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Effects of microbial preparation on production performance and rumen microbial communities of goat

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Abstract

This study explored effects of microbial preparation (MIP, developed by our research team) on production performance and body health of Boer goat to reveal the function of it in goat breeding industry. Compound yeast and enzyme preparation (CYP) was used to compare the function of MIP. Healthy male Boer goats (n = 15, BW = 25.31±4.06 kg) were allocated randomly into three groups as NC (Basal diet), MI (Basal diet + MIP) and CY (Basal diet + CYP). This study lasted for 71 days including 15 days for adaptation and 56 days for growth trial. Both MIP and CYP enhanced production performance such as average daily gain of goats, MIP enhanced the level of fat synthesis such as total cholesterol level significantly on day 28. As for rumen fermentation and microbial communities of goat, MIP decreased rumen pH. PCoA analysis showed that the rumen bacterial community on day 28 was significantly separated. In conclusion, MIP increased production performance, ameliorating rumen fermentation and shifting microflora. Our findings provide the evidence for the influence of probiotics on goat production performance as well as health condition.

Keywords: probiotic preparation; production performance; rumen fermentation; rumen microflora; goat.

Practical Application: A new probiotic product for improving goats' production performance as well as body health.

1 Introduction

Antibiotics make it possible to control many diseases and improve livestock's growth performance in animal husbandry (Zamojska et al., 2021). Nevertheless, overusing antibiotics leads to microbiological, ecological, and environmental harm (Gemeda et al., 2020). Furthermore, the residual antibiotics will enter the human body and harm health (Faber et al., 2016). Thus, banning the use of antibiotics is the general trend. In 2006, the European Union started to ban the use of antibiotics in animal husbandry to enhance animals' growth performance (Jouany & Morgavi, 2007), and China completely banned it in 2020.

Seeking a substitution to increase growth performance and ensure the health of livestock is considerably crucial (Amin & Mao, 2021). Probiotics have the potential to play an important role in this process, which are considered as good alternative products because of non-hazardous, non-polluting, non-residual and non-side-effect (Alayande et al., 2020). Probiotic preparations are the screened microorganisms from the natural environment, then isolating probiotics or probiotic growth-promoting substances that are conducive to the host and performing culture and subculture to develop live probiotics preparations (Salminen et al., 1999). Probiotics play very important role in mammals' health such as improving immunity and antioxidant capacity to prevent disease. The exopolysaccharides (EPSs)-producing *L. plantarum* YW 11 has potent immunomodulatory and antitumor activities. EPS prevented HT-29 tumor cells induced acute liver and kidney damages significantly, and promoting secretion of cytokines IL-2 and TNF-α in mice (Zhang et al., 2022). *Lactobacillus plantarum* SCS2 obviously protected against AFB1-induced oxidative stress. Thus, *L. plantarum* SCS2 is a high-quality lactic acid bacterium with antioxidant function that has the potential development of probiotic products (Long et al., 2022). *Saccharomyces cerevisiae* species screened from industrial effluents have the potential to produce Zn-enriched single cell protein that could be considered to apply to food and feed industry (Forough et al., 2022).

Adequate intake of probiotics can benefit goats' growth performance and health condition (Angulo et al., 2019; Taboada et al., 2022). Cai et al. (2021a, b) showed that the supplementation with *Saccharomyces cerevisiae*, *Clostridium butyricum* and the combination of them could ameliorate rumen fermentation and enhance growth performance of heat-stressed goats (Cai et al., 2021a, b). Saleem et al. (2017) observed lambs that received probiotics treatment shown better growth performance, higher dry matter intake, feed conversion ratio and nutrients digestibility (Saleem et al., 2017). Previous studies had proved that MIP could enhance production performance in both goats and sheep, meanwhile, shift rumen microflora and ameliorate rumen fermentation (Chaucheyras-Durand et al., 2019; Hassan et al., 2020). Microorganisms need sources of carbon and nitrogen as nutrients for growth, the chyme in the rumen contained enough fiber and protein supply the essential nutrients for probiotics. Rumen also meet the other requirements for probiotic growth, such as appropriate pH and temperature (Kareena et al., 2021).

