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Italian Journal of Animal Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

ASPA 24th Congress Book of Abstract

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To cite this article: Roberto Mantovani & Alessio Cecchinato (2021) ASPA 24th Congress Book of Abstract, Italian Journal of Animal Science, 20:sup1, 1-236, DOI: 10.1080/1828051X.2021.1968170

To link to this article: https://doi.org/10.1080/1828051X.2021.1968170

Italian Journal of Animal Science

Official Journal of the Animal Science
and Production Association

Online ISSN: 1828-051X

Taylor & Francis
Taylor & Francis Group

Italian Journal of Animal Science

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Italian Journal of Animal Science

volume 20, supplement 1, 2021

ASPA 24th Congress

Padova, September 21–24, 2021

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Padova, September 21-24, 2021

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sealant and oral administration of 200 mL of homogenate *A. arborescens*. *Aloe arborescens* was administered in the morning during the distribution of the total mixed ration for 14 days (7 days before up to 7 days after drying). For 16S rRNA-gene sequencing and volatilome analyses, rumen liquor and fecal matter were collected fourteen days before (T0) dry-off, at drying-off (T1) and seven days after dry-off (T2, only fecal samples). The V3-V4 hypervariable regions of the bacterial 16S gene was sequenced in two MiSeq (Illumina) runs with 2×250-base paired-end reads. No significant differences were observed for alpha- and beta-diversity between treatments along the three timepoints in the rumen microbiome. Conversely, according to all indices except evenness (equitability, simpson_e) the alpha diversity of the hindgut microbiome increased significantly (p-values in the range 0.002 – 0.011) in the ASIG group at T2. Regarding beta-diversity, the hindgut microbiome showed a statistically significant (p-value =0.0479) separation between treatments. Independently from sampling time and treatments, the bacterial community of the hindgut was dominated by Bacteroidetes (~40%) and Firmicutes (~48%); rumen showed prevalence of Bacteroidetes (~45%), Firmicutes (~25%) and Proteobacteria (~12%). In rumen, due to the high variability for all the metabolites no significant differences were observed between T0 and T1. In conclusion, the dietary supplementation with Aloe arborescens seems to have a sizable effect on the composition of the dairy cow gut microbiome, but not at the rumen level.

Acknowledgements
RABoLa, co-funded by the Region Lombardia D.d.s.21.12.2018 n. 19442.

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Insights into the faecal microbial phenotyping of pig using a Biolog Ecoplate method

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Gut microbiota plays a key role in the development and maintenance of heath on livestock and contributes to their productive efficiency. Although gut microbiota taxonomy characterization has been increasingly investigated, little is known about its functional characteristics and phenotyping. To fill this gap, Biolog EcoPlates™ could be applied, however, little is known about its application on pig gut samples. This study aims at evaluating the use of Biolog EcoPlatesTM to characterize the metabolic potential and activity of the microbial community and to investigate the effect of (1) different storage conditions (fresh vs. frozen) (2) optimal cell concentrations (3) specific length of storage conditions of pig faeces.
Two assays were performed aimed at evaluating differences in the metabolic activities between fresh and snap-frozen faeces at different dilutions (approximately 9×105 cells/g, 9×104 and 9×103 cells/g) and at different times of storage at –80 °C [15 h (T0) and 15 (T1), 45 (T2) and 150 (T3) days after collection]. Furthermore, the V3-V4 regions of the 16S rRNA gene were analysed to describe the biodiversity of community composition and predicted functionality.
Metabolic capacity of microbial community was detected for 31 lyophilized relevant C substrates, that were grouped by chemical classes (8 carbohydrates, 8 carboxylic acids, 4 polymers, 6 amino acids, 2 amines and 3 miscellaneous substrates). Results highlighted that snap freezing of pig faecal samples preserved the metabolic activity of the microbial community compared with fresh faeces ($p > .1$). Sample storage at –80 °C for 150 days did not affect the metabolism of the microbial community, whose activity remained stable throughout the study period ($p > .1$). The carbon source utilization by pig faecal microbiota was significantly affected by bacterial cell density ($p < .05$). A cell concentration of 104 and 105 cells/g allowed detecting the highest metabolic activity of the microbial community. Overall, after 96 h of incubation, carbohydrates were the most frequently metabolized carbon source, while amines were the least.
In conclusion, results evidenced that the functional metabolic activity of the pig faecal microbial community can be preserved without significant variation until 150 days of storage at –80 °C. The Biolog EcoPlatesTM technology represents a rapid and useful method to explore the metabolic capabilities of the microbial community in animal samples.

