PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXIX cycle, 2023-26)

Project draft

1.Field of interest

AGR 18

2.Project title

Feeding strategies to enhance dairy efficiency and environmental sustainability by modifying rumen microbiome.

3.Tutor (membro del Collegio dei Docenti)

Prof. Stefania Colombini

- Eventually: co-tutor/s

Prof. Luca Rapetti

4.Relevance of the topic and state of the art:

The livestock sector is facing different challenges and, among these, the demand for higher sustainability seems to be one of the most urgent. According to the data elaborated by Jackson et al. (2020) for the year 2017, methane (CH_4) of anthropic origin represents 51 to 61% (according to the estimation method) of total CH_4 emission. Among anthropic sources, agriculture was the most important one, contributing 29 to 38% of total emissions (natural + anthropic), and among agricultural sources, enteric and manure fermentation was the main cause (15% of total emission). Various strategies can be implied to directly or indirectly address CH_4 emissions, and these strategies can be grouped into three main categories: animal and feed management, diet formulation, and rumen manipulation strategies. Rumen manipulation through feed additives that can modify rumen microbiome to decrease methanogenesis is an active and challenging field of research, and this kind of investigation should be considered a high priority (Beauchemin et al. 2020). The ideal compound should directly inhibit methanogenesis both in a short time and persistently; not have toxic effects for animals, humans, and the environment; be cost-effective for producers, and possibly increase productivity and profitability. Among feed additives, a lot of research has been conducted on essential oils or vegetable extracts which may exert antimicrobial activities against bacteria. Essential oils have a wide spectrum of antimicrobial activity, making it difficult to target specific microbial groups. The challenge remains to identify essential oils that selectively inhibit rumen methanogenesis improving rumen microbiota with lasting effects and without depressing animal productivity. Overall, there is a need to conduct more in vivo studies to determine the efficacy of essential oils, taking into account that the favourable effects obtained in vitro are not as marked as those in vivo, potentially due to microbial adaptation.

5. Layout of the project (draft)

5.1. Materials & Methods:

In vitro screening

The research activity includes an initial phase of study of the bibliography on the most promising additives to reduce the production of enteric CH_4. This initial phase will be followed by a screening of various additives (essential oils, tannins, saponins, etc.) by in vitro tests based on the use of rumen fluid. In this phase, microreactors (120 ml) will be used and the evaluation of the digestibility of the diet, based on the production of gas, at predetermined time intervals will be done. The production of CH_4 will also be determined at the same time intervals by gas chromatography. At the end of this phase, the most promising additives will be selected to be tested in vitro with the use of macro-reactors (250 ml) which will also allow the evaluation of fermentation kinetics in terms of gas and CH4 production measured continuously.

Dairy cows feeding trial (cannulated dry cows)

The most effective feed additive (FA) will be fed to the cannulated dry cows present in the experimental farm of the University of Milan. Two cows will be fed a diet supplemented with the FA and two cows will be fed a control diet without the additive. The cows will be fed the two diets in a Latin square design in 2 experimental periods. At the end of each period, individual rumen fluid samples will be collected and used to perform in vitro incubation (total gas and methane) as previously described. Moreover, fecal and saliva samples will be collected for further microbiome DNA analyses as later described.

Lactating cows feeding trial

As the last part of the project, the FA will be fed to lactating cows housed at the experimental farm of University of Milan in Landriano. The cows will be divided into 2 groups: control vs FA diets. Dry matter intake and milk production will be determined and fecal and saliva samples will be collected to evaluate the effects on microbiome (Young et al., 2020) and to study the relationship between milk production and dairy efficiency.

DNA extraction and sequencing

Metagenomics DNA on rumen, feces and saliva samples will be extracted using Qiagen DNeasy PowerSoil Pro Kits (Qiagen, Germany). Around 12.5 ng DNA will be used to target and amplify V4 regions of 16S rRNA gene primers followed in Illumina 16S library preparation guide. The libraries will be sequenced on Illumina MiSeq platform using 250 x 2 paired-end chemistry. The 16S rRNA gene sequencing data will be analysed using DADA2 package (Callahan et al., 2016) to generate the final Amplicon Sequence Variants (ASV) table. The ASVs will be assigned taxonomy using SILVA v132 and phylogeny will be generated within QIIME2 (Quast et al., 2013; Bokulich et al., 2018; Bolyen et al., 2019). The ASV table, phylogenetic tree, taxonomy information and metadata will be imported in Phyloseq to generate a phyloseq object which was then used for other downstream analysis. Alpha diversity metrics (Shannon index) will be calculated using Phyloseq package and picante package (Kembel et al., 2010). Bray-Curtis distance derived Principal Coordinates Analysis (PCoA) will be used for beta-diversity measures and visualized by non-metric multidimensional scaling (NMDS) plot. The PERMANOVA will be run to determine the difference in community structure and composition between treatments by using the adonis function in vegan (Kembel et al., 2010).

5.2. Schedule and major steps (3 years):

Five main steps in agreement with the foreseen activities can be identified in the present project as follows:

1. Bibliography research and in vitro tests to identify the most promising feed additive (months 1-12)
2. In vivo feeding study on cannulated dry cows (months 12-18)
3. In vivo feeding study on lactating cows (months 18-24)
4. Dna analyses on rumen, faeces and saliva samples collected in the different experiments (months 18-30)
5. Papers and thesis writing (months 30-36).

6. Available funds

PNRR spoke 5. Sustainable productivity and mitigation of environmental impact of livestock systems. Task5.3.6 Precision feeding of livestock to mitigate environmental impact and reduce antimicrobials utilization. 200,000 Euros

Marie Skłodowska-Curie actions - Research Fellowship Programme: "Evaluating effects of rumen originated lipopolysaccharide on the pathogenesis of subacute rumen acidosis" 130,000 Euros

7. Literature:

Beauchemin KA, Ungerfeld EM, Eckard RJ, Wang M. 2020. Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. Animal. 14(S1):s2-s16.

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, et al. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10 57–59.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 852–857.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods, 13(7), 581-583.

Jackson RB, Saunois M, Bousquet P, Canadell JG, Poulter B, Stavert AR, Bergamaschi P, Niwa Y, Segers A, Tsuruta A. 2020. Increasing anthropogenic methane emissions arise equally from agricultural and fossil fuel sources. Environ Res Lett. 15(7).

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD. et al. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26 1463–1464.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 D590–D596.

Young J, Skarlupka JH, Cox MS, Resende RT, Fischer A, Kalscheur, KF et al. 2020. Validating the use of bovine buccal sampling as a proxy for the rumen microbiota by using a

time course and random forest classification approach. Applied and Environm. Microbiol. 86(17), e00861-20.