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ORIGINAL PAPER

Effects of lactic acid bacteria and yeast on rumen function and microbiota: An *in vitro* study optimising direct-fed microbials formulations

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ABSTRACT. This study investigated the effects of direct-fed microbials (DFM) containing *Schleiferilactobacillus harbinensis* (lactic acid bacteria, LAB) and *Pichia kudriavzevii* (yeast), applied in different combinations and doses, on rumen fermentation, nutrient digestibility, and microbial populations using an *in vitro* system. A 3 × 4 factorial design was used, with three LAB:yeast ratios (A1: 1:1, A2: 1:3, A3: 3:1) and four DFM inclusion levels (1%, 2%, 3%, and 4% v/v) with three replicates per treatment. The fermentation substrate consisted of 60% *Pennisetum purpureum* and 40% concentrate, incubated with goat rumen fluid for 48 h. Data were analysed using generalised linear mixed models, with DFM ratio and dose as fixed effects and their interaction as a random effect. DFM combinations did not alter ruminal pH or overall fermentation; however, the 1:1 ratio (A1) significantly increased butyrate, iso-butyrate, and iso-valerate concentrations ($P < 0.05$). Increasing DFM dose (up to 4%) elevated NH_3 concentration, total short-chain fatty acids, propionate and butyrate proportions, and reduced the acetate-to-propionate ratio ($P < 0.05$). Nutrient digestibility improved with higher DFM doses, with the 4% level resulting in the highest *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, and *in vitro* crude fibre digestibility values ($P < 0.05$). Microbial analysis showed that the 1:1 ratio increased *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*, whereas higher doses generally stimulated beneficial microbes and reduced methanogen populations ($P < 0.05$). In conclusion, a 1:1 combination of *S. harbinensis* and *P. kudriavzevii* at a 4% inclusion level improved fermentation profiles, nutrient digestibility, and microbial populations *in vitro*. These findings highlight the importance of both microbial ratio and dose in optimising DFM formulations to support rumen function.

Introduction

The use of direct-fed microbials (DFM) as alternatives to antibiotics (e.g., monensin and tetracycline) in ruminants has been intensively explored in recent years. These additives contain live microorganisms, such as bacteria or yeast that confer various health benefits to the host. Reported effects include enhanced immune function (Villena et al., 2018), growth

performance in beef cattle (Guimaraes et al., 2024), as well as increased milk yield, and rumen fermentation optimisation (Ji et al., 2022). However, the efficacy of DFM in ruminants depend on several factors, including dosage, frequency of administration, microbial species and strain, and dietary composition (Chaucheyras-Durand et al., 2008).

The application of DFM is increasingly recognised as an effective strategy to improve livestock health and productivity. Previous studies have investigated both single-species and multi-species DFM applications in ruminants (Jiao et al., 2017; Sanam et al., 2022). Lactic acid bacteria (LAB) play a particularly important role in maintaining microbial balance in the rumen and improving feed efficiency. LAB exert their beneficial effects through various mechanisms, including lactate production or utilisation, the synthesis of antimicrobial compounds such as bacteriocins and organic acids, competitive exclusion of pathogens, and immunomodulatory activity (Villena et al., 2018; Kraimi et al., 2019). Complementary to LAB, yeast-based DFM contribute to rumen health by stimulating the growth of fibrolytic and amylolytic bacteria while preventing lactic acid accumulation (Amin et al., 2021). Practical applications include supplementation with *Saccharomyces cerevisiae* CNCM I-4407, which has been shown to mitigate acidosis by increasing rumen pH in Holstein cattle (Kumprechtovaa et al., 2019).

Schleiferilactobacillus harbinensis (formerly *Lactobacillus harbinensis*) is a commonly isolated LAB from various fermented products, including traditional fermented vegetables (Miyamoto et al., 2025), dairy products (Mashraqi et al., 2023), and fermented fish (Susalam et al., 2024). Recent *in vitro* studies have investigated its probiotic potential for ruminants (Ardani et al., 2023), demonstrating significant antimicrobial activity against pathogens such as *Escherichia*, *Listeria*, *Morganella*, and *Vagococcus*, along with aflatoxin B1 detoxification capabilities (Jung et al., 2021; Marlida et al., 2023). These functional properties suggest that *S. harbinensis* could play a beneficial role in gut microbiome modulation and improve overall animal health and immune function.

Similarly, *Pichia kudriavzevii* has gained interest in ruminant nutrition research. Isolated from cattle rumen (Suntara et al., 2021) and fermented fish (Ardani et al., 2023), this yeast has been shown to produce biomass, secrete cellulase enzymes, as well as improve nutrient digestibility, and rumen fermentation products (Suntara et al., 2021; Ardani et al., 2023). Moreover, Goldsmith et al. (2022) observed that the administration of native rumen microbes, including *P. kudriavzevii* improved feed efficiency, although it was associated with reduced digestibility and somatic cell count. While these findings highlight the potential benefits of microbial feed additives, the precise mechanisms by which LAB and yeast influence rumen microbial ecology and host physiology require further investigation. Ji et al. (2022) reported that supplementation with *P. kudriavzevii* T17 and *Lactobacillus plantarum* Y9 did not significantly alter the overall rumen microbial community but did affect the relative abundance of specific taxa.

In the present study, *S. harbinensis* and *P. kudriavzeii*, both isolated from *budu*, a traditional fermented fish product from West Sumatra, Indonesia, were evaluated as microbial feed additives in ruminants. The experiment aimed to explore the effects of various combinations and dosages of these LAB and yeast strains on ruminal fermentation and nutrient digestibility *in vitro*. Additionally, molecular techniques were employed to characterise changes in the rumen microbial population.

Material and methods

Ethical approval

Rumen inoculum was obtained from goats at a commercial abattoir. Since the study did not involve live animal testing or interventions, and the inoculum was obtained during routine slaughterhouse operations in Padang, West Sumatra, Indonesia, ethical approval was not required, in accordance with the guidelines of the Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia.

Experimental design

This experiment was conducted at the Ruminant Nutrition and Feed Industry Technology Laboratory, Faculty of Animal Science, Universitas Andalas, Indonesia. Microbial population analyses were carried out at the Genomic and Environmental Laboratory, National Research and Innovation Agency (BRIN) in Cibinong, West Java, Indonesia. A completely randomised design was applied using a 3 × 4 factorial arrangement with three technical replicates per treatment. Factor A consisted of three LAB:yeast combinations: A1 (*S. harbinensis*:*P. kudriavzevii*, 1:1), A2 (1:3), and A3 (3:1). Factor B included four DFM inclusion levels: 1%, 2%, 3%, and 4% (v/v), resulting in 12 treatment combinations with 3 replicates each.

