

Effects of *Saccharomyces cerevisiae* supplementation on rumen microbial diversity and function in Pogasi beef cattle

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ABSTRACT. The objective of this study was to determine how *Saccharomyces cerevisiae* supplementation affects rumen microbial diversity and function in Pogasi (Peranakan Ongole Grati Hasil Seleksi) beef cattle. The treatment included a diet supplemented with 2 g of yeast per animal daily compared to a control unsupplemented diet, using a 2 × 2 Latin square design. Venn diagram analysis identified 536 core microbial genera common for all groups, while unique genera in yeast-supplemented groups indicated an effect on microbial composition and diversity. Yeast addition increased microbial richness, as shown by rarefaction curves, and improved species evenness, suggesting a more stable ecosystem. Taxonomic analysis revealed *Firmicutes* and *Bacteroidota* as the dominant phyla, with yeast supplementation inducing minor changes in microbial composition. At the class level, *Methanobacteria*, *Clostridia*, and *Bacteroidia* were predominant, with a slight reduction in *Methanobacteria* in yeast-supplemented groups, suggesting a potential inhibitory effect on methanogenesis. Functional analysis showed alterations in metabolic pathways related to fibre degradation, volatile fatty acid production, and nitrogen metabolism. These findings indicate that yeast supplementation modifies microbial structure, stabilises the rumen microbiome, and improves fermentation efficiency, potentially reducing methane emissions. This study demonstrates that yeast supplementation is a promising strategy to improve rumen function and sustainability in beef cattle production.

Introduction

High-grain diets are widely used in the livestock industry to improve productivity, but their high content of rapidly fermentable carbohydrates can cause abnormal rumen fermentation, lowering pH and leading to subacute rumen acidosis (SARA). This condition increases the likelihood of metabolic disorders and diseases such as rumen acidosis, hoof laminitis, and liver abscesses (McAllister et al., 2011; Silberg et al., 2013). Yeast, a common probiotic in ru-

minant nutrition, improves feed efficiency by optimising rumen fermentation and reducing the risk of rumen acidosis (Elghandour et al., 2020). Additionally, it helps restore microbial balance in the gastrointestinal tract during periods of digestive disorders or stress (Moré and Swidsinski, 2015). Studies have also demonstrated that yeast supplementation improves fibre degradation, stabilises rumen pH, and reduces methane emissions, further supporting its role in overall rumen function and animal health (Marden et al., 2008; Chaucheyras-Durand et al., 2016).

Yeast has been reported to improve the productive performance of ruminants including calf feed intake, weight, and growth rates (Geng et al., 2016) but also increasing weight gain of bulls fed high-grain diets (Magrin., 2018). However, other experiments found no significant effects of active dry yeast on growth performance or feed conversion efficiency in sheep (Rodriguez et al., 2015), and dairy cattle (Plata et al., 1994). These conflicting results suggest that the effects of yeast on ruminant performance are not uniform and may depend on factors such as yeast type and dosage. Although active dry yeast and yeast cultures have been extensively studied, research on the combined application of live yeast cells and yeast cultures in ruminant nutrition remains limited.

Improvements in ruminant performance associated with yeast supplementation are largely attributed to its impact on rumen microorganisms. Research indicates that yeast exerts a dual regulatory effect by suppressing lactate-producing bacteria while stimulating lactate-utilising bacteria, such as *Megasphaera elsdenii*, consequently preventing lactic acid accumulation and maintaining rumen pH stability (Wallace et al., 1993; Chaucheyras et al., 2002). Additionally, yeast promotes the proliferation of fibre-degrading bacteria, including *Fibrobacter succinogenes*, *Ruminococcus albus*, and *R. flavefaciens*, which are important for efficient fibre digestion in the rumen (Callaway et al., 1997; Mao et al., 2013). Numerous studies on dairy and beef cattle have demonstrated these effects, reinforcing the role of yeast in improving rumen fermentation and overall productivity. Therefore, the present study aimed to evaluate the effects of *Saccharomyces cerevisiae* supplementation on rumen microbial diversity and function in Pogasi beef cattle. This breed (Peranakan Ongole Grati Hasil Seleksi) has been developed through 14 years of selective breeding of Ongole Crossed (OC) cattle, initiated in 2002–2003. By the fourth generation, Pogasi cattle exhibits desirable traits, including improved feed efficiency, larger body size, yellowish-white coat, and strong adaptability, making it well suited to marginal areas with limited feed availability (Aryogi et al., 2020). Specifically, the study characterises changes in microbial composition, assesses functional alterations in metabolic pathways, and determines the potential benefits of yeast supplementation for optimising rumen fermentation efficiency and improving cattle performance.

Material and methods

The study was conducted in compliance with the Indonesian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the Animal Ethics Committee of the Indonesian Ministry of Agriculture (Balitbangtan/Lolitsapi/RM/15/2021). Detailed information on the animals and feeding management has been reported in our companion article (Marden et al., 2008). The experiment was conducted at the Beef Cattle Research Institute, Pasuruan, East Java, Indonesia. Two rumen-cannulated Pogasi steers were randomly assigned to one of two treatments in a crossover design consisting of two 21-day experimental periods separated by a 7-day wash-out. The steers were housed in individual pens and fed a diet of 40% concentrate-mix (Table 1) and 60% rice straw hay *ad libitum*. Feed was provided twice daily, at 08.00 and 16.00. Water and mineral blocks were freely available throughout the trial. The dietary treatments included (1) control (CON; basal diet without additive) and (2) yeast (YS, dosage 2 g/head/day, 1×10^{10} CFU/kg). The yeast additive was administered with the morning feed from day 1 to day 21 of each period.

