

# Effect of *Saccharomyces cerevisiae* on Rumen Fermentation Characteristics, Nutrient Degradation and Cellulase Activity of Steers Fed Diets with Different Concentrate to Forage Ratios

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**Abstract** The objective of current study was to evaluate effect of *Saccharomyces cerevisiae* (SC) on rumen fermentation characteristics and fiber degradation in steers fed diets with different concentrate levels. Ten Simmental × Local breed steers fitted with rumen fistulas were assigned to control and treatment groups. Steers were fed the same basal diets but the treatment groups received SC supplementation ( $8 \times 10^9$  cfu/h/d) following a 2-period crossover design. Each period was comprised of four phases. From the 1<sup>st</sup> to the 4<sup>th</sup> phase, steers were fed in a stepwise fashion with incremental levels of concentrate diets at concentrate to forage ratios (CTFR) of 30:70, 50:50, 70:30 and 90:10, respectively. MIXED procedure was used to analysis effect of SC supplementation on parameters. Linear and quadratic responses for dietary CTFR were assessed using orthogonal polynomial contrast statements. The results showed that with dietary CTFR increasing, rumen pH, acetate and isobutyrate molar proportion, acetate: propionate, degradation rate ( $c_{DM}$ ,  $c_{NDF}$ ) and effective degradability ( $ED_{DM}$ ,  $ED_{NDF}$ ) of CRH linearly decreased ( $L$ ;  $P < 0.01$ ); TVFA concentration, molar proportion of propionate, butyrate, valerate and isovalerate and enzyme fibrotic activities linearly increased ( $L$ ;  $P < 0.01$ ), but concentration of rumen ammonia N, D+L-lactate and rapidly degradable fraction ( $a_{DM}$ ,  $a_{NDF}$ ) of CRH presented quadratic variation ( $Q$ ;  $P < 0.01$ ). Regardless of dietary CTFR, rumen pH,  $c_{DM}$ ,  $c_{NDF}$ ,  $ED_{DM}$ ,  $ED_{NDF}$  of CRH and enzyme fibrotic activities with SC supplementation was higher compared to control groups ( $P < 0.05$ ). Overall, SC possesses the capacity to stabilize rumen pH and to enhance fiber degradation.

**Keywords:** fibrolytic activity, *in situ*, rumen fermentation characteristics, *Saccharomyces cerevisiae*, steer

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## 1. Introduction

Yeast additive obtained from *Saccharomyces cerevisiae* (SC) has been used for many years as a substitute for antibiotics to enhance rumen fermentation and improve production efficiency in ruminant production systems, especially after antibiotics were banned by the European Union [1]. Many studies have shown that SC strain CNCM I-1077 (Lallemand, France) has a positive effect on milk production and daily feed intake of dairy cows and goats [2,3,4].

One of the primary mechanisms by which live yeasts affect animal performance seems to be related to the improvement in rumen function [5]. Published results have shown that SC can function in stabilization of rumen pH [6], and in reduction of total volatile fatty acid (TVFA), ammonia N and lactic acid concentrations [5]. However,

the effect of live yeast on rumen fermentation characteristics is inconsistent [3,7,8,9]. Similarly, the effect of live yeast on nutrient degradation in the rumen is also variable. Guedes et al. reported that supplementing with live yeast increased rumen neutral detergent fiber (NDF) degradation of maize silage [10], whereas Mir and Mir (1994) reported that live yeast supplementation had no effect on rumen dry matter (DM) or NDF degradation [9]. Part of these differences may be attributed to the different basal diets, especially at different dietary concentrate levels [11].

Major studies related live yeast have focused on dairy cattle and small ruminants such as sheep and goats [2,3,4]. Little attention has been paid to the effect of live yeast on beef cattle. Therefore, the objectives of the present study were to investigate the effect of supplementation with live yeast on rumen fermentation characteristics, nutrient degradation and cellulase activity of beef cattle fed diets with different concentrate to forage ratios.