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Ruminal microbiota and ruminant have a mutualistic relationship, microbiota helps the host digest and absorb food to provide energy and small molecule nutrients for the host (Guo et al., 2021). Overall, probiotic plays an important role in rumen fermentation, rumen microflora, growth performance and health of ruminants. We hypothesized that MIP could improve growth performance, optimize rumen fermentation of goat. Thus, the aim of this work was to investigate how would MIP affect growth performance, health condition such as serum parameter, and rumen microflora.

2 Materials and methods

This study was conducted at Boer Goat Breeding Center (Shaanxi province, China) from June to September, 2020, which lasted for 71 days including 15 days for adaptation and 56 days for growth trial. The experiment was reviewed and approved by Northwest A&F University's Experimental Animal Management Committee (EAMC) and the Institutional Animal Care and Use Committee (College of Animal Science and Technology, Northwest A&F University, China) (Protocol NWAFAC1119).

2.1 Animals, diets and experimental design

Fifteen healthy 4-month Boer goats with initial BW of 25.31 ± 4.06 kg were assigned randomly into 3 groups (NC, MI and CY group, n = 5 for each group). All goats were fed basal diet shown in Table 1, goats in NC group were fed with basal diet without probiotic preparation, MI group was offered MIP 60 g/goat/day that was developed by our research team and First-feed Co., Ltd (Shaanxi, China). CY group was fed CYP 20 g/goat/day, a mature probiotic product that was developed by VTR + Bio-Tech Co., Ltd (Guangdong, China).

2.2 Feeding and management

Basal diet was formulated according to National Research Council feeding standards and feeding standard of meat-producing sheep and goats (NY/T 816-2004). Goats were fed twice each day at 8 am and 6 pm, all the animals were provided accesses to feed and water *ad libitum*. The ratio of concentrate to roughage in basal diet is 4: 6.

Table 1. Composition and nutrient level (DM basis) of basal diets (%).

Ingredients	Content	Nutrient composition[2]	Content
Corn stover	39.00	DM	88.90
Corn	22.81	CP	12.90
Alfalfa meal	16.46	Ca	0.84
Wheat bran	6.00	Total P	0.52
Cottonseed meal	5.00	CF	14.30
Rapeseed meal	4.00	EE	1.67
Soybean meal	2.77	Ash	6.72
$CaHPO_4$	1.48	ME(MJ/kg)	11.88
Premix[1]	1.50		
$NaHCO_3$	0.50		
NaCl	0.48		

[1]The premix was: Cu 500 mg, Zn 2 500 mg, Mn 2 500 mg, I 30 mg, Se 10 mg, Co 35 mg, VA 150 000 IU, VD 60 000 IU, VE 1 500 IU; [2]All data was measured except for ME. Note: Components of MIP: Bacillus subtilis ≥ 5.0×10^7 CFU/g, Bacillus licheniformis ≥ 1.0×10^8 CFU/g, yeast ≥ 1.0×10^8 CFU/g; components of CYP: Total Saccharomyces cerevisiae cells (DM) ≥ 10×10^8 CFU/g, cellulose activity ≥ 3 000 U/g, xylanase activity ≥ 2 000 U/g, β - glucanase activity ≥ 15 000 U/g, amylase activity ≥ 20 000 U/g, protease activity ≥ 2 000 U/g and some other fermented metabolites.

2.3 Sample collection and preparation

Rumen fluid samples were collected by oral intubation method from each goat on day 28 and day 56 during growth trial period, pH was measured, samples were filtered through 4 layers of gauze, then the liquid portion were collected. Subsequently, samples were homogenized and divided into 3 aliquots. The first aliquot was placed in a 5 mL cryotube and immediately placed in liquid nitrogen for subsequent microbial genomic DNA extraction and analysis. As for the second aliquot, the samples were centrifuged at 12 000 r/m for 10 min at 4 °C, and the supernatant was stored at -20 °C to determine the concentration of volatile fatty acids (VFAs). The third aliquot was for backup. Blood samples were collected from jugular vein, 10 mL for each goat, centrifuged with 3000 r/min for 15 min to collect serum for the later measurement (stored at -80°C, measurement for serum parameters was shown at 2.5).

