

The animals were monitored in order to ensure that the hay was completely ingested. Individual milk samples were collected at 0, 15, 20, 25, 30, 35, 40 days after the first buckwheat hay administration.

The rutin determination in buckwheat hay and milk was performed by a HPLC method. After 15 days of buckwheat hay administration, the lower rutin content in milk was 0.89 mg/kg. The presence of rutin in milk may constitute a non-hydrolysed aliquot or may indicate a difference between monogastrics and ruminants. This also could be in agreement with the literature which indicates that urinary and biliar excretion of flavonoids and their metabolites may be the main metabolic pathways for such compounds.

It is well known that flavonoids have biological activities and they could be used for preparing fortified foods, these compounds could be added to milk and other food.

The study in progress will be useful to evaluate the rutin carry-over from buckwheat hay to milk and the possible nutraceutical applications of this milk to improve health benefits in humans.

## 0097

### Use of donkey milk in infant feeding

Iolanda Altomonte<sup>1</sup>, Elio Novembre<sup>2</sup>, Giovanni Brajon<sup>3</sup>, Fina Belli<sup>4</sup>, Franco Corrias<sup>3</sup>, Rosario Licitra<sup>1</sup>, Federica Salari<sup>1</sup>, Lucrezia Sarti<sup>2</sup>, Mina Martini<sup>15</sup>

<sup>1</sup>Dipartimento di Scienze Veterinarie – University of Pisa, Italy

<sup>2</sup>Unità di allergologia, Dipartimento di Pediatria, Anna Meyer Children's University Hospital, Firenze, Italy

<sup>3</sup>Istituto Zooprofilattico Sperimentale del Lazio e della Toscana 'M. Aleandri', Firenze, Italy

<sup>4</sup>Unità di Nutrizione, Dipartimento di Pediatria, Anna Meyer Children's University Hospital, Firenze, Italy

<sup>5</sup>Centro Interdipartimentale di Ricerca Nutraceutica e Alimentazione per la Salute – University of Pisa, Italy  
Contact: [altomonte@vet.unipi.it](mailto:altomonte@vet.unipi.it)

There are still few literatures about the role of donkey milk (DM) in human nutrition and increasing knowledge is crucial in order to provide practical advice for DM consumption. The aim of this study was to monitor nutritional quality, hygiene and health risks, and the impact of DM in the feeding of children with cow's milk protein allergy (CMPA). DM was supplied by a farm located in central Italy, conforming with EU regulation 853/2004. Eighteen pasteurised milk samples (at 65 °C for 30 min) were taken monthly. Pasteurised DM showed a total average viable count of 4332.22 CFU/mL ( $\pm 3046.78$ ), a slightly alkaline pH ( $7.12 \pm 0.17$ ), a lactose percentage of  $6.83 \pm 0.34$ , a total protein percentage of  $1.63 \pm 0.19$ , while casein was  $0.81\% \pm 0.11$ . Fat percentage ( $0.51 \pm 0.52$ ) was lower compared to ruminant milk and about 48% of the total milk fatty acids were unsaturated. In addition, DM contained  $7.52 \pm 2.49$  g/100 g of fat of n3 linolenic acid.

Eighty-one children with CMPA referred to the Allergy Unit of the Anna Meyer Children's Hospital were recruited. They underwent to an allergological work-up including an oral food challenge (OFC) with DM; during the OFC the palatability of the milk was also evaluated. In children  $\geq 3$  years of age, DM palatability was assessed with a specific Wong-Baker modified pain scale, while in children  $< 3$  years of age it was assessed through the physician's judgment. The results of the allergological work-up showed that DM did not cause allergic reactions in the 98.7% of patients, in addition, a good palatability of the milk was found. DM was included for six months in nutritional plans for 16 children with IgE-mediate CMPA (mean age of  $20 \pm 18.4$  months at the beginning of the study) and six with Food Protein-Induced Enterocolitis Syndrome (mean age of  $5.33 \pm 1.75$  months). The daily dose of DM varied from a maximum of 1000 mL to a minimum 200–250 mL according to the age of the children. Given the low fat of DM, the diet of the children was supplemented with extra virgin olive oil (EVO) according to the age (from a minimum of 1.5 g of EVO and 1.5 g of Medium Chain Triglycerides vegetable oil in each 100 mL of milk to a maximum of 8–10 mL of EVO added to the daily meals). All the children that underwent to the nutritional plans were monitored twice (at the beginning and at the end of the study) for the auxological parameters. The results showed that DM did not change the normal growth rate of allergic children.

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## ANIMAL PHYSIOLOGY, HEALTH AND WELFARE – REPRODUCTION I

## 0098

### Quality of chicken semen cryopreserved with different N-methylacetamide concentrations combined with trehalose

Fabio Mosca, Ahmad Abdel Sayed, Maria Grazia Mangiagalli, Silvia Cerolini, Luisa Zaniboni

Dipartimento di Medicina Veterinaria, University of Milano, Italy

Contact: [fabio.mosca1@unimi.it](mailto:fabio.mosca1@unimi.it)

