





## Article

# Yeast (*Saccharomyces cerevisiae*) Culture Promotes the Performance of Fattening Sheep by Enhancing Nutrients Digestibility and Rumen Development

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**Abstract:** Paraprobiotics are potential agents for improving animal health and performance. This experiment investigated the effect of dietary supplementation of yeast (*Saccharomyces cerevisiae*) culture (YC) on the growth performance, nutrient digestibility, rumen development and microbiome of fattening sheep. Ninety male Hu sheep weighed  $38 \pm 1.47$  kg were randomly assigned to three treatments: CON diet (basal diet), LYC diet (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively), and HYC diet (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). Treatments (LYC or HYC) were sprinkled on the feed surface according to the required dosage before feeding the basal diet to each sheep throughout the trial. The trial included early (60 days) and later (30 days) fattening periods. The results showed that average daily gain and feed efficiency were higher ( $p < 0.05$ ) in the LYC group compared with CON in later and whole stages. Digestibility of DM, OM, CP, NDF and ADF were higher ( $p < 0.05$ ) in LYC and HYC compared with CON. The retained N, the utilization efficiency of N and the biological value of N were higher ( $p < 0.05$ ) in LYC compared with CON and HYC. Rumen  $\text{NH}_3\text{-N}$  was higher ( $p < 0.05$ ) in LYC and HYC. The papillary height of the rumen was higher ( $p < 0.05$ ) in LYC when compared with CON and HYC, whereas rumen wall thickness and muscular layer thickness were higher ( $p < 0.05$ ) in HYC compared with CON and LYC. The dressing percentage of LYC and HYC was higher ( $p < 0.05$ ) compared with CON. The diversity, richness and structure of rumen microbiota showed no significant difference ( $p > 0.05$ ); however, still observed remarkable increases in the relative abundance of several specific genera including *Succiniclaticum* and *Fibrobacter* with increasing doses of yeast culture. In addition, at the ASV level, ASV83, ASV123 (*Succiniclaticum*), and ASV148, ASV250 (*Fibrobacter*) were increased in YC groups. In conclusion, we confirmed that the supplementation of YC in diet could improve the growth and slaughter performance of fattening Hu sheep through improving nutrient digestion, especially nitrogen utilization, rumen microbial environment and the development of rumen epithelium, which proves the benefits of paraprobiotics in animal production.

**Keywords:** yeast culture; *Saccharomyces cerevisiae*; fattening sheep; growth performance; nutrient digestibility; rumen fermentation; rumen microbial communities



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## 1. Introduction

Statistics from the FAO showed that China's mutton production reached 5.05 million tons in 2020, ranking first in the world and accounting for about 30% of the world's total output [1]. With the intensification of the sheep industry, it is common to feed diets containing a high proportion of grains. However, with the increase of dietary concentrate

ratio, the lactic acid, and volatile fatty acids (VFA) produced by massive fermentable carbohydrates in the rumen led to changes in rumen fermentation, such as pH drop and metabolic disorders, as well as metabolic diseases such as subacute rumen acidosis and nutritional diarrhea [2], which brought colossal economic losses to production. The indoor intensive feeding system enables feed additives to be extensively used for the manipulation of rumen fermentation and the improvement of animal performance. At the same time, since 1 July 2020, China has fully implemented the “anti-resistance order” (prohibiting the addition of growth-promoting additives in diets). It led to an increased number of studies exploring other feed additives as alternatives for growth promoters in sheep [3].

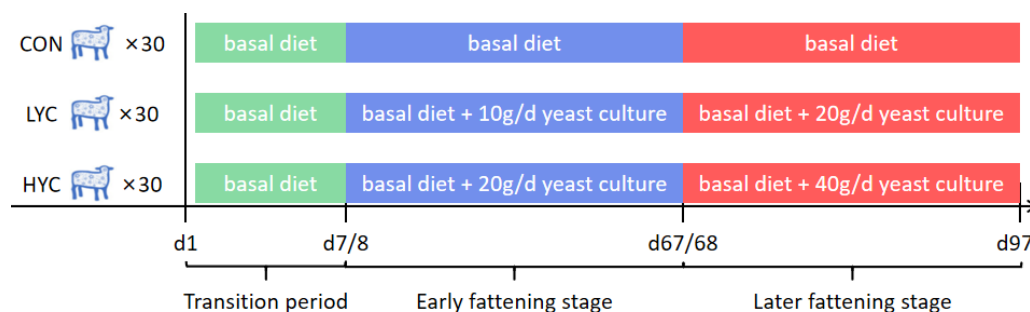
Paraprobiotics, defined as “inactivated microbial cells (non-viable) that confer a health benefit to the consumer”, hold the ability to improve the host condition. Yeast culture (YC) [4], one of the paraprobiotics, is a natural product produced from yeast fermentation. It contains, in addition to dead yeast cells, culture media and the products of yeast metabolism, including proteins, polysaccharides (mainly  $\beta$ -glucans and mannan-oligosaccharides), lipids, vitamins, peptides, amino acids, nucleotides, organic acids, and antioxidants [5]. Yeast culture supplementation in the animal diet has a range of beneficial effects, including increased structure and richness of the microbiome [6,7], feed intake [8], stabilization of rumen pH [9,10], increased feed digestion [11], enhancement of overall immunity [12,13], and consequently improved performance [14–16]. However, there are still different reports on the application effect of YC in mutton sheep. Malekkhahi et al. [17] found that there was no significant difference in dry matter intake, average daily gain or feed conversion ratio of lambs fed with 4 g/d of YC compared with the control treatment. Similarly, Tripathi et al. [18] showed that dietary YC supplementation had no significant effect on dry matter intake in lambs. As for the appropriate supplemental level of YC, studies in dairy cows [19,20] and beef cattle [21] showed that a high supplemental level of YC showed no dose effect. Ye et al. [22] added 0, 100, 200, and 300 mL YC per goat per day, respectively. The results showed that goats supplemented with YC (200 mL and 300 mL) had significantly lower ruminal pH and ammonia nitrogen compared with the control. The above studies indicate that there is a great difference in the studies on YC in fattening sheep, and the effect and appropriate supplemental level of YC is still unclear. Therefore, this experiment was conducted to study the effects of dietary yeast (*Saccharomyces cerevisiae*) culture on growth performance, carcass characteristics, nutrient digestibility, rumen fermentation and microbial communities of fattening Hu sheep under the condition of the same nutrient level, providing technical support for the application of YC in meat sheep production.