The inoculum contained *S. harbinensis* and *P. kudriavzevii*, each at 1 × 10^{10} CFU/ml, obtained from the Laboratory of Feed Industry Technology, Faculty of Animal Science, Universitas Andalas, Indonesia. *S. harbinensis* was cultured in 10 ml of MRS broth (Merck, Darmstadt, Germany) and incubated at 37 °C for 24–48 h. *P. kudriavzevii* was grown in 10 ml of yeast peptone dextrose medium at 35–37 °C for 24–48 h, following the procedure described by Ardani et al. (2023). The composition and nutritional content of the substrate are presented in Table 1.

Table 1. Ingredient and chemical composition of the substrate for *in vitro* fermentation

Ingredients	Content, %
Ingredients, % of DM	
Pennisetum purpureum cv. Mott (Odot grass)	60.0
rice bran meal	10.0
cassava pulp feed	8.00
soybean meal	4.00
palm kernel meal	6.00
corn gluten feed	4.00
copra meal	4.00
calcium	0.60
DCP	0.40
molasses	2.00
premix	1.00
Chemical composition of substrate	
DM, %	88.7
OM, % of DM	85.6
CP, % of DM	18.2
ether extract, % of DM	2.56
CF, % of DM	18.5
ash, % of DM	14.4
NFE, % of DM	34.9
TDN, %	66.9

DM – dry matter, DCP – dicalcium phosphate, OM – organic matter, CP – crude protein, CF – crude fibre, NFE – nitrogen-free extract, TDN – total digestible nutrient

Buffer solution preparation and *in vitro* rumen fermentation

McDougall's buffer solution was prepared 24 h before fermentation using the protocol of McDougall (1948). The buffer contained 9.8 g $NaHCO_3$, 3.68 g $Na_2HPO_4 \cdot 7H_2O$, 0.57 g KCl, 0.12 g $MgSO_4 \cdot 7H_2O$, and 0.47 g NaCl per 1000 ml of distilled water, and was maintained at 39 °C in a water bath. Rumen fluid was collected from a slaughtered goat at a commercial abattoir. The fermentation substrate consisted of 60% *Pennisetum purpureum* cv. Mott (Odot grass) and 40% concentrate (dry matter DM) basis). A total of 2.56 g of substrate (Table 1) was weighed into Erlenmeyer flasks, followed by 50 ml of rumen fluid and 200 ml of McDougall's buffer (1:4 v/v). DFM inoculum was then added according to the treatment. Flasks were sealed with rubber stoppers and flushed with CO_2 for approximately 2 min to ensure anaerobic conditions. *In vitro* fermentation was conducted at 39 °C with 90 rpm agitation for 48 h using a New Brunswick Scientific 126/126R incubator, following Tilley and Terry (1963).

Laboratory analysis

Fermentation parameters and digestibility

After 48 h of incubation, samples were centrifuged at 1509 *g* for 30 min at 4 °C (OHAUS Frontier FC5707, Parsippany, NY, USA). The resulting supernatant was collected and stored at −20 °C for subsequent analysis of ruminal fermentation parameters and microbial populations. Ruminal pH was determined using a calibrated pH meter (Jenway, Dunmow, UK). Ammonia (NH_3) concentration was determined with the Conway microdiffusion technique (Conway and O'Malley, 1942) using 1 ml of supernatant. Short-chain fatty acids (SCFA) were analysed in 5 ml of the supernatant using a Bruker Scion 436-GC gas chromatograph (Bruker, Billerica, MA, USA) with a 25 m × 0.32 mm column operated at 115 °C, pre-conditioned at 60 °C. Before injection, acidified rumen samples were centrifuged at 8050 *g* for 10 min at 7 °C (ThermoFisher Scientific, Waltham, MA, USA). External standards included acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate.

The solid residue was filtered through Whatman™ 41 filter paper (cat. no. 1441–125; Cytiva, Little Chalfont, UK), oven-dried at 60 °C for 24 h, and used for digestibility determination. DM was determined by drying at 105 °C for 24 h, and ash content was measured by incineration at 400–600 °C for 6 h in a muffle furnace. *In vitro* digestibility was calculated for dry matter (IVDMD), organic matter (IVOMD), and crude fibre (IVCFD) based on the Tilley and Terry method, as follows:

$$\text{IVDMD} = \frac{\text{DM sample} - (\text{DM sample } \textit{in vitro} - \text{DM blank})}{\text{DM sample}} \times 100\%;$$

$$\text{IVOMD} = \frac{\text{OM sample} - (\text{OM sample } \textit{in vitro} - \text{OM blank})}{\text{OM sample}} \times 100\%;$$

$$\text{IVCFD} = \frac{\text{CF sample} - (\text{CF sample } \textit{in vitro} - \text{CF blank})}{\text{CF sample}} \times 100\%.$$

DNA extraction and quantitative PCR (qPCR)

The relative abundance of selected rumen microbes was assessed by qPCR, targeting fibre-degrading bacteria (*Ruminococcus albus*, *R. flavefaciens*,

and *Treponema bryantii*), starch and protein-utilising bacteria (*Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, and *Prevotella ruminicola*), and methanogens (Ridwan et al., 2019). Microbial DNA was extracted using the Geneaid Genomic DNA Mini Kit (Blood/Culture Cell, Geneaid, Taipei, Taiwan) with modifications, including the addition of proteinase K (2 mg/ml) and Rnase A (10 mg/ml), followed by incubation at 60 °C for 30 min. DNA concentration was measured using a Nanodrop P-330 spectrophotometer (Implen, Munich, Germany).

qPCR was performed in 20 µl reaction volumes, containing 1 µl of microbial DNA template, 1 µl each of forward and reverse primers (10 pmol; Table 2), 10 µl SensiFAST SYBR Hi-ROX (Bioline, Meridian Bioscience, Memphis, TN, USA) and 7 µl of sterile Milli-Q water (Adawiah et al., 2025). Amplification of the 16S rDNA gene was conducted in duplicate using a QIAGEN Rotor-Gene Q Thermocycler (Qiagen, Hilden, Germany). For total bacteria, cycling conditions were: 95 °C for 3 min, 40 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 90 s, with a final extension at 72 °C for 10 min. For methanogenic archaea detection, the following programme of Zhou et al. (2022) was applied: 95 °C for 10 min, 40 cycles of 95 °C for 3 s, 60 °C for 30 s, and a final extension at 72 °C for 40 s. Relative abundance was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with control samples (rumen fluid, substrate, and buffer without DFM) as the reference.

