**Article**

*Saccharomyces cerevisiae* and *Clostridium butyricum* are Beneficial for Rumen Fermentation and Growth Performance of Heat-Stressed Goats

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**Simple Summary:** Supplementation with probiotics is one of the most effective ways to alleviate heat stress of livestock. Here, we found that the rumen fermentation and the growth performance of heat-stressed goats were improved by *Saccharomyces cerevisiae*, *Clostridium butyricum*, and their combination. Moreover, the *Clostridium butyricum* was used innovatively in heat-stressed goats, and it played a role in relieving the adverse effects of heat stress in rumen fermentation and growth performance of goats. Therefore, this study provides a reference for future studies related to applying the *Clostridium butyricum* and *Saccharomyces cerevisiae* for ruminants during heat stress.

**Abstract:** Heat stress had adverse effects on rumen fermentation and growth performance of goats. This study was to evaluate the effects of *Saccharomyces cerevisiae* (SC), *Clostridium butyricum* (CB) and their combination on rumen fermentation and growth performance of heat-stressed goats. Probiotics treatment were control (with no probiotics), 0.60% SC, 0.05% CB, and 0.60% SC + 0.05% CB (CG, SC, CB, and COM), respectively. Heat-stressed goats (n = 12, 20.21 ± 2.30 kg) were assigned to a 4 × 3 incomplete Latin square study. The dry matter intake (DMI) and body weight of goats were recorded daily. And the rumen contents and feces were collected for fermentation parameters and feed digestibility analysis, respectively. The rumen pH; rumen cellulolytic enzyme (avicelase, CMCaes, celllobiase, and xylanase) activities; the concentrations of rumen total volatile fatty acid, acetic acid, propionic acid, and acetic acid to propionic acid ratio; the DMI, average daily gain (ADG), and the digestibility of dry matter (DM), neutral detergent fiber (NDF), and acidic detergent fiber (ADF) were significantly increased (p < 0.05); while the rumen oxidation-reduction potential (ORP) was significantly decreased with SC, CB and their combination supplementation (p < 0.05) compared with that of CG. These results indicated that the supplementation with these probiotics were beneficial for rumen fermentation and growth performance of heat-stressed goats.

**Keywords:** Goats; Heat stress; *Saccharomyces cerevisiae*; *Clostridium butyricum*; Rumen fermentation; Growth performance

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**1. Introduction**

Ruminants exhibit low tolerance to heat because rumen fermentation produces large amounts of heat[1]. When heat stress occurs, a series of adverse effects include re-
duction in rumen pH, changing the composition and number of rumen microbiota, lowering the production of rumen volatile fatty acid (TVFA), altering the digestibility of nutrients, causing oxidative stress[1-5]. Finally, it has a negative impact on the production performance of goats and brings economic loss to the goat breeding industry[6].

Probiotics have been widely used both in ruminants and non-ruminants to improve feed digestion, performance, and health status[7]. Yeast is one of the probiotic which commonly used in ruminant nutrition research and production. Because its activities consume oxygen and thereby create an oxygen-free environment in the rumen, which is beneficial for the growth and reproduction of rumen anaerobic microbes, especially the majority of cellulyotic bacteria appear to be more useful in manipulating rumen fermentation[8,9]. It is indicated that the concentration of NH₃-N, TVFA, and DM, NDF, and ADF degradability were increased significantly in rumen fluid by supplementing with SC [10]. Although yeast has been widely studied and applied, few studies have focused on heat-stressed ruminants. It was reported that feeding cows active dry yeast reduced the rectal temperature of the cows and prolonged peak milk production during heat stress [11]. Furthermore, feeding an SC culture to mid-lactation dairy cows during the summer had no effects on the yield of energy-corrected milk and DMI, but it did improve feed efficiency [12]. CB is a strictly anaerobic endospore-forming gram-positive butyric acid-producing bacterium and is a good probiotic resource [13]. Most previous studies on CB have focused on its functions in monogastric animals and poultry, but few studies have focused on ruminants. A previous study showed that CB could improve the production performance of weaned piglets and chickens [14,15]. It has been reported that with an addition of 2.5×10⁶ CFU/kg, the average daily gain and feed conversion ratio were significantly increased in weaned piglets [16]. In poultry production, CB not only improved chicken production performance but also improved the fertilization rate of eggs[13]. In a study on the effects of CB on the production performance of ducks, it was found that with a diet containing 0.2% CB, the egg-laying rate increased by 28.58%, and the fertilization rate increased by 4.10%[14,17]. For ruminants, CB has the potential to improve rumen fermentation and degradability, possibly through their metabolites, which would increase the number of rumen bacteria [18]. Until now, there have been few available studies on rumen fermentation resulting from the addition of CB.

