The objective of this experiment was to investigate the potential benefits of active dry yeast (ADY) on the growth performance, rumen fermentation, nutrient digestibility, and serum parameters of weaned beef calves. Thirty Simmental crossbred male calves (body weight = 86.47 ± 4.41 kg and 70 ± 4 d of age) were randomly divided into 2 groups: control (CON) (fed basal ration) and ADY (fed basal ration and 5 g/d ADY per calf). The dietary concentrate-to-roughage ratio was 35:65. All the calves were regularly provided rations 3 times a day at 07:00, 13:00, and 19:00 and had free access to water. The experiment lasted for 60 d. The average daily gain of ADY group was higher (P = 0.007) than that of the CON group, and the ratio of feed intake to average daily gain in the ADY group was reduced (P = 0.022) as compared to the CON group. The concentration of ruminal ammonia-N was higher (P = 0.023) in the CON group than that in the ADY group, but an opposite trend of microbial protein was found between the 2 groups. Also, the ruminal concentrations of propionate and butyrate were higher (P < 0.05) in the ADY group than those in the CON group. Calves fed ADY exhibited higher (P < 0.05) crude protein and neutral detergent fiber digestibility. Supplementation of ADY increased (P < 0.05) the contents of glucose, glutathione peroxidase, superoxide dismutase, immunoglobulin A, immunoglobulin M, and interleukin 10 in the serum of calves, but an opposite trend was observed in malondialdehyde, interleukin 1 beta, and tumor necrosis factor alpha contents between the 2 groups. In conclusion, dietary supplementation with ADY could improve the growth performance, rumen fermentation, nutrient digestibility, antioxidant ability, and immune response of weaned beef calves.

© 2021 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Calves are the future of beef cattle farming, and this stage is vital to the production performance of beef cattle in the future. Early weaning of calves can improve the reproductive performance of adult cattle and speed up the ruminal development of calves (Ma et al., 2020). In recent years, early weaning was commonly conducted in rearing calves of commercial beef cattle farms. However, calves undergo changes in feeding methods and feedstuffs during weaning, thus weaning is a source of stress for calves (Lynch et al., 2010). Furthermore, the development of digestive organs, especially the rumen, of weaned calves, is immature and sensitive to changes in external environment. For this reason, weaned calves
are susceptible to pathogenic bacteria, which can lead to gastrointestinal dysfunction (Meale et al., 2017). More importantly, weaned calves lack a mature immune system that is involved in the modulation of immunity to maintain healthy growth. A previous study reported that weaning could trigger inflammatory responses of Holstein calves and cause the release of pro-inflammatory cytokines, such as interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) (Rezaadzeh et al., 2019). Ultimately, those negative effects can reduce the production performance of adult cattle. Thus, in order to raise healthy calves under a modern large-scale beef cattle farming development process, relieving the weaning stress of beef calves is an immediate problem to be solved.

Active dry yeast (ADY), derived from Saccharomyces cerevisiae, is widely used as a feed additive in animal rations. The ADY is rich in viable yeast and has positive effects on the healthy growth of dairy cows (Uyeno et al., 2016; Malekkhahi et al., 2016). The Saccharomyces in ADY belong to facultative anaerobe and can consume oxygen, which is conducive to maintaining the anaerobic environment in the rumen, thereby inhibiting the growth and reproduction of harmful bacteria (e.g. Escherichia coli and Salmonella) and providing a favourable fermentation environment (Alugongo et al., 2017). Further, the Saccharomyces can promote the growth and metabolism that ADY utilizing bacteria in the rumen and even promote the conversion from lactate to propionate so that calves can obtain more energy from their diets (Pomezky et al., 2018). In the cattle farming industry, ADY has been confirmed to be an effective alternative to in-feed antibiotics and could increase the concentration of glucose (GLU) in the serum (Ran et al., 2018). In adult ruminants (e.g. dairy cows, yaks, and goats), some studies have shown that dietary ADY supplementation was beneficial for milk production (Moallem et al., 2009), nutrient digestibility (Dehghan-Banadary et al., 2013), rumen fermentation (Desnoyers et al., 2009), and immunity (Hu et al., 2019). Other research found that ADY supplementation could increase the dry matter intake (DMI) and average daily gain (ADG) of neonatal calves with failure of passive immunity (Galvão et al., 2005). However, the effects of dietary supplementation with ADY on rumen fermentation and immune response of weaned beef calves have not been fully evaluated.