Table 1. Chemical composition (% in DM) of the basal diets

Item	Concentrate	<i>Pennisetum purpureum</i>
Dry matter	92.16	19.72
Crude protein	16.44	5.92
Crude fat	4.54	1.36
Crude fibre	11.43	19.96
Crude ash	8.99	11.66
TDN	76.96	50.20
NDF	42.61	60.09
ADF	22.73	38.82

TDN – total digestible nutrients, NDF – neutral detergent fibre, ADF – acid detergent fibre

Rumen fluid collection

On the final day (day 21) of each experimental period, representative ruminal content (100 ml) was collected through the cannula before morning feeding. Samples were manually strained through four layers of sterile cheesecloth to separate liquid and solid fractions. Equal portions of both fractions were then combined at a 1:1 ratio (w/w) to form daily composite samples, which were then stored at -80 °C for subsequent shotgun metagenomic sequencing.

Shotgun metagenomic sequencing

The rumen microbiome composition and functional potential was analysed using shotgun metagenomic sequencing. Microbial DNA was extracted from frozen rumen samples using a commercial extraction kit. DNA quality was assessed through agarose gel electrophoresis, while concentration and purity were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific, (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit fluorometer. Sequencing libraries were prepared with the Illumina Nextera XT Kit, followed by paired-end sequencing (150 bp) on the Illumina NovaSeq 6000 platform. Raw reads were subjected to quality control, trimming, and host contamination removal using FastQC and Trimmomatic. Taxonomic classification was performed using MetaPhlAn (Segata Lab., Povo, Italy) and Kraken2 (Johns Hopkins University, Baltimore, MD, USA), while functional annotation was conducted using HUMAnN3 (Huttenhower Lab., Harvard T.H. Chan School of Public Health, Boston, MA, USA) against the KEGG (Kyoto Encyclopedia of Genes and Genomes, Kanehisa Laboratories, Kyoto, Japan) and MetaCyc (BioCyc collection, SRI International, Menlo Park, CA, USA) reference databases. Microbial diversity was analyzed using QIIME2 (QIIME2 Development Team, <https://qiime2.org>). Differential abundance was assessed with LEfSe (Segata Lab.) and DESeq2 (Bioconductor community). Analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria).

Bioinformatic and statistical analysis

Raw sequencing data were demultiplexed and stored in FASTQ format, followed by quality control procedures to remove low-quality reads (Q-score ≤ 10) and contaminant sequences. High-quality reads were assembled using the Iterative De Bruijn Graph De Novo Assembler, with subsequent evaluation of contigs ≥ 500 bp performed through the Quality Assessment Tool for Genome Assemblies (QUAST). Taxonomic classification was conducted by aligning contig sequences against the NCBI reference database using Kraken software, which enabled estimation of relative abundance of microbial taxa. For downstream analysis, only microbial species demonstrating relative abundances exceeding 0.1% were considered, with comparative assessments performed across experimental treatment groups.

Results and discussion

Phylogenetic analysis (Figure 1) has indicated that yeast supplementation selectively increased the abundance of beneficial rumen microbes, especially those involved in fibre degradation and short-chain fatty acid production. The observed enrichment of *Ruminococcus* and *Fibrobacter* implied improved fibre utilisation, which is consistent with prior studies reporting increased digestibility and ruminal efficiency following yeast addition (Newbold et al., 1995; Chaucheyras-Durand and Fonty, 2001).

The observed decrease in the counts of *Proteobacteria* genera, often associated with ruminal dysbiosis under high-starch diets, supports the role of yeast in maintaining microbial balance and lowering the risk of subacute ruminal acidosis (SARA) (Pinloche et al., 2013). Concurrent enrichment of *Butyrivibrio* and *Succinivibrio* suggests increased butyrate and succinate production, important for energy metabolism and gut health in ruminants. These microbial qualitative alterations corresponded with documented improvements in rumen fermentation efficiency and cattle performance following yeast administration, confirming findings from *in vivo* growth studies and *in vitro* fermentation experiments (Desnoyers et al., 2009).

Figure 2 presents a phylogenetic tree of microbial genera identified in the rumen of Pogasi beef cattle, showing the evolutionary relationships among dominant microbial genera. The analysis identified core genera such as *Prevotella*, *Ruminococcus*, *Butyrivibrio*, and *Fibrobacter*, demonstrating a typical rumen microbiome structure. The phylogenetic profile indicates a stable and diverse microbial community characteristic of healthy ruminants. *Prevotella* was the most abundant genus, consistent with its established functions in carbohydrate fermentation and protein metabolism (Betancur-Murillo et al., 2022). The significant presence of *Ruminococcus* and *Fibrobacter* reflects their crucial role in cellulose and hemicellulose degradation (Morgavi et al., 2013).

The phylogenetic clustering of these genera indicated that control cattle maintained a balanced microbial ecosystem, with contributions from both *Bacteroidetes* and *Firmicutes* to fermentation. However, in the absence of supplementation, this microbial structure may be more susceptible to dietary stress or shifts, potentially affecting efficiency and performance under challenging feeding conditions, e.g., high-starch diets. Establishing this baseline is essential for comparison with the yeast-supplemented