In this study, it was hypothesized that feeding SC and CB to heat-stressed goats would improve their rumen fermentation and growth performance. The objectives of this study were to evaluate the effects of SC, CB and their combination on rumen fermentation in heat-stressed Chinese crossbred goats. This study will provide a scientific reference for alleviating the adverse effects of heat stress on rumen fermentation and the growth performance of goats.

2. Materials and Methods

2.1. Animals, diet, and management

Twelve female Macheng black × Boer crossbred goats from Boda Animal Husbandry Science and Technology Development Co. Ltd (Hefei City, Anhui Province, China) were used in this study. These goats aged 6.0 ± 1.0 months with a body weight of 20.21 ± 2.30 kg were kept in natural ventilation housed and individual pens. And goats were fed twice a day at 8:00 and 17:00, and had free access to water. The ingredients and nutritional composition of the diet are given in table 1. Vaccination and other prophylactic measures were described by Vatta et al. [19]. This study was conducted from June to September and was approved by the Animal Care and Use Committee of Huazhong Agricultural University (Approval code: HZAUGO-2015-007; Approval date: January 20, 2015)

| Table 1 Ingredients and nutrition (g/kg) of the basic diet fed to the goats |
### Nutrition Level

- **Dry matter**: 951
- **Organic matter**: 854
- **Crude protein**: 173
- **Neutral detergent fibre**: 434
- **Acid detergent fibre**: 257
- **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, 1,750 IU vitamin E.

#### 2.2. Probiotics feeding experimental design

The modeling processes of heat-stressed goats were described by Cai et al. [5]. Twelve heat-stressed goats were divided into four groups in a 4 × 3 incomplete Latin square design. The treatments were as follows: Control group (no probiotics; CG); *Saccharomyces cerevisiae* supplement group (0.6% DM; SC); *Clostridium butyricum* supplement group (0.05% DM; CB); combination group (0.6% DM SC combine with 0.05% DM CB; COM). The SC was obtained from Angel Yeast Co., Ltd. (Yichang, China) and had a content of 2.0×10^10 CFU/g. CB live cell product was obtained from Huijia Biotechnology Co., Ltd. (Huzhou, China) at 1.0×10^8 CFU/g. Three experimental cycles were applied in this study (3 goats were assigned to a control, to which no probiotics were provided throughout the study. The remaining nine goats were randomly assigned to three treatment groups in each experimental cycle). The grouping is described in Table 2.

#### Table 2  4×3 incomplete Latin-Square design of the experiment

<table>
<thead>
<tr>
<th>Groups</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>basal diet</td>
<td>basal diet</td>
<td>basal diet</td>
</tr>
<tr>
<td>T1</td>
<td>basal diet + SC</td>
<td>basal diet + CB</td>
<td>basal diet + combination</td>
</tr>
<tr>
<td>T2</td>
<td>basal diet + CB</td>
<td>basal diet + combination</td>
<td>basal diet + SC</td>
</tr>
<tr>
<td>T3</td>
<td>basal diet + combination</td>
<td>basal diet + SC</td>
<td>basal diet + CB</td>
</tr>
</tbody>
</table>

P1 – P3 represent the experimental cycle; SC, SB, and combination represent *Saccharomyces cerevisiae*, *Clostridium butyricum*, and their combination, respectively.