## 2. Materials and Methods

The experiment was conducted at a commercial farm in Jiangsu, China (latitude 32.01' N, longitude 119.38' E). The experimental procedure was approved by the Animal Ethics Committee of Chinese Academy of Agricultural Sciences (IFR-CAAS 20220614), and humane animal care and handling procedures were followed throughout the experiment.

### 2.1. Animals and Experimental Design

A total of 90 male fattening sheep (3- to 4-month-old,  $38 \pm 1.47$  kg body weight) were used in a 97-day trial. All sheep were randomly assigned to 3 dietary treatments: CON diet (basal diet, of which the ratio of concentrate to roughage were 65:35 and 80:20 at early and later fattening stage, respectively), LYC diet (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively), and HYC diet (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). Treatments (LYC or HYC) were sprinkled on the feed surface according to the required dosage before feeding the basal diet to each sheep throughout the trial. Each treatment had five replicates and each replicate included six sheep (Figure 1). Six sheep from each replicate were raised in one pen. The trial included a 7-day dietary transition period and a 90-day test period. Furthermore, the test period was divided into a 60-day early fattening period and a 30-day later fattening period.



**Figure 1.** The frame diagram of experimental design. CON, control (basal diet supplied with 0 g/d yeast culture at the whole stage); LYC, low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively); HYC, high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively).

2.2. YC Processing, Diets and Feeding Management

The yeast culture (YC) was a dried culture product that was produced through anaerobic fermentation of *Saccharomyces cerevisiae*. The inoculation of yeast was firstly on a liquid medium that contained molasses, urea, corn syrup, etc., with a set temperature of 30 °C. Then, the live bacteria cells in the liquid medium were cultured on a solid medium that was composed of corncob powder, corn flour, soybean meal, etc., with a set temperature of 30 °C. After dehydration, the YC products could be used for this experiment [23]. The ingredient analysis shows that YC contained: 18.0% crude protein, 8000 IU/g anti-microbial peptides, 300 IU/g digestive enzymes, 1.0% mannan, and 8% crude ash.

The basal diet was prepared according to the nutritional requirement of 250 g daily gain of mutton sheep in NRC (2007) [24]. The ingredients and chemical composition of the basic diet are presented in Table 1. The sheep were fed twice a day at 7:00 and 16:00 and had free access to water. Daily diet allotments to each pen were adjusted to allow minimal residuals (<5% of the total offered) in the feed trough just prior to the next morning feeding. The dietary offers and residuals were recorded daily. The sheep were weighed at 8, 67 and 97 days. The dry matter intake, average daily gain and feed efficiency were calculated accordingly. The floors, pens and feed troughs were disinfected, and all the sheep were earmarked, dewormed, and immunized according to normal immunization procedures before the trial.

**Table 1.** Ingredients and chemical composition of the basal diets for the fattening sheep (% , DM basis).

Ingredients <sup>1</sup> , %	Diet Formula		Nutrient Levels <sup>1</sup> , %	Contents <sup>3</sup>	
	Early Fattening Stage	Later Fattening Stage		Early Fattening Stage	Later Fattening Stage
Peanuts straw	35	20	DM	91.56	94.32
Corn	36	54	CP	15.01	14.35
Soybean meal	15	13.5	NDF	38.76	27.32
Wheat bran	6	5	ADF	21.37	11.94
Tofu residue	6	5	Ca	1.15	0.83
NaCl	0.5	0.7	TP	0.41	0.31
CaHPO <sub>4</sub>	0.8	0.8	Starch	26.33	36.86
Premix <sup>2</sup>	0.7	1	DE (MJ/kg)	12.01	12.76
Total	100	100	ME (MJ/kg)	9.78	10.42

<sup>1</sup> NaCl: sodium chloride; CaHPO<sub>4</sub>: calcium hydrogen phosphate; DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; Ca: calcium; TP: total phosphorus; DE: digestible energy; ME: metabolizable energy. <sup>2</sup> Contained followings per kg of premix: VA 1,500,000 IU, VD 220,000 IU, VE 5000 IU, Fe 5.5 g, Cu 1.25 g, Mn 4.7 g, Zn 2.4 g, Se 50 mg, I 50 mg, Co 10 mg. <sup>3</sup> All data were measured values except DE and ME, which were calculated based on Tables of Feed Composition and Nutritive Values in China 2012.

### 2.3. Digestion and Metabolism Trial

The digestion and metabolism trials were carried out from d 15 before the end of the experiment with five sheep selected from each treatment, respectively. Sheep were moved into individual metabolism crates that could separate feces and urine. Sheep were allowed a 7-day adaptation in the metabolism crates followed by a 5-day collection period [25]. During the collection period, feed intake and refusals were recorded daily. Total feces were collected and weighed daily. After thorough and uniform mixing, 10% of total feces were collected and mixed with 10 mL 12 M HCL solution. Then, 5-day samples of each sheep were pooled together. Total urine was collected into a plastic container containing 50 mL of 12 M HCL solution to prevent ammonia loss [26]. The volume was measured and recorded daily. Then, 20% of the total urine was collected each day and pooled together after the 5-day collection period. Samples of feed, feces and urine were frozen in the refrigerator at  $-20\text{ }^{\circ}\text{C}$  for further analysis. After the digestibility trial, the frozen feed, orts, and fecal subsamples were thawed overnight at room temperature, dried in an oven at  $65\text{ }^{\circ}\text{C}$  for 48 h, and then ground to pass through a 1 mm sieve. The chemical composition of the samples was analyzed according to AOAC [27] except for the neutral detergent fiber (NDF) and acid detergent fiber (ADF) whose measurement followed the method of Wang et al. [28].

The formula for calculating the apparent digestibility of nutrients in diet:

Apparent digestibility of certain nutrients in the diet (%) =  $100 \times (\text{feed intake} \times \text{the content of this nutrient in the diet} - \text{defecation amount} \times \text{nutrient content in feces}) / (\text{feed intake} \times \text{the content of this nutrient in the diet})$ .

Total excreted nitrogen (g/d) = fecal nitrogen + urinary nitrogen.

Apparent digestible nitrogen (g/d) = intake nitrogen – fecal nitrogen.

