Animal Nutrition 13 (2023) 289–301

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Active dry yeast supplementation benefits ruminal fermentation, bacterial community, blood immunoglobulins, and growth performance in young dairy goats, but not for intermittent supplementation

Jun Zhang [a], Yuntian Yang [a], Xinjian Lei [a], Yannan Wang [a], Yanhua Li [b], Zhiqiang Yang [c], Junhu Yao [a, *]

[a] College of Animal Science and Technology, Northwest A&F University, Yangling 712100, Shaanxi, China
[b] Fuping County Animal Husbandry Development Center, Fuping 711700, Shaanxi, China
[c] Shandong Institute of Feed Quality Inspection, Jinan 250010, Shandong, China

ARTICLE INFO

Article history:
Received 13 April 2022
Received in revised form
10 January 2023
Accepted 9 February 2023
Available online 17 February 2023

Keywords:
Active dry yeast
Bacterial community
Dairy goat
Growth performance
Rumen microbiota
Saccharomyces cerevisiae

ABSTRACT

This study evaluated the effects of active dry yeast (ADY) supplementation and supplementation strategies on ruminal fermentation, bacterial community, blood metabolites, and growth performance in young dairy goats. Sixty young female Guanzhong dairy goats of similar age (4.00 ± 0.50 months) and BW (19.65 ± 0.41 kg) were randomly divided into 3 groups ($n = 20$): (1) basal diet group (CON); (2) basal diet continuously supplemented with 3.0 g/goat per day commercial ADY (a proprietary strain of *Saccharomyces cerevisiae* with 5.0×10^9 cfu/g) group (CSY); (3) basal diet with intermittently supplemented ADY group (ISY; 5 d supplementation with ADY at 4.5 g/goat per day following 5 d of no supplementation). The experiment lasted 67 d with the first 7 d as an adaptive period. Rumen fluid and blood samples were collected bi-weekly. Data were analyzed using the MIXED procedure combined with the SLICE option in SAS. Specific orthogonal contrasts of ADY vs. CON and CSY vs. ISY were also analyzed. During the experimental period, ADY supplementation resulted in greater DMI ($P = 0.03$), ruminal acetate proportion ($P < 0.01$) and acetylesterase activity ($P = 0.01$), and blood contents of glucose ($P = 0.01$) and IgM ($P = 0.02$) and tended to have greater ADG ($P = 0.05$) and paunch girth ($P = 0.06$) than the CON, despite the propionate proportion ($P = 0.03$) and contents of total protein ($P = 0.04$) and IgA ($P = 0.03$) being lower. The lower ruminal NH_3–N ($P < 0.01$) and blood urea nitrogen ($P = 0.07$) contents indicated greater nitrogen utilization with ADY supplementation. ADY supplementation showed persistent effects after it was stopped because the BW at 12 months of age ($P = 0.03$) and birth weight of lambs ($P = 0.02$) were greater than the CON. However, the ISY did not show those benefits and had significantly lower relative abundances of fiber-degrading related bacteria than the CSY. In conclusion, ADY supplementation, especially continuously supplemented, may enhance ADG and ADG:DMI ratio by improving DMI, ruminal cellulolytic bacteria abundance and enzyme activity, nitrogen utilization, and immune status. These findings provide a theoretical basis for the rational application of ADY and have important practical implications for the design of nutritional strategies in growing dairy goats.

1. Introduction

As replacements for lactating dairy goats, the growth and development of young dairy goats dramatically affects the profitability and sustainable development of the dairy industry. Young ruminants have greater potential for growth and development but have lower disease resistance ability (Khan et al., 2016). Due to the unparalleled role of microbes in ruminants, establishing a stable

* Corresponding author.
E-mail address: yaojunhu2004@sohu.com (J. Yao).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2023.02.001

gastrointestinal tract microbial community has unique value in ruminants. In addition, facilitating the early growth and development of young ruminants may have long-term effects on lifetime production levels (Soberon et al., 2012; Soberon and Van Amburgh, 2013). Therefore, optimal nutrition levels and feeding regimes should be introduced in growing young ruminants.

After antibiotic additives were banned in animal feed, researchers and farmers extensively sought alternative, environmentally-friendly additives. Probiotics, which can influence gastrointestinal fermentation and microbiota, have emerged as a popular alternative for maintaining and improving livestock health and performance (Amin and Mao, 2021; Ban and Guan, 2021). As a functional and cost-effective probiotic, studies suggest that active dry yeast (ADY; *Saccharomyces cerevisiae* CNCM I-4407 or proprietary strains; 5.7×10^7 or 4.0×10^{10} or 5.0×10^{10} cfu/d per cow) alters ruminal fermentation and volatile fatty acid (VFA) production, stimulates ruminal microbial growth, and increases fiber degradation in the parturient and lactating ruminants (Jiang et al., 2017; Kumprechtova et al., 2019; Li et al., 2021). With the ability to potentially improve rumen environment and function, ADY (*S. cerevisiae* strain 1242 or proprietary strains; 8.0×10^{10} cfu/d per animal or not indicated) was also used to alleviate the effects of subacute ruminal acidosis in lactating and/or fattening ruminants (AlZahal et al., 2014; Ishaq et al., 2017; Elmhadi et al., 2022). Recently, we also found that *S. cerevisiae* product (either ADY or its fermentation products) supplementation could stimulate the development of the rumen and promote immunity resulting in greater dry matter intake (DMI) and average daily gain (ADG) in preweaning calves by conducting a meta-analysis (Zhang et al., 2022a). Villot et al. (2019) even showed that ADY (*S. cerevisiae* CNCM I-1079; 1.0×10^{10} cfu/d per animal) supplementation decreased the risk of diarrhea in preweaning calves and altered the fecal microbiota of diarrheic calves to be similar to nondiarrheic animals. In addition, ADY supplementation also had the potential to modulate host immune function possibly as a direct effect of ADY (*S. cerevisiae* CNCM I-1079; 1.0×10^{10} cfu/d per animal) supplementation or the interaction between ADY and gut microbiota on immunoglobulin production in young ruminants (Villot et al., 2019, 2020). On the contrary, the benefits of ADY supplementation can be influenced by factors including animal physiological status, basal diet, the source and dose of ADY, and supplementation strategies (He et al., 2017; Amin and Mao, 2021; Ban and Guan, 2021). Our recent meta-analysis showed that the improvement in ADG by *S. cerevisiae* product (either ADY or its fermentation products) supplementation only presented in preweaning other than postweaning calves (Zhang et al., 2022a). Due to the limited number of studies investigating the *S. cerevisiae* products supplemented in postweaning ruminants, we were unable to determine an explanation.

Unlike newborn and lactating ruminants, growing ruminants have a certain level of disease resistance but always have moderate performance due to poor feeding and management strategies. However, there is little known about ADY utilization in growing ruminants. Active dry yeast supplementation might be an opportunity to improve the performance of growing ruminants. Moreover, most studies only collected experimental data during or briefly after the ADY supplementation period, which can not reveal whether there is a persistent effect of ADY supplementation on animals. Therefore, we hypothesized that ADY supplementation would benefit growing ruminants as it would for newborn and/or lactating ruminants. The objective of this study was to evaluate if the ADY supplementation improved growth and development, altered rumen metabolism, enhanced immune ability, and had persistent benefits in young ruminants with growing dairy goats as the model. We also explored the differences between different ADY supplementation strategies. Considering the high feed cost, an intermittent supplementation ADY strategy with a lower total cost and a similar performance may be preferred over a continuous supplementation strategy.

2. Materials and methods

2.1. Animal ethics statement

This study was carried out under the recommendations of the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised 2004). All the procedures used were approved by the Institutional Animal Care and Use Committee of the Northwest A&F University.

2.2. Experimental design and animal management

This study was conducted at Aonike Dairy Goat Farm (Fuping, Shaanxi, China). Sixty healthy female Guanzhong dairy goats of similar age (4.00 ± 0.50 months old) and body weight (BW) (19.65 ± 0.41 kg) were divided into 3 groups ($n = 20$) identified by one of the following feeding regimens: (1) basal diet (CON; which mainly consisted of alfalfa hay and ground corn; the detailed ingredients and nutrient composition are listed in Table 1); (2) basal diet continuously supplemented with 3.0 g/goat per day commercial ADY (CSY); (3) basal diet intermittently supplemented with 4.5 g/goat per day commercial ADY (ISY). The ADY was a proprietary strain of *S. cerevisiae* purchased from Qingdao Aolan Mingdong Biotechnology Co., Ltd (Qingdao, Shandong, China) with 5.0×10^9 cfu/g. According to previous reports, 1.0×10^{10} and 4.0 to 8.0×10^{10} cfu/d per animal were the typical doses used for newborn and adult ruminants, respectively (AlZahal et al., 2014; Villot et al., 2019; Li et al., 2021). The amount of ADY used for supplementation in this study was between the doses used in newborn and adult ruminants. Considering the practicality and lower frequency of change in diets, we alternated 5 d of supplementation with 5 d of no supplementation until the end of the experiment in the ISY

Table 1
Ingredients and chemical composition of the total mixed ration (%, DM basis).

Item	Contents
Ingredients	
Alfalfa hay	48.5
Oat hay	12.1
Ground corn	27.6
Soybean meal	3.9
Cottonseed meal	4.7
Wheat	2.1
Limestone	0.5
Sodium chloride	0.2
Sodium bicarbonate	0.2
Calcium hydrophosphate	0.1
Premix[1]	0.1
Chemical composition	
DM	55.7
CP	16.6
NDF	38.4
ADF	25.7
Ash	7.2
ME, MJ/kg[2]	9.0

ADF = acid detergent fiber; CP = crude protein; DM = dry matter; EE = ether extraction; ME = metabolizable energy; NDF = neutral detergent fiber.

[1] Contained (per kilogram of premix; DM basis): 150,000 IU of vitamin A, 45,000 IU of vitamin D, 975 IU of vitamin E, 332 mg Cu, 611 mg Mn, 1,390 mg Zn, 12 mg I, 8 mg Se, and 7 mg Co, and was designed to meet the requirements for young growing goats.

[2] ME was calculated according to NRC (2001).

group. To ensure the supplemental ADY was completely consumed, the ADY was mixed with a small amount of total mixed ration (TMR) before feedings and was provided in equal amounts in all feedings. The remaining TMR was delivered only after the mixture was consumed. The TMR was provided three times daily at 07:00, 13:30, and 20:00. Goats were housed in separate fences (approximately 2 m^2) with individual bunks and were given free access to water. The experiment lasted 67 d with the first 7 d as an adaptive period. After 67 d, goats from the 3 groups were integrated together and fed the basal diet without ADY supplementation.