2.4 Feed proximate analyses

Dry matter (DM, method 934.01), crude fiber (CF, method 978.10), ash (method 942.05), crude protein (CP, method 989.03), ether extract (EE, method 920.39), calcium (Ca, method 927.02), total phosphorous (total P, method 964.06) was measured in accord with Association of Official Analytical Chemists (1995).

2.5 Measurement for serum parameters

The content of glucose (GLU), urea (UREA), triacylglycerol (TG), total cholesterol (TC), acetyl coenzyme A (AcCoA), beta hydroxybutyric acid (BHBA), malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC) was measured by colorimetry. The kits were purchased from Sino-UK Institute of Biological Technology (Beijing, China).

2.6 Bioinformatics analyses

The microbial DNA extraction was carried out using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.). Nucleic acid quantifier NanoDrop2000 (Life Technologies,109 Carlsbad, CA, USA) was used to determine the concentration and purity of the extracted DNA, while its quality was assessed using 1% agarose gel electrophoresis. The DNA samples that meet the requirements were subjected to MiSeq sequencing. The bacterial 16S rRNA gene universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the original sequence. Pooled the PCR products with equal molar amounts from different samples. Sequencing libraries were generated using NEXTFLEX® Rapid DNA-Seq Kit. Used fastp software to control quality of raw sequences, and used FLASH software for splicing. Processed the DADA2 plug-in in the QIIME2 process to denoise the optimized sequence after quality control. Sequences after DADA2 denoising are often referred to as ASVs (Amplicon Sequence Variants). Follow-up

data analysis was performed using the free online platform of Majorbio Cloud Platform (Majorbio , 2022) from Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China).

2.7 Statistical analyses

Finished preliminary collation of data through EXCEL, the results were processed using R (Version 4.1.3, R Core Team, Vienna, Austria) via one-way ANOVA. The normality of the data was checked with "qqPlot" function in "car" package and the homogeneity of variance was checked by the "bartlett. test" function in "stat" package. "aov" function was subjected to perform ANOVA, used "Duncan. test" function in "agricolae" package to perform hoc comparison. All the results were presented as "mean & SEM" and the criterion for significant difference is "$P < 0.05$".

3 Results

3.1 Production performance & serum parameters

Effect of MIP on production performance and serum biochemistry indexes were given at Table 2 & 3, respectively. MIP and CYP increased ADG compare to NC group ($P = 0.407$) (Table 2).

Both MIP and CYP increased the level of GLU compared to NC group on day 28 ($P = 0.128$) and day 56 ($P = 0.446$); the level of TC in MI group were higher than CY and NC group, and there was significant difference between three groups on day 28 ($P = 0.049$); MIP enhanced TG level compared to NC and CY group on day 56 ($P = 0.555$); Concerning the level of UREA, MIP had the lowest level on day 56 ($P = 0.565$); two kinds of preparations had little effect on AcCoA and BHBA level and no significant difference was observed ($P > 0.05$). As for the effect of MIP on goat antioxidant parameter, MIP enhanced antioxidant indexes on day 28 and day 56, CYP increased them on day 28, nevertheless, CYP had a completely opposite effect on day 56 (Table 3).

3.2 Rumen fermentation

The pH and VFA are the main indexes of rumen fermentation (Table 4). MIP decreased the pH, CYP increased the pH compared to NC group. There was a significant difference of pH between MI group and CY group on day 28 ($P < 0.05$); the pH on day 56 was not affected significantly by treatment ($P = 0.110$). Both CY group and MI group had a higher TVFA level than

Table 2. Effect of probiotics preparations on production performance of Boer goats (g).

Items	Treatments[1]			SEM[3]	*P*-value
	NC	MI	CY		
ADG[2] (1 d-28 d)	19.29	50.00	67.14	17.869	0.577
ADG (29 d-56 d)	67.14	117.14	117.14	17.010	0.606
ADG (1 d-56 d)	86.43	167.14	184.29	30.566	0.407

[1]Treatments: NC (basal diet without probiotic preparation); MI (basal diet with MIP, 60 g/goat/day); CY (basal diet with CYP, 20 g/goat/day); [2]ADG: Average daily gain. [3]SEM: Standard error of the mean.