The study aimed to assess the effect of different N-methylacetamide (MA) concentrations combined with trehalose on the quality of post-thawing chicken semen. Forty adult Hubbard male chicken breeders were used. All chickens were housed in individual cages at 20 °C, exposed to a 14L:10D photoperiod and fed a commercial standard chicken diet. Males were divided into four different groups (10 males/group). All the ejaculates collected within one group were pooled together, then splitted into three aliquots, each

one assigned to one treatment. Semen collection was repeated on three days, 36 semen samples (12 replicates/treatment) were collected into graduated tubes and volume and concentration were recorded. Semen aliquots were diluted to a concentration of  $1.5 \times 10^9$  sperm/mL using a Lake pre-freezing modified extender added with 0.1M trehalose. The diluted semen was cooled at 4 °C for 30 min and transferred to the laboratory. MA was added into semen aliquots to obtain 6 (M-6), 3 (M-3) and 0% MA final concentration (M-0 treatment) and  $1 \times 10^9$  sperm/mL working concentration. Semen was packaged in straws (0.25 mL), frozen for 10 min over a nitrogen bath at 3 cm of height and thawed in water bath at 38 °C for 30 s after 7 days. Sperm cell membrane integrity (SYBR-14/PI staining) and motility (SCA system) were assessed in both fresh and thawed semen. The recovery rates (%) of undamaged and motile cells after freezing/thawing were also calculated. Analysis of variance was performed considering time and MA concentration as sources of variation. Sperm quality parameters were significantly ( $p < .001$ ) decreased by the freezing-thawing process and the overall mean values were consistent to those reported in scientific reports. Significant differences ( $p < .05$ ) were found among the MA treatments: membrane integrity and sperm motility were improved in semen cryopreserved with 6% MA ( $15 \pm 0.9\%$  and  $21 \pm 1.3\%$  respectively) compared to 3% ( $7 \pm 0.9\%$  and  $15 \pm 1.3\%$ ) and 0% ( $1 \pm 0.9\%$  and  $14 \pm 1.3\%$ ) MA. Several sperm kinetic parameters and the recovery rates of undamaged, motile and progressive motile sperm followed the same trend, presenting the highest value in M-6 treatment. The present results confirmed that the treatment with 6% MA is the most effective to prevent sperm membrane cryodamage and motion impairment.

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## O099

### Influence of germ line chimerism on chicken reproductive traits

Marek Bednarczyk<sup>1</sup>, Maria Wiechetek<sup>1</sup>, Ewa Lukaszewicz<sup>2</sup>, Artur Kowalczyk<sup>2</sup>

<sup>1</sup>*Katedra Biotechnologii i Genetyki Zwierząt, UTP University of Science and Technology, Bydgoszcz, Poland*

<sup>2</sup>*Instytut Hodowli Zwierząt, Wrocław University of Environmental and Life Sciences, Wrocław, Poland*  
Contact: [bednarczyk@utp.edu.pl](mailto:bednarczyk@utp.edu.pl)

Germline chimaeras are one of the best tools for studying bird embryo development, as well as the transgenic bird's production or endangered species. The production of germline chimaeras involves the incorporation of exogenous primordial germ cells

(PGCs) into the endogenous gonadal tissue of the recipient embryo. The experiment described here was designed to study the effect of germline chimerism on reproductive traits of chicken. Chimaeras were created from purified PGCs isolated from gonads of 5.5-day old donor Green-legged Partridge-like embryos and injected into blood vessels of 3-day old recipient White Leghorn embryos. Two hundred and nineteen chickens hatched from 327 injected embryos (incubation rate – 67%), and 217 of them (99%) were raised until maturity, and germline chimerism was identified by PCR method. In 34 germline chimaeras – CH group/19 cocks and hens 14/, identified by PCR method, and in 18 control birds – C group (not manipulated), raised and housed in the same environmental conditions, 41 reproductive traits were investigated. For statistical evaluation of the results, two-way analysis of variance (ANOVA) followed by Duncan's multiple-range test was applied. Chimerism significantly ( $p \leq .01$ ) affected 9 of the studied traits. In most cases, they concerned the male traits. Sperm volume, sperm concentration, and percentage of live normal spermatozoa ( $0.338$  mL;  $365.8 \times 10^6$ /mL; and 94.6%, respectively) were lower in chimaeras, compared to the control group ( $0.427$  mL;  $452.0 \times 10^6$ /mL; and 97.6%). This impact affected also some kinematic parameters of motile spermatozoa, analysed using a computer-assisted sperm motility analysis (CASA) system. Especially, linearity coefficient (LIN), straightness coefficient (STR), and mean amplitude of lateral head displacement (ALH). In case of hens, only the per cent of late dead embryos was significantly higher ( $p \leq .05$ ) in CH group (25.8), compared to C group (16.7). In addition, one hen and two sterile cocks were identified. Our study indicated that germline chimerism may affect some reproductive abilities of chicken chimaeras. Possible reasons for the observed reproductive traits depression were discussed in the light of PGCs competition in the recipient birds.

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## O100

### How can the honey improve the post-thaw quality of turkey spermatozoa?

Michele Di Iorio<sup>1</sup>, Giusy Rusco<sup>1</sup>, Angelo Manchisi<sup>1</sup>, Silvia Carolini<sup>2</sup>, Nicolaia Iaffaldano<sup>1</sup>

<sup>1</sup>*Dipartimento di Agricoltura Ambiente e Alimenti, University of Molise, Italy*

<sup>2</sup>*Dipartimento di Medicina Veterinaria, University of Milano, Italy*

Contact: [michele.diiorio@unimol.it](mailto:michele.diiorio@unimol.it)

The most feasible biotechnologies for *ex situ* conservation of genetic resources in avian species is semen cryopreservation. However, research on turkey semen cryopreservation is not yet