2.3. Sample collection and analysis

2.3.1. Feed intake and growth performance

The feed intake was recorded daily. Samples of individual ingredients, TMR, and refusals were collected biweekly and frozen for further analysis. Feed samples were analyzed for dry matter (DM; oven method 930.15), crude protein (CP; Kjeldahl method 976.05), neutral detergent fiber (NDF; using a heat-stable amylase and expressing inclusive of residual ash), and acid detergent fiber (ADF) (Van Soest et al., 1991; AOAC, 2000).

The BW, withers height, body length (shoulders to pins), heart girth, chest width, and paunch girth were measured on d 29, 30, 59, and 60 before morning feeding, and the mean values of 2 consecutive d were used to account for day-to-day variation (Lascano et al., 2009). To evaluate the long-term effects of ADY on young dairy goats and their offspring, the BW of maternal goats at 12-months old and the total birth weight of the corresponding kids were measured at delivery.

2.3.2. Blood sample collection and analysis

Blood samples were collected on d 15, 30, 45, and 60 of the experiment from 30 goats ($n = 10$) 2 h after morning feeding. These 30 goats were randomly selected at the first sampling time point and were consistently collected at the following time points. After storing at room temperature for approximately 30 min, samples were centrifuged at 3,000 $\times$ g at 4 °C for 15 min to obtain serum, which was stored at −20 °C for further analysis. Blood metabolites, including glucose (GLU), total protein (TP), albumin (ALB), and triglycerides (TG), were quantified using a commercial kit (Hunan Yonghe-Sun Biotechnology Co., Ltd., Changsha, China) and a clinical autoanalyzer (Hitachi 7600; Hitachi, Tokyo, Japan). Globulin content was determined by subtracting the content of ALB from the content of TP. Blood urea nitrogen (BUN) was measured using reagent kits (Jiancheng Bioengineering Institute, Nanjing, China) and a Multi-Mode Microplate Reader (Synergy HT, BioTek Instruments, Winooski, VT, USA). Serum IgG, IgA, and IgM levels were determined using commercial ELISA kits (Fankewei Biotechnology Company, Shanghai, China) and a Multi-Mode Microplate Reader (Synergy HT).

2.3.3. Rumen sample collection and analysis

Rumen fluid was collected on d 15, 30, 45, and 60 of the experiment from the same 30 goats ($n = 10$), whose blood was sampled, using a flexible esophageal tube at 2 h after morning feeding. The first fraction of the rumen samples was always discarded to avoid saliva contamination. The rumen fluid was measured with a mobile pH meter (PH818; Wanchuang Electronic Products Co., Ltd., Dongguan, China) immediately after collection. Rumen liquid was then filtered through 4 layers of cheesecloth and centrifuged at 4 °C at 12,000 $\times$ g for 10 min to get a clear supernatant. One milliliter of filtered rumen fluid was analyzed for NH_3–N using a phenol-hypochlorite assay with a Multi-Mode Microplate Reader (Synergy HT) (Weatherburn, 2002). Another sample of rumen fluid (1 mL) was added to 100 μL of 25% metaphosphoric acid to determine VFA using capillary column gas chromatography with crotonic acid as the internal standard, according to a previous method (Zhang et al., 2017). The activities of carboxymethyl cellulase (CMC), microcrystalline cellulase (MCC), xylanase, and acetylesterase were determined using a colorimetric method with carboxymethylcellulose, microcrystalline cellulose, xylan, and *p*-nitrophenyl ethyl ester as the respective substrates and with a UV-1800 spectrophotometer (Shanghai Mapada Instruments Co., Ltd, Shanghai, China) (Cao and Yang, 2011).

2.3.4. Microbial DNA extraction, PCR amplification, sequencing, and analysis

Genomic DNA was extracted from the rumen samples on d 60 of the experiment using an E. Z.N.A. soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's protocol. DNA samples were quality checked, and the concentrations were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Bacterial 16S rRNA gene fragments (V3–V4) were amplified from the extracted DNA using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Zhang et al., 2020) with the following PCR conditions: initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCRs were performed with 4 μL 5 × *TransStart* FastPfu buffer, 2 μL 2.5 mM deoxynucleoside triphosphates (dNTPs), 0.8 μL of each primer (5 μM), 0.4 μL *TransStart* FastPfu DNA Polymerase, and 10 ng of extracted DNA, then adding ddH_2O to increase total volume to 20 μL. Agarose gel electrophoresis (2%) was performed to verify the size of the amplicon. Amplicons were subjected to paired-end sequencing on the Illumina MiSeq sequencing platform using PE300 from Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China).

After demultiplexing, the resulting sequences were merged with FLASH (v1.2.11) and quality filtered with fastp (0.19.6) (Magoc and Salzberg, 2011; Chen et al., 2018). Then the high-quality sequences were de-noised using the DADA2 plugin in the QIIME 2 (version 2020.2) pipeline with recommended parameters, obtaining single-nucleotide resolutions based on the error profiles within the samples (Callahan et al., 2016; Bolyen et al., 2019). These DADA2 denoised sequences are called amplicon sequence variants (ASVs). To minimize the effects of sequencing depth on alpha and beta diversity measures, the number of sequences from each sample was rarefied to 4,000, yielding an average Good's coverage of 97.90%. Taxonomic assignment of ASVs was performed using the Naive Bayes consensus taxonomy classifier implemented in QIIME 2 and the SILVA 16S rRNA database (v138). Beta diversity was measured according to unweighted UniFrac distances and displayed using principal coordinate analysis (PCoA). Analysis of similarity (ANOSIM) was used to test the significance of bacterial communities between treatments. Analyses of the 16S rRNA microbiome sequencing data were performed using the free online platform of Majorbio Cloud Platform (cloud.majorbio.com). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA810766).

2.4. Statistical analysis

The intake, growth performance, rumen fermentation, enzyme activity, and blood metabolite data were initially checked for normality and outliers using the UNIVARIATE procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC, USA). They were then analyzed using the MIXED procedure combined with the SLICE option when treatment-by-time interaction effect was significant. The model included treatment, time, and their interaction as fixed

Table 2
Effect of active dry yeast (ADY) supplementation on feed intake and feed efficiency in young dairy goats.

Item	Treatment[1]			SEM	*P*-value			*P* contrast	
	CON	CSY	ISY		Treat	Time	Treat × Time	CON vs. ADY	CSY vs. ISY
BW, kg									
Initial	19.55	19.82	19.59	0.411	0.96			0.86	0.83
Final	25.01	26.42	25.31	0.445	0.40			0.37	0.31
DMI, g/d									
d 0–30	926.3	952.2	938.7	5.98	0.22			0.13	0.36
d 30–60	1010.4	1042.7	1021.6	6.23	0.09			0.10	0.16
d 0–60	968.3^{b}	997.5^{a}	980.2^{ab}	5.34	0.02	<0.01	0.92	0.03	0.10
ADG, g/d									
d 0–30	93.1	96.9	90.0	3.38	0.71			0.97	0.41
d 30–60	103.9^{b}	139.4^{a}	110.0^{b}	4.05	<0.01			0.01	<0.01
d 0–60	98.5^{b}	118.1^{a}	100.0^{b}	2.85	<0.01	<0.01	0.03	0.05	<0.01
Feed efficiency[2]									
d 0–30	0.10	0.10	0.10	0.004	0.77			0.83	0.51
d 30–60	0.10^{b}	0.13^{a}	0.11^{b}	0.004	<0.01			0.02	<0.01
d 0–60	0.10^{b}	0.12^{a}	0.10^{b}	0.003	0.01	<0.01	0.05	0.13	0.01

ADG = average daily gain; BW = body weight; DMI = dry matter intake; SEM = standard error of means.
[1] CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.
[2] Feed efficiency = ADG:DMI ratio.

effects. Measurements at the beginning of the experiment were used as covariates in the model and were then removed from the model if they had no significant effects on the model. Specific orthogonal contrasts were analyzed to test (1) ADY vs. CON, and (2) CSY vs. ISY. Covariance structures with the lowest Akaike information criterion were used in the model (Littell et al., 1998). Variables within the microbial parameters were analyzed using the Kruskal–Wallis test and the Wilcoxon test between treatments. Results were reported as least squares means. Significance was declared at $P < 0.05$, and a trend was indicated at $0.05 \leq P < 0.10$.

3. Results

3.1. Feed intake and growth performance

The BW was similar among treatments at the onset and termination of the experiment (Table 2). ADY supplementation resulted in a greater DMI than that in the CON during the whole experimental period ($P = 0.03$), even though this difference did not appear on d 0 to 30 and d 30 to 60. DMI of the CSY and ISY was similar on d 0 to 30, d 30 to 60, and d 0 to 60. ADY supplementation produced a greater ADG on d 30 to 60 ($P = 0.01$), and tended to have a greater ADG on d 0 to 60 than the CON ($P = 0.05$). The CSY had greater ($P < 0.01$) ADG than the ISY on d 30 to 60 and d 0 to 60. ADY supplementation yielded a greater feed efficiency (FE; ADG:DMI ratio) than the CON only on d 30 to 60 ($P = 0.02$). The CSY had greater FE than the ISY on d 30 to 60 ($P < 0.01$) and d 0 to 60 ($P = 0.01$). The CSY had a greater ($P = 0.01$) body length than the ISY during the experimental period and especially on d 30 (Fig. 1B). ADY supplementation tended to produce a greater ($P = 0.06$) paunch girth than the CON during the experimental period (Fig. 1E). No significant differences were found in other body frame measurements among treatments at individual sampling time points or throughout the experiment.

3.2. Rumen fermentation parameters and cellulolytic enzyme activity

ADY supplementation produced a lower rumen pH than that in the CON only on d 30 ($P = 0.04$; Fig. 2A). ADY supplementation also yielded a lower NH_3–N concentration than that in the CON during the whole experimental period ($P < 0.01$) and especially on d 15 ($P = 0.01$; Fig. 2B). On d 60, the CSY had a lower NH_3–N concentration than the CON and ISY ($P = 0.03$; Fig. 2B). On d 30, the CSY had greater total VFA (TVFA) concentration than the ISY ($P = 0.03$); however, the comparison was the opposite on d 60 ($P = 0.01$; Fig. 2C). No significant difference was found in the overall values of TVFA. ADY supplementation produced a significantly greater acetate proportion than the CON during the whole experimental period and especially on d 15 and 45 (Fig. 2D). On d 60, the acetate proportion was lower in the CSY than in the ISY ($P = 0.03$). The groups with ADY supplementation had a lower propionate proportion than the CON on d 15 ($P = 0.03$) even though it was numerically larger in the groups with ADY supplementation on d 60 (Fig. 2E). Thus, the average propionate proportion from d 15 to d 60 d was still lower in the ADY supplemented group than the CON ($P = 0.03$). ADY supplementation yielded lower valerate proportions than the CON on d 15 ($P < 0.01$), however, the comparison was the opposite on d 60 ($P = 0.01$; Fig. 2G). Thus, no significant difference was found for the overall values of valerate proportion. ADY supplementation produced a greater isobutyrate proportion than the CON on d 60 ($P = 0.01$), but no difference was found for the overall values (Fig. 2H). The isobutyrate proportion was lower in the CSY than in the ISY based on the overall values ($P = 0.03$). ADY supplementation had no significant effect on butyrate and isovalerate proportions (Fig. 2F and I).