Statistical analysis

Generalised linear mixed models were used for statistical analysis in SPSS version 29.0 (IBM SPSS, Armonk, NY, USA). The models included fixed effects for DFM combination (Factor A) and dose level (Factor B), with their interaction (A × B) as a random effect. Degrees of freedom were calculated using the Kenward-Roger approximation. Post-hoc pairwise comparisons were conducted using contrast analysis to examine specific treatment differences. Dose-response relationships (Factor B) were evaluated through polynomial contrasts (linear and quadratic effects) using one-way ANOVA. The threshold for statistical significance was set at $P \leq 0.05$.

Results

Ruminal fermentation

The DFM combinations and dose levels showed no significant effects on ruminal pH ($P > 0.05$; Tables 3, 4). NH_3 concentration ($P = 0.165$) and total SCFA production ($P = 0.161$) were similarly unaffected by DFM treatments (Table 3). Molar proportions of acetate ($P = 0.916$), propionate ($P = 0.672$), valerate ($P = 0.104$), and the acetate-to-propionate (A:P) ratio ($P = 0.900$) were also unaffected by DFM combination. However, treatment A1 significantly increased the proportions of butyrate ($P = 0.026$), iso-butyrate ($P = 0.010$) and iso-valerate ($P = 0.036$, Table 3).

Table 2. Primer sequences and amplicon sizes for target and reference microbial populations

Target microbes	Primer (5'→3')	Size, bp	Reference
Ruminococcus albus	F: TGTTAACAGAGGGAAGCAAAGCA R: TGCAGCCTACAATCCGAACTAA	175	Stevenson and Weimer (2007)
Ruminococcus flavefaciens	F: GGACGATAATGACGGTACTT R: GCAATCYGAACTGGGACAAT	835	Tajima et al. (2001)
Selenomonas ruminantium	F: TGCTAATACCGAATGTTG R: TCCTGCACTCAAGAAAGA	513	Tajima et al. (2001)
Butyrivibrio fibrisolvens	F: GTTTTTTCGGTACAAGAT R: TTACCGCGGCTGCTGGCA	213	Mrazek and Kopecny (2001)
Prevotella ruminicola	F: GGTTATCTTGAGTGAGTT R: CTGATGGCAACTAAAGAA	485	Tajima et al. (2001)
Treponema bryantii	F: AGTCGAGCGGTAAGATTG R: CAAAGCGTTTCTCTCACT	421	Tajima et al. (2001)
Methanogen	F: CCGGAGATGGAACCTGAGAC R: CGGTCTTGCCCAGCTCTTATTC	160	Zhou et al. (2022)
Total bacteria	F: CGGCAACGAGCGCAACCC R: CCATTGTAGCACGTGTGTAGCC	130	Denman and Mcsweeney (2006)

F – forward, R – reverse

Table 3. Effects of different combinations of direct-fed microbials (DFM) on *in vitro* ruminal fermentation parameters and nutrient digestibility

Item	DFM			SEM	*P*-value
	A1	A2	A3		
Ruminal fermentation					
pH	6.79	6.86	6.89	0.043	0.223
NH_3, mg/dl	14.6	13.8	14.0	0.38	0.165
total SCFA, mM	112	100	108	5.2	0.161
acetate, %	58.4	58.0	58.4	0.95	0.916
propionate, %	23.7	22.3	22.6	0.21	0.672
butyrate, %	10.40^{a}	9.35^{b}	9.40^{b}	0.414	0.026
iso-butyrate, %	4.08^{a}	3.38^{b}	3.18^{b}	0.224	0.010
valerate, %	0.97	0.90	0.85	0.069	0.104
iso-valerate, %	1.80^{a}	1.29^{b}	1.26^{b}	0.139	0.036
A:P	2.53	2.55	2.58	0.087	0.900
Nutrient digestibility, %					
IVDMD	63.2	61.4	60.7	2.00	0.452
IVOMD	64.5	62.5	61.4	1.99	0.429
IVCFD	64.9	64.2	63.4	1.59	0.776

NH_3 – ammonia, SCFA – short-chain fatty acids, A:P – acetate to propionate ratio, IVDMD – *in vitro* dry matter digestibility, IVOMD – *in vitro* organic matter digestibility, IVCFD – *in vitro* crude fibre digestibility, SEM – standard error of the mean; A1 – *Schleiferilactobacillus harbinensis* and *Pichia kudriavzevii* (1:1 ratio), A2 – *S. harbinensis* and *P. kudriavzevii* (1:3 ratio), A3 – *S. harbinensis* and *P. kudriavzevii* (3:1 ratio); ^{ab} – values within a row with different superscripts are siginficantly different at $P < 0.05$

Dose level, on the other hand, significantly influenced several fermentation parameters (Table 4). Increasing DFM dose levels caused a linear increase in NH_3 concentration ($P < 0.001$), as well as both a quadratic ($P = 0.026$) and linear ($P < 0.001$) increases in total SCFA content (Table 4). The molar proportion of acetate decreased quadratically ($P = 0.010$), while propionate ($P = 0.001$) and butyrate ($P = 0.026$) increased linearly. The highest dose (B4) resulted in the highest concentration of total SCFA, propionate, and butyrate. A linear reduction in the A:P ratio was also observed with increasing dose ($P < 0.001$), with the largest decrease observed in B4. No significant dose effects were observed for iso-butyrate, valerate, or iso-valerate ($P > 0.05$, Table 4).

Nutrient digestibility

Different DFM combinations had no significant effects on *in vitro* nutrient digestibility ($P > 0.05$, Table 3), including IVDMD ($P = 0.452$), IVOMD ($P = 0.429$), and IVCFD ($P = 0.776$). In contrast, increasing DFM dose levels significantly affected digestibility parameters (Table 4). Dose levels had a quadratic effect on IVDMD ($P = 0.008$), IVOMD ($P = 0.004$) and IVCFD ($P = 0.019$), in addition to linear effects on IVOMD ($P = 0.047$) and IVCFD ($P < 0.001$). The highest nutrient digestibility was observed for dose B4.

Microbial population

Different DFM combinations significantly increased the relative abundance of *Prevotella ruminicola* ($P = 0.008$) and *Butyrivibrio fibrisolvens* populations ($P = 0.012$, Figure 1). In contrast, other fibrolytic bacteria, including *Ruminococcus albus* ($P = 0.439$), *R. flavefaciens* ($P = 0.184$), and *Treponema bryantii* ($P = 0.591$), *Selenomonas ruminantium* ($P = 0.941$), and methanogens ($P = 0.410$) remained unaffected (Figure 1).