## 2. Material and Methods

### 2.1. Animals, Diets and Experimental Design

Ten Simental × Local crossbred steers (450±50 kg body weight) fitted with 10-cm diameter rumen fistulas were used as experimental animals. Approval from the Animal Care and Use Committee of the College of Animal Science and Technology of China Agricultural University (ACUC-CAST, #20120806BCRC004) was obtained before commencement of this study. Each of the steers was housed in a tie-stall barn and feed, fresh water were available *ad libitum*. Steers were randomly assigned to one of two treatments: the control group received the basal diet with no SC supplementation and the treatment group received the basal diet with SC supplementation following a 2-period crossover design. Each period was comprised of four phases, each of which lasted for 17 days including 10 days for dietary adaptation, 6 days for the degradation study described below and 1 day for rumen liquor sampling. From the 1<sup>st</sup> to the 4<sup>th</sup> phase, steers were fed in a stepwise fashion with constant increases in the concentrate level. The stepwise diets (Table 1) were formulated to meet the nutrient requirements [12], with the concentrate to forage ratios (CTFR) at 30:70 (Phase 1), 50:50 (Phase 2), 70:30 (Phase 3), and 90:10 (Phase 4), respectively. The diets were well mixed and pressed into high density bales using a specialized wrapping machine before each phase. During the experiment, active dry yeast of SC (CNCM I-1077, Lallemand, France) was offered and dosed daily at 8×10<sup>9</sup> cfu/hd/d directly into the rumen through fistulas just before morning feeding.

**Table 1. Ingredients and nutrient composition of basal diets**

| Item                              | Dietary concentrate to forage ratios |       |       |       |
|-----------------------------------|--------------------------------------|-------|-------|-------|
|                                   | 30:70                                | 50:50 | 70:30 | 90:10 |
| Ingredient, % DM                  |                                      |       |       |       |
| Steam-flaked maize <sup>1</sup>   | 13.00                                | 34.00 | 54.00 | 74.00 |
| Soybean curd residue <sup>2</sup> | 15.20                                | 13.90 | 13.70 | 13.30 |
| Maize stalks                      | 57.00                                | 38.00 | 19.00 | 1.00  |
| Chinese ryegrass                  | 4.00                                 | 5.00  | 6.00  | 6.00  |
| Alfalfa pellets                   | 9.00                                 | 7.00  | 5.00  | 3.00  |
| Salt                              | 0.50                                 | 0.50  | 0.50  | 0.50  |
| Limestone                         | 0.40                                 | 0.70  | 1.10  | 1.50  |
| Dicalcium phosphate               | 0.20                                 | 0.20  | 0.00  | 0.00  |
| Magnesium oxide                   | 0.20                                 | 0.20  | 0.20  | 0.20  |
| Sodium bicarbonate                | 0.50                                 | 0.50  | 0.50  | 0.50  |
| Nutrient, % DM                    |                                      |       |       |       |
| ME, MJ/kg                         | 9.10                                 | 10.30 | 11.70 | 12.80 |
| CP                                | 12.70                                | 12.70 | 12.70 | 12.80 |
| NDF                               | 54.20                                | 42.70 | 31.60 | 20.80 |
| Starch                            | 12.20                                | 26.80 | 40.90 | 54.20 |
| Ca                                | 0.64                                 | 0.64  | 0.63  | 0.67  |
| P                                 | 0.32                                 | 0.34  | 0.33  | 0.35  |

<sup>1</sup> flaking density is 360 g/L

<sup>2</sup> a by-product comes from soybean processed into bean curd which is a kind of food in China.

### 2.2. In Situ Rumen Incubation

Chinese ryegrass hay (CRH) was ground through a 2-mm screen in a Wiley laboratory mill and then 5 g DM from the samples were placed into separate number-coded nylon bags (8 × 12 cm). The pore size of the nylon bags was 38 μm. For incubations, each steer had a total of three

bags as replicates/ sample. Rumen incubations were performed according to the method of Ørskov et al. [13] but following the gradual addition/all out schedule [14]. Samples were incubated in the rumen for 168, 96, 48, 24, 12, 6, and 0 h, respectively; for NDF, 240 h was added [15]. All bags were inserted at the same time (08:00) just before morning feed apart from the 12 h bags and 6 h bags, which were inserted at 07:00 and 13:00. After incubation, bags removed from the rumen were rinsed under a cold stream of tap water to remove excess rumen contents and to stop microbial activity. The bags were then washed with cool water without detergent in a washing machine and subsequently dried at 60°C for 48 h. The 0 h incubation samples were only washed under the same conditions. The bags were weighed and residues were pooled according to treatments and incubation times and then ground through a 1-mm screen. DM and NDF were analyzed. Data for DM and NDF disappearance at different incubation times were fitted to the following models [13]:

$$p = a + b(1 - e^{-ct})$$

$$ED = a + b \times c / (c + k)$$

where  $p$  = fraction disappearance at time  $t$ ,  $a$  = soluble or rapidly degradable fraction,  $b$  = insoluble but potentially degradable fraction,  $c$  = rate constant of degradation of potentially degradable insoluble fraction (/h),  $t$  = time of rumen incubation (h),  $k$  = rumen passage rate (/h), and ED = effective degradability.

### 2.3. Rumen Fluid Collection

Rumen samples were collected by hand from four locations in the rumen and reticulum through rumen fistulas at 3 h after morning feeding on the last day of each phase. Aliquots were filtered through two layers of cheese cloth, and immediately analyzed for pH with an electric pH meter (Model PHS-3C, Shanghai Leici Scientific Instrument Co., Ltd., China); 40 ml of filtered rumen fluid and about 50 g of the filter residue were placed into 50 ml centrifuge tubes, then all of the tubes were placed in liquid nitrogen immediately for ammonia-N, volatile fatty acid (VFA), D+L-lactate acid concentration and cellulase activity determination, respectively.

### 2.4. Measurements

Dry matter in feedstuff and their residues was measured by drying at 60°C for 48 h in a forced-air oven. NDF was measured using an ANKOM<sup>200</sup> Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA) [16]. NDF was assayed with heat stable amylase and without sodium sulfite and expressed inclusive of residual ash [17]. Samples of rumen fluid were centrifuged at 10000×g and supernatants were collected for ammonia N, VFA and D+L-lactate analysis. Concentration of ammonia N was determined using the colorimetric method according to Chaney and Marbach (1962) [18]. Concentration of VFA was determined by gas-liquid chromatography (Shimadzu GC-141 B, Kyoto, Japan) using 2-ethylbutyrate as internal standard. Concentration of D+L-lactate was determined using ion chromatography according to Mu et al. [19]. Another portion of rumen fluid was centrifuged at 2000×g and the supernatants were used for xylanase, avicelase, β-glucanase, and carboxymethyl cellulose activity

determination which was detected by the dinitrosalicylic acid (DNS) method at 540 nm wavelength using ELIASA according to Yue et al. [20] and Dashtban et al. [21]. The unit (U) of cellulase activity was defined as the amount of enzyme in 1 ml supernatant that liberates 1  $\mu$ mol of xylose or glucose per minute when temperature =39°C and pH = 6.8.

## 2.5. Statistical Analyses

SAS (1990) software was used for statistical analysis. Rumen fermentation data, degradation characteristics, and rumen fibrolytic activity data were analyzed using the MIXED procedure with the model:

$$Y_{ijkl} = \mu + \text{Period}_i + \text{Treatment}_j + \text{Diet}_k + (\text{Treatment}_j \times \text{Diet}_k) + \text{Steer}_l + \varepsilon_{ijkl}$$

where  $\mu$  represented the overall mean, *Period* represented the period (1 or 2), *Treatment* accounted for the fixed effect of yeast supplementation, *Diet* represented the fixed effect of different dietary CTFR, *Steer* accounted for the random effect of each individual animal,  $\varepsilon$  account for the unexplained random error. Linear and quadratic responses for dietary CTFR were assessed using orthogonal polynomial contrast statements.

## 3. Results

The results revealed that there were no significant differences between the 2 periods and there were no interactions between dietary CTFR and SC ( $P > 0.1$ ; Table 2– Table 5) for most parameters. Therefore, the main effects of dietary CTFR and SC supplementation are discussed independently.