ADY supplementation yielded a lower MCC activity than that in the CON on d 15 ($P = 0.02$); however, the comparison was the opposite on d 30 ($P = 0.01$; Fig. 3B). The MCC activity was greater in the ISY than in the CSY on d 30 ($P = 0.01$). However, no significant difference was found in the overall values of MCC activity. ADY supplementation produced greater acetylesterase activity than the CON during the whole experimental period ($P = 0.01$) and especially on d 45 ($P < 0.01$; Fig. 3D). The acetylesterase activity was also greater in the CSY than in the ISY ($P < 0.01$). No significant difference was found in CMC and xylanase activities among treatments at sampling times or for the entire study (Fig. 3A and C).

3.3. Ruminal microorganisms

A total of 18 phyla were detected via the taxonomic analyses of all 30 samples. Bacteroidetes, Firmicutes, Synergistetes, and Patescibacteria were the four most abundant phyla, representing 65.65%, 28.03%, 2.22%, and 1.02% of total sequences, respectively

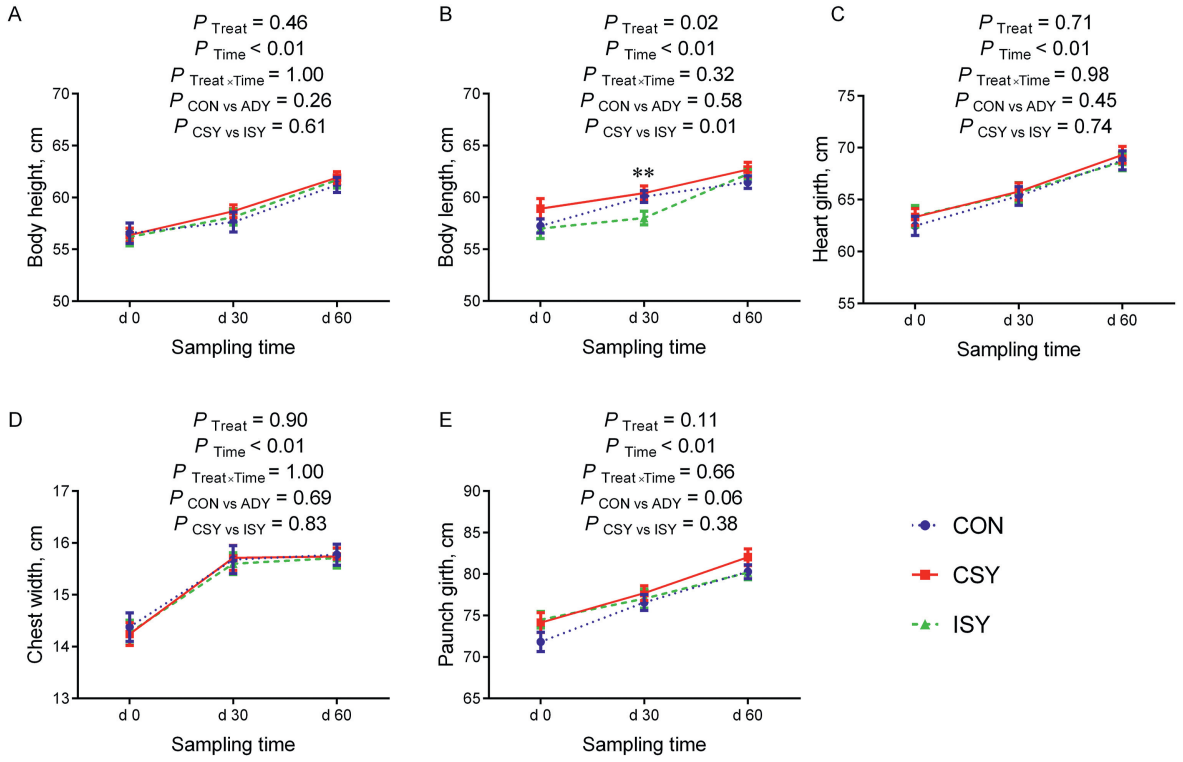

Fig. 1. Effect of active dry yeast (ADY) supplementation on body weight (A), body length (B), heart girth (C), chest width (D), and paunch girth (E) measurements in young dairy goats. The "**" at the individual sampling time point means a significant difference ($P < 0.05$) between the CSY and ISY by using contrast testing. The error bars are based on the standard error of the means. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

(Fig. 4A). At the genus level, 217 genera were detected from all samples. *Prevotella*, *Olsenella*, Rikenellaceae_RC9_gut_group, *Quinella*, Christensenellaceae_R-7_group, unclassified F082, *Fretibacterium*, *Ruminococcus*, unclassified Selenomonadaceae, Oscillospiraceae_NK4A214_group, and Prevotellaceae_UCG-003 were the ten most abundant genera, representing 47.25%, 10.45%, 7.87%, 2.65%, 2.30%, 2.21%, 1.94%, 1.89%, 1.70% and 1.43% of total sequences, respectively (Fig. 4B).

Similar values for Chao1, Shannon, and Simpson indices were observed among the 3 groups (Fig. 5A, B, and C). A PCoA analysis of overall diversity based on unweighted UniFrac metrics was performed to compare the three treatments (Fig. 5D). ANOSIM suggested a trend of different bacterial community composition between treatments CON and CSY ($R = 0.088$, $P = 0.08$) and between treatments CON and ISY ($R = 0.072$, $P = 0.06$). No significant differences were found between treatments CSY and ISY ($R = 0.071$, $P = 0.12$).

Spirochaetota was the only significantly different ($P = 0.04$) phylum among treatments. Fourteen genera were identified as significantly different among treatments. Among these, eight genera — Christensenellaceae_R-7_group, *Succiniclasticum*, norank Bacteroidales_RF16_group, Veillonellaceae_UCG-001, Erysipelatoclostridiaceae_UCG-004, *Anaeroplasma*, *Sphaerochaeta*, and norank Ruminococcaceae — had an average relative abundance of more than 0.10% (Fig. 5E). Among that, the relative abundances of Veillonellaceae_UCG-001 ($P = 0.01$) and *Succiniclasticum* ($P = 0.04$) were greater but the relative abundance of Christensenellaceae_R-7_group was lower ($P = 0.01$) in the ADY supplemented groups than in the CON. The relative abundances of norank Bacteroidales_RF16_group ($P = 0.03$), Erysipelatoclostridiaceae_UCG-004 ($P = 0.02$), *Anaeroplasma* ($P < 0.01$), and norank Ruminococcaceae ($P = 0.02$) were greater but the relative abundance of Veillonellaceae_UCG-001 was lower ($P = 0.01$) in the CSY than in the ISY.

3.4. Blood metabolites and immunoglobulin concentrations

ADY supplementation produced a greater serum glucose concentration than that in the CON during the whole experimental period ($P = 0.01$; Fig. 6A). ADY supplementation tended to result in a lower BUN than in the CON during the whole experimental period ($P = 0.07$), and had a lower BUN especially on d 15 ($P < 0.01$; Fig. 6C). The BUN concentrations were significantly lower in the CSY than in the ISY on d 15, 30, and 60. ADY supplementation produced lower TP concentrations than that in the CON during the whole experimental period ($P = 0.04$) and especially on d 45 ($P = 0.02$; Fig. 6D). The globulin concentration was lower in the CSY than in the ISY on d 30 ($P = 0.01$; Fig. 6F). ADY supplementation produced a greater ALB:globulin ratio than the CON on d 15 ($P = 0.04$), but no difference was found for the overall values (Fig. 6G). The ALB:globulin ratio was also greater in the CSY than in the ISY on d 30 ($P = 0.03$). No significant difference was found in triglyceride and ALB concentrations among treatments at any sampling times or for the entire study (Fig. 6B and E).

ADY supplementation resulted in a greater serum IgG concentration than that in the CON on d 15 ($P = 0.01$; Fig. 7A). ADY supplementation also yielded greater serum IgM concentrations than that in the CON during the whole experimental period ($P = 0.02$) and especially on d 15 ($P = 0.03$; Fig. 7B). IgM concentration was greater in the CSY than in the ISY on d 30 ($P = 0.03$). However, ADY supplementation produced lower serum IgA concentrations than that in the CON during the whole experimental period ($P = 0.03$)

