Effects of dry yeast supplementation on growth performance, rumen fermentation characteristics, slaughter performance and microbial communities in beef cattle

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ABSTRACT
This study aimed to investigate the effects of active dry yeast (ADY) on growth performance, rumen microbial composition and carcass performance of beef cattle. Thirty-two finishing beef cattle (yak × cattle-yaks), with an average body weight of 110 ± 12.85 kg, were randomly assigned to one of four treatments: the low plane of nutrition group (control), low plane of nutrition group + ADY 2 g/head daily (ADY2), low plane of nutrition group + ADY 4 g/head daily (ADY4) and the high plane of nutrition group (HPN). Supplementation of ADY increased average daily gain compared to the control group. The neutral detergent fiber and acid detergent fiber apparent digestibility in HPN group was greater than that in control group. The propionic acid concentration in the rumen in ADY2, ADY4, and HPN groups was greater than that in control group. The Simpson and Shannon indexes in control and HPN groups were higher than that in ADY4 group. At the phylum level, the relative abundance of Firmicutes in the HPN group was higher than that in ADY4 group. The relative abundance of Ruminococcaceae UCG-002 in ADY4 group was higher than that in control and HPN groups. In conclusion, supplementation ADY 4 g/head daily shift the rumen microbial composition of beef cattle fed low plane of nutrition to a more similar composition with cattle fed with HPN diet and produce the similar carcass weight with HPN diet.

HIGHLIGHTS
- The ADY can improve the utilization of nitrogen and decrease the negative impact on the environment in beef cattle.
- Cattle fed low plane of nutrition diet supplemented with ADY 4 g/head daily increased growth performance.
- Supplementation ADY 4 g/head daily in low plane of nutrition diet might be produced comparable carcass weight to HPN diet.

KEYWORDS
Active dry yeast; beef cattle; production performance; rumen microbiota; carcass performance

Introduction
Animal husbandry is now a highly developed industry, which is mainly driven by population growth, income growth, and urbanization. The rapid development of the animal husbandry is due to the increased demand for livestock products such as meat, milk, and eggs. Animal production directly or indirectly contributes 9% of total CO2 emissions, 37% of methane emissions, and 65% of nitrous oxide emissions, resulting in global warming. The China’s soybean imports are driven by demand for animal protein and edible oils, but China’s livestock production and soybean meal demand were both adversely affected by two factors. Environmental pollution and shortage of protein feed resources are the main factors that restrict the sustainable development of animal husbandry in China.

Beef cattle are an important source of high-quality protein. As the population increases worldwide, the competition between people and livestock for land, water, and food is becoming increasingly fierce.

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especially in the beef cattle industry. The elevated feed efficiency in cattle could decrease feed consumption while maintaining greater or equal production performance. In addition, cattle with high feed efficiency can not only reduce methane emissions, but also decrease fecal excretion. Therefore, improving the feed efficiency can also reduce the negative impact on the environment.

Active dry yeast (ADY) is used in ruminant nutrition as a feed additive to prevent health disorders and to improve performance. It is suggested the role of ADY in ruminant diets was to decrease the risk of low ruminal pH, resulting in fewer liver abscesses. Jiao et al. reported that dietary supplementation of ADY (1.71 × 10^10 CFU/g) promoted the digestibility of nutrients in small intestine of beef cattle fed a high-grain diet. Still, no significant effect on growth performance and carcass quality were obtained. In addition, adding 1.5 g/d ADY (1.70 × 10^10 CFU/g) to the diet did not affect the growth performance of fattening beef cattle, but decreased the rumen acidosis incidence and liver abscess.

The ADY can be used as a feed additive for ruminants to optimize rumen fermentation characteristics and prevent health problems. There is, however, little research on the effects of supplementing ADY in a low plane of nutrition dietary on rumen microbial composition and carcass performance in beef cattle. We hypothesized that the addition of ADY to a low plane of nutrition diet could change rumen microbial composition and promote beef cattle carcass performance to a comparable carcass performance with beef cattle fed high plane of nutrition diet. Maybe one reason for this is that ADY improve ruminal fermentation and feed digestion, especially fiber digestion, which would improve growth performance.

Therefore, the objectives of this experiment were to determine the effects of supplementation of ADY to low plane of nutrition diet on the growth performance, rumen microbial composition, and carcass performance of beef cattle.