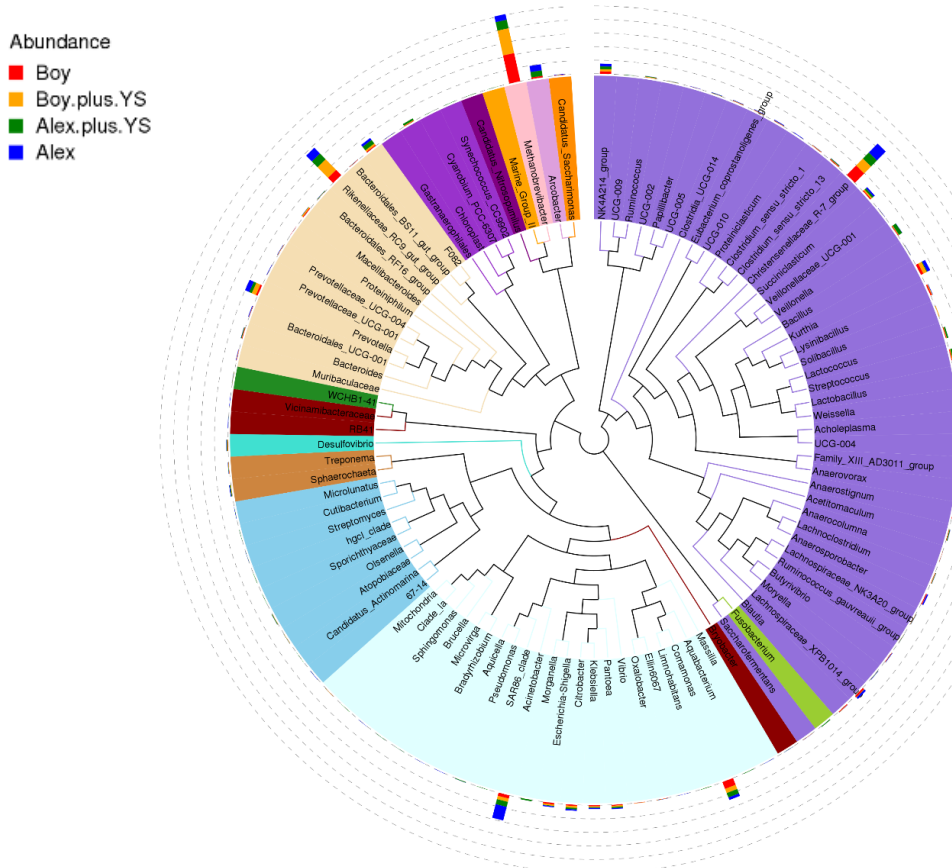


Figure 1. Rumen microbial community composition in response to yeast supplementation. The phylogenetic tree shows the evolutionary relationships between microbial genera identified in Pogasi beef cattle. The accompanying bar chart shows the relative abundance of these genera between cattle fed a basal diet (CON, red) and those supplemented with *Saccharomyces cerevisiae* (YS, blue)

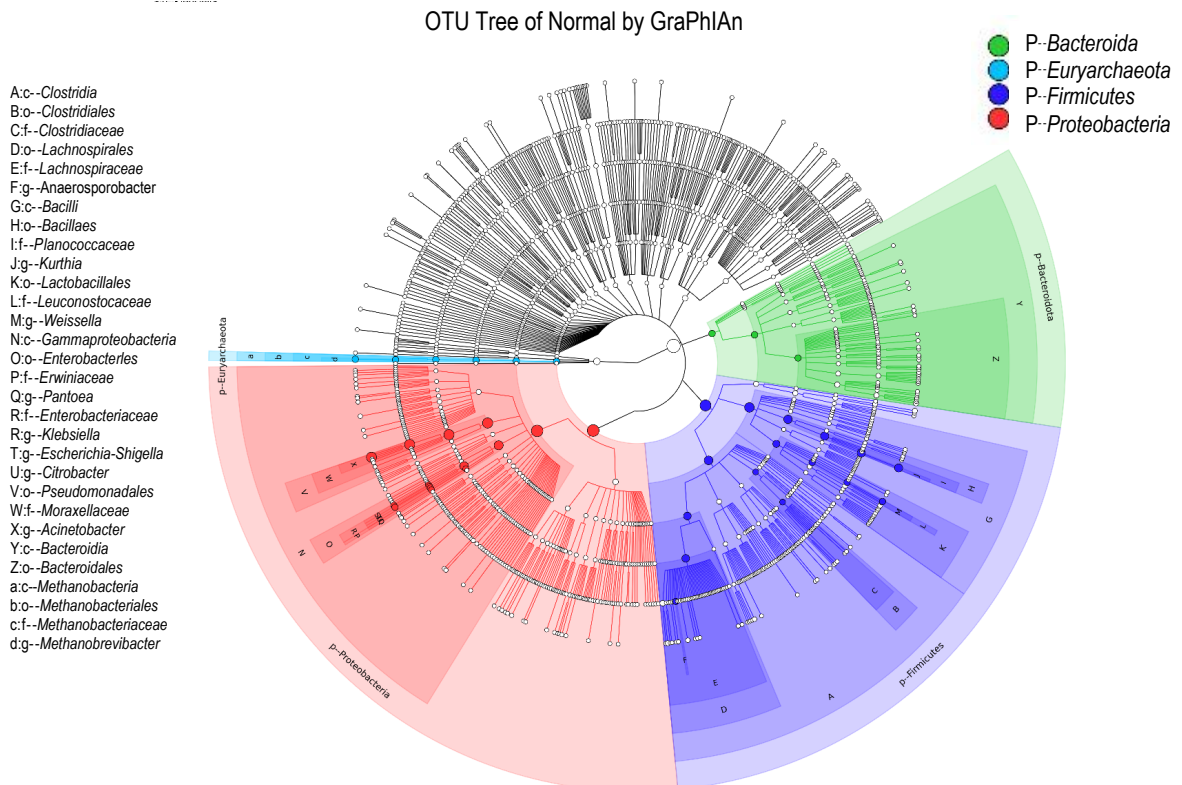


Figure 2. Phylogenetic tree of microbial genera identified in the rumen of Pogasi beef cattle fed the control diet (CON; basal diet without *Saccharomyces cerevisiae* supplementation).

group, as it enables assessment of how supplementation alters microbial composition and improves rumen health and fermentation efficiency.

The phylogenetic tree of yeast-supplemented Pogasi cattle revealed distinct compositional changes compared to the control group, with increased abundance of key functional genera including *Succinivibrio*, *Megasphaera*, and *Selenomonas*, in addition to core genera such as *Prevotella* and *Ruminococcus* (Figure 3). The increase in the abundance of *Succinivibrio* and *Megasphaera* indicated increased propionate synthesis, which improves energy metabolism in ruminants (Chaucheyras-Durand et al., 2016), while the higher counts of *Selenomonas* suggested better lactate utilisation that helps stabilise rumen pH and reduce acidosis risk (Pinloche et al., 2013). These changes in microbial community structure demonstrate how yeast supplementation promotes a more metabolically efficient rumen environment, with increased diversity and functional specialisation compared to the control group. The findings support the role of *Saccharomyces cerevisiae* in optimising rumen microbial ecosystems for better digestion and animal performance.

