Journal of Animal and Feed Sciences, 34, 4, 2025, xxx–xxx, https://doi.org/10.22358/jafs/204072/2025
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

ORIGINAL PAPER

ARTICLE IN PRESS

Effects of yeast preparation on *in vitro* rumen fermentation of low-concentrate substrate

L. Lin, J. Luan, X. Feng and Ch. Geng*

Yanbian University, Agricultural College, 133002,Yanji, China

KEY WORDS: active dry yeast, *in vitro* gas production method, yeast culture

Received: 20 January 2025
Revised: 14 April 2025
Accepted: 15 April 2025

* Corresponding author:
e-mail: cygeng1011@163.com

ABSTRACT. The purpose of this experiment was to explore the effects of yeast preparations on *in vitro* rumen fermentation of low-concentrate substrate, and to provide a theoretical basis for its application in ruminant nutrition. The study employed an *in vitro* gas production to compare the effects of four yeast preparations: active dry yeast products (ADY1 and ADY2) and yeast culture products (YC1 and YC2) using a total mixed ration (concentrate-to-roughage ratio 30:70) as the fermentation substrate. The results showed that ADY2 significantly increased ammoniacal nitrogen (NH_3-N) concentration compared to the control ($P < 0.05$). ADY1 significantly elevated acetic acid concentration while reducing valeric acid levels ($P < 0.05$). Methane production showed a numerical decrease with ADY1 and YC1 supplementation and a numerical increase with ADY2 and YC2 addition compared to control, with ADY2 and YC2 producing significantly more methane than ADY1 ($P < 0.05$). All yeast treatments significantly reduced lactic acid concentration relative to control ($P < 0.05$). These findings indicate that yeast preparations can positively influence rumen fermentation parameters, with effects varying by product type, suggesting potential for targeted applications in ruminant feeding strategies.

Introduction

Yeast is a commonly used probiotic in ruminant nutrition due to its ability to improve rumen fermentation, feed efficiency, and effectively prevent rumen acidosis (Amin and Mao, 2021). The two most common yeast-based products in animal production are yeast culture (YC) and active dry yeast (ADY). YC is produced through liquid or solid fermentation followed by concentration and drying, containing yeast cell walls, intracellular components, extracellular metabolites, and residual fermentation medium. ADY consists of live yeast cells cultured using aerobic liquid fermentation, resulting in a product containing viable yeast cells that are subsequently dehydrated and dried (Zhang et al., 2022). Although these products differ in their manufacturing processes and modes of action, they share certain similar beneficial effects when applied in ruminant nutrition (Zhang et al., 2022). Research has demonstrated that yeast preparations play many functions, including regulation of rumen microecological balance, pH stabilisation, methane (CH_4) reduction, and enhancement of immune and antioxidant capacities in animals (Bradley et al., 1994; Geng et al., 2015). Studies by Gao et al. (2022) demonstrated that supplementation with ADY and YC could alter the relative abundance of certain cellulolytic bacteria and lactic acid-utilising bacteria in the hindgut. Further supporting these findings, Ma et al. (2021) reported that the addition of ADY to the diet of weaned beef calves improved growth performance through enhanced rumen fermentation, nutrient digestibility, and immune response. Yeast preparations show

considerable promise in animal nutrition applications, yet their effects in ruminants remain inconsistent. These variations stem from multiple factors including the specific yeast strains used, basal diet composition, preparation types, and individual animal characteristics (Geng et al., 2015).

Existing research has primarily focused on comparing yeast preparation types under high-concentrate fermentation or feeding conditions (Geng et al., 2016), while comparative studies utilising low-concentrate diets remain relatively limited. This study evaluated four commonly used yeast preparations, i.e. ADY and YC preparations using an *in vitro* gas production system with a low-concentrate total mixed ration (TMR) (30:70 concentrate-to-roughage ratio). We systematically compared their effects on gas production kinetics, methane emissions, and rumen fermentation parameters. The findings provide important insights into effects of different yeast preparations under low-concentrate conditions and provide a theoretical basis for their application in ruminants.

Material and methods

All animal procedures were approved by the Yanbian University Institutional Animal Care and Use Committee (Approval ID: 20191015011).