Each experimental cycle was last for 20 days. In each experimental cycle, 5 g of external marker (chromic oxide, Cr₂O₃) was provided in the diet in two equal proportions at 8:00 and 17:00 on days 17 to 19 to determine of digestibility. Between experimental cycles, all of the goats were fed a basal diet for 15 days to eliminate the influence of previous probiotic treatment and prepare for the next experimental cycle. Rumen fluid was
collected on the last day of each experimental cycle and the methods of rumen fluid collection and pretreatment were described by Cai et al.[5]. In brief, rumen fluid was collected on the last day of each experimental cycle by a flexible stomach tube with a vacuum pump (Jin Teng GM-0.33A, Tianjin, China) 4 h after morning feed. Next, the rumen fluid was strained through four layers of gauze to remove big feed particles and then transferred the filtrate to CO2-containing bottles to maintain anaerobic conditions. The filtrate was immediately stored at -20°C for further analysis. Fecal samples were collected from the rectum of each goat before the morning and afternoon feedings at the last three days of each experimental cycle. And fecal samples from the same treatment were pooled and stored at -20°C for further analysis.

2.3. Measurements

The pH and oxidation-reduction potential (ORP) values were measured immediately after the rumen fluid was collected. Rumen fluid was centrifuged at 12,000 ×g for 15 min at 4°C. And the supernatants were used for ammonia nitrogen (NH₃-N) and volatile fatty acids (VFA) analysis. NH₃-N concentration was determined using spectrophotometry as described by Maitisaiyidi et al. [20]. The VFA concentration was determined by the gas chromatography described by Yang et al. [21]. In brief, 0.20 ml supernatant was added to 1.00 ml 25% (w/v) metaphosphoric acid and centrifuged at 10000 r/min for 10 min. Then the supernatant was injected into Chrompack CP-Wax 52 fused silica column (30m × 0.53mm × 1.00 μm) of gas chromatography equipped with flame ionization detector (Model 2010, Shimazu, Japan). The activities of avicelase, hydrolytic enzyme (CMCase), cellobiase, and xylanase in rumen fluid were determined as described by Wang and Wang [22]. DMI and body weight of heat-stressed goats was measured within each experimental cycle. DMI was calculated by subtracting the weight of food refused from the weight of that offered on the previous day. The body weight of goats was measured by an electronic weighing balance (PS-2000 Platform Scale, Salter Brecknell, Fairmont, MN, USA) in the morning before offering feed and water. The body weights were recorded at the start and end of each experimental cycle for ADG calculations. Feed and fecal samples were analyzed as described by the AOAC [23] official methods for DM (method # 930-15). NDF and ADF were determined as described by Zhang et al. [24]. All analyses were carried out in triplicate to ensure the accuracy of the test results.

2.4. Statistical analysis

Microsoft Excel 2016 was used for data collection and to calculate average values and standard errors. All the data, including rumen fermentation data, activities of enzymes, and animal growth performance were analyzed in R packages (v4.0.5) with two-way analysis of variance (ANOVA) tests followed by post-hoc Dunn test for each significant factor or interaction. P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Rumen fermentation parameters of heat-stressed goats with with probiotics supplement