The microbial composition analysis revealed differences between treatment groups (Figure 4). The control group showed typical rumen microbial dominance by *Firmicutes* and *Bacteroidota* phyla, while the yeast-supplemented group displayed a more balanced taxonomic distribution with increased relative abundance of fibrolytic and lactate-utilising genera, including *Succinivibrio*, *Prevotella*, and *Ruminococcus*. Yeast supplementation modulated rumen microbial composition, promoting greater diversity and balance among microbial populations. Specifically, the elevated abundance of *Succinivibrio* and *Prevotella* indicated better carbohydrate metabolism and protein degradation pathways, which could contribute to improved nutrient utilisation efficiency (Figures 4 and 5). The more evenly distributed microbial community in the supplemented cattle likely supports rumen pH stability, which is particularly relevant for animals receiving high-concentrate diets (Dai et al., 2023). These findings support previous research showing that *S. cerevisiae* acts as a rumen modulator, improving microbial fermentation efficiency.

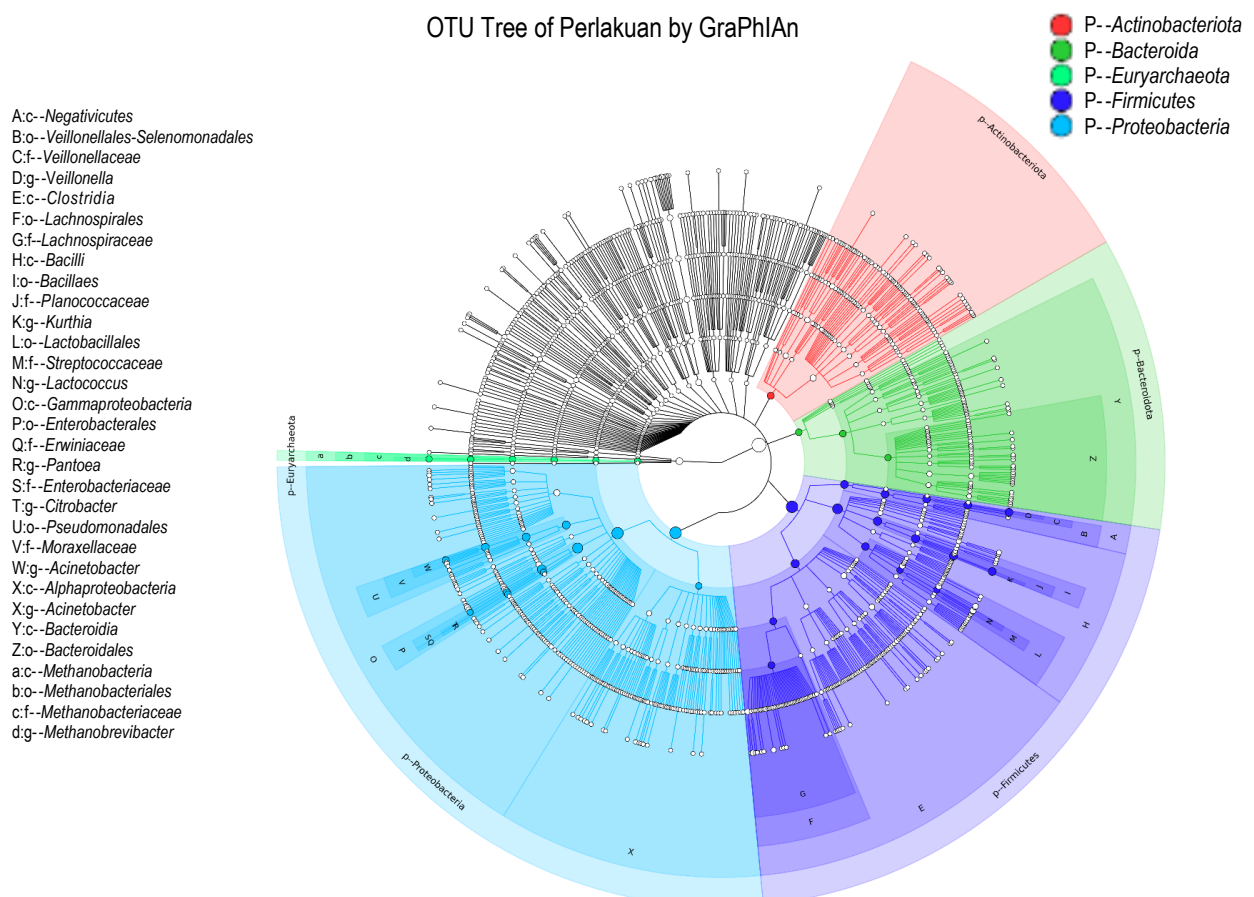


Figure 3. Phylogenetic tree of microbial genera identified in the rumen of Pogasi beef cattle supplemented with *Saccharomyces cerevisiae* (YS; basal diet with yeast supplementation)