The rumen is the main digestive and absorptive organ of ruminants for volatile fatty acids (VFA) and excess ammonia-N. The healthy development of the rumen plays an essential role in the normal growth of calves. Ruminal pH, ammonia-N, VFA, and microbial protein (MCP) concentrations are the key indexes used to assess ruminal health. As an important energy substrate produced by microbial fermentation in the rumen, VFA can provide 80% of the energy requirements of ruminants (Gabel and Sehested 1997). The development of the rumen is closely associated with the production and absorption of VFA, especially butyrate and propionate concentrations (Lesmeister et al., 2004). Previously, a study has verified that ADY could regulate the production of VFA (Desnoyers et al., 2009). As mentioned above, weaning can reduce the immunity and cause inflammatory responses in calves. Previous research in calves, yaks, and steers found that dietary supplementation of yeast products could enhance immunity and alleviate the inflammatory response (Alugongo et al., 2017; Hu et al., 2019; Kayser et al., 2019). Although ADY can improve growth performance, rumen fermentation, and nutrient digestibility of animals, study of the role of ADY as a feed additive in young ruminants, especially weaned beef calves, remains scarce. Based on previous research, we hypothesized that ADY may be an effective feed additive to improve the growth performance of weaned beef calves. Therefore, this study was performed to investigate the effects of ADY on the growth performance, rumen fermentation, nutrient digestibility, and serum parameters of weaned beef calves.

2. Materials and methods

2.1. Ethics statement

All procedures involving animal care and management were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (Chengdu, Sichuan, China).

2.2. Experimental design, diet, and management

This study was conducted at a commercial beef cattle farm (Zhangye, Gansu, China). Thirty healthy Simmental crossbred male calves after weaning were used in this study. The selected calves (BW = 86.47 ± 4.41 kg; age = 70 ± 4 d) were marked with ear tags and randomly assigned to 2 treatment groups: control (CON) (fed basal ration) and ADY (fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China; viable yeast ≥ 2 × 10¹⁰ CFU/g) per calf). The level of ADY was based on the manufacturer’s recommendation for ruminants.

Calves in the 2 groups were housed in 30 pens with 1 animal in each pen (2 m × 4 m). All the calves were regularly provided rations 3 times each day at 07:00, 13:00, and 19:00 and had free access to water. A 10-d adaptive phase was followed by 60 d of experimental period. All animals were fed the total mixed ration, and ADY was supplemented to the basal ration. The basal diet was formulated according to the Chinese Feeding Standard of Beef Cattle (NY/T 815-2004). The concentrate-to-roughage ratio was 35:65. The roughages were composed of alfalfa hay and wheat straw. The concentrates mainly included corn flour, wheat bran, cottonseed meal, soybean meal, fermented distiller’s grains, and premix. The feed compositions and nutrient levels of basal diet are described in Table 1.

2.3. Sample collection

The BW of all calves was measured on d 0 and 60 before morning feeding, and the ADG was calculated from initial and final BW. Accurate feed consumption of each calf was recorded daily and converted into DMI. Feed efficiency was determined by dividing DMI by ADG. Blood was sampled from all calves before morning feeding on d 0 and 60. Using evacuated tubes containing no anti-coagulant, blood samples were taken from the jugular vein and then centrifuged at 3,000 × g and 4 °C for 15 min to obtain serum. Serum samples were collected in 1.5-mL microtubes and stored at −20 °C. Ruminal fluid was collected on d 60 by a flexible esophageal tube (Anscitec Co., Ltd., Wuhan, Hubei, China) from all calves at 4 h after morning feeding. The first 150 mL of ruminal fluid

| Table 1 Feed ingredients and nutrient levels of the experiment diet (%) | Content | Nutrient levels | Content |
|---|---|---|---|
| Alfalfa hay | 50.00 | NGE, MJ/kg | 4.39 |
| Wheat straw | 15.00 | Crude protein | 14.25 |
| Corn flour | 14.00 | Neutral detergent fiber | 42.37 |
| Wheat bran | 6.83 | Acid detergent fiber | 31.26 |
| Soybean meal | 4.90 | Ether extract | 2.21 |
| Cottonseed meal | 3.65 | Ca | 0.74 |
| Fermented distiller’s grains | 3.50 | P | 0.41 |
| NaCl | 0.17 | | |
| Premix | 1.75 | | |

NGE – net energy for gain.

1 The premix provided following per kilogram of the basal ration: vitamin A, 8,000 IU; vitamin D, 1,200 IU; vitamin E, 50 IU; Fe, 100 mg; Zn, 60 mg; Mn, 40 mg; Cu, 10 mg; I, 0.50 mg; Se, 0.30 mg; Co, 0.10 mg.