Deposition nitrogen (g/d) = intake nitrogen – (fecal nitrogen + urinary nitrogen).

Apparent digestibility of nitrogen (%) =  $100 \times \text{apparent digestible nitrogen} / \text{intake nitrogen}$ .

Nitrogen utilization rate (%) =  $100 \times \text{deposited nitrogen} / \text{ingested nitrogen}$ .

The biological value of nitrogen (%) =  $100 \times [\text{ingestion of nitrogen} - (\text{fecal nitrogen} + \text{urinary nitrogen})] / (\text{ingestion of nitrogen} - \text{fecal nitrogen})$ .

### 2.4. Slaughter Performance and Rumen Parameter Determination

At the end of the experiment, five fattening sheep in each group were slaughtered to determine the slaughtering performance. After slaughtering, the head, hoof and viscera were removed, and the carcass was weighed after peeling. The rumen was separated, and the contents were removed and washed. Rumen tissue sections was stored in 70% ethanol until further processing after 24 h of fixing in formalin. All samples were embedded in paraffin blocks and were stained with Yihong-haematoxylin (H.E.) at the Chinese Agriculture University (Beijing, China). The length and width of the rumen papillae and stratum corneum thickness were measured using the Axiovision software of image-pro express image analysis processing system (Zeiss, Oberkochen, Germany). The ruminal digesta sample was collected and the pH value was measured immediately (Testo 206-pH2, Germany). The rumen digesta was then filtered through four layers of cheesecloth, and a 10 mL subsample of each strained fluid was collected and stored at  $-20\text{ }^{\circ}\text{C}$  for analysis of the volatile fatty acids (VFAs) and ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) as described by Huang et al. [29] and Haro et al. [30]. The sulfuric acid paper was used to draw the outline of the superior ophthalmic muscle (longissimus dorsi muscle) of the spine between the penultimate first and second ribs, and the area of the ophthalmic muscle were measured. The tissue thickness between the 12th and 13th ribs and 11 cm away from the midline of the spine (i.e., GR value) was measured with vernier caliper.

### 2.5. Next-Generation Sequencing

Then, the rumen liquid for the analysis of rumen microbial communities were kept in 10 mL tubes, immediately placed in liquid nitrogen, and then stored at  $-80\text{ }^{\circ}\text{C}$ . After the extraction of total genome DNA, bacterial 16S rRNA gene regions were amplified using PCR primers (341F:5'-CCTAYGGGRBGCASCAG-3'; 806R: 5'-GGACTACNNGGTTATCTAAT-

3'). To prevent contamination from reagents, negative controls for DNA extraction and PCR amplification were included, and no PCR products of the negative controls were detected from agarose gel. Library quality was checked using a Thermo NanoDrop 2000 UV microphotometer and 2% agarose gel electrophoresis. A Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify the library. Amplicon libraries were sequenced using an Illumina Miseq PE250 platform (Allwegene Technology Co., Ltd., Shanghai, China). The obtained raw data from the sequencer were imported into the QIIME2 platform (2021.4 released), and were processed by the Deblur program, which obtains single-nucleotide resolution based on error profiles within samples. Deblur denoised sequences are usually called amplicon sequence variants (ASVs). The number of reads from each sample was rarefied to 5556. The taxonomy of these features was assigned to the Greengenes reference database (13-8 version) classifier with 99% similarity. A feature table was generated using QIIME2's `qiime vsearch cluster-features-closed-reference` command. Determination of alpha and beta diversities and analysis of similarity (ANOSIM) was also conducted in qiime2.

### 2.6. Statistical Analysis

The data on growth performance, nutrient utilization, organ weight and meat quality were subjected to the one-way ANOVA procedure of SAS 9.1 statistical software. If the difference was significant, Duncan's method was used for multiple comparison tests.  $p < 0.05$  was the judgment standard of significant difference.

Alpha diversity of the rumen microbial data between treatments was tested using the Kruskal–Wallis test and a post hoc Dunn Kruskal–Wallis multiple comparisons with a Bonferroni adjustment were used to evaluate differences between treatments with boxplots being made in R ('ggpubr' packages). Beta diversity was visualized with a PCoA plot. Featured bacteria among treatments were identified by the linear discriminant analysis (LDA) effect size (LEfSe) with an LDA score  $> 2$  [31].

## 3. Results

### 3.1. Growth Performance

No differences in body weight were observed ( $p > 0.05$ ) among groups (Table 2). In the early fattening stage, although the YC supplementation did not affect the DMI and ADG of Hu sheep ( $p > 0.05$ ), the feed-to-gain ratio tended to be lower than that of CON ( $p = 0.082$ ). In the later and whole fattening stage, ADG and feed-to-gain ratio were significantly higher ( $p < 0.05$ ) in LYC compared with CON. In addition, in the later fattening stage, the DMI of LYC tended to be higher than that of CON ( $p = 0.076$ ); There were no differences in DMI between groups in the early and whole fattening stage ( $p > 0.05$ ).

### 3.2. Nutrient Apparent Digestibility

DM, OM, CP, NDF and ADF were higher ( $p < 0.05$ ) in LYC and HYC compared with CON (Table 3).

### 3.3. Nitrogen Metabolism

Urinary N and total N excretion of LYC were lower ( $p < 0.05$ ) than CON and HYC, but there were no differences in HYC compared with CON ( $p > 0.05$ , Table 4). The Retained N, the Utilization efficiency of N and the biological value of N were higher ( $p < 0.05$ ) in LYC compared with CON and HYC. While there were no differences among groups in the N intake, fecal N, apparent digestible N, and apparent digestibility of N ( $p > 0.05$ ).