Yeast preparations

The ADY group was subdivided into ADY1 (active yeast cells, viable count ≥20×10^{10} CFU/g) and ADY2 (CNCMI-1077, milky spherical particles, active yeast cells, viable count ≥0.8×10^{10} CFU/g). The YC group included YC1 and YC2 subgroups, both powdered yeast culture preparations. Prior to the trial, ADY samples were activated in distilled water at 37 °C for 2 h, followed by filtration to collect the supernatant. Yeast concentration in the supernatant was determined using a haemocytometer, following the dosage and methodology described by Geng et al. (2015).

Fermentation substrate

The basal substrate for *in vitro* fermentation consisted of a total mixed ration (TMR) with a concentrate-to-roughage ratio of 30:70. The complete composition and nutritional profile of the TMR are presented in Table 1. Prior to fermentation, the substrate was oven-dried and ground to pass through an 80-mesh sieve for uniform particle size.

Table 1. Composition and nutrient levels of basal diets, % DM basis

Ingredients	Content	Nutrient components	Content
Maize straw	70	NE_g[2], Mcal/kg	0.75
Maize	12.5	Crude protein	12.47
Soybean meal	16	Crude fibre	25.71
NaCl	0.5	Acid detergent fibre	32.78
$CaCO_3$	0.4	Neutral detergent fibre	52.53
$CaHPO_4 \cdot 2H_2O$	0.1	Ether extract	1.70
Compound premix[1]	0.5	Ash	6.27
Total	100	Calcium	0.69
		Phosphorus	0.30

[1] contained the following ingredients per kg of diet: IU: vit. A 85000, vit. D_3 29000; mg: vit. E 500, Cu 350, Fe 190, Zn 900, Mn 1000, Co 15, Se 10; [2] NE_g (net energy for gain) was a calculated value, while the other values were measured. NE_g was calculated based on the proportion and NE_g values of maize stalk, maize and soybean meal, according to the Table of Feed Composition and Nutritional Value of Beef Cattle NRC (2000); DM – dry matter

Experimental animals and feeding management

Three healthy Yanbian cattle (500 ± 20 kg body weight) fitted with permanent rumen fistulas were selected as rumen fluid donors. Prior to rumen fluid collection, the animals underwent a 2-week adaptation period during which they were fed the same TMR diet as used in *in vitro* fermentation experiments. The cattle were maintained on a twice-daily feeding regimen with *ad libitum* access to fresh water throughout the adaptation period.

Experimental design

This experiment employed a single-factor design with blank and treatment groups. The latter included the control group (CON), and the ADY1, ADY2, YC1, YC2 groups, with 6 replicates per group. The blank group did not contain any fermentation substrate, while the CON group received a basal diet with 200 mg of the fermentation substrate. ADY1 and ADY2 were supplemented with approximately 2.4×10^6 CFU of live yeast in addition to 200 mg basal diet, and the YC1 and YC2 groups received 200 mg basal diet supplemented with 30 mg of yeast culture. YC dosages were calculated based on manufacturer recommendations, accounting for the conversion between the 30 ml rumen inoculum reaction system and the expected rumen fluid volume of adult cattle weighing approximately 500 kg. Fermentation was terminated at 48 h, with continuous monitoring of gas production throughout the incubation period, followed by measurement of gas production parameters and fermentation characteristics.

Ruminal fermentation *in vitro*

In vitro fermentation was conducted following the method described by Menke et al. (1988). Rumen fluid was collected from three fistulated cattle prior to morning feeding, filtered through four layers of sterile gauze, pre-warmed to 39 °C, and combined with an artificial rumen nutrient solution saturated with CO_2 at a 1:2 (v/v) ratio to prepare a mixed artificial rumen culture solution. This mixture was continuously stirred under CO_2 flushing to maintain anaerobic conditions. Using an automated dispenser, 30 ml of the prepared fermentation broth was transferred into pre-warmed (39 °C) fermentation tubes (graduated from 1 to 100 ml) for subsequent experimental treatments.

Index analysis

Gas production profiles and fermentation parameters

Gas production was measured after 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, and 48 h of incubation. At each time point, the piston position was immediately recorded to determine cumulative gas volume. Net gas production was calculated by subtracting the blank group values from treatment group values (Net gas production = treatment gas production − blank gas production). These data were used to generate gas production kinetics curves. The gas production profiles were analysed using the exponential model proposed by France et al. (2000):

$$Y = b \times [1 - e^{-(t-L)}],$$

where: Y – cumulative gas production (ml) at time t (h); b – asymptotic gas production (ml); L – lag phase duration (h). Model parameters were estimated through nonlinear regression analysis in SPSS 18.0.