Table 3. Effect of probiotics preparations on serum biochemical indexes of Boer goats.

Items	Day	Treatments[1]			SEM	*P*-value
		NC	MI	CY		
GLU (mmol/L)[2]	28 d	0.24	0.93	0.61	0.145	0.128
	56 d	1.26	1.56	1.64	0.122	0.446
TC (mmol/L)[3]	28 d	1.43[b]	1.76[a]	1.61[ab]	0.057	0.049
	56 d	1.24	1.42	1.38	0.063	0.186
TG (mmol/L)[4]	28 d	0.43	0.40	0.56	0.045	0.315
	56 d	0.47	0.68	0.65	0.080	0.555
UREA (mmol/L)	28 d	7.29	7.88	8.01	0.253	0.498
	56 d	9.03	8.36	8.67	0.243	0.565
AcCoA (mmol/L)[5]	28 d	53.35	51.58	51.63	1.190	0.865
	56 d	62.15	59.80	61.11	1.348	0.746
BHBA (mmol/L)[6]	28 d	0.22	0.22	0.20	0.006	0.323
	56 d	0.24	0.23	0.21	0.007	0.236
MDA (nmol/mL)[7]	28 d	3.57	4.24	4.14	0.155	0.168
	56 d	4.35	4.43	4.28	0.387	0.950
SOD (U/mL)[8]	28 d	81.79	100.05	99.40	4.469	0.170
	56 d	78.82	88.79	74.96	3.263	0.212
T-AOC(U/mL)[9]	28 d	11.79	13.61	13.44	0.419	0.147
	56 d	10.75	10.95	10.05	0.318	0.509

[1]Treatments: NC (basal diet without probiotic preparation); MI (basal diet with MIP, 60 g/goat/day); CY (basal diet with CYP, 20 g/goat/day); [2]GLU: glucose; [3]TC: total cholesterol; [4]TG: triglyceride; [5]AcCoA: acetyl coenzyme A; [6]BHBA: Hydroxybutyric acid; Antioxidant capacity: [7]MDA: malonaldehyde; [8]SOD: superoxide dismutase; [9]T-AOC: total antioxidant capacity.

Table 4. Effect of probiotics preparations on rumen fermentation of Boer goats.

Items	Day	Treatments[1]			SEM	*P*-value
		NC	MI	CY		
pH	28 d	6.93[ab]	6.77[b]	7.14[a]	0.070	0.090
	56 d	6.80	6.50	6.80	0.067	0.110
Acetic acid (mmol/L)	28 d	48.67	49.26	50.29	2.711	0.975
	56 d	56.85	72.43	70.99	4.472	0.318
Propionic acid (mmol/L)	28 d	9.93	10.93	10.22	0.571	0.797
	56 d	12.19	13.91	14.64	0.836	0.515
Butyric acid (mmol/L)	28 d	6.66	10.06	8.80	0.720	0.147
	56 d	9.49	9.07	10.87	0.976	0.770
Isobutyric acid (mmol/L)	28 d	0.86	1.04	0.93	0.054	0.420
	56 d	0.77	0.69	0.86	0.051	0.447
Valeric aicd (mmol/L)	28 d	0.44	0.64	0.59	0.048	0.212
	56 d	0.55	0.53	0.67	0.050	0.527
Isovaleric aicd (mmol/L)	28 d	1.11	1.21	1.20	0.064	0.820
	56 d	0.84	0.73	0.95	0.065	0.434
TVFA[2] (mmol/L)	28 d	67.67	73.15	72.02	3.701	0.844
	56 d	80.69	97.34	98.96	5.862	0.408
Acetic acid /Propionic acid	28 d	4.86	4.57	4.98	0.158	0.604
	56 d	4.67	5.25	4.83	0.131	0.286

[1]Treatments: NC (basal diet without probiotic preparation); MI (basal diet with MIP, 60 g/goat/day); CY (basal diet with CYP, 20 g/goat/day); [2]TVFA: total volatile fatty acid. In the same row, values with different lower case letters mean significant difference ($P < 0.05$).