The rumen pH was significantly increased, while the rumen ORP was significantly decreased in SC, CB, and COM than that of the CG (p < 0.05). The concentrations of NH₃-N in CB was significantly higher (p < 0.05) than that of CG, SC, and COM. The concentrations of TVFA, acetic acid, propionic acid, and A/P ratio were significantly increased (p < 0.05) in SC and CB compared with that of CG and COM. The activities of rumen avicelase, CMCase, cellobiase, and xylanase in SC, CB, and COM were significantly increased (p < 0.05) compared with that in rumen of CG. The activities of rumen CMCase and xylanase in CB was significantly higher than that of SC and COM (p < 0.05), respectively. Ruminal fermentation parameters and the of ruminal cellulytic enzyme activities of heat-stressed goats with probiotics supplement are shown in Table 3.
Table 3  Ruminal fermentation parameters and the of ruminal cellulolytic enzyme activities of heat-stressed goats with probiotics supplementation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>P value</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
<td>SC</td>
<td>CB</td>
<td>COM</td>
<td>SEM</td>
<td>SC</td>
</tr>
<tr>
<td>pH</td>
<td>6.58a</td>
<td>6.72b</td>
<td>6.70b</td>
<td>6.73b</td>
<td>0.04</td>
<td>&lt;</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>-161.3a</td>
<td>-171.0b</td>
<td>-183.4b</td>
<td>-177.1b</td>
<td>7.13</td>
<td>0.045</td>
</tr>
<tr>
<td>NH3-N (mg 100 mL⁻¹)</td>
<td>9.20a</td>
<td>10.87ab</td>
<td>12.12b</td>
<td>9.81a</td>
<td>0.57</td>
<td>0.25</td>
</tr>
<tr>
<td>TVFA (mmol L⁻¹)</td>
<td>45.84a</td>
<td>54.13b</td>
<td>65.71b</td>
<td>47.00b</td>
<td>3.16</td>
<td>&lt;</td>
</tr>
<tr>
<td>Acetic acid (mmol L⁻¹)</td>
<td>19.38a</td>
<td>28.12b</td>
<td>30.77b</td>
<td>21.59b</td>
<td>2.78</td>
<td>0.007</td>
</tr>
<tr>
<td>Propionic acid (mmol L⁻¹)</td>
<td>14.08a</td>
<td>18.2b</td>
<td>20.27b</td>
<td>13.72a</td>
<td>1.64</td>
<td>0.002</td>
</tr>
<tr>
<td>Butyric acid (mmol L⁻¹)</td>
<td>12.38</td>
<td>12.80</td>
<td>14.67</td>
<td>11.69</td>
<td>1.66</td>
<td>0.061</td>
</tr>
<tr>
<td>A/P ratio</td>
<td>1.38a</td>
<td>2.13b</td>
<td>1.57b</td>
<td>1.52a</td>
<td>0.81</td>
<td>0.008</td>
</tr>
<tr>
<td>Avicelase (IU mL⁻¹)</td>
<td>1.31a</td>
<td>1.55b</td>
<td>1.82b</td>
<td>1.61b</td>
<td>0.02</td>
<td>0.050</td>
</tr>
<tr>
<td>CMCaes (IU mL⁻¹)</td>
<td>1.36a</td>
<td>2.58b</td>
<td>3.11c</td>
<td>2.57b</td>
<td>0.01</td>
<td>&lt;</td>
</tr>
<tr>
<td>Cellobiase (IU mL⁻¹)</td>
<td>2.44a</td>
<td>4.46b</td>
<td>4.71b</td>
<td>4.53b</td>
<td>0.05</td>
<td>&lt;</td>
</tr>
<tr>
<td>Xylanase (IU mL⁻¹)</td>
<td>4.54a</td>
<td>6.40b</td>
<td>7.31c</td>
<td>5.62b</td>
<td>0.10</td>
<td>&lt;</td>
</tr>
</tbody>
</table>

The data listed in the table are mean and SEM; a, b Means within a row with different superscripts letters differ significantly (p < 0.05); with no and the same superscripts letters indicate no significant difference (p > 0.05) in same row.

3.2. Growth performance of heat-stressed goats with with probiotics supplement

The DMI, ADG, DM, NDF, and ADF digestibilities were significantly increased (p < 0.05) in SC, CB, and COM compared with that of CG. The DMI and DM digestibility in CB was significantly higher than that of SC and COM (p < 0.05), respectively. The growth performance parameters of heat-stressed goats with probiotics supplement are shown in Table 4.

