Article

Prophylactic Feeding of Clostridium butyricum and Saccharomyces cerevisiae Were Advantageous in Resisting the Adverse Effects of Heat Stress on Rumen Fermentation and Growth Performance in Goats

Ligang Xue #1, Dan Wang #2, Fangyu Zhang #3 and Liyuan Cai #4,*

1 College of Animal Science and Technology, Jilin Agricultural Science and Technology University, Changchun 130118, China
2 College of Veterinary Medicine, Jilin Agricultural University, Changchun 130118, China
3 Institute of Animal Husbandry and Veterinary Medicine, Jilin Academy of Agricultural Sciences, Changchun 130033, China
4 Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
* Correspondence: dariacai@mail.hzau.edu.cn

Simple Summary: Heat stress occurs when goats are exposed to high environmental temperatures and humidity for a long period. Heat stress could adversely affect the rumen fermentation and growth performance of goats. Dietary supplementation with Clostridium butyricum, Saccharomyces cerevisiae, and their mixture were effective ways to alleviate the effects on the rumen fermentation and growth performance of heat-stressed goats. In this study, these two probiotics and their mixture were supplemented in goats for a period before heat stress. The results showed that these probiotics effectively alleviate the adverse effects of heat stress by promoting rumen fermentation and growth performance. Therefore, this study provides a reference for applying these two probiotics at the optimum timing to alleviate the adverse effects of heat stress on goats.

Abstract: This study aimed to investigate the effect of the prophylactic feeding of Clostridium butyricum (CB), Saccharomyces cerevisiae (SC), and their mixture before the onset of heat stress on the rumen fermentation and growth performance of goats, and subsequently, on heat stress status. Forty-eight male Macheng Black × Boer crossed goats (22.25 ± 4.26 kg) were divided into four groups—the control group (fed the basal diet), and the CB (0.05% CB added to the basal diet), SC (0.60% SC added to the basal diet), and Mix (0.05% CB and 0.60% SC added to the basal diet) groups—and fed for fourteen days. Then, these goats were kept in a heat stress environment (with a temperature–humidity index of 87.04) for fourteen days. Then, the parameters of rumen fermentation and growth performance were measured. The results showed that the pH values, the activities of cellulolytic enzymes (avicular, CMCase, cellobiase, and xylanase), and the concentrations of ammonia-N, total volatile fatty acid, acetic acid, propionic acid, and butyric acid were significantly increased (p < 0.05) in the rumens of the CB, SC, and Mix groups compared to those of the control group. Moreover, the average daily gain and the digestibility of dry matter, neutral detergent fiber, and acid detergent fiber were significantly increased (p < 0.05) in the CB, SC, and Mix groups compared to those of the control group. These results suggest that these two probiotics and their mixture effectively alleviate the adverse effects of heat stress on rumen fermentation and growth performance via prophylactic feeding.

Keywords: goats; prophylactic feeding; Clostridium butyricum; Saccharomyces cerevisiae; heat stress; rumen fermentation; growth performance
1. Introduction

With the prohibition of antibiotics in livestock production, probiotics are being used more widely and frequently in ruminant production to improve rumen fermentation, post-ruminal nutrient uptake, intestinal health, and performance [1–3]. There are three generally recognized action mechanisms for probiotics in ruminants: (1) When stress or disease cause a change in microecological balance in the gastrointestinal tract, increasing the abundance of harmful bacteria and decreasing the abundance of beneficial bacteria, thus affecting the growth performance of ruminants. When probiotics enter the gastrointestinal tract, they will compete with harmful bacteria in the gastrointestinal tract, inhibit the growth of pathogenic bacteria, and finally, form a stable and beneficial microecological environment [4]. (2) When probiotics enter the digestive system, a layer of the probiotic membrane will be formed on the gastrointestinal mucosa. This membrane can prevent many pathogenic bacteria from attaching to the gastrointestinal wall, prevent the absorption of toxic components, control harm caused by pathogens in the body, and maintain an excellent microecological balance [2]. (3) Most probiotics are anaerobic microbiota, and thus, they form an anaerobic environment. Thus, the number of pathogenic aerobic and facultative aerobic bacteria will decrease, making the beneficial flora become the dominant flora, thus promoting the growth of animals [5]. *Clostridium butyricum* is a strictly anaerobic endospore-forming Gram-positive butyric acid-producing bacterium and is a promising probiotic candidate [6,7]. *Saccharomyces cerevisiae* is a facultative anaerobe commonly used in ruminants [8]. These two probiotics all play a significant role in promoting rumen fermentation and improving growth performance [7,9,10].

Ruminants are the most heat-intolerant animals since their rumen fermentation produces a large amount of heat [11]. Heat stress has been shown to cause a decrease in the abundance of cellulolytic bacteria and increase the abundance of starch-decomposing bacteria [12]. Therefore, the rumen environment is disturbed, and the rumen fermentation capacity declines significantly; with a decrease in pH values, the concentration of ammonia-N, and volatile fatty acids (VFAs) increases in the rumen. Moreover, the digestibility of dry matter (DM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) has been shown to decrease, which eventually leads to a decline in the growth performance of ruminants [13–16].

