**` Title:** Use of DNA techniques to investigate rumen microbiota composition and feed efficiency in Dairy Cows.

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**Abstract**: Ruminants play an important part in the food supply chain, and manipulating rumen microbiota is important to maximizing ruminants’ production. Diet is a key factor that can manipulate rumen microbiota, and each variation of the physical and chemical composition creates a specific niche that selects specific microbes. Alteration in the chemical composition of forage, the addition of concentrates in the diet, or the inclusion of sugars or liquid feed, can induce a change in rumen microbiota. A balanced microbiota is crucial for ensuring proper fermentation of nutrients and milk production. These aspects become increasingly relevant in animal production, where milk and its derivatives are in high demand, with growing attention to high-quality and sustainable products. In this context, the current presentation aims to investigate the potential differences in the composition of the gastrointestinal microbiota in high and low-producing dairy cows fed and managed under the same conditions. A specific study was conducted at the Alma Mater University of Bologna dairy research farm involving 24 lactating cows, fed the same diet, but presenting different milk productions. Animals were then separated in two groups, high-producing cows (HY) and low-producing cows (LY). Rumen protozoa, ammonia (NH3) concentration, and volatile fatty acids (VFA) were analyzed. DNA from rumen was extracted, purified, and sequenced with Illumina MiSeq. For VFAs, acetic and propionic acid were different between groups (P ≤ 0.05), acetic being higher in LY, while propionic was higher in the HY group. No statistical difference was observed for the main protozoa genera and ammonia concentration. Ruminal microbiota composition revealed significant differences. The HY group showed a higher abundance of Lachnospiraceae (P ≤ 0.05), and Veilonellaceae (P ≤ 0.05), while Eubacteriaceae (P ≤ 0.05), and RF16 spp. (P ≤ 0.05) were higher in LY. A tendency (P = 0.10) was observed for the Prevotellaceae family, with higher values in LY compared to HY. Data obtained with the metataxonomic analysis are very interesting, since similar effects were observed in a previous in vitro study, in which we investigated the potential of molasses in modulating the rumen microbiota composition, fibre digestibility and in vitro volatile fatty acid (VFAs) production. Six different molasses-based liquid feed, equally representative of beet and cane molasses, were selected and incubated with rumen fluid for 24 hours in vitro. VFAs analysis was carried out by sampling incubation flasks at 1,2,3,4,6,8,24 h. For microbiota analysis, samples were collected after 24 h of incubation, and then subjected to DNA extraction. Obtained results showed how VFAs composition was affected by molasses inclusion: acetic acid levels were higher in the CTR group (73.5 mmol), while propionic acid being higher in beet and cane molasses treatments (19.6 mmol; 18.6 mmol, respectively). Molasses addition deeply influenced the in vitro composition of the rumen microbiota. Relative abundance of Veillonellaceae (6.48% and 8.67% + molasses compared to 4.54% in the CTR), Streptococcaceae (19.62% and 28.10% in molasses compared to 6.23% in CTR), and Fibrobacteraceae (0.90% and 0.88% in + molasses compared to 0.62% in the CTR) increased in beet and cane compared to the CTR group, while Prevotellaceae, the most predominant family in the rumen, decreased compared to CTR (37.13%, 28.88%, 49.6% respectively). The different composition of the microbial community also resulted in an improved fibre digestibility, with and increment of 17% and 20% compared to the control for beet and cane molasses, respectively. Results of both studies underline how the composition of the rumen microbiota could influence feed efficiency and production, and suggest that specific dietary addition would beneficiate microbial families positively associated with higher animal performances.