**Table 2.** Effect of dietary supplementation of YC on growth performance of fattening Hu sheep.

Items	CON <sup>1</sup>	LYC <sup>2</sup>	HYC <sup>3</sup>	SEM <sup>4</sup>	p-Value
Initial body weight, IBW (kg)	38.19	39.28	38.74	0.38	0.519
60d body weight, BW (kg)	49.07	50.41	49.66	0.48	0.526
90d body weight, BW (kg)	52.06	54.03	52.80	0.59	0.395
Early fattening stage					
Average daily gain, ADG (g/d)	184.32	188.63	185.09	4.24	0.910
Dry matter intake, DMI (g/d)	1501.56	1518.65	1509.23	3.26	0.167
Feed to gain ratio	8.21	7.89	8.05	0.11	0.082
Later fattening stage					
Average daily gain, ADG (g/d)	99.86 <sup>b</sup>	120.42 <sup>a</sup>	104.67 <sup>ab</sup>	7.31	0.036
Dry matter intake, DMI (g/d)	1466.97	1511.14	1509.6	36.80	0.076
Feed to gain ratio	14.14 <sup>a</sup>	12.49 <sup>b</sup>	14.36 <sup>ab</sup>	0.36	0.007
Whole fattening stage					
Average daily gain, ADG (g/d)	155.85 <sup>b</sup>	165.64 <sup>a</sup>	157.98 <sup>ab</sup>	0.86	0.043
Dry matter intake, DMI (g/d)	1495.74	1516.15	1509.35	16.67	0.675
Feed to gain ratio	9.69 <sup>a</sup>	9.02 <sup>b</sup>	9.22 <sup>ab</sup>	0.16	0.033

<sup>1</sup> CON: control (basal diet supplied with 0 g/d yeast culture at the whole stage). <sup>2</sup> LYC: low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively). <sup>3</sup> HYC: high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). <sup>4</sup> SEM: standard error of the mean. <sup>a,b</sup>: Means with different superscripts in the same row differ ( $p < 0.05$ ).

**Table 3.** Effect of dietary supplementation of YC on nutrient apparent digestibility of fattening Hu sheep.

Items <sup>1</sup>	CON <sup>2</sup>	LYC <sup>3</sup>	HYC <sup>4</sup>	SEM <sup>5</sup>	p-Value
DM (%)	73.62 <sup>b</sup>	78.16 <sup>a</sup>	77.13 <sup>a</sup>	0.72	0.023
OM (%)	75.29 <sup>b</sup>	82.47 <sup>a</sup>	81.33 <sup>a</sup>	1.12	0.027
CP (%)	68.22 <sup>b</sup>	74.93 <sup>a</sup>	75.23 <sup>a</sup>	0.92	0.037
NDF (%)	57.54 <sup>b</sup>	65.36 <sup>a</sup>	67.46 <sup>a</sup>	1.83	0.043
ADF (%)	49.56 <sup>b</sup>	56.85 <sup>a</sup>	55.90 <sup>a</sup>	1.06	0.035

<sup>1</sup> DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber. <sup>2</sup> CON: control (basal diet supplied with 0 g/d yeast culture at the whole stage). <sup>3</sup> LYC: low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively). <sup>4</sup> HYC: high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). <sup>5</sup> SEM: standard error of the mean. <sup>a,b</sup>: Means with different superscripts in the same row differ ( $p < 0.05$ ).

**Table 4.** Effect of dietary different supplemental levels of YC on the nitrogen metabolism of fattening Hu sheep.

Items <sup>1</sup>	CON <sup>2</sup>	LYC <sup>3</sup>	HYC <sup>4</sup>	SEM <sup>5</sup>	p-Value
N intake (g/d)	31.82	35.27	35.56	0.91	0.181
Fecal N (g/d)	10.09	10.68	11.33	0.43	0.540
Urinary N (g/d)	8.92 <sup>a</sup>	5.096 <sup>b</sup>	9.94 <sup>a</sup>	0.70	0.003
Total N excretion (g/d)	19.01 <sup>a</sup>	15.78 <sup>b</sup>	21.27 <sup>a</sup>	0.78	0.004
Apparent digestible N (g/d)	21.73	24.58	24.23	0.89	0.391
Retained N (g/d)	12.81 <sup>b</sup>	19.49 <sup>a</sup>	13.28 <sup>b</sup>	1.16	0.036
Apparent digestibility of N (%)	68.22	69.20	68.23	1.22	0.941
Utilization efficiency of N (%)	39.94 <sup>b</sup>	53.91 <sup>a</sup>	40.20 <sup>b</sup>	2.75	0.046
The biological value of N (%)	58.51 <sup>b</sup>	77.20 <sup>a</sup>	58.99 <sup>b</sup>	3.38	0.021

<sup>1</sup> N: nitrogen. <sup>2</sup> CON: control (basal diet supplied with 0 g/d yeast culture at the whole stage). <sup>3</sup> LYC: low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively). <sup>4</sup> HYC: high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). <sup>5</sup> SEM: standard error of the mean. <sup>a,b</sup>: Means with different superscripts in the same row differ ( $p < 0.05$ ).

### 3.4. Rumen Fermentation Parameters

As shown in Table 5, NH<sub>3</sub>-N was higher ( $p < 0.05$ ) in LYC and HYC compared with CON, although there were no differences in LYC and HYC ( $p > 0.05$ ). There were no differ-

ences among groups in the pH, microbial protein, TVFA, acetate/TVFA, propionate/TVFA, butyrate/TVFA, and acetate/propionate ( $p > 0.05$ ).

**Table 5.** Effect of dietary different supplemental levels of YC on the rumen fermentation parameters of fattening Hu sheep.

Items	CON <sup>1</sup>	LYC <sup>2</sup>	HYC <sup>3</sup>	SEM <sup>4</sup>	<i>p</i> -Value
pH	6.05	6.14	6.36	0.10	0.453
Microbial protein, MCP (mg/dL)	9.61	8.67	8.46	0.29	0.245
Ammonia nitrogen, NH <sub>3</sub> -N (mg/100 mL)	29.20 <sup>b</sup>	45.27 <sup>a</sup>	35.39 <sup>a</sup>	4.02	0.035
Total volatile fatty acid, TVFA (mmol/L)	21.49	24.27	23.18	1.52	0.782
Acetate (%)	42.10	46.60	45.70	1.28	0.336
Propionate (%)	14.85	16.86	16.74	0.89	0.325
Butyrate (%)	12.06	9.26	8.80	1.18	0.502
Acetate/Propionate	2.31	2.85	2.79	0.12	0.927

<sup>1</sup> CON: control (basal diet supplied with 0 g/d yeast culture at the whole stage). <sup>2</sup> LYC: low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively). <sup>3</sup> HYC: high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively).

<sup>4</sup> SEM: standard error of the mean. <sup>a,b</sup>: Means with different superscripts in the same row differ ( $p < 0.05$ ).