Table 4  The growth performance parameters of heat-stressed goats with probiotics supplementation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>P value</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
<td>SC</td>
<td>CB</td>
<td>COM</td>
<td>SEM</td>
<td>SC</td>
<td>CB</td>
</tr>
<tr>
<td>DMI (kg)</td>
<td>0.79a</td>
<td>0.84b</td>
<td>0.87c</td>
<td>0.84b</td>
<td>0.04</td>
<td>0.005</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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Supplementation with probiotics was one of the efficient ways to alleviate the adverse effects of heat stress. A previous study reported that supplementation with yeast resulted in a higher minimum, mean, and maximum rumen pH than that of the control in cows [25, 26]. In this study, the rumen pH was increased with SC, CB, and their combination supplementation, and this result is supported by the results of previous studies that the rumen pH of cows was enhanced with live yeast supplementation during the hot season [27]. These results suggested that SC was effective in alleviating the pH depression that was caused by heat stress [5]. In contrast, several studies reported that supplementation with SC could decrease rumen pH [28] or have no effect on the pH of SC [29, 30]. This may occur due to the differences in the SC source and strains. Few studies have investigated the effects of CB on rumen fermentation. It is reported that calves were adapted to a 50% high-concentrate diet for 1 week, and then CB was given to the calves once daily for 5 days at 1.5 or 3.0 g/100 kg body weight. As a result, both doses of CB improved the reduction in the 24 h mean ruminal pH in the calves. In this study, feeding heat-stressed goats CB or the combination increased the rumen pH of heat-stressed goats. The stabilizing/increasing effect of probiotics on rumen pH might be due to the effects of the probiotic on stabilizing the predominant rumen bacteria, which consume more lactate than do other microbiiota in the rumen [31]. Moreover, probiotics can promote the abundance of rumen protozoa, which could regulate lactic acid concentration [32]. The rumen ORP reflects the activity of the microbiiota and the amounts of reducing substances. It provides another insight into fermentation processes in the rumen and the mechanisms involved in the stabilization of rumen pH. It has been reported that supplementation with SC at a concentration of 1.3 mg·mL⁻¹ increased the oxygen disappearance rate by 46%–89% and decreased the rumen ORP of sheep [33]. Live yeast has been reported to be a balancer of rumen fluid ORP and effective in reducing rumen ORP [34]. In this study, supplemented probiotics could significantly decrease the rumen ORP in the rumen. This result is consistent with previous studies. These results may be attributed to the SC and CB were able to consume oxygen in the rumen and on the surface feedstuffs, which created a more oxygen-deprived environment. The results of this study suggest that SC, CB and COM may be beneficial for alleviating or removing the adverse effects of heat stress on rumen ORP. Previous studies reported that with yeast supplementation, the NH₃-N concentration increased in the rumen [9, 28, 35]. This result is consistent with that in this study. The increase in the NH₃-N concentration following probiotic supplementation may be attributed to the roles in promoting rumen microbiiota to degrade and hydrolyze protein and non-protein nitrogen in the rumen [35]. Studies also showed that yeast had no effect on the NH₃-N concentration in the rumen of cows [26]. The reasons for the different results could be due to variations in the feeding system, species, age and physiological state of the ruminants, frequency of feed-

<table>
<thead>
<tr>
<th></th>
<th>0.08&lt;sup&gt;a&lt;/sup&gt;</th>
<th>0.19&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.01</th>
<th>0.040</th>
<th>&lt;0.001</th>
<th>0.004</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG (kg)</td>
<td>DM (%)</td>
<td>50.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.63</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>38.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.13</td>
<td>52.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.59</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>37.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.06</td>
<td>49.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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4. Discussion