Table 4. Effect of different dose levels of direct-fed microbials (DFM) on *in vitro* ruminal fermentation parameters and nutrient digestibility

Item	Dose levels				SEM	*P*-value	
	B1	B2	B3	B4		linear	quadratic
Ruminal fermentation							
pH	6.86	6.86	6.81	6.86	0.050	0.662	0.532
NH_3, mg/dl	13.8^{c}	13.3^{c}	14.4^{b}	15.7^{a}	0.44	<0.001	0.026
total SCFA, mM	88.3^{c}	102^{b}	114^{a}	123^{a}	6.05	<0.001	0.215
acetate, %	59.2^{a}	58.7^{a}	59.1^{a}	56.1^{b}	1.09	<0.001	0.010
propionate, %	21.3^{c}	22.7^{b}	22.8^{b}	24.7^{a}	0.71	<0.001	0.748
butyrate, %	9.10^{c}	9.47^{bc}	10.03^{bc}	10.23^{a}	0.479	0.026	0.812
iso-butyrate, %	3.60	3.50	3.43	3.63	0.259	0.969	0.582
valerate, %	0.97	1.00	1.00	1.17	0.049	0.080	0.557
iso-valerate, %	1.80	1.70	1.67	1.83	0.023	0.160	0.286
A:P	2.77^{a}	2.57^{b}	2.57^{b}	2.30^{c}	0.100	<0.001	0.605
Nutrient digestibility, %							
IVDMD	63.4^{a}	58.5^{b}	61.7^{a}	63.4^{a}	2.31	0.130	0.008
IVOMD	64.6^{a}	58.9^{b}	62.5^{a}	65.2^{a}	2.25	0.047	0.004
IVCFD	63.5^{bc}	62.8^{c}	64.4^{b}	66.4^{a}	1.83	<0.001	0.019

NH_3 – ammonia, SCFA – short-chain fatty acids, A:P – acetate to propionate ratio, IVDMD – *in vitro* dry matter digestibility, IVOMD – *in vitro* organic matter digestibility, IVCFD – *in vitro* crude fibre digestibility, SEM – standard error of the mean; B1 – 1%, B2 – 2%, B3 – 3%, B4 – 4% (v/v ratio); ^{abc} – values within a row with different superscripts are siginficantly different at $P < 0.05$

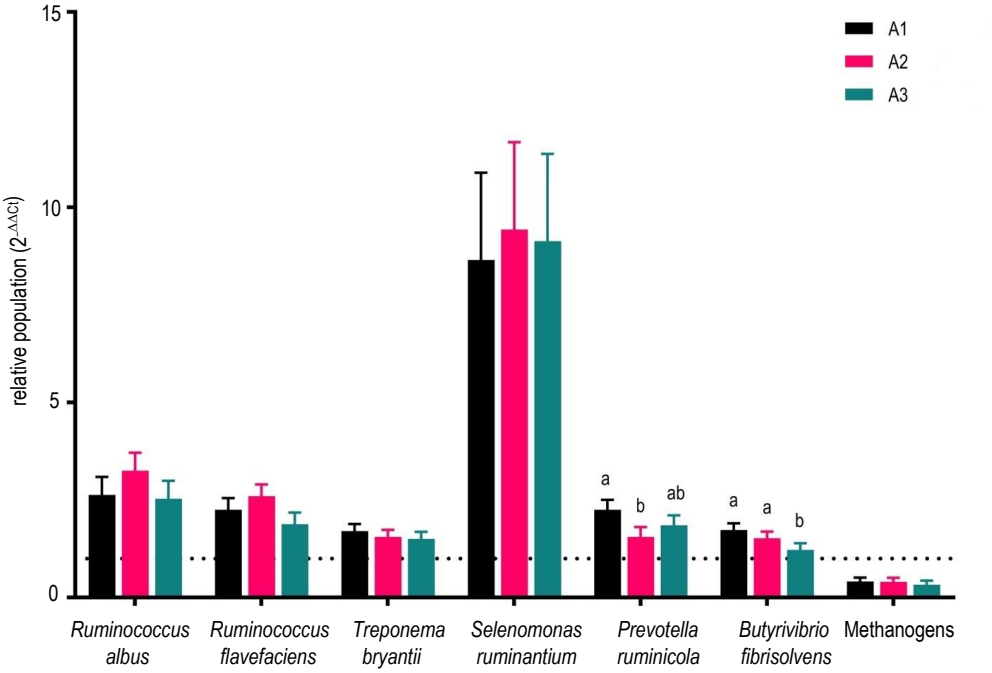

Figure 1. Relative abundance of ruminal microbial populations in response to different combinations of direct-fed microbials (DFM). Different superscripts (ab) indicate significant differences between treatments ($P < 0.05$); dashed line (------) represents the control.

DFM: A1 – *Schleiferilactobacillus harbinensis* and *Pichia kudriavzevii* (1:1 ratio), A2 – S. *harbinensis* and *P. kudriavzevii* (1:3 ratio), A3 – *S. harbinensis* and *P. kudriavzevii* (3:1 ratio)

Incrementing DFM dose levels linearly increased the relative abundance of *R. albus* ($P < 0.001$) and *T. bryantii* ($P < 0.001$; Figure 2A, 2C). *R. flavefaciens* population showed both quadratic ($P = 0.007$) and linear responses (P = 0.039)to rising doses (Figure 2B). *S. ruminantium* and *P. ruminicola* populations also increased quadratically ($P = 0.002$ and $P < 0.001$, respectively), with the highest population observed in treatment B4 (Figure 2E, 2D). No significant changes were detected for *B. fibrisolvens* ($P = 0.772$) (Figure 2F). Among all bacteria evaluated, *S. ruminantium* reached the highest relative abundance (Figure 2E). Moreover, methanogen populations decreased