### 3.1. Effect of SC on Rumen Fermentation Characteristics of Steers Fed Different Dietary CTFRs

Rumen fermentation characteristics were significantly affected by dietary CTFR ( $P < 0.01$ ). With dietary CTFR increasing, rumen pH values, acetate and isobutyrate molar proportion and acetate: propionate (A:P) linearly (L,  $P < 0.01$ ) decreased; TVFA concentration and molar proportion of propionate, butyrate, valerate and isovalerate linearly (L,  $P < 0.01$ ) increased; ammonia N and D+L-lactate concentration presented quadratic (Q,  $P < 0.01$ ) variation trend (Table 2).

Regardless of dietary CTFR, there was a difference ( $P < 0.01$ ) in rumen pH between SC supplementation and control groups (Table 2), but no differences ( $P > 0.05$ ) were observed in concentration of ammonia N, TVFA and D+L-lactate and molar proportions of individual VFA.

**Table 2. Effect of SC supplementation on rumen pH, volatile fatty acid (VFA; mmol/L), ammonia N (mg/100 mL) and D+L-lactate (mmol/L) concentrations and molar proportion of VFAs (%) of steers fed diets with different concentrate to forage ratios**

| Item             | CTFR  |       |       |       | SEM | SC   |      |      | P value |       |         | Probability |       |
|------------------|-------|-------|-------|-------|-----|------|------|------|---------|-------|---------|-------------|-------|
|                  | 30:70 | 50:50 | 70:30 | 90:10 |     | N    | Y    | SEM  | CTFR    | SC    | CTFR×SC | L           | Q     |
| pH               | 6.63  | 6.42  | 6.23  | 6.08  | 0.0 | 6.29 | 6.38 | 0.02 | <0.01   | <0.01 | 0.32    | <0.01       | 0.26  |
| Ammonia N        | 5.9   | 5.1   | 8.7   | 3.6   | 0.5 | 6.2  | 5.4  | 0.4  | <0.01   | 0.12  | 0.94    | 0.89        | <0.01 |
| TVFA             | 80.9  | 92.1  | 103.7 | 107.3 | 2.6 | 97.6 | 94.4 | 1.9  | <0.01   | 0.25  | 1.00    | <0.01       | 0.33  |
| D+L-lactate      | 9.7   | 10.0  | 8.5   | 9.9   | 0.2 | 9.6  | 9.5  | 0.1  | <0.01   | 0.53  | 1.00    | 0.53        | 0.01  |
| Molar proportion |       |       |       |       |     |      |      |      |         |       |         |             |       |
| Acetate          | 68.9  | 67.5  | 60.7  | 56.6  | 0.3 | 63.3 | 63.6 | 0.2  | <0.01   | 0.34  | 0.02    | <0.01       | <0.01 |
| Propionate       | 20.7  | 20.4  | 27.3  | 27.8  | 0.3 | 24.0 | 24.1 | 0.2  | <0.01   | 0.84  | 0.03    | <0.01       | 0.23  |
| Butyrate         | 8.4   | 9.8   | 9.3   | 12.1  | 0.3 | 10.0 | 9.8  | 0.2  | <0.01   | 0.50  | 0.23    | <0.01       | <0.01 |
| Valerate         | 0.7   | 0.9   | 1.2   | 1.6   | 0.0 | 1.1  | 1.1  | 0.0  | <0.01   | 0.49  | 0.43    | <0.01       | <0.01 |
| Isobutyrate      | 0.4   | 0.4   | 0.3   | 0.2   | 0.0 | 0.3  | 0.3  | 0.0  | <0.01   | 0.43  | 0.19    | <0.01       | 0.24  |
| Isovalerate      | 0.8   | 1.0   | 1.3   | 1.3   | 0.1 | 1.1  | 1.1  | 0.1  | <0.01   | 0.83  | 0.81    | <0.01       | 0.24  |
| A:P              | 3.3   | 3.3   | 2.2   | 2.0   | 0.0 | 2.7  | 2.7  | 0.0  | <0.01   | 0.53  | 0.10    | <0.01       | 0.06  |

N - control groups, Y- with SC supplementation; L-linear responses of dietary CTFR; Q-quadratic responses of dietary CTFR.

