility, and better amino acid profile in comparison to other grains and oilseeds. The main drawbacks of using vegetable-derived proteins in carnivorous fish feed are related to the low level of indispensable amino acids (in particular lysine, and methionine) and to the presence of a wide variety of anti-nutritional factors that could damage the intestinal tract thus reducing nutrient absorption and fish growth.

We evaluated the mitigating effects of butyrate and taurine used as feed additives on the morphological abnormalities caused by a soybean meal (SBM) based diet in the distal intestine of sea bass (*Dicentrarchus labrax)*. We used 3 experimental diets, containing the same low percentage of FM and high percentage of SBM; two diets were supplemented with either 0.2% sodium butyrate or taurine. Histological changes in the intestine of fish were determined by light and transmission electron microscopy. Infiltration of $CD45^+$ leucocytes in the lamina propria and in the submucosa was assessed by immunohistochemistry. We also quantified by real time PCR, the mRNA abundance of a panel of genes involved in the intestinal mucosa inflammatory response.

Fish that received for 2 months the diet with 30% soy protein developed an inflammation in the distal intestine, as confirmed by histological and immunohistochemistry data. The expression of target genes in the intestine was influenced by the type of fish diet. Fish fed with taurine supplemented diet displayed the lowest number of mRNA copies of IL-1β, IL-8, and IL-10 genes in comparison to fish fed with control or butyrate supplemented diets. Dietary butyrate caused an upregulation of TNFα gene transcription. Among the quantified interleukins, IL-6 was the only one, to be not influenced by the diet.

In conclusion, histological and gene expression data suggest that butyrate and taurine could have a role in normalizing the intestinal abnormalities caused by the SBM, but with different mechanisms of action.

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O144

Including marine microalgae in European seabass (*Dicentrarchus labrax*) diets: effects on digestive-absorptive functions

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Marine microalgae deserve increasing attention as fish feed ingredients or supplements, due to their nutritional value and functional properties. They were recently shown to improve intestinal morphophysiology, which is often challenged in fish fed diets high in protein-rich plant feedstuffs. The aim of this study was to evaluate the effects of supplemental dried marine microalgae on gut histology and expression of genes encoding brush border membrane enzymes and transporters, in E. seabass fed diets low in fish meal and supplying substantial levels of plant protein feedstuffs.

Two test diets (A1 and A2) were prepared by including a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass (2:1 w:w ratio) to replace 15 and 45% fish meal protein and 10 and 30% fish lipid of a control diet (C+) containing 50:50 fish to vegetable protein and lipid ratios. A negative control (C-) preparation was also formulated with a 30:70 fish to vegetable protein ratio. One hundred and forty four seabass (mean weight 204.3 ± 0.78 g) were divided among 12 tanks connected to a recirculating aquaculture system ensuring optimal rearing conditions (T, 23.8 °C; Salinity 30 ppt). Fish groups were fed the test diets to visual satiety over 105 days according to a randomized design with 3 replicates per dietary treatment. At the end of the trial, 6 fish per treatment were euthanized; the digestive tract removed, divided into pyloric caeca (PC), foregut (FG) and hindgut (HG) sections, and frozen in liquid N for gene expression analysis. Subsamples of gut tissue were also collected for histological evaluation. From histological analyses fish fed diet C- had the lowest villi thickness ($p<.05$) while those given diets including microalgae, irrespective of the inclusion level, resulted in greater villi length than that observed in either positive or negative controls. Irrespective of the dietary treatment, gene expressions of sucrase-isomaltase, PepT1, Na^+/K^+-ATPase and APN were highest in the foregut. Regardless of the gut section, gene expression of the same enzymes-transporter was higher in fish fed microalgae-containing diets ($p<.05$) when compared to both controls.

The present results indicate that even at the lower dietary inclusion level investigated here, a mix of *T. lutea e T. suecica* resulted in potentially improved digestive-absorptive functions. Further studies are needed to understand the mechanisms underlying the observed positive effects of dietary microalgae addition.

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O145

Feeding European sea bass (*Dicentrarchus labrax*) with increasing dietary fibre levels: impact on growth, blood biochemistry, gut histology and gut evacuation

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A key area of investigation for continuing to improve modern aquafeeds includes the evaluation of varying proportions and combinations of plant ingredients to identify mixtures that are more efficiently utilized by the fish. In this contest, plant ingredients will also introduce fibre component and few data for optimal dietary fibre level in marine carnivorous species are available. Thus, the effects of increasing dietary fibre level on main growth and gut health parameters in European sea bass (*Dicentrarchus labrax* L.) were studied. Five isonitrogenous and isoenergetic diets with different dietary fibre levels (1.5, 3.0, 4.5, 6.0 and 7.5%) were fed to triplicate fish groups of 60 individuals (initial weight 69 g) to satiation, over 117 days. Specific growth rate (SGR), voluntary feed intake (VFI), feed conversion rate (FCR), protein efficiency rate (PER), gross protein efficiency (GPE) and gross lipid efficiency (GLE) were determined. Furthermore, viscerosomatic index (VSI), hepatosomatic index (HSI) and fat index (FaI) were also calculated. Intestine histology examination, blood analyses of serum total protein, triglycerides, glucose, inorganic phosphorus and alkaline phosphatase were determined. In addiction gut evacuation rate was calculated. Data except for gut evacuation, were analysed by a one-way ANOVA followed by a Tukey's multiple comparison test. Regarding gut evacuation, the stomach and intestinal dry digesta content were regressed against time in order to fit to a model for calculating gastric evacuation rate and gastric evacuation time (software R version 3.1.0). At the end of the trial no significant differences ($p\geq.05$) due to fibre inclusion were observed in terms of final body weight, SGR, VFI and FCR. No significant differences among treatments were found in PER and GPE while VSI and HSI values significantly decreased ($p\leq.05$) with increasing dietary fibre levels. All the histological section examined showed a normal intestinal architecture and inflammatory and/or degenerative changes were not detected. No significant differences among treatments were found in serum blood analysis. Even if not significant, increasing fibre level led to an increasing in time required to empty the stomach while the evacuation time for foregut and hindgut was similar among treatments.

In conclusion, fibre levels up to 7.5% do not affect growth performances, nutritional status and gut integrity during the on-growing of European sea bass.

O146

Effects of dietary butyrate supplementation on histone modifications and the expression of inflammatory-related genes in European Sea bass (*Dicentrarchus labrax*)

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Bacteria that inhabit the epithelium of the animals' digestive tract provide the essential biochemical pathways for fermenting otherwise indigestible dietary fibers, leading to the production of short-chain fatty acids (SCFAs). Of the major SCFAs, butyrate has numerous positive effects on the health of the intestinal tract and peripheral tissues. The mechanisms of action of butyrate are mainly related to its potent regulatory effect on gene expression since butyrate is a histone deacetylase inhibitor that play a predominant role in the epigenetic regulation of gene expression and cell function.

We investigated in European sea bass (*Dicentrarchus labrax*) the effects of butyrate used as a feed additive on fish epigenetics as well as its regulatory role in mucosal protection and immune homeostasis through impact on gene expression. Seven target genes related to inflammatory response and reinforcement of the epithelial defense barrier (*TNFα, IL1B, IL6, IL8, IL10*, and *MUC2*) and five target genes related to epigenetic modifications (*DICER1, EHMT2, PCGF2, HDAC11*, and *JARID2A*) were analysed in intestine and liver. We also investigated the effect of dietary butyrate supplementation on histone acetylation, by performing an immunoblotting analysis on liver core histone extracts.

Results of the eight-week-long feeding trial showed no significant differences in weight gain or specific growth rate of sea bass that received 0.2% Na-butyrate supplementation in the diet in comparison to control fish. Dietary butyrate led to a twofold increase in the acetylation level of histone H4 at lysine 8, but showed no effect on the histone H3 at Lys9. The expression of four (*IL1B*, *IL8*, *IRF1*, and *TNFα*) out of seven analyzed genes related to mucosal protection and inflammatory response was significantly different between the two analyzed tissues but only *IL10* showed differences in expression due to the interaction between tissue and butyrate treatment. In addition, butyrate caused significant changes in vivo in the expression of genes related to epigenetic regulatory mechanisms such as *HDAC11*, *EHMT2*, and *DICER1*. Statistical analysis by two-way ANOVA for these genes showed not only significant differences due to the butyrate treatment, but also due to the interaction between tissue and treatment.

In conclusion, data reported here could be essentials for the identification of functional additives for fish diets in the efforts to improve the sustainability of aquaculture.

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O147

Effects of different feeds on performance of rainbow trout (*Oncorhynchus mykiss*) broodstocks

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In aquaculture, the feeding of broodstocks plays a key-role because it affects the quality of the gametes, their fecundity, hatching rate, fry survival and incidence of larval deformation, regardless of genetic selection and the strain employed. In recent years, the research of feedstuffs alternative to fish meal, in order to increase the sustainability of the productive cycle, has also been focalized on the feeding of rainbow trout (*Oncorhynchus mykiss*) broodstocks. Efforts are being concentrated on new diets able to guarantee health benefits, satisfying the essential requirements of females and males, as well as a good quality of gametes, larvae and fingerlings. Based on these assumptions, a trial was carried out employing 4-year-old broodstocks of rainbow trout in order to evaluate the effects of two feeds, containing feedstuffs of different protein and lipid source, on reproductive performance in terms of the quality of eggs, hatching rate and fingerling survival rate. Four months prior to the spawning season, broodstocks were selected and divided into two experimental Groups, with two replicates each (GF1-GF2, GV1-GV2). Groups GF were fed a diet containing fish meal and fish oil as control feed. Groups GV received a diet including legume protein feedstuffs and oil of vegetable origin (50% linseed oil instead of 50% fish oil). Proximate composition and fatty acid profile of the two feeds were analysed according to international methods. On viable eggs, incubated at 10 °C water temperature, the hatching rate and fingerling survival rate at 60 days were recorded in the different Groups. Data were submitted to one-way ANOVA and the differences between the means were evaluated by means of the Student-Newman-Keuls test (SAS, 1989) and considered significant at $p<.01$. Gamete and fingerling data were different among the batches, showing a significantly higher performance with regard to the quality of viable eggs in Groups GF compared to Groups GV. The hatching rate of GF was 92 ± 2% *versus* 71 ± 3% of GV. The survival rate of fingerlings at 60 days also showed notable differences being 74 ± 4% *vs* 49 ± 3% in GF and GV groups, respectively. Based on the results of this trial, it is possible to assume that feedstuffs containing vegetable protein and fat administered to broodstocks affected the quality of gametes and the progeny of rainbow trout negatively.

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O148

Effects of feed restriction and re-feeding on body condition, plasma metabolites and intestinal brush border enzymes activity in rainbow trout *Oncorhynchus mykiss*

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Recovery of body weight and condition as a consequence of liberal feeding after periods of fasting or feed shortage, is a well-known phenomenon in salmonid fish species. To what extent the adoption of culture protocols, alternating liberal feeding to severe feed restriction to exploit compensatory growth, could be a challenge in terms of metabolic adaptation and welfare, still remains questionable. In this context, the present study was aimed at evaluating some physiological responses of rainbow trout subjected to 3 weeks fasting or restricted feed ration and re-feeding over the following 2 weeks. Ninety-nine trout (body weight 109.1 ± 3.5 g) were randomly distributed among 3 tanks (0.5 m^3) each supplied with 8 L min^{-1} of well water at a temperature of 12.7 ± 0.1 °C. Fish groups were subjected to one of the following treatments: C, continuous feeding with a commercial trout diet at 1% body weight over 5 weeks; R, restricted ration (30% of C ration) over 3 weeks followed by 2 weeks feeding to visual satiety; F, fasting over 3 weeks followed by 2 weeks feeding to visual satiety. Three fish per group were euthanized at time 0 and after 1, 2, 4, 7, and 14 days during the re-feeding period and sampled for viscera, liver and mesenteric fat to calculate carcass yield, visceral organ or tissue weight and somatic indices. Blood and gut samples were also collected and analysed for plasma metabolites (glucose, lipid and protein levels) and the activity of intestinal brush border membrane (BBM) enzymes (disaccarases, alkaline phosphatase, γ-glutamil transaminase) in different sections (pyloric caeca, foregut, hindgut).

In comparison to the control group, i.e. continuously fed fish, a feed restriction or fasting over 3 weeks resulted in significantly reduced body and visceral to body weight ratio and in diminished activity of the intestinal BBM enzymes. Among plasma metabolites, only cholesterol was reduced. During the re-feeding period, liver and viscera weight increased more rapidly in groups R and F than in group C, resulting in significantly higher organ to body scores. The pattern of the activity of the intestinal BBM enzymes varied among the different tracts in a specific manner, but no differences were observed among treatments after 2 weeks of re-feeding.

In conclusion, a period of 14 days of *ad libitum* feeding seems to be able to restore the metabolic status in rainbow trout previously subjected to fasting or restricted feed ration over 3 weeks.

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O149

Apparent digestibility of different microalgae dried biomass in rainbow trout *(Oncorhynchus mykiss)*

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Despite a growing interest in microalgae as sustainable sources of nutrients in complete aquafeeds, little information is presently available on the nutritive value of these novel potential feed ingredients for carnivorous fish species. The aim of this study was to estimate energy and the apparent macronutrient digestibility of a panel of cultivated microalgae, using rainbow trout as a fish model.

From a basal reference diet mash, 8 test diets were obtained including finely ground dried biomass of *Arthrospira platensis* (ART), *Chlorella sorokiniana* (CHL), *Nannochloropsis oceanica* (NAN), *Nostoc sphaeroides* (NOS), *Tisochrysis lutea* (TISO), *Phaeodactylum tricornutum* (PHAE), *Porphyridium purpureum* (POR) and *Tetraselmis suecica* (TETR) at a 12:88 w:w microalgae to reference diet ratio. All diets were added with acid insoluble ash (1.0%) as an inert marker before being extruded and dried into 3 mm pellet.

The apparent digestibility coefficients (ADCs) of dry matter, protein, organic matter and energy for reference and test diets were estimated *in vivo* with juvenile rainbow trout using 9 units of three 50-L tanks, each stocked with 15 fish (52.4 ± 1.5 g), fitted with a settling column for fecal collection (Guelph system). Each diet, offered to visual satiety in two daily meals, was evaluated over three independent 10-day fecal collection periods preceded by 7 days adaptation to a new diet. ADCs were calculated by difference relative to those measured with the reference diet.

The various microalgal biomasses showed significantly different apparent digestibility values ($p<.05$). Dry matter ADCs ranged from 90.4% for TISO to 53.3% for CHL. Protein ADCs varied from 94.4% for POR to 63.9% for CHL. Organic matter ADCs ranged from 94.6 for TISO to 58.3% for CHL while gross energy ADCs varied from 93.7 for POR to 53.1% for NAN.

The results obtained here using the rainbow trout as a carnivorous fish model for digestibility, provide a useful indication of the nutritive value of different microalgae to assist in the formulation of environmental friendly fish diets. They also showed that, due to poor digestibility of certain microalgae biomass, just a few of them can tackle the sustainability challenge of the aquafeed industry as a potential and cost-effective source of nutrients and the adoption of suitable physical or enzymatic rupture treatments in some species will be needed to improve their digestibility.

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O150

Antibiotic use in a rainbow trout fattening farm in Umbria: sustainability, residues and farm environmental impact

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Umbria produces about 10% of the total national aquaculture, essentially rainbow trouts. Antibiotic use in aquaculture has a great impact on both production cost-effectiveness and public health, inside the "one health" philosophy. This study has evaluated the use of antibiotics and their residues in one Umbrian rainbow trout farm. Antibiotic consumption was evaluated with the *Defined Daily Dose (DDD)-based* approach in 2014 and 2015. Regional antibiotic consumption in aquaculture increased a lot from 2014 to 2015: +56,732% prescribed *DDDs* and +954% *DDDs*/1000 farms-*die*. In the monitored farm, a similar trend was observed: +265% prescribed *DDDs* and +23% *DDDs*/1000 treated animals-*die*. Sulfadiazine/trimethoprim (SDZ/TMP), oxytetracycline (OTC) and florfenicol (FF) were the drugs administered in 2014 and 2015, whereas SDZ/TMP and OTC the most used. Antibiotic residues were measured using liquid chromatography coupled to mass spectrometry technique in four fish and tank sediment samplings, carried out monthly (March- June 2016) from two tanks (A and B) randomly chosen. In tank A, no treatment was performed in the period November 2015-April 2016. Only in May, a treatment with SDZ/TMP was carried out. On the other hand, in tank B, treatments with FF (March 2016), OTC (April 2016) and SDZ/TMP (June 2016) were documented. Residues of OTC and SDZ/TMP were detected in fish tissues, together with flumequine and erythromycin. As expected, antibiotic concentrations progressively decreased after administration. However, in one case after three months from the treatment (June 2016 – tank B) SDZ and TMP exceeded the Maximum Residue Levels fixed by European Regulation 37/2010. Sediment analysis shows high levels of residues, particularly OTC and FF (>1 mg/kg dry weight), most likely due to their chelating properties. It is worth of note that in this matrix detectable concentrations (>0.01 mg/kg dry weight) were present even after several months from treatment ("memory effect"). Further analysis are in progress both in fish tissues and in environmental matrices. The final aim is a better understanding of the relationship between antibiotic consumption and residue persistence in both animal and environment, as to quantify antibiotic use sustainability and environmental impact. Furthermore, provided data can be used to stimulate an improvement of biosecurity and management with a lesser use of antibiotics.

O151

Environmental impact of milk production in two samples of organic and conventional farms in Lombardy

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There is an increasing interest on organic milk (OM) production in Italy even because it is considered more respectful of the environment. However, literature shows inconsistent results, where OM production is associated with higher emissions of greenhouse gases and, on the contrary, lower levels of water and land acidification (AC) and water eutrophication (EU). Considering the overwhelming increase of OM production and the special features of dairy farms in northern Italy, the environmental impacts of conventional milk (CM) and OM production systems have been compared. In 2016, eight conventional and six organic dairy farmers in the Lombardy plain were interviewed about the technical and economical results in 2015. Environmental performances have been assessed according with LCA approach. The environmental effects that have been considered were global warming (GW), AC, and EU. Functional unit was 1 kg of FPCM. Calculations have been performed by a version of LatteGHG, taking into account carbon sink and estimating AC and EU.

Results showed some differences between the rearing systems considered. Data are reported as means ± SD. Herd sizes did not differ, but variability in O herds'size (330 ± 293) was larger than C ones (317 ± 110). FPCM production was significantly ($p<.05$) higher in C than in O farms (9004 ± 113 *vs* 7736 ± 1430, kg/cow/yr). There was no difference in GW (1.24 ± 0.180 *vs* 1.37 ± 0.305, kg CO_2eq), AC (0.025 ± 0.005 *vs* 0.026 ± 0.003, kg SO_2eq), and EU (0.0109 ± 0.0019 *vs* 0.0111 ± 0.0022, kg $P_2O_4^{3-}$eq) associated to 1 kg of FPCM in C and O respectively.

In conclusion, OM production has not improved environmental categories examined, even though O farms do not use mineral fertilizers and use lower amount of off-feeds. Probably the expected benefits have been compensated by the negative effect of solid manure management, that is more frequent in organic dairy farms, and by the higher FPCM production in C farms. This survey has given an initial snapshot of organic milk production in a dairy area in Italy.

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O152

Carbon footprint from a dairy farm with combined milk production and bioenergy systems

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Milk production is a source of greenhouse gas emissions (GHG), which mostly consist of methane (CH_4) from gastroenteric fermentation and from manure management. Implementing mitigation strategies, such as electricity generation from manure anaerobic digester (AD) and photovoltaic (PV) system contributes to mitigate manure CH_4 emissions and fossil energy use for the dairy farm energy needs.

This approach allows to consequently reduce the environmental impact of milk production. In the present study to evaluate this approach, a Life Cycle Assessment (LCA) was performed to analyze the carbon footprint (CF) of milk production in a dairy farm (1368 animals), provided by an AD and PV systems.

"From cradle to farm gate" approach was chosen to detect the main environmental hotspots of milk production. The functional unit (FU) was referred to one kilogram of fat-and-protein-corrected-milk (FPCM). Beside milk product, other important co-products need to be considered are: meat and renewable energy production from AD and PV systems. AD plant was fed with a mix of manure and worse maize silage, not suitable for animal feed.

IPCC's tiered approach was adopted to associate a level of emission to each item in the life cycle inventory. A physical allocation was applied to attribute GHG emissions among milk and meat products. Renewable energy production from AD and PV systems were accounted discounting carbon credits due to the less CH_4 manure emissions and to the minor exploitation of fossil energy.

If mitigation options are not considered, CF of milk production was 1.36 kg CO_2eq/kg FPCM. Considering the integrated dairy farm with bioenergy system, the mitigation resulted from milk production by 0.29 kg CO_2eq/kg FPCM. AD had the highest reduction of GHG emissions, whereas PV system contribution in this case farm was negligible due to the small dimensions of the technology.

The results obtained on this study show that integrating dairy farms with bioenergy systems (AD and PV) is one of the successful strategies to mitigate the environmental burden of milk production. The main benefit of this approach is the offset of fossil energy use and a more efficient manure management. In this case farm a preliminary approach was adopted, nevertheless exploring different domains such as technological, environmental and territorial features, social and economic ones, allows to achieve a more integrated LCA.

O153

Carbon footprint of PDO cheeses: Grana Padano and Gorgonzola

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The aim of the study was to evaluate the carbon footprint of two PDO Italian cheeses: Grana Padano and Gorgonzola.
Grana Padano is a hard long ripening cheese, composed by 65% of dry matter, consisting of 38% protein and 24% fat. Gorgonzola cheese, instead, is a blue soft cheese with a short ripening period composed by 58.6% of dry matter (28.7% fat and 21.4% protein).
The productive process of Grana Padano was studied at a cheese factory that in 2015 processed 86,165,255 L of milk, producing 183,611 cheese wheels, with an average yield of 7.6%. For Gorgonzola, a cheese factory was studied; in 2015, it processed 12,542,552 L of milk, producing 126,910 Gorgonzola wheels with an average cheese yield of 12.5%.
The Carbon footprint was quantified using Life Cycle Assessment (LCA) method, carried out through a “cradle to cheese factory gate” point of view. All data considered were referred to 2015 and the functional unit was 1 kg of cheese. Gas emissions of milk production, at farm level, were calculated using IPCC (2009) and EEA (2009) equations, then impact categories were evaluated using IPCC (2007) method. Both economic and dry matter allocations were applied.
Assuming the economic allocation and considering the whole productive process, Global Warming Potential (GWP) was 16.9 kg CO_2 eq. per kg of Grana Padano, higher than GWP of Gorgonzola that resulted 10.7 kg CO_2 eq. Using the DM allocation, the unitary GWP resulted 10.3 kg CO_2 eq. for Grana Padano and 6.0 kg CO_2 eq. for Gorgonzola. These different values are mainly due to the lower cheese yield of Grana Padano in comparison to Gorgonzola, which implies a higher unitary value of environmental impact.
The milk production at farm was the most important contribute of the GWP using an economic allocation at cheese factory (excluding ripening and packaging): 95.6% for Grana Padano and 90.3% for Gorgonzola.
The phase of milk processing slightly contributed to GWP of both cheese but some differences were observed: a higher use of cleaning products (0.54% *vs* 0.02% of GWP) for the sanitization of the plant and use of electricity, principally for air conditioning of cheese factory (5.79% *vs* 1.64% of GWP) for Gorgonzola and Grana Padano processing respectively.
The outcomes of this study highlight how, due to the multiple products produced at the dairy plants, the choice of the allocation method deeply affects of the environmental burdens of cheeses.

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O154

In vitro study of the effects of different tannin extracts on rumen ammonia and methane production

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Several feeding strategies have been proposed to mitigate CH_4 and NH_3 production. Dietary tannins may modulate the activity of rumen microbes and modify carbohydrates fermentation, lipid bio-hydrogenation and protein degradation. However, effect on rumen fermentation may significantly differ accordingly to the nature of tannins (i.e. condensed or hydrolysable tannins). Changes in CH_4 and NH_3 accumulation during in batch fermentation may be useful to compare the effectiveness of different tannins in the modulation of rumen metabolism. Four different tannin extracts have been compared: Mimosa (MT; *Acacia dealbata*) and Gambier (GT; *Uncaria gambir*) as condensed tannins and Tara (TT; *Casealpinia spinosa*) and Chestnut (CT; *Castanea sativa*) as hydrolysable tannins. Tannins were included at 4% of DM in a diet composed by barley, wheat bran, alfalfa hay, soybean cake, molasses and vitamin mix. Control (C) diet contained the same ingredients with the addiction of 4% of bentonite. Samples of rumen liquor were collected from 5 sheep, conditioned with the C diet, using a stomach tube connected to a manual pump. Feeds (2 g) were incubated in triplicate with 200 mL of inoculum filtered into a flask under a continuous flow of CO_2. The incubator consisted of a thermostatic chamber (39-40 °C) equipped with glass fermentation vessels provided with one inlets (to release gas through a valve) and connected to an electronic pressure transducer. Gas pressure inside the vessels was recorded every 30 seconds over 24-hours. CH_4 and NH_4^+ concentration was analysed at 0, 6, 12 and 24 hours. For each treatment, the whole fermentation was replicated three times as separate batches. The total amount of gas produced during the 24-hours fermentation did not differ across treatments. The amount of CH_4 produced was significantly lower when hydrolysable tannin extracts (TT and CT) were included in the substrate, if compared to C diet (0.154, 0.156 and 0.170 ± 0.011 mmol for TT, CT and C diet, respectively). The NH_3 concentration in rumen liquor was significantly lower for samples containing tannins. Condensed tannins from MT were more effective in reducing NH_3 concentration (49.99 *vs* 136.29 ± 8.81 mg/L for MT and C diet, respectively) followed by hydrolysable tannins from CT (65.34 ± 8.81 mg/L). Overall, the results suggested that hydrolysable and condensed tannins differently affected rumen metabolism: the former being more effective on CH_4 production whereas the second on NH_3 concentration.

O155

Sustainable reduction of methane emissions from cattle's enteric fermentation

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Ruminant production generates a significant amount of anthropogenic emissions of greenhouse gases (GHG). Therefore there is the need to develop sustainable alternatives to mitigate GHG emissions by ruminants and to increase the supply of quality protein for humans in a climate change scenario. The objective of this work is to present sustainable options to mitigate methane (CH_4) production by cattle and to illustrate how productivity can be increased at the same time. We conducted several experiments to measure CH_4 emission, *in vivo* and *in vitro*, by cattle in order to estimate emission factors in the temperate (TEMP) and tropical (TROP) climate regions of Mexico followed by inventory calculation. We used the *in vitro* gas production technique, the dual tracer release flux method and the open-circuit respiration chamber method. For the *in vitro* gas production experiments we screened the potential of different local tanniferous plants to reduce enteric CH_4 formation and selected those with the highest potential. We then tested these plants *in vivo* and measured how much CH_4 was reduced by supplementing them. Finally, we used the results to propose two mitigation scenarios for each region of Mexico. *Leucaena leucocephala* and *Cosmos bipinnatus* were the plants that produced the largest reduction in CH_4 formation. In scenario 1, a moderate mitigation scenario, it was considered 16% reduction of CH_4 emission in the TEMP region and 36% in the TROP region with cattle population of 37.8 million heads, from which 22.3 are in the TEMP (emission factor 529 l/day/head) and 15.5 in the TROP (emission factor 137 l/day/head). Reduction potential resulting from the use of *C. bipinnatus* and *L. Leucocephala* over a year is 1,203Gg. In scenario 2, a high mitigation situation, it was assumed a 26% reduction of CH_4 emission in the TEMP and 36% in the TROP. The reduction potential resulting from *C. bipinnatus* and *L. Leucocephala* use in a year is 1,512 Gg. Results showed that in both scenarios the CH_4 released by enteric fermentation could be reduced by the use of the plants evaluated. Increased productivity results from higher stocking rates achieved with the use of Leucaena in TROP regions, better animal performance and lower use of concentrate feeds.

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O156

Effect of three tanniferous plants on methane emission by dairy cows

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Agriculture contributes with approximately 13% of the total global emission of greenhouse gases (GHG), from which cattle is responsible of 53%. Studies of *in vitro* experiments on the effect of tanniferous plants on reducing rumen methane (CH_4) production have been conducted to find natural alternatives to mitigate the environmental impact generated by the emissions of this GHG in the cattle industry. The objective of the present study is to evaluate the effect of *Pithecellobium dulce*, *Tajetes erecta* and *Cosmos bipinnatus* on reducing methane emission, milk production and dry matter intake in dairy cattle.
A 4 × 4 Latin square experimental design was used, where

four multiparous Holstein cows of 553 ± 72.4 kg live weigh, at their second lactation third and average milk yield of 17.3 ± 3 kg/day were used. The experiment lasted 92 days divided into four experimental periods of 23 days each. All cows had free access to maize and alfalfa-silage in a proportion 50:50, 4 kg of concentrate/day and *ad libitum* access to water. The treatments consisted in supplementation of 0.5 kg/day of the experimental plants, the cows in the control treatment did not receive any plant. Each cow received each treatment, one in each of four periods.

The experimental variables were analysed by analysis of variance for a Latin square experimental design. The Tukey's test was applied if differences between treatments were observed. A multiple correlation analysis between all variables was also run in order to find associations that help to explain CH_4 production.

Results show that no significant differences ($p > .05$) were observed between the control and the treatments for all the variables but CH_4 production. The treatment with *C. bipinnatus* reduced CH_4 production in 98.5 L/d ($<$16%) in relation with the control treatment ($p < .05$). Milk composition was also not affected ($p > .05$) by the experimental plants. The multiple correlation analysis showed significant positive associations between CH_4 L/day and DMI ($r = 0.5$, $p < .05$).

It is concluded that the inclusion of C. bipinnatus at low inclusion levels may be used to reduce CH4 production from enteric fermentation of cattle.

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O157

Phytoremediation as an innovative approach to control heavy metals output from livestock

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Heavy metals (HM) are widely found environmental contaminants as a result of anthropogenic activity. Metals are accumulating in the food chain through uptake at primary producer and represent a serious problem for health. Some HM are largely used as feed additives in livestock, also to optimize the growth performance and the excess metals are excreted. The risk of HM pollution due to the use of manure in agriculture has focused. Although using minimal supplements of trace minerals is suggested, strategies are required to reduce the environmental impact of animal productions. The aim of this study was to evaluate the ability of two plants to bioaccumulate trace elements, chosen according to results obtained in a previous investigation on HM in livestock, from contaminated water as a cost-effective plant-based approach of remediation, under controlled experimental conditions. Four pools (width 4.0 m, length 2.0 m, depth of 0.7 m; 695 L of water, 210 kg of soil) were assembled as mesocoms at the Città Studi Botanical Garden. Two of them were planted with *Typha latifolia* (TYT: treatment, $n = 30$; TYC: control, $n = 30$) and two with *Thelypteris palustris* (FPT: treatment, $n = 60$; FPC: control, $n = 60$). After 15 days of acclimation (T0) a solution of a mineral feed additive premix (final concentration: Zn 44,02 mg/L; Cu 8.63 mg/L; Mn 10.83 mg/L; Se 0.09 mg/L) was dissolved in the TYT and FPT. At T0, day 15 (T1) and day 45 (T2) samples of roots, leaves, stems, soil and water were collected, dried, mineralized and analyzed using inductively coupled plasma mass spectrometry in order to obtain HM content. No visual toxicity signs were observed during the entire experimental period. Results indicated that both plant species were able to uptake and translocate minerals into their tissues, reducing the available amount of metal in the mesocom. Metals were preferentially accumulated in rhizomes and roots: TYT and FPT plants showed a higher amount of Zn, Cu, Mn and Se if compared respectively with TYC and FPC (T1, T2). The increase was directly related to the exposure time. *T. palustris* appeared more effective than *T. latifolia* at translocating elements from solution to plant tissues. Zinc concentration was 651 ± 207.96 mg/kg dm and 177 ± 6.80 mg/kg dm respectively in *T. palustris* roots and *T. latifolia* roots. Results suggest that the evaluated plants may be candidates for the phytoremediation approach to control HM output from livestock.

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O158

EPAnHaus project: assessment of the energy use for climate control into animal houses

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Environmental conditions of livestock housing can affect the quality and the costs of the animal production. Many environmental parameters (e.g. indoor air temperature, indoor relative humidity) must be set up and controlled to maintain animal welfare, to prevent the incidence of certain diseases, and to raise productive performance of the animals. This control is generally carried out by mechanical system that entails an energy consumption for heating, cooling and ventilation, that for some livestock housing (e.g. pig and broiler buildings) may be an important part of the total running costs of the structure. Those energy consumptions and costs may be reduced by improving the thermos-physical features of the building envelope or increasing the efficiency of the used mechanical systems. To understand the effectiveness of each action regarding the envelope and/or the mechanical system, it is necessary to assess the performance of the different livestock houses.

While several investigations have been carried out for the assessment, certification and improvement of the energy performance of human-related buildings (e.g. residential and retail), in the livestock sector there was not a similar attention. For this reason, the EPAnHaus (Energy Performance of Livestock Houses) project aims at defining an energy certification scheme that considers all the energy uses related to the control of indoor environment: heating, ventilation (both for cooling and Indoor Air Quality) and cooling. This project was developed through three different steps. First, an inventory of the energy use for climate control of the animal houses across Europe was made for determining benchmark values. Then, a calculation method derived from ISO Standards on building energy performance was customized for being applied to the animal production buildings to assess the energy use for climate control. It was applied into different calculation tools (some developed in cooperation with industry). Their outputs were used for defining the energy certification scheme. Finally, measurement campaigns were carried out in some livestock houses for testing and refining the models referring to the different housing types.

The use of the certificate will make available the possibility to know the actual amount of energy consumed for the climate control and information about retrofitting actions (e.g. simple payback period and energy savings).

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O159

Feeding behaviour of Massese lamb reared indoors

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Massese breed is commonly reared for milk production, but is also able to produce lambs with good growth performances and muscular development. These lambs are slaughtered at around one month of age, in order to let milk available for cheese production. Lambs reared up to two months of age and with higher weight (about 20 kg) represent an innovative product for this breed, and their rearing and feeding system should be properly characterized. The aim of this work was to study the feeding behaviour of Massese lambs reared up to 65-70 days of age. Behavioural observations were performed during autumn and winter on a group of 12 lambs reared indoors: animals were observed from birth to slaughter, every 7 days. Focal observations were performed by scan sampling at 5 minute intervals, from dawn to dusk. Lambs were kept in collective boxes with their dams, fed by suckling milk, hay and supplemented with pelleted concentrate. The daily observation time was grouped into four time slots (morning, midday, afternoon and evening) and the percent of time devoted to the recorded behaviours within each time slot was analyzed with GLM, using diurnal time slot as discrete variable and age as intraclass (time slot) covariate.

The amount of diurnal time devoted to total feeding activity progressively increased with age ($p<.001$), and amounted to 32% at 60 days of age. The growing time dedicated to food intake was mainly due to an increasing quadratic ($p<.001$) trend in hay consumption, which raised markedly until around the 40th day of age, particularly in the morning and in the evening. Also time devoted to concentrate consumption slightly increased with age, but without statistical significance. Milk suckling occurred equally in each part of the day and decreased with age according to a quadratic regression ($p<.001$), however the presence of the dam induced lambs to continue milk intake. Rumination activity started to occur regularly around the 2nd week of age and increased linearly with age. Lambs ruminated mainly in the middle of the day and in the afternoon, as a long interval among feeding activity.

The results indicate that milk consumption during the second month of life does not show a substantial reduction, therefore lambs could be separated from ewes at around 1 month of age because they are able to access spontaneously solid food and to devote time to rumination activity. Moreover, this would allow to obtain a higher yield of milk for cheese production.

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O160

The effect of different time epoch settings on the classification of sheep behaviour using tri-axial accelerometry

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Automated monitoring of foraging behaviour in grazing sheep can help to improve efficiency of animal production. Data from tri-axial accelerometers have been used to classify feeding behaviours in ruminants. These devices sample at high frequencies, which generates vast amounts of data and carries a cost in terms of battery consumption. To overcome this, an epoch, or size of aggregation window, can be set and applied to the data stream. We evaluated the effect of different epoch settings (5, 10, 30, 60, 120, 180 and 300 s) on the precision of behaviour classification for sheep wearing the BEHARUM tri-axial accelerometer and force sensor. This device stores the data in a secure digital card, or transmits it to a remote computer. The study was conducted in spring 2016 with Sarda dairy sheep that rotationally grazed berseem clover and Italian ryegrass for 6 hours day. Sheep behaviour was recorded by a fixed video camera. On four occasions, eight sheep were equipped with the BEHARUM devices, configured to store three acceleration values per second for each axis and for the force sensor. Mean, variance and inverse coefficient of variation (ICV; mean/standard deviation) of the recorded data were calculated for each epoch. Video recordings were coded manually, with behaviour during each epoch classified as grazing, ruminating or resting. The merged sensor and behavioural data were analysed for each epoch, by multivariate statistical techniques: canonical discriminant analysis (CDA), and discriminant analysis (DA). The CDA significantly discriminated the three behaviours (Hotelling's test $p<.001$) by extracting two canonical functions. To validate the derived discriminant functions, the complete dataset was randomly divided into training and validation dataset in the proportion of 85 to 15%. This partition of the dataset was iterated 1000 times using a bootstrap procedure. At each run,

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DA was applied to the training dataset to predict behaviours in the validation dataset and errors in assignment were recorded. The DA procedure achieved correct classification rates of 79, 87, 93, 89, 87, 86 and 79 per cent for the 5-, 10-, 30-, 60-, 120-, 180- and 300-s epoch settings, respectively. The 30-s epoch setting yielded the most precise predictions of sheep behaviour.

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O161

Effect of a flushing treatment based on a glucogenic mixture on sheep thermal imaging of body temperature and fertility

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Aim of this work was to study the effect of a flushing treatment based on a glucogenic mixture (70% glycerol, 20% propylene glycol and 10% water v/v) on ewes' body temperature and fertility. Thirty late-lactation Sarda ewes were divided into four groups homogeneous for days in milk, body weight, body condition score and milk yield. The groups were randomly allocated to one of the following treatments: Control (W) dosed with 200 ml of water and Treated (G) with 200 ml of the glucogenic mixture, with two replicates per treatment. The dosing was done by gavage at 8:00 and 19:00 on days 12-15 of the ovulatory cycle. The groups were daily stall-fed concentrate (400 g/ewe) dehydrated lucerne (900 g/ewe) and ryegrass hay (900 g/ewe), and were submitted to ram effect and flushing treatment in two consecutive weeks (WK1 and WK2). Body temperatures were measured on day 14 of the cycle at 8:00 (pre-dosing), 9:00, 9:30 and 10:00 using a digital thermometer, for rectum ampulla measurement (RT), and a thermal camera to record eye surface temperature (ET). During experimental days meteorological factors were monitored daily on hourly base and temperature humidity index (THI) and hours at THI >75 (THI-hr) were computed. THI and THI-hr were compared between weeks using t tests. RT and ET was analyzed by a mixed longitudinal model with treatment (T), measuring time (H), flushing week (WK) and their interactions as fixed effects and ewe within week as random effect. A further analysis of pre-dosing temperatures was run by a GLM inclusive of T, WK, ewe fertility rate (0 or 1) and their interactions. Chi square analysis was used to evaluate the effects of T and WK on the fertility rate. THI (69.7 *vs* 69.0, $p<.01$) and THI-hr (38 *vs* 20 hr, $p<.01$) were higher in WK2 than WK1. Longitudinal analysis showed that T x WK x H interaction affected all ET and RT ($p<.01$), with maximum and mean ET and RT higher in G than W and in WK2 than WK1. Pre-dosing maximum ET and RT were higher in infertile than fertile ewes ($p<.05$), and in G than W ($p<.01$). Mean pre-dosing ET was affected also by WK ($p<.01$) and the interaction between factors ($p<.05$). The fertility rate was not affected by T or WK, although it was numerically the lowest in WK2 ($p>.05$).

Flushing based on glucogenic mixture enhances body temperature of milked ewes mated under severe heat load. Monitoring eye temperature by thermal camera can help to detect distress conditions which impairs fertility.

O162

Ovine foot-rot: a welfare and economic concern

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Lameness is considered a major disease cost in sheep husbandry. In fact, the disease is associated with direct costs, such as treatment and control costs, and with indirect costs, due to the loss of sheep productivity. Even more importantly, lameness, which causes pain and can result in the loss of the hoof, can have a dramatic impact on animal welfare. Different causes are involved in lameness in sheep and one of the most important one is ovine foot-rot.

Foot-rot is considered endemic in different countries and it is caused by *Dichelobacter nodosus*, a Gram negative, obligate anaerobe bacterium, often isolated in association with *Fusobacterium necrophorum*, another anaerobic, non-spore-forming Gram negative bacterium. This report shows *preliminary data* on the detection of *D. nodosus* and *F. necrophorum* by molecular analysis in sheep with different clinical conditions of lameness. Hoof swabs (n = 285) were analysed for *D. nodosus* and *F. necrophorum*. The swabs were collected in flocks were more than 10% of animals had foot lesions and

lameness. The samples were collected from 59 Italian farms from March 2015 to February 2017. For the detection of *D. nodosus* and *F. necrophorum*, two PCRs, one for *pnpA* and the other for *IktA* genes, respectively, were carried out. The *pnpA* gene was detected in 47.1% out of the 285 swabs ($n = 137$), 45.6% were positive for the *IktA* gene ($n = 130$), while 24.2% were positive for both *pnpA* and *IktA* gene ($n = 69$). Among the 59 farms investigated, 57.6% were positive for *D. nodosus* ($n = 34$), 74.6% for *F. necrophorum* ($n = 44$) and the co-infection was found in 52.5% of the flocks ($n = 31$). In Italy, few data are available about the epidemiology of ovine foot-rot. The study shows a high proportion of samples and farms positive for *D. nodosus* and *F. necrophorum*. These preliminary data could be pivotal to further investigate the prevalence and impact of foot-rot in sheep flocks in Italy. A proper management of the disease has not yet achieved in many farms, and in some cases, it seems obsolete. On the other hand, the control and eradication of foot-rot can be difficult and very expensive, once the infection is established in one flock. In this scenario, a prompt diagnosis is the first step towards improving an appropriate management, prophylactic and treatment protocol of foot-rot in sheep farms.

O163

Endoparasites in sheep and goats: prevalence and control strategies of mountain farms in Northern Italy?

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One of the major constraints for sheep and goat production are infections with endoparasites, in particular gastrointestinal nematodes (GIN), which are commonly controlled using medical treatments. However, its intensive use increases the risk for the development of resistant parasite populations. For mountain livestock farming, where small ruminants of various breeds are mainly kept in small herds, it remains questionable how effectively parasites are controlled. Therefore, the aim of the study was to: 1) assess the prevalence of endoparasitic infections of goats and sheep in South Tyrol and 2) evaluate the use of routinely conducted control measures. A total of 94 sheep and goat farms were surveyed with a questionnaire to collect data on farm management and control strategies against parasites. Additionally, more than 3400 individual faeces samples were collected in three consecutive seasons (autumn 2015, spring and autumn 2016) and faecal egg counts (FEC) were performed using a modified McMaster technique. Based on log-transformed FEC-values, a mixed model was used with the fixed effects of species, breed within species, age group, season and resulting two-way interactions. Results are presented as LS Means of untransformed FEC values. According to the survey, 6% of the farmers do not treat their animals with anthelminthics, while 44% do it once and 50% twice per year. Commonly the whole herd is treated (75% of the farms), while 78% of the farms never determines the infection status using faeces samples. The most common active substances used were so far ivermectin (54%), albendazole (24%) and eprinomectin (8%). Overall, 16, 23 and 24% of the samples were FEC-negative in autumn 2015, spring and autumn 2016, respectively. Goats showed an average number of eggs per gram of faeces of 542 and sheep of 424 ($p > .05$). Lambs/kids as well as adult males showed higher infections ($p < .05$) compared with adult females. In both species, differences between the breeds were found ($p < .05$). The prevalence of tapeworm-positive animals was higher in autumn (9% in 2015 *vs* 13% in 2016) than in spring (2%). In conclusion, high infections warrant the intensive use of anthelminthics. Nevertheless, through a more sustainable use of medical treatments using faeces sampling to monitor the actual infection status of the animals the risk of the development of resistant parasite populations should be reduced.

O164

Growth and morphometry of farmed fallow deer (*Dama dama* L., 1758) from birth to six months of age

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The dynamics of animal growth have been widely studied in domestic species, primarily in relation to their productive potential, but have not been equally investigated in wild species, especially regarding the early weeks of life. The aim of this work was to examine some aspects of the morphometric growth in farmed Fallow deer (*Dama dama*) fawns, from birth to the age of six months. Three male and three female fawns were artificially fed *ad libitum* with acidified dried milk for lambs and, since the 8th week of life, also with weaning pellets and alfalfa hay. Body weight and several linear measurements were recorded weekly. The ratio trunk length/chest circumference (TC) was calculated. All data were analysed separately for the sexes using Linear Regression, with age as continuous variable. After logarithmic transformation of the data, the growth of measurements in relation to body weight and height at withers was investigated using the allometric function.

Male fawns displayed a more rapid increase of live weight: males and female attained 36 kg and 32 kg at six months of

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age respectively. Stature increased by 60%, from on average 43 cm at birth to nearly 70 cm at six months of life.

Similar growth patterns for both sexes were observed in height at withers and at rump, chest length and circumference, rump length, shoulder and hip width. Rump resulted higher than withers, while shoulders and hip width had a comparable accretion; all these measures doubled their dimension in the six-months period. Differences between sexes resulted for head length, greater in males, and chest depth, greater in females; trunk length, similar among sexes at the age of six months, showed a different trend, linear in females and quadratic in males. TC ratio increased until the 3rd month of age and then reduced, expressing the preliminary elongation of the trunk followed by the development of the transverse diameters.

The allometric analysis confirmed the precocity of the head accretion and of the body heights respect to trunk elongation and its development in transverse diameters. In females emerged a precocity in the rachis elongation and a belated development of transverse diameters, especially in pelvic bones.

In this early phase of life, the most rapid growth processes involve the elongation and the volumetric development of the trunk, similarly to physiologically related domestic species (eg. lambs).

O165

Fecal sample time-points to estimate uNDF240 excretion in dairy cows

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The aim of the current study was to evaluate the effect of fecal sampling time in the determination of the undigested Neutral Detergent Fiber (uNDF240h) excretion, used as a marker for aNDFom digestibility calculation. The aim was to identify an easy way to estimate the uNDF240h excretion in a commercial dairy farm with an unique time-point sampling.

Four dairy cows, paired for lactation number (2), days in milk (184 ± 38d), milk production (35 ± 2kg), body weight (664 ± 50kg), and daily rumination time (564 ± 50 min), were housed in tie-stalls for individual dry matter intake (DMI) determination. After an adaptation period to the tie-stall (7d; the diet did not change compared to the one they were fed in the group), fecal collection started. The feces were collected every 2h (12 time-points per day), and every sample was divided in three subsamples: one was kept separate, one was composed to create a 4 time-points sample (c08-14h, c16-22, c00-06), representative for the morning, afternoon, and night excretion; the latter was used to create a composite of the whole day (c24 h). The sampling period lasted 3 days. During the whole study, DMI, rumination time, and milk production were recorded daily, while the TMR was sampled every day. The fecal and feed samples were dried (65 °C), ground, and analyzed to determine the chemical composition. The TMR was analyzed for the physical composition (Ro-Tap).

The data were analyzed statistically using JMP-12 (SAS Institute Inc., Cary NC). A mixed model was used, with collection time-points and day considered as fixed effect, while cow as random effect. The TMR during the experimental period was uniform in its physical and chemical composition. Daily DMI was 24.5 ± 2.9 kg, the rumination time 452 ± 87 min, the milk production 34.0 ± 4.0 L. The mean uNDF$_{240}$ excretion during the day was 32.4 ± 1.0% DM; some significant differences were detected comparing the 12 time-points, most of all between the morning samples (10 and 12h) and the night ones (18, 20, 22, 2, 4h; $p<.05$). The greater excretion was at 18h and the lower at 8h (feed delivery). No differences were detected comparing the 12 time-points and the 24 h composite, except for the 18h ($p=.048$).

We can conclude that the uNDF$_{240}$ excretion was not linear, with a minimum reached at feed delivery and a maximum reached 10h after. Thus, to estimate the uNDF$_{240}$ excretion in a commercial dairy with an unique time-point sampling, the samples should be taken 2-4h to 8h from the feed delivery.

O166

Transcriptome analysis reveal sex-specific gene expression in lambs supplemented with essential oils

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Essential oil (EO) dietary supplementation can be a successful strategy to improve animal health, welfare and performance due to its various beneficial effects. It is known that EOs can improve gastrointestinal functions, modulate ruminal methane emission, stimulate immune response and delay meat lipid oxidation. However, there is lack of information about EO effects on the expression of genes influencing growing performance of lambs.

In this nutrigenomic study the effect of a mix of EOs on gene expression was evaluated via RNA-seq in 16 lambs. Recruited lambs (8 males and 8 females) were divided in two homogeneous groups (EO and control) fed with or without dietary supplementation of 3 mL of cinnamon bark, eucalyptus leaf and dill seed essential oils (1:1:1) for 30 days.

Total RNA isolated from liver and muscle samples was sequenced using an Illumina platform. STAR aligner was used to map the reads to reference genome (*Ovis aries* v.3.1) and *per-gene read counts* were generated with HTSeq count.

Using DESEQ2 and WGCNA R package, differentially expressed (DE) genes were revealed and co-expression network built.

Nine DE genes (*FDR*<.05), all down regulated in the EO group, were identified in liver: *MANF, FGB, FGG, DNAJB9, HSP90B1, UFM1, SAA2, EEFE1* and *TMEM45A*. In the same group, 6 DE genes were identified in muscle (FDR <0.05); of these, 4 were down regulated (*MYLK4, ASPN*, novel Mt t-RNA and uncharacterized protein) while 2 were up regulated (*TRIM45* and *STAMBPL1*).

Cluster of co-expressed genes highly correlated to the dietary treatment were identified using WGCNA and 5 regulator genes, identified only in the liver tissue using WGCNA and

LemonTree algorithm (*DNAJB9, MANF, UFM1, CTNNLA1* and *NFX1*), were significantly influenced by the EO diet.

The results revealed that the EO diet influences the expression of genes involved in immune, inflammatory and stress responses, primary homeostasis and blood coagulation. It is worth mention that genes involved in inflammatory, immune and stress response pathways were down regulated exclusively in the liver of males fed the EO diet. Indeed, both DE genes and gene co-expression network analyses revealed that the EO diet had a sex-dependent effect.

This study shed light on the potential effects that EO dietary supplementation might have on lamb metabolism. However, future studies are necessary to understand the biological mechanisms which underlie the interaction between sex and EO metabolic activity.