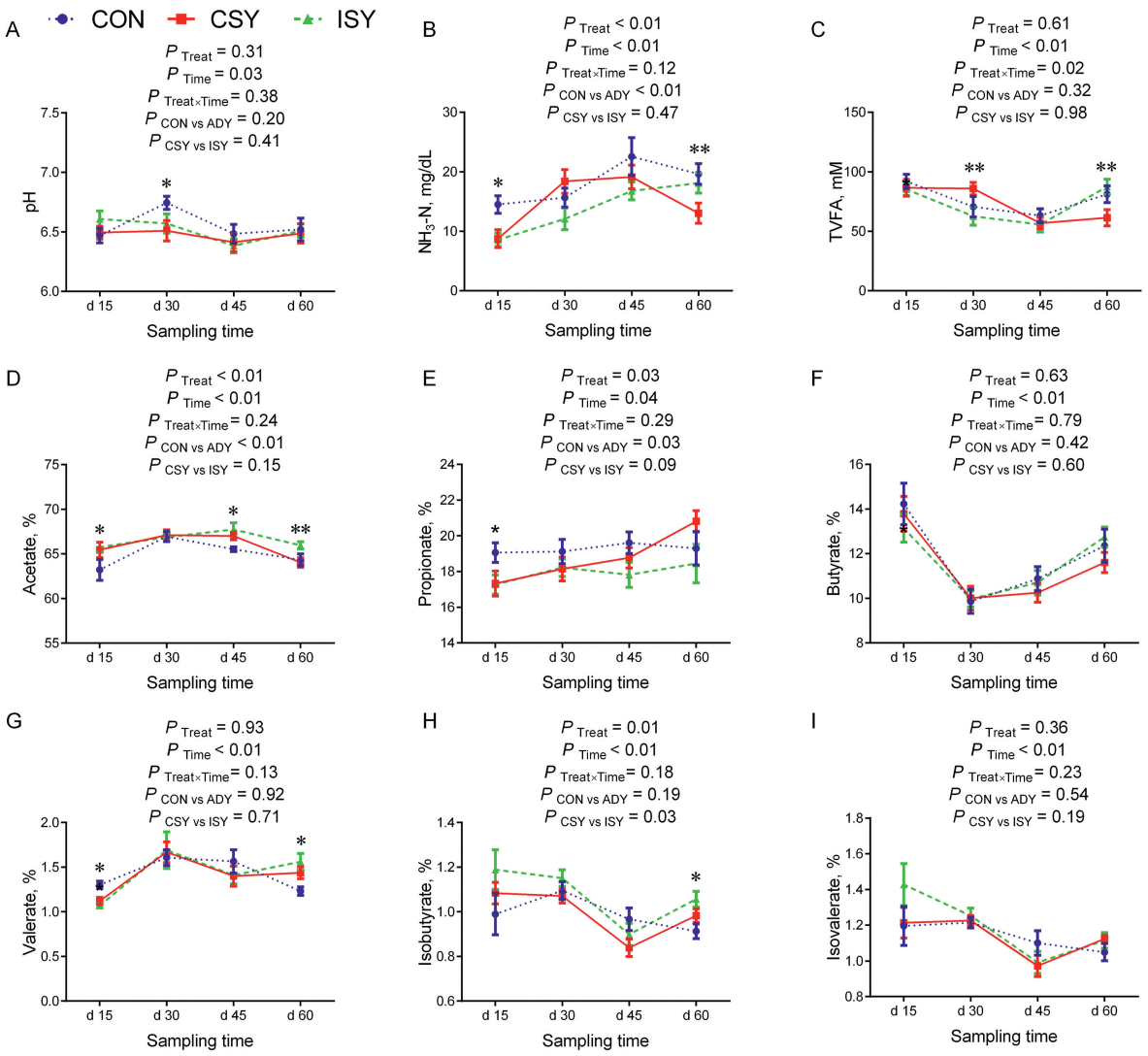

Fig. 2. Effect of active dry yeast (ADY) supplementation on rumen fermentation parameters in young dairy goats. The pH (A), contents of NH_3–N (B) and TVFA (C), and proportions of acetate (D), propionate (E), butyrate (F), valerate (G), isobutyrate (H), and isovalerate (I) are displayed. The "*" and "**" at the individual sampling time point mean significant differences ($P < 0.05$) between CON and ADY and between CSY and ISY by using contrast testing, respectively. The error bars are based on the standard error of the means. TVFA = total volatile fatty acids. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

and especially on d 60 ($P = 0.02$; Fig. 7C). IgA concentration was greater in the CSY than in the ISY on d 15 ($P = 0.01$).

3.5. Long-term effect of ADY on growth performance

ADY supplementation resulted in higher BW at birth and 12 months of age than the CON group ($P \leq 0.03$; Table 3), but the BW gains during 6 to 12 months were similar. The BW at 12 months of age and BW gains during 6 to 12 months were greater in the CSY than in the ISY ($P \leq 0.03$; Table 3).

4. Discussion

Because growth and development occurring at a young age affects later performance, feeding and management regimes for young growing ruminants are critical. Even though probiotics, such as ADY, are widely used in newborn and adult ruminants to improve health status and performance, reports of ADY application and its long-term effects in young growing ruminants are seldom found. Furthermore, the outcomes of ADY supplementation are inconsistent, which may be a result of differences in animal age, diet, physiological stage, and source and dose of ADY as well as the different supplementation strategies (Ban and Guan, 2021). Therefore, this study mainly focused on ADY supplementation and its persisting effects in growing young dairy goats. Meanwhile, the difference in ADY supplementation strategies, such as continuous or intermittent supplementations, was also investigated.

Rumen pH and VFA are essential indicators that illustrate the ruminal environment. As in previous studies (Yang et al., 2004; Bayat et al., 2015; Xiao et al., 2016), our study found no notable differences in rumen pH and TVFA concentrations with ADY supplementation despite a lower pH on d 30. However, ADY was reported to alter the rumen pH to alleviate ruminal acidosis and increase TVFA concentrations (Desnoyers et al., 2009; Chung et al., 2011; AlZahal et al., 2014). Even though the rumen pH and TVFA concentrations were similar across all treatments, the proportions of acetate and propionate were different in this study. The ADY supplementation resulted in an increased acetate proportion but a

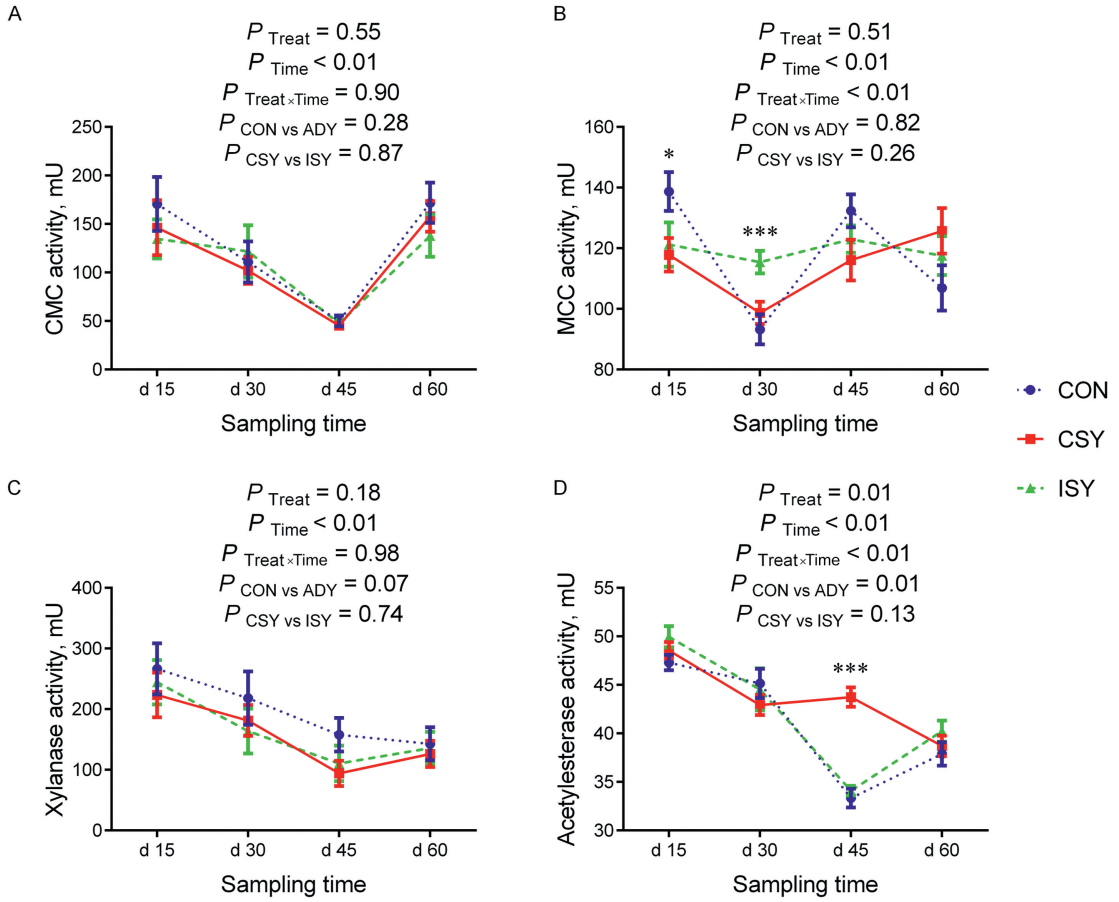

Fig. 3. Effect of active dry yeast (ADY) supplementation on ruminal carboxymethyl cellulase (A), microcrystalline cellulase (B), xylanase (C), and acetylesterase (D) activities in young dairy goats. The "*" at the individual sampling time point means a significant difference ($P < 0.05$) between CON and ADY by using contrast testing. The "***" at the individual sampling time point means significant differences ($P < 0.05$) both between CON and ADY and between CSY and ISY by using contrast testing. The error bars are based on the standard error of the means. CMC = carboxymethyl cellulase; MCC = microcrystalline cellulase. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

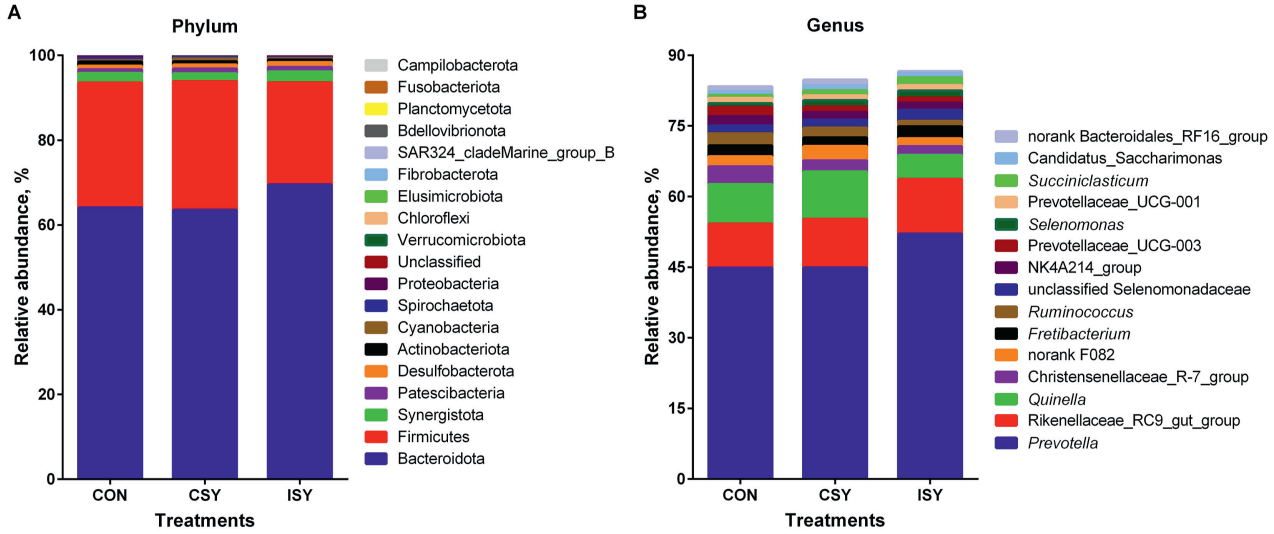

Fig. 4. Effect of active dry yeast (ADY) supplementation on ruminal bacterial community in young dairy goats at the phylum (A) and genus (B; top 15 relative abundance) levels. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