### 3.2. Effect of SC on CRH Degradation Characteristics Of Steers Fed Different Dietary CTFRs

DM degradation characteristics of CRH are shown in Table 3. With dietary CTFR increasing, rapidly degradable fraction of DM ( $a_{DM}$ ) presented quadratic (Q,  $P < 0.01$ ) variation trend; potentially degradable fraction of DM ( $b_{DM}$ ) present no significant variation trend (L,  $P = 0.10$ ; Q,  $P = 0.69$ ); degradation rate ( $c_{DM}$ ) and effective degradability of DM ( $ED_{DM}$ ) linearly (L,  $P < 0.01$ ) decreased.

NDF degradation characteristics of CRH are shown in Table 4. With dietary CTFR increasing, rapidly degradable fraction of NDF ( $a_{NDF}$ ) presented quadratic (Q,  $P = 0.01$ ) variation trend; potentially degradable fraction of NDF ( $b_{NDF}$ ) present no significant variation trend (L,  $P$

$= 0.11$ ; Q,  $P = 0.26$ ); degradation rate ( $c_{NDF}$ ) and effective degradability of DM ( $ED_{NDF}$ ) linearly (L,  $P < 0.01$ ) decreased.

Whatever CTFR, no differences ( $P > 0.05$ ) were observed for  $a_{DM}$ ,  $b_{DM}$  and  $a_{NDF}$  of CRH between SC supplementation and control treatment;  $c_{DM}$ ,  $ED_{DM}$ ,  $c_{NDF}$  and  $ED_{NDF}$  of CRH with SC supplementation was higher ( $P < 0.01$ ) compared to the control groups, but  $b_{NDF}$  with SC supplementation was lower ( $P < 0.05$ ) than control groups (Table 3 and Table 4).

In addition, relative effective DM and NDF degradability of CRH presented an increasing trend with dietary CTFR increasing and it reached 4.44% (calculated from (43.22% – 40.98%) / 43.22% × 100%) and 6.83% (calculated from (29.44% – 27.30%) / 27.30% × 100%) respectively when dietary CTFR reached 90:10.

**Table 3. Effect of SC supplementation on DM rumen degradation characteristics of Chinese ryegrass hay of steers fed diets with different concentrate to forage ratios**

| Item  | CTFR  |       |       |       | SEM | SC   |      | SEM | P value |       |         | Probability |       |
|-------|-------|-------|-------|-------|-----|------|------|-----|---------|-------|---------|-------------|-------|
|       | 30:70 | 50:50 | 70:30 | 90:10 |     | N    | Y    |     | CTFR    | SC    | CTFR×SC | L           | Q     |
| a,%   | 14.5  | 20.5  | 15.3  | 16.7  | 0.5 | 16.7 | 16.8 | 0.3 | <0.01   | 0.79  | 0.95    | 0.73        | <0.01 |
| b,%   | 56.8  | 54.7  | 58.0  | 58.6  | 0.9 | 56.8 | 57.2 | 0.6 | 0.01    | 0.68  | 1.00    | 0.10        | 0.69  |
| c,%/h | 2.4   | 1.6   | 1.9   | 1.3   | 0.1 | 1.7  | 2.0  | 0.0 | <0.01   | <0.01 | 0.06    | <0.01       | 0.20  |
| ED,%  | 46.7  | 47.2  | 45.6  | 42.3  | 0.3 | 44.8 | 46.1 | 0.2 | <0.01   | <0.01 | 0.51    | <0.01       | <0.01 |

N- control groups, Y- with SC supplementation, a- soluble or rapidly degradable fraction, b- insoluble but potentially degradable fraction, c- degradation rate of potentially degradable fraction, ED- effective degradability; L-linear responses of dietary CTFR; Q-quadratic responses of dietary CTFR.

**Table 4. Effect of SC supplementation on NDF rumen degradation characteristics of Chinese ryegrass hay of steers fed diets with different concentrate to forage ratios**