### 3.5. Development of the Rumen Epithelium

The papillary height of the rumen was higher ( $p < 0.05$ ) in LYC compared with CON and HYC, whereas rumen wall thickness and muscular layer thickness were higher ( $p < 0.05$ ) in HYC compared with CON and LYC (Table 6).

**Table 6.** Effect of dietary different supplemental levels of YC on morphology index of the rumen in fattening Hu sheep.

Items	CON <sup>1</sup>	LYC <sup>2</sup>	HYC <sup>3</sup>	SEM <sup>4</sup>	<i>p</i> -Value
Papillary height (μm)	1860.18 <sup>b</sup>	2702.88 <sup>a</sup>	2082.94 <sup>b</sup>	138.73	0.022
Papillary width (μm)	373.40	363.28	325.56	13.05	0.309
Rumen wall thickness (μm)	1474.00 <sup>b</sup>	1554.00 <sup>b</sup>	2026.28 <sup>a</sup>	99.56	0.035
Rumen epithelial thickness (μm)	147.96	156.80	131.88	5.26	0.145
Muscular layer thickness (μm)	1044.00 <sup>b</sup>	997.64 <sup>b</sup>	1475.80 <sup>a</sup>	90.20	0.043
Keratin layer thickness (μm)	39.22	52.32	36.16	3.32	0.101
Stratum granulosum thickness (μm)	31.02	32.08	33.68	0.23	0.503
Stratum spinosum thickness (μm)	69.04	73.46	69.33	2.25	0.683
Stratum basal thickness (μm)	14.84	14.44	14.56	0.14	0.509

<sup>1</sup> CON: control (basal diet supplied with 0 g/d yeast culture at the whole stage). <sup>2</sup> LYC: low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively). <sup>3</sup> HYC: high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively).

<sup>4</sup> SEM: standard error of the mean. <sup>a,b</sup>: Means with different superscripts in the same row differ ( $p < 0.05$ ).

### 3.6. Carcass Characteristics

The dressing percentage was higher ( $p < 0.05$ ) in LYC and HYC compared with CON. There were no differences in live weight before slaughter, carcass weight, eye area, and GR value among groups ( $p > 0.05$ ) during dietary different supplemental levels of YC (Table 7).

### 3.7. Rumen Microbial Communities

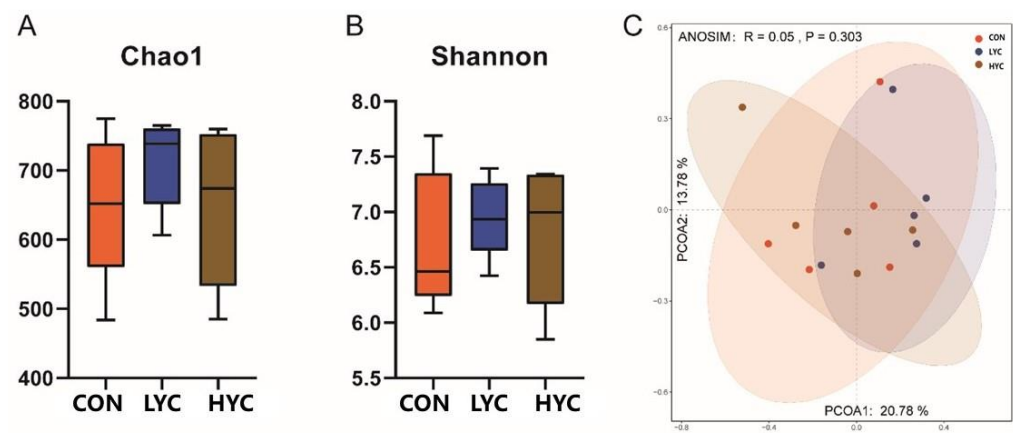
In this study, there were no significant differences in microbial richness (Chao1 index) and diversity (Shannon index) (Figure 2A,B) ( $p > 0.05$ ). Similarly, we did not observe the distinct clusters of microbial community among groups according to the PCoA plot of Beta diversity ( $p > 0.05$ ) (Figure 2C).

**Table 7.** Effect of dietary different supplemental levels of YC on carcass characteristics of fattening Hu sheep.

Items	CON <sup>1</sup>	LYC <sup>2</sup>	HYC <sup>3</sup>	SEM <sup>4</sup>	p-Value
Live weight before slaughter (kg)	55.68	56.26	55.76	0.45	0.868
Carcass weight (kg)	29.31	31.56	31.03	0.41	0.054
Dressing percentage (%)	52.62 <sup>b</sup>	56.12 <sup>a</sup>	55.66 <sup>a</sup>	0.60	0.018
Eye muscle area (cm <sup>2</sup> )	2.60	2.51	2.40	0.04	0.207
GR value (mm)	2.38	2.52	2.49	1.24	0.906

<sup>1</sup> CON: control (basal diet supplied with 0 g/d yeast culture at the whole stage). <sup>2</sup> LYC: low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively). <sup>3</sup> HYC: high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively).

<sup>4</sup> SEM: standard error of the mean. <sup>a,b</sup>: Means with different superscripts in the same row differ ( $p < 0.05$ ).