**Materials and methods**

**Experimental design, animals, and dietary treatments**

Thirty-two fattening beef cattle (yak ♂ × cattle-yak ♀), mean body weight (BW) ± standard deviation: 110 ± 12.85 kg, were randomly assigned to one of four groups with 8 replicates per group and one beef cattle per replicate. Three groups were designed as low plane of nutrition (control), and one group was regarded as a high plane of nutrition (HPN) diet treatment. Specific groups are as follows: basal diet as the control diet (no yeast), control diet + ADY (2g/head daily) (ADY2), control diet + ADY (4g/head daily) (ADY4), and HPN diet (no yeast). ADY is a typical product of *Saccharomyces cerevisiae*. The formula design referred to the feeding standard of beef cattle (NY/T 815-2004), in which the control diet (NEmf: 5.11 MJ/kg, CP: 12.00%) was designed according to the body weight of 150 kg and the daily weight gain of 1.1 kg. The HPN diet (NEmf: 5.87 MJ/kg, CP: 13.05%) was designed according to the body weight of 150 kg and the daily weight gain of 1.2 kg.

The beef cattle were given 10 days to adapt to the new environment and the treatment diets before starting the formal experiment. During the adaptation period, the beef cattle were vaccinated following standard operating procedures of the beef facility. The live yeast (2.0 × 10^10 CFU/g) was donated by Angel Yeast Co., Ltd, Yichang, Hubei, China. The ingredients and nutritional composition of the basal diet are shown in Table 1.

**Table 1. Ingredients and nutrient levels of diets (air-dry basis, %).**

| Items                  | Control | ADY2  | ADY4  | HPN  |
|------------------------|---------|-------|-------|------|
| Corn meal              | 26.50   | 26.50 | 26.50 | 39.75 |
| Wheat bran             | 3.50    | 3.50  | 3.50  | 5.25  |
| Soybean meal           | 2.00    | 2.00  | 2.00  | 3.00  |
| Cottonseed meal        | 5.30    | 5.30  | 5.30  | 7.95  |
| Urea                   | 0.20    | 0.20  | 0.20  | 0.30  |
| Soy oil                | 0.20    | 0.20  | 0.20  | 0.30  |
| CaCO₃                  | 0.70    | 0.70  | 0.70  | 1.05  |
| Ca(HPO₄)₂              | 0.20    | 0.20  | 0.20  | 0.30  |
| Na₂SO₄                 | 0.20    | 0.20  | 0.20  | 0.30  |
| NaHCO₃                 | 0.40    | 0.40  | 0.40  | 0.60  |
| NaCl                   | 0.50    | 0.50  | 0.50  | 0.75  |
| Compound premixb       | 0.30    | 0.30  | 0.30  | 0.45  |
| Distilled grain        | 30.00   | 30.00 | 30.00 | 30.00 |
| Hybrid giant napier    | 30.00   | 30.00 | 30.00 | 30.00 |
| Total (100%)           | 100     | 100   | 100   | 100   |
| Nutritional compositionc |         |       |       |       |
| Crude protein, %       | 12.00   | 12.00 | 12.00 | 13.05 |
| Acid detergent fiber, %| 27.55   | 27.55 | 27.55 | 20.46 |
| Neutral detergent fiber, % | 47.77  | 47.77 | 47.77 | 37.57 |
| Ca, %                  | 0.73    | 0.73  | 0.73  | 0.78  |
| P, %                   | 0.48    | 0.48  | 0.48  | 0.49  |

*a* Treatments: control, beef cattle fed low plane of nutrition diet without supplementing active dry yeast; ADY2 and ADY4, beef cattle fed 2 or 4 g/head daily active dry yeast on the control diet; HPN, beef cattle fed high plane of nutrition diet without supplementing active dry yeast.

*b* Supplied per kilogram of dietary DM: VA 8000 IU, VD3 2000 IU, VE 100 IU, Fe 50 mg, Cu 10 mg, Zn 60 mg, Mn 40 mg, Se 0.2 mg, I 0.5 mg, Co 0.1 mg.

*c* The value reported for the nutritional composition of diets was calculated based on the nutrient analysis from ingredient samples.

*d* NEmf was the net energy for maintenance and fattening, which was calculated reference to the equations on the Chinese Feeding standard of beef cattle (NY/T 815-2004).
Animal management and growth trial

The growth trial lasted 110 days, and all beef cattle were tethered using neck-straps in tie stalls and were individually fed with total mixed ration twice a day (0700 and 1700). During the trial (May–August, 2018), the average ambient temperature was 24.15 ± 4.78 °C, and the humidity was 64.88 ± 17.67%. The beef cattle were fed ad libitum with surplus feeds in trough anytime and had free access to freshwater.