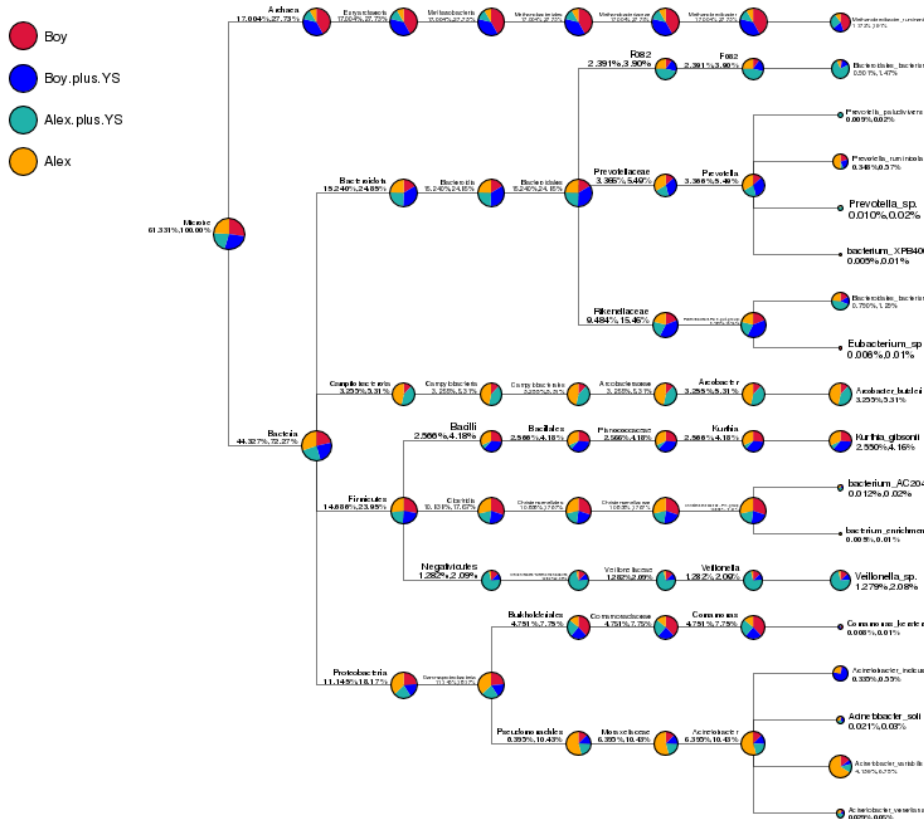


Figure 4. Taxonomic tree with corresponding pie charts illustrating the relative microbial abundance in each group: control (CON; basal diet without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; basal diet with *S. cerevisiae* supplementation) groups

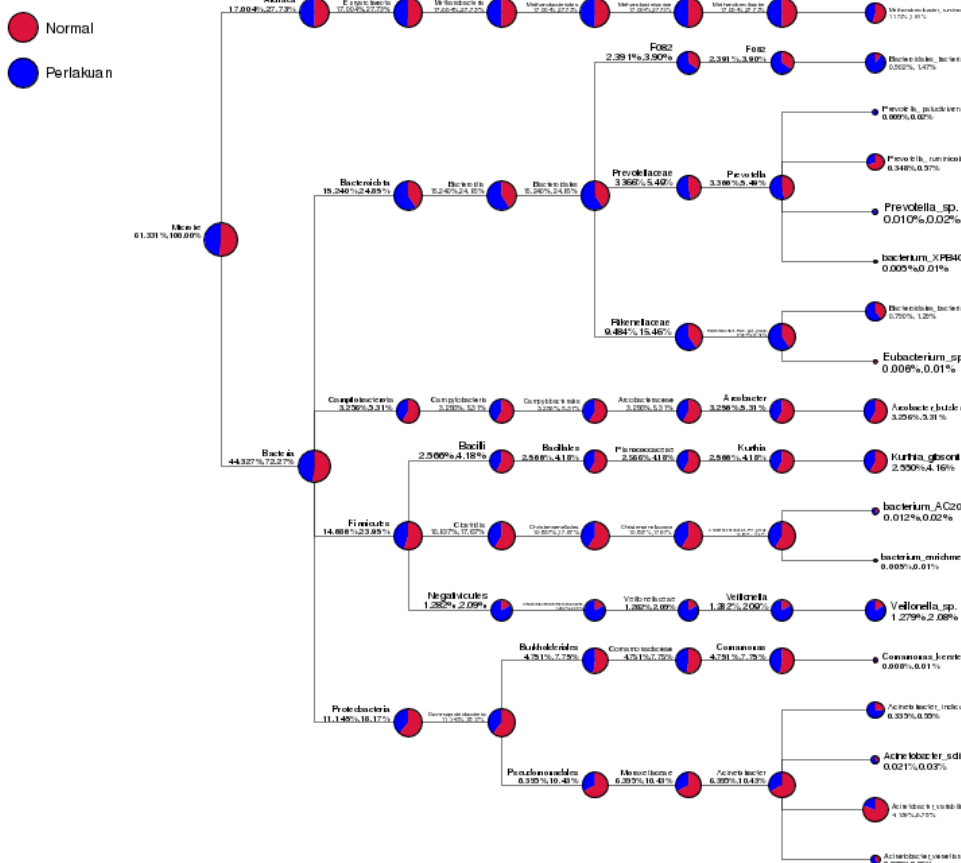


Figure 5. Taxonomic tree and relative microbial abundance in the rumen of Pogasi beef cattle, comparing the control group (CON; basal diet without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; basal diet with *S. cerevisiae* supplementation) groups

