

At 148 DIM, basal plasma glucose (62.2 vs. 51.6 mg/dL  $\pm 3.62$ ,  $p=.011$ ) and insulin (0.34 vs. 0.13  $\mu\text{g/L} \pm 0.09$ ,  $p=.036$ ) concentration were significantly higher in ewes than in goats. After glucose infusion, glucose ( $p=.06$ ) and insulin ( $p=.07$ ) concentrations were numerically greatest in ewes. The area under the glucose concentration curve, fractional glucose turnover rate and half-time were not affected by species or diet, in both stages of lactation. Quantitative Insulin Sensitivity Check Index (QUICKI) and Revised QUICKI were higher in goats than in ewes in both stages of lactation, while the Homeostasis Model Assessment (HOMA) was highest in ewes. In conclusion, the highest plasma glucose, insulin and HOMA observed in ewes suggested the presence of an insulin resistance status, which was more marked in mid than early lactation and which can be the cause of greatest body fat deposition and lowest milk yield persistency observed in this species.

## P066

### Comparison among four different bacterial DNA extraction protocols for analysing milk metagenomics

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Bovine udder is colonised by a huge number of microorganisms that constitute the intramammary ecosystem, with a specific role in modulating not only the udder homeostasis and mastitis susceptibility but also the quality of the dairy products. Therefore, information on milk microbiota composition will facilitate the dairy industry in the production of safe and high-quality products. However, generating high-quality bacterial DNA could be critical.

In the present study, bacterial DNA from healthy milk samples was isolated by four different protocols to evaluate the effect of the extraction procedures on milk microbiota composition. For the characterisation of the milk microbiota by 16S deep sequencing, 500 mL of bulk tank milk samples were aseptically collected from three different farms and bacterial DNA was extracted by using an internal laboratory protocol and three commercial kits. Bacterial DNA was then amplified using the primers for the V3–V4 hypervariable regions and sequenced in one MiSeq (Illumina) run with  $2 \times 250$ -base paired-end reads. Data analysis was

performed by using QIIME suite and SILVA 132 as a reference database for taxonomy.

The results showed that the four extraction kits performed very differently and showed a significant separation on both microbial richness (alpha-diversity) and composition (beta-diversity). In particular, the relative abundance of some genera (e.g.: *Lactobacillus*, *Acinetobacter* and *Microbacterium*) were consistently altered by the extraction method. Based on these data, then, particular attention must be kept in choosing the proper extraction method, for example, carefully evaluating eventual biases towards or against bacterial genera of interest. Moreover, we believe that, in order to define which kit best resembles the original bacterial community, an additional set of experiments with a mock community with known composition should be performed.

#### Acknowledgements

The research is funded by the framework of Bando AGER - seconda edizione Progetto 'FARM-INN' grant 2017-1130.

## P067

### Effect of weeping teats on milk microbiome in goats

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In dairy goats, variability in teat conformation is often observed, such as the presence of the so-called 'weeping teats'. This trait, observed in goat breeds selected for high milk production, is characterised by the presence of milk-secreting tissue in the wall of the teat where the milk can pass through skin pores out to the external epithelial surface and be released onto the skin surface, resulting in a 'weeping teat'. The aim of the study is to characterise the milk microbiota composition of the abnormal trait 'weeping teats' in Italian Saanen and Alpine goats in order to understand the effect of this trait on the milk microbiome.

For the characterisation of the milk microbiota by 16S rRNA-gene sequencing, milk samples were aseptically collected from three different herds for a total of 46 weeping-teats and 32 normal teats. Bacterial DNA was extracted by using an internal laboratory protocol, then amplified using the primers for the V3–V4 hypervariable regions and sequenced in one MiSeq (Illumina) run with  $2 \times 250$ -base paired-end reads. On average, 97,098 reads per sample were obtained: after quality filtering (Phred >19), 68.3% of the reads were retained for subsequent analysis. After

removing OTUs with  $\leq 10$  counts in  $\leq 2$  samples, a total of 6675 OTUs were detected. The milk microbiota was dominated by the phyla *Firmicutes*, *Proteobacteria* and *Actinobacteria*, with little differences between weeping and normal teats (e.g. 46% vs. 42% *Firmicutes*, 26% vs. 29% *Proteobacteria*, 16.7% vs. 16.9% *Actinobacteria*, respectively). Alpha diversity metrics were very similar between weeping and normal teats, with no comparison showing a significant difference.