NC group on day 28 ($P = 0.844$) and day 56 ($P = 0.408$). There was no significant difference of each kind of VFAs between three groups ($P > 0.05$). As for acetic acid, MI and CY group had higher level than NC group on day 28 ($P = 0.975$) and day 56 ($P = 0.318$); propionic acid had the same trend with acetic acid; concerning the result of butyric acid, MIP and CYP made it higher on day 28 ($P = 0.147$); MIP made it lower, meanwhile CYP had the reverse result on day 56 ($P = 0.770$). As the results of isobutyric acid, valeric acid and isovaleric acid, they had the similar trend with butyrate (Table 4).

3.3 Rumen microflora

In the rumen fluid samples, 20 phyla, 36 classes, 84 orders, 138 families, 280 genera, 585 species, 6657 ASVs were found. Rarefaction curve was given at Figure 1. As the number of reads sampled increased, the rarefaction curve that based on sobs was nearly asymptotic, which supported that the amount of sequencing data was sufficient (Figures 1A and 1B).

Effect of different probiotics preparations on alpha biodiversity of rumen microbial community was shown at Table 5. No significant difference was observed among three groups ($P > 0.05$). With regards to Sobs, both MI and CY group had higher value than NC group on day 28 ($P = 0.212$) and day 56 ($P = 0.953$), the trend of Ace and Chao 1 level was same as Sobs. As for the change of Shannon and Shannoneven, both MI and CY had the higher value than NC group among which MI had the highest level on day 28 and day 56 (Table 5). Performed PCoA analysis to represent Beta biodiversity of rumen microbial community in different groups (Figure 2). Combination the analysis of ANOSIM, there was a significant separation of rumen microflora between three groups ($R = 0.2378$, $P = 0.013$) on day 28 (Figure 2A).

4 Discussion

4.1 Production performance & serum parameter

Blood glucose provides energy for animal organs, tissues and cells of animals that is the main source of energy (Chanjula et al., 2014). It can also be used to synthesize hepatic glycogen, fat, amino acids and other substances. There are two sources of glucose in the blood, one is the absorption of carbohydrates in the gastrointestinal tract, and the other is the decomposition of hepatic glycogen (Bedford et al., 2020). In current work, MIP enhanced the level of serum glucose that was consistent with previous studies (Liu et al., 2022; Ma et al., 2021). MIP increased TC and TG level in blood better contrast to CYP, based on Liu et al. (2022) study, the result of TC was similar, nonetheless, the result of TG was opposite (Liu et al., 2022). UREA can accurately reflect the protein metabolism and to a certain extent reflect kidney function (Schmidely & Bahloul, 2022). When the host intakes excessive protein, the unutilized protein will eventually be metabolized into nitrogen, resulting the waste of dietary nutrients. As the body is in a negative nitrogen balance, it will aggravate the decomposition of protein and amino acids, causing higher urea nitrogen content in blood (Zhang et al., 2020). In our study, MIP and CYP made UREA higher on day 28 contrast to NC group, as the trial progressed to day 56, MI and CY group had the lower level of UREA than NC group, among which MIP had the better effect, this is consistent with past research (Liu et al., 2022). AcCoA is an indispensable precursor for fatty acids synthesis, which is a key factor in energy metabolism (Metallo et al., 2011; Sofeo et al., 2019). BHBA is produced by the oxidation of butyric acid in rumen epithelial cells, BHBA, as a biomarker, is considered to act an indispensable role in

Table 5. Effect of probiotics preparations on alpha biodiversity of Boer goats' rumen microflora.

Items	Day	Treatments[1]			SEM	*P*-value
		NC	MI	CY		
Coverage (%)	28 d	99.22	98.58	99.16	0.203	0.395
	56 d	98.47	98.56	98.62	0.288	0.980
Sobs	28 d	569.80	775.00	591.40	51.576	0.212
	56 d	707.40	746.00	762.40	69.300	0.953
Ace	28 d	595.61	829.81	623.59	59.669	0.227
	56 d	770.37	808.00	815.35	81.620	0.975
Chao 1	28 d	597.74	826.82	620.87	59.065	0.230
	56 d	767.65	804.22	812.09	80.920	0.976
Shannon	28 d	5.33	5.95	5.55	0.126	0.115
	56 d	5.58	5.87	5.83	0.102	0.317
Shannoneven	28 d	0.84	0.90	0.87	0.014	0.165
	56 d	0.86	0.90	0.89	0.009	0.421
	56 d	0.88[b]	0.90[ab]	0.91[a]	0.006	0.016

[1]Treatments: NC (basal diet without probiotic preparation); MI (basal diet with MIP, 60 g/goat/day); CY (basal diet with CYP, 20 g/goat/day). In the same row, values with different lower case letters mean significant difference ($P < 0.05$).