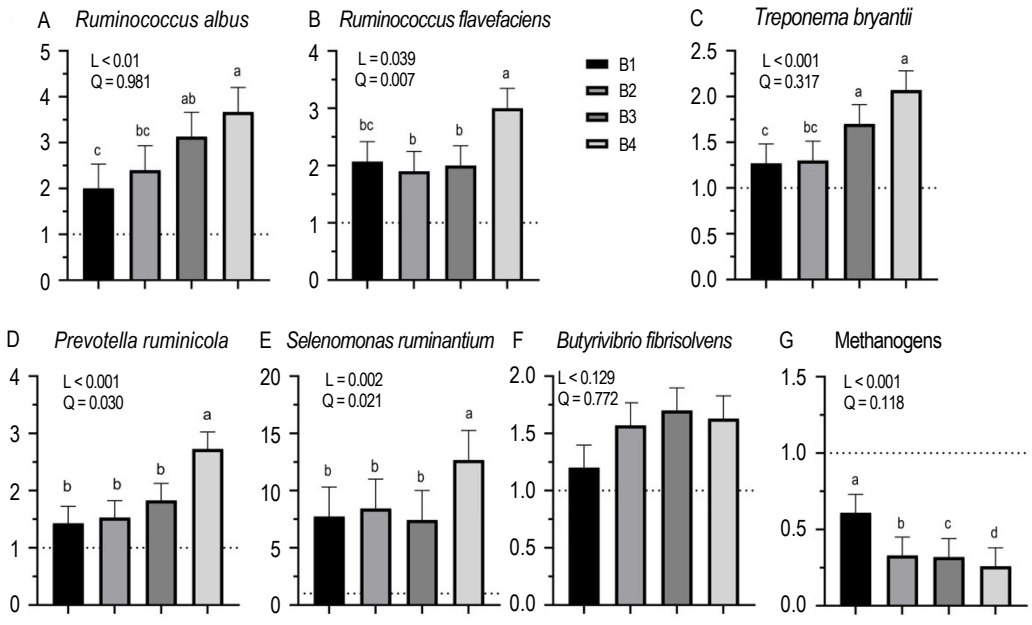

Figure 2. Relative abundance of ruminal microbial populations in response to different dose levels of direct-fed microbials. Different superscripts (a–d) indicate significant differences between treatments ($P < 0.05$); dashed line (------) represents the control.

Dose levels: B1 – 1%, B2 – 2%, B3 – 3%; B4 – 4% (v/v); L – linear; Q – quadratic

linearly with increasing doses ($P < 0.001$), with the lowest abundance observed in B4 (Figure 2G).

Discussion

Direct-fed microbials represent a well-established approach for modulating rumen fermentation and intestinal function in livestock (Guimaraes et al., 2024). Recent approaches focus on multi-species consortia, as synergistic interactions between various strains, species, and genera are considered more beneficial to the host than single-species or single-strain formulations. Previous study *in vitro* using bovine rumen fluid reported that multi-species bacterial DFM improved DM and NDF degradability (Oyebade et al., 2024). Supplementation with *Pichia kudriavzevii* T7, *L. plantarum* Y9, and *Candida glabrata* B14 has been shown to improve microbiome structure and abundance in dairy cows (Ji et al., 2022). Based on these findings, we hypothesised that DFM supplementation in various combinations and doses would positively affect ruminal fermentation, nutrient digestibility, and rumen microbiota. Our findings partially confirmed this hypothesis, demonstrating improvements in certain ruminal fermentation and digestibility parameters, leading to increased SCFA concentrations. However, no significant changes were observed in nutrient digestibility between individual DFM combinations, although higher DFM doses were generally associated with improved fermentation parameters and microbial diversity.

No interaction was observed between the DFM combinations and the doses administered in the present study. This may be explained by several factors. First, the independent modes of action of *S. harbinensis* (LAB) and *P. kudriavzevii* (yeast) likely resulted in additive rather than synergistic effects. This was consistent with the findings of Chaucheyras-Durand and Fonty (2001), who observed that bacterial-yeast synergisms primarily occurred under rumen stress conditions (e.g., pH fluctuations), which were absent in our controlled *in vitro* system. Second, *in vitro* conditions are stable and do not reflect the complexity of living organisms, which reduces biological variability and limits the likelihood of interactions between dose and combination. Zhao et al. (2022) have similarly found that synergistic effects between microbes were more apparent in complex, dynamic *in vivo* systems, where factors, such as rumen passage and absorption rates influence fermentation outcomes.

In vitro models of rumen fermentation provide a cost-effective approach for simulating *in vivo* conditions on a laboratory scale. While these models do not fully replicate the complexity of the rumen environment, their standardised conditions enable rapid assessment of treatment effects across key parameters (e.g., SCFA, gas production) within 24–72 h incubation periods (e.g., 24–72 h) (Oyebade et al., 2025). Several such studies have examined different DFM combinations, doses, incubation times, and substrates (Dhakal et al., 2023; Silva et al., 2024). However, the effects of various DFM types and doses on rumen fermentation remain inconsistent. For instance, Silva et al. (2024) observed a quadratic increase in IVOMD and total gas production, a linear increase in SCFA concentration, and reductions in methane production and the acetate-to-propionate ratio following supplementation with *Enterococcus faecium* and *Scaccharomyces cerevisiae* (5×10^9 CFU/g). Similarly, increasing doses of a *B. licheniformis* and *B. subtilis* combination (8×10^4 CFU/ml) improved total SCFA, NH_3 and gas production, with optimal effects observed at intermediate doses. Higher levels of *Bacillus* spp. reduced the molar proportion of acetate and increased that of propionate, with gas production showing a quadratic response (Maderal et al., 2022). In contrast, Madkour et al. (2018) recorded improved nutrient digestibility with a 1:1 mixture of *Phanerochaete chrysosporium* (fungus) and *B. subtilis*, but no significant changes in total SCFA or NH_3 concentration at 0, 3, and 6 hours post-feeding.

Our results are consistent with previous studies, particularly the increase in the proportions of butyrate, iso-butyrate, and iso-valerate observed with the 1:1 combination of *S. harbinensis* and *P. kudriavzevii*. However, despite these alterations in fermentation profiles, no significant effects were found with respect to nutrient digestibility, total SCFA, or NH_3 concentrations. These findings are consistent with those of Philippeau et al. (2017), who found no influence of bacterial DFM supplementation on total SCFA concentration, although they observed dose-dependent improvements in IVDMD, IVOMD, and IVCFD at 4% inclusion levels. It is possible that the 1:1 combination of LAB and yeast at this dose selectively stimulate specific beneficial microbial populations and metabolic pathways without broadly affecting overall fermentation efficiency.

The current study further supports evidence that higher DFM doses can improve nutrient digestibility

and ruminal fermentation by stimulating microbial enzyme production, which in turn modulates SCFA profiles (Oyebade et al., 2024). For example, alterations in fibre degradation can affect SCFA synthesis, which may directly change the composition and function of the rumen microbiota (Ji et al., 2022). The benefits of DFM supplementation are attributed to the supply of growth factors and the release of essential metabolites and enzymes from yeast, which stimulate the proliferation of cellulolytic and amylolytic bacteria (Ghazanfar et al., 2017). In addition, LAB supplementation promotes the growth of lactic acid-utilising bacteria that produce antimicrobial substances, reducing pathogen abundance and favouring beneficial bacteria populations (Ridwan et al., 2018; Kulkarni et al., 2022).