#### Acknowledgements

The research was funded by the University of Milano, Linea 2-Azione A, PIANO DI SOSTEGNO ALLA RICERCA 2017 – Fondi di ricerca del Dipartimento di Medicina Veterinaria.

## P068

### Gut microbiome response to dietary prevention regimes in pre-weaning piglets

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Post-weaning diarrhoea (PWD) represents the most important threat for the pig industry all over the world with a morbidity over 50% among weaned piglets during outbreaks of the disease. Given the public health concerns about the spread of multi-resistant bacteria due to the use of antibiotics in livestock, it is necessary to develop alternative strategies to restore microbial balance and control post-weaning diarrhoea in piglets. To date, the most promising alternative strategies are mainly based on the use of substances that act on bacteria indirectly by stimulating the immune system, or by improving gut health.

Therefore, the aim of our study was to evaluate the effect of an alternative treatment compared to antibiotics on supporting the health of the gut microbiota of pre-weaning piglets. Twenty-four litres were randomly divided into the following 3 treatment groups: (i) basal diet without any preventive treatments; (ii) basal diet supplemented with a mixture of garlic and oregano essential oil (500 g/ton of feed) and without any preventive treatments; (iii) basal diet not supplemented but using preventive antibiotics (cefquinome: 2 mg/kg BW for 5 days while castration and tattooing, tildipirosin: 4 mg/kg at weaning, amoxicillin: 20 mg/kg BW per os for 5 days at weaning) and antiparasitic (toltrazuril: 20 mg/kg BW at birth) treatments.

For metabarcoding analysis, rectal swab samples were individually collected at four time points (from birth to weaning) and the V3–V4 hypervariable regions of the bacterial 16S gene were sequenced in one MiSeq (Illumina) run.

Results revealed that the gut microbiota of pre-weaning piglets is dominated by the phyla *Firmicutes* (51%), *Bacteroidetes* (25%) and *Proteobacteria* (16%), which together make up for 92% of all microbes. The gut microbiota clearly changed over time: from the analysis of variance, all taxa showed significantly different relative abundances across time points, as well as alpha diversity indexes; based on the matrix of Bray–Curtis dissimilarities, samples clustered separately per time point. On the other hand, no clear differences among treatments were observed in terms of taxa abundances or alpha- and beta-diversity indexes. These preliminary results suggest that the tested treatments do not seem to exert major effects on general properties of pre-weaning piglets' gut microbiota, however, modifications of specific taxa may occur.

## P069

### Survey on mycotoxin content in feed and milk from sows

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The transmission of mycotoxins and their metabolites between animals and humans or between animals (mother-child) can occur through milk, but data for the pig are scarce. The study investigated if the intake of mycotoxins by the sow leads to a transfer of the same to piglets, through the suckled milk. Furthermore, the study evaluated the level of the main mycotoxins in farm feeds for sows.

Samples of the feed for lactating sows were obtained in 19 farms that (1) have an in-house feed mill; (2) administer the same batch of feed at least 5 days before the farrowing until the time of sampling (1st day of lactation); (3) preferably do not use chelating agents or warn of their presence. Per each farm, colostrum was obtained from 2 or 3 sows. The content of aflatoxin B1/B2/G1/G2 (AF), fumonisins (FU), deoxynivalenol (DON) and zearalenone (ZEA) of feeds was assessed by commercial ELISA kits, and confirmed by LC-MS at Laemmegroup lab.

Levels of mycotoxins present in the feeds were in general very low (10; 12; 17; 2 positive samples for AF, FU, DON and ZEA, respectively), always within the limits of the law or recommended, except