Supplementation with probiotics was one of the efficient ways to alleviate the adverse effects of heat stress. A previous study reported that supplementation with yeast resulted in a higher minimum, mean, and maximum rumen pH than that of the control in cows [25, 26]. In this study, the rumen pH was increased with SC, CB, and their combination supplementation, and this result is supported by the results of previous studies that the rumen pH of cows was enhanced with live yeast supplementation during the hot season [27]. These results suggested that SC was effective in alleviating the pH depression that was caused by heat stress [5]. In contrast, several studies reported that supplementation with SC could decrease rumen pH [28] or have no effect on the pH of SC [29, 30]. This may occur due to the differences in the SC source and strains. Few studies have investigated the effects of CB on rumen fermentation. It is reported that calves were adapted to a 50% high-concentrate diet for 1 week, and then CB was given to the calves once daily for 5 days at 1.5 or 3.0 g/100 kg body weight. As a result, both doses of CB improved the reduction in the 24 h mean ruminal pH in the calves. In this study, feeding heat-stressed goats CB or the combination increased the rumen pH of heat-stressed goats. The stabilizing/increasing effect of probiotics on rumen pH might be due to the effects of the probiotic on stabilizing the predominant rumen bacteria, which consume more lactate than do other microbiiota in the rumen [31]. Moreover, probiotics can promote the abundance of rumen protozoa, which could regulate lactic acid concentration [32]. The rumen ORP reflects the activity of the microbiiota and the amounts of reducing substances. It provides another insight into fermentation processes in the rumen and the mechanisms involved in the stabilization of rumen pH. It has been reported that supplementation with SC at a concentration of 1.3 mg·mL⁻¹ increased the oxygen disappearance rate by 46%–89% and decreased the rumen ORP of sheep [33]. Live yeast has been reported to be a balancer of rumen fluid ORP and effective in reducing rumen ORP [34]. In this study, supplemented probiotics could significantly decrease the rumen ORP in the rumen. This result is consistent with previous studies. These results may be attributed to the SC and CB were able to consume oxygen in the rumen and on the surface feedstuffs, which created a more oxygen-deprived environment. The results of this study suggest that SC, CB and COM may be beneficial for alleviating or removing the adverse effects of heat stress on rumen ORP. Previous studies reported that with yeast supplementation, the NH₃-N concentration increased in the rumen [9, 28, 35]. This result is consistent with that in this study. The increase in the NH₃-N concentration following probiotic supplementation may be attributed to the roles in promoting rumen microbiiota to degrade and hydrolyze protein and non-protein nitrogen in the rumen [35]. Studies also showed that yeast had no effect on the NH₃-N concentration in the rumen of cows [26]. The reasons for the different results could be due to variations in the feeding system, species, age and physiological state of the ruminants, frequency of feed-
ing, dose of yeast and its type, nutrient composition of the diets, and environmental conditions. In this study, supplementation of CB or the combination also caused the concentration of NH₃-N to increase significantly in the rumen of heat-stressed goats. The mechanism of the effect on N metabolism in rumen may be similar to that of SC, but few studies have been conducted on CB, which needs to be further confirmed. The results of previous studies on the effects of SC on rumen TVFA have been inconsistent. It has been reported that the concentration of TVFA in the rumen of sheep and cattle can be significantly increased by feeding active dry yeast or SC [7, 12, 34, 36-38]. Supplementing diets with SC was shown to increase propionic acid production and A/P ratio in the rumen [12, 37]. However, other studies have shown that supplementation of SC did not alter the TVFA concentration in the rumen [39]. There are few studies of the effects of CB on VFA concentration in the rumen. It is reported that CB was given once daily for 5 days (day 1-5) at 1.5 or 3.0 g/100 kg body weight, and it did not affect the ruminal VFA concentrations at either dose [31]. In this study, the TVFA, acetic acid, and propionic acid concentrations, and the A/P ratio were significantly increased with SC, CB, and their combinations supplementation compared with control group. The increase in the TVFA concentration might be due to the ability of yeast to stimulate the activities of rumen microbes, especially fibrolytic bacteria [34, 42]. Thus, the mechanism underlying the enhancement of the TVFA concentration by CB may be similar to that of SC. In addition, in this study, the A/P ratio following probiotic supplementation was increased significantly relative to the control ratio, indicating that the rumen fermentation mode may be altered by SC and CB supplementation. Furthermore, the stimulation effect of acetic acid-producing bacteria was greater than that of propionic acid-producing bacteria. The results also suggested that supplementation with SC and CB mitigated the adverse effects of heat stress on rumen VFA. Some researchers have considered that the change in VFA concentration caused by supplementation with probiotics is not worthy of attention because it will disappear once probiotic supplementation is terminated. This issue should be taken into account in production practices.