Our previous study found that a diet with *Clostridium butyricum* and *Saccharomyces cerevisiae* during the heat stress period was beneficial for the rumen fermentation and growth performance of goats [7,10,17]. However, when a hot summer is coming, goat breeders cannot accurately judge the exact time of the occurrence of heat stress. Thus, probiotics cannot be used effectively to improve rumen fermentation and promote growth. Therefore, this study aimed to investigate the effect of the prophylactic feeding of *Clostridium butyricum*, *Saccharomyces cerevisiae*, and their mixture before the onset of heat stress for a period of time on the rumen fermentation and growth performance of these goats, and subsequently, on heat stress status.

2. Materials and Methods
2.1. Goats and Probiotic Feeding Experiment

This study was approved by the Animal Care and Use Committee of the Jilin Agricultural Science and Technology University (Approval code: 20221011) and carried out from June to August. Throughout this study, the goats were fed twice daily at 7:00 and 17:00 with a 1.30 kg basal diet and free access to water. The composition and nutritional levels of the diet were set according to Mo [18]. The forty-eight male Macheng Black × Boer crossed goats (22.25 ± 4.26 kg) aged 5.0 ± 1.0 months were divided into four groups: the control group (fed the basal diet), and the CB (0.05% *Clostridium butyricum* of DM concentration added to the basal diet), SC (0.6% *Saccharomyces cerevisiae* of DM concentration added to the basal diet), and Mix (0.05% *Clostridium butyricum* and 0.6% *Saccharomyces cerevisiae* of DM concentration added to the basal diet) groups. The commercial *Clostridium butyricum* product was provided by Huijia Biotechnology Co., Ltd. (Huzhou, China) with a $2.0 \times 10^{10}$ CFU/g live cell concentration. Angel Yeast Co., Ltd. (Yichang, China) pro-
vided the commercial *Saccharomyces cerevisiae* product with a $1.0 \times 10^8$ CFU/g live cell concentration. During the feeding experiment, these goats were kept in a temperature- and humidity-controlled room where the temperature, relative humidity, and temperature humidity index (THI) were $25.0 \pm 1.0 ^\circ C$, $62.0 \pm 1.5\%$, and $73.01 \pm 2.11$ for fourteen days (control period). Then, the temperature, relative humidity, and temperature humidity index (THI) of this room were altered to $33.2 \pm 1.20 ^\circ C$, $73.3 \pm 2.3\%$, and $86.8 \pm 0.8$, respectively, to ensure that the goats were in a heat stress thermal environment for fourteen days (heat stress period). All the goat groups were fed a basal diet during the whole heat stress period. The composition and nutritional levels of the diet are given in Table 1. Five grams of Cr$_2$O$_3$ were added to the diet from days 11 to 13. The procedure of the experiment is given in Figure 1.

**Table 1.** The diet compositions and nutritional levels (g/kg) of the basal diet of goats.

| Ingredient         | Content |
|--------------------|---------|
| Alfalfa            | 552     |
| Ground corn        | 274     |
| Soybean meal       | 74      |
| Wheat barn         | 63      |
| Ca$_2$HPO$_4$      | 7       |
| Premix *           | 10      |

| Nutrition Level    |         |
|--------------------|---------|
| Dry matter         | 946     |
| Organic matter     | 858     |
| Crude protein      | 177     |
| Neutral detergent fiber | 435   |
| Acid detergent fiber| 260     |
| Ca                 | 5.9     |
| P                  | 3.2     |

*Premix contained per kg: 20.70 g Mg, 0.50 g Fe, 1 g Mn, 2 g Zn, 43 mg Se, 47 mg I, 54 mg Co, 90,000 IU vitamin A, 17,000 IU vitamin D, 1750 IU vitamin E.*

---

**Figure 1.** Heat stress modeling and sampling process of goats. Goats in the blue box are in the control period, where the environmental temperature humidity index (THI) was 73.01, and goats were in this environment for 14 days. Goats in the red box mean them in the heat-stressed period, in which the environmental temperature humidity index (THI) was 86.80, and goats in this environment for 14 days.

### 2.2. Sampling and Measurements

Fresh fecal samples were collected from the rectum of all goats before feeding on days 12 to 14 during the heat stress period, and these samples were stored at $-20^\circ C$ for further analysis. Blood samples were collected from the jugular vein of all the goats in the morning (after 24 h of fasting) on day 14 of the heat stress period. The peripheral blood
lymphocytes were isolated from whole blood using a specific kit from Solarbio Science & Technology (Beijing, China) to determine the expression levels of heat shock protein 70 (HSP 70) genes. The primer sequences, as described by Cai et al. [10] and Chaidanya et al. [19], were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The details of these primers are given in Table 2.

Table 2. The details of primer sequences of HSP 70 genes.

| Gene   | Primer Sequence | Product Length | Annealing Temperature | GenBank Accession No. |
|--------|-----------------|----------------|-----------------------|----------------------|
| B-actin| F: TCTGGCACCAACACCTTCTAC  
R: TCTTCTCACGGGTGGCCCTTG | 102 | 60 | XM 018039831.1 |
| HSP 70 | F: TGGCTTTCACCCGATAACCGGAG  
R: GTGGTGATCAGCAGCAGGAAG | 167 | 60 | NM 001285703.1 |
| HSPA 1 | F: CGACCACGGAAACCAGGCAC  
R: CGGGTGCCAGACACTTGC | 151 | 60 | NM 005677146.3 |
| HSPA 6 | F: TCTGCGCAACAGGATAAA  
R: CGCCACACGACGAGTAC | 239 | 60 | NM_001314233.1 |
| HSPA 8 | F: ACCTTTATTACCCGCGTCC  
R: CTCTATTCAGTCTCCTCCATT | 203 | 60 | XM 018039831.1 |