Dry matter intake (DMI) was individually measured based on the differences between the amount of diet offered and refused daily. The body weight was measured before morning feeding on two consecutive days at the beginning and the final day of the 110-day trial. The average daily gain (ADG) was calculated as a difference between initial and final live weight and divided by 110 days. Feed conversion ratio (FCR) was calculated as the ratio of individual dietary DMI to ADG.

Blood sample collection and analysis

Blood samples were collected from the jugular vein of the beef cattle on the morning of the last day of the trial. Tubes were centrifuged at 1200 × g at 4 °C for 15 min, and the separated serum was stored at −20 °C to be used for measurement of urea, Glucose (GLU), Triglyceride (TG), Total cholesterol (TC), High-density lipoprotein (HDL), low-density lipoprotein (LDL), non-esterified fatty acid (NEFA), Glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT). All serum indexes were determined by a HITACHI-7020 Auto-Biochemical Analyzer.

Carcass traits and rumen fermentation characteristics

At the end of the experiment, all beef cattle were shipped to a commercial abattoir for slaughter. Hot carcass weight (with kidneys removed), net meat weight, dressing percentage, and net meat percentage were recorded. Dressing percentage was calculated as hot carcass weight (HCW) divided by final BW × 100%. Net meat percentage was calculated as net meat weight divided by final BW × 100%.

Once slaughtered, the pH of the rumen content was directly measured using a glass electrode pH meter. The rumen fluid was removed from the rumen, filtered with four layers gauze, and placed in a 10 mL centrifuge tube. The collected samples were immediately frozen in liquid nitrogen, and then stored at −80 °C until further analysis. The remaining portion was stored at −20 °C for volatile fatty acid (VFA), ammonia nitrogen (NH3-N), and microbial crude protein (MCP) assay.

Digestion trial

During the last 4 days (107 – 110 d) of the trial, all feces were collected for 24 h per cattle. The collected feces were weighed and recorded every day. Apparent nutrient digestibility was determined by total collection of feces. Approximately, 100 g of the fresh fecal sample from each cattle per day was mixed with 20 mL 10% sulfuric acid and stored at −20 °C for further chemical analysis.

Analytical methods

All chemical analyses of samples were conducted in duplicate. The determination for moisture (method 934.01), CP (method 984.13) and EE (method 920.39) was referenced to standard procedures (AOAC, 2000). The method described by Van Soest et al.15 was used to determine NDF and ADF, both NDF and ADF were corrected by its ash content. The concentrations of NH3-N were measured using phenol-sodium hypochlorite colorimetric.16 The concentrations of VFAs (acetate, propionate, and butyrate) were measured using an HPLC organic acid analysis system (Shimadzu, Kyoto, Japan). The supernatant was shaken with cation exchange resin (Amberlite, IR 120B H AG, ORGANO CORPORATION, Tokyo, Japan) and centrifuged at 3500 × g for 5 min. The supernatant was passed through a 0.45 μm filter under pressure, and the filtrate was then injected into an HPLC system. The analytical conditions were as follows: column, SCR-101H (7.9 mm × 30 cm) attached to a guard column SCR(H) (4.0 mm × 5 cm) (Shimadzu); oven temperature, 40 °C; mobile phase, 4 mM p-toluenesulfonic acid aqueous solution; reaction phase, 16 mM Bis-Tris aqueous solution containing 4 mM p-toluenesulfonic acid and 100 μM ethylene-diaminetetra-acetic acid; flow rate of the mobile and reaction phase, 0.8 mL/1 min; detector, conductivity detector (CDD-6A, Shimadzu). The MCP concentration was quantified using a BCA Protein Assay Kit manufactured by Nanjing Jiancheng Bioengineering Institute.

Bioinformatics analyses (DNA extraction, sequencing)

Total genomic DNA was extracted from the rumen fluid samples using a DNeasy Power Soil Kit (Qiagen,
Table 2. Effect of dietary supplementation of active dry yeast on growth performance of beef cattle.

| Items                        | Control   | ADY2     | ADY4     | HPN      | SEM      | p-Value |
|-----------------------------|-----------|----------|----------|----------|----------|---------|
| Initial body weight (kg)    | 107.44    | 108.50   | 109.06   | 117.25   | 4.547    | .415    |
| Final body weight (kg)      | 215.72b   | 221.82b  | 224.50b  | 237.29a  | 4.661    | .021    |
| Average daily gain (kg/day) | 0.96b     | 1.03b    | 1.05b    | 1.09a    | 0.011    | <.001   |
| Dry matter intake (kg/day)  | 5.60b     | 5.72b    | 5.97b    | 6.95a    | 0.275    | .017    |
| Feed conversion ratio       | 5.74      | 5.67     | 5.68     | 6.35     | 0.267    | .262    |

Different superscript letters within a row represent significant differences (p<.05).