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O167

Herbage intake of Sarda suckler cows characterized by two different calving periods in a mountain pasture

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Beef livestock system in Sardinia is based on cattle grazing pastures all year round and calves that follow their mothers at pasture until weaning at about 6-7 months of age, when they are mainly sold to the fattening centres. The cows belonging to native, non-specialized breed, such as Sarda and Sardo-Modicana, are mated with specialized sire breeds (Limousine and Charolaise) to combine the environmental adaptability of the local cows with the growth capacity of specialized breeds, obtaining calves characterized by better growth and conformation. The study aimed to estimate the herbage intake and performance of Sarda suckler cows (mated with Charolais bulls) with different calving periods (Autumn, A and Spring, S) and hence with different energy requirements. The experiment was carried out in spring 2015 (March-June) at Foresta Burgos experimental farm (latitude 40°25'N, longitude 8°55'E, altitude 850 m a. s. l.) of AGRIS Sardegna. Two groups of 5 mature suckler cows [group A, body weight (BW) 453 ± 74 kg (means ± st. dev.) with calves born in autumn (152 ± 9 days old) and group S, BW 415 ± 37 kg with calves born in spring (46 ± 18 days old)], were compared. The cows were unsupplemented and grazed all together a natural pasture area of about 8 ha with a stocking rate of 511 kg BW/ha. In one occasion (May) herbage mass on offer was measured by cutting 8 0.5 × 0.5 m quadrats at ground level and analysed for chemical composition. Herbage intake of cows was estimated by the n-alkane method. Herbage selected by the animals was obtained by mimicking cattle feeding behaviour. Body weight of animals was assessed at the beginning and the end of the experiment. Data on herbage intake and changes of cow BW were analysed by a linear model procedure of R using calving date as fixed effect. The forage allowance was 22 kg DM/100 kg body weight and the herbage was featured by medium-low quality (DM 30.5 ± 4.7, CP 11.7 ± 1.4, NDF 50.4 ± 2.9, ADL 3.1 ± 0.2). Herbage intake (kg DM/100 kg BW), was lower in A than S (2.94 ± 0.22 *vs* 3.67 ± 0.22, lsmeans ± s.e, $p=.053$). Throughout the experimental period, the animals increased their BW with higher values in A than S (53.4 ± 5 kg *vs* 7.6 ± 5, $p<.001$). The spring calving cows, with younger calves and, therefore, with greatest energy needs for milk production, showed higher herbage intake than autumn calving cows. The latter likely directed their energy intake towards greater body weight recovery. These results can help to manage beef livestock system in Sardinia.

O168

Effects of bentonite inclusion in naturally aflatoxin contaminated diet on aflatoxin excretion and milk production of Indonesian Friesian Holstein

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The efficacy of natural bentonite in reducing aflatoxin transfer in cows routinely fed aflatoxin-contaminated diet was investigated in this study. A cross over research design was applied to eight Indonesian Friesian Holstein (IFH) cows. Cows have randomly received a diet contaminated naturally aflatoxin B1 (AFB1), containing 47 ppb AFB1 (AFB1D), and a diet with 0.1% natural bentonite inclusion (BD). After a period of 15 days, diets were switched. Milk production was recorded daily and milk samples were collected at lasted 3 days of each period for the determinations of aflatoxin M1 (AFM1) content and milk composition. AFB1 and AFM1 contents were assayed using ELISA kits and milk compositions were determined according to AOAC methods. Data was analyzed by a paired t-test using SPSS version 16.0. Results showed AFM1 content and carry-over rate (COR) of cows fed natural bentonite (BD) were significantly lower ($p<.01$) than AFB1D, namely 40 *vs* 46 ppt and 0.10 *vs* 0.11, respectively. Milk production and composition were higher in the diet with natural bentonite inclusion compare to the AFB1D, however these were not significant different ($p>.05$), namely 5.7 *vs* 5.6 L/day on milk production, 4.2 *vs* 4.0% on milk fat content, and 8.6 *vs* 8.5% on milk total solid non-fat. In conclusion, this study showed natural bentonite inclusion could alleviate the adverse effects of aflatoxin exposure on aflatoxin transfer into milk and milk production of IFH cow.

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O169

Effects of partially replacing cereal grains with soyhulls on energy partitioning in ewes and goats in mid lactation

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This study explored the possibility that dietary carbohydrates sources rich in starch or in digestible fiber differently affect the energy partitioning between milk production and body reserves accumulation of dairy ewes and goats in mid lactation, a period in which ruminants are generally highly prone to insulin action and thus to body reserve accumulation.

Thirty Sarda ewes and 26 Saanen goats were compared from 92 to 139 DIM using two dietary treatments (on DM basis): high starch (HS: 20.0% starch, 36.7% NDF, 15.5% CP) and low starch-high digestible fiber (LS: 7.8% starch, 48.8% NDF, 15.6% CP) diets. The diets contained 29% of dehydrated chopped alfalfa, 4% of chopped mature ryegrass hay, and 67% of experimental pellets fed *ad libitum*. The LS diet was obtained by substituting most of the cereal grains of the pellet of the HS diet with soyhulls.

Milk production was measured once a week. BCS was estimated every two weeks. Blood samples were collected before the morning meal at DIM 126 and 134 on 20 ewes and 20 goats (10 per diet) selected to be representative of their group for milk yield and BCS. In addition, at the end of the trial, a post-prandial blood sampling was performed at 30, 60, 120, 180 and 240 min after the morning meal. Data were analyzed with the PROC MIXED procedure of SAS for repeated measurements.

In goats the LS diet resulted in lower fat corrected milk yield (FCM) (2.53 *vs* 2.65 kg/d ± 0.05; $p<.05$), than the HS diet, with no effect on BCS whereas in ewes LS had a positive effect on FCM (1.47 *vs* 1.36 kg/d ± 0.04; $p<.01$) with a lower BCS recovery (final BCS: 3.33 *vs* 3.46 ± 0.05; $p<.05$) compared with HS.

The hormonal and metabolic status were not affected by the diets but differed between the two species both in the pre-feeding samplings and the post-prandial samplings. Goats had lower plasma glucose (48.3 *vs* 56.0 mg/dL ±1.7; $p<.0001$) and insulin (0.14 *vs* 0.38 μg/L ± 0.05; $p<.0001$) concentration and higher plasma GH (2.62 *vs* 1.37 ng/mL ±0.58; $p=0.038$) and leptin (24.72 *vs* 11.97 ng/mL ±2.13; $p<.0001$) concentration than ewes. Similar results were obtained with the post-prandial blood samplings.

In conclusion, in mid lactation the partial replacement of starchy feeds with sources of highly digestible fiber increased milk persistency in ewes but not in goats, which instead were favored by the starch-rich diet. Differences in hormones and glucose between goats and sheep confirmed the better aptitude of Saanen goat to milk production than Sarda sheep.

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O170

Effect of the presence/absence of long hay and the administration of *ad libitum* or restricted total mixed ration

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The objective of this study was to investigate in high producing cows diets, the effects of presence or absence of long hay and the administration of *ad libitum* or restricted total mixed ration (TMR) on feed intake, digestibility, ruminal pH and productive performance. Treatment one (24 hH+): feed available at the feed bunk for 24 h, with long grass hay; treatment two (24hH-): feed available at the feed bunk with no long grass hay; treatment three (19hH+): restricted TMR (19 h/d), with long grass hay; treatment four(19hH-): restricted TMR, with no long grass hay. Eight multiparous Holstein cows were assigned to a 4 x 4 Latin square design, with 3 weeks adaptation and 1 week of collection. Dry matter intake, milk production and quality, and rumination time, measured by collar, were measured daily. Rumen pH was measured continuously by ruminal bolus. Rumen liquor was collected two times point and analysed for ammonia and VFA. Fecal samples were collected at multiple time points and than weekly averaged. Diets and feces were analyzed for aNDFom, ADF, ADL, uNDF$_{240}$ and pdNDF, in order to calculate total tract fiber digestibility. During the experimental week of each period, diets were sampled daily and analyzed for chemical composition. All data were statistically analyzed with a factorial arrangement of treatments using the MIXED procedure of SAS(v9.1). The results obtained showed the diet available for 24 hours improve significantly dry matter intake (27.36 *vs* 23.27 in 24 h and 19 h $p<.05$) and tend to improve NDF intake (8.79 *vs* 7.73 in 24 h and 19 h respectively $p<.1$). Milk production tends to be higher in cows receiving long hay (37.87 *vs* 36.56 kg/d in F + and F-, respectively $p<.1$). When calculating the ECM that consider the milk fat and protein content, the difference became significant (40.26 *vs* 38.23 in F + and F- respectively $p=.01$). However it was detected for the first time as far as we know, that a restriction of TMR availability for 5 hours could led to an increase significantly in feed efficiency(1.91*vs*1.40 in 19 h and 24 h respectively $p<.05$) The pH average results obtained testify to a situation concerning the physiological pH of the rumen for the type of animals in experiments. There were no difference in rumination time, average ammonia and ruminal VFA. The conclusions obtained in this experimentation showed that in our condition the administration of TMR constantly available, associated with the presence of long hay, offers the best conditions of ruminal function and pH stability.

O171

RNA sequencing-based transcriptome profiling of dairy cows fed with a polyphenol rich grape pomace supplemented diet

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The inclusion of some agro-industrial by-products in animals' diet is becoming attractive not only for being part of an ambitious waste management and sustainability policies but also due to its possible nutritive values. The aim of this study was to evaluate the effect of grape pomace (GP) - the polyphenol-rich by-product of the wine industry – on the transcriptome of dairy cows. Twelve lactating Holstein Friesian cows, homogeneous for age and lactation period, were assigned to two groups of six animals each - in a randomized pretest-posttest control group design. The first group received a basal diet and served as a control (CTR), while the other received a 10% GP-supplemented diet for 67 days. Whole blood was collected from each group at 2 time-points [beginning (T0), and after 67 days of the GP supplementation (Tf)], then total RNA was isolated, quality-controlled, and then used for library preparation. The sequencing of twenty-four samples (2 groups × 6 animals/group × 2 time-points) resulted in an average of 17 million reads per sample. The 50bp single-end reads were quality-controlled, mapped to the *Bos taurus* reference genome (UMD 3.1 assembly), then tested for the presence of differentially expressed genes (DEGs) in the same group (CTR or GP) after (compared with before) 67-days-supplementation period (Tf *vs* T0). On average, 95.6% of the reads were mapped to the reference genome. Reads mapped to exons were counted with HTSeq-count, then analysed by the DESeq2 R package. The bioinformatics analysis evidenced a significant (adjusted $p<.1$) change in expression of 14 and 88 genes in the CTR and GP groups, respectively. Four genes were found to be overlapping between the two groups, thus they were excluded from the GP group results as being 'temporally'- and not experimentally-affected DEGs. Of the remaining 84 GP-affected genes, 73 were down-regulated, with most of them being 'ribosomal protein'-coding genes.

The functional analysis evidenced the positive enrichment of ‘defence response to other organism’ (p=.0002) biological process and the ‘interleukin signalling’ pathway (p=.0002), as well a negative enrichment of the ‘ribosome’ pathway (35 genes, $p = 5\text{x}10^{-55}$). Overall, the transcriptomic signature of GP-supplemented diet reflects an induced immune system and a suppressed ‘ribosome biogenesis’, which can be ascribed to the GP’s polyphenol-derived antioxidant effect.

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O172

Prevalence of *Escherichia coli* from lymph nodes of bovine carcasses and detection of stx1, stx2 and eaeA genes with multiplex PCR

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The lymphatic system, particularly the lymph nodes, can be a source for meat contamination due to their function as filters. During the slaughtering process most of the lymph nodes are removed from the carcass with an exception of the iliac, which remain attached to the carcass. These lymph nodes, which drain the recto-anal-junction area, represent the major site of colonization for *Escherichia coli* O157:H7 in bovine and are usually included in ground beef production. The aim of this research was to estimate the prevalence of *Escherichia coli* in bovine lymph nodes potentially involved in ground beef production and detect the presence of stx1, stx2 and eaeA genes by multiplex Polymerase Chain Reaction (PCR) after extraction of bacterial DNA. Internal and external iliac lymph nodes were collected from 467 carcasses from regularly slaughtered bovine at a commercial slaughterhouse in central Italy. *Escherichia coli* prevalence (ISO 16649-2:2001) in the lymph nodes samples was 32.33%. One of stx1, stx2 or eaeA genes was detected by PCR in 27.81% of samples; stx1 in 13.25%, stx2 gene in 14.57% and eaeA gene in 7.95%. 3.97% of samples were positive for stx1 and stx2 genes, 1.32% for stx1 and eaeA genes and 3.31% for stx2 and eaeA genes. All genes stx1, stx2 and eaeA genes were found in 0.66% of the samples. The association between each potential risk factor and prevalence was assessed using a Poisson regression model of the number of positive samples with the number of samples tested as an exposure variable. Variables associated with the outcome ($p<.20$) were then entered into a multivariable Poisson model, with number tested as exposure. A multivariate logistic regression was then performed with StatView 5 for MacOS (SAS Inst. Inc., Cary, NC, USA). Age ($p<.005$), length of travel to slaughterhouse ($p<.05$) and organs condemnations ($p<.05$) were statistically associated with *Escherichia coli* positivity in tested samples and with stx1, stx2 or eaeA positivity at PCR. These findings suggest that meat contamination does not come only from faces or the environment and focuses the attention on which methods should pair with modern trends and revisions of procedures for ante-mortem and post-mortem inspection for meat safety.

O173

Characterization of verotoxin-producing *Escherichia coli* (STEC) in dairy cattle's faeces from Pretoria, South Africa

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Shiga toxigenic *Escherichia coli* (STEC) are food born pathogens that cause diarrhoea, haemorrhagic colitis and hemolytic-uremic syndrome (HUS) in humans. Although STEC-associated illness in humans has been attributed mostly to *E. coli* O157:H7, also non-O157 STEC can cause disease and may be highly virulent. It is well documented that the *E. coli* strains differ widely in their ability to cause human disease, colonize animal carriers, and survive in the environment.

In this study 76 bacterial strains of *E. coli* isolated from rectal faeces samples of dairy cattle from three different farms in Pretoria, South Africa, were screened by polymerase chain reaction (PCR) for genes stx1, stx2 and eae, and serotyped by standard procedures. Twenty-six strains (34.2%) belonged to O-nontypable (ONT):H2 serotype, eight (10.5%) to O178:H19, eight (10.5%) to O48:H21, seven (9.2%) to O82:H8, four (5.3%) to O19:H19, four (5.3%) to O5:H7, one (1.3%) to O113:H21 and 18 (23.7%) to other serotypes. Virulence profiling of the isolates revealed that 23 strains (30.3%) were stx1-positive, 14 (18.4%) were stx2-positive, 39 (51.3%) were stx1 and stx2-positive and two (2.6%) were eae-positive. The O113:H21 serogroup was stx1-negative, stx2-positive and eae-negative. STEC overall prevalence was 51.3% and strains belonged to 25 different serotypes, including O5:H7, O8:H19, O48:H21, O60:H19, O82:H8, O88:H25, O108:H-, O113:H21, O130:H-, O130:H11, O141:H8, ONT:H2, ONT:H11 and ONT:H19 that have been associated with disease in humans. VTEC strains isolated from patients with HUS have been reported to belong to O8:H19, O48:H21, O113:H21 and O130:H11 serotypes. The finding that a stx2 strain of known human-pathogenic serogroup O113:H21 occurs in 1.3% of the isolates and the consideration that such strain, even lacking eae, has been responsible for clusters of cases of haemolytic-uremic syndrome and for sporadic cases, requires a thoughtful reasoning. In fact, although historically less common, the

severity of disease caused by these strains may be no less than that caused by recognized "enterohaemorrhagic" STEC serogroups such as O157 and O111. More widespread use of PCR- or enzyme-linked immunosorbent assay-based screening tests for the presence of STEC of any serogroup in animal samples will undoubtedly result in increased detection of similar non-O157 outbreaks in the future. This will provide more accurate data on the epidemiology of human STEC disease.

O174

Effect of lactic acid bacteria on the quality of beef hamburger under different storage conditions

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Meat products and meat preparations are very sensitive to external factors such as temperature and are good substrates for microbial growth and biochemical processes that occur during storage. The aim of this work was to study the effect of a selected lactic acid bacteria formulation (LAB) on the microbiological characteristics and colour of beef hamburgers stored at different temperatures. Two batches of hamburgers (average weight of about 100g) were prepared: one with the addition of LAB (*Lactococcus lactis* ssp. *lactis*, strain 340; *L. lactis* ssp. *lactis*, strain 16; *Lactobacillus casei* ssp. *casei*, strain 208 and *Enterococcus faecium* strain 614 in a ratio lactococci:lactobacilli:enterococci of 2:1:1; level of inoculum 10^7 cfu g^{-1}) and one without (NO LAB), in three replicates done in three different days. For each batch, a subset of samples was stored under proper conditions (4 °C) and the other at 40 °C (temperature abuse conditions, to mimic inadequate storage conditions after purchase by consumer) both for 5 hours. After 5 hours, each subset was further divided into two subgroups: one maintained at 4 °C and the other at 10 °C. All hamburgers were evaluated on day-0, day-1, day-3, and day-5 for the following microbiological parameters (*Staphylococcus* spp., enterococci, *Lactococcus* spp., *Lactobacillus* spp., total mesophilic aerobes, *Pseudomonas* spp., total coliforms) according to standard methods; moreover colorimetric measurements were performed with Colorimeter - Digital Color Picker for iOS 10, under a 6500K light, with the CIELAB system, by taking three readings for each sample. The arithmetic means within each sampling was computed, subsequently all data (geometric mean for microbiological data) were elaborated with GraphPad InStat, 3.0b and GraphPad Prism 6.0d for Mac OS X. Two way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test was performed. On day-5, all batches with LAB kept at abuse temperature and stored at 4 °C had a higher blue-yellow, green-red and lightness coordinates compared to the batches made without LAB maintained at the same conditions. On day-5 *Pseudomonas* spp. (PS103) counts were significant lower ($p<.05$) in all batches made with LAB; *Staphylococcus* spp. (BP) counts were significant lower ($p<.05$) in batches made with LAB in abuse conditions.
In conclusion, the application of the proposed LAB formulation maintains hamburgers quality standards and can be a potential tool to increase their shelf-life.

O175

Former food products safety: stereomicroscopy and computer vision for evaluation of packaging remnants contamination

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Valorisation of former foodstuffs products (FFP) as feed ingredients is part of a long-term strategy for sustainability. Processing methods to convert FFP in to feed ingredients do not usually include packaging materials pre-removal. Feed processors routinely remove the packaging from surplus food mechanically. Although, the treatment in the plant removes most of the packaging, small amounts of wrapping materials can remain in the resulting feed. In this respect, the aim of this study was to investigate the safety features of selected FFP intended for animal nutrition produced from different confectionery products. In six FFP samples, both mash and pelleted, the presence of undesired ingredients which can be identified as remnants of packaging materials has been evaluated by two different methods. The first analysis has been done by stereomicroscopy, according to published methods, based on separation of every particle that is not native to the matrix by bare eye examination. In the second one, stereomicroscopy coupled with a computer vision system (IRIS Visual Analyzer VA400), has been tested in order to evaluate the presence/absence of packaging remnants in feed materials. Results obtained have been presented as percentage of packaging material in feed, expressed as w/w in the case of the stereomicroscopic method and as a colour spectrum representing the proportion of each colour on the FFP surface, within a fixed scale of 4096 colours, in the case of computer vision system (CVS). The visual pattern recorded for each sample with CVS was processed using Statistical Quality Control (SQC) model. The stereomicroscopy approach revealed that the contamination level was below to 0.08% (w/w), within the tolerance level established by BMELV. Of note, the packaging remnants were observed mainly from the 1-millimeter sieve mesh fractions. Computer vision system, through the SQC model, revealed the possibility to rapidly detect the presence of packaging remnants in FFPs when combined with stereo-microscope. Concluding, even though the validated method (RIKILT) remain the most assured for detection and quantification of packaging materials in FFPs, it results laborious and ineffective regarding the smallest packaging remnants. In comparison, the use of CVS coupled with stereomicroscopy has shown a big potential in a rapid qualitative analysis also in low contaminated ex-food and could be considered effective in defining further analysis or investigations in FFP.

O176

Camelina cake in laying hens diet to enrich eggs with omega-3 fatty acids

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The aim of the trial was to evaluate the inclusion of 7.5% of camelina cake in the diet of laying hens on performance, egg quality characteristics, fatty acids composition and lipid oxidative stability. Thirty-two 26-weeks old Hy-Line Brown laying hens coming from the same flock were divided in two homogenous groups and allocated in enriched cages (8 replicates per treatment) at the Experimental Station (Centro Zootecnico Didattico Sperimentale) of the University of Milan in Lodi. After an adaptation week, the trial lasted 8 weeks. Diets were formulated to meet requirements suggested for the genetic line and were isoproteic and isoenergetic. Performance were recorded weekly and two eggs per replicate were sampled on day 0, 28 and 56 of the trial. On the same samples, two yolks per replicate were pooled for fatty acid composition. Samples of two eggs per replicate were also collected at the end of the trial to evaluate egg quality characteristics and oxidative stability during egg shelf life (day 9, 21 and 28 from laying); for this aim, pooled yolk were analyzed for TBARS content. Data were analyzed by MIXED procedure by SAS. No differences were observed for hens' body weights and eggs production during the trial, whereas feed intake and feed efficiency, expressed as feed intake over egg weight yield, were lower at week 6 and overall the experiment, respectively. No differences were detected for egg quality characteristics between treatments. Dietary camelina reduced by 3.5% saturated fatty acids and increased by 49% α-linolenic acid in egg yolk. No differences between treatments were evidenced for quality of eggs and TBARS yolk content during eggs shelf life. Inclusion of 7.5% of camelina cake in laying hens' diets could reduce production costs and increase nutritional properties of eggs.

Acknowledgements

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O177

Dietary administration of olive mill wastewater extract to improve broiler performance and oxidative status in chicken meat

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Olive oil extraction generates high amounts of by-products considered as potential pollutants. By-products are treated as industrial wastes or combustible material, heavy-metal absorbers and biofuel feedstock. One important alternative, considering the relatively high content in polyphenols, is the use of by-products from the olive oil industry as sources of nutrients for animals.

An olive mill wastewater polyphenols extract (OPE), obtained from aqueous waste through the use of a filtration system with progressive permeability membranes, was used as a supplement in a grower diet fed to broilers from 22 to 49 days of

age. To facilitate feed processing, OPE was preliminary mixed with a maize meal. The enriched maize meal was used in two concentrations: 16% (L-OPE) and 33% (H-OPE).

A total of 297 22-day-old female chicks (Ross 308) were assigned to 3 experimental grower diets (control, L-OPE, and H-OPE) with 3 replicates of 33 birds each. The same starter diet was administered up to 21 days of age. The birds were raised in an experimental farm, where industrial farming conditions were reproduced.

The effects of OPE extract on performance, oxidative status and quality traits of meat were evaluated. Live weight was influenced ($p<.001$) by diet at day 35, 42 and 49, with L-OPE birds showing higher values (1112.90 g, 1560.61 g and 2136.93 g, respectively) than those fed the CTR (985.87 g, 1357,50 g and 13912,22 g, respectively) and H-OPE (992.42 g, 1338.26 g, 1860.64 g respectively) diets. Average daily gain values followed the same trend in the periods 28-35 and 35-42 days.

As for meat quality, no differences were register among groups. The diet significantly affected the antioxidant status of meat: higher ORACFL values for the L-OPE and H-OPE groups (28.72 and 31.15 μmol TE g^{-1}, respectively), compared to CTR (23.10), were registered. Meat oxidative stability was modified: L-OPE and H-OPE TBARS values (0.05 and 0.04 mg MDA kg^{-1}, respectively) were higher than those recorded for CTR (0.07 mg MDA kg^{-1}). In conclusion, the results indicate that a dietary supplementation with OPE can positively affect chicken broiler performance and meat oxidative stability without any negative influence on quality traits.

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O178

Olive pomace diet supplementation of Cinta Senese swine: effect on fatty acid composition and on lipid peroxidation of sausages

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The aim the study was to evaluate the effects of stoned and dried Olive Pomace (OP) in the diet of Cinta Senese (CS) a native swine breed from Tuscany, in order to improve the protection against lipid oxidation (LO) of CS Meat Sausages (MS).

The study was carried out on 36 males of the CS swine fed according to the following dietary treatments: group C (control) without lipid supplementation, group OP received 25% stoned and dried OP in the diet; group SO, received of 3.2% high oleic sunflower oil in the diet. OP and SO diets contained the same amount of oleic acid.

For sausage manufacturing, 2 steaks corresponding to the ultimate and penultimate dorsal vertebrae were sampled. The lean and the backfat without the rind portions were mixed in a proportion of 70/30 lean/fat. The MS were stored at 4 °C for 7 days.

Fatty Acids (FA) composition, tocopherols, polyphenols, Thiobarbituric Acid Reactive Substances (TBARS) and Cholesterol Oxidation Products (COPs) were evaluated in both Raw Mixtures (RM) and MS after 7 days of storage. Aroma profile (VOCs) was also evaluated in MS.

Data regarding the RM and MS were processed separately with a randomized linear model: $y_{ij} = \mu + DT_i + R_j(DT_i) + e_{ij}$ where, y = variables; μ = common mean of all the observations; DT_i = fixed effect of the i^{th} dietary treatment (C,OP,SO); R_j = random effect of the j^{th} replication nested within DT (first and second experiment replication); e_{ij} = random error.

Data regarding VOCs were analysed with a canonical discriminant analysis (CDA).

Supplementation with OP and SO strongly affected the FA composition of swine meat. The RM from swine fed OP and SO diets were higher in polyunsatured FA (PUFA) and lower in satured FA content than C mixtures, as consequence of vegetable oil enrichment. Despite the higher content of PUFA, the LO evaluated from TBARS assay and COPs determination, resulted significantly lower (-14%, $p < 0.01$ and -66%, $p < 0.01$, respectively) in both the OP raw mixture and sausage samples. On the contrary, SO diet showed a lower effect on LO protection than OP. This effect was probably due to the transfer of antioxidant from OP to the meat, as suggested by the content of 3,4-DHPEA and α-tocopherol in the OP raw mixture. CDA of VOCs showed 3 canonical (CAN) able to discriminate: RM from MS (CAN1), PO MS from C and SO ones (CAN2), SO MS from C and PO ones (CAN3). In conclusion the enrichment of OP in CS swine diet improve the protection against LO of CS sausages and also their nutritional values.

O179

Effect of long term supplementation with *Lippia spp.* extract on meat quality parameters in heavy pigs

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In the recent year, considerable attention has been focused on the improvement of meat quality parameters. The general trend towards the reduction of synthetic substances since the restriction in 2006 of the use of antibiotic has increased the interest in studying natural substances. The aim of the study was to investigate the effects of long term dietary supplementation in pig with plant extract (PE) from *Lippia spp*., titrated in verbascoside (5 mg/kg feed), from weaning to slaughter on growth performances, nutritional profile and meat quality parameters. Eighteen pigs of initial live weight (LW) of 23.6 kg were assigned to two experimental groups: the first fed a basal diet (CON) and the second one fed a basal diet supplemented with hydroalcoholic plant extract (PE) from *Lippia spp.* for 7 months. At slaughter (169.8 kg LW), the *longissimus dorsi* (LD) muscle was sampled from all pigs ($n = 18$), vacuum packaged and stored at -20 °C for 14 days until physical and chemical analyses. The data were analysed by one way Analysis of Variance (ANOVA) using SPSS (IBM - SSPS Statistics 24). Dietary supplementation with PE did not affect growth performance, carcass traits, colour and pH of LD. Muscle drip loss was slightly higher ($p=.068$) in CON group than in pigs fed with PE (3.42% *vs* 2.93%, respectively). In addition, the total losses was lower ($p<.05$) in LD muscle from pigs fed PE than controls (17.82% *vs* 19.2%, respectively). Dietary PE supplementation decreased ($p<.05$) cholesterol content of meat (57.8 mg/100g vs 51.0 mg/100g). Dietary PE was not able to influence intramuscular collagen amount and hydroxylysylpyridinoline (HLP) concentration but the collagen stability (moles of HLP/mole of collagen) resulted higher ($p<.05$) in LD from pigs fed PE than controls (0.167 mol/mol *vs* 0.132 mol/mol respectively). The present data showed that dietary supplementation with *Lippia spp. extract* is able to improve both technological and nutritional quality of LD muscle. This dietary supplementation is able to enhance nutritional quality of pork, decreasing the cholesterol content. Future studies are required to clarify the optimal length and dosage of dietary supplementation in heavy pigs in order to verify the plant extract efficacy in dry cured products.

O180

Fatty acids and oxidative stability of meat from lambs fed linseed and brewers spent grains

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Agro-industrial by-products may be rich sources of bioactive compounds able to act as antioxidants and modulators of the lipid metabolism in ruminants. Therefore, research is focusing on their use in ruminant feeding to reduce production costs and to improve product quality. Brewers spent grain (BSG) is the typical brewery by-product, consisting of residual grain husks and compounds not soluble in the mashing and lautering processes. Residual polyphenols and vitamins are commonly recovered in BSG. The aim of this study was to assess in growing lambs the effects of including BSG in a diet enriched in n-3 fatty acids on meat's fatty acid composition and on its oxidative stability. Twenty-one weaned Appenninica male lambs were allotted into three equal groups and fed a diet with a 30:70 forage:concentrate ratio. The forage was grass hay, while each group received one experimental concentrate: control (C; based on corn and broad bean), linseed (L; containing 20% DM crushed linseed) and linseed + BSG (BSGL; containing 20% dried BSG and 20% DM crushed linseed). The dietary treatment did not affect the main growth performance parameters. Compared to the control diet, feeding linseed alone or in combination with BSG resulted in a 3, 3, 1.5 and 1.5 fold increase in the content of C18:3 n-3, C18:1 n-9, C18:2 n-6 and cis-9, trans-11 C18:2 in the intramuscular fat ($p<.05$). Lambs in the L group showed a higher level of trans-10 and trans-11 C18:1 isomers in the muscular fat ($p < .01$), compared to the C group, while the BSGL diet produced intermediate levels. Lipid oxidation, measured as TBARS values after 3 and 7 days of storage in modified atmosphere was reduced by feeding the BSGL diet ($p<.05$). At least two possible alternative or additive reasons may explain this result. On one hand, the BSGL diet contained a higher concentration of α-tocopherol, compared to the L and the C diet (41.4, 37.2 and 29.7 mg/kg, respectively). On the other hand, while the three experimental concentrates had comparable levels of total phenolic compounds (4.6 g/kg, on average), the BSGL diet had a lower content of the poorly bioavailable tannins compared to the L and C diets (0.51, 1.15 and 1.18 g/kg, respectively). In conclusion, the combination of linseed with BSG in the diet may be a promising strategy to reduce feeding costs and maintaining the beneficial effects of linseed on meat fatty acid composition, while increasing the resistance of meat to oxidative deterioration.

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O181

Consumer testing of meat from Apennine lambs fed diets containing linseed and brewers spent grain

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In recent years, the attention of consumers for healthy properties of food is increasing and research in livestock farming focuses on identifying possible feeding strategies which can ensure product of high organoleptic and nutritional quality for human consumption. Several studies have tested the use of dietary plant-derived bioactive molecules, which can be abundant in many food industry by-products. Nevertheless, while most of these studies have considered the effects of such feeds on meat and milk fatty acids, oxidative stability and other quality traits, very little is known on their impact on the consumer appreciation of the products. The present work focused on the use of brewers spent grain (BSG) and linseed in the diet for finishing Apennine lambs. In details: Apennine heavy male lamb received the same hay and three different concentrates: control (C; based on corn and broad bean), linseed (L; containing 20% DM crushed linseed) and linseed + BSG (BSGL; containing 20% dried BSG and 20% DM crushed linseed). Concentrates were isoproteic and isoenergetic. This study focuses on the Consumer Test (Central Location Test- CLT) performed on the meat. The CLT involved hundred not trained consumers and consisted both of a blind test (B) and an essay about the expectation of consumers behind information on the experimental feeding techniques, without tasting (Expected-E). Consumers expressed their pleasure as scores ranging from 1 (very unpleasant) to 9 (very pleasant) for the hedonic characters: flavour, tenderness, juiciness and overall pleasure (for B), and only overall pleasure for E. Data of the B test were analysed with ANOVA model. The BSGL meat reached higher scores than C meat in all the hedonic characters, except for juiciness (overall pleasure: 5.8 *vs* 6.4 *vs* 6.4, respectively for C, BSGL and L; $p<.05$). Furthermore, the chi square analysis of disconfirmation (comparison between B and E test for overall pleasure) showed that expectation of BSGL lamb meat was even higher compared to the its score in B test (overall pleasure expectation: 6.3 *vs* 7.9 *vs* 7.6 for C, BSGL and L, respectively), as an example of negative disconfirmation. Since the blind overall pleasure of BSGL was already high, results showed that positive image of animal-derive food can be enhanced when information on the production system of the animals is provided. This is important for red meat products which recently get bad press.

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P001

Distribution of functional variants within runs of homozygosity in four Italian cattle breeds

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Several methods have been used to identify genomic regions subjected to selective sweeps. An alternative method can make use of runs of homozygosity (ROH), defined as stretches of homozygous regions in a genome. The aim of the present study was to detect selective sweeps using ROH in four cattle breeds. Individuals of Cinisara (71), Modicana (72), Reggiana (168) and Italian Holstein (96) were genotyped with the Illumina Bovine50SNP v2 BeadChip. To identify genomic regions that were most commonly associated with ROH within each breed, the percentage of occurrences of a single nucleotide polymorphism (SNP) in ROH was calculated across animals. The genomic regions most commonly associated with ROH were identified by selecting the top 1% of the SNPs most commonly observed in ROH in each breed. This approach resulted in the identification of 11 genomic regions in the Cinisara and Italian Holstein, and 8 in Modicana and Reggiana showing increased frequency of ROH. Generally, ROH patterns differed between breeds. There were two commons genomic regions between breed pairs, and in particular one in BTA6 between Modicana and Reggiana and one in BTA10 between Cinisara and Italian Holstein. A highly homozygous region (>45% of individuals with ROH) was found only in Modicana breed in BTA6 (6:37,019,972-39,069,719) within a QTL affecting milk fat and protein concentration. In these genomic regions we identified from 126 to 347 genes for each breed. According to Panther and KEGG database, a majority of the genes was involved in multiple signaling and signal transduction pathways in a wide variety of cellular and biochemical processes. Several of these genes were also comprised in a list of genes related to phenotypes for which cattle breeds have been subjected to strong positive selection. For most genes associated with ROH islands, a biological link to traits such as milk yield and composition, reproduction, immune response, coat colour, genetic disorders and resistance/susceptibility to infectious and diseases, which are known to be under selection, can be hypothesized. These results showed that selective sweeps detected with ROH approach are shared among breeds and that scanning the genome for ROH might be an alternative or complementary strategy to detect selective sweep related with important economically traits.

P002

Litter size traits in Black Slavonian and Nero di Parma pig breeds: effects of farrowing management and sow number *per* herd

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The management of reproduction of local breeds of pigs is the key factor influencing their reproductive efficiency, genetic and biodiversity. The production of offspring and their further raising make the basis of production organization, as well as management strategies in conservation procedure for local pig breeds. The aim of this study was to analyse and evaluate Litter Size (LS) traits in two autochthonous pig breeds according to their similarity in breeding procedure, as Management of Farrowing (MF) and Number of Sows *per* Herd (NSH), to improve their reproductive performance and self-sustainability. Data analysis included 2026 parities of Black Slavonian (BS) and 906 of Nero di Parma (NP), from the 1st to 10th parity (PAR). The LS traits referred to Total Number of Born (TNB), Number of Born Alive (NBA) and Number of Weaned (NW) piglets. Data analysis was performed using GLM (SAS). Five effects with two double interactions were included in the model of calculation to analyse the possible factors influencing between-breed differences in reproductive efficiency. The following effects were tested: breed (BR), PAR, four season of farrowing (SF) according to calendar year, NSH (three levels: 1-3, 4-10 and 10 reproductive sows *per* herd) and MF (two levels: controlled farrowing in roofed premises and farrowing at uncontrolled and non-roofed sites) with interaction in the scalar equation as BR*MF and BR*NSH. The results were expressed as Least Squares Means ± SE. Pooled farrowing analysis showed TNB to be lower in BS (6.93 ± 0.08) compared with NP sows (8.31 ± 0.13), with significant effects ($p < .05$) of BR, PAR, MF, BR*NSH and BR*MF. The NBA was 6.48 ± 0.09 in BS *vs* 8.01 ± 0.15 in NP sows, with significant effects ($p < .05$) of BR, PAR, MF, BR*NSH and SF. The analysis of NW with 6.24 ± 0.09 in BS and 6.47 ± 0.15 in NP sows pointed to significant effects ($p < .05$) of PAR, SF, MF, BR*NSH and BR*MF. Accordingly, differences between the breeds could be ascribed to the higher number of controlled farrowing in population of NP *vs* BS with the best results in LS within a group from 4 to 10 sows per herd.

P003

A methodology for the parentage diagnosis of the Italian Brown breed

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Parentage is a measure of the genetic similarities between two related individuals: it is defined as the possession of genes identical by descent. Since the amount of common genes determines phenotypical similarities, e.g. morphological and production-related features, an accurate parentage test is crucial in the selection process. The advent of genomic analyses has paved the use of SNPs information to accurately investigate parentage. However, the current transition from traditional to genomic selection needs methods able to combine different sources of genetic information. In particular, since genomic information is not available for all animals, there is the practical and economic need to cross-examine parentage of genotyped offspring and parents with microsatellites information only. Two steps are necessary to overcome this issue: firstly, to assign microsatellite information from SNPs data of a genotyped animal and secondly, to use microsatellites for the lineage verification.

The objective of this study was to design a method capable to analyse microsatellites data that validates pedigree information. The data was provided by National Brown Cattle Breeders' Association (ANARB) and was made up of 49,828 cattle with microsatellite information from SNP data, 37,262 cattle with official microsatellite data and a pedigree database with 2,399,305 cattle.

The first stage was to create an algorithm that cross-examined 12 microsatellites per animal along with the microsatellites from the presumed parents, to check the correctness of the pedigree.

The procedure was developed by using the software R, which has permitted to deal with large databases. The conditions used for the parentage diagnosis followed the ISAG protocol and the ICAR guidelines. The accuracy of the method was checked by comparing the results obtained by microsatellite analysis with the official parentage data for the 37,262 animals where official analyses were available. The comparison of the results between the proposed method and the available official verifications led to an accuracy of 96.2%. Consequently, the procedure has allowed more than 12,000 new parentage verifications and the correction of 600 pedigree information.

This procedure is useful for direct verification, without further external laboratory testing, of parentage compatibility when different sources of information are already available: SNPs and microsatellites.

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P004

Biodiversity in tench populations of Sicily

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Previous studies on mtDNA revealed the existence of genetic variability in Italian tench populations. With this study we extended the analysis to tench populations of Sicily, with the aim of verifying if the long geographical isolation affected their genetic differentiation.

A total of 72 fin samples were collected from six wild tench populations living in rivers (Alcantara, ALC; Irminio, IRM; Prainito, PRA; Sant'Elia, SEL) or water basins (Cesarò, CES; Santa Ninfa, NIN) located in different areas of Sicily. The PCR-RFLP technique was used to analyse the variability at the same four mtDNA segments considered in the previous studies (ND1, ND6, cyt*b* and D-loop) to make it possible the comparison of all the Italian populations studied so far. MEGA and Arlequin softwares were used for data analysis.

Three haplotypes (H1, H3, H5, according to the nomenclature used in the previous studies) were found in the Sicilian populations: H1 was present in all the populations (frequency: 0.4 ÷ 1.0); H3 was observed in ALC, CES, IRM and PRA, while H5 was found only in PRA. Therefore H1 was confirmed as the most widespread haplotype in tench; H3, exclusive of Italian populations, had been also reported in tench of the Central Italy, suggesting a common phylogenetic history; surprisingly, tench living in Prainito river share the H5 haplotype with fish from Valagola lake (Autonomous Province of Trento, Northern Italy). The more ancestral origin of H3 with respect to H5 could explain in part its wider distribution. All the haplotypes found belong to the haplogroup A, indicating that the analysed populations derive from the same maternal lineage emerged after expansion from isolated glacial refugia, according to phylogeographic structure common to many freshwater fish. As for the genetic variability, estimated by the haplotype (H) and nucleotide (π) diversity, NIN and SEL were monomorphic, while IRM, PRA and ALC showed a considerable level of variability (H: 0.60 ÷ 0.67; π: 0.029 ÷ 0.037). In general, the Sicilian populations exhibit a higher haplotype diversity compared to the other Italian populations studied until now (mean H value: 0.36 *vs* 0.26), while show similar values for the mean π value (0.018 *vs* 0.020). Stocking from outside Sicily could have contributed to the high variability of some Sicilian populations.

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P005

Lipofection conditions of CHO-K1 cells with the use of two transposon systems

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This study aimed to elaborate CHO-K1 cells transfection conditions with the use of two transposon systems: piggyBac and Tol2 by lipofection method. Firstly, we optimized transfection conditions for Tol2 transposons. We compared effect of cells density (1×10^5 cells/ml and 5×10^5 cells/ml), amount of lipofectant Xtreme HP DNA (1-8μl) and plasmid constructions: pCMV-Tol2 with pL-miniTol2-OVA5IFN, pCMV-Tol2 with pL-miniTol2-OVA5Egfp and pL-OG-OVAIFNEnh-Egfp (control vector) on viability of cells. To select transfected cells we used G418 at concentration 400μg/ml, which was determined based on antibiotic kill curve. The viability of cells was the marker of transfection efficiency and was examined with the use of flow cytometry FACS Aria after iodide propidium (PI) staining. Secondly, using the most effective conditions of lipofection, we transfected cells with plasmids piggyBac transposons: pCMV-sPBo (Transposagen) with pLPB-NeoOVA5fEIFN and pCMV-sPBo with pLPB-NeoOVA5fEegfp. Significant differences in the viability of transfected CHO-K1 cells were calculated with a three-factor analysis of variance followed by Bonferroni and Games-Howell tests.

The highest transfection efficiency was obtained for concentration 1×10^5 cells/ml. In comparison to control (32%), the highest lipofection efficiency for Tol2 transposon vectors was achieved for cells transfected with 0.5μg pCMV-Tol2 with 1μg pL-miniTol2-OVA5IFN and 0.5μg pCMV-Tol2 with pL-miniTol2-OVA5Egfp and 1μl Xtreme HP DNA (62.0 and 60.1%, respectively). The same conditions of lipofection used to transfect the cells for pCMV-sPBo with pLPB-NeoOVA5fEIFN and pCMV-sPBo with pLPB-NeoOVA5fEegfp resulted in cells viability 76.8 and 77.9%, respectively. The percentage of live cells after transfection with the use of control vector at density 1×10^5 cells/ml and 5×10^5 cells/ml was 67.3% and 36.7%, respectively. The EGFP expression after transfection for 1×10^5 cells/ml and 5×10^5 cells/ml was 32.3 and 11.2%, respectively. These elaborated transfection conditions will be used to transfect chicken primordial germ cells (PGCs) in the next step of our project.

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P006

Genetic variability detected at the (c-type) milk lysozyme encoding gene in donkey

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Lysozyme is known to be a natural antimicrobial agent since it catalyses the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. It inhibites the development of many pathogens bacteria, thus making the milk somewhat selective in regards to the milk bacteria content. Three major distinct types of lysozymes have been identified: chicken-type (c-type), invertebrate-type (i-type), and goose-type (g-type). In particular, there are at least 4 non-stomach lysozyme genes in ruminants (i.e., mammary gland, kidney, trachea, intestinal). Lysozymes in ruminants and equine milk are considered to be the c-type because of their similarity to chicken egg white lysozyme. The c-type lysozyme content in donkey's milk varies during the different stages of lactation with a mean value of 1.0 mg/mL and proved to be higher than that in bovine, ovine, caprine (traces), whereas it was very close to mare's milk. In the equine species, the c-type lysozyme encoding gene (4 exons) maps on chromosome 6 and transcribes a mRNA of 1329bp, coding for a protein of 148aa. To our knowledge, no information on genetic variability has been reported so far at this *locus* in donkey. Consequently, in order to detected variability, total RNA was extracted from milk somatic cells of 6 unrelated Ragusana donkeys reared in Central Italy. The mRNA fragment comprised between the last 84nt of exon 1 and the first 285nt of exon 4 was amplified by RT-PCR and sequenced. Primers (For GCAAGGTCTTTG-AAAGATGT and Rev ACCAGCATTAGTTCTATTCG) were designed using as template the genomic donkey sequence (EMBL ID: NW_014638180). The obtained sequence (465bp) is relative to the cDNA tract spanning the last 64nt of exon 1 to the 236thnt of exon 4. Stop codon is located at the 65th-67thnt of exon 4. Sequences comparison showed a transition G→A at the 160thnt of exon 2 (NW_014638180:g-1784688C > T) responsible for the aa change Arg^{90}→Gln. The presence of the codon CGA at exon 2 of the donkey milk lysozyme encoding gene might represent the ancestral condition of the gene in *equidae*, as it has also been found in other donkey and male sequences. The identification of this SNP could represent the first report of polymorphism at this *locus* in donkey. Next step of the research will be the analysis of a large number of samples in order to establish the frequency of this mutation in donkey species and to evaluate if and how the new genetic variant may influence functional and biological properties of donkey's milk.

P007

First SNP discovery in ACACA gene and association study with milk yield in Mediterranean river buffalo

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Table 1 Genotyping data and effects of *ACACA* SNP genotypes on milk yield.

Locus	Trait	Genotype		
ACACA	Genotype distribution	CC (430)	CT (112)	TT (9)
	N. lactations	854	223	19
	N. records	5603	1498	135
	Average milk quantity	8.36	8.18	7.32

The *ACACA* enzyme catalyses the first committed step of fatty acid synthesis in mammalian cytosol, the carboxylation of acetyl-CoA to malonyl-CoA, leading to the biosynthesis of long-chain fatty acids. To our knowledge no information on DNA genetic variability at *ACACA locus* has been reported so far in buffalo species. Consequently, in order to detected polymorphisms at Italian Mediterranean river buffalo *ACACA locus* and test possible associations with milk yield, we analyzed 551 subjects belonging to 14 farms, located in Salerno and Caserta province. A total of 7096 records for milk yield measured monthly with an automatized milk recording system on 1096 lactations were used. The DNA regions of the *ACACA* gene spanning partial exons 1 of 10 individual samples, randomly chosen, were amplified and sequenced using primers (GACAGTTTCTGACCTTTTGGTG and AGACCTCTCTG-CTTCCAA) designed on the genomic buffalo sequence (EMBL acc. no. NW_005785166). Sequence comparison showed a transvertion C→T at position 34 of the exon 1 (5' UTR) (NW_005785166:g4381303G > A). The genotyping of DNA samples was performed at the KBiosciences (http://www.kbioscience.co.uk) laboratory. The major allele had a relative frequency of 0.88 and the *locus* was in Hardy–Weinberg equilibrium. A mixed linear model procedure of SAS 9.1 (SAS Institute) was used for the association analysis between genotypes and milk yield. The model included fixed effects of the genotype, farm, calving season, days in milk, parity and the random effect of the animal. None of the three genotypes had significant associations with milk yield (Tab. 1). In conclusion, in this study, we report the first SNP identification at the *ACACA locus* in Mediterranean river buffalo. Although we found no association between the detected polymorphism and milk yield, our work provides a starting point for studies of the future possible association between *ACACA* variation and other milk phenotypic traits in buffalo.

P008

The interleukin-10 polymorphism g.3936 G > A is uncoupled with bovine tuberculosis susceptibility in water buffalo (*Bubalus bubalis*)

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Mycobacterium bovis (Mb), the causing agent of bovine tuberculosis (bTB), is an intracellular pathogen highly adapted to the host condition. In cattle, several studies regarding the influence of host genetic makeup, have highlighted the influence of polymorphisms on progression and/or resistance to bTB. For this reason, we decided to investigate the role of a pleiotropic immune mediator, named interleukin-10 (IL10) on susceptibility to bTB in water buffalo (*Bubalus bubalis*). IL10 is secreted by several kind of cells belonging to the innate immune response and is expressed during TB infection. It is involved in phagosome maturation and in the regulation of pro-inflammatory cytokine induction as well. These two aspects indicate that IL10 gene play a critical role in susceptibility and pathogenesis of bTB. To test our hypothesis, we extracted the DNA from blood samples of 184 buffaloes (59 case and 125 controls) reared in 5 herds located in Campania region (South Italy). A 300bp region spanning the exon 5 of the *IL10* gene (NW_005783511) in 10 cases and 10 controls chosen randomly was amplified and sequenced. Sequence comparisons showed a transversion g.3936G > A responsible of the substitution p.Arg152Lys in the primary protein sequence. To test the possibility that g.3936G > A polymorphism is a bTB associated marker we genotyped all samples by mean of AS-PCR.

Subjects carrying the genotype AA were more represented in the cases group (16 out of 59; frequency: 0.37) compare to controls groups (20 out of 125; frequency: 0.19), Thus, when we compared the AA *vs* GG ratio between case and control subjects by Fisher's exact test, the odds ratio (OR) was 2.26; the 95% confidence interval (CI) was 0.970 – 5.299; *p*-value (two sided) = 0.078. Although we have demonstrated that the polymorphism g.3936 G > A is uncoupled with susceptibility in water buffalo, our work offer a good starting point for further investigate this polymorphism in a wider sample group.

P009

Effects of diets rich in β-glucans on chromosome stability of peripheral blood lymphocytes of pigs

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β-glucans are non-digestible glucose polymers which can be isolated by cell walls of fungi, bacteria, algae and cereal grains and that stimulate the growth of bifidogenic and lactic acid bacteria in the gastro-intestinal tract. It has been demonstrated that they exert a lot of health-promoting effects like immunostimulation and they are often applied as feed additive to enhance the immune response. Aim of this pilot study is to investigate the effects of β-glucans rich diets on chromosome stability of peripheral blood lymphocytes of pigs. 20 Landrace × Large White gilts have been divided in four groups (A, B, C and D) and administered four diets: (A) Corn; (B) β-Glucan enriched barley and Corn; (C) β-Glucan enriched barley, Corn and Faba beans; (D) β-Glucan enriched barley, Corn and peas. All diets were iso-energetic (14.27 ± 0.04 MJ/kg) and iso-protein (14.36 ± 0.16% a.f.) and administered *ad libitum* for 40 days (10 d adapt +30 d treat). At the day 40th of the treatment peripheral blood was collected and per each animal two type of lymphocytes cultures were set up: one for Sister Chromatid Exchanges test (SCE) and one for Chromosome Aberrations (CA) and aneuploidy tests. At least 100, 50 and 35 metaphase plates for each animal were observed for aneuploidy, CA (chromatid and chromosome breaks) and SCE tests, respectively. Average differences were evaluated using SPSS software with Tukey-test and results were confirmed with Bonferroni test. Percentages of cell with aneuploidy were 7.83, 11.86, 8.00 and 8.25 in group A, B, C and D respectively. The mean number of total CAs were 2.83 ± 2.86 (A), 1.14 ± 1.2 (B), 1.33 ± 0.58 (C) and 1.50 ± 1.29 (D), while the mean number of SCEs/cell were 5.37 ± 2.69 (A), 4.41 ± 2.26 (B), 5.54 ± 2.61 (C) and 5.00 ± 2.55 (D). The statistically significant differences are: mean SCEs/cell between group B and A ($p < .001$) and group B and C ($p < .05$). Despite the small number of animals employed in this study results indicate that β-glucans rich diet exert the reduction of mean SCEs/cell value in healthy pigs and a better functionality of the DNA-replication mechanisms in peripheral blood lymphocytes, fundamental for proper immune response. To confirm healthy effects of β-Glucans enriched diets and their benefits on animal welfare it is necessary to test them on a higher number of animals.