TRIzol® Reagent (Life Technologies, CA, USA) was used to extract total RNA from the peripheral blood lymphocytes following the manufacturer’s instructions. Reverse transcription was performed using the Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer’s instructions. An SYBR RT-PCR Kit (Bio-Rad, Hercules, CA, USA) in conjunction with a real-time fluorescent quantitative PCR system (Life Technologies, Carlsbad, CA, USA) was used for the RT-PCR. The conditions of PCR reaction were 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 50 °C for 45 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Each sample was analyzed in triplicate, and the levels of relative expression were quantified using the $2^{-\Delta\Delta Ct}$ method [20]. For measurement of the concentration of cortisol, blood samples were centrifuged at 3000 rpm for 10 min to obtain serum. A cortisol assay kit provided by the Nanjing Jiancheng Bioengineering Institute (Nanjing, China) was used to measure the concentration of serum cortisol following the manufacturer’s instructions.

Rumen fluids were collected from all goats using a soft plastic stomach tube with a GM-0.33A vacuum pump (Jinteng, Tianjin, China) after feeding for 4 h in the morning on day 14 of the heat stress period. The values of pH and oxidation-reduction potential (ORP) were determined immediately using a pH meter and an ORP meter (Orion Technology Co., Ltd., Massachusetts, USA), respectively. Then, the rumen fluids and supernatants were collected. As described by Maitisaiyidi et al. [21], ammonia nitrogen (NH$_3$-N) was measured using an ultraviolet-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Yang et al. [22] described that volatile fatty acids (VFAs) were measured using gas chromatography. In brief, 1.0 mL of 25% (w/v) metaphosphoric acid was added to 0.20 mL of ruminal supernatant and centrifuged at 10,000 r/min for 10 min. Then, the liquid supernatant was injected into a 30 m × 0.53 mm × 1.00 um Chrompack CP-Wax 52 fused silica column in a gas chromatograph equipped with a Model 2010 flame ionization detector (Shimazu, Kyoto, Japan).

During the heat stress period, the amounts of feed given and left were recorded for each goat to calculate the daily matter intake (DMI), and the body weights were recorded at the start and end of this period to calculate the average daily gain (ADG). The dry matter (DM) was measured in the feed and feces according to method #930.15 in the AOAC [23], and the digestibility (%) of DM was calculated as: DM content in feedstuff—DM content in feces)/ DM content in feedstuff × 100; this was as described by Goering and Van Soest [24]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured in the feed and feces. The digestibility (%) of these two parameters was calculated as: NDF or ADF content in feedstuff—NDF or ADF content in feces)/NDF or ADF content in feedstuff × 100.
2.3. Statistical Analysis

The data were analyzed using the “stats” package in R studio (v.4.0.2) (Allaire, Wall Township, NJ, USA). To reveal significantly different physiological parameters, gene expression levels, and cortisol concentrations between the control and heat stress periods, a two-tailed Student’s t-test analysis was performed. To reveal the significantly different parameters among the probiotic-supplemented groups, two-way analysis of variance (ANOVA) tests followed by a post hoc Dunn test for multiple pairwise comparisons were performed. p values of less than 0.05 were considered statistically significant.

3. Results

3.1. Parameters for Evaluation of the Occurrence of Heat Stress in Goats

In the heat stress period, the goats’ skin temperature, respiratory rate, and pulse were significantly increased (p < 0.05); however, there were no significant differences (p > 0.05) in the rectal temperature of goats compared to the control period. The skin temperature, rectal temperature, respiratory rate, and pulse of the goats are given in Table 3.

Table 3. The skin temperature, rectal temperature, respiratory rate, and pulse of the goats between control and heat stress periods.

| Periode | Parameters | Control | Heat Stress | SEM | p Values |
|---------|------------|---------|-------------|-----|----------|
| Rectal temperature (°C) | 39.51<sup>a</sup> | 39.44<sup>b</sup> | 0.11 | 0.232 |
| Skin temperature (°C) | 33.11<sup>a</sup> | 36.54<sup>b</sup> | 1.23 | 0.047 |
| Pulse (beats/min) | 76.30<sup>a</sup> | 85.12<sup>b</sup> | 6.53 | 0.002 |
| Respiratory rate (breaths/min) | 25.43<sup>a</sup> | 33.1<sup>b</sup> | 4.25 | 0.032 |

The different lowercase superscripts in the rows indicate significant differences (p < 0.05); the same lowercase superscripts in the rows indicate no differences (p > 0.05).

To further confirm that the goats were experiencing heat stress, the HSP 70 genes in the rumen fluid and blood were determined. The expression of HSP 70 and HSPA 1 was significantly increased (p < 0.05; Figure 2A) in rumen fluid and blood, respectively; but there were no differences in the expression of HSPA 6 and HSPA 8 in the blood of goats between the control period and the heat stress period (p > 0.05; Figure 2A). Additionally, the cortisol concentrations were significantly increased in the serum of goats under heat stress compared to those in the control period (p < 0.05; Figure 2B).