*Treatments: control, beef cattle fed low plane of nutrition dietary without supplementing active dry yeast; ADY2 and ADY4, beef cattle fed 2 or 4 g/head daily active dry yeast on the control diet; HPN, beef cattle fed high plane of nutrition dietary without supplementing active dry yeast.

Valencia, CA, USA) according to the manufacturer’s instructions. DNA concentration and quality were checked using a Nano Drop Spectrophotometer. DNA was diluted to 10 ng/µL using sterile ultrapure water and stored at −80°C for downstream use. PCR Primer 16S V4: 515F (5’-GTGCGCACG MGCCGC GGTAA-3’) and 806R(5’-GGACTACH VGGGTWTC TAAT-3’) 16S rRNA genes were amplified using the specific primer with 12 nt unique barcode.17,18 The PCR mixture (25 µL) contained 1× PCR buffer, 1.5 mM MgCl₂, each deoxynucleoside triphosphate at 0.4 µM, each primer at 1.0 µM, 0.5 U of KOD-Plus Neo (TOYOBO) and 10 ng template DNA. The PCR amplification program consists of initial denaturation at 94°C for 1 min, followed by 30 cycles (denaturation at 94°C for 20 s, annealing at 54°C for 30 s, and elongation at 72°C for 30 s), and a final extension at 72°C for 5 min. Three replicates of the PCR reactions for each sample were combined, and the PCR products were purified using Gel Extraction Kit (Omega Bio-Tek, USA). DNA was quantified using Qubit 2.0 Fluorometer (Thermo Scientific). The PCR products from different samples were pooled with equal molar amounts. Library preparation and sequencing libraries were generated using TruSeq DNA PCR-Free Sample Prep Kit following the manufacturer’s recommendations, and index codes were added. The library quality was assessed on the Qubit®2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was applied to paired-end sequencing (2 x 250 bp) with the Illumina Hiseq apparatus at Rhonin Biosciences Co., Ltd.

To maintain the Phred quality score of the reads, low-quality sequences were trimmed using Trimmomatic and Usearch before assembly with the paired-end assembler.19,20 The minimum abundance cutoff was set at 0.5% abundance. UPARSE was used to cluster the sequences into OTUs (operational taxonomic units) as well as choose the representative sequence of each OTU at 97% similarity followed by the removal of chimeras and singletons by UCHIME.21 Four alpha diversity indices (Simpson, Shannon-Wiener, Chao1, and phylogenetic distance) were calculated. Principal component analysis (PCA) was applied to decrease the dimensions of original community data. The OTU table, rarefaction dilution curves and beta diversity analysis were performed using R programming tools (version 3.3.0).

Statistical analysis

The Mixed model (SAS 9.4 Institute Inc., Cary, NC, USA) included treatment as fixed effect and the beef cattle as random effect. For repeated measures, various covariance structures were tested with the final choice exhibiting the lowest value for Akaike’s information criteria. Differences between treatments were declared significant at p < .05.

Results

Growth performance

The effect of dietary supplementation of ADY on the growth performance of beef cattle is shown in Table 2. The HPN group had a greater final body weight than that in the control group (p=.021). The yeast addition had no significant effect on final body weight compared with control (p>.05). However, the ADY group also had no difference with HPN (p>.05). Compared to control, the supplementation of ADY increased ADG (p<.001), and no difference was obtained between ADY4 and HPN group (p>.05). The DMI of HPN group was greater than that in control and ADY2 groups (p=.017), and no difference was observed between ADY and control (p>.05), and the DMI of ADY4 group had no difference with HPN group (p>.05). No significant effect was detected on FCR among the four groups (p=.262).