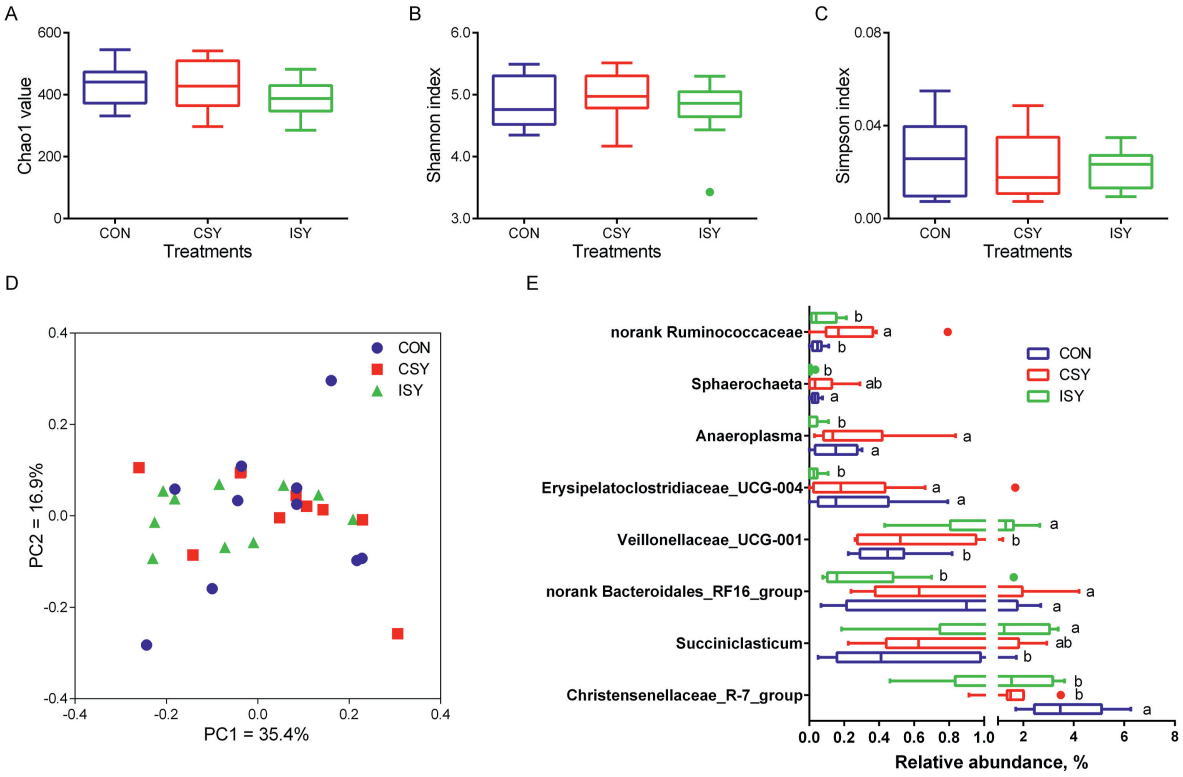

Fig. 5. Changes in the ruminal bacterial community among treatments in young dairy goats. The alpha diversity of the ruminal bacterial community was estimated by Chao1 (A), Shannon (B), and Simpson (C). The beta diversity was estimated by principal coordinate analysis (PCoA; D). Only significantly different bacterial genera with an average abundance of more than 0.10% were displayed (E). Boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Samples with a relative abundance for a given genus exceeding those values are represented as points beside the boxes. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

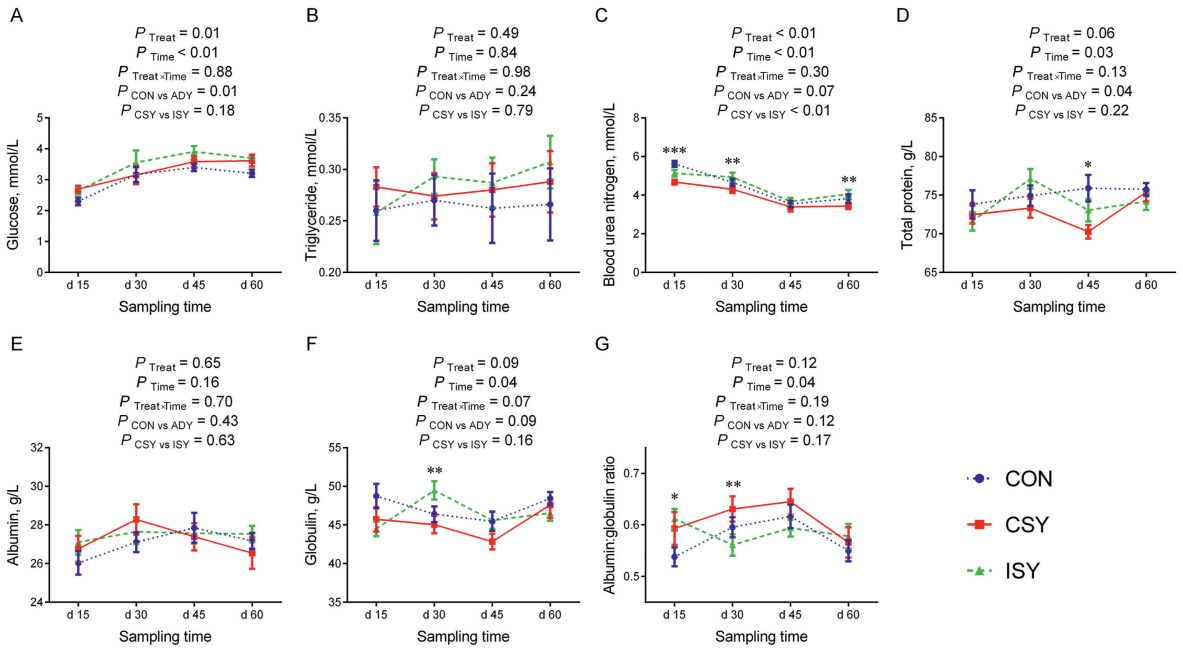

Fig. 6. Effect of active dry yeast (ADY) supplementation on blood metabolites in young dairy goats. The "*" and "**" at the individual sampling time point mean significant differences ($P < 0.05$) between CON and ADY and between CSY and ISY by using contrast testing, respectively. The "***" at the individual sampling time point means significant differences ($P < 0.05$) both between CON and ADY and between CSY and ISY by using contrast testing. The error bars are based on the standard error of the means. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

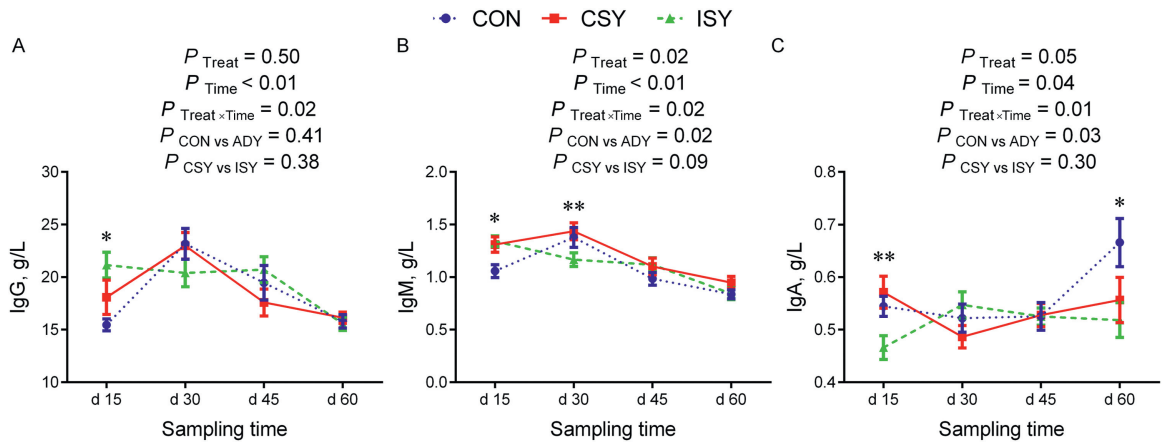

Fig. 7. Effect of active dry yeast (ADY) supplementation on blood immunoglobulin concentrations in young dairy goats. The "*" and "**" at individual sampling time point mean significant differences ($P < 0.05$) between CON and ADY and between CSY and ISY by using the contrast testing, respectively. The error bars are based on standard error of the means. CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

Table 3
Long-term effects of active dry yeast (ADY) supplementation in young dairy goats.

Item	Treatment[1]			SEM	*P*-value	*P* contrast	
	CON	CSY	ISY		Treat	CON vs. ADY	CSY vs. ISY
Body weight at 12 months, kg	36.30[b]	41.67[a]	37.04[b]	0.716	<0.01	0.03	0.01
Body weight gain during 6 to 12 months, kg	11.57[b]	15.37[a]	11.81[b]	0.676	0.04	0.16	0.03
Birth weight of lambs, kg	2.74	3.68	3.42	0.171	0.05	0.02	0.56

SEM = standard error of means.
[a–b] Means within a row with different superscripts differ significantly ($P < 0.05$).
[1] CON, basal diet; CSY, basal diet continuously supplemented with ADY (5.0×10^9 cfu/g; Qingdao Aolan Mingdong Biotechnology Co., Ltd., Qingdao, China) at 3.0 g/goat per day; ISY, basal diet intermittently supplemented with ADY at 4.5 g/goat per day.

decreased propionate proportion. Previous studies have reported an increase in ruminal acetate following yeast supplementation in calves and dairy cows (Malekkhahi et al., 2016; Amin and Mao, 2021; Sun et al., 2021). The increased acetate proportion observed both in our study and other studies could be due to the benefits of yeast on cellulolytic bacteria growth and then fiber degradation (Amin and Mao, 2021). The decreased ruminal NH_3–N concentration with ADY supplementation indicates improved nitrogen utilization, which may be related to ADY increasing the cellulolytic bacteria that prefer using NH_3–N as their N source (Hristov et al., 2010; Kamal et al., 2013).

How ADY supplementation affects the rumen microbial community depends on both the animal and ADY related factors (Xiao et al., 2016; Jiang et al., 2017; Bach et al., 2019). As in previous studies (Jiang et al., 2017; Bach et al., 2019), we found no significant differences in alpha diversity indices (Chao1 and Shannon) among treatments. The four most common bacterial phyla, Bacteroidetes, Firmicutes, Synergistetes, and Patescibacteria, were observed in all the treatments and accounted for a total of 96.9% 16S rRNA gene reads. The same rumen bacteria have been reported as the dominant phyla in previous studies (Zhang et al., 2017, 2020; Chen et al., 2021b). The only bacteria significantly different at the phylum level, Spirochaetota, has good potential for degrading hemicellulose like pectin, even though its role in the rumen is not fully understood (Liu et al., 2015; Gharechahi et al., 2021). Spirochaetota was found to be more abundant in the rumen of more concentrate-fed ruminants (Chen et al., 2021a). A higher relative abundance of Spirochaetota in the CON was consistent with lower ruminal cellulolytic enzyme activity. Overall, we found ADY supplementation had limited effects on the rumen bacteria at the phylum level.