Figure 2. The indicators to evaluate the occurrence of heat stress in goats. (A) The expression levels of Hsp 70 family member genes in blood and rumen fluids (n = 6). (B) The serum cortisol concentrations in goats in the control and heat stress periods (n = 12). Data are expressed as the means ± SEM. * p < 0.05 and ** p < 0.01, indicating significant differences between the control and heat stress periods.
3.2. Prophylactic Feeding of Probiotics Improved Rumen Environment and Enhanced Rumen Fermentation of Heat-Stressed Goats

The pH values were significantly increased in the rumen of the CB, SC, and Mix groups compared with that of the control group \((p < 0.05)\). Meanwhile, the ORP values were significantly increased in the rumen of the CB, SC, and Mix groups compared with that of the control group \((p < 0.05)\). The concentration of \(\text{NH}_3\)-N was significantly increased \((p < 0.05)\) in the CB, SC, and Mix groups compared with that of control group. Additionally, the concentrations of TVFA, acetic acid, propionic acid, and A/P ratio were significantly increased \((p < 0.05)\) in the CB, SC, and Mix groups compared with that of control group. The activities of rumen avicelase, CMCase, cellobiase, and xylanase in the CB, SC, and Mix groups were significantly increased \((p < 0.05)\) compared with those in the rumen of control group. Additionally, the activities of these four rumen enzymes in the Mix group were significantly higher than those in the CB and SC groups \((p < 0.05)\). The parameters of rumen fermentation and the activities of the ruminal cellulolytic enzymes of heat-stressed goats with probiotic supplements are shown in Table 4.

Table 4. The parameters of rumen fermentation and the activities of ruminal cellulolytic enzymes of heat-stressed goats with probiotic supplements.

| Parameters                          | Treatment       | SEM  | \(p\) Value |
|-------------------------------------|-----------------|------|-------------|
|                                    | Control | CB   | SC | Mix |                |
| pH                                 |          |      |    |     | <0.001 | <0.001 | <0.001 |
| ORP (mV)                           | −161.3   | −191.0 | −193.4 | −197.1 | 8.24   | 0.044 | <0.001 | 0.002 |
| \(\text{NH}_3\)-N (mg 100 mL\(^{-1}\)) | 9.17     | 10.89 | 12.23 | 13.81 | 1.47   | 0.041 | 0.021  | 0.041 |
| TVFA (mmol L\(^{-1}\))             | 32.84    | 51.22 | 52.68 | 52.98 | 6.45   | 0.043 | <0.001 | <0.001 |
| Acetic acid (mmol L\(^{-1}\))      | 14.38    | 24.12 | 24.77 | 25.59 | 3.08   | 0.009 | <0.001 | <0.001 |
| Propionic acid (mmol L\(^{-1}\))   | 10.08    | 15.22 | 16.24 | 15.73 | 3.44   | 0.004 | 0.023  | <0.001 |
| Butyric acid (mmol L\(^{-1}\))     | 8.38     | 11.90 | 11.67 | 11.69 | 2.68   | 0.044 | 0.040  | 0.056 |
| A/P ratio                           | 1.43     | 1.59  | 1.53  | 1.63  | 0.55   | 0.007 | 0.049  | 0.022 |
| Avicelase (IU mL\(^{-1}\))         | 1.30     | 1.56  | 1.61  | 1.81  | 0.02   | 0.040 | <0.001 | <0.001 |
| CMCase (IU mL\(^{-1}\))            | 1.34     | 2.51  | 2.58  | 3.12  | 0.01   | <0.001 | <0.001 | <0.001 |
| Cellobiase (IU mL\(^{-1}\))        | 2.44     | 4.43  | 4.51  | 4.73  | 0.05   | <0.001 | <0.001 | <0.001 |
| Xylanase (IU mL\(^{-1}\))          | 4.54     | 6.43  | 7.21  | 7.62  | 0.10   | <0.001 | <0.021 | 0.043 |

The different lowercase superscripts in the rows indicate significant differences \((p < 0.05)\); the same lowercase superscripts in the rows indicate no differences \((p > 0.05)\).

3.3. Prophylactic Feeding of Probiotics Improved Growth Performance of Heat-Stressed Goats

The DMI, ADG, and digestibility of DM, NDF, and ADF were significantly increased \((p < 0.05)\) in the CB, SC, and Mix groups compared with those of the control group. Additionally, the Mix group exhibited a more significant effect in enhancing these growth performance parameters. The parameters of the growth performance of heat-stressed goats with probiotic supplements are shown in Table 5.

Table 5. The parameters of growth performance in heat-stressed goats with probiotic supplementation.

| Parameters | Groups | SEM | \(p\) Value |
|------------|--------|-----|--------------|
|            | Control | CB  | SC | Mix |             |
| DMI (kg)   | 0.70    | 0.85 | 0.85 | 0.88 | 0.12        |
| ADG (kg)   | 0.08    | 0.15 | 0.17 | 0.19 | 0.04        |
| Digestibility of DM (%) | 51.57 | 59.48 | 60.64 | 63.34 | 6.63        |
| NDF (%)    | 39.23   | 47.40 | 50.31 | 51.02 | 3.09        |
| ADF (%)    | 35.28   | 45.30 | 48.60 | 49.29 | 3.47        |

The different lowercase superscripts in the rows indicate significant differences \((p < 0.05)\); the same lowercase superscripts in the rows indicate no differences \((p > 0.05)\).
4. Discussion

In the hot summer, heat stress is an inevitable issue of intensive goat production in the Jianghuai region of China [12,16]. Heat stress has adverse effects on rumen fermentation and the growth performance of ruminants [11,16,25]. Our previous studies showed that the parameters—including the pH value and the concentrations of NH$_3$-N, TVFA, acetic acid, propionic acid, butyric acid, and cellulolytic enzyme activity—was significantly decreased in heat-stressed goats. Moreover, the parameters of growth performance, including DMI, ADG, and the digestibility of DM, NDF, and ADF, were significantly decreased in heat-stressed goats [16]. Dietary supplementation with probiotics was a good way to alleviate the adverse effects of heat stress on livestock [26,27].