Blood indexes

The effect of dietary supplementation of ADY on blood biochemical indices of beef cattle is presented in Table 3. Compared to the HPN group, the
The concentration of urea in the ADY4 group was lowered ($p < .005$). However, the ADY4 group was not significantly different from the control and ADY2 ($p > .05$). The concentration of LDL in ADY4 group was greater than that in HPN group ($p = .033$), no difference was observed among control and ADY group. The activity of GPT in control and ADY4 groups was greater than that in HPN group ($p = .001$), no difference was obtained between ADY4 and HPN groups ($p > .05$). The ADF ($p = .043$) and NDF ($p = .022$) digestibility in HPN group were greater that in control group, and no difference was obtained among the ADY2, ADY4, and HPN groups ($p > .05$). Supplementation of ADY and HPN diet had no significant effect on DM and EE digestibility ($p > .05$).

### Nutrient apparent digestibility

The effect of dietary supplementation of ADY on nutrient apparent digestibility of beef cattle is displayed in Table 4. The CP digestibility in HPN group was higher than that in control and ADY2 groups ($p = .001$), and no difference was obtained between ADY4 and HPN groups ($p > .05$). The ADF ($p = .043$) and NDF ($p = .022$) digestibility in HPN group were greater that in control group, and no difference was obtained among the ADY2, ADY4, and HPN groups ($p > .05$). Supplementation of ADY and HPN diet had no significant effect on DM and EE digestibility ($p > .05$).

### Ruminal fermentation characteristics

Table 5 shows the effect of dietary supplementation of ADY on ruminal fermentation parameters of beef cattle. The concentration of NH$_3$-N was lowered by ADY supplementation ($p = .003$) compared with that control. The levels of total VFA ($p = .001$) and acetic acid ($p = .019$) in HPN group were greater than that in ADY4 group. However, no difference was obtained among the control, ADY2, and ADY4 groups ($p > .05$). The concentration of propionic acid in ADY2, ADY4, and HPN groups was greater than that in control group ($p < .001$). No significant difference was observed in pH, MCP, and acetic acid/propionic acid ($p > .05$).

### Carcass performance

As shown in Table 6, the live weight before the slaughter of HPN group was higher than that in control group ($p = .023$), but there was no significant difference observed among ADY2, ADY4, and HPN groups. The carcass weight of HPN group was significantly higher than that in control and ADY2 groups ($p = .001$). However, no significant difference was obtained between ADY4 and HPN groups. The net

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### Table 3. Effect of dietary supplementation of active dry yeast on blood biochemical indices of beef cattle.

| Treatmentsa | Control | ADY2 | ADY4 | HPN | SEM | p-Value |
|-------------|---------|------|------|-----|-----|---------|
| Urea (mm/L) | 5.31ab  | 5.17b | 5.03ab | 5.45a | 0.079 | .005 |
| GLU (mm/L)  | 2.85    | 3.21 | 2.98 | 3.17 | 0.153 | .345 |
| TG (mm/L)   | 0.21    | 0.19 | 0.17 | 0.15 | 0.012 | .054 |
| TC (mm/L)   | 2.22    | 2.36 | 2.56 | 2.23 | 0.172 | .569 |
| HDL (mm/L)  | 1.04    | 1.16 | 1.17 | 0.99 | 0.139 | .168 |
| LDL (mm/L)  | 0.20ab  | 0.21b | 0.26  | 0.17pb | 0.019 | .033 |
| NEFA (mm/L) | 0.05    | 0.05 | 0.05 | 0.05 | 0.006 | .715 |
| MCP (mg/dL) | 146.07b | 147.32 | 147.86b | 146.53a | 6.016 | .001 |
| GOT (U/L)   | 54.17   | 50.00 | 47.33 | 44.33 | 2.560 | .059 |
| GPT (U/L)   | 23.00ab | 20.33b | 24.00ab | 17.50ab | 1.052 | .001 |

Different superscript letters within a row represent significant differences ($p < .05$).

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### Table 4. Effect of dietary supplementation of active dry yeast on nutrient apparent digestibility of beef cattle.

| Treatmentsa | Control | ADY2 | ADY4 | HPN | SEM | p-Value |
|-------------|---------|------|------|-----|-----|---------|
| DM (%)      | 67.50   | 70.23 | 70.16 | 68.92 | 1.144 | .314 |
| CP (%)      | 52.24b  | 52.71b | 53.48ab | 54.45a | 0.344 | .001 |
| EE (%)      | 67.26   | 70.60 | 70.78 | 67.59 | 1.512 | .228 |
| ADF (%)     | 43.71b  | 46.55ab | 46.85ab | 50.59ab | 1.565 | .043 |
| NDF (%)     | 46.60b  | 51.42ab | 51.67ab | 52.58ab | 1.344 | .022 |

Different superscript letters within a row represent significant differences ($p < .05$).