During fermentation, LAB and yeast can synergistically produce health-promoting metabolites. Microbial interactions in the rumen occur not only with substrate but also among microorganisms. Jiang et al. (2017) demonstrated that increasing the dose and viability of *Saccharomyces cerevisiae* improved DM and NDF digestibility, likely promoting the growth of cellulolytic bacteria (e.g., *Ruminococcus* and *Fibrobacter succinogens*) and amylolytic bacteria (e.g., *Ruminobacter* and *Selenomonas ruminantium*) in the rumen. The observed changes in SCFA proportions, such as increased propionate and decreased acetate concentrations, indicate changes in fermentation processes induced by DFM. Elevated propionate levels are likely due to succinate conversion, a reaction aided by ruminal microorganisms, including yeast, involving coenzyme-A biosynthesis (McCoun et al., 2021). The combination of *S. harbinensis* and *P. kudriavzevii* in the present study appears to provide an effective synergistic interaction. This finding aligns with McCoun et al. (2021), who reported that probiotic formulations containing *S. cerevisiae* and LAB reduced lipid peroxidation products, an effect attributed to the antioxidant properties of LAB and their synergistic interaction with *S. cerevisiae*. These findings further support the use of multi-strain DFM formulations to improve fermentation processes.

In the present study, DFM supplementation altered rumen microbial populations. The DFM combinations significantly increased the relative abundance of *Prevotella ruminicola* and *Butyrivibrio fibrisolvens* populations, with the 1:1 ratio showing the most optimal effect compared to other treatments. In contrast, the remaining target microbial populations were not significantly affected by the DFM ratio (Figure 1). However, DFM dose levels exerted a broader impact on the rumen microbiome, influencing nearly all populations examined. Higher doses increased the abundance of *R. albus*, *R. flavefaciens*, *T. bryantii*, *S. ruminantium*, *P. ruminicola*, while reducing methanogen populations (Figure 2). These results indicate that dosage is a key factor in shaping the rumen microbiota.

A previous study by Ji et al. (2022) demonstrated that yeast and LAB did not significantly alter the rumen microbiome but modified the relative abundance of specific microorganisms. LAB produce lactic acid in the rumen, which stimulates lactate-utilising bacteria such as *S. ruminantium* and *Megasphaera elsdenii*. This process increases propionate production either through direct lactate conversion or indirectly via succinate pathways (Beauchemin et al., 2003). Yeast supplementation has also been associated with increases in fibrolytic bacterial populations, likely due to its oxygen-scavenging properties, which help maintain anaerobic conditions and stable pH, supporting cellulolytic activity (McAllister et al., 2001). McCoun et al. (2021) also reported that *S. cerevisiae*-based DFM changed the abundance of *Prevotella 1* and *Prevotellaceae UCG-001*. Similarly, in the present study, DFM inclusion increased the population size of *R. flavefaciens*, *R. albus*, and *Butyrivibrio fibrisolvens*, all of which are involved in the degradation of cellulose, pectin, and xylan (Palmonari et al., 2024).

The increased relative abundance of *R. albus*, *R. flavefaciens*, *T. bryantii*, *S. ruminantium*, and *P. ruminicola* indicates a higher fermentation efficiency within the rumen ecosystem. A study by Phestcha et al. (2021) reported that yeast inclusion increased *R. flavefaciens* and *Fibrobacter succinogens* populations in feedlot steers. Other members of the genus *Prevotella*, such as *P. ruminicola* and *P. bryantii*, also play a role in protein degradation and interact synergistically with cellulolytic bacteria (Palmonari et al., 2024). *R. albus* and *R. flavefaciens* are key fibrolytic bacteria that break down plant cell walls, increasing fibre degradation and acetate production (Phestcha et al., 2021). These species produce acetic, butyric, lactic, formic and fumaric acids as fermentation end products (Palevich et al., 2019). The genera *Ruminococcus* and *Butyrivibrio* with dipeptidyl peptidase activity hydrolyse peptides into dipeptides in the rumen, which are further deaminated into amino acids (AA), resulting in free AA accumulation and increased NH_3 production (Monteiro et al., 2022). *T. bryantii* contributes to secondary fermentation by metabolising

oligosaccharides and promoting microbial interactions. *S. ruminantium* is a lactate-utilising and propionate-producing bacterium that helps stabilise rumen pH and shift fermentation towards propionate synthesis, which is an energetically favourable volatile fatty acid (VFA) in terms of animal performance (Chen et al., 2025). Meanwhile, *P. ruminicola* demonstrate versatile substrate utilisation, degrading both non-structural carbohydrates and peptides to produce butyrate and microbial protein (Palmonari et al., 2024).

This study demonstrated consistent reductions in methanogen population in all treatment groups (Figures 1, 2). This decrease could be attributed to the activity of LAB, which secrete antimicrobial peptides such as bacteriocins and possess the ability to metabolise and detoxify various toxins (Jeyanathan et al., 2014). The observed methanogen decline suggests potential mitigation in methane emissions, reflecting a more energy-efficient fermentation process. Methanogens utilise hydrogen for methanogenesis, a process that diverts energy away from the host's metabolism. Suppressing the development of these bacteria allows redirecting hydrogen towards alternative sinks, such as propionate production by *S. ruminantium* (Philippeau et al., 2017; Jeyanathan et al., 2019). This microbial transformations increase VFA production and supports sustainable rumen function. The present findings are consistent with previous studies suggesting that targeted modulation of the rumen microbiota can reduce energy losses and improve rumen fermentation (Jeyanathan et al., 2019). However, the specific mechanisms by which DFM affect methanogen populations remain unclear and require further research.

Conclusions

This study assessed the effects of different combinations and inclusion levels of direct-fed microbials (DFM) on ruminal fermentation, nutrient digestibility, and microbial populations under *in vitro* conditions. The 1:1 ratio combination of *Schleiferilactobacillus harbinensis* and *Pichia kudriavzevii* at a 4% inclusion level increased ruminal fermentation, nutrient digestibility, and rumen microbial communities. While positive effects were observed, not all parameters were significantly affected, indicating that DFM efficacy depends on microbial interactions and dose supplemented. The results also suggest that higher DFM doses may lead to more pronounced responses.