CH_4 production after 48 h of fermentation

At the 48-h time point, the culture tubes were transferred to an ice water bath to terminate microbial activity. Using a gas-tight syringe (1000 µl), 500 µl of headspace gas was sampled from the rubber tubing for methane analysis. The CH_4 content in total gas was determined using gas chromatography (GC-1120; Sunny Hengping Instrument, Shanghai, China).

Fermentation parameters at 48 h

Rumen culture tubes were immediately transferred to ice water to terminate fermentation. The pH of the fermentation broth was measured using a calibrated ST3100 pH meter (Ohaus, NJ, USA). For volatile fatty acid (VFA) analysis, 1 ml of fermented rumen fluid was mixed with 0.2 ml of 25% (w/v) metaphosphoric acid solution (containing ethyl 2-butyrate) and centrifuged at 10000 rpm for 15 min. The supernatant was analysed using gas chromatography (GC-1120; Sunny Hengping Instrument, Shanghai, China) according to the method of Broderick and Kang (1980). Ammonia nitrogen (NH_3-N) and lactic acid concentrations were determined spectrophotometrically using a CMax Plus microplate reader (Molecular Devices, San Jose, CA, USA) following established protocols (Baker and Summerson, 1941; Li and Meng, 2006).

Organic matter digestibility and metabolizable energy

The organic matter digestibility (OMD) and metabolisable energy (ME) were calculated using the equations described by Menke et al. (1979):

$$OMD\ (\%) = 14.88 + 0.889GP + 0.45CP + 0.651A$$
$$ME\ (MJ/kg\ DM) = 2.20 + 0.136GP + 0.0574CP,$$

where: DM – dry matter; GP – net gas production at 24 h (ml/200 mg DM); CP – crude protein content (%); A – crude ash content (%).

Statistical analysis

The experimental data were analysed using one-way analysis of variance (ANOVA) in SPSS 18.0, while multiple comparisons were carried out using Duncan's multiple range test. Differences were considered statistically significant at $P < 0.05$.

Results

Gas production and fermentation kinetics

The cumulative gas production profiles of low-concentrate diets supplemented with different yeast preparations are presented in Table 2. None of the yeast treatments significantly influenced total gas production at any measured time point compared to the CON group ($P > 0.05$).

The effects of different yeast preparations on gas production parameters of low-concentrate diets are shown in Table 3. The theoretical maximum gas production of all groups was comparable to the gas production at 48 h of fermentation, with no significant differences between the treatment groups ($P > 0.05$). However, all yeast-supplemented groups demonstrated significantly shorter lag times (L) before gas production initiation compared to CON ($P < 0.05$).

Table 2. Effects of yeast preparations on gas production of low-concentrate diet

Time, h	Groups					SEM	*P*-value
	CON	ADY1	ADY2	YC1	YC2		
4	11.63	12.98	13.03	14.50	15.85	0.67	0.396
8	22.63	27.13	26.42	26.75	28.88	0.85	0.271
12	29.38	35.63	34.00	34.00	36.25	1.06	0.342
16	33.50	40.70	38.83	38.78	40.13	1.31	0.516
24	40.25	48.13	47.25	47.63	49.08	1.45	0.386
36	47.88	56.13	54.67	55.38	57.08	1.52	0.398
48	52.00	59.88	58.00	59.63	61.20	1.62	0.490

CON – control group without yeast preparation, ADY1 and ADY2 – treatment groups with active dry yeast supplementation, YC1 and YC2 – treatment groups with yeast culture supplementation; SEM – standard error of the mean; $P > 0.05$ (not statistically significant)

Table 3. Effects of yeast preparations on gas production parameters in low-concentrate diet

Items	Groups					SEM	*P*-value
	CON	ADY1	ADY2	YC1	YC2		
Theoretical maximum gas production/ml	54.27	60.36	59.27	61.83	62.61	1.46	0.484
LAG, h	1.19^{a}	0.66^{b}	0.63^{b}	0.37^{b}	0.48^{b}	0.12	0.037

CON – control group without yeast preparation, ADY1 and ADY2 – treatment groups with active dry yeast supplementation; YC1 and YC2 – treatment groups with yeast culture supplementation; SEM – standard error of the mean, LAG – lag time of gas production; ^{ab} – values within a row with different superscript letters differ significantly ($P < 0.05$)

Fermentation kinetics at 48 h

The effects of yeast preparations on the 48-h fermentation parameters of the low-concentrate diet are presented in Table 4. No significant differences were observed in the pH value of rumen fluid in the treatment groups compared to the CON group ($P > 0.05$). Similarly, there were no significant differences in NH_3-N concentration between the groups ($P > 0.05$). While the total volatile fatty acid (TVFA) content in the ADY1 and YC1 groups increased compared to the CON group, no significant differences were found in TVFA levels between the groups ($P > 0.05$).