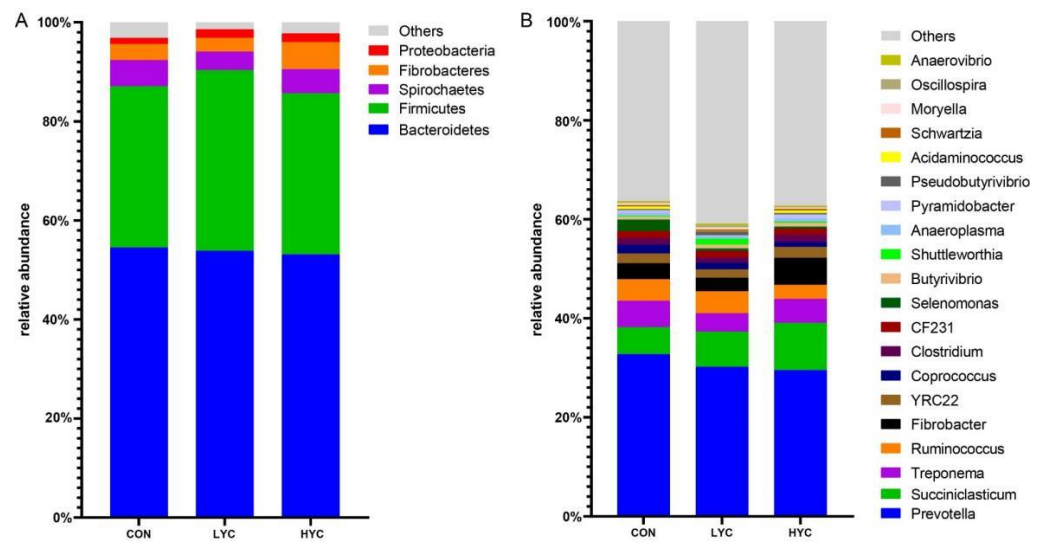


**Figure 2.** Effect of yeast culture supplementation on microbial diversity and structure. CON, control (basal diet supplied with 0 g/d yeast culture at the whole stage); LYC, low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively); HYC, high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). (A) the richness of rumen microbiota. (B) The diversity of rumen microbiota. (C) The principal coordinate analysis (PCoA) based on the bacterial Bray–Curtis in each sample.

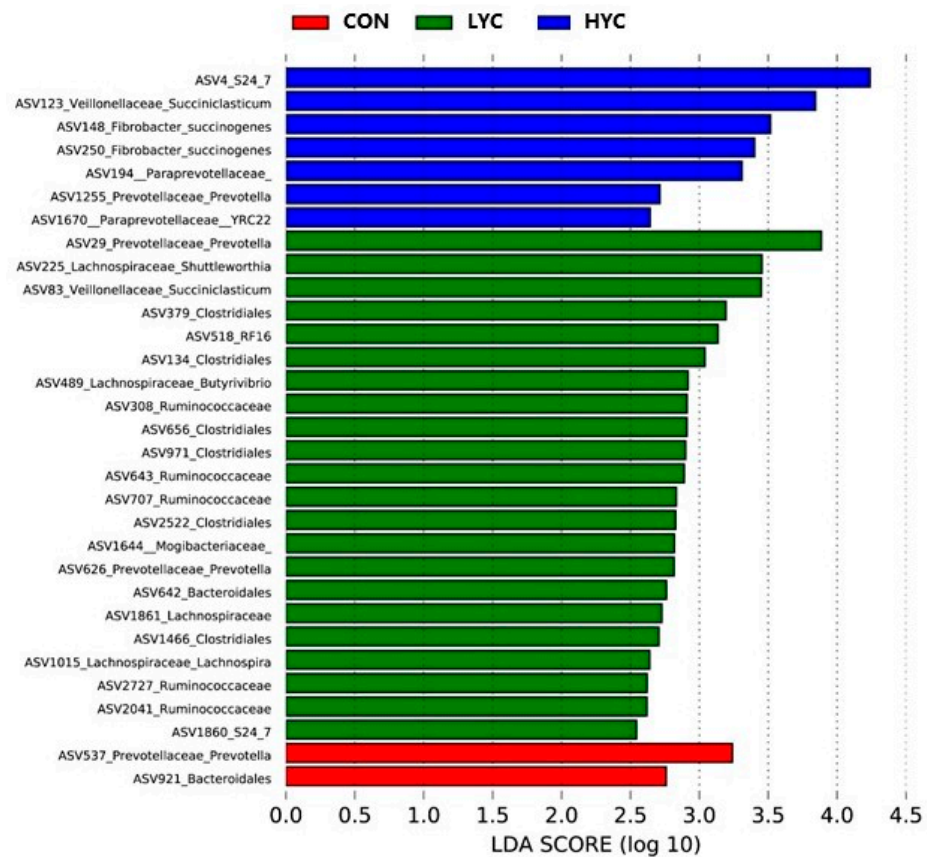
In terms of the rumen composition, at the phylum level, Firmicutes and Bacteroidetes were the dominant phyla across the three groups (Figure 3A). At the genus level, *Prevotella*, *Succinivlasticum*, *Treponema*, *Ruminococcus* and *Fibrobacter* were the top 5 genera. The relative abundance of *Prevotella* decreased with yeast culture supplementation (33.72% vs. 30.17% vs. 29.51%), while the abundance of *Succinivlasticum* and *Fibrobacter* showed an opposite pattern (5.46% vs. 7.11% vs. 9.52%; 3.24% vs. 2.75% vs. 5.47%) (Figure 3B).

To deeply understand the microbial composition affected by YC, linear discriminant analysis (LDA) effect size (LEfSe) analysis was performed to identify the featured microbiota at the ASVs level (Figure 4). *Prevotella* (ASV537) and *Bacteroidales* (ASV921) were identified as the featured bacteria in the CON group. *Prevotella* (ASV29), *Shuttleworthia* (ASV225), and *Succinivlasticum* (ASV83) were the featured bacteria in the LYC group. In the HYC group, the bacteria including S24\_7 (ASV4), *Succinivlasticum* (ASV123), *Succinogenes* (ASV148, ASV250), and *Prevotella* (ASV1255), were higher.



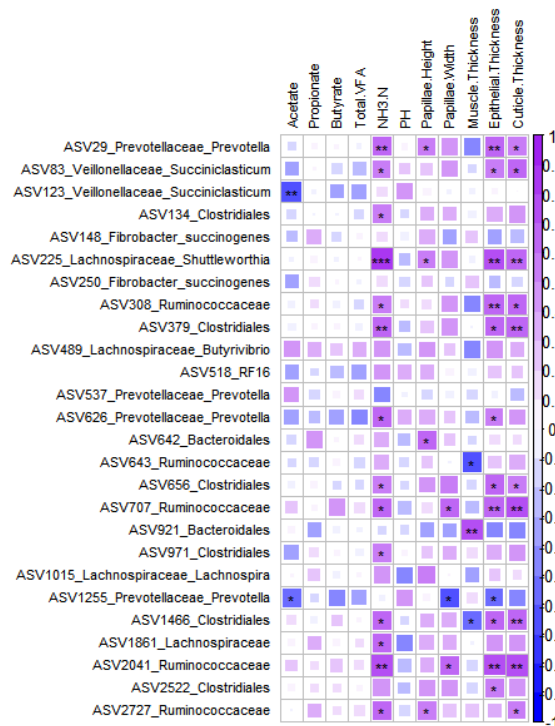


**Figure 3.** The composition of rumen microbiota at phyla level (A) and genus level (B). CON, control (basal diet supplied with 0 g/d yeast culture at the whole stage); LYC, low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively); HYC, high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively).