A prophylactic feeding strategy was applied in this study. Prophylactic feeding means supplementation with probiotics for a period of time before heat stress occurrence to investigate the effects of these probiotics on rumen fermentation and growth performance. This is closer to the practice of goat production. In this study, the pH values in the rumen were significantly increased with dietary supplementation with Clostridium butyricum, Saccharomyces cerevisiae, and their mixture. This result is consistent with previous studies that supplement cows with live yeast in the summer [7,10,28–30]. Few studies have investigated the effects of Clostridium butyricum on ruminal pH values. A previous study reported that supplemented calves, which were fed a 50% high-concentrate diet for one week with Clostridium butyricum at 1.5 or 3.0 g/100 kg body for five days, the 24 h mean ruminal pH value was increased [31]. This result suggests that these two probiotics could effectively resist the pH decrease caused by heat stress [16]. This function could be explained by the fact that that Clostridium butyricum and Saccharomyces cerevisiae could produce organic acids, vitamins, and other nutrient factors in the rumen, which could promote the activity of lactic acid-utilizing bacteria [32]. Additionally, yeast could promote the utilization of soluble sugars by gut microbiota, and then, reduce lactic acid production [30]. Therefore, these probiotics have an effect on rumen pH stabilizing. Moreover, the abundance of rumen protozoa may be enhanced by these two probiotics, which could also lower the ruminal lactic acid concentration [33]. However, previous studies also reported that supplementation with Saccharomyces cerevisiae has no effect on pH [34–37] or could decrease ruminal pH [36]. These differences could be due to the sources, strains, or doses, the timing of feeding of probiotics, the animal species, or their health status [12]. In this study, supplementation with Clostridium butyricum, Saccharomyces cerevisiae, and their mixture could enhance the production of VFAs, including TVFA, acetic acid, propionic acid, butyric acid. Previous studies have shown inconsistent results regarding the effects of Saccharomyces cerevisiae on VFAs. Previous studies reported that supplementation with Saccharomyces cerevisiae or active dry yeast could significantly increase the concentration of TVFA, acetic acid, propionic acid, butyric acid, and the A/P ratio in the rumen [7,38–43]. However, a previous study has shown that supplementation with Saccharomyces cerevisiae has no effect on VFA concentration in the rumen [44]. Until now, few studies have reported the effects of Clostridium butyricum on ruminal VFA production. It was reported that Clostridium butyricum improves the ruminal VFA production of heat-stressed goats in vitro and in vivo [7,10]. However, in calves, supplementation with Clostridium butyricum at 1.5 or 3.0 g/100 kg (body weight) did not affect ruminal VFA production [36]. In this study, supplementation with Clostridium butyricum and its mixture with Saccharomyces cerevisiae could enhance the concentration of TVFA, acetic acid, propionic acid, butyric acid, and the A/P ratio. The increased trends of VFA concentration due to the effects of these two probiotics promote the activities of ruminal fibrolytic bacteria [12,42,45]. In this study, supplementation of the A/P ratio was increased compared to that of the control, suggesting that probiotics may change in fermentation mode in the rumen [16]. The increase in the A/P ratio in this study is attributed more to the increased extent of acetic acid than that of propionic acid. One opinion has been held which states that the change in VFA concentration through supplementation with probiotics is temporary; once probiotic supplementation is terminated, this rising
effect disappears [7]. This problem should be taken into consideration in nutrition studies and the production practices of ruminants.

In this study, the DMI and ADG were significantly increased with dietary supplementation with Clostridium butyricum, Saccharomyces cerevisiae, and their mixture. This result is consistent with previous studies that state that supplementation with yeast at 0.2 g/day increased feed intake in the early lactation stage of dairy goats [46]. However, another previous study reported that there was no effect on DMI on cows in summer with Saccharomyces cerevisiae supplementation [28]. There are few studies that investigate the effect of Clostridium butyricum on DMI, especially in ruminants under heat stress. Moreover, our study shows that supplementation with Saccharomyces cerevisiae could increase ADG in heat-stressed goats. This result is consistent with previous studies that found that with Saccharomyces cerevisiae supplementation, the ADG increased both in cows and goats [7,47–49]. Similarly, Clostridium butyricum was found to have positive effects on the ADG of farm animals [7,10,50]. In this study, Clostridium butyricum, Saccharomyces cerevisiae, and their mixture have positive effects on the digestibility of DM, NDF, and ADF. These results were similar to previous studies that found that the digestibility of DM, NDF, and ADF in sheep and goats was improved through supplementation with Saccharomyces cerevisiae [7,36,44]. Previous studies scarcely investigated the effects of Clostridium butyricum on these parameters of digestibility. As a promising candidate for microbial additives [50], it is necessary to evaluate the effects of Clostridium butyricum on the digestibility of DM, NDF, and ADF. In this study, supplementation with Clostridium butyricum could promote the digestibility of DM, NDF, and ADF in heat-stressed goats.