At the genus level, *Prevotella*, *Olsenella*, Rikenellaceae_RC9_gut_group, *Ruminococcus*, and Prevotellaceae_UCG-003 were the common and dominant bacteria in our study, which was consistent with previous studies (Bach et al., 2019; Zhang et al., 2022b). Among the significantly different bacterial genera, Christensenellaceae_R-7_group had the highest relative abundance and represented an average of 2.63% ASVs among samples. The family Christensenellaceae is related to structural carbohydrate fermentation with acetate and butyrate as end products (Morotomi et al., 2012), but we did not observe this relationship in this study. As in previous studies (Jewell et al., 2015; Bach et al., 2019; Huang et al., 2021), we found that the relative abundance of Christensenellaceae_R-7_group was negatively correlated with the ruminal proportion of valerate ($r = -0.63$, $P < 0.01$), indicating that members of Christensenellaceae might have some differences from those reported. *Succiniclasticum* and Veillonellaceae_UCG-001 were both higher with ADY supplementation. *Succiniclasticum* is specialized in fermenting succinate, converting it to propionate (Shabat et al., 2016; Zhang et al., 2017), but no significant correlation between *Succiniclasticum* and propionate was found in this study, suggesting that the changes of *Succiniclasticum* herein were not significant enough to induce any difference associated with rumen fermentation. Although propionate is a primary fermentation product of the Veillonellaceae family (Myer et al., 2015; Lyons et al., 2017), the relative abundance of Veillonellaceae_UCG-001 was significantly positively correlated with ruminal acetate ($r = -0.53$, $P < 0.01$), which was consistent with previous studies (Lyons et al., 2017; Li et al., 2019). This suggested that Veillonellaceae_UCG-001 might have a function that differs from other members in the Veillonellaceae family.

Norank Bacteroidales_RF16_group, Erysipelatoclostridiaceae_UCG-004, *Anaeroplasma*, and norank Ruminococcaceae were significantly decreased in the ISY group. Bacteroidales have a broad range of plant polysaccharide-degrading genes and have more potential for fiber digestion. Unclassified Bacteroidales are more abundant in ruminants fed more forage (Henderson et al., 2015; Hu et al., 2021). The function of Erysipelatoclostridiaceae_UCG-004 and *Sphaerochaeta* is still unclear, but they might facilitate plant fiber degradation (Caro-Quintero et al., 2012; Xie et al., 2018; Qian et al., 2019). Ruminococcaceae is more prominent in forage-fed ruminants, and the members of Ruminococcaceae, especially *Ruminococcus*, are known to be involved in cellulose degradation (Morrison and Miron, 2000; Ozbayram et al., 2017; Zhang et al., 2017). We speculate that norank Ruminococcaceae has a similar function to other Ruminococcaceae. The decreased relative abundance of these potential fiber-degrading bacteria might be consistent with the numerical lower ruminal CMC and MCC activities in the ISY compared with the CSY on d 60. The greater relative abundance of *Anaeroplasma* and *Asteroleplasma* in the CSY suggests that ADY has oxygen-consuming characteristics as *Anaeroplasma* and *Asteroleplasma* are obligate anaerobes (Joblin and Naylor, 2002; Alugongo et al., 2017). One of the most plausible mechanisms of ADY working in the rumen is that ADY could eliminate dissolved oxygen and enhance lactate metabolism within the rumen, creating an optimal environment for cellulolytic microbes (AlZahal et al., 2014). It is assumed that yeast affects the growth of lactate-producing bacteria (*Streptococcus* and *Lactobacillus*), lactate-utilizing bacteria (*Megasphaera*), cellulolytic bacteria (*Ruminococcus* and *Fibrobacter*), and/or amylolytic bacteria (*Ruminobacter* and *Selenomonas*) (Jiang et al., 2017; Bach et al., 2019; Amin and Mao, 2021). However, we did not observe any significant differences in these bacteria from ADY supplementation. This discrepancy could be related to the difference in animal physiological stages and basal diets, as well as the source and dosage of yeast products (Ban and Guan, 2021).

Cellulolytic enzymes, secreted by rumen microbes, are crucial to fiber degradation in the rumen, a process unique to ruminants. Cellulolytic enzyme activity reflects how well fiber is degraded. Increased acetylesterase activity with ADY supplementation might be consistent with greater relative abundances of the above-mentioned cellulolytic bacteria, which might confer further benefits for the digestibility of NDF and ADF. Similarly, previous studies reported improved digestibility of NDF and ADF in both young and adult ruminants supplemented with ADY (Li et al., 2021; Ma et al., 2021). The increased acetylesterase activity also might be related to the growth of rumen fungi as ADY can stimulate the growth of *Neocallimastix* which has greater acetylesterase activity (Chaucheyras-Durand et al., 2008; Cao and Yang, 2011; AlZahal et al., 2014).

As in previous studies (Dehghan-Banadaky et al., 2013; Li et al., 2021), blood glucose concentrations were elevated in ADY-supplemented ruminants. Although ADY supplementation did not increase the rumen propionate proportion and concentration (13.51 mM vs. 15.58 mM) in this study, the higher blood glucose concentration might be due to the increased total propionate production in the rumen resulting from more DMI. On the other hand, elevated glucose concentration can be a result of promoted gluconeogenesis, intestinal glucose absorption, or both. A previous study also reported that ADY facilitates gluconeogenesis in ruminants, but the specific molecular mechanism still needs further investigation (Ma et al., 2021). Increasing fiber degradation in the rumen might also produce more substrates for gluconeogenesis in the liver. Post-ruminal digestion of feeds might be affected, especially when ADY passes through the rumen (Jiao et al., 2017; Ran et al., 2018). The BUN indicates nitrogen balance — a lower BUN suggests better nitrogen utilization. Combined with the lower ruminal NH_3–N concentration, the lower BUN in the ADY-supplemented groups suggested improved nitrogen utilization, as demonstrated in previous studies (Dehghan-Banadaky et al., 2013; Issakowicz et al., 2013), which might also contribute to the greater growth performance. Although the TP was lower with ADY supplementation and globin was lower in the CSY group on d 30, they were still within the normal ranges of healthy animals in similar studies, suggesting no negative influences on the hepatic metabolism of goats with ADY supplementation (Li et al., 2021; Sun et al., 2021).

Serum levels of IgG, IgM, and IgA serve as indicators for adaptive immune status, as they constitute approximately 90%, 9%, and 1%, respectively, of all immunoglobulins (Zaworski et al., 2014). IgG and IgM bind to antigens to activate complement proteins that kill pathogens, whereas IgA agglutinates pathogens which interfere with bacterial adhesion, as well as possessing anti-inflammatory properties (Zaworski et al., 2014). Greater IgM concentrations in the ADY supplementation group suggested improved resistance to pathogens, consistent with a previous study of weaned beef calves (Ma et al., 2021). Our recent meta-analysis also showed that *S. cerevisiae* products (either ADY or its fermentation products) increased serum IgG concentration by decreasing fecal pathogen colony counts, especially *Escherichia coli*, in young ruminants (Zhang et al., 2022a). Although the blood IgA concentration was slightly lower with ADY supplementation, the observed immunoglobulin concentrations were within normal physiological range, indicating no specific disease occurrence (Zaworski et al., 2014; Liu et al., 2020; Wu et al., 2021). In addition, the lower IgA concentration in the blood did not necessarily mean decreased bacterial adhesion ability, as a previous study reported significantly increased secretory IgA in the ileum and colon of calves supplemented with ADY (*S. cerevisiae boulardii* CNCM I-1079) despite numerically decreased serum IgA concentration (Villot et al., 2020). According to previous studies, ADY not only can reduce the adherence of pathogenic bacteria to inhibit them from colonizing the intestinal mucosa but also can compete with the binding sites of some toxins in the digestive tract (Ban and Guan, 2021; Ma et al., 2021). This suggested that quality feed and management strategies enhanced immunity and promoted the health of goats in this study.

As in previous studies (Bayat et al., 2015; Phesatcha et al., 2021), we found an increase in DMI with ADY supplementation. In ruminants, the dietary fiber content and fiber digestibility greatly influence DMI (de Souza et al., 2019). Based on our observation and previous studies (Li et al., 2021; Ma et al., 2021), the greater ruminal cellulolytic enzyme activity and relative abundance of fiber degradation-related bacteria might increase the degradation of feed in the rumen, which further improves intake. On the other hand, ADY supplementation can decrease the meal interval in dairy cattle, which increases DMI (Bach et al., 2007; Olagaray et al., 2019). Previous studies also reported the benefits of ADY on ADG and FE in neonatal, weaned, and finishing ruminants (Kamal et al., 2013; Hassan et al., 2016; Ma et al., 2021). The long-term effect of ADY on growth performance might be due to facilitating the development and maturation of the gastrointestinal tract and ecosystem. Previous studies have suggested that ADY establishes a healthy gastrointestinal ecosystem by preventing pathogens from binding to the gastrointestinal tract or accelerating its development in young ruminants (Chaucheyras-Durand and Fonty, 2009; Xiao et al., 2016; Ma et al., 2020). Similarly, a previous study reported persistent effects in calves by inhibiting the colonization of harmful bacteria in the rumen (Chang et al., 2022). From a holistic view of animal physiological state, better growth performance was associated with improved feed intake, rumen fermentation, microbial community, nutrient digestibility, gastrointestinal tract health, whole body metabolism, and immunity. Few studies have examined the long-

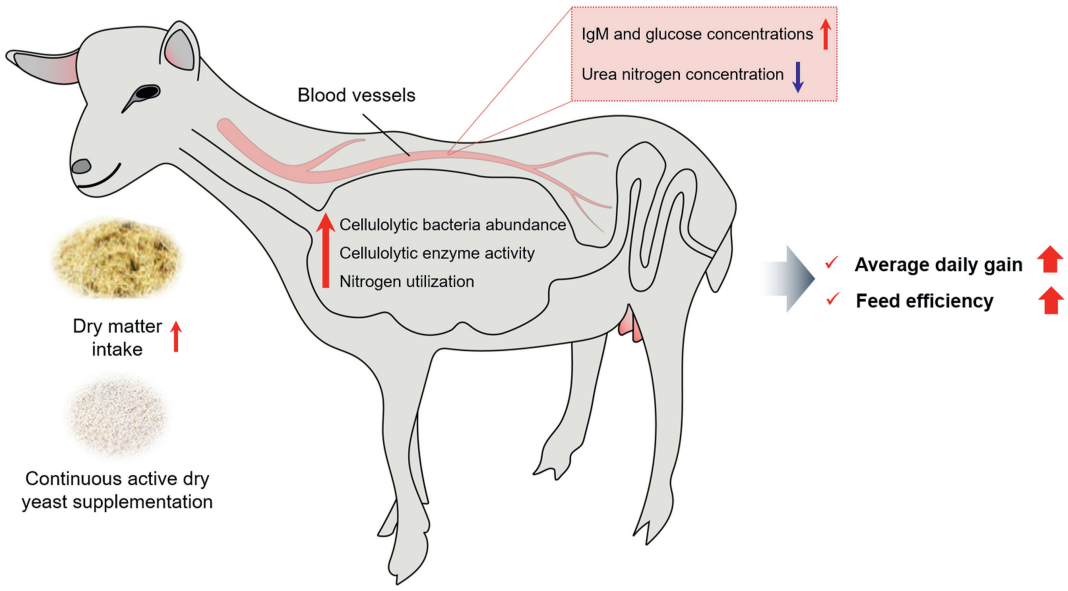

Fig. 8. An overview of the means whereby continuous active dry yeast supplementation might facilitate the average daily gain and feed efficiency in young dairy goats. Cellulolytic bacteria included norank Bacteroidales_RF16_group, Erysipelatoclostridiaceae_UCG-004, *Anaeroplasma*, *Sphaerochaeta*, and norank Ruminococcaceae. Cellulolytic enzyme was represented by acetylesterase. Nitrogen utilization was represented by ruminal NH_3–N and blood urea nitrogen concentrations. Immune status was represented by blood IgM concentration. Red arrow indicates an increase, and blue arrow indicates a decrease.

term effects of probiotics and prebiotics on young ruminants. We suggest that future studies should focus on how ADY supplementation persistently affects gastrointestinal tract health, nutrient digestibility, immune status, and then growth performance.