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P010

Complex transcripts pattern identified at river buffalo DGAT1

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DGAT1 has been recognised as strong functional QTL for the milk fat content in cattle. In river buffalo, *DGAT1* has been investigated mainly for the characterization of the gene itself and for the identification of the K232A mutation similarly to what has been done in cattle. No investigation has been carried out at transcripts level so far. Aim of this study was to analyse the transcript profile of *DGAT1* in lactating buffaloes.
Milk samples were collected from 8 unrelated buffalos (at the 3rd lactation and 120 days from calving), reared in Piedmont region and belonging to one farm. Total RNA was isolated from milk somatic cells using TRIzol. The reverse transcription was performed using an oligo dT_{18}, whereas the PCR reaction was accomplished using the following primers 5′-ATGGGCGACCGCGGCGG-3′ and 5′-TCAGGTGCCGGC-TGCCGG-3′, corresponding to the nucleotides 1-17 (exon 1) and complementary to the base pairs 1453-1470 (exon 17) of the buffalo *DGAT1* cDNA (EMBL ID: DQ120929). The PCR products were purified and cloned. Recombinant clones were randomly chosen and screened by PCR. All amplicons different in size (bp) were purified and sequenced in both directions. The obtained sequences were compared with NCBI sequences by BLAST.
A total of 147 recombinant clones were analysed. The sequence analysis showed a complex mRNA pattern with a total of at least 6 transcripts. The most represented mRNA was that correctly assembled (86 out of 147 clones, 58.5%), 1470bp long and coding for a functional protein of 489aa (amino acids). The following mRNA population (1425bp) was skipped of the exon 12 (21.09%). Despite this deletion, the mature mRNA did not undergo any frame-shift and the termination codon was kept as in the normal isoform. The putative protein 474 aa long is different from other predicted buffalo *DGAT1* isoforms available in NCBI. Part of the transcripts were deleted of the last 66bp of the exon 8 (1404bp; 7.48%). This alternative splicing is consequence of the incorrect identification of a splice donor site directly in the exon 8 and it is responsible of a protein isoform 22 aa shorter. Minor transcripts are represented by mRNAs with the insertion of the intron 13 (1557bp; 5.44%), populations skipped of the exon 16 (1407bp; 4.76%) and transcripts characterised by the contemporary out-splicing of the exons 6 and 7 and the insertion of the intron 13 (1337bp; 2.72%). The investigation at DNA level will likely clarify the variability found at mRNA level.

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P011

Establishment of a crossbreed Simmental × Holstein experimental herd and first assessment of heterosis effects on technical and biological parameters

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Parallel to the large increase in productivity, dairy bovine breeds show deterioration of functional traits such as fertility, health, and longevity. Crossbreeding may help to overcome these problems because crosses express heterosis. A long term research has been established at CREA-PCM with the aim to implement a rotational breeding scheme between the Holstein and the Simmental breeds in order to evaluate both the genomic basis of heterosis and the possibility to increase meat production from dairy farms. The experimental herd is made by three groups of lactating cows: purebred Holstein, purebred Simmental and Crosses, in the expected relative proportions of 1:1:2. During the project the following data have been recorded: weight at birth, weight, price and morphological scoring at sale for male calves, health treatment on all calves, weight and age at puberty for heifers, reproductive events (inseminations, calving, abortions, early embryonic losses). Cows have been monitored by an activity meter system to detect heats. Since the beginning of the experiment, four years ago, a total of 276 calves were born and, out of those, 103 females have been kept as replacement: 53 crossbred, 18 purebred Simmental and 32 purebred Holstein. Among calves requiring veterinary treatments, crossbreed animal sicked less and required less time to resolve illness compared to purebreds. Male calves of the three genetic groups showed statistically significant differences for weight at birth, weight at sale and morphological scores. Following the experimental design, the first F1 heifers were artificially inseminated with either Simmental or Holstein semen. Weight at birth, weight and age at puberty of F1 heifers showed intermediate values between Holstein and Simmental heifers. Moreover the pregnancy rate at first insemination indicated an heterosis value of +8% compared to purebreds. Effects of

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heterosis for milk production traits and longevity will be assessed when more recording will be available. Genome wide analysis will be carried out to identify chromosomal regions likely to be associated with heterosis. Beyond the scientific interest, the experiment has a strong demonstration content.

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P012

Signals of adaptive introgression between European taurine and indicine cattle revealed by local ancestry inference

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European taurine cattle (*Bos taurus*) was domesticated in the Fertile Crescent around 8,000 year BC and later colonised Europe, Asia and Africa following the agriculture wave. A second domestication involving the ancestors of the modern humped zebu cattle (*Bos indicus*) occurred 2,000 years later in the Indus valley, in Central Asia. Admixture between taurine and indicine species occurred extensively in the past, the indicine species sometimes contributing to taurine's genetic pool with a better adaptation to tropical climate and diseases, and an improved ability to thrive with very poor fodder. Interestingly, a small percentage of indicine ancestry can be detected in several modern taurine breeds. With the aim to identify the putative adaptive nature of such indicine *x* taurine introgression, we analysed Illumina BovineHD SNP genotypes of 16 Chianina cattle sampled in central Italy, along with 187 individuals from six reference breeds of taurine (3) and indicine (3) origin. Local ancestry investigations involving nine reference population combinations were performed and smoothed using the CIWI (Consistently Introgressed Windows of Interest) analytical framework, able to identify concordant and reference-independent genomic regions of a given ancestry. Among the CIWIs of indicine ancestry identified in Chianina, the strongest signal was recorded in chromosome 18. Haplotype homozygosity-based selection sweep analysis evidenced signatures of selection occurring within the same genomic region. Here, we infer the putative adaptive nature of this ancestral indicine genome portion, and suggest its association to indicine cattle's superior ability to efficiently use poor quality fodder.

P013

A pipeline for variants discovery using next-generation DNA sequencing data

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Recent advances in next generation sequencing (NGS) technology provide a cost-effective approach to large-scale resequencing of livestock samples in order to study several biological phenomena. NGS produces millions of short DNA sequences that require an unbiased way to make possible comprehensive searches for variation to identify putative causative mutations for economically important traits. The aim of this work was to present a bioinformatics pipeline analysis for variants discovery in ovine genome. A total of 30 individuals belonging to Valle del Belice dairy ewes was used for whole genome sequencing of pooled libraries prepared using Illumina Nextera Kit. Paired-end sequencing was carried out in an 8-lanes flow-cell of the Illumina HiScanSQ platform yielding a total of 1,159,664,912, 101 bp length reads. The left and right raw reads were separated into two files, and converted to the fastq format using CASAVA 1.8. The whole procedure was split in different workflows, in order to give more flexibility to end-users. One workflow is aimed to verify the quality of the raw sequencing reads using FastQC and FASTX-Toolkit, in order to keep bases with Phred quality Score greater than 20 and to trim the reads with poor quality. Another step aligns the reads to the *Ovis aries* 3.1 reference genome using BWA-mem with standard parameters. The resulting SAM file was converted in BAM file using the SAMtools software, then unmapped and duplicate reads were removed using the CleanSam and MarkDuplicate commands of the Picard software. Therefore, to get more accurate base qualities, Genetic Analysis Tool Kit (GATK) was used to locally realign reads such that the number of mismatching bases due to indels is minimized across all the reads (IndelRealigner) and to detect systematic errors in base quality scores (BaseRecalibrator). In the last workflow SNPs and indels are identified using mpileup command of SAMtools software. The resulting BCF file is passed to "bcftools view" tool to be filtered and converted into VCF format. Finally, for variants annotation the SNPSift software was used. A total of 6,357,170 variations, of which 5,265,739 SNPs and 1,091,431 indels, were discovered. About 77% of the SNPs were present in the *Ovis aries* dbSNP v147 while the remaining were novel SNPs. The discovered SNPs must be validated and then could be used to several applications as phylogenic analysis, genome-wide association studies or genomic selection.

P014

Effect of *CSN1S1* G allele on some characteristics of milk produced by Cinisara cows

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In bovine milk about 37-40% of total casein is represented by αs_1-casein. The *CSN1S1* G allele is characterised by a less efficient protein synthesis (about 40%) compared with the other known alleles because of the insertion, in the 19th exon, of a Long Interspersed Nuclear Element relict. This allele is responsible for a reduced mRNA stability. The lower proportion of αs_1-casein in milk is associated with faster formation, higher firmness and greater resistance to compression of the curd.

The purpose of this study was to analyse the G allele distribution at the *CSN1S1* locus in Cinisara breed and its influence on some milk traits. The Cinisara is a small Sicilian breed for which, recently, recovery and safeguard actions were accomplished. Milk obtained from this breed is mainly used to produce the *Caciocavallo Palermitano*, a typical stretched-curd (pasta filata) cheese. This product represents an example of link between breed, breeding environment, and traditional dairy technology, factors highly appreciated by consumers today. We typed 192 lactating cows, by means of PCR-RFLP analyses, in order to identify *CSN1S1* G carriers. Individual milk samples of 14 cows (low αs_1-casein) and 23 control cows (normal αs_1-casein) were analysed for total nitrogen (TN) and non-casein nitrogen (NCN) according to FIL-IDF standard procedures, and for fat by an infrared method (Combi-foss 6000, Foss Electric, Hillerød, Denmark). Coagulation properties were measured using the Formagraph. Control cows were chosen in the same herds of G carrier cows and had comparable lactation stage and number. Data were analysed using ANOVA procedure in which the fixed factor of *CSN1S1* was evaluated. The frequency of the *CSN1S1* G allele in the 192 analyzed cows was 0.039 (1 homozygous and 13 heterozygous cows). The analyses of milk traits and clotting parameters show that also in Cinisara the *CSN1S1* G allele is associated with significantly lower protein ($p < .049$), casein ($p < .046$), and casein index ($p < .039$). Moreover the milk of cows with *CSN1S1* G allele show a tendency to a lower percentage of fat ($p < .072$). As a consequence, the *CSN1S1* G allele is associated with significantly lower coagulation time (r) ($p < .019$), and tendentially lower curd firming time (k_{20}) ($p < .057$), and higher curd firmness (a_{30}) ($p < .063$). These results confirm that *CSN1S1* G allele considerably affects milk traits. Further analyses are necessary to evaluate the influence of this allele on yield and composition of *Caciocavallo Palermitano* cheese.

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P015

The genetic structure of Hispano-Arabe horse in the Spanish context

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The Hispano-Arabe horse is the result of the crossed between two breed, the Spanish Pure Breed and Arab Breed and for this particularity to analyze the genetic variability within the Spanish context is necessary. Twenty-five molecular markers were analyzed in 40 random samples from the Hispano-Arabe equine breed, along with 20 other equine races from the Iberian Peninsula. The parameters of genetic diversity estimated in the Hispano-Arabe breed are at the highest compared to the levels obtained in the set of breed analyzed.

The average F_{ST} distance of this breed with respect to the rest of equine races analyzed was 0.07. As expected, a very close relationship was found between the Hispano-Arabe horse and the Arabian and Purebred Spanish breeds. The F_{IS} value obtained shows an adequate number of heterozygotes ($F_{IS} = 0.02$). The results of the genetic differences by F_{ST} calculated between pairs of populations, as well as the dendrogram of relations obtained from the matrix of distances F_{ST}, show the same behaviour. The Hispano-Arabe breed formed a cluster within Arab and Purebred Spanish breeds. The northern breeds of the Peninsula formed a second well-differentiated group, and finally different groups were formed among the other races clearly separated from each other and from the other races analyzed. The results observed in this study have important implications for the use, conservation and genetic improvement of the Hispano-Arabe breed. The Hispano-Arabe horse is a valuable genetic resource with a high level of genetic diversity and the analyzes show that there is a genetic differentiation of the rest of populations. In terms of genetic structure, the Hispano-Arabe breed is a population that begins to be genetically homogeneous, suggesting that the breeding program must be properly managed to maintain the overall genetic and allelic diversity of the population.

P016

Capramur proyect: genomic analysis of the genetic determination of milk yield, composition and body condition and viability in Murciano-Granadina goats

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The Murciano-Granadina breed is, without a doubt, the main goat dairy breed of Spain, with an average production of 530 Kg milk per lactation. In the current project, we aim to undertake the study of the genomic architecture of milk and body condition traits and some sanitary aspect in Murciano-Granadina goats in order to take profit of such information in the context of genetic improvement activities. We will select 1300 animals (1200 female and 100 male) using pedigree (at least 3 complete generation) and genetics information (breeding value and phenotypic score) and at the same an extended phenotype set will be collected: milk production (Kg of milk, protein, fat) as well milk quality parameter (casein and globulin quantification), linear score and measurement recording and finally total milk cell count score and white cells series characterization by laser flow cytometry. On the whole sample, we will carry out the genotyping of the 1300 animals using the Illumina 50K chip, then using this information and phenotypic records we will select 100 divergent animals for the mRNAseq analysis. RNAseq approach will be used to compare the mammary expression of mRNAs and non-coding RNAs at four lactation stages (before lactation as well as beginning, mid and latelactation). This experiment will be useful to identify genes whose expression is activated in the mammary gland as a consequence of the establishment of lactation. By combining the RNAseq and GWAS approaches, we expect to identify genomic regions associated with the phenotypic variation of traits under analysis. On the other hand, we will analyse phenotypes related with biological efficacy by using a two-tier strategy. First, we will use a bayesian methodology to detect regions of the goat genome presenting transmission ratio distortion (alleles that segregate in non-mendelian proportions). Moreover, we will estimate genomic inbreeding coefficients and we will identify genomic regions affected by inbreeding depression for the traits under study. Such data could be employed in gene assisted selection schemes. Besides, we will evaluate the usefulness of genomic information to predict the genetic potential of candidates to breeding.

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P017

Preliminary results of the project “backcross recovery and conservation of the Iberian pig variety Manchado de Jabugo”

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The Iberian pig variety Manchado de Jabugo originated in the second half of the nineteenth century, in the province of Huelva, from various crosses between black and red varieties of the Iberian breed, with the participation of other foreign populations. Traditionally it is linked to the dehesa, a particular environment of the southwest of the Iberian Peninsula, although with expansion in recent years to other areas of Spain. It is a rustic animal that takes advantage of the extensive pasturage and is able to provide hams and other cured products of great acceptance. Due to its current census situation (74 composed by 13 males and 61 female), the province government of Huelva and the University of Córdoba started the project entitled "*Implementation of the backcross recovery and conservation program of the Iberian pig Manchado de Jabugo*". Using backcrossing with a phylogenetically close, based on historical information, Iberian pig variety (Villalón pig), the project aimed to increase the real and effective size of the Manchado de Jabugo pig variety. Twenty-five microsatellite specific have been used in 74 animal samples born between the years 2013 and 2016 in order to monitor the genetic diversity of second and third generation animals and to know the evolution of the levels of diversity of the new animals from the Manchado de Jabugo (MJ) × Villalón (Vill) line. Besides we carried out individual genetics assignment analysis of third generation animals to the Manchado de Jabugo variety using the Structure software v 2.3.4. The genetic variability observed in the third-generation MJ × Vill strain is lower than that found in the Villalón pure line, with observed heterozygosity levels of 0.39, being similar to those found in the pure Manchado de Jabugo reference population. In general, there is a decrease in the levels of variability of the animals of the 3rd generation with respect to the animals of the 1st and 2nd generation. The individual assignment values show that the animals of the first generation have very different values being between 4.60% and 91.60%. In the second generation, the values are generally higher, and in the third generation of backcrossing the animals are assigned to the Manchado de Jabugo Iberian pig with higher coefficients, close to those presented by pure animals.

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P018

A genomic landscape of mitochondrial DNA insertions in the rabbit nuclear genome

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Nuclear DNA sequences of mitochondrial origin (*Numts*) are derived by insertion of DNA fragments of the mitochondrial genome (mtDNA), from both coding and non-coding regions, into the nuclear genome. *Numts* are fossil sequences present in the nuclear genome of many eukaryotes, contributing to shape their genomic architecture and evolution. In this study, we provided a first picture of *Numts* in the rabbit (*Oryctolagus cuniculus*) genome. The rabbit reference nuclear genome (OryCun2.0) was aligned with the reference linearized mitochondrial DNA sequence of using LAST software. A total of 216 *Numts* were identified in the assembled rabbit genome including scaffolds. Inserted nuclear mtDNA fragments ranged in size from 54 bp to 6228 bp and covered about 0.002% of the assembled nuclear rabbit genome. Three *numts* not inserted in repeated regions were validated by PCR and then by sequencing in several rabbit breeds (Bristle White, Burgundy Fawn, Californian, Giant Grey, Hungarian Giant, Tan and Vienna White), in Sardinian wild rabbits and in hares of different species (*Lepus europaeus*, *L. timidus* and *L. capensis*). One *numt* showed insertion polymorphisms (i.e. presence or absence of the insertion) in some wild Sardinian rabbits and domestic breeds. The other two *numts* showed only the insertion in all analysed rabbits and hares. This is the first study that reported information of *numts* in the OryCun2.0 rabbit genome version and identified a polymorphic *numt* region in this species that might be useful to track evolutionary and domestication processes that contributed to differentiate rabbit populations.

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P019

Comparative analysis of metabolomics profiles of plasma and serum in pigs

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Metabolomics, defined as the study of small molecules within a biological matrix, is a powerful technique that can describe the biological processes underlying complex phenotypes. Different matrices can be used in metabolomics studies, but biofluids derived from blood, such as serum and plasma, are the most frequently used. Serum is the supernatant fluid that is obtained from the blood that is allowed to clot. Plasma is obtained by adding an anticoagulant to the blood and then separating the cellular components from the liquid portion. These methods of preparation lead to some differences in protein and metabolite levels that might be relevant for subsequent uses and interpretation of metabolomic data. In this study, we obtained a first evaluation of these differences by comparing metabolomic profiles of paired plasma and serum samples from 24 pigs (12 Italian Large White and 12 Italian Duroc pigs) analysed for about 200 metabolites of five biochemical families (amino acids, biogenic amines, hexoses, acylcarnitines and glicerophospholipids) by targeted LC-MS/MS. Pearson's correlation coefficient (r), Wilcoxon signed-rank test and Delta [Delta = (serum − plasma)/serum)] between plasma and serum were computed for each metabolite both within breed and considering all pigs. A strong correlation ($r \geq 0.7$) between the two biofluids was obtained for 60% of metabolites when considering all pigs together. This correlation was observed for a larger number of metabolites (66%) in Italian Large White than in Italian Duroc (45%) pigs. Considering all pigs, 28% of metabolites differed significantly at the Wilcoxon signed-rank test ($p < .05$, Bonferroni corrected), with metabolite concentrations higher in serum than in plasma. These metabolites belonged to all the metabolic classes included in the analysis except for the class of hexoses. Amino acids and acylcarnitines were the two metabolic classes having exclusively a higher concentration in serum than in plasma. Results we obtained in pigs were also compared with data coming from a similar study in humans that covered 72% of pig metabolites. Considering the Delta values of the metabolites with significant differences between plasma and serum in pigs, similar differences between these two biofluids were observed for 70% of these metabolites also in humans. These results add useful information for the metabolomic characterization of the pig and for a better definition of this livestock species as animal model.

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P020

Mining 116 pig genomes to identify putative functional mutations in candidate genes for economically relevant traits

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The bigdata era in livestock genomics, generated by next generation resequencing of a large number of individual genomes, is opening new opportunities to mine public nucleotide archives and identify mutations that could putatively affect economic relevant traits. One bottleneck in genomic data mining is derived by the difficulties in handling large amount of sequence data. In this study, we developed an efficient pipeline to manage and mine 116 individual pig genomes retrieved from the European Nucleotide Archive and from proprietary datasets generated from pigs of 20 different breeds. All these pig genome datasets were searched for polymorphisms in 135 annotated candidate genes. Genes were selected based on their roles in biological processes involved in disease resistance, meat quality and carcass traits, growth and morphological traits. Short reads from these genomes were first aligned using *bowtie* to a customized reference sequence generated from Sscrofa10.2 genome version, including sequence of the selected genes (with depth ranging from 4 to 40X for each genome). Variant calling was performed with *samtools* software. The Ensembl Variant Effect Predictor tool was used to inspect the consequences of the identified variants and potentially deleterious mutations were detected with SIFT. A total of 102,957 variants were identified. Among them, 2393 were in coding region and included 725 synonymous, 483 missense mutations, 21 stop codon gains/losses and 49 frameshift events. A total of 1263 variants among all identified polymorphisms were not previously available in any other public repository. Principal component analysis of the identified single nucleotide polymorphisms clustered pig breeds according to their origin (European *vs* Chinese breeds). The constructed pipeline provided the possibility to efficiently mine whole genome resequencing datasets and to identify mutations that could have a relevant role in defining genetic variability for economically important traits in pigs.

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P021

Genome wide association studies for meat quality, carcass and performance traits in Italian Duroc pigs

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In Italy, about 70% of pigs are raised for protected designation of origin (PDO) productions. The most important Italian PDO products are Parma and San Daniele dry-cured hams that are obtained from legs of heavy pigs slaughtered at about 160 kg live weight. Italian Duroc boars are usually used as terminal sires in crossbreeding programs for the production of heavy pigs destined for these PDO hams. Therefore, this breed is particularly relevant in the Italian pork PDO production chains. This works describes the first genome wide association studies (GWAS) in the Italian Duroc breed carried out for seven production traits (ham weight loss at first salting, HWLFS; visible intermuscular fat, VIF; feed conversion ratio, FCR; average daily gain, ADG; backfat thickness, BFT; lean cuts, LC; and ham weight, HW) included in the selection program of the National Pig Breeder Association (ANAS). A total of 574 performance tested pigs were genotyped with the Illumina PorcineSNP60 BeadChip. Random residuals (RR) were used as trait values in GWAS using Genome-wide Efficient Mixed Model Association (GEMMA) software. A total of 154 suggestively significant, including 27 significant single nucleotide polymorphisms (SNPs) were associated with one or more production trait. The traits with the largest number of SNPs in these lists were HWLFS (43 SNPs) and BFT (29 SNPs). Among all these SNPs, eleven were shared with two or more traits. Annotation of SNPs showed a large heterogeneity of functions suggesting that these complex traits are affected by many different biological mechanisms involving a large number of genes.

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P022

Signs of de-domestication in local pig breeds: evidences from variability in genes affecting phenotypic and morphological traits

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The domestication process has fixed phenotypic and morphological traits in many pig breeds as result of selective breeding towards more productive or differentiated populations compared to wild boars from which domestic pigs are originated. Coat colour and size/shape of the animals (including vertebral number) are among the first traits that have contributed to this differentiation. Several alleles of the porcine melanocortin 1 receptor (*MC1R*) gene determine the variability associated to the *Extension* (*E*) coat colour locus: E^+, the wild type allele; and four main domestic alleles. A missense mutation (p.P192L) in the nuclear receptor subfamily 6, group A, member 1 (*NR6A1*) gene has been already reported to be the causative mutation for an important QTL affecting the number of vertebrae in pigs. The wild type allele (p.192P) has been shown to be fixed in wild boars, whereas the alternative mutated allele has been reported to be fixed in commercial populations. In this study, we investigated 500 pigs from six Italian local breeds (84 Apulo-Calabrese, 124 Casertana, 83 Cinta Senese, 74 Mora Romagnola, 75 Nero Siciliano and 60 Sarda) and 44 pigs of the East Balkan Swine breed. All animals were genotyped or sequenced to define the genotype at the *MC1R* and *NR6A1* genes. Results showed that all breeds were not fixed for one specific allele at one or the other gene. In particular, the wild type *MC1R* allele (E^+) was observed in all breeds (Apulo-Calabrese, 0.12; Casertana, 0.18; Cinta Senese, 0.01; Mora Romagnola, 0.18; Nero Siciliano, 0.17; Sarda, 0.30; East Balkan Swine, 0.28). The wild type *NR6A1* allele was observed in Apulo-Calabrese, 0.10; Casertana, 0.07; Nero Siciliano, 0.28; and East Balkan Swine, 0.19). Observed variability in "domestic" genes in local breeds might be derived by a combination of different events: occasional (due to the extensive production systems in which these populations are usually raised) or deliberate recurrent crossbreeding with wild boars, crossbreeding with other pig breeds and relaxed directional selection towards domesticated traits.

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P023

Identification of genomic regions affecting the hairless and tail shape phenotypes in Casertana pigs

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Casertana is an autochthonous pig breed mainly raised in the Central-South of Italy that is characterized by a typical hairless phenotype. However, despite hairless is the most important breed-specific phenotype, variability for this trait is present in the breed population, that includes also pigs with hairs. Some animals of this breeds have a characteristic sign of domestication (curly tail) whereas other show a strait tail. The shape of the tail is a domestication trait might reduce tail-biting. In this study, we carried genome wide association studies (GWAS) in the Casertana breed by i) comparing pigs completely or almost completely hairless (n. 81) versus pigs having hairs (n. 15) and ii) comparing pigs with strait (n. 19) versus curly tail (n. 53). Different total numbers were due to the fact that, for some animals, phenotypic information was not available. All pigs were genotyped with the Illumina PorcineSNP60 BeadChip. Genotying data were processed with PLINK software and 47018 single nucleotide polymorphisms (SNPs) were used to carry out an association analysis with GEMMA software. Results of the GWAS for the presence/absence of hairs showed a few regions with highly significant SNPs on porcine chromosome 4 (SSC4), SSC7 and SSC15, suggesting that the hairless phenotype might be determined by more than one major locus with a more complex inheritance behaviour than previously thought. Genomic regions including significant SNPs contain genes already reported to affect alopecia or hair follicle development in humans and other species. Results of the GWAS for tail shape showed a high significant SNP signal on SSC12 in a gene desert region, according to the Sscrofa10.2 genome annotation. Our study contributed to clarify the genetic mechanisms affecting the hairless and the tail shape phenotypes in pigs.

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P024

Effect of muscle hypertrophy on the carcass traits in Marchigiana beef cattle breed

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The Marchigiana is one of the most important Italian beef cattle breed; it is famous for its large body size and favourable dressing percentage. A mutation (g.874G > T, in exon 3) in the *GDF8* gene was identified in this breed. This mutation is responsible of the double muscling phenomenon: the homozygote "GG" yields a "normal" phenotype, the homozygote "TT" yields a double muscled body shape but sometimes it causes survival problems of calves at birth; the heterozygote genotype produces an extremely muscled body without defects. Due to growing consumer demand for animal products, nowadays growth and carcass traits are kept in high consideration by the breeders.

In this study the effect of the causative mutation in the *GDF8* gene (at heterozygous condition) on the carcass traits was investigated. On 337 Marchigiana bullocks genotyped at the *GDF8* locus (285 normal, 52 heterozygous) 11 traits (live weight, carcass weight, dressing percentage, SEUROP conformation and fattening, shank circumference, weights of head, skin, tongue, stomachs and intestines) were registered at slaughtering. All the animals were sacrificed, between 16.7 and 24.3 months of age, in Senigallia abattoir (Ancona, Italy) during a three year period (2004-2006). The statistical model (PROC GLM) considered the genotype at *GDF8* as fixed effect and age at slaughtering as covariate. The frequencies by genotype of the SEUROP gride scores (conformation and fattening) were estimated by SAS software.

The effect of the *GDF8* genotype was significant ($p > .05$) on the following traits: dressing percentage, SEUROP conformation and fattening, head weight, skin weight, tongue weight, stomachs weight, intestines weight).

As expected, the heterozygous animals show a higher carcass weight (454.77 ± 6.4 *vs* 441.66 ± 2.7 kg), a better dressing percentage (64.58 *vs* 62.11%), the lowest head weight (30.31 *vs* 31.14 kg), skin weight (62.76 *vs* 67.94 kg) and intestines weight (23.75 *vs* 25.00 kg) than normal ones. The heterozygous animals registered also the highest tongue weight (1.84 *vs* 1.77 kg in the normal), confirming a general superior muscle development.

The SEUROP scores in the heterozygous animals highlighted a good muscular conformation (E score for the 65% of the sample *vs* 37% in the normal) and a low fat carcass (2 score for the 75% of the sample *vs* 57% in the normal).

In conclusion the heterozygous animals could represent an opportunity to improve the productive performance in Marchigiana breed.

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P025

Milk quality of six goat breeds maintained in the same environmental conditions

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The aim of this study was to evaluate the differences and the quality of milk in six goat breeds maintained in the same management conditions. The trial was carried out in the experimental farm of CREA (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bella, Potenza, Italy) and a total of 446 goats from six different breeds were selected (74 Garganica, 72 Girgentana, 75 Jonica, 74 Maltese, 76 Red Mediterranean and 75 Saanen goats). Milk yield was recorded 15 days apart throughout lactation from each animal during both morning and afternoon milking. Milk samples were collected and analyzed for protein, fat, lactose, betahydroxybutyrate (BHB) and the total amount of saturated (SFA), mono (MUFA) and polyunsaturated (PUFA) fatty acids by infrared spectroscopy. Data were analyzed by 2-way repeated measures ANOVA.

The mean fat content of the Saanen milk (3.29 ± 0.1) was significantly ($p < .05$) lower compared to the other breeds (3.93 ± 0.1, 4.50 ± 0.1, 4.12 ± 0.1, 4.05 ± 0.1 and 4.17 ± 0.1 in Garganica, Girgentana, Jonica, Maltese and Siriana, respectively), in particular at the end of lactation, while the Garganica milk showed the higher ($p < .05$) protein content (3.77 ± 0.1 *vs* 3.19 ± 0.1, 3.35 ± 0.1, 3.13 ± 0.1, 3.47 ± 0.1 and 3.44 ± 0.1, in Garganica *vs* Girgentana, Jonica, Maltese, Saanen and Siriana, respectively). A progressive increase in fat and protein content was recorded throughout lactation, together with a decrease of lactose. Interestingly, BHB levels showed a significant increase at the end of lactation in all breeds, except for the Saanen. The metabolic strength for

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encountering high milk fat demand in this period, together with the lower dry matter intake at the end of lactation, may be accounted for the negative energy balance observed in this phase. SFA content showed a progressive raise over the lactation period, as well as MUFA and PUFA except for Saanen in which the concentration was stable throughout the lactation. In conclusion, milk quality recorded in the local breeds considered in this study was considerably better than that recorded in Saanen.

P026

The Italian Official Semen Control (OSC)

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In Italy, livestock selection and reproduction are regulated by laws establishing the "Official Semen Control" (OSC), which is assigned to the "Istituto Sperimentale Italiano Lazzaro Spallanzani" (IS). Therefore, the Institute acts as technical executor of the Ministry and Regions for the protection of farmers with regard to "genetic quality" and "fertilizing capacity" of frozen semen distributed in Italy. To this end, the OSC, certified UNI EN ISO 9001:2008, ensures that frozen semen supplied by semen production and/or importing organizations (Centers) belongs to the sire identified on the straws (Identification check - VCI) and is correctly self-certified for quality (Self-certification check - VCA). The program, started in 1996, involves frozen semen of many species (bovine, equine, swine and buffalo) distributed in Italy (produced at national level or imported) by random sampling of at least 10% batches.

The VCI is accomplished by comparing the DNA of a sire extracted from a reference sample (blood or semen) with the DNA obtained from the frozen sperm using microsatellites according to ISAG panels. In case of mismatch (incorrect identification) the Center must destroy the entire batch. The VCA involves the comparison of number of progressively motile sperm (NPMS) certified by the Center with the NPMS obtained by IS, using an algorithm derived from an extensive study of the variability within the batches produced by different Centers. If the VCA is classified as incorrect, the Center must modify the self-certification with the data of the OSC.

In the years, more than 44,200 batches and 43,400 comparisons have been processed: 32 incorrect identifications and 657 incorrect self-certifications have been issued. Considering the reduction of incorrect VCA accounted over the years for bovines, the most relevant species in Italian animal breeding, the trend reported indicates an increasing capacity of the Centers to evaluate their product even if great variability is still present for importation.

The OSC is producing data of considerable interest to those involved in reproduction technologies: on the one hand, it allows a quantitative monitoring of the frozen semen produced in Italy or imported from foreign countries, on the other, it allows to gather useful information on the quality of semen. Moreover, OSC has allowed to create a cryobank with the genetic material of more than 14.700 sires used in artificial insemination in Italy.

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P027

Wood's model application in six different goat breeds

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The Wood's model of the lactation curve is a useful toolto analyse dairy goats performances and although it has been successfully applied, some differences have been recorded among breeds. Therefore, aim of this work was to record milk yield and quality in different goat breeds maintained in the same management conditions and to investigate the differences in the lactation curve trends among breeds, using Wood's model. The trial was carried out in the experimental farm of CREA (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Potenza, Italy) and a total of 446 goats (parity ranging from 2nd to 4th) from six different breeds were selected. In particular, 74

Garganica (Gar), 72 Girgentana (Gir), 75 Jonica (Jon), 74 Maltese (Mal), 76 Red Mediterranean (Red) and 75 Saanen (Saa) goats. Milk yield was recorded every 15 days throughout 150 lactation days from each animal during both morning and afternoon milking and samples for each day were collected. Milk samples were analyzed for protein, fat and lactose by infrared spectrophotometry. Furthermore, milk yield was standardized at 35g fat/kg milk. Wood's model showed to fit well all curves considered in all breeds, with R^2 values ranges from 0.9281 (Red) to 0.9605 (Saa) for milk yield and higher than 0.7681 for milk constituents. Considering milk yield, Gar and Jon goats showed the lowest a values ($p < .01$), Gar and Gir goats showed the latter days in milk (DIM) peak ($p < .01$). Gar and Gir were characterized by a low initial production (a), by a higher increasing rate (b) and a latter peak day. Moreover, Pearson's correlations showed a negative correlation between a and b, c, estimated peak (b/c) and persistency index ($-(b + 1)\ln(e)$) ($p < .01$), implying that higher initial yields (milk, fat, protein or lactose) are associated with lower rates of increase. Based on this, animals characterized by higher initial production levels and higher a values, tend to exhibit lactation curves characterized by short ascendant phase (lower b values), earlier peaks DIM and lower persistency. Gar and Gir Wood's parameters are characterized by the highest b values. The b *parameter* has great importance in the shape of curves. Its absolute value determines the magnitude of the curvature of each milk traits curve considered. In conclusion, Wood's model could represent a good way to select dairy goats, particularly of some minor breeds, in order to make them most economically advantageous and profitable.

P028

Alternative splicing site disruption as strong candidate for white dominant phenotype in an Italian Trotter

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Since performance and color are not correlated, race horses are generally not affected by coat color selection: still the appearing of an unusual coat can pique interest. Recently, in Thoroughbred and Standardbred studbooks, a number of individuals characterized by dominant white (W) coat pattern, born white from solid pigmented parents has emerged. This phenotype is uncommon for these breeds, where bay and chestnut or grey are the norm, and did not appear on a population of 32.000 (UET report, 2015) trotter reared in Europe per year. Dominant white is characterized by depigmented skin areas from 50% up to the entire body surface.

Here we present results from the whole genome sequencing of Via Lattea, a solid white Italian trotter born in Assisi in 2015, as the first documented case in Europe.

Sequencing generated over 1 billion reads gaining over 35x coverage in at least 85% of the genome. GATK suite and current best practices were applied for the variant calling analysis and more than seven million variants were called at high quality in comparison to the reference genome (equcab2). Genes known to affect coat color with white alterations (*KIT*, *PAX3*, *MITF* and *EDNRB*) were particularly investigated. SIFT algorithm revealed one mutation in intron 15 of *KIT* gene that disrupts a splicing signal, compromising the correct mRNA formation. Sanger sequencing confirmed NGS data and the absence of mutation in parents, therefore the mutation is a spontaneous one, carried in heterozygous state in Via Lattea.

P029

Start-up of a conservation plan of the Altamurana sheep breed

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Altamurana is an endangered-maintained Apulian sheep breed worthy of preservation because of its significant features concerning history, robustness, tolerance to tick borne diseases, and unusual hemoglobin polymorphism. In 2013, the University of Bari undertook a research project aiming at reporting on the status of the breed. In the following years, the Alta Murgia National Park funded a program with the specific purpose of monitoring: (a) number of local farms raising Altamurana breed; (b) population size; (c) productive, reproductive and health parameters; (d) stakeholder involvement and sensitization about biodiversity issues. In this paper are reported the results of a four-year action plan. Firstly, statistics of the Altamurana breed were derived. Census size data were obtained with farm-by-farm detail. Eight flocks were found, summing up to 507 heads. The information collected included number of sires and dams, age, health status (body condition scores [BCS], hematological and biochemical parameters, gastro-intestinal parasitism), and screening of hemoglobin polymorphism. Average flock size ranged 30 to 145 animals; total rams numbered 54¸ while the ewes numbered 453, resulting in an effective population size (Ne) of 193 individuals. Records for BCS, haematology and biochemistry results, and the faecal test to check for intestinal parasites generally depicted a healthy pattern of the flocks. Analysis of allele frequencies for the β-globin locus and those related to the α-globin haplotypes confirmed the presence of all the variants reported in previous studies, pointing that no losses of rare alleles occurred, despite the small population size. The general positive attitude of farmers towards the breed preservation brought the census recorded in autumn 2016 to number 780 heads of which 112 rams. Unfortunately, the severe winter strongly affected the population. Several cold-related deaths occurred in the flocks; most of the lambs selected to replace the animals to be culled died; moreover, the flocks had to be confined in the barn for a month, making the cost for feeding unsustainable and leading to the slaughter of many males. Currently; total rams number 39, while the ewes number 665 with subsequent reduction of effective population size to 147 individuals. This shows the fragility of the system and the fact that an effective conservation plan needs further external support, as the regional biodiversity contribution cannot cover these extra costs.

P030

Application of SNP reduction approaches and random forest for the identification of population informative markers in cosmopolitan and local cattle breeds

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In livestock, single nucleotide polymorphism genotyping arrays have been used to differentiate breeds and populations for several downstream applications, including breed allocation of individuals, breeds of origin of crossbred animals, authentication of mono breed products, comparative analyses of selection signatures among several other uses. We already tested a combination of principal component analysis (PCA), used as pre-selection method, and random forest (RF) used as classification method to assign cosmopolitan Italian breeds with no or very low error rate. In this work, we increased the number of breeds and approaches, to have a more comprehensive view of the strategies available and the applicability to local Italian breeds. A total of 3304 animals belonging to cosmopolitan cattle breeds (2091 Holstein, 738 Brown and 475 Simmental) and 311 animals belonging to three Italian local cattle breeds (71 Cinisara, 72 Modicana and 168 Reggiana) were genotyped with the Illumina BovineSNP50 BeadChip array (Illumina). Several methods of SNPs pre-selection (Delta, F_{ST} and PCA) in addition to RF classifications were evaluated. From these classifications, two panels of 96 and 48 SNPs that contained the most discriminant SNPs were created for each pre-selection method. The results showed that the 96 SNP panels were generally more able to discriminate all breeds, while for the 48 SNP panels the error rate increased mainly for autochthonous breeds, particularly for Cinisara. This was probably a consequence of limited selection pressure, admixed origin, and ascertain bias on the construction of the SNP chip. Several selected SNPs are located nearby genes affecting breed-specific traits (e.g. coat color and stature) or associated to production traits. The 96 SNP panel obtained after a preselection chromosome by chromosome, and used in the previous work with cosmopolitan breeds only, could identify informative SNPs that were particularly useful for the assignment of minor breeds. This panel reached the lowest value of out of bag (OOB) error in the RF test even in the Cinisara, whose value was quite high in all other panels. Moreover, this panel contained also the lowest number of SNPs in linkage disequilibrium. Our results showed the usefulness and power of the combination of PCA pre-selection and RF also for the discrimination of cattle breeds.

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P031

Cholesterol supplementation reduces cryocapacitation damages in bovine sperm

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The quality of frozen semen is still a major factor affecting both in vivo and in vitro fertility, and hence has a great impact on genetic improvement. It is known that cryopreservation induces sperm premature capacitation reducing the functional life span of sperm. Altering the lipid composition of sperm plasma membrane affects the ability of sperm to capacitate and react to cryopreservation. Therefore, aim of this study was to evaluate the effect of cholesterol loaded cyclodextrins (CLC) on the capacitation status of bovine frozen/thawed sperm. Four ejaculates from 4 breeding bulls (Centro Tori Chiacchierini, Civitella D'Arna, Italy) were selected, split in 2 aliquots and diluted at 37 °C with BULLXcell extender, containing 0 (control) and 1.5 mg/mL CLC, to a final concentration of 30×10^6 sperm/mL. Cyclodextrins were loaded with cholesterol as described earlier (Purdy and Graham 2004. Cryobiology. 48(1): 36-45). The aliquots were frozen according to standard procedures. At thawing, sperm motility was evaluated by phase contrast microscopy while viability and capacitation status by Hoechst 33258/Chlortetracycline (CTC) assay (Neild et al. 2003. Theriogenology.59:1693–705). Spermatozoa were classified as: dead when nuclei showed bright blue fluorescence over the sperm head and into one of three CTC staining patterns: pattern F, with fluorescence over the entire sperm head (non capacitated); pattern B, with a fluorescence-free band in the post-acrosomal region (capacitated); pattern AR, with no acrosome and a thin band of fluorescence along the equatorial segment (acrosome reacted). Cell recorded as dead were not assessed for their CTC-staining pattern. Data were analyzed by Student *t* test. No differences between control and 1.5 mg/mL CLC were recorded in both sperm motility (74.4 ± 1.7 and 77.8 ± 1.5, respectively) and viability (80.9 ± 1.9 and 85.2 ± 2.1, respectively). A remarkable reduction of sperm cryocapacitation was observed in the CLC-treated group compared to the control, as shown by the increased percentage of pattern F sperm (33.6 ± 3.1 and 70.8 ± 2.0; $p < .01$) and the decreased percentage of pattern B sperm (65.7 ± 3.1 and 28.8 ± 2.0; $p < .01$). However, no differences were detected in pattern AR that remained low in both groups (0.7 ± 0.3 and 0.4 ± 0.2 in the control and CLC groups, respectively). In conclusion, the inclusion of CLC in the extender strongly decreases cryocapacitation improving the quality of bovine frozen/thawed sperm.

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P032

Population structure and genetic variability in Maremmano horse via pedigree analysis

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The Maremmano is an indigenous Italian breed, probably descending from the native horses of the Etruscans (VI century b.C.); the Studbook was acknowledged in 1980, and it includes 12,368 horses born from that year up to 2015. Demographic and genetic parameters of this population have been computed by "Endog v 4.8" program and in-house software.

The pedigree completeness of this population is very good: more than 90% at the third parental generation, and the completeness is still more than 70% at the fifth generation; in the average, the number of generations traces back to a maximum of 10.5, with generations 3.3 complete generations and 5.7 equivalent complete generations. The average generation interval is 10.08 ± 4.5 years, ranging from 9.81 ± 4.32 (mother-daughter) to 10.40 ± 4.71 (father-daughter), and stallions are in use for longer periods than mares.

The effective number of founders (f_e) is 74 and the effective number of ancestors (f_a) is 30, so that the ratio f_e/f_a is 2.47; the founder genome equivalents (f_g) is 13.72, with a ratio f_g/f_e equal to 0.18. The mean of the genetic conservation index is 5.55 ± 3.37, and it ranges from 0.81 to 21.32. The average inbreeding coefficient is 2.94%, with a yearly increase of 0.1%, and the average relatedness coefficient is 5.52%. The effective population size (N_e) computed by individual increase in inbreeding is 68.1 ± 13.0; the N_e on equivalent generations is 42.0 and this value slightly raises to 42.2 when computed by Log regression on equivalent generations.

The analysis confirmed the presence of seven traditional male lines; in 2015, the most contributing represented stallion newborn foals is Ingres (4.3% of "blood percentage"), then Otello (4.1%), Ajace (3.3%) and Ussero (1.2%): the three "minor lines" (Le Brun, Ognon and Noris) are below 0.5% each. At the population level, the Ajace's male line founders represent 18.1% of the Maremmano genetics: after this, Otello's line founders represent 13.6%, then Ussero's founders (6.0%) and Ingres' ones (5.5%); all the "minor lines" are below 1% for this parameter.

The percentage of Thoroughbred blood in the foals born in 2015 is 20.3%: this percentage increased 0.21% per year since 1980: in particular, it raised more than twice (0.51%/year) till 1993, with only minor fluctuations after that year.

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P033

Polymorphisms at *PITX2* gene and effects on milk quality of Argentata dell’Etna goat breed

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The paired-like homeodomain transcription factor 2 (PITX2) is a member of paired-like homeodomain transcription factor (PITX) family and is able to act as a transcriptional regulator of multiple genes. The PITX2 is expressed very early during the development of the pituitary gland, thus constituting one of the first genetic markers of the development. Since the *PITX2* gene was found to affect both the Wnt/beta-catenin pathway and the POU1F1 (pituitary-specific positive transcription factor 1) pathway associating with milk traits, it is supposed to affect milk traits. Recently, the effects of some genetic variants of *PITX2* gene on milk trait were reported in two Chinese dairy goat breeds. This study analyzed the genetic polymorphism at *PITX2* gene (Acc. Num. AC_000163) in 48 Argentata dell’Etna goats. Three SNPs, namely P1 (g.18353T > C), P6 (g.18161G > C) and P7 (g.18322C > A), mapping at IVS1 + 110TNC, Pro42Pro and IVS1 + 79CNA, were genotyped by the RsaI, SmaI and MspI aCRS–RFLP or PCR–RFLP methods, respectively. The association between genetic variants, milk yield and chemical composition of the individual milk was also investigated. The individual milk samples were analyzed for fat, protein, casein, lactose and urea percentage, somatic cells by infrared system and fatty acids composition by gas chromatography. The experimental data were processed by the analysis of variance (ANOVA general linear model) . Only one (P1 g.18353T > C) out of three SNPs investigated was found to be polymorphic in Argentata dell’Etna. Three genotypes g.18353TT (33), g.18353TC (14) and g.18353CC (1) and two haplotypes GCT and GCC (AC_000163 n.18161, n.18322, n. 18353) were detected. The allele g.18353C and the related haplotype GCC had a frequency of 0.17 and 0.31, respectively. In 47 Argentata dell’Etna goat (the only one g.18353 CC goat was removed from the dataset), the allele g.18353T was significantly associated with an higher percentage of fat (4.79% *vs* 4.38% respectively in TT and TC goats; $p = .016$) and protein (4.20% *vs* 3.94% respectively in TT and TC goats; $p = .028$). The fatty acids composition was not affected by the PITX2 polymorphism, but there was an increase in the total trans fatty acids content in TT goats (2.49 *vs* 2.19, $p = .059$). These preliminary data seem to be promising because they reveal a significant relationships between genetic polymorphism of *PITX2* gene (AC_000163 locus: g.18353T > C) and the milk traits in *Argentata dell’Etna* goats.

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P034

Genome-wide methylation profiles changes associated with stress exposure in cattle

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Stress has a detrimental impact on dairy cattle health, welfare and productivity. Its impact on gene expression, metabolism and immune response has been investigated, but little is known on the epigenetic mechanisms mediating the effect of stress at the cellular and organism level.

To assess how different levels of stress exposure affect epigenetic regulation mechanisms in cattle, we investigated genome-wide DNA methylation in 20 Italian Red Pied dairy cows falling in the plus- and minus-variant tails of the distribution of milk cortisol concentration (MC), a neuroendocrine biomarker of stress in dairy cows, measured in 180 animals belonging to the same farm in the framework of the Gen2Phen Italian project. The ‘low-’ and ‘high-cortisol’groups of animals had MC <370 pg/ml and >810 pg/ml, respectively. Their methylome was analysed by Reduced Representation Bisulfite Sequencing, which provides single-base resolution methylation profiles across the whole genome.

To date, 20 animals (10 low- and 10 high-MC) have been sequenced and an average of 22.5 million reads were produced per sample, with varying mapping efficiency (18.0–33.6%). The two groups showed similar proportion of methylation at CpG sites, while they differed at non-CpG sites.

Significant methylation changes were observed in 360 regions and 95 genes. KEGG pathway analyses indicated that these genes were mainly involved in glucocorticoids metabolism, stress responses, cellular defense and calcium signaling pathways.

These preliminary results suggest that stress response in livestock is mediated by epigenetic regulation, provide target biomarkers to assess the effect of stress-mitigation management procedures and candidate genes for the selection of stress-tolerant animals.

P035

A GWAS in the autochthonous Italian Valdostana Red Pied cattle population based on a high resolution Copy Number Variant (CNV) scan

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CNVs are an important source of genomic structural variation, recognized to affect phenotypic variation in many mammalian species especially related to regulation of complex disease. Recently CNVs have been mapped in several cattle populations and they have been shown to be located also in relevant gene families affecting productive, functional and health traits. Valdostana Red-Pied, an autochthonous Italian dual purpose cattle population, did not undergo strong selection for production traits as compared to Holstein or Brown Swiss and can thus deliver valuable information on structural variation and its association with complex traits in an Italian local cattle breed. CNVs have been mapped from the log R ratio (LRR) and B allele frequency obtained genotyping 143 bulls with the Illumina BovineHD bead chip. After PennCNV-CNVRuler analysis, a total of 368 CNVRs were found with a MAF threshold above 0.05 (165 gains and 204 losses). Official EBVs for cheese yield (IRC) and cheese yield + muscularity (IRCM) were provided by A.N.A.Bo.Ra.Va. In addition, the traits milk yield (MILK), fat yield (FAT), fat % (FAT%), protein yield (PROT), protein % (PROT%), muscularity (MUSC) and udder (UD) were analyzed. After PennCNV-CNVRuler analysis, a total of 368 CNVRs were found with a MAF threshold above 0.05 (165 gains and 204 losses). Our results indicate that the 27% of the CNVRs here identified resulted to be significantly associated with functional and productive traits on several chromosomes. Some of these regions overlap known chromosome regions previously identified in other breeds using different experimental designs for the same traits on BTAs 2, 5, 9, 18, 23 and 29. Data were generated as part of the FP7 project QUANTOMICS contract n. 2226642.

P036

Identification of genomic regions of recent selection for productive and reproductive traits in Italian Holstein bulls

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The impressive genetic improvement of milk production obtained in cosmopolitan dairy breeds during the last 65 years has been accompanied by a reduction in fertility, due to negative genetic correlation between those traits. Aim of this work is to identify genomic regions recently selected for dairy and reproductive traits in ~3000 Italian Holstein Bulls born between 1987 and 2007.