These two probiotics can improve digestibility because they are good sources of vitamins, organic acids, and minerals, which promote the abundance of cellulolytic bacteria and fungi in the rumen and may improve fiber digestion [15,51].

Despite the fact that Saccharomyces cerevisiae and Clostridium butyricum can have positive effects on the growth performance of ruminants, their effects may vary in various studies. This discrepancy is due to factors such as differences in the composition of diets, the number of live cells of probiotics, supplement levels, and management strategies [51]. In this study, the activities of cellulolytic enzymes were significantly increased in probiotic-supplemented groups, which could be a reason for the improvements in the digestibility of DM, NDF, and ADF.

5. Conclusions

Prophylactic feeding with Saccharomyces cerevisiae, Clostridium butyricum, and their mixture could improve the rumen environment and fermentation by increasing pH values and VFA concentrations in the rumen. Moreover, the digestibility of DM, DNF, and ADF was increased by these probiotic supplements. Thereafter the growth performances were enhanced by increasing the DMI and ADG of heat-stressed goats. Therefore, in practical goat production, prophylactic supplementation with Saccharomyces cerevisiae and Clostridium butyricum can be an effective way to alleviate the adverse effects on rumen fermentation and growth performance in heat-stressed goats.

Author Contributions: Conceptualization, L.C. and L.X.; methodology, L.X.; software, L.X.; validation, D.W. and L.X.; formal analysis, D.W. and L.X.; investigation, L.C., D.W., F.Z. and L.X.; resources, L.X.; data curation, L.C. and L.X.; writing—original draft preparation, L.X.; writing—review and editing, L.X. and L.C.; visualization, L.X.; supervision, L.C.; project administration, L.X.; funding acquisition, L.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Jilin Provincial Department of Science and Technology (Grant No. 20220202062NC) and the Jilin Agricultural Science and Technology University (Grant No. 20217002).

Institutional Review Board Statement: This study was approved by the Animal Care and Use Committee of the Jilin Agricultural Science and Technology University (Approval code: 20221011).

Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.

Conflicts of Interest: There are no conflict of interest to disclose for this study.

References

1. Uyeno, Y.; Shigemori, S.; Shimosato, T. Effects of prebiotics/prebiotics in cattle health and productivity: Minireview. Microbes Environ. 2015, 30, 126–132. [CrossRef] [PubMed]

2. Nie, L.; Zhang, A.Z.; Jiang, N.; Yang, Z.N. Application of probiotics in the production of juvenile ruminants. Fed. Res. 2017, 9, 11–14.

3. Zapata, O.; Cervantes, A.; Barreras, A.; Monge-Navarro, F.; González-Vizcarra, V.M.; Estrada-Angula, A.; Urias-Estrada, J.; Corona, L.; Zinn, R.; Martínez-Alvarez, I.; et al. Effects of single or combined supplementation of probiotics and prebiotics on ruminal fermentation, ruminal bacteria and total tract digestion in lambs. Small Rumin. Res. 2021, 204, 106538. [CrossRef]

4. Chu, J.; Liu, K.C.; He, Q.X.; Han, L.W. Mechanisms of yeast in monogastric animals and ruminants. Vet. World 2009, 36, 14–17.

5. Wu, D. Studies on Compound Microbial Feed Additives. Master’s Thesis, Lanzhou Jiaotong University, Lanzhou, China, 2015.

6. Wang, R.Z.; Sun, Y.J.; Chen, W.; Huang, Y.Q.; Jiang, Z.H.; Yang, Q.F.; Meng, G.M.; Chen, X.P. Effects of Clostridium butyricum culture. J. Dairy Sci. 2009, 82, 257–260. [CrossRef]

7. Cai, L.Y.; Yu, J.K.; Hartanto, R.; Qi, D.S. Dietary supplementation with Saccharomyces cerevisiae, Clostridium butyricum and their combination ameliorate rumen fermentation and growth performance of heat-stressed goats. Animals 2021, 11, 2116. [CrossRef]

8. Retta, K.S. Role of probiotics in rumen fermentation and animal performance: A review. Int. J. Livestock Prod. 2016, 7, 24–32.

9. Miller-Webster, T.; Hoover, W.H.; Holt, M.; Nocek, J.E. Influence of yeast culture on ruminal microbial metabolism in continuous culture. J. Dairy Sci. 2002, 85, 2009–2014. [CrossRef]

10. Cai, L.Y.; Rudy, H.; Zhang, J.; Qi, D.S. Clostridium butyricum improves rumen fermentation and growth performance of heat-stressed goats in vitro and in vivo. Animals 2021, 11, 3216. [CrossRef]

11. Tajima, K.; Nonaka, I.; Higuchi, K.; Takusari, N.; Kurihara, M.; Takenaka, A.; Mitsumori, M.; Kajikawa, H.; Aminov, R.I. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 2007, 2, 57–64. [CrossRef]

12. Cai, L.Y.; Xu, J.K.; Zhang, J.; Qi, D.S. The effects of slatted floors and manure scraper systems on the concentrations and emission rates of ammonia, methane and carbon dioxide in goat buildings. Small Rumin. Res. 2015, 132, 103–110. [CrossRef]