Compared with the CSY group, the adversely affected parameters in ruminal bacterial relative abundance and growth performance in the ISY group might be due to a disturbance in gastrointestinal microbes, digestion, or both by the intermittent ADY supplementation. A previous study asserted that even if the rumen pH and TVFA concentrations return to their pre-exchange values after a near–total exchange of ruminal contents, it takes 14 to 61 d for the ruminal bacterial community composition to regain a similar profile (Weimer et al., 2010). After a 4-day dietary challenge, 83.3% of Holstein heifers experienced digestive upset, as indicated by > 50% feed intake decreases (Moya et al., 2009). Even when *S. cerevisiae* culture products are used as dietary supplements to stabilize ruminal fermentation, they do not significantly affect the incidence or the time to cause the digestive upset. The required time for stabilization varies with intake, ruminal fermentation parameters, the composition of digested matter, nutrient digestion, and blood metabolites and can range from 4 to 30 d when diets are shifted (Sun et al., 2010; Machado et al., 2016; Qiu et al., 2021). In our study, even though the disturbance from the intermittent pattern was not as severe as the near–total exchange of ruminal contents, the 5-day interval may not have been enough for the animal to recover. In addition, since certain *S. cerevisiae* strains cannot colonize the rumen for a long period of time (Chaucheyras-Durand et al., 2012), the intermittent ADY supplementation, which was designed to decrease the feed cost, was not a suitable supplementation strategy for young growing dairy goats under this circumstance. Future studies investigating the mechanism whereby intermittent ADY supplementation influences animal performance are also warranted.

5. Conclusions

ADY supplementation enhances ADG and FE primarily by improving DMI, ruminal cellulolytic bacteria abundance, cellulolytic enzyme activity, nitrogen utilization, and immune status in young growing dairy goats (Fig. 8). Moreover, goats fed ADY exhibited persistent effects on the BW at 12 months of age and even the birth weight of lambs. However, these benefits did not all occur with intermittent ADY supplementation compared with continuous ADY supplementation, indicating that the ADY supplementation strategy had significant effects on the performance of young growing dairy goats. Overall, continuous ADY supplementation, rather than intermittent ADY supplementation, can be used as an effective feed additive and supplementation strategy to promote the healthy and efficient growth of young growing dairy goats.

Author contributions

Junhu Yao designed the experiments. **Yuntian Yang**, **Xinjian Lei**, and **Jun Zhang** performed the experiments. **Jun Zhang** and **Yuntian Yang** analyzed the experimental data and wrote the draft manuscript, **Junhu Yao**, **Xinjian Lei**, **Yannan Wang, Yanhua Li,** and **Zhiqiang Yang** reviewed and edited the manuscript. The authors read and approved the final manuscript.

Availability of data and materials

Raw sequence reads for all samples described above were deposited into the NCBI Sequence Read Archive (SRA) database (No. PRJNA810766).

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant number 32102570), the Key R&D Program of Shaanxi Province in China (grant number 2022ZDLNY01-11), the Chinese Universities Scientific Fund (grant numbers 2452020188 and 2452022252), and the fellowship of China Postdoctoral Science Foundation (grant number 2021M702691). The authors declare that they have no competing interests. We would also like to thank members of Prof. Yao's lab and Aonike Dairy Goat Farm for their assistance in animal care and sampling.

References

Alugongo GM, Xiao J, Wu Z, Li S, Wang Y, Cao Z. Review: utilization of yeast of *Saccharomyces cerevisiae* origin in artificially raised calves. J Anim Sci Biotechnol 2017;8:34.

AlZahal O, Dionissopoulos L, Laarman AH, Walker N, McBride BW. Active dry *Saccharomyces cerevisiae* can alleviate the effect of subacute ruminal acidosis in lactating dairy cows. J Dairy Sci 2014;97:7751–63.

Amin AB, Mao S. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: a review. Anim Nutr 2021;7:31–41.

AOAC. Association of official analytical chemists. 17th ed. Arlington, VA, USA: AOAC International; 2000.

Bach A, Iglesias C, Devant M. Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Anim Feed Sci Technol 2007;136:146–53.

Bach A, Lopez-Garcia A, Gonzalez-Recio O, Elcoso G, Fabregas F, Chaucheyras-Durand F, et al. Changes in the rumen and colon microbiota and effects of live yeast dietary supplementation during the transition from the dry period to lactation of dairy cows. J Dairy Sci 2019;102:6180–98.

Ban Y, Guan LL. Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. J Anim Sci Biotechnol 2021;12:109.

Bayat AR, Kairenius P, Stefanski T, Leskinen H, Comtet-Marre S, Forano E, et al. Effect of camelina oil or live yeasts (*Saccharomyces cerevisiae*) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. J Dairy Sci 2015;98:3166–81.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852–7.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–3.

Cao YC, Yang HJ. Effect of roughage fibre content on fibrolytic activities and volatile fatty acid profiles of *Neocallimastix* sp YAK11 isolated from rumen fluids of yak (*Bos grunniens*). Anim Feed Sci Technol 2011;170:284–90.

Caro-Quintero A, Ritalahti KM, Cusick KD, Loffler FE, Konstantinidis KT. The chimeric genome of Sphaerochaeta: nonspiral *spirochetes* that break with the prevalent dogma in spirochete biology. mBio 2012;3:000255-e112.

Chang M, Wang F, Ma F, Jin Y, Sun P. Supplementation with galacto-oligosaccharides in early life persistently facilitates the microbial colonization of the rumen and promotes growth of preweaning Holstein dairy calves. Anim Nutr 2022;10:223–33.

Chaucheyras-Durand F, Chevaux E, Martin C, Forano E. Use of yeast probiotics in ruminants: effects and mechanisms of action on rumen pH, fibre degradation, and microbiota according to the diet. In: Probiotic in animals; 2012.

Chaucheyras-Durand F, Fonty G. Influence of a probiotic yeast (*Saccharomyces cerevisiae* CNCM I-1077) on microbial colonization and fermentations in the rumen of newborn lambs. Microb Ecol Health Dis 2009;14:30–6.

Chaucheyras-Durand F, Walker ND, Bach A. Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. Anim Feed Sci Technol 2008;145:5–26.

Chen H, Wang C, Huasai S, Chen A. Effects of dietary forage to concentrate ratio on nutrient digestibility, ruminal fermentation and rumen bacterial composition in Angus cows. Sci Rep 2021a;11:17023.

Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018;34:i884–90.

Chen X, Su X, Li J, Yang Y, Wang P, Yan F, et al. Real-time monitoring of ruminal microbiota reveals their roles in dairy goats during subacute ruminal acidosis. NPJ Biofilms Microbiomes 2021b;7:45.

Chung YH, Walker ND, McGinn SM, Beauchemin KA. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. J Dairy Sci 2011;94:2431–9.

de Souza RA, Tempelman RJ, Allen MS, VandeHaar MJ. Updating predictions of dry matter intake of lactating dairy cows. J Dairy Sci 2019;102:7948–60.

Dehghan-Banadaky M, Ebrahimi M, Motameny R, Heidari SR. Effects of live yeast supplementation on mid-lactation dairy cows performances, milk composition, rumen digestion and plasma metabolites during hot season. J Appl Anim Res 2013;41:137–42.

Desnoyers M, Giger-Reverdin S, Bertin G, Duvaux-Ponter C, Sauvant D. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J Dairy Sci 2009;92:1620–32.

Elmhadi ME, Ali DK, Khogali MK, Wang H. Subacute ruminal acidosis in dairy herds: microbiological and nutritional causes, consequences, and prevention strategies. Anim Nutr 2022;10:148–55.

Gharechahi J, Vahidi MF, Bahram M, Han JL, Ding XZ, Salekdeh GH. Metagenomic analysis reveals a dynamic microbiome with diversified adaptive functions to utilize high lignocellulosic forages in the cattle rumen. ISME J 2021;15:1108–20.

Hassan AA, Salem AZM, Kholif AE, Samir M, Yacout MH, Hafsa SHA, et al. Performance of crossbred dairy Friesian calves fed two levels of Saccharomyces cerevisiae: intake, digestion, ruminal fermentation, blood parameters and faecal pathogenic bacteria. J Agric Sci 2016;154:1488–98.

He ZX, Ferlisi B, Eckert E, Brown HE, Aguilar A, Steele MA. Supplementing a yeast probiotic to pre-weaning Holstein calves: feed intake, growth and fecal biomarkers of gut health. Anim Feed Sci Technol 2017;226:81–7.

Henderson G, Cox F, Ganesh S, Jonker A, Young W, Global Rumen Census C, et al. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep 2015;5:14567.

Hristov AN, Varga G, Cassidy T, Long M, Heyler K, Karnati SK, et al. Effect of *Saccharomyces cerevisiae* fermentation product on ruminal fermentation and nutrient utilization in dairy cows. J Dairy Sci 2010;93:682–92.

Hu C, Ding L, Jiang C, Ma C, Liu B, Li D, et al. Effects of management, dietary intake, and genotype on rumen morphology, fermentation, and microbiota, and on meat quality in yaks and cattle. Front Nutr 2021;8:755255.

Huang S, Ji S, Suen G, Wang F, Li S. The rumen bacterial community in dairy cows is correlated to production traits during freshening period. Front Microbiol 2021;12:630605.

Ishaq SL, AlZahal O, Walker N, McBride B. An investigation into rumen fungal and protozoal diversity in three rumen fractions, during high-fiber or grain-induced sub-acute ruminal acidosis conditions, with or without active dry yeast supplementation. Front Microbiol 2017;8:1943.

Issakowicz J, Bueno MS, Sampaio ACK, Duarte KMR. Effect of concentrate level and live yeast (*Saccharomyces cerevisiae*) supplementation on Texel lamb performance and carcass characteristics. Livest Sci 2013;155:44–52.