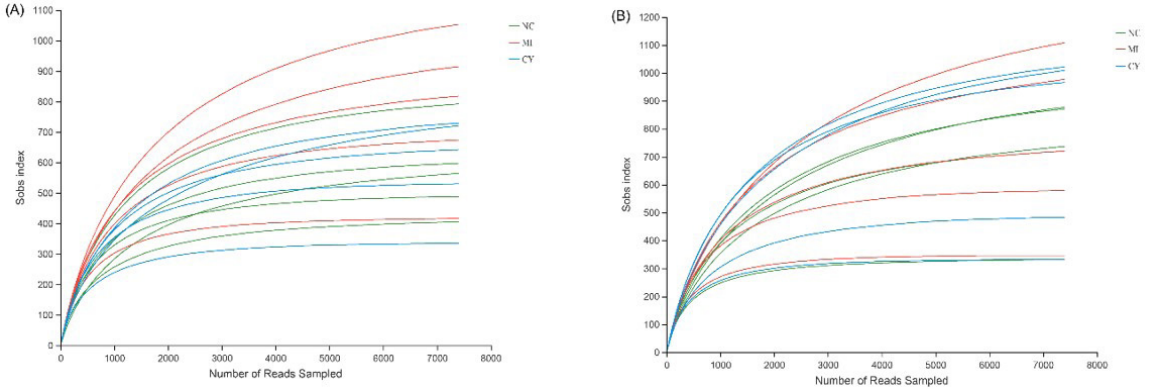

Figure 1. Rarefaction curve of rumen bacterial communities based on the 16S RNA gene sequences on 28 d (A) and 56 d (B).

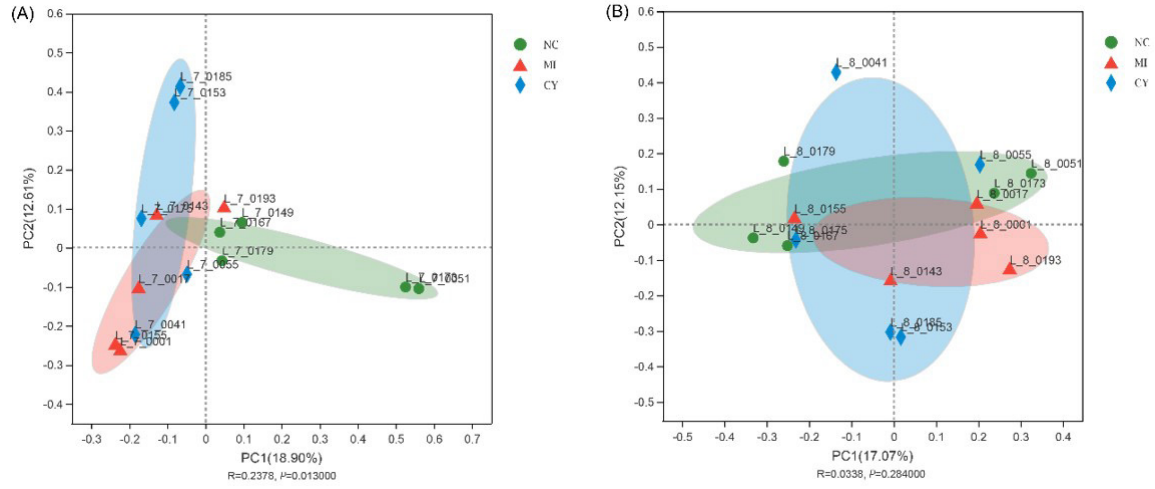

Figure 2. PCoA of rumen bacterial community in ASV level on 28 d (A) and 56 d (B).

the development of rumen epithelium metabolic functions (Elolimy et al., 2018; Abdelsattar et al., 2022).