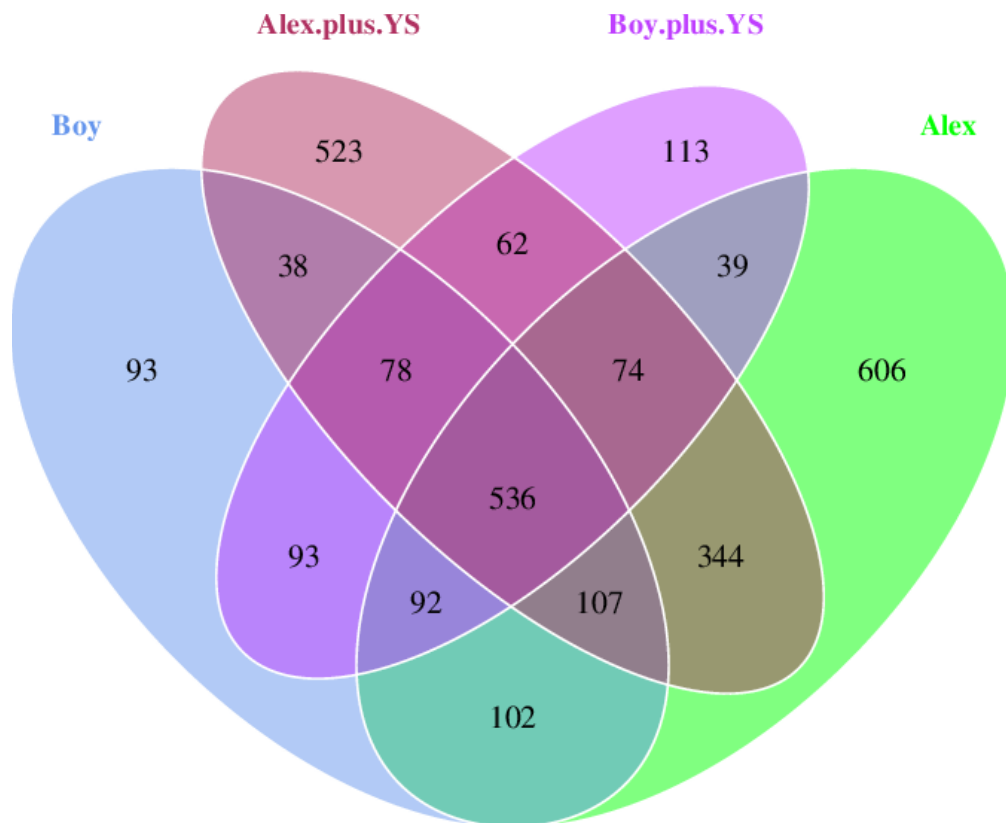


Figure 6. Venn diagram showing microbial taxa identified in the rumen of Pogasi beef cattle, comparing control (CON; without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; with *S. cerevisiae* supplementation) groups

The Venn diagrams (Figures 6 and 7) illustrate the shared and unique microbial genera identified in the treatment and control groups. The central overlapping region (536 genera) represents the core rumen microbiota, which remained stable regardless of treatment. Unique genera in the control groups (93 and 606, respectively) and the yeast-supplemented groups (113 and 523, respectively) showed distinct

microbial compositions influenced by experimental conditions, including host genetic differences and the effects of yeast supplementation. Additionally, the diagram illustrates microbial interactions between yeast-supplemented and non-supplemented groups. A total of 78 genera were common for the two yeast-supplemented groups, suggesting that supplementation induced consistent alterations in

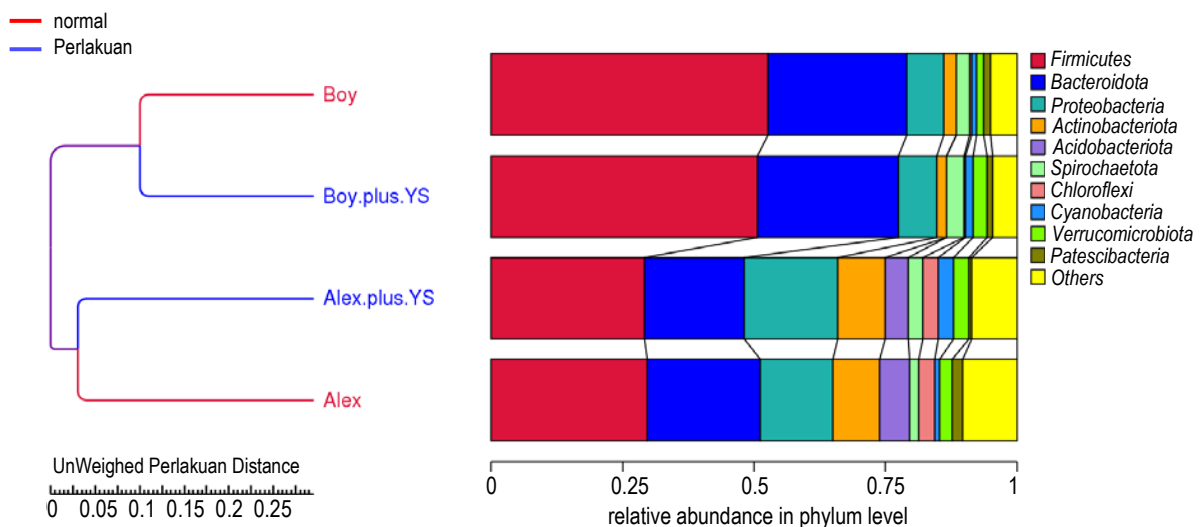


Figure 7. Venn diagram showing microbial taxa identified in the rumen of Pogasi beef cattle. Numbers indicate unique and shared microbial taxa between control (CON; without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; with *S. cerevisiae* supplementation) groups

the rumen microbial community. In contrast, the presence of unique genera in the control groups reflects the role of the host in shaping microbial composition in the absence of yeast supplementation. These findings align with prior research demonstrating that rumen microbial communities are influenced by both host factors and dietary interventions, such as yeast supplementation, which can improve fermentation efficiency and alter microbial diversity (Newbold et al., 1995). Yeast has also been reported to support fibrolytic and volatile fatty acid (VFA)-producing microbes, contributing to a more stable rumen environment (Oeztuerk, 2009). Additionally, microbial diversity plays a key role in maintaining rumen function and adaptability, as noted by Weimer et al. (2010), who examined ecological interactions between rumen microbes and their responses to dietary changes.

Figure 8 compares the relative abundance of microbial phyla in individual groups, emphasising how yeast supplementation alters community composition. Dominant phyla were *Firmicutes* (red) and *Bacteroidota* (blue), which play critical roles in fibre degradation and VFA production, and are prevalent in all groups. *Proteobacteria* (orange) increased slightly in supplemented groups, suggesting roles in nitrogen metabolism or secondary fermentation pathways. Minor phyla, including *Actinobacteriota*, *Spirochaetota*, and *Chloroflexi*, showed variability, potentially reflecting microbial specialisation influenced by yeast. These changes, particularly the increased abundance of *Firmicutes* and *Bacteroidota*, are consistent with previous studies on yeast effects in ruminal fermentation (Oeztuerk, 2009).

The findings indicate that yeast supplementation consistently altered microbial composition, as evidenced by phylogenetic clustering and differences in phylum abundance. Similarity between yeast-supplemented groups demonstrated a uniform treatment effect on microbial communities, regardless of group differences. These alterations likely contributed to improved rumen fermentation efficiency, as yeast can promote microbial growth and activity by providing essential nutrients, stabilise rumen pH by reducing lactic acid accumulation, and modify fermentation processes towards lower methane production and increased propionate generation (Newbold et al., 1995; Oeztuerk, 2009).