Illumina BovineSNP50 and BovineHD SNP chip genotype data from SELMOL, PROZOO and INNOVAGEN projects were used. Estimated Breeding Values (EBVs) of the genotyped animals were provided by the Italian Holstein association (ANAFI) for 32 productive, morphological and reproductive traits. Genotypes were quality-checked and medium-density data were imputed to high density using BEAGLE (v.3). The working dataset comprised 2,918 animals and 613,956 SNPs. Contrasting groups of bulls, identified as minus- or plus-variant for each EBV over the 20-year span were previously analyzed to find chromosomes containing genomic regions with the highest differences in allele frequency between groups, and thus supposed to be under strong directional selection. On these chromosomes, runs of homozygosity (ROHs) were investigated on plus- and minus-variant bulls for 13 production and fertility EBVs (bull's and cow's calving easiness, body condition score, calving interval, combined longevity, days at first service, somatic cells count, milk, protein and lipid yield, protein and lipid content). Delta ROH (ΔH) scores for each SNP between plus- and minus-variant groups for each EBV were calculated to detect regions with the highest variation. Genes with the highest differences in allele frequencies between minus and plus variant groups and with the highest ΔH were identified. About 500 genes on nine chromosomes (BTA 1, 2, 4, 5, 7, 9, 20, 26 and 29) were detected of which 39 showed signals of selection both for dairy and reproductive traits. These signals were classified as discordant ($N = 12$) or concordant ($N = 27$) effects on productive and reproductive traits. In addition, these genes are located into QTLs for both dairy and reproduction traits.

This approach seems promising for the identification of genomic regions containing genes that show recent selection both for dairy and reproductive traits. In the genomic evaluation of the animals, the SNPs associated with these genes deserve to be weighted for their peculiar effect on EBVs.

P037

The CA.RA.VA.N project: toward implementation of a modern dromedary selection system

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In desert and semi-desert areas, camel farming plays a pivotal role in conservation of rural societies and valorisation of natural resources through multifunctional livestock production systems. Rapid, though fragmentary and disorganized, changes are currently ongoing in the camel sector, with urban growth and changing diets contributing to accelerate the commercialisation of camel products and, consequently, production intensification and radical changes in camel management practices. Dromedary national or local breeder associations have been recently established in some Maghreb countries, however animal identification and phenotype recording are still in an early stage. A recent project named CAmel tRAnsnational VAlue chaiN (CA.RA.VA.N) has been financed by EU-Arimnet2 through a common pot instrument, involving partners from Spain, Algeria, Morocco, Tunisia, Italy and France, aiming to transfer and apply modern technologies to the various field of the dromedary sector, in order to generate sustainable development opportunities. A specific task will be the implementation of a modern organization system for animal identification, genealogy and phenotypic recording, linear assessment of morpho-functional traits, DNA banking and genotyping, adopting an integrated transnational participatory approach. During the first stage, analysis and standardization of guidelines and practices for work, milk, meat, and reproductive phenotyping in dromedaries and for their individual identification and genealogical recording will be carried out. Later on, a transnational pilot project for the implementation of the above standardized practices, the development of linear assessment protocols and the initial utilization of new-generation genotyping technologies in Southern Mediterranean countries (Morocco, Algeria, Tunisia) will be launched, in collaboration with several breeder associations. An additional pilot project for the development of linear assessment protocols to evaluate the work aptitude of the dromedaries through body and functional (gait, trot and canter) characteristics related to work performance will take place in Canary Islands in collaboration with the Asociación de Criadores de Camellos Canarios. The project will also provide a valuable opportunity for scientific exchange and networking in the dromedary sector, and to increase visibility of a still generally neglected livestock species.

P038

Whole genome analysis of the Lupo Italiano

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The Lupo Italiano (Italian Wolfdog) is a domestic dog (*Canis lupus familiaris*) breed created in 1966 by crossing of Apennine grey wolves (*Canis lupus italicus*) to German

Shepherd dogs (GSD). The breed has an official studbook with management protocols, under the control of the Ministry of Agriculture. The breed is characterized by strength, resistance, and strong capabilities in learning, as well as participation in search and rescue activities. Considering the depth and completeness of genealogical information, the high levels of inbreeding, and the presence of the wolf as a recent ancestor, the Lupo Italiano can be used as a model to investigate the effects of population structure and selection on wolf-dog hybridization. The aim of this work is to compare the genetic background of the Italian Wolfdog with that of the GSD, village dogs, grey wolves from the Apennines, and other dog populations, with a specific goal of detailing introgression between the Lupo Italiano, wolf and GSD.

Three hundred and seventy-seven individuals were genotyped using a high density chip containing more than 170K SNPs. Genotypes for Lupo Italiano were provided by University of Milano and National Institutes of Health in Bethesda, MD. Those for the Apennine wolves were produced by ISPRA, and those for GSD, village dogs and grey wolves were publicly available (Dryad, Shannon et al. 2015). Samples and loci were quality checked, and then analyzed using Multi-Dimensional Scaling (MDS). The relationship matrix based on pedigrees was compared with the genomic relationship matrix (GRM), calculated using GCTA64 software. In addition, the genomic heterozygosity-based inbreeding coefficient has been estimated using PLINK v1.9 software. Reynolds distances were computed to define the relationships among the five populations. In addition, the Lupo Italiano, wolves and GSD were studied at the chromosomal level. As expected, the closest population to the Lupo Italiano is the German Shepherd dog (Reynold genetic distance 0.25), whereas the farthest is the Apennine Wolf (0.40).

Results are expected to provide a clear picture of the genomic structure of the Lupo Italiano and its hybridization history. Beside scientific interest, these findings will allow the breeder association (AAALI) to better manage their animals and conserve breed genetic variation.

Acknowledgements

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P039

Polymorphisms in genomic region of *PTX3* gene and their association with somatic cell score breeding value in Italian Holstein

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Using a target re-sequencing approach, we identified SNPs in a region of 20kb of BTA1 containing the Pentraxin3 (*PTX3*) gene, a candidate gene for mastitis resistance in Holstein cattle breed. *PTX3* plays a role in the regulation of innate resistance to pathogens and the consequent inflammatory reactions. *PTX3* is a secreted prototype protein of the long pentraxin group synthesized by a variety of tissues and cells and in particular by innate immunity cells in response to proinflammatory signals and Toll-like receptor (TLR) engagement. A TruSeq Custom Amplicon Assay was designed to resequence the region including *PTX3*: 12 kb upstream of the promoter and 1 kb downstream of the 3′-UTR (Miseq NGS technology). The DNA of 95 Holstein bulls chosen using a selective genotyping approach according to their somatic cell score breeding value (SCS-EBV), was extracted, amplified with the custom assay and sequenced. An individual average coverage threshold of 20X was considered, resulting in 55 high and 35 low SCS-EBV individuals retained. All data analyses were carried out in R environment. On a total of 66 identified SNPs, only 20 with minor allele frequency (MAF) higher than 0.05 were considered: four were in exon 3 and 16 in intron 2. All these SNPs were already recorded in the NCBI database. Allele and genotype frequencies were calculated in the two groups (high and low SCS-EBV) and a Mann-Whitney test was used to detect frequency difference between groups, while the genotype-EBV association was assessed with Kruscal-Walls Test. The SNP effect was estimated using the heteroscedastic effects model (HEM) (Shen et al. 2013) using the package bigRR (Shen et al., 2013). The genotype distribution of eight SNPs was different between groups and was significantly associated with SCS-EBV ($p < .05$) with a mean HEM effect of 0.032 (0.015-0.037); remarkably, only one of these eight SNPs maps in exon 3 (rs136063049 G/T). An additional SNP in exon 3 (rs 378618076) gave a different genotypes distribution but the association genotype/EBV was not significant ($p = .055$) even though its HEM value (0.97) was the highest. In conclusion, nine SNPs were identified as candidate markers for genetic resistance to mastitis in Italian Holstein. Further investigations could be useful to understand the role of intronic regions including SNPs: in fact, some identified SNPs are localized in two non-coding segments of 600bp which could be involved in RNA splicing or in gene expression regulation.

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P040

Preliminary characterization of a microsatellites panel in the major histocompatibility complex in Curraleiro cattle breed

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The majority of cattle in Brazil is represented by *Bos indicus*, of Nellore and Gyr breeds (*Bos indicus species*) but there are also several cattle breeds, derived from the animals carried by Spanish and Portuguese settlers during the XIV and XV centuries. These animals have been reared during the past 500 years in the tropical conditions and for this reason they have a natural adaptability to the environment and to the tropical disease. Two examples are the Curraleiro (from the Portuguese name currais, yard) and Pantanero (from Pantanal, a marsh region of South West Brazil) reared typically in central part of Brazil in semiarid environment and in South West tropical region respectively, in the majority of cases they represent the only means of subsistence of local people by producing meat in economically marginal areas. Through routine sanitary assessment of 2000 animals, high variability was detected in serological parameter and antibody response to tuberculosis, paratubercolosis, and leucosis. Under the hypothesis that molecular variability existed in the genes with a role in the immune system and in disease resistance, a study was designed for the molecular characterization of the bovine major histocompatibility complex (BoLA). The assessed sample included 1200 Curraleiro animals from 21 herds and 300 Pantanero, from 5 herds. We used the following set of published 15 microsatellites markers linked to Class I, II and III Histocompatibility Complex in cattle, located on BTA23: RM185, DBRP1, 312, 171; 1870, 415, BDR3, 1687, LA54, 1268, AGER and 1778 (available at oaktrust.library.tamu.edu; Krista L. Fritz Phd dissertation). Furthermore, microsatellite SLC11a1 (BTA2) was also included because linked to the Solute carrier 11A1 gene, which was reported to be associated to tuberculosis and tick resistance in cattle. All marker resulted polymorphic with a total number of alleles of 9.67 ± 5.65 ranging from 2 of locus 415 and 19 of 1687 locus while observed and expected heterozygosity of 0.64 and 0.71 respectively. The panel showed sufficient polymorphic content and will be used in association analysis for disease resistance.

P041

Follow-up on the genealogical and demographic characterization of the Murgese horse

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The black, or rarely roan, Murgese is a mesomorph, baroque-type horse, autochthonous from the arid and rocky Murgia hills of Apulia (Southern Italy) appreciated for good temperament, hardiness and surefootedness, strong limbs and hooves. After agricultural mechanization, the Murgese decreased in numbers, and it was mainly kept for production of locally consumed meat. Only recently (October 2008), the Murgese Studbook was established in order to improve effectiveness of the breeding strategy. In previous studies, we addressed variability and evolution of morphological traits, and performed preliminary pedigree analysis of the Murgese horse. Here, data including 4550 records belonging to individuals (1003 males and 3447 females) registered in the Studbook from its foundation to mid 2014, out of which 4276 with both father and mother known, were analyzed by ENDOG v. 4.8. The maximum number of generations was 14 (mean 8.84), the maximum number of complete generations was 6 (mean 3.28), and 5.08 the mean equivalent generation. The number of individuals registered per year in the Studbook increased from its foundation to 2008 (240 newly registered animals). In the years 2009-2013, on average, 220 animals were registered per year. A total of 81 animals were registered in the first half of the 2014. The average inbreeding coefficient was 4.46% (9.02% in the last complete generation), with 100% of animals being inbred already starting from the 3rd complete generation. The average relatedness coefficient was 8.51% (11.55% in the last complete generation). Popular sires and dams were identified. Sixty-one animals had ≥20 offspring (top sires: Nume and Everest, with 96 and 91 registered offspring, respectively). Seven ancestors were able to explain 50% of the population. Number of founders was 325 but the effective number of founders was 37. The effective number of ancestors was 20. Generation interval was 11.7 ± 10.9 years. The above results, if compared to values obtained in 2007, when the data set was composed by records for which no DNA parentage control of individuals was available, highlight a critical increase of the inbreeding coefficient (1.22% in 2007) and the mean average relatedness (2.45% in 2007), which are worthy of careful consideration for a sound reproduction management, and balanced selection decisions.

P042

Colostrum fatty acid composition in sows belonging to three different Italian pig breeds

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Sow colostrum represents the first nourishment for piglets and an essential vehicle to stimulate piglets' immune response immediately after birth. In the past years, the majority of the studies carried out on colostrum matrix were aimed at investigating the different immunoglobulins quantity and quality. To date, thanks to new technological approaches such as the gas-liquid chromatography, it is possible to improve our knowledge of colostrum bioactive compounds and fatty acids (FA). In the present study, six pools of colostrum gathered from sows belonging to three different Italian pig breeds were analysed and their fat contents and FA compositions compared. The six pools were constituted as following: i) 7 Duroc sows (D); ii) 21 Landrance (L): iii) 21 Large White (LW), and the colostrum of these pools belong to different farrowing parities; iv) colostrum taken from 7 fourth parity Large White sows (LW4); v) colostrum from 6 second parity Italian Large White sows (LW2); vi) colostrum from 7 fourth parity Landrance sows (L4). Three independent samples were prepared for each pool. The determination of the main lipid classes (triglycerides, diglycerides, monoglycerides, free and esterified sterols, and free FAs) and the total FA composition of the lipid matter obtained by liquid-liquid extraction (chloroform:methanol, 2:1, v/v), was carried out. The obtained lipid compositions were analysed using ANOVA and Tukey's honest significance test was carried out at a 95% confidence level ($p < .05$). Fat content results are reported as percentage. Large White pool LW2 showed a lower amount of fat (expressed as g/100g of colostrum) compared to D and L pools (2.33% in LW2 and 3.63% in both D and L pools) and a higher content of free FAs (g/100g of fat) compared to all other pools (1.70% in LW2 *vs* 0.87% in D and L, 0.73% in LW, 0.90% in LW4 and 0.80% in L4, $p < .01$). Furthermore, LW pool presented higher amount of conjugated linoleic acid (expressed in g/100g of total FAs) compared to all other pools ($p < .001$) and an increased content of omega3 FAs (g/100g of total FAs) than LW2 and L4 (2.41% in LW *vs* 1.72% in LW2 and 1.67% in L4, $p < .05$). The identified differences represent an interesting starting point for further studies aimed at relating variations in pig colostrum fat content and FA composition with the different maternal aptitudes.

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P043

Functional characterization and small RNA sequencing of cryopreserved semen in high and low fertility bulls

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Individual bulls differ in their ability to fertilize oocytes due to physiological and molecular characteristics of spermatozoa. The combined use of advanced techniques, as standard semen quality assessment together with sperm molecular investigation, is a promising approach to achieve a better understanding of sperm functions and to predict bull fertility.

The aim of this study was to evaluate the relationships among frozen bull sperm parameters, assessed by computer-assisted semen analysis (CASA) and flow cytometry (FCM), and miRNAs expression in high and low fertility bulls.

Ten frozen semen doses from 10 Italian Holstein-Friesian bulls, 5 of high and 5 of low fertility according to the estimated relative conception rate (ERCR), were analyzed in two different breeding seasons (Sep-Oct and Feb-Mar), by using CASA system for motility and sperm kinetic parameters assessment and FCM for sperm viability, acrosomal status and DNA integrity evaluation. The semen doses were thawed

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and pooled; high (HM) and low (LM) motile sperm fractions were isolated by Percoll gradient. For each bull total RNA was extracted from HM fraction and small RNA libraries were generated using Illumina Truseq Small RNA Preparation kit. Libraries were sequenced on a single lane of Illumina Hiseq 2000.

Sperm cells were successfully fractionated in HM and LM populations achieving a better quality ($p < .05$) in the HM population respect to raw semen and LM population. Fertility level affects raw semen quality for almost all of the variables, significantly for total and progressive motility and for two DNA integrity indicators (degree of chromatin abnormality and percentage of sperm with high green fluorescence), showing a superior quality in high fertile bulls. Season seems not to affect sperm quality. A backward stepwise multiple regression analysis was applied in order to define a model with high relation between semen quality parameters of the HM population and ERCR. A prediction model that explained almost 80% ($R^2 = 0.78$, $p < .05$) of the variation in the conception rate was identified. The model includes five variables: total and progressive motility, curvilinear velocity, degree of chromatin abnormality and percentage of DNA fragmented sperm. miRNA profiling didn't change in sperm collected in different seasons, whereas 9 *Bos taurus* miRNAs, and 6 new candidate miRNAs were found to be statistically different (False Discovery Rate <0.05) between high and low fertility bulls. Target genes of 9 known miRNAs were predicted, and pathways potentially affecting sperm fertility were identified. Among miRNAs, 5 targeted 469 genes involved in different biological pathways such as regulation of meiosis I and cell division.

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P044

Effect of a diet enriched in PUFA and polyphenols on the expression of genes involved in lipids metabolism in pigs

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The content of saturated, monounsaturated and polyunsaturated fatty acids (FA) in pig muscle is very important not only for the consumers' health but also for the pig processing industry. Breed, diet and genetics are the main factors controlling FA composition of pig meat even if molecular processes and genes controlling lipids metabolism are not yet well known. We performed the present study to improve the knowledge of diet influence on the transcription of genes controlling lipids metabolism. The effects on gene expression of a diet enriched in polyunsaturated fatty acids (PUFA) and polyphenols *vs.* a control diet were compared. The transcription level of 30 genes involved in FA metabolism and fat deposition in pigs was analysed. Two groups of 12 Italian Large White pigs each, balanced for sex, were reared until the average weight of 149 kg (±10.7 kg). The first group (G1) was fed a standard diet while for the second group (G2) the diet was supplemented with linseed and grape extract. Samples of *longissimus dorsi* were collected at slaughter and stored at -80 °C until RNA extraction. Preliminary data have been obtained for 11 genes (*ACACA, ACDC, ADIPOR1, ADIPOR2, CHREBP, ELOVL6 isoform 1* and *2, FADS2, FASN, LXRA, PLIN3, SCD*) using qRT-PCR and *B2M, HPRT1, POLR2A* as normalizing genes. Moreover, the pigs have been genotyped for a SNP in the promoter region of the *ELOVL6* gene (*ELOVL6*:c.-480C > T). The expression levels of this set of genes were compared between the two diets using the Exact Wilcoxon Test in R software. Partial results show that the diet supplementation with PUFA and polyphenols influences the transcription of genes involved in the long chain FA synthesis and in lipids utilization. In particular, both *ELOVL6 iso1* and *iso2* expressions are higher in G2 than in G1 ($p = .04$ and $p = .03$, respectively). The same trend is observed for the higher transcription level of *ACDC* gene in G2 than in G1 group ($p = .06$). The ratio between the *ELOVL6* isoforms (iso2/iso1) is more elevated in the *ELOVL6* CT when compared to TT ($p = .03$) and to CC ($p = .06$) genotypes. The study is currently underway on the remaining genes. At the end of the analysis of the full set of genes, the correlated gene expressions in both diets and the effects on FA profile of muscle and meat quality traits will also be considered.

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P045

Selection model for resistance to scrapie in small populations: the case study of Brianzola sheep breed

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Scrapie is a transmissible spongiform encephalopathy (TSE) affecting the central nervous system of sheep and goats. Susceptibility to scrapie is associated to polymorphisms of the prion protein (*PrP*) gene. The European states have introduced specific breeding programmes to select for TSE-resistant individuals in order to increase the frequency of the ARR resistant haplotype and to reduce the susceptible alleles VRQ (EC Regulation n. 999/2001; decision 2003/100/EC). The Lombardy region, with the D.d.s. July 26, 2016 made it mandatory to comply with the genetic selection plans. However, selecting for resistance to scrapie in small populations may increase the risk of inbreeding depression, with even further reduction of the effective population size.

The aim of this work was to validate an economical advantageous chip of molecular Single Nucleotide Polymhorphism (SNP) markers in the Brianzola sheep breed, a small local population at risk of extinction. The chip was, then, used in a selection model for estimating the genetic distances among potential scrapie-resistant breeders.

A number of 128 SNP markers were tested among those suggested by the International Sheep Genomics Consortium (ISGC). The chip, which can be used with the technology QuantStudio®, was tested in 200 ewes selected on a random base. A total of 80 markers with the best informativeness were individuated on the basis of heterozygosity, minor allele frequency and call-rate. The Wright's fixation indices (F) for locus were calculated with particular interest for F_{ST}, and the gene differentiation at individual loci was evaluated using the exact test G (Fischer method).

Thereafter, 835 male and female breeders, located in 23 farms, were genotyped for the SNPs panel and for the *PrP* gene. The frequency of resistant genotypes (ARR/ARR) was low, being 4.8% in the tested males and 2.4% in the females. The mean genetic distance, calculated as Euclidean distance, was 6 (range 0 to 8.2) and the average inbreeding value was above 10%.

The results of the *PrP* gene genotyping and SNPs markers provided useful indications to farmers for maximizing the genetic distances within groups with genotypes resistant to scrapie.

The SNP panel can now be used to monitor the progress of genetic variability and to estimate the genetic distance in the Brianzola breed for the choice of most appropriate resistant homozygous ARR and heterozygotes broodstocks, while simultaneously decreasing the risk of inbreeding depression.

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P046

Genetic characterization of sheep breeds from the Arabian peninsula through genome-wide SNP markers

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Due to a strategic position, the nomadic Bedouin life-style and the Arab expansion, the Arabian peninsula may have played an important role in sheep dispersal from the domestication center. Currently, three major sheep breeds are documented in KSA - Kingdom of Saudi Arabia (Najdi, in eastern KSA; Naeimi, in western KSA, also known as Awassi; and Herri) and one in Oman (Omani), all fat-tailed and adapted to arid environments. In this study, 50K SNP genotypes were produced for 10 Omani, 6 Najdi, 7 Naeimi, and 7 Herri sheep samples. Multi-Dimensional Scaling analysis of sheep from KSA and Oman in a world-wide context (3279 samples from more than 100 domestic and feral breeds and populations) revealed a clear east-west genetic gradient along the first dimension, and a certain closeness among Najdi, Herri and Omani, with Naeimi being more differentiated and placed in a more "western" position. ADMIXTURE analysis on the world-wide dataset highlighted that Omani and Herri were more close to Afec-Assaf (Israel), Najdi more close to Local Awassi (Israel), and Naeimi close to Local Awassi and Mohgani (Iran) and, to a lesser extent, to Qezel (Turkey). After discarding the most distant breeds (those displaying less than 10% of the genomic component peculiar to Gulf sheep breeds), ADMIXTURE analysis was repeated on the dataset that included the Gulf breeds and other 22 breeds from Turkey, Israel, Iran, India, Egypt, Ethiopia, European Mediterranean (Italy, Cyprus) and Balkan countries (Serbia, Montenegro, Macedonia). At $K = 10$, a major component was shared among sheep breeds from Italy and Balkan countries, also observed, at lower extent (20-30%) in Egyptian Barki. A secondary component was shared among sheep breeds from Iran, Oman, Saudi Arabia, Israel, Turkey, Egypt. The Ethiopian Menz genomic component was shared with Herri (>40%), Najdi (around 25%), Omani and Egyptian Barki (15-20%). The Indian Deccani genomic component was shared with Omani (14-19%). Our results point to a complex scenario of genetic relationships in the Middle East, with Gulf sheep partly influenced by northern stocks (Naeimi), partly connected to Horn of Africa (Najdi and Herri), and partly related to eastern Asiatic stocks (Omani).

P047

Signatures of merinization through analysis of local genome ancestry

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Merino and Merino-derived sheep breeds are widely distributed across the world and, despite the collapse of the wool industry in the last decades, they still represent an economically and historically important genetic resource. The most distinctive traits in Merino, and most Merino-derived sheep breeds, are long and soft white bright fleece, well-formed staple, consistent and pronounced crimp throughout the fleece, high fibre density in fleece; fine fibre diameter; presence of skin wrinkles and wool-covered face and legs, and presence of spiralled horns. We previously investigated the genetic influence of Merino in the context of a global collection of domestic sheep breeds using data from the OvineSNP50 BeadChip to provide a general picture of the relationships between Merino and other sheep populations and reconstruct the history of Merino and merinization. Moving from a global to a local genome perspective, we tested for the presence of "merinization" signatures in various Merino populations. Results of the LAMP_ANC approach in the LAMP package, which assumes priors on ancestral allele frequencies, are presented here for Chinese Merino, supposing the breed originated from admixture between a Merino and a not-Merino population, with Rambouillet and Tibetan selected as representatives of the most probable ancestors based on a preliminary world-wide survey. Significant peaks (i.e., local excess of Rambouillet ancestry) were observed at 37 regions in 20 autosomes. At *Ovis aries* (OAR) chromosomes 1, 2, 3, 4, 10, 13, 14, 19 and 25 the signatures were fixed (100% local "Merino" ancestry). Overlapping signals at OAR1, 3, 10, 19 and 25 were observed also when considering Australian Merino. Interestingly, at OAR10, a GWAS study for wool traits in Chinese Merino detected a significant SNP for fibre diameter at position 30042791 (Oar_v4.0), well within both our Chinese Merino (28628167.35035511) and Australian Merino (28857230.30295323) signature regions. The region encompasses several genes of which the best candidate is the evolutionary conserved *Furry* gene (*FRY*), whose disruption in *Drosophila* has been shown to provoke abnormally branched bristles and strong multiple-hair cell phenotype, with clusters of epidermal hairs and branched hairs. Further studies are needed to confirm the role of *FRY* in sheep.

P048

Genome-wide characterization of local cattle breeds from central and western Mediterranean

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Following-up on previous studies that investigated genetic relationships among several world-wide cattle breeds using genome-wide SNP data, the aim of this study was to focus on the central and western part of the Mediterranean. Notably, 50K genotypes from 30 Marismeña (Spain); 24 Guelmoise (Algeria); 46 Brune de l'Atlas Fauve, 15 Brune de l'Atlas Grise, and 15 Blonde du Cap (Tunisia); 29 Modicana and 30 Cinisara (Sicily); 24 Podolica Italiana (Southern continental Italy); 34 Chianina and 24 Romagnola (Central Italy); 23 Modenese, 24 Reggiana, 23 Alpine Grey and 50 Pezzata Rossa Italiana (Northern Italy); 50 Bruna Italiana and 50 Frisona Italiana were considered. When included in a world-wide dataset, the three Tunisian, the Algerian, the two Sicilian, the Podolica, the Chianina and the Romagnola were the taurine breeds showing the highest closeness to the Indian zebu group (358 animals from 27 breeds, from a previously published study) in the MDS plot of the IBS distance. These results were also confirmed by ADMIXTURE analysis ($K = 2$). This relatively lower influence of zebu material into cattle breeds from Northern Italy may suggest a major maritime-mediated route of dispersal of zebu material into Southern Europe and Northern Africa. Interestingly, breeds from the Iberian peninsula showed lower evidence of zebu introgression, suggesting that zebu introgression was likely not mediated by Moors, and possibly occurred at earlier times. Clear evidence of more recent introgression of material from improved dairy cattle breeds (Bruna Italiana and Brown Swiss) into the two Brune de l'Atlas populations from Tunisia highlights the need for urgent conservation of this cross-border cattle.

P049

GWA study for backfat fatty acid composition in pigs detects SNPs involved in the phenotypic variations of porcine subcutaneous fat quality

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Pork production has to carefully consider fatty acid (FA) composition of meat and carcass to respond both to consumers' and industry requirements, which do not always agree. A high level of unsaturated FA is healthier for consumers but can lead to an increased worsening and deterioration of organoleptic quality. The right balance between unsaturated and saturated FA can be considered a convenient and suitable result to achieve. The inclusion in selection schemes of markers or causative variants linked to phenotypic variation of pig carcass FA composition could be an important step to reach this aim. Anyway the knowledge of genes involved in this trait is still poor and incomplete. In order to obtain genetic markers and genomic regions associated with pig backfat FA composition, a population of about 800 Italian Large White individuals was used to perform a Genome Wide Association (GWA) study. The pigs were genotyped using Illumina PorcineSNP60k, quality control was performed on PLINK and markers were mapped using Sus scrofa assembly build 10.2. Finally, the obtained data were analysed using GenSel, a Bayesian statistics based software. Several interesting chromosomal regions were found on chromosomes (SSC) 1, 5, 7, 8, 9, 10, 11, 14, 16, 17 and 18, where markers associated to the different FA were located. Interestingly, these data partly agree with literature and provide also new SNPs in genes associated to FA phenotypic variability. Strong associations were found in regions of SSC8 located at 119-122 Mb (with markers displaying Bayes Factors >12) and SSC14 at 121-124 Mb (with MARC0006531 Bayes Factor = 11,7) for palmitic, palmitoleic, stearic and oleic acids. Known genes related to *de novo* FA synthesis, such as *ELOVL elongase 6* and *Stearoyl-CoA Desaturase Δ9* (*SCD*), are located in these regions. Moreover, linoleic, arachidic, omega 6 and omega 3 FA were mainly associated with regions harbouring genes related to lipids digestion and intestinal fat absorption and utilisation (such as *Amyloid beta precursor protein binding family B member 1 interacting protein-APBB1IP-* and *Pancreatic lipase- PNLIP*). The present

GWA study identifies interesting genomic regions associated with the phenotypic variations of backfat FA composition and the results indicate the likely involvement of distinct biological pathways leading to the different FA deposition.

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P050

Lipases and their co-expressions in porcine muscle tissue samples divergent for intramuscular fat deposition

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Pig selection efforts have mainly focused on increasing carcass muscle deposition, leading to a decrease in intramuscular fat (IMF) and backfat deposition. An excessive reduction of IMF and carcass fat depots can impact negatively on meat quality and a better knowledge of the gene networks associated with IMF and backfat deposition and fatty acid (FA) composition is a key step for a more efficient improvement of these traits. The present study is aimed at testing the associations between IMF deposition and some genes with a key role in intracellular lipids hydrolysis. Different lipases were taken into account (such as *Hormone sensitive lipase – LIPE*, *Lipoprotein lipase – LPL*, *Adipose triglyceride lipase - ATGL*), in addition to *Perilipins* (*PLIN1-5*) and some genes involved in *de novo* FA synthesis. A gene expression study was performed on 47 samples of *semimembranosus* muscle, chosen among a population of 950 Italian Large White pigs for their extreme and divergent IMF content (25 pigs with low IMF *vs* 22 with high IMF deposition). Three normalising genes were used (*B2M*, *YWHAZ* and *HPRT1*), and the expression levels among the two groups divergent for IMF content were compared using Student's t test. The expression levels were also analysed using partial correlations and the strongest correlations were found between *Fatty acids synthase* gene (*FASN*) and *Stearoyl-CoA Desaturase* $\Delta 9$ (*SCD*) and between *PLIN1* and *LIPE* genes, both with $r > 0.7$. The lipases *ATGL* and *LPL* were differentially expressed between the two groups ($p < .01$ and $p < .001$, respectively) and their overexpression in high IMF samples could be due to a more active lipid metabolism (both biosynthetic and catabolic) characterising these samples. The same trend was evident also in *FASN*, which was more expressed in high IMF pigs ($p < .05$). Furthermore, an association study between a SNP located in *PLIN5* gene downstream region (variant ID: rs327694326) and *LIPE* mRNA was performed: *LIPE* expression was quantified through qRT-PCR in ILW samples with divergent genotypes and its transcription levels were compared using Student's t test. Samples with TT genotype at the analysed SNP were associated to a higher expression of *LIPE* gene. The obtained results add new data to the poorly known expression patterns involved in porcine intramuscular lipids metabolism.

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P051

Improving anaerobic digestion of lignocellulosic biomass through ultrasound treatment

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Anaerobic digestion, when used to exploit the livestock waste and agricultural by-products, serves both as additional income and to recycle biomass and reduce nitrogen emissions. However, the presence of lignin in substrates such as manure limits the fermentations of biomass and the efficiency of the process. The aim of this study was to verify whether an ultrasound treatment could improve the degradability of the lignified fraction of biomass during anaerobic digestion.

Straw samples (6 for each thesis) were treated with ultrasound for 5 (S5) and 10 minutes (S10) and were compared with untreated straw which acts as a control (CON). The sonication was carried out by steeping 5 g of straw in 100 mL of distilled water and using an ultrasonic processor (400 W, 24 kHz) with a 22 mm diameter probe. Half of the samples were analyzed for chemical composition after the treatment, whereas the remaining samples were subjected to anaerobic fermentation in stirred bottles (500 mL) at 39 °C and 125 g of inoculum obtained by a biogas plant. In addition to straw samples, 3 samples of cellulose (5 g) and 3 samples of inoculum (125 g) were subjected to anaerobic digestion. The anaerobic digestion lasted 48 days, until the daily gas production was less than 1% of the cumulated gas production. After anaerobic digestion, the samples were subjected to chemical analysis for the determination of the degradation of nutrients, and the production of gas was determined by μFlow system. The experiment was replicated.

All data were subjected to ANOVA using treatment as a fixed effect. After sonication the samples treated showed significant differences in DM (10.3 *vs* 8.98 *vs* 7.92, $p = .03$) and EE (1.21 *vs* 0.61 *vs* 0.03, $p = .01$) for CON, S5 and S10, respectively. Anaerobic digestion showed no difference in the degradability of DM (87.8), CP (86.2) and NDF (85.9) between the various theses, but led to a higher ADL degradation in sonicated samples, equal to 62.6 *vs* 73.6 *vs* 71.5, $p = .05$. Gas production, against expectations, was lower in sonicated samples than in the control and was equal to 3935, 1917 and 2313 mL, $p < .05$ for CON, S5 and S10, respectively. Concluding, sonication leads to a better degradation of lignified component of biomass, but decreases gas production, probably because of the release of substances which inhibit methanogenic Archaea. This limit may be probably overcome eliminating the water used during the sonication prior to conducting the anaerobic digestion.

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P052

Effect of rearing system on meat quality of Lucanian black pigs

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The Lucanian black pig is an autochthonous genotype present in South Italy; so far this breed has been little investigated and more recently it is at risk of genetic erosion. Thus, several projects aimed to rescue and rediscover this swine native breed in order to evaluate its potential exploitation in sustainable production systems in rural marginal areas of the Basilicata region.

This study was carried out to evaluate the effects of the rearing system (intensive *vs* extensive) on 12 castrated male Lucanian black pigs, weaned at about 60 days. The intensive (I) group ($n = 6$) was permanently housed indoor and fed *ad libitum* with a commercial pelleted feed. The extensive (E) group ($n = 6$) was reared outdoor on a deciduous oak wood and received supplementary feeding (1 kg/head/day) at housing. Pigs were slaughtered at 9 months of age. They were weighed immediately before being fasted (final live weight) and slaughtered (pre-slaughter weight). Hot carcass weight was recorded within 45 min after slaughtering. After 24 h at 0-4 °C, the carcasses were split into two halves; the right one was dissected into commercial cuts. The ham, loin and shoulder were separated into lean, fat and bone. Meat samples of the *longissimus dorsi* (Ld) were analysed for physical parameters and chemical and fatty acid composition. Data were processed by analysis of variance (GLM procedure of SAS, 1999/2000). Means were compared by Student's t test.

The final live weight was slightly higher in the E group (124.25 *vs* 116.50 kg). Outdoor reared pigs showed a higher incidence of lean of the leg (75.5 *vs* 70.8%, $p < .01$). Extensive rearing increased markedly ($p < .05$) L (55.63 *vs* 51.31), a (5.31 *vs* 3.03) and b (12.86 *vs* 10.76) meat colour parameters, probably due to feeding on grass, leafy greens, tubers and roots. Chemical composition of the Ld muscle did not differ between groups except for intramuscular fat, that was markedly higher in the E pigs (3.6 *vs* 1.8%, $P < 0.05$). Feeding high energy acorns may be held responsible for this result. Meat of the E group showed a lower amount of saturated fat (36.20 *vs* 39.78%, $p < .05$) and a higher

concentration of PUFAs (11.61 *vs* 15.32%) and ω3 fatty acids (1.55 *vs* 1.09%), with benefits for consumers' health.
It may be concluded that outdoor rearing system in woods positively influences the nutritional value of pig meat mainly by improving the fatty acid composition. Moreover, this rearing strategy has great advantages in terms of economic sustainability.

P053

Use of untreated whey for animal feeding

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The aim of this study was to verify the suitability of untreated whey for animal feeding. The trial was carried out in a dairy farm equipped with small cheese plant, where *mozzarella* cheese, from raw cow milk, and *ricotta*, from whey, were produced. A commercial starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was added to milk for cheese production. A mix (50:50 V/V) of whey and *scotta*, resulting from *ricotta* production, was prepared. The effects of temperature and addition of *Lactobacillus helveticus* on the kinetic of acidification of the mix were evaluated. In order to simulate the storage at the farm (room temperature), the mix was incubated in three replicates at 45 and 55 °C, that are intermediate values between those of whey (25 ÷ 30 °C) and *scotta* (75 ÷ 80 °C), when they flow from the plant. pH was measured at 0, 24 and 48 h. The mixture was then administered, as supplement (4 l/d/head) to 15 weaned crossbred calves, within 24 hours after processing. Group's weight was determined at beginning and ending of the trial, thirty days later. Individual body condition score (BCS) was monitored weekly, classifying animals in two categories (BCS $\geq$3 or $<$3). Moreover, we compared the control mix (C), containing only LAB from cheese starters and the experimental one (E), added by *Lactobacillus helveticus*. Both E and C were incubated at 42 °C (the optimal growing temperature for *Lactobacillus helveticus*) in three replicates and pH was monitored until 48 h. Owing to a limited sample size, an explorative data analysis using the Npar1way procedure of SAS (Kruskall-Wallis test, *p*-level $=$0.05) was performed to test for pH differences between C and E according to the storage time (0, 24, 48 h). The 45 °C mix showed the most favorable kinetic of acidification reaching pH around 4.0 after 24 h and pH 3.5 within 48 h. No significant differences emerged in pH values of C and E. Though no statistical analysis was performed, due to a limited number of data and the lack of a control group, animals showed regular weight gain in modulus ($>$1 kg/day on average), when compared with the literature. BCS resulted steadily $\geq$3, with one exception, and no category shift was observed. It is concluded that to provide untreated fresh mix of whey and *scotta* to veal had no negative impact on their overall condition. Further in-depth studies are required to establish whether any microbiological risk is associated with short-term (1–3 days) storage of whey and *scotta*.

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P054

Performance *in vivo e post mortem* of Marchigiana and Romagnola breed

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Marchigiana, Romagnola and Chianina breeds are included in the PGI mark "White Cattle of Appennines". Thirteen Marchigiana (M) and fifteen Romagnola (R) cattle were reared in the same farm and fed first cut hay (88.2% DM, 6.2% CP, 1.2% EE, 34.9% CF, 63.4% NDF, 44.9% AFD, 7.1% ADL, 7.8% ash) *ad libitum*, and concentrate (87.8% DM, 16.5% CP, 2.0% EE, 5.2% CF, 23.6% NDF, 9.6% ADF, 3.4% ADL, 8.4% ash) given 10 kg/head/d. Beef cattle, reared under extensive "cow-calf" production system, during the experimental period, were subjected to weight controls and average daily gain (ADG) was calculated; then cattle were slaughtered and carcass weight was recorded; also, pH was measured between 8-9 thoracic vertebra after slaughter. Meat samples were collected between fore and hind quarter of half carcass and analysed for chemical composition and cooking loss. Data were analysed by ANOVA (JMP) to evaluate breed effect. Statistical analysis did not show differences due to breed for all parameters but cooking loss. The average live weight at the beginning of the trial was 435 kg for M and 401 kg for R, (both were 12 months old) and then animals were slaughtered at estimated mean live weights of 703 kg for M and 699 kg for R. The ADG was 1.3 kg for M and 1.2 kg for R, and the mean carcass weight was 450 kg and 433 kg respectively.

The ADG of carcass was 0.750 kg for M and 0.696 kg for R. For the classification of carcasses muscularity and fatness, according to EC grid, 7.7% of M carcasses were assigned to class AE3, 84.6% to AU3 and 7.7% to AU2, whereas for R breed 80.0% were assigned to class AU3, 6.7% to AU2 and 13.3% to AR3. The average protein content of meat samples was 20.1% for M and 19.8% for R, the fat content (3.5%) was the same for both breeds. Only cooking loss showed significant differences ($p < .05$) between the two breeds, with mean values of 14.7% and 18.4% respectively for M and R beef cattle.
Overall, Marchigiana and Romagnola groups here investigated did not differ for *in vivo* and *post mortem* performance parameters, as well as for meat chemical composition, whereas a difference between breeds has observed for cooking loss.

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P055

Use of Infrared Termography (IRT) in laboratory animal environment: preliminary note

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Framing the work in view of the principle of the 3Rs (Replace, Reduce, Refine) the objective of this experimental test is to assess what benefits are obtainable by the application of infrared imaging technique (IRT) to analyze the status of laboratory animal welfare. This technique still needs to be validated for the study of thermoregulation in rodents used for experimental purposes. The rodents' breeding environment should be constantly monitored and the individually ventilated cages (IVC) offers several advantages. The purpose of the trial was to evaluate the environmental differences between open cages and IVCs. The evaluation was performed with aid of a TVS 500 EX camera. Twelve female nude mice, of 6 weeks of age, housed within the enclosure of Institute "Mario Negri" in Milan were randomly divided into 4 cages, 2 of which IVCs and 2 open cages. The animals were fed ad libitum with a standard diet and had free access to water. The room temperature was set at 22 ± 2 °C with a relative humidity of 52% and 18 - 20 air changes per hour. The air changes in a conventional cage are 5 - 10 per hour. In IVC, controlled air changes were 75 per hour, preserving room humidity and CO2 percentage. Measurements were collected weekly from the 9th to the 12th week of age. The cages were placed under a vertical laminar flow hood and, after removing the cover, animals were video-recorded for 4 minutes. Values obtained from the interscapular area, where the Brown Adipose Tissue (BAT) is located, and the base of the tail were recorded. As for the BAT and tail measurements, the most remarkable difference between cages was recorded at the first week of observation, namely the 9th week of life of the mice. This could be due to the fact that inside the IVC the temperature was two degrees higher than that recorded in the room: the higher temperature detected in the BAT of mice housed in open cages could reflect an higher activation of the tissue in response to a lower environmental temperature. In this case we observed a reduction of the temperature of the tail due to vasoconstriction of the vessels. In conclusion, the present research demonstrates the possibility of using IRT to evaluate the skin temperature and BAT of nude mice in different environmental conditions.

P056

BEEF CARBON: demonstration actions to mitigate the carbon footprint of beef production in France, Ireland, Italy and Spain

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In Italy, agriculture accounts for 7% of two greenhouse gases (CH_4 and N_2O) emissions and beef production is considered one of the most contributors of GHG, included also CO_2 emitted in on- and off-farm feed production. Public concern on this economic sector has imposed to study mitigation strategies and to demonstrate that reduction of GHG emission in beef sector is possible.
The EU has founded the "BEEF CARBON ACTION PLAN" project, which aims to reduce the carbon footprint of beef production by 15% (119 000 tons CO_2 eq) over the next 10 years in four European countries.

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The "LIFE BEEF CARBON" (*Demonstration actions to mitigate the carbon footprint of beef production in France, Ireland, Italy and Spain)* project has the purpose to promote innovative livestock farming systems, ensuring the technical, economic, environmental and social sustainability of beef farms.

The project, coordinated by the French Institut de l'Elevage (IDELE), has 26 beneficiaries and involves about 2000 demonstrative farms, corresponding to six different production systems: (1) calf to weaning system producing weaners (9-10 months old) or (2) bulls/heifers (18 months old) and culled suckler cows; (3) fattening system producing young bulls and heifers (18 months old) or (4) beef steers (over 30 months old), (5) heifers (over 28 months old) and (6) dairy system producing culled dairy cows.

About 170 innovative farms will be identified where GHG mitigation strategies will be applied and their effect on GHG emissions will be evaluated. These strategies will refer to animal, soil, waste management and energy consumption.

The functional unit will be 1 kg of live weight gain and the environmental categories are: global warming, eutrophication, acidification, water consumption and the contribution of beef farms to the rural landscape in 100 demonstrative beef farms (production system 1, 2 and 3) located in Piemonte and Veneto. In 20 innovative farms the effect of the mitigation strategies for carbon footprint reductions and their economic feasibility will be evaluated. The activity will be carried out in collaboration with ASPROCARNE, UNIVARVE and CRPA of Reggio Emilia.

To evaluate the environmental impact of these farms, "Calcul des AUTOMATISE Performances Environnementales en Elevage de Ruminants" (Niveau 1 and 2) developed at the IDELE will be used.

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P057

Comparison between cow milk and soymilk combining nutritional values and greenhouse gas data

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Carbon Footprint (CF) of food products commonly report results using functional units (FU) of mass or volume. While these functional units appear to facilitate comparison between different products, they fail to account for substantial differences in nutritional value. This study was aimed to compare CF intensity of cow milk and soymilk in relation to their nutritional values. Biological value (BV) of proteins, net protein utilization (NPU), fat content and energy content, were used for the environmental comparison. Nutritional emissions were calculated starting from the CF of cow milk and soymilk reported from environmental product declarations (EPDs) and expressed as kg of carbon dioxide equivalents ($kgCO_{2eq}$) per litre of product. The environmental performance of the two products were quantified as laid down in the general rules of the International EPD system and in the specifications of the product category rules (PCR), using the life cycle assessment (LCA) method, regulated by the ISO 14040 series International standards. One litre of cow milk packed in polyethylene terephthalate (PET), with 670 kcal, 3.35% of protein and 3.75% of fat, showed a CF intensity of 1.390 $kgCO_{2eq}$, whereas one litre of soymilk packed in flexible polylaminate, with 480 kcal, 3.3% of protein and 2.1% of fat, accounted for 0.950 $kgCO_{2eq}$. Emission intensity for litre of product was about 32% greater in cow milk compared to soymilk. When milk corrected for fat and protein content (FPCM) was considered as FU, the difference in emission intensity was reduced of about 56%. When BV of protein content was considered as FU, the gap between two milks was reduced and cow milk CF was about 15% greater than soymilk, the gap was further reduced to 7% when the NPU was considered as FU. The differences in emission intensity between the two milks was lower (about 5%) when energy content was considered as FU. Finally, a different trend was found on the basis of fat content, in this case CF of soymilk resulted 18% greater than cow milk. These results suggest that the sustainability of agro-food products can change significantly when considering as FU the nutritional values. In this case the greenhouse gas emissions associated to animal food products would retain lower environmental impact, approximately lying within the range of that of most foods of vegetal origin, because of the greater biological value of proteins and higher content of fat.

P058

Accumulation of manganese, copper and silver in honey bee organism, depending on the concentration and the type of the pesticide

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Intensive agriculture requires continuous improvement of systems, weed control and pests. It is estimated that approximately 1/3 of crops are pollinated by the honey bee (*Apis mellifera* L.), which are exposed to pesticides of different groups. The aim of the study was to examine the effect of selected pesticides on the accumulation of elements (copper, manganese and silver) in the bodies of these insects. We selected manganese and silver because they negatively affect the behavior and lead to disturbances in chitin synthesis, while copper is a natural component of the hemolymph of bees. The study used the worker bees at the age of 10 days, which were placed in treated cages (50 × 150 × 150 mm; 100 honey bees/cage). They were maintained in laboratory conditions (temperature 25 ± 2 °C, humidity 75%) for 7 days. Three pesticides were used in the experiment: fungicide (F - $C_{16}H_{22}ClN_3O$), insecticide (I - $C_9H_{10}ClN_5O_2$), herbicide (H - $C_3H_8NO_5P$), while the control group (K) was not treated. Honey bees were fed sugar syrup (60% w/w) with the addition of the pesticides for the experimental groups. Three different concentrations of pesticides were used: one practical concentration comparable to that used in field conditions (F – 0.62 g, I- 0.75 ml, H-4 ml); a 1.5 fold higher concentration than the practical dose (FH – 0.93 g, IH – 1.13 ml, HH – 6 ml); a 0.5-fold lower concentration than the practical dose (FL – 0.31 g, IL - 0.38 ml, HL – 2 ml) each was dissolved in 1000 ml of syrup. Dead honey bees were collected in labeled containers and frozen (-20 °C). The analysis of elements was carried out using the technique of F-AAS. Data were analysed using a one-way ANOVA and comparisons were performed using the Kruskal – Wallis test. The concentration of manganese in the bees was higher in the I group, compared to the FH group (69.88 ± 2.39 *vs* 54.22 ± 10.22 mg/kg DM; $p < .05$), and was not related to the composition of the pesticide. The concentration of copper was numerically higher in the IH group compared to the K group (25.29 ± 0.38 *vs* 21.98 ± 1.03) mg/kg DM), although not significant ($p > .05$). Similarly, the silver content in the K group was numerically lower compared to the HH group (0.20 ± 0.06 *vs* 0.28 ± 0.23) mg/kg DM), with no statistical significance ($p > .05$). It is concluded that pesticides influence the content of these elements in the organism of honey bees. Such condition, especially in the case of manganese, can adversely influence the condition of bees.

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P059

The use of *Azospirillum* as a tool for promoting sustainable production of silage maize: a preliminary evidence under field condition

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The aim of this on-farm study was to evaluate the use of N_2-fixing rhizobacteria *Azospirillum* in maize cropping as a strategy to reduce the use of nitrogen fertilization. The trial was conducted on an irrigated plain farm. Seeds of *Zea maize* were inoculated with a commercial product (Graminosoil srl®) based on *Azospirillum* spp. live cells at the dose of $5x10^{-4}$ cell form unit/kg seed. The treatments were MC (not inoculated maize seeds, fertilization: N 250 kg ha^{-1}) and MA (inoculated maize seeds, none fertilization) that were arranged in 2 contiguous areas, 1 ha each. Maize was harvested at the milky–waxy ripening stage. Measurement of the yield components and of the plant traits were made at harvesting, on a 3 strips (10 m long) per plot. For each strip, 50 plants were manually cut at 10 cm above the ground and weighted in order to determine biomass yield. Moreover, 3 sub-samples, each consisting of 3 plants, were collected to evaluate the plant traits. To this purpose, plants were separated in culm, cob and leaves and the following traits were recorded: culm height, number of green leaves, leaf area, weight of the culm, cob, and leaves. Leaf Area Index (LAI) were calculated based on the leaf area and the plant density for unit area (m^2). Finally, 3 samples/plot of freshly chopped forage were collected in order to determine the dry matter content (DM) and chemical composition

(Foss NIR systems 6500, Silver Springs, MD). Data were analyzed by using one-way ANOVA, with treatment as factor. By comparing plant traits recorded for MC and MA, the plant height (294 *vs* 298 cm), number of green leafs (15.7 *vs* 15.9), leaf area (0.63 *vs* 0.64 m^2), and LAI (4.76 *vs* 4.77) were not influenced by the treatment (p>.05). Likewise, no significant differences (p>.05) could be detected for the weights of culms (412.0 *vs* 430.4 g), cobs (416.9 *vs* 430.9 g), and leaves (141.7 *vs* 142.5 g). As a consequence, the total biomass yields from MC and MA (72.92 *vs* 75.28 Mg ha^{-1}, respectively) were not statistically different (p>.05). In regard of fresh forage chemical composition, no differences were found in terms of DM (34.6 *vs* 34.7%), crude protein (6.9 *vs* 6.9%DM), crude fibre (21.8 *vs* 21.9%DM), ether extract (1.0 *vs* 1.0%DM), ash (4.8 *vs* 4.9%DM), and NDF (50.7 *vs* 50.8%DM). Based on these preliminary results, seed's inoculation with *Azospirillum* may represent a viable tool for improvement of environmental effects of maize.