The VFA profile analysis revealed distinct effects of yeast preparations on the proportion of individual volatile acids. Specifically, the acetic acid content in the ADY1 group was significantly higher than in the CON group ($P < 0.05$). However, no significant differences were observed for propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid between the groups ($P > 0.05$). The ratio of acetic acid to propionic acid was significantly higher in the ADY1 group than in both the CON group and other treatment groups ($P < 0.05$).

Table 4. Effects of yeast preparations on fermentation parameters of low-concentrate diet

Items	Groups					SEM	*P*-value
	CON	ADY1	ADY2	YC1	YC2		
pH	6.39	6.36	6.33	6.36	6.35	0.098	0.997
NH_3-N, mg/dl	32.13	32.45	37.26	34.67	32.00	0.69	0.058
TVFA, mmol/l	65.36	68.52	58.20	68.56	65.65	1.36	0.084
Acetic acid, %	58.32^{b}	60.28^{a}	57.39^{b}	57.40^{b}	58.16^{b}	0.32	0.015
Propionic acid, %	23.71	23.09	24.15	24.45	24.06	0.16	0.059
Isobutyric acid, %	1.55	1.58	1.72	1.63	1.57	0.031	0.481
Butyric acid, %	10.59	9.77	10.74	10.98	10.59	0.15	0.095
Isovaleric acid, %	3.46	3.19	3.69	3.22	3.32	0.074	0.196
Valeric acid, %	2.37	2.09	2.32	2.32	2.30	0.041	0.210
A/P	2.46^{b}	2.61^{a}	2.38^{b}	2.35^{b}	2.42^{b}	0.028	0.013

CON – control group without yeast preparation, ADY1 and ADY2 – treatment groups with active dry yeast supplementation, YC1 and YC2 – treatment groups with yeast culture supplementation; SEM – standard error of the mean, NH_3-N – ammoniacal nitrogen, TVFA – total volatile acids, A/P – acetic acid/propionic acid; ^{ab} – values within a row with different superscript letters are different of significantly at $P < 0.05$

CH_4 and lactic acid production

Yeast supplementation significantly altered CH_4 and lactic acid production in the low-concentrate diets (Table 5). While ADY1 and YC1 reduced 48-h CH_4 production by 39.43 and 5.53%, respectively, compared to CON, these changes were not statistically significant ($P > 0.05$). Conversely, ADY2 and YC2 increased CH_4 production by 48.32 and 31.96% relative to CON ($P > 0.05$), with both groups showing significantly higher CH_4 output than ADY1 ($P < 0.05$). All yeast treatments significantly decreased lactic acid concentrations compared to the control group ($P < 0.05$).

Table 5. Effects of yeast preparations on methane and lactic acid production in low-concentrate diet

Items	Groups					SEM	*P*-value
	CON	ADY1	ADY2	YC1	YC2		
Lactic acid, mg/dl	0.55^{a}	0.46^{b}	0.46^{b}	0.47^{b}	0.45^{b}	0.011	0.038
Methane, %	19.68^{ab}	11.92^{b}	29.19^{a}	18.59^{ab}	25.97^{a}	1.83	0.015

CON – control group without yeast preparation, ADY1 and ADY2 – treatment groups with active dry yeast supplementation, YC1 and YC2 – treatment groups with yeast culture supplementation; SEM – standard error of the mean; ^{ab} – values within a row with different superscript letters are significantly different at $P < 0.05$

Organic matter digestibility and metabolizable energy

The predicted OMD and ME values, calculated from 24-h gas production data, are presented in Table 6. All yeast-supplemented groups showed numerically higher OMD and ME values compared to the CON group, although these differences were not statistically significant ($P > 0.05$).