**Figure 4.** The featured bacteria identified by LEfSe analyses. CON, control (basal diet supplied with 0 g/d yeast culture at the whole stage); LYC, low dose yeast culture (basal diet supplied with 10 and 20 g/d yeast culture at the early and late stages, respectively); HYC, high dose yeast culture (basal diet supplied with 20 and 40 g/d yeast culture at the early and late stages, respectively). ASVs in this figure was significant ( $p < 0.05$ ) and had an LDA Score  $> 2$ , which was considered a significant effect size.

To detect the correlations between these featured bacteria and rumen phenotypes (VFAs, pH, NH<sub>3</sub>-N, rumen epithelial morphology) (Figure 5). Spearman correlation matrix was used to explore their relationships. Only *Prevotella* (ASV1255) and *Succiniclasticum* (ASV123) showed negative correlations with Acetate ( $p < 0.05$ ,  $|r| > 0.5$ ), while other bacteria did not construct significant correlations with VFAs. In contrast, many featured bacteria had associations with NH<sub>3</sub>-N and epithelial morphology. For instance, *Prevotella* (ASV29) and *Shuttleworthia* (ASV225) were positively correlated with NH<sub>3</sub>-N, papillae height, epithelial thickness and keratin layer thickness ( $p < 0.05$ ,  $|r| > 0.5$ ). *Clostridiales* (ASV379, ASV656, ASV1466), *Ruminococcaceae* (ASV308) showed positive association with NH<sub>3</sub>-N, epithelial thickness and cuticle thickness ( $p < 0.05$ ,  $|r| > 0.5$ ). In addition, *Ruminococcaceae* (ASV643) and *Clostridiales* (ASV1466) were negatively correlated with muscle thickness ( $p < 0.05$ ,  $|r| > 0.5$ ).



**Figure 5.** The associations between signature bacteria and rumen phenotypes. The colors of the squares range from blue to purple to indicate negative (closer to -1) to positive (closer to 1) correlations. The degree of significance is indicated by the symbol \*, 0.01 < \*  $p < 0.05$ ; 0.001 < \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

#### 4. Discussion

Yeast culture (YC) is a natural product produced from yeast fermentation [4]. Studies in ruminants showed that YC can improve feed digestibility, and promote feed intake and growth performance [10,15]. However, due to the different production processes of YC, the quality of products is also different. Currently, there is no unified supplemental amount in practical application. Different management modes and different dietary compositions lead to inconsistent application effects of YC in ruminants. In this experiment, although dietary YC did not improve the average daily gain and dry matter intake of early fattening stage Hu sheep, the feed-to-gain ratio was improved during the trial, which indicated the effects of YC on growth performance. Consistently, improved nutrient digestion, rumen NH<sub>3</sub>-N, and utilization efficiency of N in YC groups were observed. The development of the rumen epithelium was found in lambs supplemented with YC. Additionally, significant changes in rumen microbiota caused by YC supplementation were identified. Our results revealed

that YC application in meat sheep production is important and can be an alternative to antibiotics.

The effect of YC on ruminant performance is controversial. It was found that YC had no significant effect on the average daily feed intake, average daily gain and the feed-to-gain ratio of beef cattle when 40 g/d YC was added to the diet with the ratio of concentrate to roughage of 60:40 [32]. Similarly, Bayat [33] and Kawas [34] found that dietary YC had no effect on the growth performance of dairy cows and lambs. In this experiment, similar results were observed. However, after the ratio of concentrate to roughage was adjusted from 65:35 to 80:20 in the later fattening stage, LYC trended to increase dry matter intake, increased the average daily gain, and decreased the feed to gain ratio significantly, which is consistent with Tripathi and Karim [35]. In the whole fattening period, low supplemental level of YC improved the growth performance of fattening sheep, but high supplemental level showed no significant effect, which was similar to the dose effect of high supplemental level of YC in cows [19,20] and beef cattle [21], which may be related to the  $\beta$ -glucan concentration in YC. It was reported that the active components of the cell wall in YC were mainly composed of  $\beta$ -glucan (30–34%), mannan oligosaccharides (30%), glycoproteins (22%) and chitin [36]. The content of mannan oligosaccharides in the YC used in this study was  $\geq 1\%$ . According to the dose estimation, the content of  $\beta$ -glucan in LYC and HYC was 55.6 and 111.1 mg/kg in the early fattening stage, and 111.1 and 222.2 mg/kg in the late fattening stage, respectively. Zhou [37] found that adding 75 mg/kg  $\beta$ -glucan promoted the growth performance of calves, while added 100 and 200 mg/kg  $\beta$ -glucan decreased the growth performance of calves. These results indicate that the high supplemental level of YC may lead to the high content of  $\beta$ -glucan, which may have adverse effects on animal health. This may be due to overfeeding YC, whose active ingredients may stimulate phagocytosis of white blood cells and increase the production of reactive oxygen species, inflammatory mediators and cytokines [38].

As a safe and environmentally friendly feed additive, YC plays a certain role in improving the apparent digestibility of nutrients in mutton sheep. Malekxhahi et al. [17] reported that the apparent digestibility of CP and NDF of fattening sheep was significantly increased by adding 4 g/d YC in the high concentrate diet. Song et al. [15] found that fiber digestibility increased with the supplementation of YC. Similarly, Lei [39] and Jiang [40] reported that YC increased the apparent digestibility of fiber in beef cattle and dairy cows. In this study, dietary YC supplementation increased the digestibility of DM, OM, CP, NDF and ADF in group LYC and HYC compared with the control group. The common finding is an increase in fiber digestibility with the supplementation of YC, which may be due to an increase in the number of cellulose-degrading bacteria or the metabolites in YC that stimulate ruminal cellulolytic bacteria [41–43]. Another possibility is that YC may stimulate anaerobic fungi colonizing fiber to make the fiber more accessible to fiber-degrading bacteria rather than having a direct effect on the proliferation of these bacteria [44].