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P060

Ventilation and evaporative cooling of the resting area in free stalls dairy barn

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A study during the summer season evaluated the effect of different cooling systems of resting area on behavioural and productive responses of Italian Friesian dairy cows kept in an experimental free-stall barn located in Piacenza province. The study involved 30 lactating dairy cows subdivided into two groups, homogenous for calving number, parities, and milk yield, and kept in two pens with external paddock. A deep-bedded sand, consisted of about 20 cm of fine sand, was used in the resting area of both pens. The same cooling system was applied in the feeding area in both pens, and a different cooling system in the resting area was applied to the two pens. In one pen the simple ventilation (SV) was applied by using two fans (one for each free-stall row) switched on at 25 °C. In the other pen the ventilation was combined with misting (SW) by using three misters (delivery rate of 26 L/h) switched on at 27 °C (working time of 60 s every 2 min). Temperature and relative humidity indoor were continuously recorded in the two pens. Breathing rate, rectal temperature, milk yield, and milk characteristics (fat, protein, and somatic cell count) were measured. Behavioural activities (standing and lying cows in the different areas, as well as the animals in the feed bunk) were recorded. The data were processed using analysis of variance. Mild to moderate heat waves during the trial were observed. On average, the breathing rate was greater (not significant) in SV compared with SW cows (60.2 and 55.8 breath/min, respectively), and mean rectal temperature remained below 39 °C in both groups during the trial (on average 38.7 and 38.8 °C in SV and SW, respectively). During the hotter periods of the trial, the time spent lying indoor in the resting area was greater in SW (11.8 h/day) than SV (10.7 h/day), with significant differences ($p < .05$) during evening and night. Considering the time spent outdoor by the cows, mainly lying during evening and night, the total lying time was 14.6 h/day in cows of both treatments. The time spent standing indoor without feeding was 3.8 and 4.3 h/day in SW and SV, respectively, with significant differences ($p < .05$) during evening and night. Milk yield was better maintained during hotter period in SW compared with SV, with a slightly greater somatic cell count in the former. In conclusion, the adoption of the cooling system by means of evaporative cooling also in the resting area reduces the alteration of time budget caused by heat stress.

P061

Towards a sustainable wool chain in Apulia: dream or possible reality?

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Apulia is a region in Southern Italy with traditional vocation for sheep breeding, homeland of the fine-wool endangered-maintained Gentile di Puglia sheep breed. The region currently counts 53945 heads, mainly open-fleece, coarse-wool, pure- or cross- bred animals. In the past decades, sheep wool has lost most of its commercial value; wool price is usually too low to cover the shearing costs. Unsold wool also has to be disposed expensively as a special waste. FAO proclaimed 2009 as International Year of Natural Fibres. On the wave of this renovated interest, in 2010, the Murgia Viva Consortium, established in 2008 by 9 breeders, aiming to improve the value for farmers in sheep production, gathered 30 local farmers to conjointly collect and sell wool in block, at a better price than usual. In 2012, a 3-years project (PartnerSheep) financed by the Alta Murgia National Park allowed to extend the initiative to 108 farmers, for a total of 55.000 kg of greasy wool. In the same year, the Apulia Region financed a project (Pecore Attive) for the valorization of the wool from local Apulian sheep breeds through hand-made felting that later on evolved into a concept design startup for wool-based products. In 2016, a 2-years project (PLAUTO) has been financed by the Apulia Region to extend the campaign of wool collection to the whole region, in order to create two interprovincial centres for collection, pre-selection, grading and market distribution of the wool, to agree on procedural guidelines for wool production, and to test possible applications of wool to the textile, construction and horticultural sectors. After only six years, self-sustainability of the above initiatives is yet far from being a reality; notwithstanding, they had a major role in promoting networking and integration among stakeholders, which alone can be already considered a great achievement for a sector generally poorly aggregation-prone.

P062

Facing carbon emission mitigation of dairy sheep supply chain: estimation of a baseline trend

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This work showed the values of GHG emissions baseline estimated for the Sardinian dairy sheep sector. The estimation was carried out to target future strategies of effective mitigation within a specific project of LIFE + Program 2014-2020 for Climate Action. Sheep farming plays a large socio-economic role in some specific economies. However, sheep significantly contributes to livestock greenhouses gas emissions (GHG) worldwide. In fact, emission intensities of sheep products (kg of CO_2 eq/kg of milk or meat) usually reach much higher values than cow and goat products both for their lower production levels and higher milk solid content. Within the Mediterranean area, Sardinia (Italy) is one of the first producers of sheep milk and the top sheep cheese exporter in the world. Despite the small surface, Sardinia shows high number of farms (about 12.000), a broad variety of production systems and stocking rates, with dairy local breeds. Considering these features Sardinia was targeted as the best context for demonstrative actions of GHG mitigation for dairy sheep supply chain by the project SheepToShip LIFE. It aimed to: (i) implement the most viable and feasible strategies of GHG mitigation at farm and processing plant level; (ii) plan future policy regulation of the regional government to get emission reduction in the next 10 years. Data reported by Atzori et al. (2014; LAR, 4(1):2–5) from Italian inventories and FAO estimates described in Table 1 of the same work, were considered for the baseline calculation referred to the year 2015. Sardinian sheep sector, estimated as average of 10 years, accounted for 3.3 million sheep heads and 3.15 million liters of produced milk. Emissions were equal to 3.2 (kg of CO_2 eq/kg of milk) from animal and manure emissions of methane and N_2O. Other emissions to farm gate (feed, energy and secondary emissions) reached 26.9% of total emissions following FAO estimates. Processing and post farm emissions were considered equal to 10% of total emissions based on Sardinian site-specific studies. The cumulative emissions from the whole dairy sheep supply chain in Sardinia in the reference year resulted equal to 1565 ktons of CO_2 equivalent that might be attributed for a 80% to milk and 20% to meat. This value is intended to cover emissions from “cradle to dairy plant gate”.

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P063

Measuring the profitability of the Apulian native Altamurana ovine breed for better conservation programmes

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Altamurana is a triple attitude breed (milk, meat and wool) selected for centuries by the harsh Murgia environment. In the ‘80s, it gave way to more productive specialized breeds and since ‘90s it may be considered an endangered-maintained breed. The preservation of this local and historical breed allows not only the achievement of a better balance of the livestock ecosystems, but also the enhancement of the traditional related products (milk, cheese, wool, etc.). To this end, the 2014-2020 Rural Development Programme of Apulia provides funds to farmers for the preservation of the Altamurana (sub-measure 10.1.5). The premium, 200 €LSU^{-1} year^{-1} per animal, is supplied to farmers who undertake to breed the Altamurana heads for five years. The payment is calculated on the additional costs and income losses consequent to the breeding of Altamurana with respect to the ordinary breeds. In the period 2013-2016 a further indirect support came from a research project funded by the Alta Murgia national Park.

In this context, we analysed the profitability of Altamurana breed and compared it with the economic performances of Comisana, one of most popular dairy sheep. We focused on the gross margin, which corresponds to total revenues minus operating costs (EU FADN methodology). In particular, revenues were referred to milk and other products revenues, while operating costs were distinguished between specific costs (cultivation, feed, veterinary, etc.) and non-specific costs (upkeep of machinery, taxes on land and buildings, etc.).

Result highlighted several important aspects of Altamurana and in particular, compared to the Comisana breed: the lower milk and meat profitability, respectively of 64% and 37% and a minor incidence of the feeding and veterinary costs (42% and 25%, respectively). Finally, the gross margin per head for the Altamurana breed was just 3.69 euros compared to the Comisana one (41.93 euros). Omitting the EU premium, the gross margin was even negative, namely −16.31 euros.

These findings showed the need of proper conservation plan based on external support. However, the sensitivity analysis pointed out that several marketing strategies concerning the sale of milk, meat, wool and transformed products could be implemented. These products, as obtained by a local ovine native breed, could exploit higher market prices, ensuring better profitability performances and cancelling the differences with the Comisana breed.

P064

Start vs cessation of ruminant farming activity from 2010 to 2015: the paradigma of Umbria

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Cattle and small ruminant sector has currently suffered a general decline in profitability, because of the increasing management costs, different productivity levels, feed costs price increase, lower income from farms products, poor monetization of by-products (hide, bone, wool etc.) and other factors, resulting in a decrease in the number of farms.

The aim of this work was to evaluate the evolution of the number of farms starting or ceasing the ruminant farming activity during the period 2010 - 2015, in Umbria.

The data were obtained from the Banca Dati Nazionale of Anagrafe Nazionale Zootecnica established by the Ministry of Health at the Istituto Zooprofilattico Sperimentale Abruzzo and Molise. Both new and closed cattle, sheep and goat farms were considered for the analysis in the period from 1 January 2010 to 31 December 2015. The consistency difference (farms from 1 to 19 cattle heads *vs* farms with 20 or more cattle heads) between started and closed cattle farms was evaluated for each year using odds ratio (OR) and confidence intervals (CI95%); a chi-square for trend was used to asses the trend in years. A $p < .05$ was considered significant.

The cattle farms that begun their activity have been on average 197 per year, but the total number of cattle farms was changed from 3.656 in 2010 to 2.970 in 2015 (beef production farms was about 90% of total cattle farms), with an average decrease of 4% per years. This is probably due because in 2010, 2011, 2012, 2014 closed farms have been more than 300. The chi-square for trend test analysis put in evidence a not statistically significant trend in the increase of the number of closed farms over the years.

Small cattle farms (<20 heads) had closed more frequently than medium-big ones (≥20 heads) every year, with a greater odds ratio in 2010 (OR 3.3; CI95% 1.7 – 6.4; *p*-value 0.0001); only in 2013 there were no difference (OR 1.4; CI95% 0.8 – 2.4; *p*-value 0.18). These results seems to show more problems for small cattle farms to be competitive on the market.

The number of sheep farms is the same period did not change (about 3000), while goat farms recorded in 2015 were 709 showing a 57.6% increase if compared to the number enrolled in 2010. This growing is probably a consequence of actions taken by the Council of Agricolture Ministers and by the Commission after the call of European Parliament to ensure a profitable and sustainable future for sheep and goat farms.

P065

Microbiological characteristics of *mozzarella* type cheese from raw ewe milk

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The aim of this study was to produce *mozzarella* type cheese from raw ewe milk so that curd acidification might develop at both high and low temperatures.

Four replicates per *mozzarella* processing were performed in small cheese plant. Starters based on thermophilic (T) lactic acid bacteria (LAB) or thermophilic and mesophilic (TM) LAB were added. Curd was let acidify under whey until it reached pH 5.0. During the stretching phase, hot water at about 85-90 °C was used. Cheese was then molded in shape of about 200 g, firmed in cold water, salted in saturated brine for 2 hours and stored at 4° C into governing liquid with 2-3% of NaCl. Samples of milk, curd, T and TM cheese at day 1 were submitted to the following microbiological analyses: *Salmonella* and *Listeria monocytogenes*, used as food safety markers, coagulase-positive staphylococci and β-glucuronidase-positive *E. coli*, used as hygiene markers, *enterobacteriaceae*, coliforms at 30 °C and total microbial count (TMC) at 30 °C. At day 5, coliforms and TMC analyses were performed on cheese samples. Differences were tested by GLM procedure using the factorial model including the fixed effect of starter type and storage time of cheese.

Both T and TM curds were successfully stretched at pH 5.0, but the length of the acidification phase was affected by the kind of starter. TM curd was ready for stretching in four hours and a half on average, while T curd in seven hours on average, starting from whey draining. Pathogens were absent in milk, curd and both kind of cheeses. Average counts of microorganisms considered as hygiene markers, coagulase-positive staphylococci and β-Glucuronidase-positive *E. coli*, were, respectively, 2.019 and 1.803 log 10 cfu $\times$ g^{-1} in milk samples, 2.375 and 3.319 log 10 cfu $\times$ g^{-1} in curd ones. These microorganisms resulted not detectable in T and TM cheeses. *Enterobacteriaceae* and coliforms decreased from curd to fresh cheeses, respectively, by 71% and 72%. After cheese storage, coliforms did not increase in TM *mozzarella*, while they increased (48%), but not significantly, in T cheese. In conclusion, results of this study showed that the high temperatures reached during stretching phase of *mozzarella* processing strongly contributes to the safety of the resulting cheeses. Moreover, in colder seasons, the use of starter based on TM LAB allowed the reduction of curd acidification time. The different curd acidification time did not affect significantly microbiological characteristics.

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P066

The Net Waterfootprint: a proposal to calculate the water consumption of animal products

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Water can be classified among blue water (man-managed freshwater of lakes and aquifers); green (soil rainwater which eventually evaporated through plants); and grey water (polluted). Water FootPrint (WFP) is an indicator of the water used for the production of a unit of goods or services. In 2017 the International Dairy Federation published *The IDF guide to water footprint methodology for the dairy sector*. It suggest to estimate milk WFP with a Life Cycle Assessment for blue water consumption and then to apply local adjustments for water stressing index. It allow consider how milk production will limit water availability for other uses. For the US dairy production the guide reported WFP that varies with the watersheds from 517 to 0.9 L of blue water/kg of milk.

We propose an alternative method, the Net Waterfootprint (WFP$_{net}$) to estimate the WFP of the foods at farm gate accounting for green and blue water. Green water is calculated considering the differential evapotranspiration (ΔET) between the total ET of a crop or pasture used for animal feeding and the ET of a hypothetical scenario of a natural cover (e.g. a natural grassland under Mediterranean conditions 3000 m^3/ha of ET) from the same land surface, which represents the natural substitute to the human activity. The blue water is consumed for irrigation and for animal drinking and servicing). The values of WFP$_{net}$ of sheep milk were simulated for two standard flocks raised on intensive (INT) or extensive (EXT) production systems (250 *vs* 150 lt/year of milk per present ewe; respectively). INT vs EXT used more concentrate (1.0 *vs* 0.7 kg of DM/kg of milk) and higher percentages of produced and purchased feeds from irrigated land (75% of DM *vs* 35% of grain DM). Three scenarios of increasing water use efficiency (WUE) due to irrigation techniques were also simulated. WFP_{net} of sheep milk for decreasing values of (WUE) was equal to 218, 421, 796 L of water/kg of milk in INT, and to -135, 10, 280 L of water/kg of milk in EXT, respectively. The WFP_{net} method showed: (i) increasing values for increasing use of blue water; (ii) lower values for EXT scenarios that showed low DM conversion efficiency but were slightly dependent from blue water; (iii) negative values for scenarios where the irrigated crops had higher WUE than grasslands and pastures. The development of the WFP_{net} concept is still incomplete. This work should stimulate further discussions and encourage calculations of local WFP.

P067

Wooden breast myopathy in broiler chickens: does myowater have a role on hardness?

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Wooden Breast (WB) myopathy is characterised by tissue damage and reparative fibrosis, so far considered the major reason for the hard texture of affected chicken breasts. Recently, hard breasts are detected without fibrosis, opening new hypotheses on the reasons for hardness, such as the role of myowater, as differences in muscle structure can affect water properties. This study investigated the role of myowater on hardness by integrating low-field nuclear magnetic resonance (NMR) relaxometry with texture analysis (compression test). From 34-day-old chickens, 48 WB (diffuse hardness at touch) and 48 normal (N) breasts were selected. Four stripes per breast (1 cm x 1 cm x 4 cm, ca 5 g) were cut parallel to fibres and analysed (Maran Benchtop Pulsed NMR Analyser; magnetic field strength: 0.47 T). Transverse relaxation (T_2) was measured with the Carr-Purcell-Meiboom-Gill sequence and plots resulted from the distributed exponential analysis of data. Relaxation time constants and proportions of protons exhibiting those relaxation times were determined for the three water populations: T_{2B} (H_2O closely associated to macromolecules/proteins), T_{21} (H_2O trapped into myofibrillar matrix) and T_{22} (extramyofibrillar water). Samples were then subjected to 80% single cycle compression test (Brookfield CT3 texturometer; load cell: 25 kg; trigger load: 0.3 N; test speed: 0.83 mm/s) to measure compression force. One-way ANOVA (fixed effect: muscle condition) and Pearson correlation analysis between texture and NMR measurements were performed (SAS 9.1.3). WB breasts had longer T_{2B} and T_{21} relaxation times than N breasts (T_{2B}: 0.82 *vs* 0.50 ms; T_{21}: 21.0 *vs* 13.6 ms; $p<.0001$). The proportions of T_{2B} and T_{21} populations were lower in WB than in N samples (T_{2B}: 4.47 *vs* 5.29%; T_{21}: 88.5 *vs* 93.4%, $p<.0001$) in favour of T_{22} (7.05 *vs* 1.26%, $p<.0001$), demonstrating an increase in the most loosely bound water compartment T_{22} in WB breasts. WB were harder than N breasts (24.8 *vs* 23.0 N, $p=.0314$). Only in WB group, hardness was significantly ($p<.0001$) correlated with T_{2B} ($r = 0.48$), T_{21} ($r = 0.46$) and T_{22} ($r = 0.41$) time constants, whereas texture was not correlated with the proportion of T_{2B}, T_{21}, T_{22} populations within the groups. Up until now, it is not clear, which water population (T_{2B}, T_{21}, or T_{22}) contains the extracellular water of the altered and swollen extracellular space in WB samples. However, a role of myowater properties on hardness is demonstrated from this study.

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P068

Morphological evaluations of *pectoralis major* muscle in two chicken strains

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Poultry production is one of the larger, growing and successful industry. Chicken meat is the cheapest and most consumed meat in western countries. This success is due to deep changes in feeding plan, environmental conditions, veterinary care and mainly to the genetic of chickens, selected for fast-growing rate and the size of breast muscle. Such muscle hypertrophy may contribute to the onset and development of muscular disorders. Relevant metabolic changes in breast muscle as the presence of Giant Fibers (GF) with high cross-sectional area and hypercontracted fibers (HF) have been reported. Beside histological changes, also the quality and taste of breast meat of meat-type chickens has been affected (e.g. wooden breast). The aim of the study was to assess the morphological and immunohistochemical features of pectoralis major in two chicken strains, respectively with slow- and fast-growing rate. The experiment included 20 cockerels belonging to Ross 308 ($n = 10$) and Leghorn ($n = 10$) strain and slaughtered at 45 or 100 days of age. Samples from pectoralis major muscle were taken and processed for histology and immunohistochemistry. The capillary density was assessed with von Willebrand factor antigen; the presence and severity of muscle lesions, the presence of HF and GF, the number of fibers were also evaluated. All the samples of Ross chickens showed different features of myopathy represented by single-cell necrosis, lymphocytes, plasma cells and macrophages inflammatory infiltrates, fibers regeneration, HF and angulated fibers, fiber splitting and increased internal nuclei, whereas only few Leghorn samples (40%) had minor signs of myopathy. Furthermore, the number of GF per field (200X) was higher in fast-growing strain than in slow-growing one (7.89 *vs* 1.77). The number of capillaries per muscular area was also higher in slow-growing strain. These results support previous studies in breast muscle that showed a reduced blood supply by capillary vessels and a higher presence of GF in fast-growing strains. Moreover, we found different features of myopathy associated with GF. Some of them were represented by HF, splitting fibers and internal nuclei are also characteristic of human muscular dystrophies (eg. Duchenne). Accordingly, muscle hypertrophy of meat-type chickens favoured fiber dystrophy that may develop breast muscle anomalies such as white stripping and wooden breast responsible for significant economic losses.

P069

Effects of dietary insect meal from *Hermetia illucens* on the expression of genes related to methionine metabolism in rainbow trout (*Oncorhynchus mykiss*)

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With most wild fish capture fisheries at or above maximum sustainable yield, aquaculture cannot rely any more on oceanic resources for the manufacturing of aqua feeds and such feed options are just not sustainable. This means that fishmeal (FM), will increasingly be used in combination with other ingredients in fish diet. Plants already deliver the majority of the protein to farmed fish diets due to the abundance, and low cost. However, inclusion of vegetable meals in aqua feeds is limited since they could lead to a number of adverse effects. Therefore, research on new protein sources is needed. FAO indicates insects as an innovative and high potential source of protein to be employed in aqua feed manufacturing, due to their high nutritional value, especially in terms of crude protein content. Insect-based meal could thus become a sustainable and commercially viable alternative to FM in aquaculture. One of the most promising insect species for commercial exploitation is the black soldier fly (BSF), *Hermetia illucens*. The larvae of BSF grow on different organic substrates consuming twice their weight each day and the prepupa contain a very high percentage of protein (36-48%) on a dry matter basis. However, the BSF larvae are characterized by a relative deficiency in some indispensable aminoacids such as methionine (Met), cysteine, and threonine yet having a better aminoacid profile than the vegetable meal. Accordingly, we evaluated the effect of dietary inclusion of BSF larvae meal, as replacement for fishmeal, on fish growth performances and the transcript levels of genes involved in Met resynthesis (BHMT, SAHH) and net Met loss (taurine synthesis) (CBS) in rainbow trout (*Oncorhynchus mykiss*) liver.

Results of the feeding trial (3 months) showed no significant differences in specific growth rate of fish that received 25 or 50% of FM/BSF meal substitution in comparison to control (CTRL) fish that did not receive insect-meal in the diet. SAHH gene was affected by the diet, showing higher expression in CTRL group in comparison to BSF25 and BSF50 groups. The expression of BHMT was not different in fish fed with different diets. Finally, the highest expression of CBS gene was found in the CTRL group as compared to BSF25 one, whereas the expression of CBS in the BSF50 group was similar to that of fish fed with other diets.

In conclusion, *H. illucens* meal is an innovative raw material that seems to be promising as an alternative to FM in trout diets.

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P070

A comparison of water distribution and protein oxidation between poultry and rabbit meat

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In the last years, proton Nuclear Magnetic Resonance (NMR) relaxometry has been successfully applied to study water mobility and distribution in pork. In addition, although neglected for decades, the impact of protein oxidation on meat quality traits has been recently reviewed. Within this context, considering the incomplete knowledge on water distribution and mobility in white meats and the lack of information concerning protein oxidation, this study aimed at provide reference values on chicken, turkey and rabbit meat. For this purpose, rabbit (*longissimus lumborum* muscles from 11 weeks-old males slaughtered at 2.7 Kg), chicken (*pectoralis major* muscles from medium-size 44 days-old male broiler slaughtered at 3.0 Kg) and turkey (*pectoralis* *major* muscles from 20 weeks-old male birds slaughtered at 21 Kg) meat samples ($n = 8$/specie) were selected 24 h *post-mortem* and used to assess ultimate pH, colour ($L^*a^*b^*$), NMR relaxation properties and protein oxidation. In detail, proton transverse relaxation (T_2) decay curves were recorded, at the operating frequency of 20 MHz, with a Bruker (Milan, Italy) Minispec PC/20 spectrometer, while protein carbonylation was assessed following a novel 2,4-dinitrophenylhydrazine (DNPH)-based method. Data were analysed using one-way ANOVA with Tukey's multiple comparison test. Overall, ultimate pH values and colour were consistent with previous studies. If compared to both chicken and turkey, rabbit meat exhibited a remarkably higher proportion of the extra-myofibrillar water fraction (2.4 and 2.8 *vs* 8.4%; $p < .001$) and a consequent decrease in the intra-myofibrillar one (93.7 and 93.3 *vs* 88.5%; $p < .001$). However, although increased, the extra-myofibrillar water in rabbits appeared to be more tightly bound (lower T_2) in comparison to both chicken and turkey meat (130.3 *vs* 286.3 and 210.6 ms; $p < .001$). These dissimilarities might be related to the different muscle fibre characteristics (*e.g.* type, size, *post-mortem* acidification behaviour, etc.). As for protein oxidation, significantly higher carbonyls content were found in turkey and rabbit in comparison with chicken meat (3.10 and 3.60 *vs* 1.11 nmol/mg of protein; $p < .001$). The lower protein oxidation level observed in chicken meat might be likely related to its lower iron content if compared to both turkey and rabbit meats. In conclusion, providing reference values, this study improved the current knowledge concerning poultry, turkey and rabbit meat quality characteristics.

P071

Hepatic metabolites S-adenosyl methionine (SAM) and S-adenosyl homocysteine (SAH) as signals of methionine nutritional deficiency in rainbow trout (*Oncorhynchus mykiss*) fed on diets with partial replacement of fishmeal with insect (*Hermetia illucens*) meal

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The metabolism of the indispensable amino acid methionine mainly occurs in the hepatic tissue of fish, where the enzyme methionine adenosyltransferase (MAT) is highly functioning. In several methylation reactions that occur in liver, the activated methionine, *S*-adenosyl methionine (SAM), serves as a donor of methyl groups for methyltrasferases and is converted to *S*-adenosyl homocysteine (SAH). Therefore, the SAM/SAH ratio represents an indicator of cellular methylation status being also very important for homocysteine (Hcy) metabolism. Hcy is involved in the methionine regeneration (transmethylation) and in the trans-sulfuration pathway, which produces cystathionine, cysteine, glutathione, and taurine.

In aquafeeds that use high percentages of vegetable meal to replace fishmeal (FM), the methionine could be a limiting amino acid that must be added to the fish diet. Similarly, the insect meal in particular that from black soldier fly (BSF), *Hermetia illucens*, is characterized by a relative deficiency in some indispensable aminoacids including methionine yet having a better aminoacid profile than the vegetable meal. Therefore, we evaluated the effect of dietary inclusion of BSF larvae meal as replacement for FM on the concentration of SAM, SAH and SAM/SAH ratio in rainbow trout (*Oncorhynchus mykiss*) liver.

We set a 90-day feeding experiment with 360 trouts. Two fish groups were fed with increasing FM/BSF meal substitutions [25% (BSF25) and 50% (BSF50)] without any methionine supplementation and a third control group was fed a FM-based diet (BSF0). SAM and SAH concentrations were analyzed by HPLC.

Hepatic SAM concentrations resulted significantly higher in BSF50 group (65.71 ± 5.86 nmol/g) compared to BSF0 (59.39 ± 2.44 nmol/g) and BSF25 (61.17 nmol/g) groups. On the contrary, the lowest hepatic SAH concentration was measured in BSF50 group (17.28 ± 1.53 nmol/g), whereas in the other two groups, SAH concentrations increased linearly with decreasing hepatic SAM concentrations. Therefore, SAM/SAH ratio increased linearly with the percentage of dietary BSF inclusion.

In conclusion, BSF_meal could be a potential candidate for FM substitutions in rainbow trout feeds; it seems to maintain high levels of hepatic SAM and to promote the removal of Hcy through trans-sulfuration or transmethylation pathway.

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P072

Growth performance and carcass traits of rabbits raised in cages or in a free range system from 30 to 100 days of age

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In recent years there has been a decline in intensive production of rabbit meat in Italy for the high production costs and the attention of producers has focused on the semi-intensive or extensive production to produce high quality meat. The aim of this study was to ascertain the effect or rearing technique on growth performance and carcass traits of rabbits from weaning (30 d) to 100 days of age. A total of 36 California × New Zealand White rabbits (average weight 1079.1 g ± 181.7, sex ratio 1:1) were equally divided into 2 groups (farmed in cages or in a free range system). Both in cage or in the free range system, rabbits were divided in 6 replicates (3 rabbits/replicate). In the cages, rabbits had around 800 cm^2/head; in the free range system 2000 (in the area there were shelters and trees but no grass). Both groups fed *ad libitum* the same diet consisting on alfalfa hay and a cereal mix (1/3 barley, 1/3 oat, 1/3 bran). Weekly, the rabbits were weighted and the feed intake was calculated by the difference from a pre-weighed amount of feed and the remaining one at the end of the week. Mortality rate was recorded weekly. At 100 days rabbits were slaughtered and carcass traits were evaluated. The results on growth performance and carcass characteristics were analyzed by a two-way ANOVA (main factors were the raising system, the sex and their interaction); the differences among means were analyzed by Tukey test. Both groups of rabbits were in good health condition and no death were observed along the trial. Growth performance (including feed intake and feed conversion ratio) and most of carcass traits were unaffected by dietary treatments. However, the meat to bone ratio obtained from dissection of left hind leg of rabbits was lower in free range rabbits (4.9 *vs* 6.6, respectively in free range and caged rabbits, $p < .05$) and this result was mainly affected by the higher incidence of bone (16.6 *vs* 13.0% of left hind leg, $p < .05$) in free range rabbit, indicating a positive effect of this farming system on bone structure. In conclusion, this study has shown no differences between the two farming systems and, in addition, free range system had a positive effect on the bone structure, representing a valuable alternative to the cage system.

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P073

Effect of equilibration time for semen cryopreservation of endangered Mediterranean brown trout (*Salmo cettii*) inhabiting the Biferno river (Molise region, South Italy)

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Sperm cryopreservation is useful for the conservation of endangered fish species. This procedure has effectively been described as safe for the *ex situ* preservation of biodiversity by facilitating the storage of gametes in a gene bank.
The success of fish semen cryopreservation depends on interplay among many factors (freezing medium, cryoprotectant (CPA) and its concentration, equilibration time, and freezing and thawing rate). The choice of equilibration time is certainly among the most important factors for an effective semen freezing protocol. The equilibration time is the period of time necessary for the CPA to permeate the sperm. Accordingly, the objective of this study was to determine the effects of two equilibration times on post-thaw semen quality of Mediterranean brown trout from the Biferno river. Twenty autochthonous Mediterranean brown trout males (*Salmo cettii*) were captured from the Biferno river (Molise region) by electro-fishing. Semen was collected by gentle abdominal massage. Ejaculates were pooled (4 ejaculates/pool), 6 pools were used. Each pool was divided into two subsamples and diluted 1:3 in freezing extender containing 300 mM glucose, 10% egg yolk and 10% dimethylsulfoxide. The extended semen was loaded in 0.25 mL plastic straws and equilibrated at 4 °C for 10 or 30 min (equilibration time). At the end of each period, the semen was frozen by exposure to liquid nitrogen vapor (5 cm above the liquid nitrogen surface) for 10 min; then, the straws were plunged into liquid nitrogen (-196 °C). Semen samples were thawed at 30 °C for 10 s. Sperm motility, spermatozoa movement duration (SMD), sperm viability (SyBr-PI) and sperm DNA integrity (Acridine

orange) were assessed in both fresh and frozen semen.
Semen quality parameters recorded in fresh semen show a good quality soon after collection. After thawing, no significant effect of the equilibration time (10 *vs* 30 min) was observed for all the sperm quality parameters assessed. However, slightly higher values were observed with 10 min equilibration compared to 30 min: sperm motility = 33.5% *vs* 31.8%, SMD = 40.7% *vs* 38.7%, viability = 36.3% *vs* 35.9%. These results lead us to assume that the equilibration time of 10 minutes is sufficient to allow dehydration of sperm cells. The development of an effective freezing protocol is required to create a sperm cryobank to support the *ex situ in vitro* conservation of the original population of Mediterranean brown trout in the Biferno river.

P074

Comparison of two basic extenders on the *in vitro* post-thaw quality of rabbit semen

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Semen cryopreservation is a strategic tool to secure genetic diversity. Research efforts have focused on developing freezing protocols to improve the cryopreservation of rabbit semen by the reduction of sperm cryoinjuries. Since a lack of knowledge about the use of basic extenders, we tested the effect of a commercial extender (Cortalap®) compared to Tris-citrate-glucose (TCG) on the *in vitro* post-thaw quality of rabbit semen. Six pools of semen (4 ejaculates/pool) were collected from 40 adult rabbit bucks of Bianca Italiana breed from the Italian Rabbit Breeders Association (ANCI-AIA, Volturara Appula (FG), Italy), an aliquot from each pool was used for the analysis of fresh semen, the remaining part of pooled semen was cooled at 5 °C for 90 minutes. Each pool was split into two equal aliquots, and each of them were diluted to a ratio 1:1 (v:v) with a freezing extender composed of TCG or Cortalap® both containing 16% of dimethylsulfoxide and 0.1 M sucrose. The diluted semen was packaged into 0.25 mL plastic straws, equilibrated at 5 °C for 45 min. Semen was frozen at heights of 5 cm above liquid nitrogen for 10 min, then straws were transferred into liquid nitrogen for storage at -196 °C. Sperm samples were thawed at 50 °C for 10 seconds. Sperm motility (phase contrast microscopy), viability (SyBr-PI), acrosome integrity (FITC-PSA) and DNA intactness (Acridine orange) were examined on fresh and post-thawed sperm. Sperm variables among the fresh and cryopreserved semen were compared by ANOVA, followed by Duncan's comparison test, the level of significance was set at $p \leq .05$. Results showed that the cryopreservation process impaired the post-thaw quality of rabbit semen compared to fresh semen ($p < .05$). However, the quality of frozen-thawed semen was affected by the freezing extender. In fact, the post-thaw semen quality was significantly improved in semen samples diluted in the Cortalap® extender compared with TCG for total and progressive motility (43.4 ± 1.4 *vs* 36.8 ± 1.5 and 36.5 ± 1.1 *vs* 30.2 ± 1.9), sperm viability (52.5 ± 1.8 *vs* 44.6 ± 2.1) and acrosome integrity (37.5 ± 0.6 *vs* 30.9 ± 1.2). The present results show that the Cortalap® extender provided better *in vitro* condition to preserve sperm integrity (membrane and acrosome) and function (motility) during the cryopreservation process. However, further studies are needed to confirm these results *in vivo*.

P075

Advantages and disadvantages of mechanical separation process applied to fishery industry

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Fish represent an important source of nutrients, such as essential fatty acids and minerals, so numerous technological strategies have been adopted for exploiting the huge amount of no directly marketable specimens without altering fish nutritional quality. Recently, mechanical separation process (MSM) have been demonstrated to be successfully applied to fish by-products for increasing processing yield. The present study aims to compare the impact of mechanical separation with manual mincing applied on farmed European sea bass, gilthead sea bream, rainbow trout, and wild Atlantic horse mackerel by evaluating technological yield and nutritional quality of obtained meat. Eighteen fish for each species were subdued to the two different treatments. MS process yield was found higher than the manual one when applied to sea bass, sea bream, and trout (58, 63%, 70% respectively against 39, 40, and 53%). Horse mackerel instead was better separated by manual operation. MS seemed to slightly (around 2%) but significantly increase water content and decrease minerals in all the species except sea bass. Interestingly, even Ca tended to decrease confirming the efficiency of the mechanical separation process in muscle separation from the skeletal part of the fish, which is the main source of Ca. Hopefully, lipid fraction of the considered species remained

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unaltered. Few differences in C18:2n6 (1.18 *vs* 1.00 g/100 g total fatty acids), and C22:1n11 (0.27 *vs* 0.20 g/100 g total fatty acids) were found for horse mackerel minced and MSM meat. Globally, seawater species confirmed to be richer in PUFA n3 than freshwater species being around 55, 33, and 38 g/100 g total fatty acid in mackerel, sea bass and sea bream, whereas 21 g/100 g total fatty acids were found in trout. Processing may promote oxidative damages as confirmed by TBARS content of MSM samples, however sea bass and sea bream were found the most susceptible by doubling their TBARS content compared to minced samples. Trout and horse mackerel were mostly unaffected even if TBARS values of the latter exceeded 6 mg/100g muscle. maybe because of both intrinsic lipid susceptibility and killing/handling procedure after fishing. In conclusion, mechanically separation process can be adopted in order to exploit unmarketable fish, however not all the species shown to be equally prone. Globally, sea bass and trout were the most suitable from a technological and qualitative perspectives.

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P076

On-growing turbot (*Scophthalmus maximus*, Linnaeus 1758) in a land-based aquafarm in Southern Italy: preliminary results

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Turbot (*S. maximus*) is considered a possible candidate to increase the number of farmed fish species and contribute to diversify Italian aquaculture production. Rearing techniques, developed in France and Spain, highlighted good growth performances for this species, while breeding attempts in Italy, carried out in the past, failed due to the high water temperatures reached in the tanks during the fattening cycle in the summer. The present study aims to evaluate the feasibility of turbot rearing in a land-based aquafarm located in the Campania region, South of Italy. 7000 turbot juveniles (initial weight 7.5 g) were purchased from a specialized French hatchery (France Turbot) and placed in a rectangular concrete raceway (27x7 m) at the Soc. Coop. “Acquamarina” located in Villa Literno (CE, Italy) in December 2015. The tank was adapted to the species and fitted with a shading net. Fish were fed commercial diets (Biomar, Spain). Dissolved oxygen (>8 mg/l) and temperature were monitored daily, while pH (7.3 ± 0.1), salinity (37‰) and TAN (<5.5 mg/l) were determined monthly. The farm sources its water from wells, with a constant temperature of 18 °C, with slight seasonal fluctuations (16 °C-20 °C) in tank, still contained within the ranges tolerated by the species thanks to a proper management of water renewal and to the shading. In addition, the low pH and temperature values allowed un-ionized ammonia to be kept under control and at acceptable levels for the species (0.04 mg/l).”

Monthly, a sample of fish, representative of the population, was weighed to determine the average weight of the subjects, the FCR and the SGR. At the beginning and during the trial, a sample of subjects was sacrificed to evaluate the whole body and the fatty acid profile of the fillets. After 445 days of rearing, fish reached an average weight of 770 g, with a FCR of 1.15 and a SGR of 1.04. These performances allow us to hypothesize the achievement of the minimum commercial size (1 kg in Italy) within 18 months of rearing. These preliminary results suggest that in particular environmental conditions and adopting appropriate farming solutions, on-growing turbot in Italy is feasible. This could act as a catalyst to revitalize a sector, such as fish farming, which has been in difficulty in recent years especially in the Campania region.

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P077

Sensory comparison of mussels (*Mytilus galloprovincialis*) farmed in the Adriatic and Sardinian Sea

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Italy, with Spain and France, is a top producer of molluscan shellfish in the EU; the national production of bivalve mollusc (111,000 tons) accounts for 63% of the Italian aquaculture size and mainly consists in farmed Mediterranean mussels (*M. galloprovincialis*). Mussels have an interesting nutritional value making them ideal for the human diet; however, being cultured in extensive systems, their quality entirely depends on natural resources. Recent studies highlighted that mussels from different Spanish and Portuguese production sites can be distinguished by biometric, nutritional and sensory characteristics (Olivera *et al.*, 2014). Such differences could help to register seafood products by a Protected Designation of Origin. The experiment aims at a quick, explorative sensory profiling of mussels cultured in different Italian rearing sites.

M. galloprovincialis of commercial size, from Arborea (OR), Marano Lagunare (UD) and Goro (FE) after heat treatment (85 °C for 10 minutes) *Sous vide* cooking and chilling, were analyzed using Flash Profiling in both the conventional method (CFP), with a free generation of sensory attributes, and the guided method (GFP), with a selection of sensory attributes from a precompiled list. Products were evaluated by two panels of 8 untrained assessors each, during two parallel sessions of sensory analysis, comprising three replicates each. Data collected with the FIZZ software (Biosystem, Couternon, F) were subjected to GPA by Senstool software (O&P Product Research BV, 1998).

The same average number of attributes, 13, were considered by the two methods, but GFP proved more reliable (Permutation test) in providing the sensory profile of mussels originating from different areas. The experiment showed significant differences in flavor characteristics between mussel groups; "sweetness" was the prevailing perceived feature of mussels cultured in the Adriatic Sea, while "salty", "brackish", "marine" and "seaweed" were the most recurrent sensory attributes selected to describe the mussels farmed in the Sea of Sardinia.

P078

The effect of the season on the nutritional quality of wild white sea bream (*Diplodus sargus*)

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White sea bream *(Diplodus sargus)*, an omnivorous Mediterranean species of the *Sparidae* family, shows a modification of fillet fatty acids ratio in relation to environmental conditions (i.e. water temperature, salinity, diet). On 12 white sea bream captured in Naples Bay at the end of May 2016 (spring) and the end of September 2016 (summer), we determined proximate composition and fatty acid profile of lipid on raw fillet and Warner Bratzler shear force (WBSF) on cooked fillet. The season was considered as a fixed factor in the ANOVA analyses. At dissection, the digestive content of stomach and gut presented some clamps, algae and various debris. The mean body weight and length were significantly higher ($p < .01$) in the summer group (mean ± SD: 294.8 ± 78.7 g and 23.15 ± 1.33 cm, respectively) than the spring group (149.9 ± 57.5 g and 18.65 ± 1.57 cm, respectively). The proximate composition of fillets did not change with the season, except for higher lipid contents observed in summer compared to spring (1.8% ± 0.7 *vs* 0.8% ± 0.4, $p < .05$, respectively). However, in the May sampling, a better fatty acid profile was assessed. Indeed, the most noteworthy difference was the higher proportion of EPA (eicosapentaenoic acid; 7.15 ± 1.18 *vs* 4.13 ± 1.46, $p < .01$, respectively), DHA (docosahexaenoic acid; 29.65 ± 5.99 *vs* 14.18 ± 2.53, $p < .01$, respectively) and DPA (docosapentaenoic acid; 2.87 ± 0.62 *vs* 1.20 ± 0.47, $p < .05$, respectively). In addition, the level of n3 series fatty acids was higher in fish captured in spring. No statistical differences were detected on total saturated and monounsaturated fatty acids. Finally, the rheological parameters of the cooked fillets did not show seasonal changes. We conclude that the nutritional quality of fillets is influenced by the environmental conditions.

P079

The real annual per capita consumption of fish in Italy

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To determine the real annual per capita consumption (RACC) of fish (excluding shellfish) in Italy between 2010 and 2014, the production data (UNIMAR-MIPAF for aquaculture, IREPA for captures) and data of the balance of fish exchanges

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between Italy and the rest of world (Coeweb-ISTAT) were utilized. The non-edible portions (head, bones, viscera, etc.), dependent on the kind of product and the ingredients in some commercial products (*i.e.* salt, covering medium, etc.) were considered.

The study overcame difficulties due to the complexity and heterogeneity of the fish commercial sector, characterized by a high number of taxonomic units and commercial categories, by different methods of preservation and transformation. In the year 2013, in Coeweb-ISTAT, n. 334 commercial categories were identified; in the case of capture and aquaculture productions, the official statistical sources reported 65 and 28 taxonomic entities, respectively. To calculate the meat yield the following methods were used for different products:

-fish marketed as "whole fish": the fillet yields found in literature were used, integrated with the "Conversion factors from landed to nominal weight ", used to convert processed fish into fresh fish when it is processed on ships, which consider different coefficients for different species and different types of treatment applied to the fish (evisceration, decapitation, filleting, skinning);

- categories identified as "meat, even minced", "filleted", "in pieces" (fresh or chilled): the values were directly derived;

-category "frozen" (in forms of whole fish, fillets, meat): the incidence of the glazing (average 7.5%) was considered;

-smoked products, dried, salted or in brine products: specific correction factors were particularly difficult to achieve, so they were not applied;

-fish in form of preserves and/or preparations: the real quantities of fish contained were calculated by applying the minimum thresholds established by the current EU regulatory framework. In the absence of specific regulations, correction factors were obtained through a survey carried out ad hoc.

The estimated RACC of fish meat in Italy in the 2010-2014 period resulted on average 6.50 kg, corresponding to ~70% of the apparent consumption of fish, 9.28 kg/per capita/year, which deviate significantly from the apparent consumption data reported in official statistics, including not only fish but also crustaceans and molluscs.

P080

Use of a motivational cage to evaluate the social relationship in colony-reared rabbit does

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Alternative housing systems can be considered important forms of social enrichment, but grouping rabbits can also cause stress, fightings and wounds. Motivational tests permit to check animal needs contributing to proper animal handling. This preliminary study aimed to evaluate whether changes in the housing environment leads to improvements in welfare conditions or in the motivation toward social contact or self-exclusion of colony-reared rabbit does.

Three successive experiments were carried out:

EXP 1: eight rabbits does (4/cage) were housed in 2 motivational cages separated in 4 compartments by easy to open, transparent doors.

EXP 2: the same 4 individual areas were equipped with 4 environmental enrichments: straw, sand and hiding box, respectively; the 4th area is empty and served as a control To reach these resources, rabbits had to pay entry cost, since doors were loaded every 48 hours by increasing weights.

EXP 3: eight rabbit does (4/cage) were reared in the same colony cages with a central group area (G zone) or 4 individual areas, divided as follows:

-zone SS1-SS2: two zones equipped with straw replaced every 8 days, at the end of each video recording cycle.

-zone FS1-FS2: two zones equipped with straw replaced every 2 days.

A computer system software (OBSERVER XT) and video-camera (NOLDUS XT) were used to analyze the animal choices. Data were analyzed with the ANOVA procedure considering the fixed effect of the preference for isolation/group (Exp 1), isolation/cage choice (Exp 2) or straw quality (Exp 3). Rabbit does showed almost the same percentage of time spent in group or isolation without (51% *vs* 49%, respectively) or with (55% *vs* 45%, respectively) weight on push-doors.

Putting additional weight on push-doors generally modified rabbit behavior by reducing visit number and increasing visit length to isolation areas. Isolation area equipped with straw remained the preferred area independently of the applied weight followed by hiding box and control. Rabbits does spent approximately the same time in isolation and in group: impediments requiring an extra effort to achieve isolation (i.e. weighed doors) lightly increased social contact.

Acknowledgements

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P081

Heart rate and heart rate variability as indicators of animal welfare and coping style of dairy cattle

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Heart rate (HR) and heart rate variability (HRV), providing information on the balance of the sympato-vagal system, have been used as indicators of emotional state and coping style in humans, horses and lab animals; although scientific and technical knowledge on the topic have grown a lot in recent years, the use of HR and HRV in livestock sciences is still limited.

Polar Equine S625x and S810i^{TM} (POLAR$^{®}$) were used to assess feasibility of using HR and HRV in field experiments involving dairy cows housed in free stall barns. Heart rate was recorded for 24 h on 17 cows, 6 primiparous (PR) and 11 pluriparous (PL), 6 in early and 11 in late lactation; 17 cows, 9 PR and 8 PL, in early lactation, were monitored to estimate the effect of parity on HRV measured at milking; 23 cows, PR and PL, at different stages of lactation, were monitored to estimate the correlation between HRV and number of steps at milking.

Cows in early lactation showed higher ($p < .001$) daily mean HR, but the effect of the hour of day was the same for both early and late lactation cows ($p < .0001$). Diurnal pattern of HR was not affected by parity, whether was strongly shaped by the feeding routine.

At milking, higher HR and lower vagal tone were recorded in PR than in PL Tab. (1), suggesting that PR, in early lactation, were less adapted to milking stress. Number of steps at milking resulted correlated ($p < .05$) with some parameter in the

Table 1 Effect ($p < .05$) of parity on the parameters in the time domain of HRV analysis.

Parameters description, unit	PR	PL
Mean heart rate, beats/min	93.0 ± 2.0	86.3 ± 2.0
Mean interval between two consecutive heart beats (R-R intervals), ms	648.5 ± 13.6	699.4 ± 14.4
Square root of the mean squared differences between successive R-R, ms	3.8 ± 0.5.5	5.7 ± 0.6

frequency domain of HRV analysis: power of low frequency band (rho = 0.47), power of high frequency band (rho = -0.47), ratio of low to high frequency power (rho = 0.49).
In our conditions, 24 h recording was a good tool to define individual basal level of HR and variations due to the daily routine management; HRV efficiently detected expected differences in coping with stressful conditions (PR *vs* PL at milking) and was correlated to a known indicator of stress response (steps at milking). Implementing sensors of HRV to be used at farm level could be useful to monitor the individual response of cows to the internal and external challenges.

P082

On-farm animal welfare assessment in beef cattle: an integrated approach

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The present study aims (i) to evaluate beef cattle welfare with an integrated approach as housing conditions and management practice change and (ii) to investigate the correlations between the parameters used and their applicability. A total of 15 Piemontese breed bulls reared in a tied stall housing system (farm A) and 15 Piemontese, 15 Blonde d'Aquitaine (from France) and 15 cross-breed (from Ireland) reared in pen for groups (farm B, C and D, respectively) were considered. A welfare protocol including a checklist and the "RIBECA" scoring system (formulated by CRPA) was prepared for the assessment of animal based measures *(e.g. BCS*, Avoidance distance test, ocular and nasal discharge, diarrhea) and resource based measures *(e.g.* appropriate housing and feeding, space, temperature*)*. Some blood parameters were also analyzed: WBC, % of neutrophils, lymphocytes, monocytes, eosinophils, basophils, RBC, HGB, HCT, N/L ratio (ADVIA®120, Siemens), total protein, albumin, CK, creatinine, AST, LDH, cholesterol, glucose, triglyceride (ILab Aries, Instrument, Werfen) and dROMs (dROMs test, DIACRON INTERN. IT). Observations and blood samples were performed 2 times: 3 weeks after the arrival of the animals on the fattening farm (T0) and after 5 months (T1). Comparisons between animal groups within T0 and T1 were performed by One-way ANOVA and Kruskal-Wallis test, while data between T0 and T1 within each group were analyzed by Student's t and Wilcoxon tests (GraphPad Prism® software, $p < .05$).
Animal-based score was not significantly different between the farms, while total welfare score was "poor" for farm A (tied stall system), "fair" for farms B and D and "very good" for farm C. Irish cross-breed were the most fearful. Blood findings suggest that Irish cross-breed showed the worst adaptation (WBC, neutrophils, the N/L ratio, albumin, CK, glucose, creatinine and LDH increased at T1; $p < .01$) whereas Blonde d'Aquitaine cattle were well adapted (WBC, monocytes, eosinophils and basophils decreased at T1; $p < .01$, dROMs were significantly lower in this group; $p < .01$). The two groups of Piemontese bulls did not show significant differences.
The results obtained in the present study suggest that parallel use of both animal-based and resource-based measures is preferable for animal welfare assessment.

Acknowledgements

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P083

Identification of differentially expressed microRNAs in the peripartum of dairy cows: potential indicators of welfare and metabolic efficiency

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Genetic selection pressure on dairy cows has increased the incidence of metabolic disorders with important consequences on welfare and longevity. MicroRNAs (miRNA), a family of small non-coding RNAs with a key role in the regulation of gene expression, are interesting for characterizing the molecular basis of immune imbalances. The aim of this work was to investigate differences in miRNAs expression in dairy cattle during the highly susceptible to stress period of the peripartum. Forty metabolic and immunological parameters, classically used as indicators of increased risk of peripartum-related diseases, were recorded in six Holstein Friesian cows during 4 time-points of the peripartum (-21 ± 2; -3 ± 1;

+3 ± 1; +21 ± 2, days). Animals were housed in a highly producing farm monitored according to the model of the National Reference Centre for Animal Welfare. Based on the simultaneous variation found in the levels of non-esterified fatty acids, albumin, cholesterol and haptoglobin, cows were separated into a control group (values within the reference range) and a group with altered metabolic values. The miRNAs were extracted from the buffy coat of 24 blood samples relatively to the 4 time-points and sequenced by Illumina HiSeq 2000. Standard count based bioinformatic pipelines (EdgeR) were applied to reveal expression changes in miRNAs between the two groups. Results identified 30 differentially expressed miRNAs in the group with altered metabolic values compared to control with False Discovery Rate (FDR) at $p < .05$. Among them, the bta-mir-2415, bta-mir-126, bta-mir-6522, bta-mir-423, and bta-mir-10b are of particular interest as potential early indicators of a risk of imbalance because they resulted to be differentially expressed 3 weeks before the birth. The corresponding Fold Change (FC) were: $\text{logFC} = -2.98$ ($FDR = 0.0039$); $\text{logFC} = 3.93$ ($FDR = 0.0040$); $\text{logFC} = 2.14$ ($FDR = 0.003$); $\text{logFC} = -1.32$ ($FDR = 0.016$); $\text{logFC} = 3.25$ ($FDR = 0.021$). Preliminary bioinformatic analysis indicated putative target genes involved in pathways of cytokines, protein kinase C, membrane transport, and mitochondrial processing (total score >250, total energy < -10).