Table 6. Effects of yeast preparations on organic digestibility and metabolisable energy value of low-concentrate diet

Items	Groups					SEM	*P*-value
	CON	ADY1	ADY2	YC1	YC2		
OMD, %	60.35	67.35	66.57	66.90	68.20	1.29	0.386
ME, MJ/kg DM	8.39	9.46	9.34	9.39	9.59	0.20	0.386

CON – control group without yeast preparation, ADY1 and ADY2 – treatment groups with active dry yeast supplementation, YC1 and YC2 – treatment groups with yeast culture supplementation; SEM – standard error of the mean, OMD – organic matter digestibility (% of total substrate on DM basis), ME – metabolizable energy; DM – dry matter; $P > 0.05$ (not statistically significant)

Discussion

Gas production parameters

In vitro gas production reflects rumen microbial degradation activity and feed degradation kinetics, with established strong correlations (r = 0.98) to *in vivo* dry matter degradation and metabolisable energy values (Menke et al., 1979). In this study, low-concentrate fermentation substrates were used to evaluate the effects of different yeast preparations on *in vitro* gas production. The results demonstrated that both ADY and YC treatments increased cumulative gas production compared to the unsupplemented control, with YC groups showing the most pronounced stimulation. This suggested that both ADY and YC positively influenced the utilisation of substrate nutrients. The YC group's higher gas production could be attributed to the fact that it provided not only yeast cellular components but also fermentation-derived metabolites that served as both microbial stimulants and supplemental nutrients for bacterial populations. This could be the primary reason for the highest gas production observed in the YC group.

The lag time (LAG) represents the initial period before gas production begins, reflecting the microbial adaptation phase to the substrate. Studies have demonstrated that increasing the organic matter (OM) and crude protein (CP) content in the substrate can shorten fermentation lag time (Haddi et al., 2003), which improves ruminal feed degradation efficiency (Menke et al., 1979). In the current study, all yeast-supplemented groups showed significantly reduced LAG values compared to the control, demonstrating that both ADY and YC preparations effectively stimulated early microbial activity and improved substrate utilisation in the low-concentrate diet.

Fermentation parameters

Rumen pH serves as a critical indicator of rumen health and is closely related to dietary composition and additives. The physiologically optimal range of ruminal fluid pH is between 6.2 and 7.2 (Gang et al., 2024), and is essential for maintaining microbial populations and supporting efficient anaerobic fermentation. Deviations from this range can disrupt microbial ecosystems and impair feed digestion. Our findings are consistent with previous work by Mao et al. (2013), demonstrating that yeast supplementation (both ADY and YC) maintained rumen pH within normal physiological ranges without causing significant fluctuations.

NH_3-N is an important indicator of rumen nitrogen metabolism, reflecting microbial protein synthesis and degradation potential. The optimal NH_3-N concentration range of 10–50 mg/dl (Luan et al., 2023) provides sufficient nitrogen precursors for microbial growth while avoiding excessive prote-

olysis. In our study, all treatment groups maintained NH_3-N concentrations within the normal physiological range (32–38 mg/dl), demonstrating that yeast supplementation preserved adequate nitrogen availability for rumen microorganisms. However, the effect of yeast preparation on rumen NH_3-N concentration appears inconsistent, as evidenced by contrasting findings in the literature. For instance, Zeng (2020) reported significant yeast-induced modifications in NH_3-N and microbial protein concentrations in yak rumen fermentation studies, suggesting that dietary composition (particularly concentrate-to-forage ratio) may mediate yeast effects on nitrogen metabolisms. However, contrasting results were reported by Hristov et al. (2010), who found no significant effects of ADY and YC preparations on NH_3-N concentration in rumen fluid. In the present study, NH_3-N concentrations in the ADY1, ADY2, and YC1 groups showed a numerical increase compared to the CON group, though the differences were not statistically significant. The observed increase in NH_3-N concentration indicated enhanced protein utilisation by rumen microorganisms. Notably, the ADY2 group demonstrated the most pronounced numerical increase in NH_3-N concentration, indicating that this specific yeast preparation may be particularly effective in optimising protein metabolism under low-concentrate dietary conditions.