2 NGE was calculated according to the Chinese Feeding Standard of Beef Cattle (NY/T 815-2004).
samples were discarded in order to avoid saliva contamination (Shen et al., 2012). Immediately, the ruminal pH was measured with a portable pH meter (Anscitech Co., Ltd., Wuhan, Hubei, China). Ten milliliters of ruminal fluid were squeezed through 4 layers of cheesecloth and transferred into sterile tubes and stored at –20 °C for subsequent analysis.

Beginning at 00:00 on d 57, fecal samples (about 300 g) of all calves were collected at 6-h intervals for 3 d by stimulating the rectum to cause defecation. The sampling time was moved forward 2 h daily (12 samples in total). The specific sampling time points were as follows: (d 57, 00:00, 06:00, 12:00, and 18:00; d 58, 22:00, 04:00, 10:00, and 16:00; d 59, 20:00, 02:00, 08:00, and 14:00) (Zhao et al., 2017; Ma et al., 2021). In the meantime, the fresh basal diets and orts were collected daily. The daily feed, orts, and fecal samples were mixed per calf, subsampled, and stored at –20 °C.

At the end of the experiment, all the samples were thawed (the 100 g fecal samples were mixed with 10 mL of 10% sulphuric acid) and dried at 65 °C for 20 h to a constant weight (Zeng et al., 2018). The dried samples were ground to pass through a 1-mm sieve (Tianguan Drying Equipment Co., Ltd., Hebi, Henan, China) for later analysis.

2.5. Statistical analysis

Data were based on each calf as the experimental unit, and the normality and homogeneity of data were tested first. Then, all data were analyzed by the independent sample t-test of the SPSS statistical software (Version 20.0 for Windows; SPSS, Chicago, USA). Data are shown as means and standard error of mean (SEM). A significance level was indicated at $P < 0.05$, and a trend was declared at $0.05 < P < 0.10$.

3. Results

3.1. Growth performance

Effects of ADY on growth performance of beef calves are presented in Table 2. The initial and final BW did not show an obvious difference ($P > 0.05$) between the CON and ADY groups. The ADG of the ADY group was higher ($P = 0.007$) than that of the CON group. The DMI was similar ($P > 0.05$) between the 2 groups. However, the DMI-to-ADG ratio in the ADY group was lower ($P = 0.022$) as compared to the CON group.

3.2. Rumen fermentation

Table 3 shows the effects of ADY on rumen fermentation of beef calves. Ruminal pH was similar ($P > 0.05$) and averaged 6.54 and 6.61 in the CON and ADY groups, respectively. The concentration of ammonia-N in the CON group was higher ($P = 0.023$) than that in the ADY group, but an opposite trend of MCP was found between the 2 groups. The concentrations of acetate and total VFA were not affected ($P > 0.05$) by ADY supplementation. However, the propionate and butyrate concentrations were higher ($P < 0.05$) in the ADY group than those in the CON group. Dietary supplementation with ADY decreased ($P = 0.002$) the molar proportion of acetate and increased ($P = 0.006$) the molar proportion of butyrate. Compared to the CON group, a slightly increased ($P = 0.089$) molar proportion of propionate was observed in the ADY group. Furthermore, the acetate-to-propionate ratio was lower ($P = 0.020$) in the ADY group as compared to the CON group.

3.3. Nutrient digestibility

The differences in nutrient digestibility of beef calves are presented in Table 4. Notably, the apparent digestibility of DM, OM, and EE were not different ($P > 0.05$) between the 2 groups. However, calves fed ADY had significantly increased ($P < 0.05$) the CP and NDF digestibility. Additionally, the ADY group exhibited a slightly higher ($P = 0.078$) ADF digestibility as compared to the CON group.

Table 2: Effects of ADY on growth performance of weaned beef calves1.

| Item      | Treatments | SEM | $P$-value |
|-----------|------------|-----|-----------|
| Initial BW, kg | 95.67 | 96.07 | 0.800 | 0.808 |
| Final BW, kg | 164.40 | 169.07 | 1.456 | 0.110 |
| ADG, g/d | 1,145.56 | 1,216.67 | 13.602 | 0.007 |
| DMI, kg/d | 6.19 | 6.29 | 0.048 | 0.294 |
| FE | 5.41 | 5.18 | 0.051 | 0.022 |

ADY = active dry yeast; SEM = standard error of mean; BW = body weight; ADG = average daily gain; DMI = dry matter intake; FE = feed efficiency; DMI/ADG.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.
Table 3
Effects of ADY on rumen fermentation of weaned beef calves1.