The miRNAs identified in the present work are candidates as potential biomarkers associated with critical moments of the production cycle and can enhance the understanding on the ability to respond more efficiently to critical phases.

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P085

Applicability of infrared thermography of chimpanzees in semi-natural conditions: a preliminary study

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Infrared imaging thermography (IRT) is a technique widely used in wildlife studies and the assessment of animal welfare is among its fields of application. The heat emitted from superficial capillaries changes as blood flow is under control of the autonomic nervous system. For this reason, cutaneous temperature on selected areas can be considered as a good indicator of the health status and welfare of a particular individual.

These changes in heat emitted can be quantified using IRT. The system consists of an infrared camera FLIR A65 (640 × 512 pixel, uncooled microbolometer detector, thermal sensitivity 0.05 °C) with a 7° and 13° angle of view germanium tele lenses and a dedicated laptop.

In the case of chimpanzees' studies, most of the available data derive from subjects kept in small enclosures, with little ecological validity. The aim of this research was to develop an infrared method allowing the acquisition of thermographic videos and images of animals at long distance. The system was tested on a group of eleven chimpanzees, in semi-natural conditions, housed at Parco Natura Viva in Bussolengo (Verona). The study ran between April and December 2016. Images and videos were shot at distance greater than 10 meters, during the everyday chimpanzees' life. The accuracy and repeatability of measurements was that typically reserved to the image acquisition with closer subjects at indoor conditions.

Through this system it was possible to detect temperature variations in face layers by distinguishing the facial features of the subject.

Thus, despite the distance from the animals, thanks to the good resolution of the system, the cutaneous temperatures were detected. In conclusion, IRT could be able to non-invasively detect different autonomic responses of the chimpanzees to different situations, suggesting that this system could be a valuable tool to study the chimpanzee's behavior and welfare at long distance.

P086

Evaluation of adhesion molecules in bovine neutrophils and monocytes during early lactation

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The periparturient period is characterized by dramatic changes in the efficiency of the bovine immune system.

Numerous studies have documented changes in many aspects of both innate and adaptive immunity that can affect the susceptibility to new diseases in the transition cow. Higher susceptibility to infections is accompanied by reduced migration of blood neutrophils and monocytes to the site of infection, and can be identified by lower expression of adhesion molecules on cells surface. The aim of this study was to examine the changes in expression of adhesion molecules (CD62L and CD11b) of leukocytes at early lactation in Friesian cows. At three points (calving, +15 and +35 day) jugular blood samples from twenty-nine cows were collected. Blood was incubated with saturating amounts of PE-conjugated anti-CD62L mAb (clone FMC46) and APC-conjugated anti-CD14 mAb (clone TÜK4), to determine the expression of L-selectin on neutrophils and monocytes; with FITC-conjugated anti-CD11b mAb (clone CC126) to determine the expression of CD11 on neutrophils. Flow cytometric assay was performed on FC500 cytometer (Beckman Coulter Inc). Monocytes were identified as $CD14^{+}$ cells; neutrophils were identified based on forward and side-scattered characteristics. The percentage of positive cells for each marker was calculated after plotting FITC and PE fluorescence associated histogram, the mean fluorescence intensity (MFI) was computed in the geometric linear mode. Flow cytometry data were analysed using Kaluza Analysis 1.3 Software (Beckman Coulter, Inc). Statistical comparisons involved Student's t-test and Proc GLM in SAS (SAS Institute Inc.,Cary, NC, USA) to assess differences between cows and lactation stage. The results showed significant differences in MFI of CD62L on neutrophils and monocytes between the three stages ($p < .05$); no significant difference was found in MFI of CD11b on neutrophils. The percentage of neutrophils and monocytes $CD62L^{+}$ and neutrophils $CD11b^{+}$ showed no significant differences between stages. Furthermore, a great individual variability of CD62L expression was found within cows, with MFI on neutrophils ranged from 20.45 to 90.47 and MFI on monocytes ranged from 37.40 to 95.27. Lower variability of CD11b expression was found, with MFI on neutrophils ranging from 48.7 to 90.20. Because of the significant individual variability, these preliminary results indicate that L-selectin could be used as immunological marker to evaluate disease resistance.

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P087

Milk intake in relation to growth in hand-reared fallow deer (*Dama dama L.*, 1758) fawns

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In wild Cervids, a substantial forage consumption starts at about one month of age, however nutrition in the first days of life is crucial for the survival and growth of fawns, and little is known about milk intake in the post-natal period. In order to describe some growth performances in fallow deer (*Dama dama*), this study examined the consumption of milk and the increase in body weight in the first 45 days of life of hand-suckled fawns.

Six fawns, three males and three females, were fed with colostrum in the first 3 days of life and then artificially fed *ad libitum* three times a day (8 am, 12 pm, 18 pm) with acidified dried milk for lamb. The feed was composed by 60% of dried skim milk (46.6% lactose, 22.6% crude protein, 18.5% crude fat, 7.3% ash, 0.2% crude fibre) and complemented with dried whey, coconut oil, lard, tallow, corn starch, and soy lecithin. Milk was prepared by dissolving 150g of feed in 1 litre of water until the 4th week of life, then feed concentration was augmented to 180g/l. Intake was measured by weighting milk before and after each meal, and body weight was measured weekly. Milk consumption and average daily gain were analysed with a Generalised Linear Model (GLM).

The daily milk intake (g milk/kg body weight$\cdot$day^{-1}) in male fallow deer fawns was higher than in females ($p = .003$). The maximum milk intake occurred around the 18th day of age, when male and female fawns consumed respectively 199.12 and 160.24 g milk/kg body weight$\cdot$day^{-1}. Then, intake declined to 145.44 and 133.11 g/kg day^{-1} at the age of 45 days. Both male and female fawns displayed a similar ingestive behaviour among the three daily meals: milk consumption was the highest in the morning and the least in the midday, while an intermediate amount of milk was ingested in the late afternoon. Average daily gains were 181.75 g/day for male fawns and 130.9 g/day for females, and slightly increased during the study period ($p = .053$). Male fawns displayed a greater, but not significant, precocity in body weight growth and attained 11.94 kg at 45 days, while in females of the same age body weight was 10.12 kg.

This study revealed that dried skim milk for lambs represents a suitable feed for fallow deer fawns when maternal milk is not available. In the first 45 days of life, milk intake and average daily gains are higher for male fawns. Live weight growth, however, is similar for the sexes suggesting a greater efficiency of food conversion for female fawns in fallow deer.

P088

Use of thermography (IRT) as a pre-diagnostic technique in subclinical bovine mastitis: a pilot study

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The bovine mastitis is one of the major sources for Italian dairy farms. Goal of this work is to verify if it is possible use infrared thermography (IRT) as a non-invasive tool for the early diagnosis of subclinical mastitis.

Udder skin temperature has been detected both from the lateral (TL) than from the rear of the breast (TP). 380 Holstein cows from 3 different farms in the North of Italy were selected. A portable infrared camera G120EX with microbolometer sensor (320 × 240 pixels) was used. The measurements carried out concurrently with monthly DHI control performed by local Breeders Association during the summer in 2016.

Somatic cell count SCC in milk is approved as marker to assess inflammation; a cutoff of 200,000 cell/mL was used to settle infected cows. Udder temperature values obtained by IRT were compared with those of SCC given from DHI control using a statistical software (SPSS 24.0).

The SCC was correlated with the maximum temperature of the udder taken laterally TL ($p < .05$, Rho Spearman = -0.181).

In infected animals (SCC >200.000 cell/mL), the average value of TP was 38.5 ± 0.8 °C and that of TL was 38.3 ± 1.0 °C. In healthy animals (SCC <200.000), average value of TP was equal to 38.6 ± 0.8 °C and that of TL was equal to 38.6 ± 0.8 °C.

Increasing the number of subjects is necessary to confirm the data obtained and investigate the meaning of a negative correlation factor.

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P089

Thermographic monitoring of nests of nude mice in their early life: technical note

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We hypothesized to use Infrared Thermographic Technique (IRT) to detect the phenotype of athymic Nude-Foxn1 nu/nu and nu/+ mice from the very first hours of life, assuming that the presence (in nu/+ mice) or absence (in nu/nu mice) of hair follicles could completely shield or partially reduce the emitted radiation.

The experimental phase of three months was carried out at the IRCCS Istituto Mario Negri in Milano, in compliance with institutional guidelines and international laws. Were used: Mice: 3 females Hsd: Nude-Foxn1nu athymic (nu/+) 6 weeks of age and 3 males Hsd: Nude-Foxn1nu athymic (nu/nu) 6 weeks of age; Cages: 3 IVCages working under positive pressure, at 75 air changes/hour; Bedding: autoclaved hard wood; Food (Standard Diet Harlan) and water (autoclaved tap water) were administered ad libitum. Hood Laminar Flow: CS5 changing station. Nesting material: autoclaved wooden wool (aspen).

Thermographic measurements were conducted 2 times per day (10:00 and 14:00) starting from day 0 (day of birth) to day 5 in 2 nests (40 images in 10 minutes). For each shot the camera detects two image formats: ".IRI" (thermography) and ".JPEG" (visible) as a control. The instrument display allows to see in real time the quality of thermographic images thus we preferred to make the filming manually instead of automatically in order to optimize the image's quality. Statistical analysis was performed on the data obtained by the nests at different ages: day 0, 1, 2, 3, 4 and 5. We were not able to distinguish between nu/nu and nu/+ mice because they stayed all huddled together but we observed an increase in nests temperature between day 0 and day 5 where animals, mainly nude pups, are mostly exposed to heat loss. In absence of effective thermogenic processes, the higher temperature measured in 5 days old nests can be attributed to animals body size. The physiological thermoregulation (BAT activity) and behavioral thermoregulation (huddling) do not allow autonomy thermoregulatory but help to slow the dissipation heat; the only way that the 0 - 5 days old pups can increase their body temperature is the transmission of heat from parents.

In this paper we have provided clear and replicable data about the usefulness of IRT in animal welfare monitoring.

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P090

Evaluation of antioxidant effects of pomegranate peel extract on bovine mammary epithelial cells

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Nutraceuticals can be considered valid supplement to the classical pharmacology. In particular, molecules and/or compounds contained in plants (or their products), may have positive effects on preventing oxidative stress, modulating the immune system and have antimicrobial effects. The present study was aimed to assessing the antioxidant effects of pomegranate peel extract on bovine mammary epithelial cells. pomegranate fruits (*Punica granatum* L. cv. “Wonderful”) were purchased from the local market and, after washing, arils were discarded and peels were dried in the dark at 35 °C until constant weight. pomegranate peels were powdered and distilled water was added (10 g/100 mL). After 24 hours infusion in magnetic stirring, the mixture was centrifuged, filtered and lyophilized. Bovine mammary epithelial cells (BME-UV1) were chosen as cell line for our studies. To evaluate cytotoxic effect and maximum tolerable concentration after different exposure times, the extract was re-suspended in cell medium and its different dilutions (1000, 100, 10, 1, 0.1 µg/mL) were replaced to standard culture medium. After 24 hours, 48 hours and 7 days of treatment, cytotoxic effects were evaluated using methyl tetrazolium (MTT) assay. Concentrations of 10, 1 and 0.1 µg/mL were not lethal at all exposure times. Antioxidant capacity of the extract was evaluated measuring reactive oxygen species (ROS) production. Cells were treated with sub-lethal concentrations of extract and with same exposure times used for the cytotoxicity assay. After 24 hours, 48 hours and 7 days of treatment, hydrogen peroxide (50 µM) was added as oxidative agent and ROS concentration was determined by 2′,7′-dichlorodihydrofluorescin diacetate reaction, using a microplate reader (Tecan Sunrise$^{\text{TM}}$). In conclusion, the extract used in the study, in particular at concentration of 10 µg/mL, showed a strong protective effect against oxidant agents. pomegranate peel may thus be a good candidate to be tested in *in vivo* studies as a dietary supplement against oxidative stress.

P091

Limiting the impact of electromagnetic fields on the family honeybee - preliminary results

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The honey bee (*Apis mellifera* L.) is one of the most important elements of food production. Worker bees during work on flowers are exposed to a variety of threats. Among them we can distinguish pesticides, adverse weather conditions and electromagnetic fields (EMF). The right amount of worker bees from the same family, this is necessary to maintain in high condition. This is only possible once the limited impact of these risks on the honeybee family. The aim was to determine the effect of limiting the electromagnetic field on the reconstruction of wax foundation by the family of the bee and age of brood. The study used 15 families of bee Carniola breed reared in the same condition (free from diseases) and placed in mini plus hives. In order to reduce the influence of an external EMF, hives covered with copper foil with a thickness 0.2 mm (10 of them). The control group consisted of 5 colonies in the same type of hive without foil. Prepared hives subjected to measurement of the internal electromagnetic field using a measuring instrument type TENMARS (TM-196). Following the isolation of found to 70% reduction of the impact of external electromagnetic. Bees were deposited on the frames of wax foundation. We evaluated the percentage of rebuilding this frames every 7 days. The results clearly indicate that reducing the impact of electromagnetic fields through the use of copper foil reduces the speed of reconstruction comb foundation by family insulated foil in comparison with the attempt to control 15%. In addition, the frames from the experimental group were observed that some of the cells is not correctly built (they accounted for about 125 (5%) cells) and is not used by the bees for storing food and brood rearing. Such changes were not observed in the control group 2450 (98%) cells were built properly, 50 cells (2%) marginal cells. Brood in the experimental groups was in differed age (4 - 16 days) on individual frames and distributed unevenly. Each bee colony in the end of the experiment was occupied 5 frames. Statistical analysis has demonstrated the link between the insulation and the hive mistake in the honeycomb, and the unrecognized connection between the insulation and the differences in age brood. All this shows that there is a correlation between the electromagnetic field and the rebuilding comb from wax foundation by bees. Determining the size of the impact of the electromagnetic field on the honeybee family requires further analysis and insightful.

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P092

Development of a reference tool based on physiological measurements to validate the models of animal welfare assessment in dairy farms

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The animal welfare assessment in dairy farms is a very complex and controversial task. The main problems are related to the subjectivity of welfare definition, that complicates the development of models for welfare assessment at farm level. This should be obtained through the choose of representative indicators, their evaluation with numerical scores, and the aggregation of the scores in a model that provides an overall welfare score (OWA). The Integrated Diagnostic System Welfare (IDSW), considers both indirect and direct indicators, and calculates a standardized (0-100 scale) OWA. The indirect indicators are included into two clusters: environmental cluster and feeding cluster, and each contributes 30% to the OWA. The direct indicators are included into the third cluster, the animal cluster (AC), which contributes for the remaining 40%. This score should be validated comparing it with the best welfare conditions, which can't be certainly identified, due to the subjectivity of the welfare definition. Thus, the IDSW was applied in 6 dairy farms, and the results were compared with blood physiological indices to obtain an objective validation of the results. Blood samples were collected from 10 early lactating cows per farm and energy, protein, mineral metabolism indices, and enzymes activities and markers of inflammatory conditions were determined. For each subject the deviation from the reference range was calculated for each blood parameter, and the results were aggregated in an average farm value (AFV). The AFV of each blood parameter was aggregated to obtain an overall farm blood index (BI). A higher BI indicates a greater deviation of hematic parameter from the reference range, thus more severe alterations of biological functions, reflecting worst welfare conditions. Farms were ordered, and best and worst farm were identified, in accordance with BI values (9.81 and 11.99 in best and worst farm respectively). The results showed an inverse relationship in comparison to both AC (33.67 and 28.39 in best and worst farms respectively) and global IDSW scores (70.40 and 78.45 in best and worst farms respectively), indicating a good relationship between IDSW forecasts and BI observations. Milk yield ranged between 21.9 to 33.7 kg/d, and greater values were observed in best farms. The results obtained in this study, seem promising in order to develop methods, based on physiological indicators, to validate the models of welfare assessment at farm level.

P093

Influence of serum γ-globulin concentration at 24 h after birth on lamb growth in natural rearing system

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In ruminants, colostrum is the unique source of acquired immunity for protection in early life. The inadequate absorption of colostral Ig leads to failure of passive transfer that expose ruminant neonates to the development of disease. In addition, passive transfer of immunity seems to have predictive value for health and productivity outcomes in lambs before and after weaning. The aim of this study was to evaluate the influence of serum γ-globulin concentrations at 24 h after birth on pre-weaning growth performance in dairy lambs reared under *ad libitum* intake of colostrum and milk. The study was performed in Catanzaro area (Italy) on 10 multiparous Sarda ewes and their singleton lambs. Lamb body weights (BW) were recorded at lambing and weekly until 28 d after birth. Blood samples were collected by jugular-vein puncture before morning feeding at 24 h after birth. Total serum proteins were measured by refractometer and serum γ-globulin concentrations were measured by capillary electrophoresis (Minicap Sebia). Data were subjected to PROC CORR of SAS in order to evaluate the association between serum γ-globulin concentrations at 24 h and BW at 7, 14, 21, and 28 d after birth. Serum γ-globulin concentration at 24 h ranged from 0.2 to 4.4 g/dL with an average of 3.11 g/dL and a SD of 1.36 g/dL. Correlation analysis between serum γ-globulin concentrations at 24 h and lamb BW at 7, 14, 21, and 28 has led Pearson coefficients of 0.49, 0.58, 0.71, and 0.51, respectively. Serum γ-globulin concentrations were significantly associated with lamb BW at 21 d of life ($p = .015$), whereas no significant

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association was detected with the other values. However, an increase of correlation coefficient values was observed until 21 d of life. Results of the present study suggest that passive transfer status of lambs evaluated at 24 h after birth affected pre-weaning growth of lambs reared under a natural non-intensive system, particularly within 21 d of life. Thus, lambs with adequate passive transfer of antibodies against pathogens may develop more efficient metabolic systems that contribute to growth.

P094

Cross-sucking and mortality in buffalo calves

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Cross-sucking can be defined as an oral abnormal behavior expressed by calves and directed towards various body regions of co-specifics. This behavior can result in inflammation or injuries at the prepuce, teats or navel. We aimed to verify whether this behavior is also responsible for an increased mortality risk of calves, which represents a major problem in buffalo farming. Therefore, we assessed the incidence and the potential risk factors for cross-sucking and mortality of calves in 33 buffalo farms. Three trained assessors collected the data through a questionnaire including resource and management measures. These measures were collected using both direct observations and interview with farmers. The farm was used as experimental unit. For each categorical risk factor an ANOVA was performed to assess the association with the outcome variables, whereas for continuous risk factors the association was tested using Pearson correlation coefficients. The mortality rate was defined as the incidence of calves dying from 24 h to the weaning age (83.40 ± 19.93 days; mean ± SD), which occurred at a mean weight of 80.00 ± 18.31 kg. The mean per cent mortality rate was 13.17 ± 8.54, whereas cross-sucking was observed in 90% of farms and the mean per cent incidence was 20.40 ± 25.71. Cross-sucking tended to be positively correlated with the age of separation from the mother ($n = 20$, $r = 0.39$, $p = .08$) and negatively associated with space allowance ($n = 20$, $r = -0.38$, $p = .11$). A positive correlation between cross-sucking and calf mortality rate was observed ($n = 20$, $r = 0.45$, $p = .06$). Calves fed acidified milk showed a lower mortality rate (8.86 ± 2.18) as compared to dam-rearing (16.32 ± 2.62) and non acidified milk replacer (17.13 ± 2.97; $n = 33$; $p = .04$). The administration of water in buckets after milk feeding also tended to reduce the mortality rate (9.93 ± 2.10) as compared with empty buckets (16.32 ± 2.71) and no buckets (17.22 ± 3.63; $n = 33$; $p = .10$). In conclusion, cross-sucking and mortality appear to be associated and appropriate management strategies should be applied in order to reduce the incidence of cross-sucking and mortality.

P095

Identification and use of cross reactive monoclonal antibodies to characterize leukocyte subsets in water buffalo (*Bubalus bubalis*)

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Although buffaloes (*Bubalus bubalis*) are a major component of the livestock industry worldwide until now very few studies have been conducted to develop or identify the monoclonal antibodies (mAb) reagents needed to fully characterize the immune system of water buffalo. The aim of the present study was to identify and use a panel of cross reactive monoclonal antibodies (mAbs) to characterize and compare leukocyte subsets in Italian and Egyptian water buffalo. Two different trials were performed. The first trial was conducted with blood collected from three young (2 months of age) and three adult male (2 years of age) (River type - Egyptian Mediterranean breed). The second trial was conducted with three adult lactating buffaloes (River type - Italian Mediterranean breed).

A total of 80 mAbs, 79 from the Washington State University Monoclonal Antibody Center (WSUMAC, USA), anti-CD335 from Pierce-Thermo Fisher Scientific, were screened by flow cytometry. 74 mAbs recognize 43 different orthologous molecules expressed on Italian and Egyptian buffalo leukocytes on major histocompatibility complex (MHC) I and II molecules, CD2, CD3, CD4, CD6, CD8, CD11a, CD11b, CD11c, CD14, CD18, CD21, CD25, CD26, CD28, CD40, CD44, CD45R0, CD49d, CD62L, CD69, CD80, CD86, CD163, CD172a, CD209, CD335, $\gamma\delta$ T cells, workshop cluster 1 (WC1) uniquely expressed on a subset of $\gamma\delta$ T cells, IgG$_1$ and sIgM, IL23R, and a set of mAbs under study that recognize molecules with upregulated expression on activated T cells: ACT1, ACT2, ACT16, and ACT36. The identification of these mAbs now provide an opportunity to compare the immune responses to pathogens affecting cattle and buffalo and determine the immunological basis for differences in susceptibility to diseases affecting both species.

Acknowledgements

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P096

Evaluation of blood plasma catecholamine concentration in Martina Franca donkeys: effect of age and sex

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The aim of this study was to evaluate plasma catecholamine levels (adrenaline, noradrenaline and dopamine) in healthy donkeys, with no evident stressor components, and investigate the effect of sex and age on their values. A total of 358 Martina Franca donkeys were used for this study. They were 224 females and 134 males, age ranged from 4 months to 24 years. Animals were subdivided in four age categories: lower than 12 months, from 13 to 36 months, from 37 to 120 months and over 121 months old. All the donkeys were clinically normal and had not received any medications during the last month. Indwelling jugular catheters were placed at least 12 h before the start of sampling. The 16 gauge catheters were placed aseptically into the left jugular vein of each animal, and fixed to the skin with adhesive tape. The catheter was heparinized (1000 U/ml). Venous blood for analysis was placed in chilled glass tubes containing EDTA (Vacutainer®) and the collection procedures lasted at most 15 seconds. The concentrations of dopamine, adrenaline, and noradrenaline in blood samples were determined by HPLC according to the guide of the data sheet of the kits (Eureka Serotonin and Catecholamine HPLC detection KITs, Chiaravalle, Ancona, Italy). The detection limit was 0.03 nM for all three monoamines. The data set was subjected to analysis of variance (ANOVA) using the GLM by SAS software, considering age, sex and the interaction between them as independent variables. Moreover, Pearson’s correlation coefficients between each catecholamine were also evaluated. All the data were expressed as last squares means. Significance was set as $p < .05$. Confidence intervals were calculated for all parameters with a significance of 0.05. Noradrenaline ranged from 239.98 to 255.07 ng/L, adrenaline ranged from 116.73 to 126.31 ng/L, dopamine ranged from 189.80 to 220.38 ng/L and noradrenaline/adrenaline ratio from 2.27 to 2.51.

Sex did not affect the investigated parameters. Donkeys older than 36 months showed lower values of adrenaline ($p < .001$) and higher values of noradrenaline/adrenaline ratio ($p < .05$) than younger animals. Probably, younger animals showed to be more sensible to the contact with human, considering that older animals are more used to be close to people, and this could explain the higher content of adrenaline in younger donkeys.

P097

Gene expression profiling in response to experimental *Staphylococcus aureus* infection in mammary gland tissues of two divergent lines of goat for high and low milk somatic cell scores

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Staphylococcus aureus is a frequent and costly disease for the dairy ruminants and it is responsible for chronic, subclinical, clinical and gangrenous mastitis. Disease response is a complex trait under multi-genic control, which makes it difficult to develop appropriate genetic selection strategies for improving immune response, also because we have very few information about the genetic basis of resistance and functional complexity of the host pathogen interaction during infection. The number of somatic cells in milk is correlated with intra-mammary infection and cattle breeders used somatic cell scores (SCS) in genetic selection for resistance to mastitis. For goats, the relevance of SCS as a predictor of udder health parameters and susceptibility against mastitis is still unclear.

The aim of the present study was to investigate in caprine mammary gland tissue the response to an experimental infection with *S. aureus* between two divergent lines of goat selected for high and low milk somatic cell scores (HSCS and LSCS, respectively).

For this study, the left mammary glands of six primiparous alpine goats (three from HSCS and three from LSCS, respectively) were inoculated with one *S. aureus* strain, while right udder halves were infused with sterile solution. Thirty hours post inoculation, tissue samples were examined by histopathological and immunohistochemical analyses and total RNA was extracted and analyzed by the bovine 90K CustomArray (Combimatrix).

Normal tissues of LSCS line revealed mild interstitial lymphoplasmacytic inflammation and lesser numbers of alveolar macrophages than HSCS. Infected tissue samples showed more severe lymphoplasmacytic inflammation and intra-alveolar macrophage accumulation with maximal luminal macrophage presence in HSCS. Only infected tissues showed neutrophilic intra-alveolar inflammation; interstitial microabscesses were observed in HSCS. Moreover, differences in gene expression were found between HSCS and LSCS both in normal and in infected tissues. In normal tissues 16 genes were obtained belonging to "energy production", "molecular transport and amino acids metabolism" networks. In infected tissues 20 differentially expressed genes, encoding for host defense against microbial infection and anti-inflammatory activity, were detected. These preliminary results represent a primary approach for further studies on understanding the genetic basis of mastitis in the early stage.

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P098

Carcass measurements for ethnic allocation in cattle

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Animal breed identification is since long time one of the main aspects in the production and marketing of beef, also shown on the labels for consumer’s guarantee. To quickly verify, at slaughterhouse, the truthfulness of some of the declared parameters, this study aimed to use carcass's measurements to correctly assign them to different genetic types.

The trial was carried out on 36 carcasses obtained from 6 homogeneous groups of male cattle of different breeds (P, Piemontese; L, Limousine; C, Charolaise; B, Brown; F, Friesian; PxF, crossbred PxF), slaughtered at similar age (approx. 18 months). After slaughtering, half carcass weight and linear measures (side and leg length, maximum and minimum leg width, chest depth) were recorded and data analysed by Linear Discriminant Analysis procedure (IBM SPSS Statistics 22.0, SPSS Inc., Chicago, IL), to evidence differences among breeds.

In the graphic result of the discriminant analysis applied to the half carcass weight and carcass measures the different groups of animals were easily identified. In fact, the 94.4% of the original grouped cases were correctly classified: 83.3% of P (16.7% ascribed to L), 100.0% of L, 100.0% of C, 100.0% of B, 100.0% of F, and 83.3% of PxF (16.7% ascribed to F). Moreover, the discriminant Function 1 explained the 74.1% of the total variance, the Function 2 the 15.9% (90.1% as cumulative variance), the Function 3 the 7.6% (97.7% as cumulative variance), and the Function 4 and 5 the remaining 2.3%. In detail, Function 1 was mainly affected by chest depth and maximum leg width, while Function 2 was mainly affected by carcass length and minimum leg width, Function 3 was mainly affected by minimum leg width and half carcass weight, Function 4 was mainly affected by maximum leg width, and Function 5 was mainly affected by maximum leg width, carcass length and half carcass weight.

Then, the use of the discriminant analysis applied to the cattle carcass measurements seems to be effective for correctly allocate the carcasses to the genetic type of origin.

P099

Determination of some water-soluble vitamins in donkey milk

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Donkey milk chemical composition is very similar to human milk. In fact, donkey’s milk is characterized by a low casein content which makes it a good substitute for cow's milk in cases of Cow's Milk Proteins Allergy (CMPA). Furthermore, the high content of lactose makes this milk useful for the treatment of osteoporosis in adults, whereas in infants, it promotes bone mineralization and the development of the intestinal bacterial flora. In this work, the content of some water-soluble vitamins in donkey's milk (vitamin B_1 or thiamine, vitamin B_2 or riboflavin, vitamin B_9 or folic acid and vitamin C) has been determined. Milk samples were collected weekly over a 3 months period from 12 cross-bred donkeys in mid-lactation reared in the same farm; samples were stored at -20 °C till the performing of chemical analysis. Different standard solutions of the vitamins were used in order to obtain the respective calibration lines; the concentrations of thiamine, riboflavin and folic acid in donkey’s milk were determined by RP-HPLC using the equation of the corresponding calibration line. The following results were obtained: vitamin B_1 2.55 ± 0.01 mg/L, vitamin B_2 0.04 ± 0.01 mg/L; folic acid was found only in traces. Vitamin C has been determined using the kit ENZYPLUS EZA 941+ L - Ascorbic Acid, and the result showed that the average concentration of vitamin C in donkey's milk is 57 mg/L. From these preliminary studies, other additional nutraceutical properties of donkey's milk were highlighted, since the presence of vitamin B_1 and vitamin C seems to be quite high compared to bovine milk, in which the average content is respectively 0.80 mg/L for vitamin B_1 and 18 mg/L for vitamin C. Vitamin B_1 acts as a cofactor of cytosolic transketolase, an enzyme of the pentose phosphate pathway which provides the pentose phosphate group for the synthesis of nucleic acids and NADPH for the synthesis of lipids. Vitamin C is a powerful antioxidant and is essential in numerous enzymatic reactions and in the collagen formation, the recommended daily intake of vitamin C is 60 milligrams. Finally, studies have shown that intake of vitamin C from the milk may be associated with a reduced risk of atopic dermatitis in children. The determination of other water-soluble and fat-soluble vitamins is ongoing, in order to obtain a complete profile of the vitamins content in donkey's milk.

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P100

Effect of dietary flaxseed supplementation on liver Δ6-desaturase activity in two chicken genotypes

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Humans have low ability to elongate and desaturate essential polyunsaturated fatty acids (EFA), into long chain (LCP). Accordingly, human diets must contain suitable level of LCP, mainly of *n-3* series, which are considered low in western diets. The main source of LCP *n-3* is fish, however, the world fish stocks are dropping and aquaculture is not fully sustainable. Thus, the capacity of terrestrial animals in metabolizing EFA into LCP is a relevant goal, and such metabolic process requires an efficient activity of the Δ-6 desaturase enzyme (Δ6d), which desaturate, respectively fatty acids of *n-6* and *n-3* series (linoleic acid - LA or α-linolenic acid- ALA), into arachidonic acid (AA) or eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). Flaxseed is a rich source of ALA and it is widely used in animal diets to improve the fatty acid composition of meat. The objective of the study was to determine the effect of dietary supplementation of flaxseed on liver Δ6d activity of two chicken strains having different growing rates. A total of sixty chickens of slow-growing (Livorno) and fast-growing (Ross 308) genotypes were reared in the experimental farm of Perugia University. Thirty birds per strains fed diet supplemented with 10% flaxseed (flaxseed group) and the other fed standard diet (control group). Ten chickens per group were slaughtered at 110 and 80 days respectively, for Livorno and Ross and the liver was taken for analyses. The enzyme activity (expressed as ratio between precursor and direct metabolites) was detected on liver microsomes using alternatively LA or ALA as substrates for assessing the enzyme preference. Data were analyzed by PROC ANOVA with diet, genotype and interaction as fixed effects.

The results showed that both diet and genotype affected liver Δ6-desaturase activity; in particular, the fast-growing strains, fed standard diet, had Δ6d activity prevalently oriented toward LA (LA/ALA 7.1). The slow-growing had higher preference for ALA (LA/ALA 5.7) and the administration of flaxseed increased 3.5 folds the direct metabolite respect to only 2.5 fold of the fast-growing. As a consequence, ALA was more efficiently desaturated by slow- than fast-growing strain. On the light of these preliminary results, it is possible to conclude that the relationship between genotype and desaturation ability is a critical point to obtain an improvement of lipid profile (>LCP *n-3, n-3/n-6*) using a dietary flaxseed supplementation.

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P101

Dietary supplementation of selenium-enriched olive leaves in fattening rabbits: 1. Productive performance, carcass and meat characteristics

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Nowadays, great attention is given to use of by-product in the animal feeding to reduce the cost and the environmental impact of the diets. A good strategy could be the recycling of agricultural by-products, which normally, required an expenditure of resources for disposing. In the present study two different type of olive leaves, enriched or not with selenium (Se) were used, because it could exert an antioxidant defense both in the herbaceous plants, increasing the resistance to water stress, both in animals, improving the health status. Then, the main objective of this study was to evaluated the effect of olive leaves, treated or not with Se, on productive performance of growing rabbits. Thirty weaned New Zealand White rabbits (30 days of age) were allocated in bicellular wire net cages and assigned to three homogeneous groups (970 ± 80 g b.w.; 10 animals/group): control (C) fed standard diet, olive leaves group (OL) fed standard diet +10% dried olive leaves and selenium group (SE) fed standard diet +10% dried olive leaves enriched with 2.17 mg/kg of sodium selenate. The feed was provided *ad libitum.* The productive performance (daily weight gain, daily feed intake) were registered. At 75 days of age the rabbits were slaughtered and the carcass (carcass weights and sizes, edible organs weight, fat content and distribution) and *longissimus lumborum* meat characteristics (physical: pH, colour, WHC, cooking loss; chemical: moisture, crude proteins, ether extract, ash) were evaluated on refrigerated samples (24 h at 4 °C).

Data were analyzed with a linear model considering the fixed effect of dietary treatment. Results of this trial showed that the diets did not affect the productive performance of rabbits and the physio-chemical characteristics of meat. However, the rabbits fed olive leaves showed carcass with lower lumbar circumference and intrascapular fat amount. This study confirms the possibility of using olive leaves, enriched or not with selenium, in rabbits feeding and make possible to continue the study (see the companion paper presented at this Congress) evaluating the effect of selenium-enriched olive leaves supplementation on *in vivo* and meat oxidative status of rabbit.

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P102

Dietary selenium-enriched olive leaves supplementation in fattening rabbits: 2. *In vivo* and meat oxidative status

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Recent studies have shown the positive effects of selenium in animal and human nutrition for its role as essential component of glutathione peroxidase enzyme. The incorporation of olive leaves added with Se in feeds could be a suitable strategy to increases the antioxidant content of diet aiming to improve the health and oxidative status of animals. The aim of this study was to assess the influence of dietary supplementation of 10% olive leaves, treated or not with Se, on the oxidative status of plasma and rabbit meat.
Thirty New Zealand White weaned rabbits of 30 days of age were divided in 3 groups (10 animals/group) and subjected to different dietary treatments: control feed (C) provided *ad libitum*; control feed +10% olive leaves (OL) and control feed +10% olive leaves treated with 2.17 mg/kg of Se (SE). At 75 days of age rabbits were slaughtered and blood samples were taken from carotid vein. After carcass refrigeration for 24 h at 4 °C, *longissimus lumborum* muscle was removed. The antioxidant content (tocotrienols, tocopherols, lutein, zeaxanthin and carotene) was carried out on olive leaves, and experimental diets. The oxidative status of plasma and meat (TBARS, carbonyls and vitamins) was also determined. Data were analyzed with a linear model with the fixed effect of dietary treatment. No differences were found on the antioxidant content and lipid oxidative status of plasma; however, the groups fed olive leaves showed a higher protein oxidation ($p < .01$) respect to control (2.98 and 2.69 *vs* 0.35 nmol/mg protein in SE, OL and control, respectively). In the meat the carbonyls were also higher with olive leaves enrichment, conversely the lipid oxidation was lower in such groups probably due to the balance of antioxidant defense. Indeed, the α-tocotrienol content was lower ($p < .05$) in the OL and SE meat respect to the control (25.70 and 38.99 *vs* 63.08 ng/g, respectively). On the other hand, the α-tocopherol content was higher ($p < .05$) in SE meat samples (892.34 *vs* 650.46 and 570.35 ng/g in SE, control and OL, respectively), probably for an indirect protective effect operated by selenium. In conclusion, the present study suggests that the dietary Se enrichment, improved the oxidative status of rabbits, whereas remain unclear its metabolic route. Further studies are needed to understand whether the higher protein oxidation is directly related to the olive leaves addition and to find which compounds are responsible of such effect.

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P103

Lipid supplement in the finishing diet of beef: effects on the nutritional profile and fatty acids composition of *longissimus dorsi* muscle

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There is a considerable attention in enhancing *n-3* polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) in ruminant meat and milk, due to their healthy effects in humans. In fact, the Western countries diet is characterized by a high intake of saturated fatty acids (SFA) and *n-6* PUFA. Therefore, a considerable attention has been focused in the manipulation of fatty acid composition in meat. Dietary lipid

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supplementation in beef positively modify muscle fatty acid composition; in particular, extruded linseed and fish oil are able to increase *n-3* PUFA and CLA content. The aim of the present study was to determine the effects of a lipid supplement (Vitality®, Zoofarma, Villafranca di Verona, Italy) on the nutritional profile and fatty acids composition of *longissimus dorsi* (LD) muscle in the finishing diet of beef. One hundred Charolaise beef were divided in two experimental groups: the control group (CON) received a basal diet composed (on DM basis) by 3.7 kg of corn silage, 1.3 kg of straw and 4.3 kg of concentrate (corn, soybean meal, wheat bran, beet pulp, distiller grain, protected fatty acid and mineral and vitamin premix). In the second group (TR) the protected fatty acid was replaced by 350 g/kg DM per animal/day of the lipid supplement (Vitality®). The lipid supplement contains extruded linseed, protected linseed oil and protected fish oil. After 6 months, beef were slaughtered (19 month of age) and LD muscle were collected from 20 animals per treatment. The samples were stored under vacuum and frozen at -20 °C pending analyses. Dietary treatment did not affect ($p>.05$) colour parameters and nutritional label. Inclusion of the lipid supplement decreased ($p<.001$) cholesterol (54.62 ± 0.7 mg/100 g muscle in CON *vs* 40.23 ± 1.7 mg/100 g muscle in TR) and SFA content in LD muscle. The n-3 PUFA (3.13 ± 0.1% of total FA in TR *vs* 1.06 ± 0.07% of total FA in CON) and CLA content (1.32 ± 0.02% of total FA in TR *vs* 0.43 ± 0.04% of total FA in CON) were significantly higher ($p<.001$) in treated group than in control group. The present data show that the inclusion of this lipid supplement in Charolaise bulls diets improve the fatty acid profile of beef and decrease cholesterol content. This feeding strategy is suggested to enhance the nutritional value of beef meat, improving the n-3 PUFA supply and consequently the consumer's health. However, assessments on meat oxidative stability and sensory characteristics should be evaluated.

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P104

Zingiber officinale reduced lipid oxidation and increased antioxidant capacity in cooked pork burgers

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Ready-to-eat products represent an important percentage of food production for their high usage and acceptance by consumers. Burgers are one of the most used meat product for their ease of consumption. Ginger (*Zingiber officinale* Roscoe) is one of the most common spice used worldwide, as a condiment for food and beverage. Ginger flavour is a mix of spicy, peppery and sweet with a strong pungent characteristic. Ginger powder contains several antioxidant molecules as gingerol, paradol, shogaols, zingerone, zerumbone, terpenoids as well flavonoids and phenols. The objective of this research was to evaluate the influence of ginger powder in pork burger (100 g, six burgers per formulation). Three formulations were prepared: one formulation was used as control (only meat) and two formulations were supplemented with ginger powder at 1 or 2%. Lipid oxidation and antioxidant capacity were determined in cooked burgers (baked in preheated oven at 163 °C to an internal temperature about 71 °C). Burgers were analysed for lipid oxidation (TBARS - thiobarbituric acid reactive substances) and antioxidant capacity as ABTS (2,2-azino-bis-(3 ethylbenzothiazoline-6-sulfonic acid)), DPPH (1,1-diphenyl-2-pircydrazyl), and FRAP (ferric reducing ability).

In order to quantify antioxidant capacity (ABTS, DPPH and FRAP) cooked meat were treated with ethanol. Control burgers showed to be more sensitive to lipid oxidation with higher value of TBARS than the other two formulations added with ginger (0.06, 0.02 and 0.02 mg equivalent of malondialdehyde per kg of sample respectively for control, 1% and 2% ginger burgers; $p<.05$). Both percentages of ginger powder showed to enhance ABTS, DPPH and FRAP values from control burgers ($p<.05$ for ABTS and DPPH, $p<.001$ for FRAP). Furthermore, in the FRAP evaluation burgers with 2% of ginger showed to gain more antioxidant activity than burgers with 1% of spice.

Burgers added with both percentages of ginger showed to be less sensitive to lipid oxidation and gain highest antioxidant capacity.

P105

Performance and meat quality in light lambs fed with organic or conventional commercial concentrates

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Organic animal production implies that livestock feed should be composed of ingredients that come from organic farming, avoiding the use of certain additives and medication. We tested whether feeding a local commercial certified organic concentrate (ORG, n 11) from weaning to slaughter (41 days) affected animal performance and product quality compared with a local commercial conventional concentrate (CON, n 13). Both concentrates were based on barley, maize and soya; but they differed in the type of dietary components (i.e. in CON, maize and soya were genetically modified) and some additives, which were only present in one group (i.e. ORG contained diatomaceous earth, whereas CON included a synthetic antioxidant). A general linear model was used, with diet as the fixed factor. Animal performance, pH, meat colour and cooking losses were similar between the two groups (p>.05). Carcass conformation scores were higher ($p<.05$) with the organic feed (O to O+) compared to the CON group (P- to P), but fatness remained unchanged (-2 to +2, p>.05), according to the European carcass classification. A sensory panel of 12 trained members found ORG meat more tender ($p<.001$), juicier ($p<.01$) and with less residue ($p<.001$) than CON, but the groups did not differ in odour and flavour intensities (p>.05). Supporting the sensory results, shear force values tended ($p=.069$) to be higher for CON animals. With regard to fatty acid composition (% of total fatty acids), *n*-3 fatty acids were higher in ORG meat ($p<.01$), including long chain fatty acids (eicosapentaenoic and docosahexaenoic acids) and α- and γ- linolenic acids. Consequently, PUFA: SFA and *n-6/n-3* ratios were higher and lower in ORG *vs* CON, respectively. In general, the organic concentrate enhanced carcass and meat texture and provided a healthier fatty acid profile of the meat. Most of the differences are thought to come from differences in the dietary components of the both commercial concentrates.

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Beef colour measurement with an RGB camera

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Spectrocolorimeters are the most commonly used instruments for measuring meat colour because they provide readings in the device independent CIE$L^*a^*b^*$ colour space which corresponds well with the colour perception by humans. These instruments, that can accurately measure colour surface of a sample uniform and rather small, cannot completely represent the surface characteristics when it is not uniform and highly textured, as in case of the meat. In recent years, digital cameras have been used to measure meat colour since they provide some advantages over a conventional colorimeter, namely, the possibility of analysing the entire surface of meat. As the digital cameras use the RGB colour model, which is a device dependent colour space, it is necessary to derive a conversion matrix to estimate CIE$L^*a^*b^*$ parameters from RGB measurements. Eighty *longissimus thoracis* steaks were cut into 5×5×4 cm samples. After blooming, the samples were photographed using a NIKON 3100 digital camera. All the images were captured under controlled conditions with a reference grayscale target, used to correct white balance. The RGB colours of meat were measured from JPEG images using ImageJ software. An area of 3.0 cm^2 at the centre of each sample was evaluated. Immediately after, the true colour of each sample was measured by a MINOLTA CM-600d spectrocolorimeter and the results were expressed in the CIE$L^*a^*b^*$ space model. The conversion of RGB colour values to $L^*a^*b^*$ values was carried out using a quadratic model which considers the influence of the square of R, G and B variables on the estimation of $L^*a^*b^*$ values. R-square, root mean square error and the mean normalized error were used for measurement of differences between the values achieved with ImageJ and the spectrocolorimeter. Moreover, the predicted colorimeter colour coordinates from digital images were compared to real colorimeter values using CIE colour difference equation (ΔE*). The real and estimated values showed coefficients of determination of 0.67, 0.41 and 0.33 for L^*, a^*, and b^*, respectively. The root mean square error between ImageJ and spectrocolorimeter was 1.48, 1.11 and 0.80 for L^*, a^* and b^* values, respectively. The model showed an error of 1.16% and a standard deviation of 0.92. The ΔE* was equal to 2.5. The proposed method achieves a promising performance, however the acquisition of the images need some adjustments to improve the accuracy of the model.

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P107

Sensory properties of goat cheese from Friuli Venezia Giulia farms

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The aim of the study was to evaluate the sensory characteristics of goat cheeses produced with autochthonous or commercial starters in different farms of Friuli Venezia Giulia region. Autochthonous starters, obtained by milk from different goat farms and breeds, were lyophilized after selection based on their acidic and proteolytic activity. Twelve types of goat cheeses were obtained in three different farms (X, Y and Z) using two different starters (A, autochthonous; C, commercial). Cheeses were analysed after 14 and 28 days of ripening. Quantitative descriptive analysis (DA) and temporal dominance sensation analysis (TDS) were performed by a trained panel. Then, the likings of 76 consumers who habitually eat this type of cheese were assessed, using a labelled affective magnitude (LAM) scale. Consumers were also asked to group the cheeses according with their similarities and dissimilarities (mapping test, MT).

PCA was performed on DA and TDS data to highlight the intrinsic sensory similarities and differences between cheeses; those from Y farm were characterized by an animal like flavour, bitter and acid taste and astringency, while X and Z farms produced cheeses having sweet taste and milky flavour. Autochthonous starter enhanced milk and sweet taste.

Two clusters of consumers were identified by preference mapping: the biggest comprising who appreciated the sweet and milky notes, the smallest including those that preferred the bitter, astringent and animal flavour. As showed by MT, consumer clearly recognised the differences among farms, while those between A and C starters were less clearly detectable.

In conclusion, even if the autochthonous starter is a good approach for strengthening the territorial linkage of goat cheeses, their perceived sensory attributes appear more associated to farm influence, involving the confounded effects of goat breed, animal feeding and management, milk quality and cheese making process.

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P108

Total substitution of soybean meal with *Hermetia illucens* meal in laying hens: effect on lipid composition of yolk

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Eggs are a cheap source of high quality nutrients and FAO projects a production of 89 million tones of eggs in 2030. At present, ingredients for poultry feed include soybeans and several other grains but the continuous increase of prices has been enhancing the need for alternative feed. In this context, insects are considered a viable and attractive solution for animal feeding, including chickens. Insects indeed are natural food sources for poultry although the information about the effect of the substitution of soybean with insect meal on the quality of eggs in terms of lipid composition of yolk are scarce. The present study aims to fit this gap by testing a 100% replacing diet with *Hermetia illucens* defatted meal. One-hundred and eight Lohman Brown Classic laying hens were farmed for 21 weeks and divided into 2 groups fed two isoproteic and isoenergetic diets, differing in the main protein source: corn-soybean meal based diet (SB group) or *Hermetia illucens* larve defatted meal (HI group). Results on lipid composition of feeds showed that insects meal contained 29% and 18.9% of C18:2n6 (linoleic acid, LA), and C18:1n9 (oleic acid), therefore lower than the values obtained for soybean (36.5 and 20.5%). C12 was the fatty acid which mostly characterized insect meal being around the 8% against the 1.3% of soybean. Globally, insect meal was higher in saturated (32 *vs* 24.7%), and *n-3* fatty acids (5.9 *vs* 5.7%) and lower in *n-6* (32 *vs* 39%) than soybean. Nevertheless, feeds did not affect yolk fatty acids composition. Indeed, despite the lower percentage of *n-6* in insect feed than in soybean, HI yolks contained significantly higher *n-6* (17 *vs* 14%). Furthermore, yolks of both groups were extremely rich in oleic, C16, LA, C18:1n7, and C16:1n7, accounted for around 65% of total fatty acids. Probably, desaturase enzymes involved in the conversion of LA showed a reduced activity in HI group than in SB, as revealed by the significant higher content of LA (11.72 *vs* 9.45%) and lower C22:5n6 (0.49 *vs* 0.76%). Nevertheless, docosahexaenoic acid (C22:6n3, DHA), the final product of desaturase chain, was not affected amounting to 1.2% in both groups. In conclusion, despite the high presence of saturated fatty acid in *Hermetia illucens* meal, fatty acid composition of yolk did not show to be significantly damaged especially concerning polyunsaturated *n-3* and *n-6* fractions.

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Chemical and fatty acids composition of fish roes

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The global crisis of wild caviar production, due to the block of catch quotas granted by the CITES, caused by the steady decline of world sturgeon populations and the limited production from aquaculture, that has not made up for the caviar demand, has allowed the creation of a market for cheap caviar substitutes. Various fish species are used for the production of caviar substitutes, whose eggs shows different characteristics from a nutritional point of view. The aim of present study was to investigate the chemical composition and fatty acid profile of fish roes deriving from seven different species, collected in the Italian market. The survey involved 12 samples of salmon eggs (*Oncorhynchus keta*), 11 of lumpfish (*Cyclopterus lumpus*), 3 of rainbow trout (*Oncorhynchus mykiss*), 3 of pike (*Esox lucius*), 3 of cod (*Gadus morhua*), 3 of Alaska pollock (*Theragra chalcogramma*) and 3 of capelin (*Mallotus villosus*). The eggs proximate composition (AOAC standard methods) and fatty acid profile (GC analysis) were determined. The data were processed by SPSS 22 statistical package. Salmon eggs (217.3 ± 32.7 mg) were the biggest while the smallest resulted the cod eggs (0.16 ± 0.08 mg). Generally, eggs of greater size showed the higher protein and lipid content. Protein content ranged from the value of 29.6 ± 0.8 g/100 g of salmon to the 8.1 ± 0.6 of capelin, while lipid content ranged from 12.8 ± 0.6 of salmon to 2.8 ± 0.6 of Alaska pollock. With regard to the fatty acid composition, the fish eggs showed some peculiarities. EPA and DHA (their sum ranged from 46.6% of total fatty acid of pollock to the 20.9% of pike) were dominant in all sea species (salmon, capelin, cod, pollock and lumpfish) while pike and trout presented a higher amount of oleic acid (21.6% and 27.1% respectively). Eggs from trout, the only farmed species, presented also a significant amount of linoleic acid (9.4%) if compared with all the other species. This content probably derived from the vegetable oils utilized in feed formulation. This study contributes to the chemical characterization of caviar substitute present on the Italian market. Salmon eggs showed a chemical composition that was more similar to caviar, according to our previous results obtained from caviar samples coming from four different farmed sturgeon species. Regarding fatty acid profile, fish eggs presented a valuable content of essential fatty acids of the *n-3* series, which were higher if compared to the more expensive caviar.