Jewell KA, McCormick CA, Odt CL, Weimer PJ, Suen G. Ruminal bacterial community composition in dairy cows is dynamic over the course of two lactations and correlates with feed efficiency. Appl Environ Microbiol 2015;81:4697–710.

Jiang Y, Ogunade IM, Qi S, Hackmann TJ, Staples CR, Adesogan AT. Effects of the dose and viability of *Saccharomyces cerevisiae*. 2. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. J Dairy Sci 2017;100:325–42.

Jiao PX, Wei LY, Walker ND, Liu FZ, Chen LY, Beauchemin KA, et al. Comparison of non-encapsulated and encapsulated active dried yeast on ruminal pH and fermentation, and site and extent of feed digestion in beef heifers fed high-grain diets. Anim Feed Sci Technol 2017;228:13–22.

Joblin KN, Naylor GE. The ruminal mycoplasmas: a review. J Appl Anim Res 2002;21:161–79.

Kamal R, Dutt T, Singh M, Kamra DN, Patel M, Choudhary LC, et al. Effect of live *Saccharomyces cerevisiae* (NCDC-49) supplementation on growth performance and rumen fermentation pattern in local goat. J Appl Anim Res 2013;41:285–8.

Khan MA, Bach A, Weary DM, von Keyserlingk MAG. Invited review: transitioning from milk to solid feed in dairy heifers. J Dairy Sci 2016;99:885–902.

Kumprechtova D, Illek J, Julien C, Homolka P, Jancik F, Auclair E. Effect of live yeast (*Saccharomyces cerevisiae*) supplementation on rumen fermentation and metabolic profile of dairy cows in early lactation. J Anim Physiol Anim Nutr 2019;103:447–55.

Lascano GJ, Zanton GI, Suarez-Mena FX, Heinrichs AJ. Effect of limit feeding high- and low-concentrate diets with Saccharomyces cerevisiae on digestibility and on dairy heifer growth and first-lactation performance. J Dairy Sci 2009;92:5100–10.

Li L, Qu M, Liu C, Xu L, Pan K, OuYang K, et al. Effects of recombinant swollenin on the enzymatic hydrolysis, rumen fermentation, and rumen microbiota during in vitro incubation of agricultural straws. Int J Biol Macromol 2019;122:348–58.

Li Y, Shen Y, Niu J, Guo Y, Pauline M, Zhao X, et al. Effect of active dry yeast on lactation performance, methane production, and ruminal fermentation patterns in early-lactating Holstein cows. J Dairy Sci 2021;104:381–90.

Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci 1998;76:1216–31.

Liu J, Pu YY, Xie Q, Wang JK, Liu JX. Pectin induces an in vitro rumen microbial population shift attributed to the pectinolytic *Treponema* group. Curr Microbiol 2015;70:67–74.

Liu T, Chen H, Bai Y, Wu J, Cheng S, He B, et al. Calf starter containing a blend of essential oils and prebiotics affects the growth performance of Holstein calves. J Dairy Sci 2020;103:2315–23.

Lyons T, Boland T, Storey S, Doyle E. Linseed oil supplementation of lambs' diet in early life leads to persistent changes in rumen microbiome structure. Front Microbiol 2017;8:1656.

Ma J, Shah AM, Shao Y, Wang Z, Zou H, Kang K. Dietary supplementation of yeast cell wall improves the gastrointestinal development of weaned calves. Anim Nutr 2020;6:507–12.

Ma J, Wang C, Wang Z, Cao G, Hu R, Wang X, et al. Active dry yeast supplementation improves the growth performance, rumen fermentation, and immune response of weaned beef calves. Anim Nutr 2021;7:1352–9.
Machado MG, Detmann E, Mantovani HC, Valadares SC, Bento CBP, Marcondes MI, et al. Evaluation of the length of adaptation period for changeover and cross-over nutritional experiments with cattle fed tropical forage-based diets. Anim Feed Sci Technol 2016;222:132–48.
Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 2011;27:2957–63.
Malekkhahi M, Tahmasbi AM, Naserian AA, Danesh-Mesgaran M, Kleen JL, AlZahal O, et al. Effects of supplementation of active dried yeast and malate during sub-acute ruminal acidosis on rumen fermentation, microbial population, selected blood metabolites, and milk production in dairy cows. Anim Feed Sci Technol 2016;213:29–43.
Morotomi M, Nagai F, Watanabe Y. Description of *Christensenella minuta* gen. nov., sp. nov., isolated from human faeces, which forms a distinct branch in the order *Clostridiales*, and proposal of *Christensenellaceae* fam. nov. Int J Syst Evol Microbiol 2012;62:144–9.
Morrison M, Miron J. Adhesion to cellulose by *Ruminococcus albus*: a combination of cellulosomes and Pil-proteins? FEMS Microbiol Lett 2000;185:109–15.
Moya D, Calsamiglia S, Ferret A, Blanch M, Fandino JI, Castillejos L, et al. Effects of dietary changes and yeast culture (*Saccharomyces cerevisiae*) on rumen microbial fermentation of Holstein heifers. J Anim Sci 2009;87:2874–81.
Myer PR, Smith TP, Wells JE, Kuehn LA, Freetly HC. Rumen microbiome from steers differing in feed efficiency. PLoS One 2015;10:e0129174.
NRC. Nutrient requirements of dairy cattle. 7th ed. Washington, DC: National Academy Press; 2001.
Olagaray KE, Sivinski SE, Saylor BA, Mamedova LK, Sauls-Hiesterman JA, Yoon I, et al. Effect of *Saccharomyces cerevisiae* fermentation product on feed intake parameters, lactation performance, and metabolism of transition dairy cattle. J Dairy Sci 2019;102:8092–107.
Ozbayram EG, Kleinsteuber S, Nikolausz M, Ince B, Ince O. Effect of bioaugmentation by cellulolytic bacteria enriched from sheep rumen on methane production from wheat straw. Anaerobe 2017;46:122–30.
Phesatcha K, Chunwijitra K, Phesatcha B, Wanapat M, Cherdthong A. Addition of active dry yeast could enhance feed intake and rumen bacterial population while reducing protozoa and methanogen population in beef cattle. Fermentation-Basel 2021;7:172.
Qian W, Ao W, Jia C, Li Z. Bacterial colonisation of reeds and cottonseed hulls in the rumen of Tarim red deer (*Cervus elaphus yarkandensis*). Antonie Leeuwenhoek 2019;112:1283–96.
Qiu Q, Gao C, Su H, Cao B. Rumen fermentation characteristics require more time to stabilize when diet shifts. Animals 2021;11:2192.
Ran T, Shen YZ, Saleem AM, AlZahal O, Beauchemin KA, Yang WZ. Using ruminally protected and nonprotected active dried yeast as alternatives to antibiotics in finishing beef steers: growth performance, carcass traits, blood metabolites, and fecal *Escherichia coli*. J Anim Sci 2018;96:4385–97.
Shabat SK, Sasson G, Doron-Faigenboim A, Durman T, Yaacoby S, Berg Miller ME, et al. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J 2016;10:2958–72.
Soberon F, Raffrenato E, Everett RW, Van Amburgh ME. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J Dairy Sci 2012;95:783–93.
Soberon F, Van Amburgh ME. Lactation Biology Symposium: the effect of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: a meta-analysis of current data. J Anim Sci 2013;91:706–12.
Sun X, Wang Y, Wang E, Zhang S, Wang Q, Zhang Y, et al. Effects of *Saccharomyces cerevisiae* culture ruminal fermentation, blood metabolism, and performance of high-yield dairy cows. Animals 2021;11:2401.
Sun YZ, Mao SY, Zhu WY. Rumen chemical and bacterial changes during stepwise adaptation to a high-concentrate diet in goats. Animal 2010;4:210–7.
Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.
Villot C, Chen Y, Pedgerachny K, Chaucheyras-Durand F, Chevaux E, Skidmore A, et al. Early supplementation of *Saccharomyces cerevisiae boulardii* CNCM I-1079 in newborn dairy calves increases IgA production in the intestine at 1 week of age. J Dairy Sci 2020;103:8615–28.
Villot C, Ma T, Renaud DL, Ghaffari MH, Gibson DJ, Skidmore A, et al. *Saccharomyces cerevisiae boulardii* CNCM I-1079 affects health, growth, and fecal microbiota in milk-fed veal calves. J Dairy Sci 2019;102:7011–25.
Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Anal Chem 2002;39:971–4.
Weimer PJ, Stevenson DM, Mantovani HC, Man SL. Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. J Dairy Sci 2010;93:5902–12.
Wu S, Li X, Chen X, Zhu Y, Yao J. Optimizing the growth and immune system of dairy calves by subdividing the pre-weaning period and providing different milk volumes for each stage. Anim Nutr 2021;7:1296–302.
Xiao JX, Alugongo GM, Chung R, Dong SZ, Li SL, Yoon I, et al. Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: ruminal fermentation, gastrointestinal morphology, and microbial community. J Dairy Sci 2016;99:5401–12.
Xie X, Yang C, Guan LL, Wang J, Xue M, Liu JX. Persistence of cellulolytic bacteria fibrobacter and treponema after short-term corn stover-based dietary intervention reveals the potential to improve rumen fibrolytic function. Front Microbiol 2018;9:1363.
Yang WZ, Beauchemin KA, Vedres DD, Ghorbani GR, Colombatto D, Morgavi DP. Effects of direct-fed microbial supplementation on ruminal acidosis, digestibility, and bacterial protein synthesis in continuous culture. Anim Feed Sci Technol 2004;114:179–93.
Zaworski EM, Shriver-Munsch CM, Fadden NA, Sanchez WK, Yoon I, Bobe G. Effects of feeding various dosages of *Saccharomyces cerevisiae* fermentation product in transition dairy cows. J Dairy Sci 2014;97:3081–98.
Zhang C, Zhang J, Yu Z, Zhou G, Yao J. Effects of supplementation with *Saccharomyces cerevisiae* products on dairy calves: a meta-analysis. J Dairy Sci 2022a;105:7386–98.
Zhang J, Shi H, Wang Y, Li S, Cao Z, Ji S, et al. Effect of dietary forage to concentrate ratios on dynamic profile changes and interactions of ruminal microbiota and metabolites in Holstein heifers. Front Microbiol 2017;8:2206.
Zhang J, Zheng N, Shen W, Zhao S, Wang J. Synchrony degree of dietary energy and nitrogen release influences microbial community, fermentation, and protein synthesis in a rumen simulation system. Microorganisms 2020;8:231.
Zhang T, Mu Y, Zhang R, Xue Y, Guo C, Qi W, et al. Responsive changes of rumen microbiome and metabolome in dairy cows with different susceptibility to subacute ruminal acidosis. Anim Nutr 2022b;8:331–40.