Figure 8 shows the relative abundance of microbial phyla in rumen samples from the control and treatment groups. The dominant phyla in all groups were again *Firmicutes* and *Bacteroidota*, which play essential roles in rumen fermentation, fibre degradation, and VFA production. *Firmicutes* had the highest relative abundance, followed by *Bacteroidota*, while *Proteobacteria*, *Euryarchaeota*, and *Campylobacterota* were present in lower proportions. Minor phyla, including *Spirochaetota*, *Actinobacteriota*, and *Fusobacteriota*, displayed minimal variation between groups, indicating a relatively stable presence in the rumen microbiome regardless of supplementation.

The addition of yeast influenced the composition of the rumen microbial community. Supplemented groups showed slight shifts in the proportions of minor microbial phyla, indicating that yeast could increase microbial diversity or favoured the growth of specific taxa. These findings are consistent with previous studies that yeast selectively stimulates

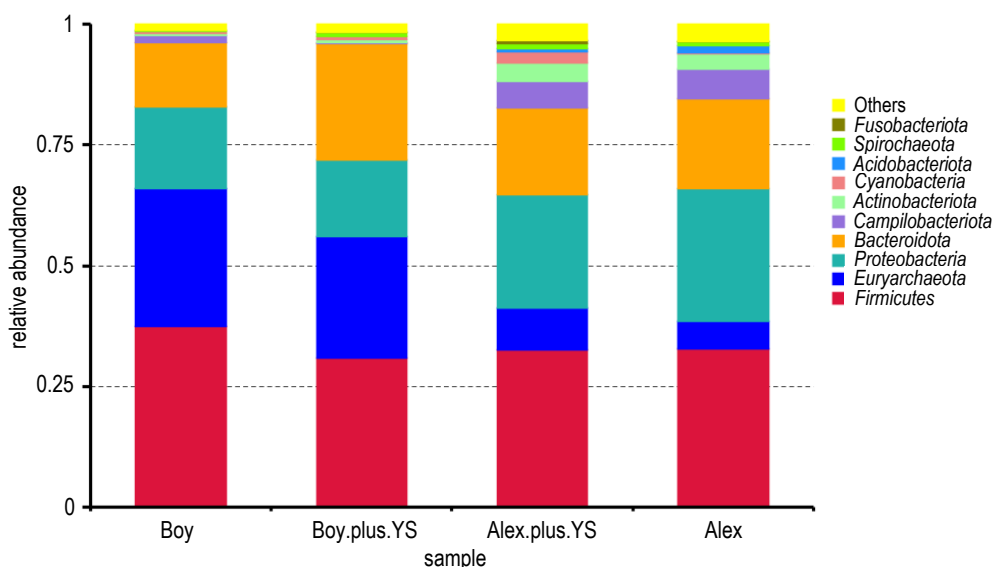


Figure 8. Relative abundance of the ten most prevalent bacterial phyla in the rumen of Pogasi beef cattle. A comparison between control (CON; without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; with *S. cerevisiae* supplementation) groups

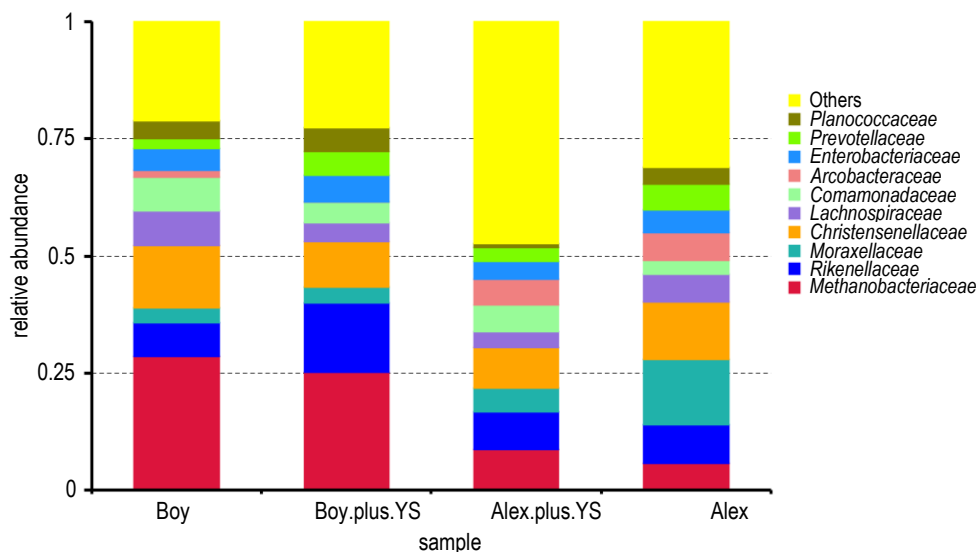


Figure 9. Relative abundance of the ten most prevalent bacterial families in the rumen of Pogasi beef cattle. A comparison between control (CON; without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; with *S. cerevisiae* supplementation) groups

previous studies that yeast selectively stimulates beneficial populations and improve rumen fermentation efficiency (Newbold et al., 1995; Oeztuerk, 2009). The detection of Euryarchaeota, a phylum containing methanogenic archaea, points to the involvement of microbial communities in methane production, which may be modulated by yeast supplementation (Chaucheyras-Durand et al., 2016).

Figure 9 illustrates the relative abundance of microbial families in Pogasi beef cattle in individual groups. The dominant family in all groups was *Methanobacteriaceae*, followed by *Rikenellaceae*, *Lachnospiraceae*, and *Prevotellaceae*, which are essential for rumen fermentation and methane production processes. *Methanobacteriaceae*, a methanogenic archaeal family, generates methane through hydrogenotrophic pathways. Its relative abundance remained high in all groups but was slightly reduced in yeast-supplemented cattle, indicating suppressive effect of yeast on methanogenesis, consistent with earlier findings (Newbold et al., 1995; Chaucheyras-Durand et al., 2016). *Rikenellaceae* and *Lachnospiraceae*, involved in fibre degradation and VFA production, indicated active carbohydrate fermentation in all groups (Mao et al., 2013; Pitta et al., 2014). *Prevotellaceae*, known for their role in degrading protein and simple carbohydrates, maintained stable proportions, reflecting its established role in rumen fermentation (Henderson et al., 2015). Minor families, including *Christensenellaceae*, *Moraxellaceae*, and *Planococcaceae*, varied slightly between supplemented and control groups, suggesting that yeast may influence microbial diversity and activity, contributing to more efficient fermentation (Marden et al., 2008; Oeztuerk, 2009).