P110

Variation in composition and colour indices of sheep raw milk influenced by productive practices and feeding strategy

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Over the past few years, there has been a great development of the sheep dairy sector in Spain, and a wide variability in the production outcomes of farms has been detected, although most of the farms have similar environmental and economic constraints. Milk quality evaluation is important not only for cheese production, but also for other dairy products. Therefore, the aim of this work was to evaluate the effect of some factors on bulk milk characteristics of Manchega sheep reared in La Mancha region, with special attention to casein and urea contents and color indices. Factors of variation such as season, milk yield, the inclusion (or not) in a breeding program and the feeding strategy of breeds have been considered. The study included 77 farms distributed along La Mancha region, in Spain. Data were collected from interviews using a questionnaire that included questions properly selected to obtain a general description about production practices and feeding strategy. In total, 308 analytical determinations of milk characteristics including fat, protein, total solids, casein, lactose, pH and urea content, color indices and somatic cell count (SCC) were analyzed by automated methods. Data were analyzed with GLM procedure analysis of SAS version 9.3 to analyze the relationship between factors of variation and milk characteristics. Season showed a significant ($p < .05$) effect on the major components of milk and urea content, having lower values, and hence less luminous milk in Spring and Summer. However, it is important to remark the stability of the total casein/fat ratio of bulk milk from Manchega sheep throughout the year, which is an important factor for the cheese making process. Moreover, the inclusion in a breeding program was linked with lower values ($p < .05$) of bulk milk SCC. Besides, our study showed a decrease of the major components of milk ($p < .05$)

for indoor feeding groups, an increase ($p < .05$) of urea content when by-products were used and higher ($p < .05$) milk production when silage was included. The results of the present work indicate that the season, the breeding program and the feeding strategy influence the production level, composition and color of sheep bulk. In turn, these changes have an impact from a technological point of view. A comprehensive knowledge of these factors is still required, in particular, a better optimization of feeding management according to the productive level of the breed.

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Effect of dietary regimen and of feeding maltodextrins and dextrose on meat and fat quality of immunocastrated pigs

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This research aimed to verify the effects of restricted *vs ad libitum* feeding and of the dietary inclusion of maltodextrins (M) and dextrose (D) on the qualitative traits of muscle *longissimus dorsi* (LD) and on fatty acid (FA) composition of backfat lipids of immunocastrated intermediate pigs.

To this goal, 36 male pigs (Italian Duroc × Italian Large White) were used. The animals, after having received the second immunocastrating injection at 162 days of age, had been allocated for 5 weeks, till slaughtering [144.51 ± 9.70 kg live weight (l.w.)], to one of the three experimental dietary treatments: basal diet given either *ad libitum* (CL), or restricted at 7.5% l.w.$^{0.75}$ (CR), or with M and D (3.5% + 3.5%, balanced for energy and protein levels) provided *ad libitum* (MD). At slaughter, a sample of LD muscle and backfat were collected from each left-half carcass, at the last rib level. At 24 h *post mortem* on the LD samples, pH, colour (CIE$L^*a^*b^*$, 1976) were determined, further, protein (CP) and intramuscular lipid (IMF) contents (on the fresh and cooked muscle), drip and cooking loss, oxidative stability (TBARS) and tenderness (WBSF) were measured. Moreover, FA composition of backfat was analyzed.

The data were submitted to ANOVA with the dietary treatment as independent variable. The two degrees of freedom of the treatment effect were split up *a priori* in two orthogonal contrasts: CR *vs* the average of CL + MD and CL *vs* MD. The CR pigs, compared with *ad libitum* subjects, exhibited lower IMF ($p < .05$), both in the fresh (2.75% *vs* 3.41%) and cooked muscle (3.83% *vs* 5.15%), higher CP in the fresh muscle (24.04% *vs* 23.36%, $p < .05$) and higher Hue value ($p < .05$), indicating a slightly decoloured meat. Indeed, CR group showed a higher content of polyunsaturated FA (15.50% *vs* 13.23%, $p < .01$) and a lower content of saturated FA (41.46% *vs* 42.91%, $p < .05$) in backfat lipids. The feeding regimen did not affect pH, drip loss, cooking loss, TBARS and tenderness of LD muscle. No difference was found between CL and MD pigs, except for the ω-6/ω-3 ratio in backfat lipids, lower in MD (15.54 *vs* 16.70, $p < .01$).

Thus, in our experimental conditions, the dietary inclusion of M and D did not markedly affect meat quality characteristics and the FA composition of backfat of immunocastrated intermediate pigs fed *ad libitum*, whereas the dietary regimen exerted a considerable influence, especially on CP and IMF contents of LD and on lipid FA composition of subcutaneous adipose tissue.

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Smart near infrared spectroscopy on frozen milk samples can discriminate grass-fed from conventional milk

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The real origin of milk is sometimes doubtful, even at the *km-zero* level for locally appreciated productions. The aim of the research was to authenticate the origin of milk on farms as coming from cows fed grass or hay diets. For this purpose, 43 milk samples, originating from different farms in Piedmont (Italy) that use a prevalent diet of grass or hay for their dairy cows, to supply milk for milk and cheese production for the local market (G), and from more intensive farms, that adopt a total mixed ration to feed their cows for milk destined for dairies (C), were collected. The samples were frozen and the top surface of each sample was examined using a smart new NIR miniaturized web-based wireless spectrophotometer (SCIO, range 740-1030 nm), in which three spectra were retained per sample. Chemometrics of the 331-point spectra was performed using categorical discrimination, integrated within the SCIO Lab proprietary software, AKA (Also Known As) the confusion matrix, which includes a mathematical pre-treatment of the spectra, that is, SNV (Standard Normal Variate) and the 1st derivate. The reclassification capacity has been considered as the reference of the performances. The discrimination of the origin of the milk was found to be very reliable. In fact, the chemometric results showed that the AKA confusion matrix assigned an 83% reclassification ability to the G memberships and 82% to the C memberships.

The advantage of the method is represented by the small amount of sample that is needed for the analysis and the originality of the method concerns the fact that all the samples, even when fresh, were frozen before scanning, thus preventing the instrument from being damaged due to the effects of the liquids. Moreover, this preparation could substantially improve the quality in spectra scans of liquids and fluids (such as milk, oil and wastes) that are not scannable by means of NIR devices that work in transflectance mode.

This smart instrument seems to offer several advantages, concerning the on farm authentication of milk as deriving from a prevalent grass feeding diet. On farm calibrations for raw labelling of farm cheeses (moisture and energy in particular) could be conducted, thanks to the easy processing, by providing the necessary reference analyses in concerted networks or simply using standard samples for qualitative assessments.

P113

CASTRUM project highlights that Italian PDO and PGI products with pig meat require the castration of males

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Male pig castration is mainly carried out to avoid boar taint in meat. Even if surgical castration of male piglets has been a traditional practice for centuries, in recent years the practice has been questioned based of animal welfare considerations. The CASTRUM project, including 10 project partners throughout Europe, has recently evaluated and reviewed the alternatives to surgical castration for pigs used in traditional pork products in European Union. Here we reported information recovered for the Italian pig production system focused on the analysis of the protected designation of origin (PDO) and protected geographical indication (PGI) specification rules and relevant literature. In Italy there are 22 PDO and 19 PGI based on pig meat: 28 of them explicitly require castration of male pigs. Furthermore, 34 out of 41 PDO + PGI products require a slaughter weight above 140 kg. Of the remaining 7 products, 3 require a minimum age of at least 9 months at slaughter.

At the slaughter weights and ages required for the PDO and PGI rules, male pigs usually are sexually mature, and they begin to accumulate androstenone and skatole in their adipose tissue. As consequence, typical pig products would be compromised because of meat off flavours.

This is the reason why the production rules of 90% of traditional Italian products exclude meat from entire male pigs. Literature also highlights the differences between meat from entire male pigs and meat from castrated pigs on meat quality traits important for traditional products. Lower fat thickness and higher proportion of unsaturated fatty acids make the meat more prone to oxidation, a critical factor that affects many traditional products, firstly the dry cured hams, needing firm fat and low water losses during the long maturation period.

Besides technological properties of pig meat, male pigs show aggressive behavior against conspecifics on farm and during transport, so reducing the welfare status of the group. Also, aggressive behavior at slaughtering leads to glycogen consumption, insufficient *post-mortem* drop of pH, reduced proteolytic activity, water holding capacity, tenderness and lightness.

PDO and PGI production rules and the available literature exclude the possibility to make typical products by meat from entire male pigs. Welfare concerns on surgical castration could be faced by using analgesia or local anesthesia administered by pig farmers.

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P114

Effect of extruded linseed, vitamin E and polyphenols pig dietary supplementation on microbial growth of meat during refrigerated storage

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Meat is an important source of essential nutrients for human, it is a source of lipids, protein and vitamins but it is a highly perishable product. The shelf-life of meat depends on several factors such as storage condition, processing, packaging and distribution that can cause evident spoilage. Microbial contamination is influenced by the condition of animals prior to slaughter, slaughterhouse practices, extent of handling and subsequent storage. Temperature is the main factor used to decrease growth of spoilage bacteria on meat. The aim of this work was to study the effect of vitamin E (VE) and polyphenols (P) dietary supplementation on microbial growth of pork coming from pig fed with 5% extruded linseed from about 80 kg live weight till to slaughtering (144.7 ± 7.2 Kg).

Twenty-four Large White pigs were fed with four different diets: control group (C) a basal barley/soybean based diet (VE 10 ppm and selenium 0.1 ppm); L group, 5% extruded linseed with VE 10 ppm and selenium 0.1 ppm; LE group, 5% extruded linseed with 200 ppm VE and 0.3 ppm selenium; LP group, 5% extruded linseed with 800 ppm of total P added by grapes (10.45% of P) and oregano (25% of P) extract (2.87 g and 2 g per kg feed, respectively), VE 10 ppm and selenium 0.1 ppm.

At 24 h *post mortem* (*p.m*), *longissimus dorsi* muscle was sliced and packed under modified atmosphere (35% CO_2, 65% O_2) and stored at 2 ± 1 °C in darkness for 4, 6, 8 and 12 days. Starting from six loins for each treatment group, two homogenized sub-samples, each consisting of chops from three different loins, were created. Each sampling was replicated twice.

Samples (10 g) were homogenized in 90 ml of sterile physiological solution in a stomacher. Mesophilic aerobic bacteria and *Enterobacteriaceae* were quantified by plating onto appropriate media. At 24 h *p.m*, the microbial load was similar in L (50 CFU/g), LE (75 CFU/g), and LP (60 CFU/g) groups, but lower compared with C (875 CFU/g) group. At the end of storage time, the microbial loads for L, LE and LP groups were about 10^3 CFU/g, without significant differences among them. Instead, the final value for C group was about 10^4 CFU/g. The results show that dietary polyphenols, vitamin E and linseeds supplementation might increase the shelf-life of pork meat packaged under modified atmosphere. However, taking into consideration the microbial load evolution, groups L and LP showed a less pronounced increase of the viable count during the entire storage period.

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Stracchino cheese: an alternative use of Buffalo milk

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Stracchino cheese has a longer shelf-life and is more evenly sold throughout the year than mozzarella cheese. Therefore, it may represent an alternative product for dairy buffalo enterprises. This study aims to characterise buffalo Stracchino cheese (BS) through sensory profiling, assess consumer liking and compare this product with a traditional Stracchino cheese obtained using milk from dairy cattle (CS). A quantitative–descriptive analysis (QDA) was performed by a trained 9-member panel (7 females and 2 males) in quadruplicate, whereas 80 consumers (average age of 38 years, equally distributed with respect to gender) evaluated product liking. Consumers expressed an overall liking and a liking accordcing to appearance, taste/flavour and texture and rated their liking on a 9-point hedonic scale labelled at the left end with “extremely unpleasant” (score = 1), at the right end with “extremely pleasant” (score = 9). In addition, the effect of information about milk type on consumer expectations (expected liking) was assessed. Buffalo Stracchino cheese showed lower milk ($p < .05$) and sweetness ($p < .001$) odour/flavour and higher sourness and oiliness ($p < .001$) intensities than CS. Stracchino appearance was affected by milk type in terms of whiteness and shininess ($p < .001$), with higher intensities perceived for BS. The higher oiliness perceived in BS may be due to the higher fat content of Buffalo milk, whereas the higher level of perceived whiteness may be attributed to the lack of β-carotene in Buffalo milk. Consumers rated both products (6.80 ± 0.17 *vs* 6.58 ± 0.7, NS, for CS and BS, respectively) at scores well above the neutral point (5 = neither pleasant nor unpleasant). Therefore, we can assume that both products were characterized by a good eating quality. Milk type did not affect overall liking or liking in terms of taste/flavour (6.58 ± 0.14 *vs* 6.83 ± 0.14, for CS and BS, respectively), texture (6.90 ± 0.17 *vs* 6.69 ± 0.17, for CS and BS, respectively) and appearance (6.80 ± 0.17 *vs* 6.58 ± 0.17, for CS and BS, respectively), whereas the expected liking was significantly affected by the information on milk type (7.0 ± 1.5, $p < .10$) with higher scores than those expressed in blind conditions (i.e. without information). Our findings suggest that consumers may favourably receive buffalo Stracchino cheese, which may be conveniently included by dairy Buffalo enterprises in their product range.

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Effects of milk production level on milk quality in Italian Mediterranean buffaloes

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The average milk yield of the Italian Mediterranean buffalo (8.34 kg/day/head) is the highest in the world with an average of 8.37% and 4.80% of fat and protein, respectively. Due to the increasing economic relevance of the dairy buffalo industry, interest is great in improving the production level of animals maintaining at the same time good and stable values of milk quality parameters over the seasons. Aim of this study was to investigate the milk quality among buffalo cows with different production levels. From March to August 2016, 60 lactating buffaloes were monthly monitored for milk yield and composition. All buffaloes were kept in loose housing system and fed the same total mixed ration distributed once a day. The individual milk yield of the two-daily milking was recorded and milk samples were collected. Milk samples were analyzed to measure fat, protein, casein, lactose, urea, and cryoscopy index using Fourier Transform InfraRed (Milkoscan FT^{plus}), calibrated with appropriate buffalo standards. Buffaloes were grouped in quartiles according to production level: Lower (LO): <7.6 kg/d; Intermediate Lower (INLO): 7.6 to 10.2 kg/d; Intermediate Higher (INHI): 10.3 to 12.8 kg/d; Higher (HI): >12.8 kg/d. Data were analyzed with PROC MIXED by SAS software including the parity (1, 2, and 3+) and the classes of days in milking (1 = 0 - 30 d; 2 = 31 - 60 d; 3 = 61 - 90 d; 4 = 91 - 120 d; 5 = 121 - 150 d; 6 => 150 d) of buffaloes as covariate.

As expected, the average milk protein was significantly higher ($p < .05$) in LO group (4.95%) than INLO (4.76%), INHI (4.55%) and HI (4.48%) groups. The fat content showed a reverse trend over the production level, showing an average content significantly higher ($p < .001$) for the LO group (8.83%) and the lowest for the HI (7.82%) group. Lactose showed instead a reverse trend: the lowest average value for the LO (4.66%) and the highest for the HI (5.06%) group. Urea levels were not influenced by milk production level, although the urea milk content showed an increasing trend ($p < .001$) from March (35.86 mg/dL) to August (53.38 mg/dL). Results of this study point out that milk production level has a consistent effect on milk quality in mostly for protein and fat. Whereas, urea level was not affected by production level over the months. From these results, we also conclude that the buffalo cow diet should ensure a sufficient proteins percentage, not only to meet requirements, but also taking into consideration the energy level.

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P117

Infra vitam and *post mortem* performance of ancient autochthonous genetic type pigs: comparison between Cinta Senese, Siciliano, and Suino Nero Lucano pigs

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The aim of this study was to evaluate and compare the performance *infra vitam* and *post mortem* of ancient autochthonous genetic type (AAGT) Cinta Senese (CS), Siciliano (S), and Suino Nero Lucano (SNL) pigs. A total of 30 autochthonous pigs, 10 for each genetic type, all raised on the same farm of the province of Potenza (Southern Italy) under a semi-wild system, were used in this experiment. On all pigs (range from 1 to 480 days) were evaluated the performance *infra vitam*: birth weight (kg), trend of live weight (LW) in relation to age (kg/day), average daily gain (ADG; kg/day), Biological Efficiency (BE = ADG/LW; g/Kg) and Livestock Efficiency (LE = $ADG/LW^{0.75}$; g/Kg), slaughter weight (kg) and *post mortem*: slaughter yield (%, carcass weight/LW) and carcass composition (% meat, % "fat + rind", and % bone; Total carcass dissection). Data were analyzed according to GLM procedure (SAS Institute, 1996). CS pigs showed the highest birth weight, growth performance (range from 1 to 480 days), and ADG ($p < .05$), while SNL pigs showed tendentially the lowest values for all parameters considered. No significant differences were detected among the three AAGT studied for BE and LE, even if S pigs showed higher BE value while CS pigs presented higher LE value.

At slaughter (480 days), CS showed the highest weight (119.71 kg), while SNL presented the lowest weight (96.10 kg). The average slaughter yield was about 80%; it was higher in CS pigs (80.5%) compared to SNL (77.5%) and S (72.9%) pigs. As regards to carcass composition, SNL pigs provided a higher meat quantity (37.2%), in particular meat for dry-cured products as *salame* and *soppressata* (28.6 and 8.6%, respectively; $p < .05$), and a lower "fat + rind" quantity (29.0%) compared to other AAGT considered ($p < .05$). CS pigs presented a higher bone quantity (12.07%), while S pigs showed a lower meat and bone quantity (29.5 and 10.2%, respectively) and a higher "fat + rind" quantity (40.3%).

P118

Microbiological and chemical dynamics during manufacturing and ripening of pasteurized goat's cheese in a dairy farm

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The aim of this study was to evaluate the microbiological and chemical dynamics of the cheese produced from pasteurized goat's milk during the first stage of ripening.

Seven cheesemaking of "Caprino cheese" were performed in a small cheese plant, located in the province of Rome.

Goat raw milk was pasteurized (64 °C × 18 minutes) in tank. Starters based on thermophilic lactic acid bacteria were added to pasteurized milk (40 °C), after 10 minutes rennet was added.

Ripening was at 9 °C and a relative humidity of 86% for 30 days.

Alkaline phosphatase was determined on pasteurized milk (Fluorophos®). Curd (day 0), and cheese (days of ripening 1, 7, 14, 28) were collected and submitted to the following microbiological analyses: Colony count at 30 °C, *Enterobacteriaceae*, *E. coli* and Coagulase-positive staphylococci.

Fat, protein, total solid and moisture, were determined using an infrared analyzer FoodScan™ (Foss, Hillerød, Denmark).

All data are presented as means and standard deviation. Mean and standard deviation were calculated by MedCalc Software.

Alkaline phosphatase in pasteurized milk was on average 382 ± 82 mU/L.

The average colony count at 30 °C ranged from 6.95 ± 0.53 log cfu $\times$ g^{-1} in curd to 9.50 ± 1.5 log cfu $\times$ g^{-1} in cheese at 28 days of ripening. *Enterobacteriaceae* ranged from 3.79 ± 2.51 log cfu $\times$ g^{-1} in curd to 6.87 ± 0.4 log cfu $\times$ g^{-1} in cheese at 7 days of ripening.

E. coli increased from 1 to 14 days of ripening (1.48 log cfu $\times$ g^{-1} to 4.04 log cfu $\times$ g^{-1} respectively), while it was not found in the curd.

Coagulase-positive staphylococci were found in curd (1.30 log cfu $\times$ g^{-1}) and in 1 day cheese (2.53 log cfu $\times$ g^{-1}) of one only trial.

The average chemical composition of goat cheese was: fat (21.1 ± 4.1%), protein (13.2 ± 7.9%), total solid (43.1 ± 11.3%) and moisture (56.9 ± 11.2%), similar to other cheese produced in the Mediterranean area.

The presence of high count of *Enterobacteriaceae* and *E. coli* in stored cheese suggests giving greater attention of hygiene practices during cheese making and storage of cheese.

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P119

Indigenous proteolytic enzymes in buffalo milk and fresh cheese

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The aim of the present research was to evaluate the level of indigenous proteolytic enzymes in buffalo milk obtained from cows fed with flaxseed supplementation. The experiment involved 36 Mediterranean buffalo cows during mid lactation; animals were allocated to three experimental groups homogeneous for age, body weight, parity, milk production and milk composition. Groups were: 1) no flaxseed supplementation - CON; 2) low flaxseed supplementation (500 g/die) – LFS; 3) moderate flaxseed supplementation (1000 g/die) – MFS. The experiment lasted 7 weeks with the first two weeks used as adaptation to the feed supplementation and the measurements were made during the last 5 weeks. Buffalo cows were housed in cement paddocks with free access to water and were fed twice daily. The total amount of flaxseed was given before the morning feeding to each buffalo cow of the FS groups checking that each animal consumed the total quantity of food and supplement given. At the end of the feeding treatment, bulk milk from each group was used for cheese production. Three cheese-making trials were performed in triplicate: thermized milk was coagulated using liquid rennet, after molding and pressing, the curds were held at 80 °C for 3 h. Cheeses were sampled and analyzed in duplicate at 7 d of ripening.

Fat, protein and casein were influenced by flaxseed supplementation. Protein and casein resulted higher in CON milk, intermediate in MFS milk and lower in LFS milk. Level of plasmin, plasminogen-derived plasmin, elastase were affected by flaxseed supplementation ($p < .05$). Plasmin concentration was lower in flaxseed supplemented groups than in control group (4.36 ± 0.08, 4.09 ± 0.08, 4.03 ± 0.08 mg/L ± SEM in CON, LFS, and MFS, respectively) whereas an opposite result was found in plasmin activity derived from the activation of plasminogen by urokinase activator which was lower in control group than in flaxseed supplemented groups (1.01 ± 0.09, 1.32 ± 0.09, 1.35 ± 0.09 mg/L ± SEM in CON, LFS, and MFS, respectively). Fresh cheese highlighted differences in plasmin and plasminogen levels; the highest levels for plasmin-plasminogen system were found in cheeses made from the group receiving the moderate flaxseed supplementation. These results suggested that PL activity in cheese from buffalo cows receiving moderate flaxseed supplementation has greater potential in contributing to cheese ripening and may be advantageous for flavour and texture development of buffalo cheese.

P120

Replacement of nitrates and nitrites by plant extracts in Italian pork salami

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Nitrates and nitrites are commonly added in cured pork meat as inhibitors of the growth of poisoning microorganisms (*e.g.*, *Clostridium botulinum*) and as color stabilizers of the pork meat, but they can form hazardous N-nitroso compounds. The objective of this preliminary study was to assess the replacement of nitrates and nitrites commonly added to pork salami by using two different natural additives under industrial conditions (Umbria Food Valnerina S.r.L, Italy). Two different experimental treatments were conceived as possible replacements of the potassium nitrate/sodium nitrite mix (0.225 g/kg, the control - C): *Spinacia oleracea* extract (E5105; 0.1 and 17.9 g/kg as $NaNO_2$ and $NaNO_3$, respectively) (SE) and *Bixa orellana* extract (annatto, E160b) (AN). The SE and AN salami samples were obtained adding 12.5 g/kg of spinach extract or 0.23 g/kg of annatto at the mixing of all the commercial ingredients used in industry. Three lots of salami (about 6 cm OD, 1.1 kg) were made, in successive weeks, for each experimental thesis (C, SE, AN). Each lot/thesis combination consisted of a total of 12 units that were sampled, in double, after 0, 1, 5, 15 and 22 days. On each sub-sample, the following analytical determinations were carried out: enterobacteria (EBC) and lactic acid bacteria (LBC) counts, pH, water activity (aw) and thiobarbituric reactive species (TBARS). The TBARS were also assessed on 22-days salami sub-samples, minced and maintained at 4 °C for 0, 2 and seven days. As expected, except the TBARS, all the variables investigated were affected by the sampling time. The treatment did not affect the EBC (overall mean 4.47 Log_{10} CFU/g) but, the LBC was higher ($p < .05$) in AN (7.89 ± 0.25 $\log_{10}$ CFU/g) than in the SE samples (7.52 ± 0.27 $\log_{10}$ CFU/g). Treatment affected ($p < .001$) the TBARS concentration that was 72.4 ± 2.2 μg MDA_{eq}/kg in SE, 47.6 ± 1.9 μg MDA_{eq}/kg in AN, and 33.8 ± 1.5 μg MDA_{eq}/kg in C samples. The effect of refrigerated aging on lipid oxidation was significant ($p < .05$) only for the AN treatment after two (126.7 ± 51.6 μg MDA_{eq}/kg) and 7 days (227.7 ± 54.8 μg MDA_{eq}/kg) if compared to C (44.8 ± 8.1 and 100.9 ± 46.7 μg MDA_{eq}/kg at 2 and 7 days, respectively). These preliminary findings suggest that annatto and spinach extract can be of interest for replacing or reducing the use of nitrites in the salami production. Identification of the right plant extract-to-nitrite replacement rate and the sensorial evaluation of products are matters of ongoing researches.

P121

Influence of slaughtering procedures and carcass processing on meat quality of hunted wild boar

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Wild boar (*Sus scrofa*) populations have increased in the last few decades and frequently can interfere with human agricultural activities. To avoid this, plans to control wild boar population through hunting are implemented, resulting in an increased availability of meat for consumption.

Meat of hunted wild ungulates undergoes several steps from the field to the consumer, implying a possible decline in meat quality, particularly related to storage systems in the period between slaughter and arrival to slaughterhouse for carcass processing, while to strengthening the game-meat sector, it is essential to ensure that the meat is of top quality. This means that good practices must be observed across the whole production sector both by hunters and by cutting plants.

The aim of this study was to compare the physiochemical quality of wild boar meat from animals that have undergone different treatments in the period between evisceration and carcass processing.

Twenty five wild boars undergone three different treatments post hunting were analysed: A group: the animals were eviscerated, skinned and quartered at room temperature within 2 hours from killing by untrained persons; B group: the animals were eviscerated directly but transported to the slaughterhouse within 1 hours to complete the grooming and quartering after 12 hours of refrigeration at 2 °C; C group: immediately transported to the slaughterhouse for evisceration and skinning, with dissection and portioning carried out post rigor, after 30 hours of refrigeration at 2 °C.

A sample of *longissimus dorsi* muscle of each carcass was removed after dissection, stored under vacuum packaging and analysed after 4 days to evaluate the physico-chemical quality. Significant differences ($p < .001$) among A, B and C groups for pH (from 5.74 to 5.53) and lightness (from 33.86 to 51.68) were reported. The B and C groups did not show significant differences in shear force (3.58 kg) and TBARS (0.75 mg/kg), while significant differences ($p < .05$) was detected in A group compared to the others regard to shear force (6.63 kg) and TBARS (1.49 mg/kg). There were no differences for the other colour parameters, raw and cooking water loss and proximate composition. We can conclude that qualitative characteristics of wild boar meat depend also by first processing after evisceration, slaughtering and aging temperature.

P122

Milk composition, coagulation properties and cheese yield in Sarda and Maltese goats reared in semi-extensive system

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In the last century the Maltese goat has been imported to Sardegna, both to be reared as a pure breed and to be crossbred to improve milk yield of the local goat population. This decision was taken by farmers without a preliminary study of the adaptability of this breed to the local farming system. Despite in this region milk of both breeds is totally used for cheese production, no comparative evaluation of milk cheesemaking ability was performed. Nowadays, few farmers rear the Maltese goat as purebred while, especially in low-input sustainable system, the Sarda goat farming is growing. The aim of this study was to compare milk yield, composition, coagulation properties and cheese yield (%CY) in Sarda and Maltese goats reared in the Sardinian semi-extensive system. Individual milk samples were collected from 114 Sarda and 104 Maltese multiparous goats reared in 14 farms presenting similar management. Daily milk yield for the same sampling day was recorded. Milk was analysed for fat and protein content (FT6000, Foss Electric A/S, Hillerød, Denmark). The 9-MilCA method, a recent modification of the standard lactodynamographic analysis (Formagraph®), was used to measure rennet coagulation time (RCT), curd firming time (k_{20}), curd firmness at 30 and 60 minutes after rennet addition (a_{30} and a_{60}, respectively), and %CY as the ratio between the weights of fresh curd and raw milk. Daily cheese yield (dCY) was computed as the product of multiplication between daily milk yield and %CY. A GLM procedure (SAS software) was performed to test the effects of the breed, herd and stage of lactation. Daily milk yield was higher in Maltese (1,614 *vs* 1,017 g/day), whereas Sarda goats resulted greater in milk protein (4.05 *vs* 3.35%) and fat (5.23 *vs* 4.03%) content. Milk from Maltese goats showed a shorter RCT (11.21 *vs* 12.88 min) as well as a slower k_{20} (6.56 *vs* 3.55 min). The more favourable coagulation properties for Sarda goats resulted in a better curd firmness (41.1 *vs* 31.2 mm and 36.7 *vs* 19.3 mm for a_{30} and a_{60}, respectively). Sarda showed also a higher %CY than Maltese (17.24 *vs* 15.37%, respectively), although the highest dCY was recorded for the Maltese (248 *vs* 175 g/day). These results evidenced that the selection in Sarda breed should be directed towards improving milk yield within the breed, while keeping its good chemical and coagulation properties.

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P123

Epigallocatechin-3-gallate and hydroxytyrosol protect against oxidative damage and improve anti-inflammatory response in bovine mammary epithelial cells (BME-UV1)

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Epigallocatechin-3-gallate (EGCG), the major catechin of green tea and hydroxytyrosol (3,4-dihydroxyphenylethanol, HTyr) present in virgin olive oil, have received increasing attention due to their wide health benefits. Despite the fact that they have been linked to mastitis prevention in rats, there is no evidence that clarifies their effect in bovine mammary gland. Therefore, the aim of the present study was to evaluate the anti-oxidative and anti-inflammatory effects of ECGC and HTyr in bovine mammary epithelial cell line (BME-UV1). In order to assess the actual role of these phenolic compounds, highly pure samples were tested. Then, fresh HTyr was synthetized in our laboratories by a patented procedure while a green tea extract containing a high content of EGCG (94%) was purchased. The levels of mammary oxidative stress and inflammatory responses were assessed by measuring the oxidative stress indicators and the levels of inflammatory cytokines. To evaluate the cellular antioxidant response, glutathione (GSH/GSSH), and γ-glutamylcysteine ligase activity was measured after 48 h from addition of 50 μM EGCG and 50 μM of HTyr. Malondialdehyde (MDA) production was also assessed. Reactive oxygen species production after 3 h of H_2O_2 exposure was assessed to evaluate and to compare the potential protection of EGCG and HTyr against H_2O_2-induced oxidative stress. The anti-inflammatory activity of EGCG and HTyr was further investigated by determination of bovine pro-inflammatory cytokines (*TNFα*, *IL1β* and *IL6*) mRNA abundance after treatment of cells for 3 h with 20 μM of lipopolysaccharide (LPS). EGCG and HTyr treatments induced higher concentrations of intracellular GSH compared to control cells, matched by an increase of γ-glutamyl-cysteine ligase activity mainly in cells treated with HTyr. Interestingly, EGCG and HTyr prevent oxidative lipid damage in the BME-UV1 cells by a reduction in intracellular MDA levels. EGCG and HTyr were able to enhance cell resistance against H_2O_2-induced oxidative stress. The mammary expressions of *TNFα*, *IL1β* and *IL6* were evaluated by real time PCR analysis. It was found that EGCG and HTyr decrease the levels of the three inflammatory cytokines. These results indicated that EGCG and HTyr provide dual protection because they were able to attenuate oxidative stress and inflammatory responses, suggesting that these compounds are potential natural alternatives for the control of bovine mammary gland inflammation.

P125

Use of a partial least squares (PLS) regression to predict the glycaemic index of raw cereal grains commonly utilized in pig nutrition

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A modern evaluation of the nutritional parameters for raw cereals entering pig diets requires information on rates of starch digestion. For pig nutrition, several authors introduced a novel approach based on the glycaemic index (GI) classification. In addition, the *in vitro* glycemic index (pGI) has been successfully utilized as alternative to *in vivo* GI values for expressing kinetics of starch digestion through the gastrointestinal tract, pGI being highly related to *in vivo* GI data. The objective of this work was to reduce the predictor dimensionality and to develop a model able to forecast the pGI of raw cereal grains commonly utilized in pig nutrition. A survey on 92 raw cereal grains was carried out and samples from corn ($n = 18$), barley (19), oat (7), wheat (8), triticale (8), rice (8), sorghum (8) and high moisture corn (16) were analysed for chemical and digestion traits using well-established *in vitro* digestion methods. A partial least squares (PLS) regression with a one-leave-out cross validation method was used to reduce the dimensionality of the original predictors ($n = 15$, being both chemical or Englyst's starch hydrolysis values) by projecting the independent variables into latent constructs. In a first step of model development, the importance of independent variables in predicting the pGI values was assessed by plotting factor loadings of both dependent and independent variables on the first two components and by verifying for each predictor the variable influence on projection values adopting the Wold's criterion as well as the entity of standardized regression coefficients. Zein, crude fiber, amylose, rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS) starch contents were retained as important terms in the PLS model and they were used to develop a reduced-variable PLS (rPLS) regression. Despite a slight decrease in coefficients of determination of PLS *vs* rPLS models (i.e., $R^2 = 0.84$ *vs* 0.79, respectively) and an increase in error terms of regressions (i.e., $\sqrt{MSE} = 7.99$ *vs*

9.43, respectively), both models were adequate to forecast the pGI of raw cereal grains commonly utilized in pig nutrition. In concert with chemical composition and classical criteria (as the ileal or the faecal digestibility), the adopted models may be useful for the estimation of the pGI to provide valuable feed formulation tools for screening the nutritional value of raw cereal grains.

P126

Effect of plant oil addition to dairy goat diet on milk fat contents of trans-9 C16:1 and C16:2

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Milk fat has a great variety of fatty acids derived from ruminal and mammary gland metabolism. Some milk fatty acids are quantitatively minor, but might be relevant from the human health point of view. The aim of the present study was to contribute to the knowledge of changes in the contents of *trans*-9 C16:1 and C16:2 when differently unsaturated plant oils are fed to dairy goats. *Trans*-9 C16:1 has recently been attributed positive effects on insulin resistance and risk of type II diabetes, probably due to its elongation in the organism to vaccenic acid and ulterior desaturation to rumenic acid, while C16:2 could act as a substrate for the synthesis of long chain ω-6 fatty acids. The data obtained in three experiments were used. The oils tested in each experiment were high oleic sunflower oil (HOSO), regular sunflower oil (RSO) and linseed oil (LO). In each experiment, four treatments were tested: control diet (CTR) without added oil, or the same diet added with 30, 48 or 66 g/d of oil. At the beginning of each experiment, 3 mid-lactation Malagueña goats, kept in individual cages were randomly assigned to each one of the four treatments. The basal diet comprised a pelleted concentrate and alfalfa hay and was the same in all the treatments and experiments. Milk fatty acid composition was determined by gas chromatography on the lipid fraction extracted and subjected to methylation. Data were analyzed with a nested mixed model by using the MIXED procedure of SAS UE 3.5. Linear trends and least-squares mean differences were tested by polynomial and orthogonal contrasts. Supplementation with 30 to 66 g/d of RSO or LO had a positive and linear effect ($p<.05$) on the content of *trans*-9 C16:1 in milk fat. The HOSO only increased ($p<.05$) the milk fat content of *trans*-9 C16:1 at a supplementation level of 66 g/d, and the response was also linear ($p<.05$). The content of C16:2 in milk fat only increased ($p<.05$), compared to CTR, when the animals consumed LO. This change was linear ($p<.05$) and correlated ($p<.05$) with that of *trans*-9 C16:1. The present work indicate that the increase of *trans*-9 C16:1 content in goat milk fat, after plant oil supplementation, depends on the unsaturation degree of the oil and is higher when the amount of supplied oil increases. There is a positive relationship between the consumption of LO and the contents of C16:2 in milk fat and also between the contents of C16:2 and *trans*-9 C16:1 in milk fat, which deserves further research.

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Effects of supplemental methionine and lysine levels on growth performance, blood serum parameters, immune responses, and carcass traits of broilers

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Supplementation of corn-soybean based diets with synthetic methionine (Met) and lysine (Lys) is a common practice in poultry nutrition. This study investigated effects of Met and Lys excesses on growth performance, blood serum parameters, immune responses, and carcass traits of broilers. Ross 308 broilers ($n=270$) were randomly allocated to 9 iso-energetic and iso-nitrogenous dietary treatments (3 replicates/treatment and 10 birds/replicate) based on 3 levels of Met and Lys (100, 110, and 120% of Ross recommendations) for starter, grower, and finisher periods. Antibody titers against avian influenza, Newcastle disease (NDV), and sheep red blood cell were measured. Blood serum samples were analyzed for lipid profile and uric acid, glucose, total protein, albumin, globulin, alkaline phosphatase (ALP), Ca, and P levels. At slaughtered, carcass traits were evaluated. Carcass yield and relative weights (as % of eviscerated carcass) of abdominal fat, anatomical parts, gut tracts, and organs related to immune system were calculated. A completely randomized block design was adopted and main effects (Met and Lys) were arranged in a 3×3 factorial approach. When broiler requirements for Met and Lys were 100% satisfied, maximum growth performance (weight gain, feed, energy, and protein intakes and efficiencies) was achieved. The Lys inclusion at

110% of Ross specification tended to decrease feed, energy, and protein efficiencies (quadratic, $p = .06$) and pre-slaughtered weight (quadratic, $p = .10$) whereas increased serum uric acid concentration (quadratic, $p = .05$). Increasing Lys levels decreased serum P concentration (linear, $p = .02$) and immune response against NDV after the first vaccination (linear, $p = .09$) whereas increasing Met levels tended to increase serum concentration of cholesterol linked to high density lipoproteins (linear, $p = .09$). Met × Lys interaction were observed on serum glucose ($p = .01$) and ALP (tendency, $p = .06$), relative weights of rectum ($p = .02$), liver ($p = .02$), and bursa of Fabricius (tendency, $p = .06$). Results showed that optimal growth performance, blood serum parameters, immune responses, and carcass traits can be achieved if Met and Lys requirements of Ross 308 broilers are satisfied according to the Ross recommendations. Levels of Met and Lys higher than NRC specifications (approximately +10% on average over grower and finisher periods for Met and over starter, grower, and finisher periods for Lys) could be necessary for comparable results.

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P128

Efficiency of *in vivo* ear flap NIR scan in the detection of differences related to diet or pregnancy status in young rabbit does

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The aim was to determine whether *in vivo* spectroscopy of the ear flap was able to detect the diet-related differences and pregnancy status, with its prognostic in forward or backward perspective.

Methods. Young grey Carmagnola rabbit does in groups of eight were fed diets enriched with: linseed at 5% (A); linseed at 5% and hazelnut skins at 1.5% (B); palm oil at 1.8% (C); and a standard diet (D), respectively. After the zero-point (P0) before A.I. a second point (P1) was fixed at d 21. A smart-NIR device (SCIO, 740-1030 nm) was used. Chemometric analyses of the 331-point spectra were performed by using WinISI 1.5, without math pretreatment. Modified Partial Least Squares method was fitted to dummy binary variables. Outliers were identified once and excluded when $t > 2.0$. R^2 coefficient in cross-validation was retained as reference parameter. In parallel, for binary categorical discrimination, the proprietary software SCiO lab based on AKA (Also Known As) confusion matrix was used. Results. The NIR spectra were able to clearly perceive changes from a standard pre-experiment D diet to the A, B and C diets, with R^2 around 0.60, while the value was lower for diet D (0.29). This first outcome is favorable for the repeatability of the method and for a discernment of nutritional factors. Prognostic of gestation in backward mode (scan at P1) was significant with mild NIR relationship (0.35), not suitable for practical uses, while in forward mode prediction (scan at P0) result was poor (0.20). The feeding treatments with different fatty acid profiles were instead more accurately perceived in the NIR spectra, especially for the C group, which was discriminated from the A and B groups with a R^2 value of 0.71, which corresponds to a 75% reclassification as AKA. Interestingly, the antioxidant compound of the B diet was enhanced for a non-pregnant status (0.85 *vs* 0.12 when pregnant) and this could indicate the presence of interactions between growth and pregnancy requirements, as assessed from skin variations. Conclusions. At the present state of knowledge, the miniaturized NIR device looks promising, mainly for the assessment of body fat composition. As far as the feeding experiment is concerned, it should be pointed out that it is rare to obtain model R^2 values as high as those obtained during this preliminary work for a classic design, even multivariate ones, without extreme diets. The skin is an unbiased mirror of health and nutritional status of rabbits.

P129

Effects of arbuscular mycorrhizal fungi and low fertilizer supply on forage quality, milk traits and profitability

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The combined effect of arbuscular mycorrhizal fungi and low fertilizer supply on crop yield, *in vitro* rumen degradability of DM (IVDMD) and NDF (IVNDFD), and milk traits was assessed. Seeds of maize (*Zea mays* L.) and sorghum (*Sorghum vulgare* Pers.) were inoculated with a commercial product based on Glomus spp. and were sown in an irrigated plain buffalo farm (maize) and a dryland hill dairy farm (sorghum). The treatments (C control, E experimental) were the following: MC (not inoculated maize seeds, fertilization: N 250 kg ha^{-1}, P 100 kg ha^{-1}), ME (inoculated maize seeds, fertilization: N 125 kg ha^{-1}), SC (not inoculated sorghum seeds, fertilization: N 120 kg ha^{-1}, P 50 kg ha^{-1}), SE (inoculated sorghum seeds, fertilization: N 60 kg ha^{-1}). For each treatment, 2 plots, 1.5 ha each, were arranged. The forages were harvested as silages, and were used in 2 feeding trials carried out on 32 lactating cows (sorghum) or 40 buffaloes (maize). After 10 d of adaptation to diet, for 6 d milk traits (yield and chemical composition) of each animal were measured, along with determination of dry matter intake (DMI). Data were analyzed using ANOVA for repeated measures, with treatment as factor. A significant improvement ($p < .05$) of biomass yield (Mg ha^{-1}) was found for both experimental maize (76.3 *vs* 65.7, respectively for ME and MC) and sorghum (46.3 *vs* 36.1 respectively for SE and SC). SE silage resulted in less ($p < .05$) NDF (39.8 *vs* 42.0% DM) and more ($p < .05$) crude protein (11.7 *vs* 9.7) content, but no significant differences between ME and MC could be detected for chemical composition. In similarity, IVDMD was significantly ($p < .05$) higher in SE compared to SC (72.2 *vs* 70.1), whereas no differences were observed among MC and ME ($p > .05$). For both forages, experimental treatments did not influenced IVNDFD ($p > .05$) as well as DMI and milk traits of both buffaloes and cows. The economic benefit analysis showed that experimental managements improved the crop profitability due to increased salable products and to reduced costs of fertilizers. Finally, energy efficiency was improved in experimental crops as a consequence of both reduction of energy inputs from fertilizers and the reduction of energy outputs. Results indicate that combining mycorrhizal inoculation with low rate of fertilizers may help to increase eco-efficiency and profitability in forage production without affecting the feed quality and the livestock productivity.

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P130

Proximate composition and microbiological characterization of fresh mealworms (*Tenebrio molitor* L.)

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The increasing demand for high-quality proteins requests intensive farming of meat and milk animals, thus leading to a strong environmental impact. The exploitation of edible insects as a valuable large-scale, animal-based commodity, can represent a valid alternative due to their richness in high-quality proteins, good lipids, micronutrients, B-group vitamins and fibre. Moreover, insects are very efficient in transforming vegetable into animal biomass compared to ruminants, converting their feeding substrate into proteins of high biological value. In this study, laboratory-reared fresh mealworms larvae, wheatmeal used as feeding substrate and frass (excrements with substrate residues) were analysed for moisture, protein, fat, fibre, Nitrogen Free Extract – NFE, ash, and for microbial counts, according to official methods. For larvae also the energy value was estimated with the Atwater coefficients.

The chemical composition of larvae (% DM) showed protein values 3-4 fold the limit (10g/100g) for labelling a food as "high source of protein". Differently from meat, a modest content of crude fibre was also measured, thus resulting in a potential positive effect in human digestion when mealworms are used as food. Energy content was in agreement with recent values reported in scientific literature, even though the observed slight differences on proximate composition of larvae may be ascribed to the modest protein level of wheatmeal. Regarding microbiological analyses, in wheatmeal a mean (± s.d.) load of 5.5 ± 0.4 log colony forming units (cfu)/g was discovered for total mesophilic aerobes (TMA), while viable counts for *Enterobacteriaceae* (ENT) were below detection limit (1 log cfu/g). In fresh larvae the counts of TMA and ENT showed means of 8.3 ± 0.3 and 6.7 ± 0.8 log

Table: Chemical composition (min-max) of wheatmeal, mealworms and their frass (% DM)

		Wheatmeal	*T. molitor* larvae	Frass
Moisture	%	11.00-11.28	60.16-63.86	12.36-13.30
Protein	%	15.55-15.62	39.03-44.09	17.81-21.80
Fat	%	3.19-3.35	33.96-35.36	1.02-1.86
Fibre	%	8.75-9.08	5.76-6.57	17.77-20.67
NFE	%	66.77-67.03	11.81-17.40	46.47-51.66
Ash	%	5.32-5.34	3.57-4.04	10.52-11.08
Energy	kcal/100 g	Not Determined	529-536	Not Determined

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cfu/g, respectively; whereas in frass, mean counts of 8.4 ± 0.4 and 5.9 ± 1.6 log cfu/g for TMA and ENT, respectively, were observed. The high loads of microorganims detected in the samples suggest to apply a heat treatment to mealworms before any consumption.

P131

Effect of rumen-protected methionine and choline supplementation to dairy goats during dry period on metabolic profile, milk yield and composition

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In high yielding goats, late pregnancy is characterized by strong inflammatory conditions and severe negative energy balance, which can affect subsequent performance if not properly counteracted. In this phase, liver performs many essential functions related to digestion, metabolism and acute phase reaction. The molecules donor of methyl groups can aid the metabolism, nevertheless, it is debated the role of each molecule which release methyl groups. The aim of the study was to check the effect of delivery of 10 g of rumen-protected choline (Ruprocol®, Vetagro, RE - I) (COL group) alone or associated with 10 g of rumen-protected methionine (Timet®, Vetagro, RE - I) (MC group) in the last month of pregnancy, on dairy goats metabolism and milk yield in the first month of the following lactation. The test was conducted in a commercial herd located in the Po Valley on 33 pregnant Alpine goats: 16 in COL and 17 in MC. During the dry period, the diet included 350 g of concentrate and straw *ad libitum.* Straw was substituted with grass hay few days before kidding. During lactation, goats received 500 g of mixed concentrate at each milking. Blood samples were collected weekly from about 40d before parturition and till 10d after. Goats were milked twice a day and milk samples were collected on 7, 14, 28 days in milk. The supplementation of methionine and choline respect to choline alone did not cause effects at metabolic level and glucose, NEFA, β-hydroxybutyrate, cholesterol and urea resulted similar between groups. Conversely, MC goats showed a lower inflammation after kidding as haptoglobin resulted lower (0.43 *vs* 0.77 g/L, $p < .05$) and Zn higher (8.94 *vs* 7.76 μmol/L, $p < .05$). This suggests that liver was less involved in the synthesis of the acute phase protein. During first 28 d of lactation average milk content of fat (4.53 vs 3.84%, $p < .08$), total protein (3.7 *vs* 3.4%, $p < .05$) and casein (2.88 *vs* 2.67%, $p < .05$) resulted higher in MC. Nevertheless, because the slightly higher milk yield in COL (3.1 *vs* 2.9 L/d, NS), total solid outputs resulted similar between groups. The average linear score of somatic cell counts (SCC) resulted similar, but goats with SCC higher than 1000 μL were 54% in COL and only 18% in MC. Adding Met to choline to the diet of goat during the dry period has not determined improvement of energy metabolism, but it has attenuated the inflammatory events at kidding and this could have determined mild positive consequences in term of milk quality.

P132

Phylogenetic analysis of methanogens in the hindgut of donkey

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Donkeys are herbivores and, like other *equidae*, derive varying amounts of energy from the microbial fermentation of feeds in the large intestine. Methanogens are members of the microbial community inhabiting the hindgut and they can be differentiated from the other organisms, since they all produce methane as a metabolic end-product. To date the research on the gut microbiota of donkey is surprisingly scarce nevertheless this knowledge is essential for maintaining the gut homeostasis and to understand the possibility to reduce the methane greenhouse effect.

To assess the diversity of the methanogenic community along the donkey hindgut.

The total DNA was extracted from lyophilized samples of caecum, colon and rectum content of one female healthy donkey maintained on pasture of natural meadow, with not any supplementation necessity. The archaeal DNA was amplified by specific primers targeting 16S rDNA gene and the PCR products were cloned into plasmid vectors pCR4. The positive clones were re-amplified by M13 primers and the resulting PCR products were further subjected to the RFLP (Restriction Fragment Length Polymorphism) analysis using the restrictases MluI and MboI. The representatives of the different restriction patterns underwent the sequence analysis.

A total of 335 clones generated from six archaeal 16S rDNA gene cloning libraries of the different segments of the donkey hindgut were examined and the RFLP analysis revealed 9 ribotypes. The RFLP and the phylogenetic analysis revealed interesting variations among the different parts of the digestive tract and the sequenced clones were classified in 3 clades: *Methanobacteriales*, *Methanomassiliicoccales* and *Methanomicrobiales*, with the dominance of *Methanomicrobiales*. The distribution of the methanogens along the donkey hindgut was dependent on the part of the digestive tract. *Methanomicrobiales* (*Methanocorpusculum*-like clones) dominated in the caecum, left and right dorsal colon and faeces, while *Methanobacteriales* (*Methanobrevibacter*-like clones) absolutely dominated in the right and left ventral colon.

The absence of the *Methanobrevibacter* species and the dominance of the *Methanocorpusculum* species in the donkey caecum reflect specific fermentation processes in each part of the digestive tract and indicate possible differences in the metabolic activities among the different hindgut segments of the donkey.

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P133

Growth and post mortem performance in Saanen goat kids fed with polyphenols: preliminary results

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The aim of this study was to evaluate the effects of a diet supplementation with polyphenols, derived from oil extract on Saanen goat kids performance either in vivo (age at slaughtering, daily weight gain "DWG", body weight) and post-mortem (weight of carcass, rheology, colour and fatty acids profile). The trial was performed on 18 Saanen females kids naturally weaned at 69 days of age and fed with hay *ad libitum* and concentrate to meet growing requirements (forage concentrate ratio 65:35; concentrate crude protein 16%). The animals were divided into 2 homogeneous groups, according to age and weight (LW) at weaning: Group A (n = 9) was used as control group, while Group B (n = 9) received the same diet supplemented with 3.2 mg of polyphenols. The kids were weighed every 10 days and DWG was calculated. After 78 days the animals were slaughtered and pH was recorded after 45 minutes (hot) and 24 hours. Samples of *longissimus dorsi* muscle were collected from each carcass to evaluate the proximate composition of the meat. Data were analyzed by ANOVA for repeated measures.