Concerning to the antioxidant capacity. T-AOC is a comprehensive indicator reflecting the functional status of antioxidant system (Mao et al., 2019). SOD can catalyze the disproportionation reaction of superoxide ion free radicals, removing harmful substances such as free radicals generated by animal metabolism, which is an important symbol reflecting the antioxidant capacity of animals (Giorgio et al., 2020). MDA can attach polyunsaturated acids in biological membranes, which destroy the integrity of the cell membrane, consequently causing lipid peroxidation. Therefore, MDA content is an index reflecting lipid peroxidation in the body (Angrimani et al., 2019). Compare to CYP, MIP increased the antioxidant capacity, having positive effect on goats' body health (Jia et al., 2018).

4.2 Rumen fermentation

The pH of rumen fluid is mainly determined by the content and ratio of rumen VFA, rumen VFAs are produced by microbial fermentation of carbohydrates and are absorbed, utilized by animals as a source of energy (Del Bianco Benedeti et al., 2018; Gleason et al., 2022). pH is a key detector for ruminant health such as it could be indicator for subacute ruminal acidosis (SARA) (Mensching et al., 2020). The normal range for rumen fluid is 5.50 to 7.50, too high pH will affect the absorption of VFA by the rumen epithelium, while too low pH is not conducive to the feed fermentation in the rumen (Padilla, 2022). Similar result to Deng et al. (2018) research, we found MIP decreased the rumen pH, however, Chang et al. (2021) research result was quite the opposite (Deng et al., 2018; Chang et al., 2021), the addition of yeast cultures to high-concentration diet could enhance the pH value of rumen fluid and his result was consistent with Thrune et al. (2009) and Dias et al. (2018) works (Thrune et al., 2009; Dias et al., 2018).

4.3 Rumen microflora

Alpha diversity is subjected to investigate the community diversity within the samples. Sobs, chao, ace are the marks of community richness, these indexes in treatment group were higher than NC group that meant probiotic made community richness higher. Shannon index could reflect community diversity. Shannoneven is a symbol of community evenness, coverage could reflect the community coverage within the samples. In this work, MIP increased the rumen microbial richness, diversity that was consistent to Jia et al. (2018) study. Metagenomics has enabled the discovery of a large number of unculturable microorganisms, as well as more new functional genes or new genomes, greatly enhancing our understanding of the composition of their microbial communities. Biçer et al. (2021) performed the comparison of commercial and traditional kefir microbiota using metagenomic analysis, proving the latter has higher microbial diversity compared to former (Biçer et al., 2021). As for the microflora, Krokmach possesses specific and distinctive characteristics, therefore, Dimov (2022) conducted the study about analyzing the unusual microbiota of the traditional Bulgarian dairy product Krokmach via metagenomics study. The result demonstrated microbiota's composition was quite specific at species level---high content of *Exiguobacterium* (Dimov, 2022). Méndez-Romero et al. (2021) characterized the physicochemical and the microbiota composition of the artisanal Fresco cheese from Sonora, the result for characterization of microbiota illustrated more of 80 genera, of which the LAB as *Lactococcus*, *Streptococcus*, *Lactobacillus* and *Leuconostoc* were the most abundant may be considering for future items (Méndez-Romero et al., 2021). Based on these research, the high-throughput sequencing provides the access for us to identify the bacterial diversity deeply, supplying the standard for food quality control and safe consumption as well.

5 Conclusions

Goats received MIP treatments showed higher production performance. Both MIP and CYP enhanced level of serum parameters such as glucose, lipid metabolism and some other biochemical indexes, among which MIP performed better. MIP could enhance richness, diversity and evenness of rumen microflora, which meant probiotics preparations probably ameliorate rumen fermentation status and shift rumen microbial community.

Conflict of interest

No potential competing interest was reported by all authors.

Availability of data and material

All the datasets used and/or analyzed in this current work are available from 1-st author (Kaixin Yuan). The sequence data related to microbial community had been published on NCBI SRA (Accession: PRJNA863373).

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