| Item                  | Treatments | SEM  | P-value |
|-----------------------|------------|------|---------|
|                       | CON        | ADY  |         |
| pH                    | 6.54       | 6.61 | 0.053   | 0.524 |
| Ammonia-N, mg/dL      | 9.48       | 8.64 | 0.189   | 0.023 |
| MCP, mg/mL            | 2.27       | 2.51 | 0.058   | 0.036 |
| Acetate, mmol/L       | 56.58      | 54.55| 0.829   | 0.229 |
| Propionate, mmol/L    | 14.30      | 15.46| 0.269   | 0.029 |
| Butyrate, mmol/L      | 8.30       | 9.35 | 0.177   | 0.002 |
| Total VFA, mmol/L     | 83.04      | 84.06| 0.980   | 0.611 |
| Acetate-to-propionate ratio | 4.00   | 3.55 | 0.098   | 0.002 |

ADY = active dry yeast; SEM = standard error of mean; MCP = microbial protein; VFA = volatile fatty acids.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.

Table 4
Effects of ADY on nutrient digestibility of weaned beef calves (%)1.

| Items                | Treatments | SEM  | P-value |
|----------------------|------------|------|---------|
|                       | CON        | ADY  |         |
| DM                   | 79.39      | 81.23| 0.926   | 0.331 |
| OM                   | 69.93      | 72.24| 0.962   | 0.235 |
| CP                   | 70.73      | 74.93| 0.902   | 0.017 |
| EE                   | 82.14      | 80.97| 0.953   | 0.551 |
| NDF                  | 62.19      | 67.01| 0.787   | 0.001 |
| ADF                  | 61.43      | 64.03| 0.741   | 0.078 |

ADY = active dry yeast; SEM = standard error of mean; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.

Table 5
Effects of ADY on serum biochemical indexes of weaned beef calves1.

| Item                  | Treatments | SEM  | P-value |
|-----------------------|------------|------|---------|
|                       | CON        | ADY  |         |
| Day 0                 |            |      |         |
| TP, g/L               | 61.25      | 60.28| 0.788   | 0.547 |
| ALB, g/L              | 28.61      | 27.87| 0.741   | 0.625 |
| GLB, g/L              | 32.64      | 32.41| 0.419   | 0.790 |
| GLU, mmol/L           | 4.21       | 4.15 | 0.057   | 0.643 |
| UN, mmol/L            | 3.34       | 3.40 | 0.060   | 0.622 |
| TG, mmol/L            | 0.293      | 0.285| 0.005   | 0.416 |
| ALT, U/L              | 12.39      | 12.09| 0.216   | 0.247 |
| AST, U/L              | 42.41      | 43.40| 0.669   | 0.466 |
| ALP, U/L              | 53.15      | 51.89| 0.751   | 0.412 |
| Day 60                |            |      |         |
| TP, g/L               | 59.55      | 60.73| 0.813   | 0.475 |
| ALB, g/L              | 28.79      | 28.29| 0.762   | 0.748 |
| GLB, g/L              | 30.76      | 32.45| 0.566   | 0.138 |
| GLU, mmol/L           | 4.13       | 4.35 | 0.051   | 0.032 |
| UN, mmol/L            | 3.38       | 3.16 | 0.059   | 0.061 |
| TG, mmol/L            | 0.293      | 0.291| 0.004   | 0.867 |
| ALT, U/L              | 12.00      | 12.34| 0.198   | 0.393 |
| AST, U/L              | 43.18      | 42.35| 0.644   | 0.531 |
| ALP, U/L              | 51.36      | 50.14| 0.753   | 0.426 |

ADY = active dry yeast; SEM = standard error of mean; TP = total protein; ALB = albumin; GLB = globulin; GLU = glucose; UN = urea nitrogen; TG = triglyceride; ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.

3.4. Serum biochemical index

Effects of ADY on serum biochemical indexes of beef calves are presented in Table 5. On d 0, the contents of TP, ALB, GLB, GLU, UN, TG, ALT, AST, and ALP were similar (P > 0.05) between the CON and ADY groups. Likewise, no significant difference (P > 0.05) of TP, ALB, GLB, TG, ALT, AST, and ALP contents was observed between the 2 groups on d 60. However, ADY supplementation increased (P = 0.032) the GLU content in the serum of beef calves. Also, the UN content in the ADY group was slightly lower (P = 0.061) than that in the CON group.