These findings confirm the potential of DFM supplementation to improve ruminal fermentation and nutrient utilisation. However, further research is required to confirm these effects under *in vivo* conditions and to optimise direct-fed microbials (DFM) formulations for practical application in ruminant production.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

Adawiah A., Meryandini A., Ridwan R., Fidriyanto R., Sarwono K.A., Wiryawan K.G., 2025. The rumen microbiome and metabolome profile of Ongole crossbreed cattle fed probiotics and protected amino acids. Trop. Anim. Health Prod. 57, 148, https://10.1007/s11250-025-04400-z

Amin A.B., Mao S., 2021. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review. Anim. Nutr. 7, 31–415, https://doi.org/10.1016/j.aninu.2020.10.005

Ardani L.R., Marlida Y., Zain M., Jamsari J., Fassah D.M., 2023. Lactic acid bacteria and yeast strains isolated from fermented fish (Budu) identified as candidate ruminant probiotics based on *in vitro* rumen fermentation characteristics. Vet. World 16, 395–402, https://doi.org/10.14202/vetworld.2023.395-402

Beauchemin K.A., Yang W.Z., Morgavi D.P., Ghorbani G.R., Kautz W., Leedle J.A.Z., 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. J. Anim. Sci. 81, 16281640, https://doi.org/10.2527/2003.8161628x

Chaucheyras-Durand F., Fonty G., 2001. Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive *Saccharomyces cerevisiae* CNCM I-1077. Reprod. Nutr. Dev. 41, 57–68, https://doi.org/10.1051/rnd:2001112

Chaucheyras-Durand F., Walker N.D., Bach A., 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed. Sci. Tech. 145, 5–26, https://doi.org/10.1016/j.anifeedsci.2007.04.019

Chen Q., Sha Y., Liu X. et al., 2025. A study on the differences in rumen microbiota-liver gluconeogenesis-mitochondrial interaction between Tibetan Sheep and Hu Sheep in the Qinghai-Tibet Plateau. Animals 15, 1603, https://doi.org/10.3390/ani15111603

Conway E.J., O'Malley E., 1942. Microdiffusion methods. Ammonia and urea using buffered absorbents (revised methods for ranges greater than 10 µg. N). Biochem. J. 36, 655–661, https://doi.org/10.1042/bj0360655

Denman S.E., McSweeney C.S., 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiol. Ecol. 58, 572–82, https://doi.org/10.1111/j.1574-6941.2006.00190.x

Dhakal R., Copani G., Cappellozza B.I., Milora N., Hansen H.H., 2023. The effect of direct-fed microbials on *in-vitro* rumen fermentation of grass or maize silage. Fermentation 9, 347, https://doi.org/10.3390/fermentation9040347

Ghazanfar S., Khalid N., Ahmed I., Imran M., 2017. Probiotic yeast: mode of action and its effects on ruminant nutrition. In: A. Morata, I. Loira (Editors). Yeast - Industrial Applications. Intech Open. London (UK), pp. 179–202, https://doi.org/10.5772/intechopen.70778

Goldsmith K., Lefler J., Embree M., VandeHaar M.J., 2022. The effect of supplementing native rumen microbes on milk production of dairy cows. JDS Commun. 4, 31–34, https://doi.org/10.3168/jdsc.2022-0250

Guimaraes O., Preedy G., Fox J.T., Cappellozza B.I., Davis T.C., Schutz J.S., Theurer M.E., 2024. A novel direct-fed microbial impacts growth performance and supports overall health of feedlot cattle. Ruminants 4, 267–279, https://doi.org/10.3390/ruminants4020019

Jeyanathan J., Martin C., Eugène M., Ferlay A., Popova M., Morgavi D.P., 2019. Direct-fed microbials fail to reduce methane emissions in primiparous lactating dairy cows. J. Animal Sci. Biotechnol. 10, 41, https://doi.org/10.1186/s40104-019-0342-9

Jeyanathan J., Martin C., Morgavi D.P., 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. Animal 8, 250–261, https://doi.org/10.1017/S1751731113002085

Ji Y., Dong X., Liu Z., Wang W., Yan H., Liu X., 2022. Effects of bovine *Pichia kudriavzevii* T7, *Candida glabrata* B14, and *Lactobacillus plantarum* Y9 on milk production, quality and digestive tract microbiome in dairy cows. Microorganism 10, 842, https://doi.org/10.3390/microorganisms10050842

Jiang Y., Ogunade I.M., Qi S., Hackmann T.J., Staples C.R., Adesogan A.T., 2017. Effects of the dose and viability of *Saccharomyces cerevisiae*. 1. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. J. Dairy Sci. 100, 325–342, https://doi.org/10.3168/jds.2016-11263

Jiao P.X., Liu F.Z., Beauchemin K.A., Yang W.Z., 2017. Impact of strain and dose of lactic acid bacteria on *in vitro* ruminal fermentation with varying media pH levels and feed substrates. Anim. Feed Sci. Tech. 224, 1–13, https://doi.org/10.1016/j.anifeedsci.2016.11.005

Jung J.Y., Han S.S., Kim Z.H., Kim M.H., Kang H.K., Jin H.M., Lee M.H., 2021. *In-vitro* characterization of growth inhibition against the gut pathogen of potentially probiotic lactic acid bacteria strains isolated from fermented products. Microorganisms 9, 2141, https://doi.org/10.3390/microorganisms9102141

Kraimi N., Dawkins M., Gebhardt-Henrich S.G., Velge P., Rychlik I., Volf J., 2019. Influence of the microbiota-gut-brain axis on behaviour and welfare in farm animals: a review. Physiol. Behav. 210, 112658, https://doi.org/10.1016/j.physbeh.2019.112658

Kulkarni, Nitish, Chethan H., Srivastava, Rashika, Gabbur, Anil, 2022. Role of probiotics in ruminant nutrition as natural modulators of health and productivity of animals in tropical countries: an overview. Trop. Anim. Health Pro. 54, https://doi.org/10.1007/s11250-022-03112-y

Kumprechtovaa D., Illek J., Julien C., Homolka P., Janccik F., Auclair E., 2019. Effect of live yeast (*Saccharomyces cerevisiae*) supplementation on rumen fermentation and metabolic profile of dairy cows in early lactation. J. Anim. Physiol. An. N. 103, 447–55, https://doi.org/10.1111/jpn.13048

Livak K.J., Schmittgen T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25, 402–408, https://doi.org/10.1006/meth.2001.1262

Maderal A., Fernandez-Marenchino I., Cuervo W. et al., 2022. PSI-19 Effect of increasing supplementation of *Bacillus* spp. on in vitro ruminal fermentation and nutrient digestibility. J. Anim. Sci. 100, 359, https://doi.org/10.1093/jas/skac247.655

Madkour M.A., Khattab H.M., El-Bordeny H.M., Mattar B.E., 2018. Evaluation of direct-fed microbial supplementation to improve utilization of the low quality roughages in ruminants. Arab. Univ. J. Agric. Sci. 26, 1869–1878, https://doi.org/10.21608/ajs.2018.31656

Marlida Y., Nurmiati N., Husmaini H., Huda N., Anggraini L., Ardani L.R., 2023. The potential of lactic acid bacteria isolated from ikan budu (fermented fish) to inhibit the growth of pathogenic fungi and detoxify aflatoxin B1. Vet. World 16, 1373–1379, https://doi.org/10.14202/vetworld.2023.1373-1379

Mashraqi A., Eshetea B.B., Eribo B., 2023. Draft genome sequences of probiotic candidate Schleiferilactobacillus harbinensis isolated from fermented milk "Laban". Microbiol Resour. Announc. 12, e0104322, https://doi.org/10.1128/mra.01043-22