Similar LW (17.9 ± 2.9 *vs* 18.6 ± 3.8 kg in Group A and B, respectively) and mean DWG (0.08 ± 0.0, 0.10 ± 0.0 g/day in Group A and B respectively) were recorded in both Groups at the end of the trial. Carcass yield was similar among groups either at 45 minutes (10.0 ± 1.5, 10.0 ± 2.3 kg in Group A and B) and 24 hours (9.7 ± 1.5, 9.7 ± 2.2 kg in Group A and B). Similarly, no differences were observed in pH values (meanly 6.8 at 45 minutes and 5.8 at 24 hours) and meat proximate composition. A significantly ($p < .01$) lower shear myofibrillar force was found in Group B compared to Group A (0.68 ± 0.4 *vs* 3.12 ± 0.5 kg/cm^2). Meat colour was similar between groups throughout 7 days of refrigeration. However, deoxymyoglobin concentration tended to be higher ($p = .10$) in Group B on day 7 (6.85 ± 10.1 *vs* 13.05 ± 13.7%), although similar methyoglobin and oxymyoglobin contents were estimated. A lower ($p < .01$) saturated (49.2 *vs* 39.51% in Group A and B, respectively) and higher ($p < .05$) monounsaturated (34.35 *vs* 42.22% Group A and B, respectively) fatty acid concentration was observed in Group B compared to Group A. Furthermore, the indexes of thrombogenicity (2.13 *vs* 1.38 in Group A and B, respectively; $p < .01$) and atherogenic (1.28 *vs* 0.69 in Group A and B, respectively; $p < .05$) were lower in Group B. In conclusion, polyphenols integration in the diet of weaned goat kids improved the meat texture and fatty acids profile.

P134

SCD1 and DGAT1 polymorphism in Modicana cows and their interaction with feeding system. Effects on milk fatty acid composition

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Steroyl-CoADesaturase (SCD1) introduces a Δ9 double bond in a wide spectrum of fatty acids (FA), while Diacylglycerol O-acyltransferase 1 (DGAT1) catalyses last step in triacylglycerides synthesis. In cattle mutation of the genes encoding for these enzymes has been associated with fat yield and composition. Taking into account the key role of pasture feeding in milk FA profile, the aim of the research was to evaluate the effects of p.A293V polymorphism at *SCD1* gene (Acc.

Num. AC_000183.1) and p.K232A at *DGAT1* gene (Acc. Num. AY065621) in Modicana cows and their interaction with feeding system on milk fat composition. Individual blood samples (118 from a semi-intensive (SI) and 70 from an extensive (EX) farm of Modicana dairy cows) were collected. In the whole sample three SCD1 (TT, TC and CC) and three DGAT1 (p.232KK, p.232KA, p.232AA) genotypes were found. The alleles g.10329T and p.232A were found to be most frequent in *SCD1* and *DGAT1* (0.73 and 0.91, respectively). Deviation from Hardy-Weinberg equilibrium was observed only in SCD1 locus ($\chi^2 = 35.14$ $p \leq .001$). Based on genotypes at SCD1 (TT, CC) and DGAT1 (p.232AA, p.232AK) loci, 26 cows in the SI farm and 24 cows in the EX farm balanced for parity, day in milk and milk yield, were selected. In the SI farm, cows were fed 5 kg of vetch and oats hay, 5 kg of concentrate and 2h/die grazing on natural pasture. In the EX farm, cows were fed vetch and oats hay (5 kg) and 8h/die grazing on natural pasture. Individual FA composition of monthly milk was analysed by GLM procedure for repeated measures of SPSS software. On average the SCD1 genotype significantly ($p < .05$) increased percentage of C6:0 (1.81 *vs* 1.61), C8:0 (1.30 *vs* 1.12), C10:0 (3.11 *vs* 2.70), t-11 C18:1 (1.60 *vs* 1.36) in CC cows. Moreover, some significant interactions were also found between SCD1 polymorphism and feeding system. In the EX system CC cows showed higher ($p < .05$) C4:0 ÷ C12:0 and *t*-11 C18:1 than TT cows. The same trend was not found in the SI system. DGAT1 polymorphism did not affect milk FAs. However, in the EX system, significantly ($p < .05$) higher C4:0 (2.39 *vs* 2.11%) and lower *t*-9 *c*-11 C18:2 (CLA) and CLA ratio (respectively, 0.81 *vs* 0.99% and 1.24 *vs* 1.44) were found in the p.232AK cows, compared to p.232AA cows. The same trend was not evident in the SI feeding system. These interactions should be explained assuming that the feeding system could have resized the polymorphisms effects due to the different FA profile of the diet.

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P135

Effect of a diet enriched with linoleic acid (hemp seeds) or α-linolenic acid (linseed) on the goat milk transcriptome and miRNAs

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Nutritional strategies, such as supplementation with feed rich in unsaturated fatty acids, can increase the healthy property of milk in dairy ruminants, even if their effect throughout the pathways/genes involved in these processes are not properly defined. The next-generation RNA sequencing technology allows the whole transcriptome characterization under given conditions, thereby providing deeper knowledge of the cells activity under such conditions. MicroRNAs (miRNAs) have recently been described to be potent post-transcriptional regulators in fatty acid and cholesterol metabolism by targeting lipid metabolism genes. In this study, next generation RNA-sequencing was used to investigate the goat milk transcriptome and miRNAs following supplemental feeding enriched with linoleic acid (hemp seeds) or alpha-linolenic acid (linseed), in order to point out the general biological mechanisms underlying the effects related to milk fat metabolism.

Eighteen pluriparous Alpine goats fed with the same pre-treatment diet for 40 days were, then, arranged to three dietary treatments consisting of control (C), linseed (L) and hemp seeds (H) supplemented diets. Milk samples were collected at 40 (Time point = T0) and 140 days of lactation (Time point = T1), and RNA was extracted from the pelleted milk cells. Gene expression analysis was conducted by Illumina RNA sequencing. A total of 677 and 218 genes were highly differentially expressed ($p \leq .001$, Fold Change >1.5 and FDR $\leq .05$) in H and L group, respectively. Functional analyses using Ingenuity Pathway Analysis (IPA) evidenced that modifications in feeding strategies affected key transcription factors regulating the expression of several genes involved in milk fat metabolism, e.g. SREBPs (Sterol regulatory element binding proteins), PPARs (Peroxisome proliferative activated receptor) and RXR (retinoid X receptor). Moreover, 64 and 6 differentially expressed miRNAs were uncovered in H and L group, respectively, and their potential functions were also predicted by IPA.

This study provides noteworthy insights into the molecular changes occurring as a result of fat supplementation in diets and broadens our understanding of the relationship between nutrients variation and phenotypic effects.

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Determination of theobromine and caffeine in complementary feedingstuffs for horses

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Caffeine and theobromine are widespread environmental substances in horse feeds. They are well absorbed when administered by oral intake and may appear in blood and urine. These compounds, if present even at low concentration in feed, may produce positive results in the anti-doping controls. Therefore, the aim of the present work was to develop and validate an LC-MS method for the simultaneous determination of caffeine and theobromine in complementary feeding stuffs for horses. A chromatographic separation, using caffeine C13 as internal standard, was developed using a C18 Synergie Polar® column, with methanol and 0.1% formic acid in water as mobile phases on 500 µg samples of feed. Triple quadrupole mass spectrometer Agilent 6410, equipped with an ESI source was operated in positive ion mode. The analytes were determined by a Selected Reaction Monitoring (SRM) method by acquiring three transitions. For the validation of the method the following parameters were evaluated: Limit of Detection (LOD), Limit of Quantification (LOQ), repeatibily limit (r) for ion ratio, extraction recovery, and linearity. Obtained results outlined a LOD value of 0.2 µg/g for both the compounds, with a LOQ of 1 µg/g for caffeine and 6 µg/g for theobromine. Repeatibility limit ($r = t \times Sr \times \sqrt{2}$) was found to be 2.99% for caffeine and 1.15% for theobromine, while extraction recovery was 78% and 70% respectively. Linearity showed an R^2 value of 0.996 for both the tested molecules. The obtained results allow to routinely apply the developed method to check the complementary feeding stuffs for horses for the presence of caffeine and theobromine even at low concentrations.

P137

High dietary calcium levels in low phosphorus diets affects intestinal microbiota and the inflammatory gene expression response in the intestinal mucosa of weaning pigs

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Calcium is usually supplemented in pigs diet with animal and mineral sources as plant ingredients. However, Ca increase in diet has been negatively associated to growth performance due to its capacity of reducing the phytase efficiency and P digestibility. In the present work, the effects of low and high dietary calcium content on intestinal microbiota and gene expression of piglets were evaluated. Eighteen pigs (28 d old, average BW = 7.2 ± 0.24 kg) were divided in two groups, individually fed one of the two diets containing low (0.45% - of feed, LCa) or high (0.95% - feed bases, HCa) Ca level for 14 days, and then euthanized to obtain mid-jejunum tissue and cecum digesta. Total mRNA was extracted from 100 mg of mid-jejunum samples and hybridized on Affymetrix Porcine Gene 1.1 ST array strips. The Affymetrix Transcripts ID’s were associated to 13494 Human gene names, based on Sus scrofa Ensembl and the gene set analysis were performed. Normalized enrichment score (NES) was calculated for each gene set, and statistical significance was considered when False Discovery Rate % <25 and p-values of NES <.05. Bacterial DNA was extracted from colon and V3-V4 region were sequenced on MiSeq Illumina. Sequence data were processed using QIIME software based on open reference OTU picking strategy and taxonomically assigned using GreenGenes v13.8 database. Alpha and Beta diversity analysis were performed using vegan package and the phyla differential abundances were assessed using fitFeatureModel in MetagenomeSeq package on R v3.3.0. The transcriptomic profile of pigs fed LCa diet indicates a lower activation of the inflammatory pathways (IFNα and IFNγ) on the contrary pigs fed HCa diet had cell cycle regulation, RNA transcription and DNA replication pathway enriched. Multivariate Ordination of colon microbial profile highlighted two clusters that clearly differenced the two diets ($p = .01$). 15 different phyla differed between diets ($p < .05$) and in particular HCa increased the abundance of Euryarchaeota phylum. Moreover, HCa

increased the abundance of Bacteroides genus ($p < .05$). In conclusion, the high buffering capacity of HCa diet, favours microbial genera associated with gut dysbiosis, and could explain the expression of genes related to the inflammatory response, which may cause more energy expenditure with detrimental effect on growth.

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P138

Suitability of oil seed cakes as livestock feeds: preliminary results

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Increased pressure for land use and higher cereal grain prices have resulted in significantly higher feed costs for ruminant livestock. On the other hand, in the last few years, an increasing supply of new byproduct feeds, many derived from oil extraction became available. In this work, four of these byproducts, among the most diffused in Campania Region, were analyzed with the goal of evaluating if they would be suitable as livestock feeds. The following cakes were evaluated: Po, Pomegranate (*Punica granatum*); Th, Thistle (*Cynara cardunculus*); To, Tobacco (*Nicotiana tabacum*); He, hemp (Cannabis sativa). For each cake, three samples of different origin were analyzed in duplicate according to the Weende (dry matter DM; crude protein CP; fat; ash), Van Soest (neutral and acid detergent fibre, NDF and ADF; acid detergent lignin; ADL) and CNCPS (soluble proteins, SP; non-protein nitrogen, NPN; neutral and acid detergent insoluble protein, NDIP and ADIP; starch) schemes. For all samples ash content (%DM) was below 10% (overall mean 5.1 ± 1.2 sd), ranging from 4.0 ± 0.1 of Po to 7.1 ± 0.1 of He. The fat content (%DM) was relatively high for almost all cakes (7.5 ± 0.6, 15.7 ± 4.6, and 10.2 ± 0.8, respectively for Th, To, and He), except Po (0.9 ± 0.1). The CP levels (%DM) were quite variable, being 14.7 ± 0.3, 20.0 ± 0.6, 37.1 ± 2.5 and 29.0 ± 2.8, respectively for Po, Th, To and He. In addition, SP (%CP) largely fluctuated, being respectively 2.8 ± 1.8, 54.9 ± 2.3, 31.0 ± 1.5 and 14.5 ± 1.6. By contrast, the levels of NPN %CP (overall mean 2.1 ± 0.7), and NDIP %CP (16.7 ± 2.7) were quite constant among and within the cakes. Limited variability was observed also for ADIP %CP (9.1 ± 1.4) that it is assumed the unavailable protein fraction. Starch content (%DM) was not detectable in To, and was generally low (5.6 ± 0.4, 5.0 ± 1.6, 5.6 ± 2.5, respectively for Po, Th and He). Regarding the fiber, all cakes, but Po, showed relatively limited contents of NDF %DM (71.9 ± 0.7, 46.4 ± 2.9, 48.8 ± 2.6, 51.1 ± 2.6, respectively for Po, Th, To and HeC), and ADF %DM (50.8 ± 0.6, 32.8 ± 2.1, 32.9 ± 1.8, 32.3 ± 1.5). High ADL (%DM) level were found for Po and To (11.1 ± 0.1 and 11.4 ± 0.4), whereas for the other cakes the values were 5.2 ± 2.0 (Th) and 6.3 ± 0.8 (He). Overall, Th, To, and He cakes showed interesting nutritional features in terms of protein and fiber content suggesting their potential utilization supplement for ruminants, whereas the use of Po may be not recommended as a feedstuff.

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Use of camelina seed (*Camelina sativa*) by-products in diets for feedlot lambs: effects on productive results

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The by-products of camelina seed (*Camelina sativa*) oil extraction are husks and meal, both rich in neutral detergent fibre (52-60% and 32-35%, respectively). Camelina meal is also rich in crude protein (34-37%). In the present work, the effect of the inclusion of camelina by-products in the concentrate feed of feedlot lambs was studied. A total of 105 male lambs of the Manchego breed (35 ± 7 d of age and 13.9 ± 1.7 kg of initial body weight), kept in a commercial farm, were used. The lambs were randomly assigned to one of three treatments (5 replicates of 7 animals per treatment): Control (CON, concentrate based on cereals and soybean meal, and similar to commercial concentrates), Camelina (CAM, where camelina meal replaced 50% of the crude protein from the soybean meal in the control feed), and Fibrous (FIB, concentrate without cereals nor soybean meal, with camelina meal and husks, and 20% less energy). Body weight and feed intake were recorded on a weekly basis for 42 d. On day 42, two lambs from each replicate having the body weight closest to the average of the replicate were tagged to track their carcasses. All the animals were sent to a commercial slaughterhouse, where the carcasses were graded and their hot and cold weights were recorded. Data were analysed using the GLM procedure of SAS UE 3.5 with the treatment as fixed effect and the initial body weight as covariate. Feed intake and feed conversion ratio were 23% higher ($p < .001$) in FIB than in CON and CAM treatments, which were no different between them. There were no differences ($p > .05$) in the final body weight, average daily gain and carcass grades. The cost of the total feed consumed per lamb in the 42-d period was lower in CAM (7.19 €) and FIB (7.45 €) than in CON (7.79 €). In conclusion, the concentrates that included camelina by-products had no negative effects on the productive performance of feedlot lambs and were economically more favourable. Hence, they could be feasible alternatives to typical cereal-soybean meal based concentrates, which are more expensive and do not take advantage of the capacity of ruminants to digest fibrous feeds.

Acknowledgements

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Precision feeding based on TMR system equipped with NIR analyser in dairy cows farm

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The aim of this study was to evaluate the consistency of the total mixed ratio (TMR) and the milk feeding cost in dairy farms equipped with a precision feeding system. The study was carried out on seven dairy farms equipped with the dg precisionFEEDING™ (PF) installed on the TMR wagon. The system, based on a near infrared (NIR) scanner, performs a weight-adjustment operation re-calculating the target weight on the base of the difference between actual DM measured in real time on each feed by the NIR scanner and their theoretical one. A cross-over (two consecutive periods of 14 and 12 weeks each) was applied, and the system in three herds was switched ON in the 1st period and OFF in the 2nd, and in the other herds was OFF in the 1st and ON in the 2nd. The PF system measured daily in all herds the DM of each silage, and the weight-adjustment of silages to be loaded was performed only when the system was ON. The DM effectively delivered every day to the lactating cows was calculated. These results were compared with the data of the recipe. Feeds samples were collected to measure DM, crude protein (CP), starch, and neutral detergent fibre (NDF). Milk yield, feeding cost and income over feed cost were calculated on a weekly basis. The data were processed using analysis of variance. The 5th and 95th percentile of the DM delivered daily was respectively 97 and 109.8% of the recipe when the PF was OFF, and was respectively 99.7 and 105.7% of the recipe when the PF was ON. The majority of the greater values was also a consequence of the operator error, with a tendency to load more concentrate than the value of the recipe. The CP, starch and NDF content of the TMR delivered was closer to values of the recipe when PF was ON compared with OFF. On average the absolute difference between CP of the TMR delivered and the CP of the recipe was 4.2 and 5.5% with PF ON and OFF, respectively; for starch the difference was 10.9 and 12.8%, and for NDF was 4 and 11% for PF ON and OFF, respectively. A progressive reduction and increase of the feeding cost per kg of milk was observed with PF switched ON and OFF, respectively. The difference became significant ($p < .05$) from the 7th week until to the end of the period. The maximum difference between ON and OFF was observed at the end of both periods (0.171 and 0.196 €/kg of milk with system ON and OFF, respectively). This means an increase of the income over feed cost with this PF system of 0.025 €per kg of milk.

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Microbial community structure and VFA profile in the rumen liquids from a continuous culture fermenter and in vivo

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Continuous culture fermenters (CCF) are often used to study rumen fermentation in vitro as an alternative to in vivo trials. Here we have compared the characteristics of the fermentation liquid (FL) from a CCF with the correspondent rumen inoculum (RI). The RI was collected at the slaughterhouse from 24 bulls (8 animals in each of three separate sessions) and used as inoculum for the CCF. The diet fed to bulls was used as a substrate for the fermenters and FL samples were collected on day 6, 7 and 8 of fermentation. RI and FL samples were analyzed for VFA concentration and 16S rDNA amplicon sequencing was used to characterize the bacterial population. Total VFA concentration was lower in FL compared to RI (56.6 *vs* 114.6 mM, $p < .01$). Propionate, butyrate and isovalerate were higher ($p < .01$, 0.05 and 0.01, respectively) in the FL compared to the RI (26.6 *vs* 19.2, 13.1 *vs* 11.3 and 3.45 *vs* 1.38 mol/100mol, respectively), while acetate was lower (55.3 *vs* 66.6 mol/100mol, $p < .01$). Variability in VFA content was estimated by the error (E) from a statistical analysis, which considered the type of fluid within the session. The molar percentage of propionate, isobutyric, butyric, isovaleric and total VFA in the FL had E values (13, 18, 11, 19 and 9%, respectively) lower than for RI (17, 25, 16, 45 and 15%, respectively), while similar values were obtained for acetate (5%). Ion Torrent 16S rDNA amplicon sequencing generated 2.57M high-quality sequences clustered into 4,918 unique OTU's with 11,199 sequences per sample after normalization. At the Phylum level, the *Firmicutes* did not vary between fluids, averaging 25.1%, *Bacteroidetes* decreased by about 20% in the FL (45.0 *vs* 55.3%, $p < .01$), whereas, *Proteobacteria*, *Tenericutes* and *Spirochaetes* increased in FL compared to RI (11.3 *vs* 7.6%, $p < .05$; 2.7 *vs* 0.8 $p < .05$; 11.8 *vs* 5.0%, $p < .01$, respectively). There was no effect on *Fibrobacteres* (averaging 2.1%). Both the Shannons and Simpson index of diversity was lower in FL than in RI (4.90 *vs* 5.63, and 0.973 *vs* 0.984, $p < .01$, respectively) as was the Chao1 estimate of total species richness (1268 *vs* 1965, $p < .01$). Although, the differences in VFA concentrations and microbial populations between RI and FL were statistically significant, the studied CCF environment favored maintenance and growth of the major bacteria phyla found in RI.

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Differential gene expression analysis in broiler chickens in response to dietary larvae Mealworm meal inclusion

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Recent studies on the exploitation of larvae meal inclusion in broiler chickens diet report a significant increase of breast muscle, carcass quality and growth performance. The purpose of this study was to investigate the effects of Mealworm (*Tenebrio molitor*, TM) meal inclusion in broiler chicken diet on global gene expression of four tissues, namely breast muscle, liver, jejunum and caecum. Isonitrogenous and isoenergetic diets were formulated with 0% (control group) and 15% (test group) of TM meal inclusion. 80 one-day-old male broiler chicks (*Ross 708*) were reared in 10 pens (eight birds/pen): the pens were divided in two groups including five experimental replicates for each diet. The birds were slaughtered at 53 days of age. The tissue samples were collected and stored in RNA Later solution (Ambion) for gene expression analysis. RNA-seq was carried out on Illumina NGS analyzer. The results showed that 117, 118, 119 and 182 RNA transcripts were upregulated and 120, 116, 168 and 126 RNA transcripts were downregulated in breast muscle, liver, jejunum and cecum respectively. In all tissues 25% of downregulated differentially expressed genes (DEG) had a fold-change > four; while the 25% of upregulated DEGs had a fold-change higher than four in breast and liver and > two in intestinal mucosa. A gene ontology analysis showed that only half of DEGs were involved in known biological processes including protein metabolism (30 DEGs), gene expression (30), signal transduction (15), and immune system (21). An expression pathway analysis with Reactome showed that the pathway with minor False Discovery Rate (FDR) was the striated muscle contraction in breast and the peptide chain elongation in the other tissues. The ubiquitin B gene (*UBB*), that plays an important role in protein's metabolism, was downregulated (-0.8) in breast and upregulated (+0.57) in liver, whereas the expression of this gene did not change in intestinal mucosa. The differential expression of UBB might suggest an increase of non-lysosomal intracellular protein degradation related to a fast protein turnover in liver and a slow protein turnover in breast. In addition, the ribosomal protein (RPLP) profile, related to peptide chain elongation, was similar: -0.45 mean fold-change in breast and +0.5 in liver while a moderated increase in intestinal mucosa was observed. No alterations in RNA expression were detected that could discourage the use of larvae mealworm inclusion in broiler chicken diet.

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Dietary supplementation with linseed and vitamin E affects sheep immune response in transition period

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The transition period represents a critical stage in sheep farming. Compared to other periods of the production cycle, transition ewes are exposed to high levels of oxidative stress, which can compromise the immunological status of the animals. If the management of the diet is not correctly performed, negative effects on health status, productivity and farm profitability can be easily observed. A number of bioactive compounds can be added to the diet to reduce oxidation and improve immune system. Linseed and vitamin E are commonly used to increase the dietary level of polyunsaturated fatty acids and decrease the oxidative stress, respectively, but few studies have investigated their effects on ewes during transition period. In the present study, 26 Sarda ewes and 22 Lacaune ewes were randomly assigned to one of the three experimental groups: 1) control feed with no supplementation (CTR), 2) CTR supplemented with 22% of extruded linseed (L), 3) CTR supplemented with 22% of extruded linseed and vitamin E (320 ppm) (LE). All concentrates were isoenergetic and isonitrogenous and were administered (from 400 g/d in late pregnancy to 600 g/d in early lactation) with alfalfa hay ad libitum. Antibody production against *Salmonella abortus ovis* after vaccination were evaluated in blood samples collected at 0, 7, 14, 21, 28 and 80 days from vaccination. To evaluate the relationship between diet and antibody production, the odds ratio (OR) analysis and the Pearson chi-square test (χ^2) were performed using the Stata 11.2 software. Significance was declared for $p < .05$. Antibody titers were grouped in two major classes: 1) "HIGH", with values greater than or equal to 1:320; and "LOW", which included values below 1:320. An influence of the dietary treatment on antibody production was detected at 14 days after vaccination: both LE ($OR = 14.4$, $p < .001$) and L titers ($OR = 5.4$, $p < .05$) were higher than those observed in the CTR group. The difference between CTR and LE group was confirmed when analyzing data with the breed included in the model (Lacaune: $OR = 15$, $p < .05$; Sarda: $OR = 13.33$, $p < .05$). The present study shows interesting results concerning the immune state of dairy ewes in the transition period, but further studies are required to better understand the relationships among oxidative status, metabolic pathways and animal welfare.

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TNF-α and adiponectin evolution in lactating ewes and goats and interactions with dietary carbohydrates

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In lactating animals, TNF-α has been associated with reduced galactopoietic activity of growth hormone, increased body fat deposition, and reduced adiponectin concentration. This work assessed possible differences between ewes and goats in TNF-α and adiponectin and their interaction with the type of dietary carbohydrate (starch or highly digestible fiber) used during the lactation.

Twenty-four healthy Sarda ewes and 24 healthy Saanen goats were compared in early and mid-late lactation. After parturition, both species were fed a high starch diet (20.4% starch, 35.5% NDF, 16.2% CP, DM basis). At 52 ± 3 DIM, 8 ewes and 8 goats were slaughtered. At 92 ± 11 DIM, the remaining 16 ewes and 16 goats were divided in two subgroups per species and fed either a high starch diet (HS: 20.0% starch, 36.7% NDF, 15.5% CP, on DM) or a low starch-high digestible fiber diet (LS: 7.8% starch, 48.8% NDF, 15.6% CP, on DM), obtained by partially replacing cereal grains with soyhulls. At

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159 ± 15 DIM all animals were slaughtered. Plasma, and subcutaneous (SAT) and mesenteric (MAT) adipose tissues were sampled and analyzed for TNF-α and adiponectin by using species-specific ELISA test (kits: E06T008, E14T0008, E14A0125, E14A0125. Blugene, Shanghai, China) for sheep and goats. Data were analyzed with PROC GLM procedure.

In early lactation, the ewes had higher blood TNF-α (34.0 *vs* 2.6 pg/ml ±5.1; mean ± SEM; $p < .0001$) and adiponectin (22.7 *vs* 14.6 ng/ml ± 1.5; $p = .001$), TNF-α in MAT (36.8 *vs* 19.6 pg/mg ± 3.6; $p = .012$), and adiponectin in MAT (3.2 *vs* 2.6 ng/mg ± 0.1; $p < .001$) and lower TNF-α (5.2 *vs* 42.3 pg/mg ± 5.9; $p < .0001$) and adiponectin (2.6 *vs* 7.9 ng/mg ± 0.72; $p < .0001$) in SAT than the goats.

In mid-late lactation, the ewes still had higher TNF-α in blood ($p < .0001$) and in MAT ($p = .003$) and lower TNF-α ($p < .0001$) and adiponectin ($p < .0001$) in SAT compared to the goats in the same stage, whereas blood adiponectin was higher in goats ($p < .0001$) and adiponectin in MAT did not vary with species. Moreover, the HS diet increased blood TNF-α compared to the LS diet ($p = .016$) in both species but did not affect adiponectin.

The pattern of TNF-α suggests that adipose tissue synthesis was more intense in ewes than in goats in both lactation stages and in the HS than LS dietary groups in mid-late lactation. The behavior of adiponectin was less clear. In addition, MAT seemed to be more metabolically active in ewes than goats, while the contrary occurred for SAT.

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From lab to farm: using fermentation rates (kd) of corn silage to improve diet formulation of dairy cows

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The CNCPS model estimates nutrient supply using combinations of feed degradation rates (kd) and passage rates (kp). Lab measured kd vs. average values from literature and kp settings used in the feeding formulation might affect the model output. This work quantified the nutrient supply given by the Large Ruminant Nutrition System (LRNS 1.0.33 www.nutritionmodels.tamu.edu; based on the CNCPS, 5.0.40) when feed library or measured kds of the corn silage of seven dairy cow diets from different farms, were used. Only corn silage was preliminary analysed, because it is usually the largest and most variable part of the TMR within farms. The kd values of corn silage CHO fraction in the LRNS feed library (kd starch, B1: 35.0%/hr; kd potentially digestible NDF, B2: 6.0%/hr; LRNS_kd) or those of the farm silage measured in vitro (LAB_kd) were compared. All the other kds were kept constant as LRNS library. Nutrient supply was also compared by combining the LAB_kd with the kp predicted by i) the LRNS (Fox et al., 2004; LRNS_kp) or ii) the NorFor (Volden and Larsen, 2011; NorFor_kp), the latter lower than the former. The null hypothesis that absolute differences between model outputs from different kd or kp rates were equal to zero was tested (paired t-test). The kd of the silage CHO B1 was estimated with rumen-based starch degradability test, whereas the CHO B2 kd was measured *in situ*. A first order kinetic model was applied by using seven hours of incubation times for B1, 48 and 288 hours (the latter to estimate indigestible NDF fraction) for B2. Mean model rates were: LAB_kd B1 25.1 ± 2.7%/hr; LAB_kd B2 2.2 ± 0.7%/hr; LRNS_kp 5.1 ± 0.4%/hr; NorFor_kp 3.9 ± 0.4%/hr. The LRNS kd and kp gave the highest values of metabolizable energy (ME) and protein (MP) intake and concentrations, microbial supply and allowable milk (Table 1). The use of LAB_kd instead of LRNS_kd markedly reduced all these values. The LAB_kd jointly with NorFor_kp vs. LAB_kd and LRNS_kp, caused a less important reduction. When LAB_kd and NorFor_kp replaced the library rates ME, MP supply and allowable milk were reduced, but with smaller extent than when only the LRNS_kd was replaced. Evaluations with real data should support the optimum kp choice for LAB kd practical uses.

Table 1 LRNS estimates for different settings of degradability (kd of CHO B1 and B2 fractions) and passage rate (kp).

Variable	LRNS output with LRNS_kd and LRNS_kp	Difference LAB_kd - LRNS_kd	Difference LAB_kd + Norfor_kp - LAB_kd + LRNS_kp	Difference LAB_kd + NorFor_kp - LRNS kd and kp
ME intake, Mcal/d	58.97 ± 4.39	− 2.92 ± 1.05**	− 0.87 ± 0.36*	− 2.05 ± 1.00*
MP intake, g/d	2529 ± 180	− 163 ± 45**	− 67 ± 28*	− 96 ± 44*
Diet ME, Mcal/kg DM	2.64 ± 0.14	− 0.13 ± 0.05**	− 0.04 ± 0.02**	− 0.09 ± 0.04**
Corn silage ME, Mcal/kg DM	2.59 ± 0.06	− 0.33 ± 0.09*	− 0.09 ± 0.02**	− 0.24 ± 0.10**
Microbial yield, g/d	3811 ± 213	− 418 ± 126**	− 161 ± 56**	− 257 ± 119**
ME allowed milk, kg/d	37.67 ± 3.73	− 2.49 ± 1.03**	− 0.77 ± 0.30**	− 1.71 ± 0.96*
MP allowed milk, kg/d	38.31 ± 4.17	− 4.76 ± 1.23**	− 1.81 ± 0.65**	− 2.94 ± 1.34**

*$p < .01$
**$p < .001$

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Effect of Myrtus berries by-product on milk fatty acid profile in Sarda ewes

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Use of ingredients containing polyphenols in ruminant diet could be useful to modulate biohydrogenation processes of polyunsaturated fatty acids (PUFA), with the aim to improve the fatty acid (FA) profile of milk, in particular to increase concentration of conjugated linoleic acid (CLA) and PUFA, and to decrease that of saturated fatty acids (SFA). The residue of Mirto liqueur production, resulting from the maceration process of myrtle (*Myrtus communis L.*) berries, is a by-product rich in polyphenols. This study evaluated the effect of dietary inclusion of exhausted myrtle berries (EMB) on milk FA profile in sheep. With this aim, 18 Sarda dairy ewes were assigned to three dietary treatments consisting of: a control diet (CON), a basal diet supplemented with 50 g/d per animal of EMB (M50), and a basal diet supplemented with 100 g/d per animal of EMB (M100). Individual milk samples of morning milking at day 2, 6, 8 and 12 of the trial were used for the analysis of FA composition, using a gas-chromatograph coupled with a flam-ionization detector. Data of group of FA were analyzed with the PROC MIXED procedure of SAS. The model included the fixed effects of dietary treatment, sampling date and their interaction, and the random effect of animal. The interaction dietary treatment per sampling date did not affect any group of FA, that were (except total CLA and odd and branched-chain FA) all influenced by sampling date, even if no defined pattern can be individuated. The ratio between PUFA *n-6* and PUFA *n-3* *(n6:n3)* was higher in M100 compared with M50 and CON groups ($p < .05$). The milk concentration of trans fatty acids (TFA) increased in M100 compared with CON and M50 groups ($p < .05$). Milk samples from M100 group showed a higher concentration of total CLA compared with M50 and CON. The supplementation of EMB had no significant effect on milk concentration of short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), SFA, PUFA *n6*, PUFA *n3*, monounsaturated FA (MUFA), PUFA, unsaturated FA (UFA) and odd and branched-chain FA (OBCFA). In conclusion, the supplementation of EMB had a slight effect on milk FA profile with an interesting increase of CLA content in milk of ewes receiving the highest dose of by-product.

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Feed value of dried distiller's faba bean seeds (Faba bean var. equine) with solubles

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Biofuels from renewable energy sources (RES) are necessary for environmental and economic sustainability. Due to the risk of depletion of conventional energy resources, recently RES is in the centre of European policy. Legumes are important sources of oil, fiber, and protein-rich food and feed. They also provide nitrogen to agro-ecosystems. Potentially the legume seeds are a good source of RES (bioethanol). Starch content ranges from 22 to 45% and protein content can be as high as 35%.

The aim of the study was to determine potential feed value of faba bean dried distiller's seeds with solubles (faba bean DDSS) and possibilities of its inclusion to livestock diets.

Research material was six faba bean DDSS -by products of faba bean seeds fermentation for bioethanol. Experimental material was pre-treated in accordance with following experimental scheme: E -with enzymes (α-amylase), PE -acid (phosphoric acid) and enzymes, CE -alkali (calcium hydroxide) and enzymes. After the pre-treatment and mashing, media obtained were filtered and subjected to ethanol fermentation with 2g/kg of rehydrated *Saccharomyces cerevisae* yeast in non-sterile conditions. After fermentation ethanol from media was evaporated by immersing flasks in boiling water bath for 15 min and then cooled to room temperature.

The proximate analysis (DM, CP, CF, CA, EE), fiber fractions NDF and ADF, minerals (Ca, P, Na, Mg) and amino acids profile were determined. The faba bean seeds pre-treated samples affected chemical composition of DDSS clearly.

Statistic differences between groups were verified using two-way analysis of variance (ANOVA).

Obtained data shows that the pre-treatment affected significantly crude ash content ($p<.01$) respectively in FE 2.01%, FPE 4.22%, FCE 7.63% of CA, what directly reflected in Ca, Mg and P content in DDSSs. The highest content of Ca was noted in FCE 16.51% compared to FPE and FE respectively 6.89% and 0.98%. Similarly P content, the highest content of

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the element was found in FCE 15.44% compared to FPE 9.15% and FE 3.61%. The lowest Mg content was stated in FPE 0.94% and the highest in FCE 1.46%. Obtained data showed that the pre-treatment affected protein and lysine content ($p < .05$) significantly, but there was no significantly differences in methionine content between various DDSS.

Taking into consideration chemical composition faba bean DDSS appears to be a valuable feed material for livestock nutrition but to determine actual feed value of these by-products in vivo or in vitro researches are needed.

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Impact of pre-treatment Sucrocorgo 304 bagasse on the nutritional value and the kinetics of drying

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Changes in climate conditions, which can be observed also in the areas of Central Europe, are forcing local farmers in looking for alternative plants, which will enable in difficult conditions to get a high yield of satisfactory quality biomass. Sorghum is well-know due to it's wide application possibilities and is used in food and feed, the raw material for the production of paper, alcohol and biofuels. Also, by-products of sorghum are a source of numerous organic compounds.

The aim of the study was to determine the effect of pre-treatment and drying methods and with various feed value storage time of the dried Sucrocorgo 304 bagasse (solid and liquid fraction) on the nutritional value, kinetics of drying and phenolic content.

The research material was sorghum bagasse: solid (SF) and liquid fraction (LF) subjected pre-treatment acid prehydrolysis (ASD) or base prehydrolysis (BSD). The solid fractions were dried by: convection (CD) and microwave-vacuum (VMD), and the liquid fraction was spray-dried (SD).

Type of pre-treatment had no influence on the drying kinetics of sorghum biomass. The drying method of solid sorghum fractions influenced the drying time. According to own research microwave-vacuum drying reduces the drying time more than 12-fold compared with convection drying. The method of drying solid sorghum fractions did not affect the content of total polyphenols in sorghum by-products stored at different temperatures.

Polyphenol content was expressed as mg of gallic acid equivalents GA/g DM. Polyphenol content in the fresh samples obtained from the distillation differed significantly ($p < .05$). The highest content of polyphenols was stated in sample LF BSD 18.05 mg GA/g DM. Slightly lower polyphenol content was to LF ASD 12.67 mg GA/g DM and about half content determined in samples SF BSD and SF ASD respectively 9.60 and 7.82 mg GA/g DM.

Storage of dried samples for a period of 6 months resulted in a total polyphenols content decrease. The decrease in pholyphenol contents in samples stored at 5 °C was lower than in samples stored at 23 °C.

P150

Bioavailability of phosphorus from various fodder phosphates in lambs

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Phosphorus is one of basic minerals essential in animals feeding for cellulose digestion by rumen microorganisms, for microbial protein synthesis and for playing an important role in almost all energy and nutrients metabolic pathways.

The aim of the present study was to investigate the availability, in lambs (POLISH MERINO × ROMANOWSKA × BERRICHONE crossbreed), of P from various fodder phosphates. Forthy-eight male lambs (2.5 months) were divided into 3 groups and were fed with concentrate feeds and meadow hay (0.2 and 0.4 kg/day/head, respectively). Each group received a diet with a different source of P: monocalcium (MCP), dicalcium (DCP) or calcium-sodium phosphate (CSP). All of experimental animals ingested similar amounts of N: 35.8-36.6 g/day/head. After 70 days of the trial, six lambs (average body weight 40 kg) were selected from each group and used for the digestibility and nitrogen balance experiment. Protein and fat digestibility indices were determined. The apparent digestibility coefficients were determined by subtracting the total amount of nutrients excreted in faeces from the amount of nutrients ingested, which were assumed as absorbed, and dividing this value by amount of nutrients that were ingested. Apparent absorption of N and minerals (P, Mg, Ca) were calculated in the same way. Data were analysed using a one way ANOVA. Data obtained indicated a numerically higher, although not significant, P absorption in lambs that had received DCP in comparison with P bioavailability in animals in other groups (56.8, 54.7 and 51.3 for DCP, MCP and CSP, respectively; $p>.05$). Data concerning apparent digestibility of nutrients and N balance indicate that feed phosphates application in concentrate mixtures had no significant ($p>.05$) influence on digestibility coefficients of CP (78.5%), EE (68.2%), CF (74.3%) and NFE (89.4%). Data indicate a numerically higher, but not significant, retention of Mg and Ca in the DCP group (35.2%, 72.0%). Similarly, DCP had the highest numerical absorption of Mg and Ca (57.0% and 70.6%), although not significantly.

The slight increase in nutrients digestibility coefficients in DCP animals could be explained by a better P supply to rumen microorganisms, a higher apparent absorption of P and a more effective microbial processes increasing availability of nutrients in animals.

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Chemical composition and quality of two varieties of sorghum forages ensiled with various silage additives

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Sorghum is on the fifth place among the most important cereals in the world, with potential applications in the food, energy and feed industries. Sorghum is resistant to drought and it is widely cultivated in subtropical climates where water availability is a limiting factor in crop production. Climate changes, with the occurrence of periodic drought, are challenging European countries, including Poland. Therefore, research is focusing on the possibility of cultivating plants alternative to more water-demanding crops, such as corn.

The aim of this study was to compare different sorghum varieties ensiled with different additives. Research material consisted of two sorghum varieties: S1 (code 12FS901) with dual usage (fodder and grain); and S2 (code NK251) with mainly grain usage. Each plant was ensiled on a laboratory scale in mikrosilos (approx. 1.5 kg of green fodder) using silage additives according to this experimental design: 0 (control, without additive), 1 (bacterial inoculant containing *Lactobacillus buchneri* and *L. plantarum*), 2 (bacterial, enzymatic (glucanase and xylanase) and chemical additives (formic acid)). Therefore, the following 6 silages were prepared: S1-0, S1-1, S1-2, S2-0, S2-1, S2-2.

The proximate analysis (dry matter DM, crude protein CP, crude fiber CF, crude ash CA, ether extract EE), fiber fractions (NDF and ADF) were determined, by standard chemical methods (AOAC 2006). To evaluate silage quality pH, volatile fatty acids (VFA) and aerobic stability were determined (AOAC 2006).

Data were analysed using a one-way ANOVA. Regardless of the additives, DM, CA and CP content were higher ($p<.05$) in all silages prepared from sorghum S2 (37.1%, 6.2% and 7.2%, respectively) in comparison to S1 (33.8%, 4.5% and 6.3%). Conversely, S1 had higher NDF and ADF than S2 (51.75% and 33.38% *vs* 47.98% and 27.3% respectively; $p<.05$). Lactic acid was higher in all S2 silages compared to S1 silages (respectively 76.35% VFA and 52.64% VFA; $p<.01$). Accordingly, the pH in all S1 silages was higher than S1 (4.53 *vs* 4.15, respectively; $p<.05$). All silages were aerobic stable during the 5-days aeration. Therefore, sorghum variety affect the chemical composition of the silage.

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Effects of glycerol esters (Butirflex®) on metabolic profile of Limousine young bulls

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Supplementation of glycerol esters (GE) had positive effect on animal welfare and was able to reduce the antibiotic treatment in monogastrics. Studies in ruminants are not conclusive; therefore, the aim of the present study was to evaluate the effect of GE on metabolic profile of young beef cattle. The GE product (Butirflex®) resulted by esterification of glycerol with butyric, caprylic, capric and lauric acids. The production has been pushed into monolaurin (up to 9%) for keeping the product in the liquid state. Forty-five Limousine young bulls were used. Average body weight of the animals at the beginning of the study was 345 kg and were randomly subdivided into two experimental groups: 18 subjects as negative control (C), and the remaining 27 (T) received 40 g/head/d of GE. The rearing and feeding conditions were the same for both groups. The GE were fed mixed with the TMR. At arrival the animals were vaccinated with a quadrivalent vaccine and were treated with a broad-spectrum antibiotic. The vaccine was repeated after 30 days. Blood samples were taken from all subjects, always in the morning, before the first vaccination and at the booster (after 30 days). The blood was

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immediately centrifuged and the plasma was frozen at −20 °C until analysis. Parameters expression of oxidative metabolism (superoxide dismutase, glutathione peroxidase, ROS), energy (glucose, NEFA, beta-OH butyrate, cholesterol), protein (urea, creatinine), mineral (Ca, inorganic P, Mg, Na, K, Cl), liver function (albumin, AST, gamma-GT, LDH, ALP, total bilirubin) and the inflammatory state (haptoglobin, Zn, ceruloplasmin, globulins) were determined. Data were analysed using the GLM procedure of SAS by factorial model with interactions including group, subject within group, days of control as main effects, and an error term. Least squares means were separated with the PDIFF procedure of SAS. Significance was declared at $p < .05$. No substantial differences were found between the two groups with respect to the observed mortality (1 subject per group), while the percentage of antibiotic treatment (mainly made necessary for breathing problems), within 30 days of the beginning test, was greater ($p < .05$) in the C group. From the analysis of metabolic profiles substantially it emerges a positive effect of administration of GE on the inflammatory state. In particular, treated subjects showed lower ($p < .05$) values of ROS, haptoglobin, glutathione peroxidase and greater ($p < .05$) levels of Zn.

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Influence of rumen fluid sampling techniques on assessment of fermentation parameters and microbial profile in the bovine rumen

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Variations in rumen microbial populations affect fermentations and methane emissions. Differences exist between liquid and solids, and between rumen locations. Therefore it is important to understand, and we aimed to verify, the extent to which the rumen sample collection methods affect the fermentation and microbial profile of the samples obtained from dairy cattle.

Samples of rumen fluid were obtained from three cannulated non lactating dairy cattle fed a high forage diet by three collection methods (grab sampling through rumen cannula (FIS), rumenocentesis (RC) and oral stomach tube (OGT)) before feeding (T0) and, two weeks later, 6 hours after feeding (T6). The samples (18 in total) were analysed for physico-chemical characteristics and microbial community diversity. Time points and collection technique affected rumen pH (7.06 and 6.74 for T0 and T6, respectively, $p < .001$; 6.72, 6.84 and 7.14 for FIS, RC and OGT, respectively, $p < .01$) and volatile fatty acids (VFA) concentrations ($p < .01$) but not their molar ratios ($p > .05$). Microbiota composition was qualitatively and quantitatively evaluated using culture-independent methods (PCR-DGGE, and Real-time PCR). Qualitative analysis of the Eubacteria population revealed individual profiles that differed between the sampling times but not between the sampling techniques. qPCR revealed a reduction of Eubacteria content in ruminal fluids collected from cows using the OGT ($p < .05$), not associated to a lower content of Archaea and Protozoa. Samples collected by RC give the best approximation to samples collected through FIS. Collection by OGT is associated with risks of saliva contamination and the sampling location is uncertain. Nevertheless, this does not affect proportion of VFAs and microbial profile.

This study demonstrates that sampling technique affects the comparability of the results obtained by different monitoring techniques. Understanding the effects of sampling technique is fundamental for a better understanding of biology and biochemistry of methane emissions, manipulation of the ruminal microflora and the diagnosis of digestive disorders.

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P154

A focus on the project: bioconversion of fruit and vegetable waste to earthworm meal as novel food source

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Recycling and redevelopment of organic wastes from the food chain is a priority for a more healthy and environmental-friendly food production. Bioconversion of organic waste in a novel food source can be an alternative to waste disposal contributing to reduce GHG emissions and responding to global food demand.

This project integrates various expertise to plan the food chain based on vegetable waste bioconversion to earthworms as potential new food. Among the terrestrial invertebrates, utilization of earthworms may be an answer ecologically, economically and socially acceptable as an alternative protein source. Vegetable waste discarded directly from the producers, complies with the relevant EU feed regulations for food producing animals.

The project follows a multi-factor approach based on demand-driven innovations and the inclusion of various partners/actors (University of Bari and IULM, CREA-FLC, SMEs). The sustainability of the entire cycle will be evaluated considering the environmental, economic, ethical and social impacts.

The main original elements of the proiect are to:

- propose *Eisenia foetida* as foodstuff
- predict the acceptability and intention of eating the new food source by a questionnaire
- evaluate of the ethical/social impact
- utilize the fruit and vegetable waste as safe, cheap and sustainable substrate of rearing
- propose an innovative and cost-effective production system
- evaluate the environmental aspects of this new process
- evaluate the HACCP of the production chain
- ensure the quality and safety of the food derivative product
- elaborate an industrial code of practices/standards
- safeguard EU animal welfare
- provide information useful for the regulatory frameworks
- propose earthworm meal for particular nutritional purposes or claim (e.g. low glycemic index, reducing blood cholesterol, high protein, source of [name of vitamin/s] and/or [name of mineral/s], high polyunsaturated fat ….)
- train sensory panel for consumer evaluation.

The first results of this project from the joint industrial stakeholders and scientists collaboration show how earthworms can be used as food source. Furthermore, our study highlights the motivational domain in promoting the intention to eat annelids-based products as new food. Some implications for practice are discussed.

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P155

Wheat bran as dietary tool to improve dairy production, oxidative status of lactating cows and food sustainability indexes

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Wheat bran (WB), an inexpensive by-product widely available in southern Italy, is largely used as component of feed for ruminants, contributing to decrease the use of food suitable for human consumption, thus to improve the sustainability of livestock production. However, the potential benefits of WB, due to its content in polyphenolic compounds, mainly consisted of ferulic acid, in improving rumen conditions and the antioxidant balance of animals, and also providing animal products with functional properties, are not yet well known. Accordingly, this experiment was carried out for 100 days with 36 lactating Italian Simmental cows divided into 3 groups receiving one of 3 concentrates including WB at 0% (WB0), 10% (WB10) or 20% (WB20), formulated to be isoenergetic and isoproteic. During the trial, the group feed intake and the individual milk production were monitored, and cheesemaking of bulk milk were carried out. Statistical analyses were performed using MIXED (individual data) and GLM (cheese traits) procedures in SAS 9.2. Milk yield was similar among groups throughout the trial. Milk from WB20 group resulted slightly higher in casein and curd firmness (a_{2r}). In cows fed WB, the higher intake of polyphenols, especially ferulic acid, was responsible of a higher blood content of polyphenols, which had an impact on reactive oxygen metabolites (ROMs), resulted significantly higher in WB0 cows (115 *vs* 106 and 1045 U. Carr in WB0, WB10 and WB20; $p \leq .05$). WB20 cheeses showed, compared to WB0, a tendency to have greater total polyphenol content (4.21 *vs* 3.65 mg GAE/g, $p \leq .10$), lower number of peroxides (1.04 *vs* 1.30 mEqO$_2$/kg, $p \leq .05$) and higher antioxidant capacity (1848 *vs* 1518 μmol FeSO$_4$/g, $p \leq .10$), with intermediate values in WB10. WB20 diet, due to WB low cost, reduced the feeding cost for cow and for kg of milk yield, in comparison with the WB0 diet. In addition, the WB20 group showed the best indexes heFCE (human edible feed conversion efficiency = milk/human edible feed) and NFP (net food production = human edible food/milk), expressed as crude protein or gross energy. In conclusion, the WB ingested by dairy cows, at a level of about 12% of total DM intake seems lead to several benefits, such as the improvement of oxidative status of cows, milk quality, shelf-life characteristics and nutraceutical properties of cheese, as well as it can contribute to reduce the feeding cost per unit of product and limit the human-animal competition for feeding sources.

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Tenderness development and proteomic changes in lamb meat: effect of quinoa and/or linseed supplementation

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It is well known that dietary supplementation can improve meat nutritional quality, on the contrary, no data are available on the effects of enriched n3 supplementation on tenderness variation. This study aimed to evaluate the effect of dietary supplementation on linseed and/or quinoa on tenderness and on proteome of lamb meat. Thirty-two Italian Merino male lambs after weaning (42 ± 2 days of age) were distributed into 4 groups with different diet: control (CO) with no supplemental fat, linseed (LS), quinoa (QS) and QS + LS diets. pH at 1, 3, 6 and 24 h, total collagen content, Warner Bratzler shear force (WBSF), texture profile analysis and changes of myofibrillar and sarcoplasmic proteins were estimated. Proteolysis was investigated by SDS-PAGE (8-18%), western blotting and two-dimensional electrophoresis (2DE) coupled to mass-spectrometry. All data were subjected to an analysis of variance using the GLM procedure including fixed effect due to dietary supplementation. pH was affected by diet supplementation showing the highest values at 1 and 3 h post-mortem in LS ($p < .01$), while QS and QS + LS groups showed intermediate pH values. Meat from control group showed higher values ($p < .01$) in the collagen content than meat from other groups. Tenderness was affected by diet supplementation showing the lowest values of WBSF ($p < .001$), hardness ($p < .01$), gumminess ($p < .01$) and chewiness ($p < .01$) in meat from linseed group. SDS-PAGE analysis revealed that meat from QS, LS and QS + LS groups were characterized by lower values of myosin heavy chain (MHC; $p < .001$) and higher values of 190-130 kDa ($p < .05$) bands than control group. In linseed group proteomic analysis revealed a degradation of desmin ($p < .05$) and TnT proteins complex, together with the highest Myosin Light Chain 2 (MLC2; $p < .01$) and 32-25 kDa ($p < .001$) bands. Proteins separation with 2DE revealed a major number of spots and phosphorylation isoforms of fast MLC2 patterns in meat from linseed group. On the contrary, meat obtained by lamb fed quinoa showed a major number of spots ascribed to sarcoplasmic proteins and fragments of MHC. Data suggest that diet supplementation may act on meat tenderness and on proteolytic pattern of myofibrillar fraction.

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