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### Table 5. Effect of dietary supplementation of active dry yeast on ruminal fermentation parameters of beef cattle.

| Treatmentsa | Control | ADY2 | ADY4 | HPN | SEM | p-Value |
|-------------|---------|------|------|-----|-----|---------|
| pH          | 5.85    | 6.18 | 6.33 | 6.21 | 0.057 | .185 |
| NH$_3$-N (mg/dL) | 7.70b  | 7.42b | 7.38b | 7.45ab | 0.065 | .003 |
| MCP (mg/dL) | 260.07  | 217.32 | 223.36 | 220.85 | 6.121 | .282 |
| Total VFA (mm/L) | 74.82ab | 75.13ab | 73.12b | 76.63a | 0.081 | .001 |
| Acetic acid (mm/L) | 53.01ab | 52.58ab | 49.99b | 53.56a | 0.775 | .199 |
| Propionic acid (mm/L) | 12.58ab | 13.67a | 14.02a | 13.82a | 0.018 | .001 |
| Butyric acid (mm/L) | 9.23    | 8.88 | 9.11 | 9.24 | 0.167 | .610 |
| Acetic acid/Propionic acid | 4.21   | 3.83 | 3.52 | 3.88 | 0.092 | .235 |

Different superscript letters within a row represent significant differences ($p < .05$).

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### Table 6. Carcass performance

As shown in Table 6, the live weight before the slaughter of HPN group was higher than that in control group ($p = .023$), but there was no significant difference observed among ADY2, ADY4, and HPN groups. The carcass weight of HPN group was significantly higher than that in control and ADY2 groups ($p = .001$). However, no significant difference was obtained between ADY4 and HPN groups. The net
meat weight of HPN group was significantly greater than that in the other three groups \( (p < .001) \). No significant difference was obtained in dressing percentage \( (p = .448) \) and net meat percentage \( (p = .346) \) among the four groups.

Rumen bacterial communities

Rarefaction curves obtained from bacterial sequences is shown in Fig. 1. As can be seen from Fig. 1, the curve of each sample was nearly asymptotic, which indicated that its sequencing depth has substantially covered the entire microbial population. In total, 804,875 bacterial sequences were produced, and based on 97% sequence similarity, the sequences were clustered into 32,215 OTUs. Distribution of valid sequences and OTUs were presented in Supplementary Table S1. The alpha diversity indexes (Chao1, phylogenetic distance, Simpson and Shannon) of the rumen bacterial community in each group are presented in Fig. 2. The Chao1 index and the phylogenetic distances index did not differ among the four treatments, but the Simpson \( (p = .014) \) and Shannon \( (p = .045) \) indices of the control and HPN group were greater than ADY4 group. At the genus level, ADY4 and HPN group can be clustered together with a distance of 0.04, and mingled with ADY2 with a distance of 0.08, then minged with the control with a distance of 0.16, indicating that the microbial composition of ADY4 and HPN have certain similarities (Fig. 3).

The bacteria with relatively high abundance in rumen fluid are shown in Table 7. At the phylum level, the relative abundance of Bacteroidetes and Firmicutes was dominating, account for about 85% of the total bacteria. Compared with the HPN, the relative abundance of Firmicutes in ADY4 group was lowered \( (p = .015) \). At the genus level, the relative abundance of Ruminococcaceae UCG-002 of ADY4 was greater than that in control and HPN groups \( (p = .004) \). In addition, the relative abundance of Ruminococcaceae UCG-002 in ADY2 group was also greater than that in control group \( (p = .004) \).

Discussion

As a kind of yeast preparation, ADY is widely used in ruminant animals. Most studies have shown that the addition of yeast preparation to beef cattle diets could improve growth performance.\(^{21,22}\) In present study, cattle fed low plane of nutrition diet supplemented with ADY 4 g/head daily significantly increased ADG, and achieved to a comparable level with cattle fed with a high plane of nutrition diet, which was in agreement with the research of Geng et al.\(^ {21}\) Previous studies have also shown that the supplementation with ADY could increase DMI of ruminants.\(^ {23,24}\) However, some studies showed that DMI could be decreased by ADY supplementation.\(^ {25,26}\) Our results indicated that dietary supplementation with ADY had no significant effect on DMI, which was consistent with the result reported by Vyas et al.\(^ {27}\) The reason for the inconsistencies may be due to the different yeast strains and ration used in the trials.\(^ {28}\) However, the present study found that the DMI of the HPN group was significantly higher than the control group. Yuangklang et al.\(^ {29}\) reported that the DMI of beef cattle increased considerably due to the increase in dietary protein. Arriola et al.\(^ {30}\) also reported that increase the amount of concentrate in diets could increase the feed intake of the cattle, which was consistent with our study.