VFA are the main energy source for rumen microorganisms and the host animal, with their production and profile being largely determined by dietary composition (Yuan et al., 2019). Under typical fermentation conditions, acetate constitutes the predominant VFA, followed by propionate and butyrate (Luan et al., 2023). Current research presents inconsistent findings regarding the effects of yeast preparations on rumen VFA levels, potentially due to the complex interplay of factors influencing rumen fermentation, such as dietary composition, the type of yeast preparation used, and the physiological status of the animals. Research demonstrates variable effects of yeast supplementation on rumen VFA profiles depending on animal species and diet composition. An *in vivo* study demonstrated that ADY supplementation significantly increased propionate production in lambs (Liu et al., 2024) and elevated TVFA concentrations in early lactation dairy cows (Kumprechtová et al., 2019). Similarly, Halfen et al. (2021) showed that YC supplementation significantly increased TVFA levels and improved rumen fermentation characteristics in Holstein dairy cows. In contrast, *in vitro* studies under the same experimental conditions showed that while ADY had no significant effect on TVFA concentration or the proportion of individual VFA in a high-concentrate fermentation substrate, YC supplementation significantly increased acetic acid concentrations and the acetic acid/propionic acid ratio (Geng et al., 2016). The present study used a low-concentrate fermented diet (concentrate to forage ratio of 30:70) and observed distinct effects of yeast preparations on VFA profiles. The ADY1 treatment significantly increased both acetic acid concentration and the acetate-to-propionate ratio, whereas ADY2 showed no significant influence on total or individual VFA production. Similarly, neither YC formulation substantially altered VFA parameters. These findings have demonstrated that the effects of yeast preparations are influenced not only by the type of yeast preparation (ADY and YC), but also by the specific variety (ADY1 and ADY2) and the substrate to concentration ratio. These differences may explain the inconsistent results observed in practical applications.

CH_4 and lactic acid production

Rumen microbial fermentation of carbohydrates generates CH_4, representing both an environmental concern due to its greenhouse gas potential and an energy loss for the host animal, accounting for 2–12% of dietary gross energy (Johnson and Johnson, 1995). Our results revealed divergent CH_4 production trends among yeast treatments: ADY1 and YC1 showed numerical decreases, while ADY2 and YC2 exerted an opposing effect compared to the control. However, these changes did not reach statistical significance. The findings concerning the effects of yeast preparations on CH_4 emissions remain inconsistent across studies. Chung et al. (2011) showed that ADY reduced CH_4 emissions from dairy cows, while Geng et al. (2016) found no significant effect of ADY on CH_4 production. Lila et al. (2004) concluded that YC had no significant effect on CH_4 generation, while Qiao and Shan (2006) documented increased CH_4 levels. More recently, Sookrali and Hughes (2022) demonstrated a 20% reduction in peak CH_4 emissions from concentrate feeds when using a combined yeast culture and enzymatically hydrolysed yeast (YC + EHY) formulation. The variability in research findings regarding yeast supplementation effects on methane production reflects the complex interplay of multiple factors. Differences in yeast product formulations, specific strain characteristics, basal diet composition, and experimental methodologies (particularly between *in vivo* and *in vitro* systems) all contribute to these inconsistent results.

This complexity highlights the need for more comprehensive studies employing standardised protocols to better understand and predict the effects of yeast additives on ruminant methane emissions.

Rumen lactic acid concentration serves as an important indicator for evaluating yeast preparation efficacy in mitigating subacute ruminal acidosis (SARA), with normal physiological levels typically below 1 mmol/l (Feng, 2006). Our results demonstrated lactic acid concentrations of 0.45–0.55 mg/dl in all treatment groups, well within this normal range. These findings align with previous reports showing lactic acid reduction potential of both ADY (de Poppi et al., 2021) and YC (Ren et al., 2020) supplements. The effects of yeast preparations on rumen lactic appears to be related to both changes in lactic acid production and the abundance of lactic acid-utilising bacteria (Mao et al., 2013). Consistent with these findings, this study observed that both ADY and YC reduced lactic acid levels, which indicated that their inclusion in the diet effectively inhibited the production of rumen lactic acid.

OMD and ME

OMD represents the proportion of dietary organic nutrients assimilated during digestion, while ME reflects the biologically available energy for maintenance and production functions. In this study, we estimated these parameters using 24-hour gas production data from the *in vitro* fermentation system. Although statistical analysis revealed no significant differences between the experimental groups and the control, all yeast-supplemented groups showed numerically higher OMD and ME values compared to CON. This consistent trend suggests that both ADY and YC formulations may enhance nutrient utilisation efficiency in low-concentrate diets.