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Effect of diets containing *Hermetia illucens* on rainbow trout microbiota: DGGE and NGS approaches

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Hermetia illucens (Diptera: Stratiomydae; H) larvae are commonly studied as aquafeed ingredient due to their nutritional composition comparable to that of fishmeal. Different dietary formulations are reported to have different effects on fish gut microbiota, which, in turn, modulate fish digestion, immunity, energy balance, social behaviour and more. The previous studies reported contradictory results regarding the effect of insect-based

diets on the microbiota of salmonids. Moreover, to the author's knowledge, the effect of dietary full-fat insects on fish microbiota has not been addressed up to now.

To fill the above-mentioned gap, the aim of this study was to elucidate the effect of a diet containing full-fat H larvae meal (H50, 50% substitution level of fishmeal with full-fat H larvae) on the gut microbiota of rainbow trout (*Oncorhynchus mykiss*) in comparison to a control diet containing fishmeal (H0). Microbial DNA from pyloric caeca mucosa (PC), pyloric intestine content (PIC), mid intestine mucosa (MI) and mid intestine content (MIC) from five fish samples per diet was extracted and subjected to denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing (MiSeq, Illumina; HTS). Irrespective of the diet, the analysis of selected DGGE bands (excision, sequencing and closest relative search on BLASTN, NCBI) showed that the microbial communities were dominated by *Bacillus* sp. and *Staphylococcus* sp., with sparse *Streptococcus* sp., *Mycoplasma* sp. and *Shigella* sp. Bacterial relative abundances resulting from HTS analysis showed the domination of Proteobacteria in all samples (up to 85% relative abundance), followed by Firmicutes, Actinobacteria and Bacteroidetes. Fusobacteria were almost only found in MIC and PIC extracted from fish fed H0 diet. The differences between dietary groups were not captured by alpha-diversity (observed OTUs, Shannon's entropy, Pielou's evenness, tested with a Kruskal–Wallis test) or beta-diversity indices (unweighted unifrac, Jaccard, robust Aitchison metrics, plotted on PCoA and tested with a PERMANOVA).

Further studies with a higher number of replicates might be able to find significant differences between dietary treatments and are needed to postulate a clearer hypothesis on the microbiota structure and diversity in fish fed dietary insects. That knowledge would pave the way to unravelling the functions of the microbiota and to understand the mechanistic laws at the root.

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Anthocyanins dietary supplementation in lambs: effects on gut microbiome

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In ruminants, the gastrointestinal tract is colonized by highly concentrated and variable populations of microorganisms, particularly bacteria, archaea, fungi, ciliated protozoa and viruses. Age, diet and management influence bacterial community both for proportions and for diversity. At the first stages after lambing, lambs are functional monogastric animals and low rumen bacterial activity leads to a greater influence of the diet on the gut microbiome composition. The aim of the present study is to assess the effect of anthocyanins on the fecal bacterial microbiome and microbiota of lambs. A total of 44 Merino male lambs of 25 days were randomly divided in a control group (CG; $n = 22$) and anthocyanins group (AG; $n = 22$). All lambs were fed with alfalfa hay and starter ad libitum and only the AG received a red orange and lemon extract with an 85% anthocyanin concentration (90 mg/kg live weight calculated each two days). Lambs were slaughtered at 40 days and fecal samples were sterile collected from rectum and frozen at –20 °C until analysis. Analysis of fecal microbiome was carried out by metabarcoding analysis of 16S rRNA. After reads denoising, sequences were aligned against SILVA rRNA sequence database using MALT, and taxonomic binning was performed with MEGAN. Regardless of the dietary treatment, Proteobacteria and Firmicutes were the predominant bacterial phyla identified. The amount of Firmicutes was 10% and 15% in the AG and CG respectively. Moreover, the amount of Actinobacteria was almost two-fold in the AG than CG. At genus level, Acinetobacter percentage of number of reads recorded double values in the AG than CG, while an increase of Psychrobacter and Streptomyces was observed in AG compared to CG. Dietary supplementation of anthocyanins reduced the relative abundance of Enterobacteriaceae as Escherichia coli and Salmonella compared to the CG. These results are consistent with some studies carried out on lambs and using other phenolic compounds. Results indicate that the dietary supplementation with anthocyanins in lambs inhibits the growth of some potential pathogenic gram-negative bacteria. These outcomes encourage further studies aiming to deepen knowledge on this topic, as a potential way for reducing the use of antimicrobial substances, as well as improving animals' health and welfare status.

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Multi-omics approach to assess the effects of a dual mode synbiotic supplementation on gut health and performance of broiler chickens

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Feeding pre- and pro-biotics to broilers immediately after hatch and during growth has led to positive health and performance outcomes. Such strategy could synergistically affect gut health establishment and preservation. Hence, this hypothesis was tested by