**Analysis of environmental factor effects on rumen microbiome multi-omics traits in beef cattle**

**Application:** The study highlights that breed, diet, and additives were essential factors shaping the composition of a major number of ruminal metabolites, microbial genes, and microbe genera, but for some these specific interactions between factors were also significant with sometimes large effect value.

**Introduction:** Productivity of ruminant animals depends on the anaerobic microbial ecosystem to ferment and convert indigestible forages into premium meat products (Leahy et al., 2010). A comprehensive meta-analysis evaluated 98 enteric methane mitigation options (Arndt et al., 2022). Of the five solutions that decreased absolute methane emissions while maintaining animal productivity, only three were able to boost animal productivity while reducing product-based methane emissions. A lack of knowledge of rumen microbiomes and environmental interactions may be partially responsible for the inconsistency of the outcomes obtained from various methane mitigation strategies. The study aims to understand the impact of environmental factors, including host genetics, diet, and additives, on the rumen microbiome from a multi-omics perspective.

**Material and methods:** The study used metabolome and metagenomic data from rumen samples of 30 beef cattle recorded by SRUC (Roehe et al., 2016). The rumen samples corresponded to four different beef cattle breeds, two diets, and four feed additives. The detailed experimental design is as previously reported (Rooke et al., 2014). Metabolome: 115 metabolites with relative concentrations>0.001% were selected. Metagenome: 1461 microbial genes with relative abundance>0.001% were selected, and 1178 microbial taxonomies (genus level) with relative abundance>0.001% were selected. The study used the SPSS Generalized Linear Models module to treat each multi-omics trait (e.g., metabolite/microbial gene/microbial genus) as a dependent variable. The contribution and significance(*α*=0.05) of the main effects and interaction effects of environmental factors for each dependent variable were then obtained. Boxplots show the distribution of main effects and interaction effects contribution (*P*<0.05) for each omics trait.

**Results:** For the metabolite, Figure 1 shows that Diet had the lowest contribution of all main effects. Among interaction effects, Breed\*Diet\*Additive significantly affected the greatest number of metabolites, but the overall contribution was at a low magnitude. The interaction effect with the highest average impact contribution was Breed\*Additive.

Breed significantly contributed to more than 40% of variations of 267 microbial genes. Diet significantly affected the relative abundance of 607 microbial genes, which was 42% of the microbial genes in our dataset. Among the interaction effects, Diet\*Additive affected the highest number of microbial genes and expressed the highest overall contribution.

For microbial taxa, Breed explained 34.5% of the relative abundance of 258 microbial genera, which was the highest among the main factors. Diet was the factor that significantly affected the largest number of microbial genera. Among the interaction effects, Diet\*Additive significantly affected most microbial genera. Breed\*Addtive had a higher overall contribution among interaction effects.

**Conclusions:** The Breed effect showed the largest average contribution to multi-omics traits. Diet significantly affected equal or more levels of each multi-omics trait but at a lower effect value than Breed. Generally, the interactions between factors affected lower levels of each multi-omics trait, than the main factors, but sometimes with large effect value, as found for Breed\*Additive effect on the variation of 13 metabolites.

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