Conclusions

In summary, the present study demonstrated that both active dry yeast (ADY) and yeast culture (YC) preparations improved rumen fermentation characteristics under low-concentrate conditions, as evidenced by reduced gas production lag time and decreased lactic acid concentrations. These results suggest that the addition of yeast preparations exerts positive effects on rumen substrate utilisation while supporting rumen health. However, there were some differences in the effects of yeast preparation groups on the proportion of individual volatile acids, NH_3-N concentration and CH_4 production in the rumen. These variations may explain the inconsistent effects observed in practical applications. Future research should focus on exploring the relationship between the types, varieties and dietary composition of yeast preparations and their application effects, while expanding the relevant data to provide a solid foundation for their scientific application.

Funding

This work was funded by the National Natural Science Foundation of China (grant numbers 32060763 and 31660669), and the Jilin Science and Technology Development Program (grant numbers YDZJ202203CGZH042 and 20220202048NC).

Conflict of interest

The Authors declare that there is no conflict of interest.

References

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, 31–41, https://doi.org/10.1016/j.aninu.2020.10.005

Baker S.B., Summerson W.H., 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138, 535–554, https://doi.org/10.1016/S0021-9258(18)51379-X

Bradley G.L., Savage T.F., Timm K.I., 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. *boulardii* on male poult performance and ileal morphology. Poult. Sci. 73, 1766–1770, https://doi.org/10.3382/ps.0731766

Broderick G.A., Kang J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy. Sci. 63, 64–75, https://doi.org/10.3168/jds.S0022-0302(80)82888-8

Chen X., Xiao J., Zhao W. et al., 2024. Mechanistic insights into rumen function promotion through yeast culture (*Saccharomyces cerevisiae*) metabolites using *in vitro* and *in vivo* models. Front. Microbiol. 15, 1407024, https://doi.org/10.3389/fmicb.2024.1407024

Chung Y.-H., Walker N.D., McGinn S.M., Beauchemin K.A., 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J. Dairy. Sci. 94, 2431–2439, https://doi.org/10.3168/jds.2010-3277

De Poppi A.C., Lazzari G., Gomes A.L.M. et al., 2021. Effects of feeding a live yeast on rumen fermentation and fiber degradability of tropical and subtropical forages. J. Sci. Food. Agric. 101, 6220–6227, https://doi.org/10.1002/jsfa.11273

Feng Y.L., 2006. The limitation of digestible protein in the small intestine of dairy cattle and its balance of amino acid (in Chinese). Chin. J. Anim. Nutr. 18, 63–68, https://doi.org/10.3969/j.issn.1006-267X.2006.02.001

France J., Dijkstra J., Dhanoa M.S., Lopez S., Bannink A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br. J. Nutr. 83, 143–150, https://doi.org/10.1017/S0007114500000180

Gang G., Gao R., Zhao H., Xu Y., Xing Y., Jin X., Hong L., Yan S., Shi B., 2024. Effects of water extracts of *Artemisia annua* L. on rumen immune and antioxidative indexes, fermentation parameters and microbials diversity in lambs. Front. Microbiol. 15, 1485882, https://doi.org/10.3389/fmicb.2024.1485882

Gao K., Geng C., 2023. Comparison of rectum fecal bacterial community of finishing bulls fed high-concentrate diets with active dry yeast and yeast culture supplementation. Anim. Biosci. 36, 63–74, https://doi.org/10.5713/ab.22.0215

Geng C.Y., Ren L.P., Zhou Z.M., Meng Q.X., 2015. Yeast preparation: application effects in ruminants and possible mechanisms (in Chinese). Chin. J. Anim. Nutr. 27, 1011–1020, https://doi.org/10.3969/j.issn.1006-267x.2015.04.002

Geng C.Y., Zhao L.P., He L.W., Zhou Z.M., Meng Q.X., 2016. Comparison of the effects of active dry yeast and yeast culture on rumen fermentation parameters (in Chinese). China Anim. Husbandry Vet. Med. 43, 2931–2938, https://doi.org/10.16431/j.cnki.1671-7236.2016.11.018

Haddi M.L., Filacorda S., Miniai K., Rollin F., Susmel P., 2003. In vitro fermentation kinetics of some halophyte shrubs sampled at three stages of maturity. Anim. Feed Sci. Technol. 104, 215–225, https://doi.org/10.1016/S0377-8401(02)00323-1

Halfen J., Carpinelli N., Del Pino F.A.B., Chapman J.D., Sharman E.D., Anderson J.L., Osorio J.S., 2021. Effects of yeast culture supplementation on lactation performance and rumen fermentation profile and microbial abundance in mid-lactation Holstein dairy cows. J. Dairy. Sci. 104, 11580–11592, https://doi.org/10.3168/jds.2020-19996