| Item  | CTFR  |       |       |       | SEM | SC   |      | SEM | P value |       |         | Probability |       |
|-------|-------|-------|-------|-------|-----|------|------|-----|---------|-------|---------|-------------|-------|
|       | 30:70 | 50:50 | 70:30 | 90:10 |     | N    | Y    |     | CTFR    | SC    | CTFR×SC | L           | Q     |
| a,%   | -0.9  | 2.7   | -1.6  | -1.1  | 0.6 | -0.6 | 0.1  | 0.4 | <0.01   | 0.25  | 0.89    | 0.05        | 0.01  |
| b,%   | 65.7  | 66.9  | 69.6  | 68.9  | 0.8 | 68.7 | 66.9 | 0.5 | <0.01   | 0.02  | 0.31    | 0.26        | 0.11  |
| c,%/h | 2.4   | 1.4   | 1.8   | 1.2   | 0.1 | 1.6  | 1.8  | 0.0 | <0.01   | <0.01 | 0.49    | <0.01       | 0.07  |
| ED,%  | 35.9  | 35.4  | 33.5  | 28.5  | 0.3 | 32.6 | 34.1 | 0.2 | <0.01   | <0.01 | 0.54    | <0.01       | <0.01 |

N- control groups, Y- with SC supplementation, a- soluble or rapidly degradable fraction, b- insoluble but potentially degradable fraction, c- degradation rate of potentially degradable fraction, k- passage rate, ED- effective degradability; L-linear responses of dietary CTFR; Q-quadratic responses of dietary CTFR.

### 3.3 Effect of SC on Cellulase Activity of Steers Fed Different Dietary CTFRs

Dietary CTFR significantly affected ( $P < 0.01$ ) xylanase, CMCase, avicelase, and  $\beta$ -glucanase activity and which linearly (L,  $P < 0.01$ ) increased with dietary CTFR increasing (Table 5).

Regardless of dietary CTFR, compared with control groups, no difference ( $P = 0.10$ ) was observed for CMCase in SC supplementation groups; while xylanase and avicelase activity were higher ( $P < 0.05$ ) and  $\beta$ -glucanase activity showed an increasing tendency ( $P = 0.05$ ) with SC supplementation (Table 5).

**Table 5. Effect of SC supplementation on rumen cellulase activity (mU) of steers fed diets with different concentrate to forage ratios**

| Item               | CTFR  |       |       |       | SEM | SC    |       | SEM | P value |       |         | Probability |      |
|--------------------|-------|-------|-------|-------|-----|-------|-------|-----|---------|-------|---------|-------------|------|
|                    | 30:70 | 50:50 | 70:30 | 90:10 |     | N     | Y     |     | CTFR    | SC    | CTFR×SC | L           | Q    |
| Xylanase           | 200.8 | 241.3 | 235.1 | 249.8 | 4.9 | 224.1 | 239.5 | 3.5 | <0.01   | <0.01 | 0.10    | <0.01       | 0.24 |
| CMC-ase            | 30.2  | 38.7  | 37.2  | 38.0  | 1.6 | 34.6  | 37.4  | 1.2 | <0.01   | 0.10  | 0.91    | <0.01       | 0.07 |
| Avicelase          | 104.8 | 140.0 | 141.1 | 190.5 | 8.2 | 134.4 | 153.7 | 5.8 | <0.01   | 0.02  | 0.30    | <0.01       | 0.58 |
| $\beta$ -glucanase | 74.2  | 94.1  | 98.6  | 116.5 | 6.2 | 89.7  | 102.0 | 4.4 | <0.01   | 0.05  | 0.74    | <0.01       | 0.99 |

N- control groups, Y- with SC supplementation, L-linear responses of dietary CTFR; Q-quadratic responses of dietary CTFR.

## 4. Discussion

### 4.1. Effect of Dietary CTFR on Rumen Fermentation Characteristics

Variation trend of rumen pH and concentration of VFA and D+L-lactate with dietary CTFR increasing was consistent with other studies [22] and which was easy to understand. When dietary CTFR was higher, more readily fermentable carbohydrates (mainly starch) were ingested into the rumen, which brought about more organic acid production such as VFA and D+L-lactate contributing to lower rumen pH [5].