A previous study found that the digestibility of DM in sheep was improved by supplementation with SC [30]. It is reported that NDF digestibility increased by 10.5% with yeast supplementation at 5 g/day in goat kids [39]. Similarly, in this study, SC effectively increased the digestibilities of DM, NDF, and ADF, respectively. To our knowledge, few studies have evaluated the effects of CB on rumen digestibilities of DM, NDF, and ADF. CB is a good probiotic resource [42]. Therefore, it is important to evaluate the role of CB in rumen digestibilities of DM, NDF, and ADF. In this study, we found that live CB had positive effects on the digestibilities of rumen DM, NDF, and ADF of heat-stressed goats. Supplementation with probiotics, which consumed oxygen in the rumen, were found to provide a more favorable environment for the growth and reproduction of rumen cellulolytic bacteria, which increased the digestibility of fiber [9, 42]. In addition, SC and CB are good sources of vitamins and minerals [43, 44] which also benefit the growth and reproduction of rumen cellulolytic bacteria and fungi and may therefore improve fiber digestion. In this study, supplementation with SC, CB, or their combination significantly increased the DMI of heat-stressed goats. This result is similar to that of a previous study showed SC increased feed intake in the early lactation stage of lactating dairy goats after 15 weeks of yeast supplementation at 0.2 g/day, DMI was increased from 2.35 kg/day to 2.71 kg/day [45]. However, another study showed that supplemented with SC had no effect on the DMI of cows under summer heat stress conditions [25]. There are few studies on the effect of CB on DMI. This study showed that SC, CB, or COM improved the ADG of goats. In previous studies in cows and goats, SC supplementation was found to increase ADG [46-48]. However, other studies that supplemented with SC alone or a combination of SC and L. sporogenes found ADG values similar to control values. In addition, it has been reported that the use of CB in the diet (2.5×10⁸ cfu/kg feed) of weaning piglets and chickens can improve weight gain and feed efficiency [16]. Similarly, CB was
found to have positive effects on the growth performance of broiler chickens: the addition of $2 \times 10^7$ CFU or $3 \times 10^7$ CFU/kg of CB in the diet improved ADG [42]. Thus, although SC and CB can have beneficial effects on growth performance, their effects are varied. This variation can be attributed to factors such as a variation in basal diet (hay, straw, and forage), the number of live cells of probiotics, dosage, and feeding strategy [49]. Studies have shown that SC can consume oxygen and thereby create an oxygen-free environment in the rumen, which is beneficial for the growth and reproduction of rumen anaerobic microbes [50], especially the majority of cellulolytic bacteria. As a result, the production and activities of cellulolytic enzymes are enhanced. In this study, the activities of avicelase, CMCase, cellobiase and xylanase were increased by supplementation with SC, CB or their combination. However, the effects on the activities of these enzymes varied among supplementation levels. This result might have been due to the different effects of these probiotics on bacteria that produce the various cellulolytic enzymes. The results of this study suggest that supplementation with SC and CB alleviated the adverse effects of heat stress on the activities of cellulolytic enzymes.

5. Conclusions

The SC, CB, and their combination supplemented to the diet ameliorate rumen conditions by increasing pH and decreasing ORP, and enhance the rumen fermentation functions by increasing digestibility of nutrients and improve the VFA production, and thereafter improve the animal growth production of heat-stressed goats. Therefore, supplementation with these probiotics can be effective measures to alleviated adverse effects of heat stress on goats.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

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