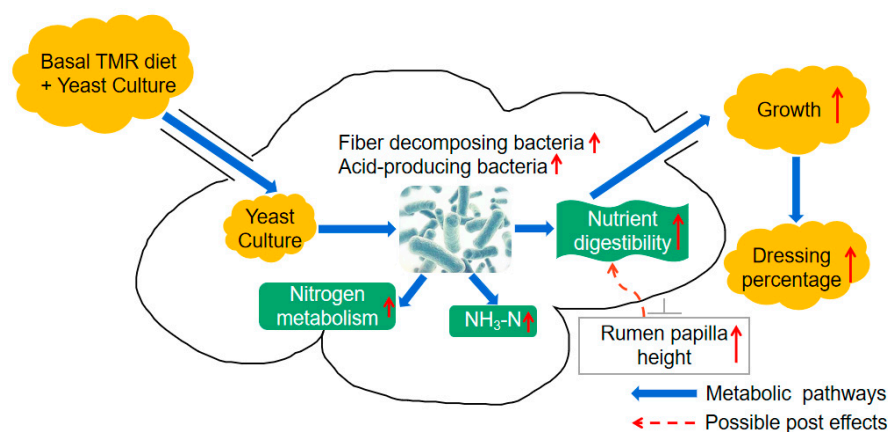
Part of the nitrogen intake is mainly used for protein synthesis, and the other part is discharged with the metabolic products, which constitute the balanced metabolism of nitrogen and the dynamic balance system of protein. This study found that rumen  $\text{NH}_3\text{-N}$ , the retained N, utilization efficiency of N and biological value of N were improved by YC. Similarly, Previous studies showed that YC supplementation in diet can increase the yield of rumen microbial protein, increase the available amino acids in the small intestine and promote protein deposition [45]. The increase in  $\text{NH}_3\text{-N}$  concentration indicates that rumen microbes have enhanced utilization of protein, which may be one of the important reasons why YC improves the growth performance of animals. Malekxhahi [17] and Cole [46] also found that dietary YC supplementation can significantly improve retained N. The reason may be that the addition of YC reduces the circulation of microbial nitrogen in the rumen, accelerates the emptying speed of nutrients to the rear intestinal segment, increases the circulation of total nitrogen in the duodenum, improves the absorption and utilization of nitrogen-containing substances, reduces the discharge of nutrients from feces and urine [47], and promotes the absorption and deposition of nitrogen. Although YC is widely used in

practical production, due to its complex composition, the nutritional regulation mechanism of YC on ruminants is not clear, which still needs to be further explored.

VFA is the product of carbohydrate degradation by rumen microorganisms in rumen and is an important energy source for ruminants. In this experiment, YC addition had no significant effect on TVFA and the proportion of acetic acid, propionic acid and butyric acid in TVFA in the rumen fluid of fattening sheep in each group. Previous studies on dairy cows [48,49], mutton sheep [17] and beef cattle [50] also obtained similar results. The addition of YC improved nutrient apparent digestibility, but rumen VFAs did not change significantly. This may be because there is a dynamic balance between rumen VFA production and absorption.

Rumen morphological development reflects the development of the animal body, and also affects the digestion and absorption of nutrients [51]. Our study found that the supplementation of yeast culture improved papillary height, rumen wall thickness, and muscular layer thickness of the rumen in fattening Hu Sheep. Lesmeister [52] found that the addition of yeast culture in a dairy calf starter at 2% slightly improves rumen development in dairy calves.  $\beta$ -glucan, an important component of YC, acts as a substrate for immune response. It may activate the humoral immune system to produce antibodies, which attach to the intestinal mucosa, protect intestinal villi from loss and promote villi development to a certain extent [53]. It is reported that yeast cell walls ( $\beta$ -glucan and  $\alpha$ -mannose) have protective effects on the intestinal mucosa, which competitively inhibit the binding of pathogenic bacteria to villi and prevent antigen and other substances from contacting with villi, thus promoting the normal development of villi [36].

The rumen microbiota is essential for the digestion of plant fibers and contributes to the variation of production and health traits in ruminants [54,55]. In this study, the diversity, richness, and structure of rumen microbiota were not affected by YC. A previous study related to yeast culture also found that the  $\alpha$  diversity and  $\beta$  diversity were not affected by the dietary treatments, which is consistent with our result [56]. This might be due to the rapid absorption of yeast culture in the rumen and the functional redundancy of rumen microbiota maintaining the steady state of ruminal microecology [57,58]. Moreover, the remarkable increases of several specific genera, including *Succiniclasicum* and *Fibrobacter* were caused by YC supplementation. Consistently, at the ASV level, *Succiniclasicum* (ASV83, ASV123) and the members under *Fibrobacter* (i.e., ASV148, ASV250) were increased in YC. These two genera are cellulolytic bacteria and short-chain fatty acid producers [59], which may explain the increased nutrient (especially for NDF and ADF) digestion in YC groups. Interestingly, the VFA in the rumen was not affected by YC, which might be due to the rapid absorption of VFA by developed rumen morphology in YC groups. Moreover, positive correlations between *Succiniclasicum* and  $\text{NH}_3\text{-N}$  were observed in our study, which was also found in a previous study [54]. The strong correlation between microbiota and  $\text{NH}_3\text{-N}$  represented that it is feasible to increase the utilization rate of rumen nitrogen by regulating the related microbiota. However, the roles of *Succiniclasicum* in nitrogen utilization were still unclear. A previous study assumed that *Succiniclasicum* restrains the nitrogen metabolism and absorption of the host. Future studies need to classify the effects of *Succiniclasicum* in rumen nitrogen. Taken together, we thought yeast culture supplementation increased the abundance of some nutrient decomposers without altering the rumen microbial community and these bacteria further promoted the rumen nitrogen cycle and rumen epithelium development (Figure 6).



**Figure 6.** Putative schematic illustration of supplementing yeast culture to promote rumen development and growth performance of fattening Hu sheep. ↑ increase.

## 5. Conclusions

In summary, the supplementation of yeast culture in diet could improve the growth and slaughter performance of fattening Hu sheep through improving nutrient digestion especially nitrogen utilization, rumen microbial environment and the development of rumen epithelium. Our findings provide insights that yeast (*Saccharomyces cerevisiae*) culture (one type of paraprobiotics) could be an alternative approach to improve ruminant performance.

**Author Contributions:** J.W. and G.Z. contributed to animal trial, data collection and evaluation, laboratory and statistical analysis, and writing; J.C. contributed statistical analysis and critical manuscript review; Y.Z. contributed to manuscript review and data visualization; N.Z. contributed to study design, feed formulation, and critical manuscript review. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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