13. Maloyi, G.M.O.; Kanui, T.I.; Towett, P.K.; Wambugua, S.N.; Miarona, J.O.; Wanyoike, M.M. Effects of dehydration and heat stress on food intake and dry matter digestibility in East African ruminants. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2008, 2, 185–190. [CrossRef] [PubMed]

14. Yadav, B. Impact of heat stress on rumen functions. Vet. World 2013, 6, 992–996. [CrossRef]

15. Chen, Y.Q. Application of yeast in dairy feed. Feed Res. 2011, 2, 22–24.

16. Cai, L.Y.; Yu, J.K.; Hartanto, R.; Zhang, J.C.; Yang, A.; Qi, D.S. Effects of heat challenge on growth performance, ruminal, blood and physiological parameters of Chinese crossbred goats. Small Rumin. Res. 2019, 174, 125–130. [CrossRef]

17. Cai, L.Y.; Hartanto, R.; Xu, Q.B.; Zhang, J.; Qi, D.S. Saccharomyces cerevisiae and Clostridium butyricum could improve b-vitamin production in the rumen and growth performance of heat-stressed goats. Metabolites 2022, 12, 766. [CrossRef] [PubMed]

18. Mo, F. Evaluation and Application of Nutritional Requirements of Ruminants and Nutritional Value of Feed; China Agricultural University Press: Beijing, China, 2011.

19. Chaidanya, K.; Soren, N.M.; Sejian, V.; Bagath, M.; Manjunathareddy, G.B.; Kurien, K.E.; Varma, G.; Bhatta, R. Impact of heat stress, nutritional stress and combined (heat and nutritional) stresses on rumen associated fermentation characteristics, histopathology and HSP70 gene expression in goats. J. Anim. Behav. Biometeorol. 2017, 5, 36–48. [CrossRef]

20. Ramakers, C.; Ruijter, J.M.; Deprez, R.H.; Moorman, A.F. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. 2003, 339, 62–66. [CrossRef]

21. Maitisayidi, T.; Yibureymu, A.; Yang, K. Determination of ammonia-nitrogen in ruminal fluid treated with methanol by alkaline hypochlorite-phenol spectrophotometry. Xinjiang Agric. Sci. 2012, 3, 565–570.

22. Yang, W.Z.; Beauchemin, K.A.; Rode, L.M. Effects of grain processing, forage to concentrate ratio, and forage particle size on rumen pH and digestion by dairy cows. J. Dairy Sci. 2001, 84, 203–216. [CrossRef]

23. AOAC. Official Methods of Analysis, 18th ed.; AOAC Int.: Gaithersburg, MD, USA, 2005.

24. Goering, H.K.; Van Soest, P.J. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications); USDA Agricultural Handbook: Washington, DC, USA, 1970; p. 379.

25. Uyeno, Y.; Sekiguchi, Y.; Tajima, K.; Takenaka, A.; Kurihar, M.; Kamagata, Y. An rRNA-based analysis for evaluating the effect of heat stress on the rumen microbial composition of Holstein heifers. Anaerobe 2010, 1, 27–33. [CrossRef] [PubMed]

26. Gan, F.; Ren, F.; Chen, X.X.; Lv, C.H.; Pan, C.L.; Ye, G.P.; Shi, J.; Shi, X.; Zhou, H.; Shituleni, S.A.; et al. Effects of selenium-enriched probiotics on heat shock protein mRNA levels in piglet under heat stress conditions. J. Agric. Food Chem. 2013, 61, 2385–2391. [CrossRef]

27. Shah, M.; Zaneb, H.; Masood, S.; Khan, R.U.; Mobashar, M.; Khan, I.; Din, S.; Khan, M.S.; Rehman, H.U.; Tinelli, A. Single or combined applications of zinc and multi-strain probiotic on intestinal histomorphology of broilers under cyclic heat stress. Probiotics Antimicrob. Proteins 2020, 12, 473–480. [CrossRef] [PubMed]
28. Bach, A.; Iglesias, C.; Devant, M. Daily ruminal pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. *Anim. Feed Sci. Technol.* 2007, 1, 146–153. [CrossRef]

29. Moallem, U.; Lehrer, H.; Livshitz, L.; Zachut, M.; Yakoby, S. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. *J. Dairy Sci.* 2009, 92, 343–351. [CrossRef] [PubMed]

30. Thrune, M.; Bach, A.; Ruiz-Moreno, M.; Stern, M.D.; Linn, J.G. Effects of *Saccharomyces cerevisiae* on ruminal pH and microbial fermentation in dairy cows: Yeast supplementation on rumen fermentation. *Livest. Sci.* 2009, 124, 261–265. [CrossRef]

31. Qadis, A.Q.; Goya, S.; Ikuta, K.; Yatsu, M.; Kimura, A.; Nakanishi, S.; Sato, S. Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of holstein calves. *J. Vet. Med. Sci.* 2004, 76, 877–885. [CrossRef] [PubMed]

32. Chaucheyras-Durand, F.; Walker, N.D.; Bach, A. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Anim. Feed Sci. Technol.* 2008, 145, 5–26. [CrossRef]

33. Galip, N. Effect of supplemental yeast culture and sodium bicarbonate on ruminal fermentation and blood variables in rams. *J. Anim. Physiol. Anim. Nutr.* 2006, 90, 446–452. [CrossRef]

34. Lascano, G.J.; Zanton, G.I.; Heinrichs, A.J. Concentrate levels and *Saccharomyces cerevisiae* affect rumen fluid-associated bacteria numbers in dairy heifers. *Livest. Sci.* 2009, 126, 189–194. [CrossRef]

35. El-Waziry, A.M.; Ibrahim, H.R. Effect of *Saccharomyces cerevisiae* on cell wall constituents digestion in sheep fed berseem (Trifolium alexandrinum) hay and cellulase activity. In Proceedings of the International Conference on the Arabian Oryx in the Arabian Peninsula, Riyadh, Saudi Arabia, 20–22 August 2007; p. 142.