Ammonia in the rumen is a pool with several inputs and exits. Ammonia is derived from degradation of dietary protein and dietary non-protein nitrogen (NPN), from hydrolysis of urea recycled to the rumen and from degradation of microprotein (MCP). Ammonia disappears from the rumen pool due to uptake by microbes, absorption through the rumen wall and flushing to the omasum. Changes in any of these six factors will alter the concentration of ammonia in the rumen [23,24]. With dietary CTFR increasing, rumen bacteria increases and more ammonia N is needed to synthesize MCP, which results in lower ammonia N. However, the increased level

of concentrate leads to more acid matter which can conversely liberate ammonia as ammonium ion ( $\text{NH}_4^+$ ). The ammonium ion inhibits ammonia absorption as well as facilitating ammonia N in blood recycling to the rumen, which results in a higher ammonia N concentration in the rumen [23]. Therefore, rumen ammonia N concentration fluctuated with variation of concentrate level.

### 4.2. Effect of SC Supplementation on Rumen Fermentation Characteristics

Chaucheyras-Durand et al. reported that live yeast can scavenge unnecessary oxygen in the rumen and provide some bioactive substance [5]. Due to the metabolic activity of live yeast, ciliate entodiniomorphid protozoa are stimulated with more starch granules engulfing and lactate-metabolizing bacteria such as *S. bovis* are restrained. Consequently, concentration of VFA and D+L-lactate decreased and rumen pH increased [25,26,27,28].

In the present study, effect of SC supplementation on rumen pH, concentration of TVFA and D+L-lactate was consistent with some previous studies [6,10,29,30] but inconsistent with others [3,31] which reported that SC had a negative effect on rumen pH or improved TVFA concentration.

Some previous experiments showed that SC supplementation altered acetate: propionate [10,28,31] or

proportions of branched-chain VFA (isobutyrate and isovalerate) [32,33], which means that rumen fermentation patterns was altered. However, no differences ( $P > 0.05$ ) of individual VFA including branched-chain VFA molar proportions between SC and control groups were observed in current experiment.

Previous studies indicated that yeast additives either stimulate rumen ammonia N uptake by bacteria, which allows better growth of these species in the rumen, or they stimulate the growth of cellulolytic bacteria, which could use more rumen ammonia N to synthesize cellular nitrogenous components [34], hence the concentration of rumen ammonia would be decreased by live yeast [8]. However, no significant reduction for rumen ammonia N was observed between SC supplementation and control groups in the present experiment, and the result was consistent with previous studies [10,33].

#### 4.3. Effect of Dietary CTFR on Fiber Degradation

From above discussion of effect of dietary CTFR on rumen pH, effect of which on fiber degradation characteristics is easy to understand. Fiber is degraded by fiber-degrading bacteria, but the major fiber-degrading bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus* and *R. flavfaciens* are sensitive to low pH [35]. With dietary CTFR increasing, rumen pH linearly decreased, fiber-degrading bacteria activity was restricted [5], consequently fiber degradation rate and effective degradability linearly decreased.

However, in present experiment, cellulase activity linearly increased with dietary CTFR increasing which is contrary to our knowledge and the experimental results (fiber degradation rate linearly decreased). The main reason may be related to the pH value when cellulase activity was detected.

#### 4.4. Effect of SC Supplementation on Fiber Degradation

Besides the positive effect of SC on rumen pH, SC still possesses the capacity to scavenge oxygen traces in the rumen which leads to lower redox potential [36,37]. Hence, suitable acidity and favorable ecological conditions were created for the growth and activities of anaerobic microorganisms, consequently the number or activity of fiber-degrading bacteria were enhanced, which is beneficial to improve DM and NDF degradation [38]. In the present study, cellulase activity was proved to be increased with SC supplementation (Table 5), and whether the number of fiber-degrading bacteria could be enhanced will be evaluated in a future study.

However, an improvement with SC supplementation in rumen fiber degradation was not always observed. Some previous studies reported that SC supplementation improved rumen fiber degradation [10,34], which is consistent with the present study, whereas others reported there was no effect with SC supplementation on fiber degradation [39,40]. Similarly, improvement of SC on cellulase activity was not always observed [41]. The inconsistent results may be related to physiological status, breed of animals, feed composition or concentrate to forage ratio, yeast strain and dosage and more research

needs to be done in the future to evaluate effect of SC on fiber degradation under different conditions.

## 5. Conclusions

Dietary CTFR had a significant effect on rumen fermentation characteristics, fiber degradation and rumen cellulase activity. SC possesses the capacity to stabilize rumen pH and to enhance fiber degradation and cellulase activity.

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