The relative abundance analysis revealed that the rumen microbiota of Pogasi beef cattle was dominated by genera such as *Methanobrevibacter*, *Prevotella*, *Rikenellaceae_RC9_gut_group*, and *Christensenellaceae_R-7_group*, with variations observed between the control (CON) and yeast-supplemented (YS) groups (Figure 10). Supplementation with *S. cerevisiae* was associated with a numerical reduction in *Methanobrevibacter*, the main hydrogenotrophic methanogen, which is consistent with earlier reports that yeast supplementation can suppress methanogen activity by redirecting hydrogen toward propionate synthesis rather than methanogenesis (Chaucheyras-Durand et al., 2008; Patra, 2012).

In parallel, the YS group showed relative increases in *Prevotella* and *Christensenellaceae_R-7_group*, genera involved in fibre degradation and volatile fatty acid production, suggesting enhanced fibrolytic activity and improved rumen fermentation efficiency (Chaucheyras-Durand and Fonty, 2001; Marden et al., 2008). Similarly, the enrichment of *Rikenellaceae_RC9_gut_group*, which has been linked to the utilization of complex polysaccharides, supports the hypothesis that yeast supplementation favours carbohydrate-degrading bacteria, thereby enhancing energy extraction from feed (Petri et al., 2013). Taken together, these shifts indicate that *S. cerevisiae* supplementation promoted a more efficient and stable rumen microbial community, characterized by lower methanogen abundance and greater prevalence of fibrolytic and carbohydrate-utilizing taxa. Such microbial restructuring may contribute to reduced methane production and improved nutrient utilization, aligning with previous findings in both dairy and beef cattle where yeast supplementation

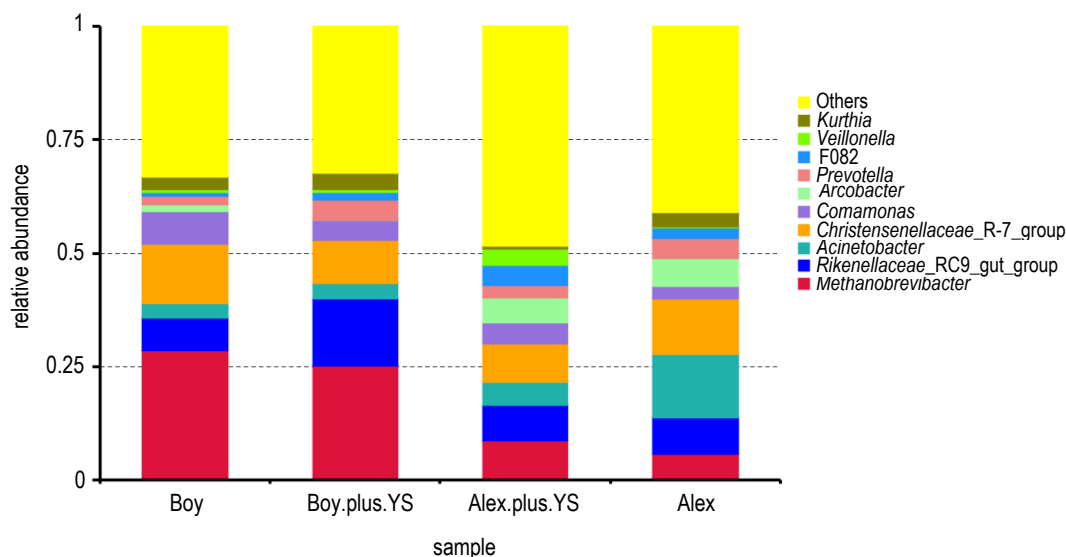


Figure 10. Relative abundance of the ten most prevalent bacterial genera in the rumen of Pogasi beef cattle. A comparison between control (CON; without *Saccharomyces cerevisiae* supplementation) and yeast-supplemented (YS; with *S. cerevisiae* supplementation) groups

stabilized rumen pH, stimulated fibrolytic microbes, and improved animal performance (Newbold et al., 1995; Chaucheyras-Durand et al., 2008).

Yeast supplementation resulted in a reduction of the methanogenic family Methanobacteriaceae, alongside subtle changes in other microbial families. This aligns with previous findings that yeast can suppress methanogenesis and alter rumen microbial dynamics, thereby improving fermentation efficiency and reducing greenhouse gas emissions (Miller and Berry, 2008; Patra and Saxena, 2010). In addition to taxonomic changes, yeast supplementation affected functional microbial pathways related to fibre degradation, VFA production, and nitrogen metabolism. These changes suggests that yeast not only supports rumen stability and fermentation efficiency but also modifies microbial interactions, favouring populations that contribute to more efficient nutrient utilisation and lower methane output. These findings provide a detailed understanding of how yeast promotes beneficial microbial populations and optimises rumen function, highlighting its potential to improve ruminant productivity while mitigating environmental impacts.

Conclusions

This study demonstrated that supplementation with *Saccharomyces cerevisiae* in Pogasi beef cattle markedly changed rumen microbial diversity and function. Yeast supplementation increased microbial richness and evenness, altered community composition, particularly by reducing the relative abundance of methanogenic archaea such as *Methanobacteriaceae*, and affected key metabolic

pathways related to fibre degradation, volatile fatty acid production, and nitrogen metabolism. These taxonomic and functional alterations indicate improved fermentation efficacy and a potential reduction in methane emissions. The results support the role of yeast as a promising nutritional strategy to stabilise the rumen microbiome, improve fermentation processes, and promote sustainable beef cattle production. Future studies are needed to assess long-term effects and confirm these results under different dietary and management conditions.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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