Hristov A.N., Varga G., Cassidy T., Long M., Heyler K., Karnati S.K., Corl B., Hovde C.J., Yoon I., 2010. Effect of *Saccharomyces cerevisiae* fermentation product on ruminal fermentation and nutrient utilization in dairy cows. J. Dairy. Sci. 93, 682–692, https://doi.org/10.3168/jds.2009-2379

Johnson K.A., Johnson D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492, https://doi.org/10.2527/1995.7382483x

Kumprechtová D., Illek J., Julien C., Homolka P., Jančík 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. Anim. Nutr. 103, 447–455, https://doi.org/10.1111/jpn.13048

Li Y., Meng Q., 2006. Effect of different types of fibre supplemented with sunflower oil on ruminal fermentation and production of conjugated linoleic acids in vitro. Arch. Anim. Nutr. 60, 402–411, https://doi.org/10.1080/17450390600884401

Lila Z.A., Mohammed N., Yasui T., Kurokawa Y., Kanda S., Itabashi H., 2004. Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation *in vitro*. J. Anim. Sci. 82, 1847–1854, https://doi.org/10.2527/2004.8261847x

Liu H., Li F., Ma Z., Ma M., Ungerfeld E., Zhang Z., Weng X., Liu B., Deng X., Guo L., 2024. Effects of active dry yeast supplementation in *in vitro* and *in vivo* nutrient digestibility, rumen fermentation, and bacterial community. Animals 14, 2916, https://doi.org/10.3390/ani14192916

Luan J., Feng X., Yang D., Yang M., Zhou J., Geng C., 2023. Effects of medium-chain fatty acids (MCFAs) on i*n vitro* rumen fermentation, methane production, and nutrient digestibility under low-and high-concentrate diets. Anim. Sci. J. 94, e13818, https://doi.org/10.1111/asj.13818

Ma J., Wang C., Wang Z., 2021. Active dry yeast supplementation improves the growth performance, rumen fermentation, and immune response of weaned beef calves. Anim. Nutr. 7, 1352–1359, https://doi.org/10.1016/j.aninu.2021.06.006

Mao H.L., Mao H.L., Wang J.K., Liu J.X., Yoon I., 2013. Effects of *Saccharomyces cerevisiae* fermentation product on in vitro fermentation and microbial communities of low-quality forages and mixed diets. J. Anim. Sci. 91, 3291–3298, https://doi.org/10.2527/jas.2012-5851

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W., 1979. The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubarted with rumen liquor in vitro. J. Agric. Sci. 93, 217–222, https://doi.org/10.1017/S0021859600086305

Menke K.H., Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Develop., 28, 7–55

Qiao G.H., Shan A.S., 2006. The Effect of direct-fed microbial culture on methane production *in vitro* and production performance in dairy cattle (in Chinese). China Anim. Husbandry Vet. Med. 33, 11–14, https://doi.org/10.3969/j.issn.1671-7236.2006.05.003

Ren S.N., Jin Y.Q., Zhao F.F., 2020. Regulating effects of yeast culture on subacute ruminal acidosis fermentation model *in vitro* in goats (in Chinese). Chin. J. Anim. Nutr. 32, 2263–2272, https://doi.org/10.3969/j.issn.1006-267x.2020.05.035

Sookrali A.A., Hughes M.P., 2022. Influence of combined yeast culture and enzymatically hydrolyzed yeast on in vitro ruminal fermentation in contrasting feed substrates. J. Sci. Food Agric. 102, 3628–3635, https://doi.org/10.1002/jsfa.11709

Yuan J.W., Wan X., 2019. Multiple-factor associative effects of peanut shell combined with alfalfa and concentrate determined by *in vitro* gas production method. Czech J. Anim. Sci. 64, 352–360, https://doi.org/10.17221/94/2019-CJAS

Zeng Y., 2020. Effects of yeast culture supplementation in diets on rumen fermentation parameters and microflora of house-feeding yak (in Chinese). Chin. J. Anim. Nutr. 32, 1721–1733, https://doi.org/10.3969/j.issn.1006-267x.2020.04.031

Zhang X., Zhang T.Z., Qu M.R., 2022. Research progress and application of active dry yeast and yeast culture in ruminants (in Chinese). Chin. J. Anim. Nutr. 34, 20–29, https://doi.org/10.3969/j.issn.1006-267x.2022.01.003