So far, there are few studies focused on the effect of ADY on carcass performance of beef cattle. Gleghorn et al.\(^ {31}\) reported that with the increase of CP concentrations in the diet, the carcass weight, dressing percentage, and net meat production increased. Our study showed that the addition of ADY increased the final body weight so that its carcass weight increased to a comparable level with the HPN group accordingly, and no significant difference between ADY4 and HPN groups was detected, which was consistent with the previous studies.\(^ {22,23}\)

The diagnostic results of serum biochemical analysis provided information on the function and nutritional status of almost all organs, as well as disease...
progress, which are closely related to the performance of animals. In present study, all serum indexes were not significantly influenced by ADY supplementation, however, the serum urea concentration in HPN group was significantly higher than ADY4 group, which might indicate that the HPN group cattle could not utilize nitrogen as efficiently as ADY4, and another reason is that dietary CP concentration in HPN group was higher than other groups. Geng et al. reported that the urea content of finishing bulls could not be affected by ADY supplementation, which was consistent with the present study. GPT and GOT are two important enzymes of liver function, and their activity has a great relationship with the growth and development performance of animals. If the activity of two enzymes in the blood were elevated, it might be caused by liver damage. In this study, compared with the control group, the supplementation of ADY had no significant effects on serum GOT and GPT, indicating that ADY had no adverse effect on the liver function. Different from this, reports showed that the addition of ADY could result in fewer liver abscesses.

Our results showed that the CP, NDF, and ADF digestibility in HPN group was significantly greater than the control group. However, the yeast addition had no significant effect on CP, NDF and ADF digestibility, though the NDF and ADF digestibility were increased numerically but not statistically difference was obtained. Vyas et al. reported that the addition of probiotics (Propionibacterium) to beef cattle diet had no significant effect on the digestibility of DM, NDF, and ADF. Sanchez et al. also reported that the addition of probiotics (Propionibacterium acidipropionici P1691) to the low-quality coarse fodder had no significant effect on the digestibility of NDF in beef cattle. These results were consistent with our study. It is suggested that adding ADY to the diet could change the composition of rumen bacteria, thereby increasing the digestibility of fiber. Although the digestibility of NDF and ADF in our study were not increased, probably due to the measurement of total tract apparent digestibility of nutrients, while Jiao et al. reported that the addition of ADY could increase the nutrients digestibility in the small intestine.

NH₃-N is the main precursor for microbial protein synthesis in the rumen. The addition of ADY in the present study decreased NH₃-N concentration. This might indicate that ADY help rumen bacteria effectively synthesize MCP by using NH₃-N. However, Vyas et al. reported that the addition of ADY (4 g/head daily) increased the concentration of NH₃-N, probably due to the stimulation of the proteolytic
activity of ruminal bacteria with supplementation of yeast, resulting in deamination of amino acids and production of NH₃ in the rumen. VFA in the rumen, acting as an important source of energy for ruminants, is mainly derived from the fermentation of carbohydrates, which also provides energy for the synthesis of microorganisms in the rumen. The previous study reported that ADY could not affect the total VFA production in the rumen. In contrast, other studies have shown that yeast preparation could increase the proportion of propionic acid, while decreased the proportion of acetic or increased the proportion of acetic acid. In present study, there was no difference in the concentration of rumen total VFA after ADY supplementation, indicating that changes in microbial composition and fermentation capability are too subtle to cause changes in total VFA concentration. However, the VFA concentration in the HPN group was significantly greater than that of the ADY4 group, indicating that there was a gap in the amount of VFA production between the HPN dietary group and the yeast supplementation group. Interestingly, the addition of ADY increased the propionic acid molar proportion, and this means that the cattle might utilize energy more efficiently because cattle mainly produce glucose by gluconeogenesis using propionic acid as a substrate. Propionic acid production is accompanied by less production of carbon dioxide and methane than does the production of acetic acid.