McAllister T.A., Beauchemin K.A., Alazzeh A.Y., Baah J., Teather R.M., Stanford K., 2001. Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci. 91, 193211, https://doi.org/10.4141/cjas10047

McCoun M., Oyebade A., Estrada-Reyes Z.M., Pech-Cervantes A.A., Ogunade I.M., 2021. Effects of multi-species direct-fed microbial products on ruminal metatranscriptome and carboxyl-metabolome of beef steers. Animals (Basel) 11, 72, https://doi.org/10.3390/ani11010072

McDougall E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43, 99–109, https://doi.org/10.1042/bj0430099

Miyamoto M., Seto Y., Hai H.D., Teshima T., Bo S.Y., Kabuki T., Bing Y.L., Nakajima H., 2005. *Lactobacillus harbinensis* sp. nov., consisted of strains isolated from traditional fermented vegetables "Suan cai" in Harbin, Northeastern China and *Lactobacillus perolens* DSM 12745. Syst. Appl. Microbiol. 28, 688–694, https://doi.org/10.1016/j.syapm.2005.04.001

Monteiro H.F., Lelis A.L.J., Fan P, Calvo A.B., Lobo R.R., Arce-Cordero J.A., Dai X., Jeong K.C., Faciola A.P., 2022. Effects of lactic acid-producing bacteria as direct-fed microbials on the ruminal microbiome. J. Dairy Sci. 105, 2242–2255, https://doi.org/10.3168/jds.2021-21025

Mrazek J., Kopecny J., 2001. Development of competitive PCR for detection of *Butyrivibrio fibrisolvens* in the rumen. Folia Microbiol. (Praha) 46, 63–65, https://doi.org/10.1007/BF02825888

Oyebade A.O., Arriola K., Queiroz O., Cappellozza B.I., Vyas D., 2024. Effects of direct-fed microbials supplementation on *in vitro* and ex vivo ruminal fermentation and nutrient degradability in lactating Holstein dairy cows. Transl. Anim. Sci. 8, txae162, https://doi.org/10.1093/tas/txae162

Palevich N., Kelly W.J., Leahy S.C., Denman S., Altermann E., Rakonjac J., Attwood G.T., 2019. comparative genomics of rumen *Butyrivibrio* spp. uncovers a continuum of polysaccharide-degrading capabilities. Appl. Environ. Microbiol 86, e01993-19, https://doi.org/10.1128AEM.01993-19

Palmonari A., Federiconi A., Formigoni A., 2024. Animal board invited review: The effect of diet on rumen microbial composition in dairy cows. Animal 18, 101319, https://doi.org/10.1016/j.animal.2024.101319

Phesatcha K., Phesatcha B., Chunwijitra K., Wanapat M., Cherdthong A., 2021. Changed rumen fermentation, blood parameters, and microbial population in fattening steers receiving a high concentrate diet with *Saccharomyces cerevisiae* improve growth performance. Vet. Sci. 8, 294, https://doi.org/10.3390/vetsci8120294

Philippeau C.A., Lettat C., Martin M., Silberberg D.P., Morgavi A., Ferlay C., Berger P.N., 2017. Effects of bacterial direct-fed microbials on ruminal characteristics, methane emission, and milk fatty acid composition in cows fed high- or low-starch diets. J. Dairy Sci. 100, 2637–2650, https://doi.org/10.3168/jds.2016-11663

Ridwan R., Bungsu W.A., Astuti W.D., Rohmatussolihat R., Sari N.F., Fidriyanto R., Jayanegara A., Wijayanti I., Widyastuti Y., 2018. The use of lactic acid bacteria as ruminant probiotic candidates based on in vitro rumen fermentation characteristics. Buletin Peternakan. 42, 31–36, https://doi.org/10.21059/buletinpeternak.v42i1.23317

Ridwan R., Rusmana I., Widyastuti Y., Wiryawan K.G., Prasetya B., Sakamoto M., Ohkuma M., 2019. Bacteria and methanogen community in the rumen fed different levels of grass-legume silages. Biodiversitas 20, 1055–1062, https://doi.org/10.13057/biodiv/d200417

Sanam M.U.E., Detha A.I.R., Rohi N.K., 2022. Detection of antibacterial activity of lactic acid bacteria, isolated from Sumba mare's milk, against *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli.* J. Adv. Vet. Anim. Res. 9, 53–58, https://doi.org/10.5455/javar.2022.i568

Silva T.H., Ncio B.R., Magnani E., Reolon H.G., Timm T.G., Ama, Meurer G.W., Cappellozza B.I., Branco R.H., Paula E.M., 2024. Evaluation of direct-fed microbials on *in vitro* ruminal fermentation, gas production kinetic, and greenhouse gas emissions in different ruminants' diet. Front. Anim. Sci. 5, 1320075, https://doi.org/10.3389/fanim.2024.1320075

Stevenson D.M., Weimer P.J., 2007. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl. Microbiol. Biotechnol. 75, 165–174, https://doi.org/10.1007/s00253-006-0802-y

Suntara C., Cherdthong A., Wanapat M., Uriyapongson S., Leelavatcharamas V., Sawaengkaew J., Chanjula P., Foiklang S., 2021. Isolation and characterization of yeasts from rumen fluids for potential use as additives in ruminant feeding. Vet. Sci. 8, 52, https://doi.org/10.3390/vetsci8030052

Susalam M.K., Harnentis, Marlida Y., Jamsari, Ardani L.R., 2024. The effect of probiotics consortium isolated from fermented fish (Budu) on broiler performances and meat quality. IJVS 13, 100–107, https://doi.org/10.47278/journal.ijvs/2023.066

Tajima K., Aminov R.I., Nagamine T., Matsui H., Nakamura M., Benno Y., 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microbiol. 67, 2766–2774, https://doi.org/10.1128/AEM.67.6.2766-2774.2001

Tilley J.M.A., Terry R.A., 1963. A Two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18, 104–111, https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Villena J., Aso H., Rutten V.P.M.G., Takahashi H., van Eden W., Kitazawa H., 2018. Immunobiotics for the bovine host: Their interaction with intestinal epithelial cells and their effect on antiviral immunity. Front. Immunol. 9, 36, https://doi.org/10.3389/fimmu.2018.00326

Zhao W., Abdelsattar M.M., Wang X., Zhang N., Chai J., 2023. *In vitro* modulation of rumen fermentation by microbiota from the recombination of rumen fluid and solid phases. Microbiol. Spectr. 11, e03387-22, https://doi.org/10.1128/spectrum.03387-22

Zhou M., Hernandez-Sanabria E., Guan L.L., 2022. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microbiol. 75, 6524–6533, https://doi.org/10.1128/AEM.02815-08