36. Desnoyers, M.; Giger-Reverdin, S.; Bertin, G.; Duvaux-Pontier, C.; Sauvant, D. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk productivity of ruminant. *J. Dairy Sci.* 2009, 92, 1620–1632. [CrossRef]

37. Hossain, S.A.; Parnerkar, S.; Haque, N.; Gupta, R.S.; Kumar, D.; Tyagi, A.K. Influence of dietary Supplementation of live yeast (*Saccharomyces cerevisiae*) on nutrient utilization, ruminal and biochemical profiles of Kankrej calves. *Int. J. Appl. Anim. Sci.* 2012, 1, 30–38.

38. Oeztuerek, H. Effects of live and autoclaved yeast culture and sodium bicarbonate on ruminal fermentation in vitro. *J. Anim. Feed Sci.* 2009, 18, 142–150. [CrossRef]

39. Schingoethe, D.J.; Linke, K.N.; Kalscheur, K.F.; Hippen, A.R.; Rennich, D.R.; Yoon, I. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. *J. Dairy Sci.* 2004, 87, 4178–4181. [CrossRef]

40. Kˇrižova, L.; Richter, M.; Tˇrinacty, J.;ˇRiha, J.; Kumprechtová, D. The effect of feeding live yeast cultures on ruminal pH and redox potential in dry cows as continuously measured by a new wireless device. *Czech J. Anim. Sci.* 2011, 56, 37–45. [CrossRef]

41. Stella, A.V.; Paratte, R.; Valnegri, L.; Cigalino, G.; Soncini, G.; Chevaux, E.; Dell’Orto, V.; Savoini, G. Effect of administration of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminant. *J. Dairy Sci.* 2009, 92, 343–351. [CrossRef] [PubMed]

42. Kawas, J.; García-Castillo, R.; Garza-Cazares, F.; Fimbres-Durazo, H.; Olivares-Saenz, E.; Hernández-Vidal, G.; Lu, C. Effects of sodium bicarbonate and yeast on productive performance and carcass characteristics of light-weight lambs fed finishing diets. *Small Rumin. Res.* 2007, 67, 157–163. [CrossRef]

43. Yang, C.M.; Cao, G.T.; Ferket, R.R.; Liu, T.T.; Zhou, L.; Zhang, L.; Xiao, Y.P.; Chen, A.G. Effects of probiotic, *Clostridium Butyricum* on growth performance, immune function, and cecal microflora in broiler chickens. * Poult. Sci.* 2012, 91, 2121–2129. [CrossRef] [PubMed]

44. Schingoethe, D.J.; Linke, K.N.; Kalscheur, K.F.; Hippen, A.R.; Rennich, D.R.; Yoon, I. Feed efciency of mid-lactation dairy cows fed yeast culture during summer. *J. Dairy Sci.* 2004, 87, 4178–4181. [CrossRef]

45. Haddad, G.; Goussous, S.N. Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. *Anim. Feed Sci. Technol.* 2005, 118, 342–348. [CrossRef]

46. Lesmeister, K.E.; Heinrichs, A.J. The effect of feeding live yeast cultures on ruminal pH and redox potential in dry cows as continuously measured by a new wireless device. *Czech J. Anim. Sci.* 2011, 56, 37–45. [CrossRef]

47. Krijt, L.; Richter, M.; Trinacty, J.; Riha, J.; Kumprechtová, D. The effect of feeding live yeast cultures on ruminal pH and redox potential in dry cows as continuously measured by a new wireless device. *Czech J. Anim. Sci.* 2011, 56, 37–45. [CrossRef]

48. Kawas, J.; García-Castillo, R.; Garza-Cazares, F.; Fimbres-Durazo, H.; Olivares-Saenz, E.; Hernández-Vidal, G.; Lu, C. Effects of sodium bicarbonate and yeast on productive performance and carcass characteristics of light-weight lambs fed finishing diets. *Small Rumin. Res.* 2007, 67, 157–163. [CrossRef]

49. Yang, C.M.; Cao, G.T.; Ferket, R.R.; Liu, T.T.; Zhou, L.; Zhang, L.; Xiao, Y.P.; Chen, A.G. Effects of probiotic, *Clostridium Butyricum* on growth performance, immune function, and cecal microflora in broiler chickens. * Poult. Sci.* 2012, 91, 2121–2129. [CrossRef] [PubMed]

50. Yi, Z.H. Advances in research and application of probiotic *Clostridium butyrate*. *Feed Res.* 2012, 2, 4–17.

51. Soren, N.M.; Tripathi, M.K.; Bhatt, R.S.; Karim, S.A. Effect of yeast supplementation on the growth performance of Malpura lambs. *Trop. Anim. Health Prod.* 2013, 45, 547–554. [CrossRef]