In this trial, with the increase of ADY addition level, the relative abundance of Bacteroides increased...
and the relative abundance of Firmicutes decreased, but the difference was not significant. Of note, the relative abundance of Firmicutes in HPN group was significantly higher than that in ADY4 group, which may be related to greater consumption of concentration by beef cattle under the condition of confinedness, which increased the relative abundance of Firmicutes. At the genus level, *Prevotella* is the dominating flora in the beef cattle rumen in the four treatment groups, and followed by *Succiniclasticum* and *Prevotellaceae UCG-004* which was consistent with Myer et al.\(^41\) The dietary yeast supplementation increased the relative abundance of *Ruminococcaceae UCG-002*, which was consistent with previous studies claiming *Saccharomyces cerevisiae* favor the establishment of cellulolytic bacteria in the rumen.\(^42\)–\(^44\) The *Ruminococcaceae* plays key roles in cleaving the cellulose and hemicellulose components of plant material.\(^45\) The increased bacterial number resulting in improved fiber digestibility has been one of the beneficial effects of yeast supplementation in ruminants.\(^46\) Patra et al.\(^47\) reported that the reduction in the relative abundance of *Ruminococcaceae* would lead to a decrease in fiber digestibility. We observed increased NDF and ADF digestibility at least more than 7% with ADY supplementation, though it was not statistically significant. The increased fibrous material digestibility may be attributed to the increased relative abundance of *Ruminococcaceae UCG-002*.

### Table 7. Bacteria with relatively high abundance and significant difference in rumen fluid (%)

| Items                  | Treatments\(^a\) | SEM | \(p\)-Value |
|------------------------|-------------------|-----|-------------|
| Phylum                 | control ADY2 ADY4 HPN |     |             |
| Bacteroidetes          | 55.92 58.83 60.47 54.81 2.162 | .258 |
| Firmicutes             | 30.47\(a\) 26.67\(b\) 23.58\(a\) 33.75\(a\) 2.098 | .015 |
| Euryarchaeota          | 3.93 4.27 4.89 3.61 1.537 | .943 |
| Proteobacteria         | 2.78 2.74 3.17 2.34 0.468 | .674 |
| Kiritimatiellaeota     | 1.56 1.16 1.68 1.03 0.399 | .684 |
| Genus                  |                   |     |             |
| Prevetell 1            | 12.26 18.16 13.56 19.40 3.445 | .504 |
| Succiniclasticum       | 12.52 12.11 9.07 12.73 1.760 | .438 |
| Prevotellaceae UCG-004 | 5.24 5.53 3.58 4.47 0.851 | .389 |
| Rikenellaceae RC9 gut group | 5.65 5.26 5.05 3.08 0.719 | .148 |
| Methanobrevibacter      | 3.27 3.94 4.36 3.55 1.580 | .965 |
| Bacteroides            | 2.42 2.84 2.36 2.60 0.191 | .317 |
| Prevotellaceae UCG-003  | 1.66 2.84 1.42 1.91 0.254 | .464 |
| Ruminococcaceae UCG-002 | 0.79\(b\) 2.36\(b\) 2.50\(a\) 1.03\(d\) 0.356 | .004 |
| Christensenellaceae R-7 group | 1.86 0.73 1.03 1.21 0.281 | .401 |
| Prevotellaceae UCG-001  | 1.16 1.03 1.03 1.46 0.185 | .340 |
| Ruminococcaceae UCG-005 | 1.11 1.13 0.87 1.21 0.275 | .844 |
| p-1088-a5 gut group     | 1.49 0.78 1.39 1.17 0.342 | .494 |
| Prevetell 9             | 1.45 0.89 0.74 0.85 0.351 | .498 |

Different superscript letters within a row represent significant differences \((p < .05)\).
The abundance cutoff in this table was set at 0.5%.

\(^a\)Treatments: control, beef cattle fed low plane of nutrition dietary without supplementing active dry yeast; ADY2 and ADY4, beef cattle fed 2 or 4 g/head daily active dry yeast on the control; HPN, beef cattle fed high plane of nutrition dietary without supplementing active dry yeast.

Conclusions

Supplementation ADY 4 g/head daily increased the relative abundance of genus *Ruminococcaceae UCG*
002 in the rumen and the yield of propionate. Supplementation ADY 4 g/head daily in low plane of nutrition diet produced comparable carcass weight to HPN diet.

Ethical approval
All techniques concerning animal care and management were in harmony with and permitted (Code-SYXK-Chuan-2014-184) by Sichuan Agricultural University, Chengdu, Sichuan, China.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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