3.5. Serum antioxidant index

On d 0, the GSH-Px, SOD, MDA, and T-AOC concentrations were similar (P > 0.05) between the CON and ADY groups (Table 6). On d 60, dietary supplementation with ADY significantly increased (P < 0.05) the concentrations of GSH-Px and SOD in the serum of beef calves. However, an opposite trend of MDA was found between the 2 groups. The T-AOC activity in the ADY group tended to be higher (P = 0.068) than that in the CON group.

3.6. Serum immunoglobulin

The contents of IgA, IgG, and IgM in serum are presented in Table 7. No obvious difference (P > 0.05) of IgA, IgG, and IgM contents was observed between the CON and ADY groups on d 0. However, on d 60, ADY supplementation significantly increased (P < 0.05) the IgA and IgM contents in the serum of beef calves. A slight improvement (P = 0.077) of IgG content was found in the ADY group as compared to the CON group.

3.7. Serum cytokine

The contents of IL-1β, IL-6, IL-10, and TNF-α in serum did not show a significant difference (P > 0.05) between the CON and ADY groups on d 0 (Table 8). Notably, on d 60, the concentrations of IL-1β and TNF-α in serum of the ADY group were obviously reduced (P < 0.05) as compared to the CON group, whereas the IL-10 concentration displayed an opposite trend between the 2 groups. Moreover, calves fed ADY tended to have a lower (P = 0.079) IL-6 content.

Table 6
Effects of ADY on serum antioxidant indexes of weaned beef calves1.

| Item                  | Treatments | SEM  | P-value |
|-----------------------|------------|------|---------|
|                       | CON        | ADY  |         |
| Day 0                 |            |      |         |
| GSH-Px, U/mL          | 197.27     | 199.18| 3.406   | 0.785 |
| SOD, U/mL             | 120.29     | 119.65| 1.945   | 0.873 |
| MDA, mmol/mL          | 6.46       | 6.26  | 0.124   | 0.430 |
| T-AOC, U/mL           | 10.83      | 10.42 | 0.202   | 0.325 |
| Day 60                |            |      |         |
| GSH-Px, U/mL          | 192.38     | 207.80| 3.805   | 0.040 |
| SOD, U/mL             | 122.36     | 131.01| 2.171   | 0.084 |
| MDA, mmol/mL          | 6.58       | 6.01  | 0.128   | 0.023 |
| T-AOC, U/mL           | 11.08      | 11.90 | 0.227   | 0.068 |

ADY = active dry yeast; SEM = standard error of mean; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malondialdehyde; T-AOC = total antioxidant capacity.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.
IgG $\equiv$ ADY with the toxins produced by pathogenic bacteria and ADY can competitively bind to the binding sites in the gastrointestinal tract, which is helpful for reducing the occurrence of disease. In addition, ADY is an important precursor of gluconeogenesis (Aschenbach et al., 2010). The positive effects of ADY on MCP may be that ADY promotes the growth and reproduction of microbes related to protein utilization (Dias et al., 2018). The positive effects of ADY on the rumen fermentation of weaned beef calves may be that ADY can promote the utilization of ammonia-N by ruminal microbiota and then synthesize more MCP. Interestingly, the MCP concentration of the ADY group was higher than that of the CON group, which was in line with the ammonia-N results. The microbial community plays a critical role in the utilization of ruminal ammonia-N and synthesis of MCP. Yeast products can promote the growth and reproduction of microbes related to fiber degradation and protein utilization (Dias et al., 2018).

### Table 7

| Item | Treatments | SEM | P-value |
|------|------------|-----|---------|
|      | CON | ADY |      |
| Day 0 |   |   |   |
| IgA | 314.19 | 323.51 | 5.017 | 0.362 |
| IgG | 520.65 | 511.39 | 5.443 | 0.405 |
| IgM | 18.39 | 17.99 | 0.209 | 0.344 |
| Day 60 |   |   |   |
| IgA | 318.83 | 339.46 | 4.321 | 0.014 |
| IgG | 518.66 | 538.15 | 5.512 | 0.077 |
| IgM | 18.75 | 19.79 | 0.252 | 0.037 |

ADY $\equiv$ active dry yeast; SEM $\equiv$ standard error of mean; IgA $\equiv$ immunoglobulin A; IgG $\equiv$ immunoglobulin G; IgM $\equiv$ immunoglobulin M.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.

### Table 8

| Item | Treatments | SEM | P-value |
|------|------------|-----|---------|
|      | CON | ADY |      |
| Day 0 |   |   |   |
| IL-1β | 80.30 | 81.23 | 0.780 | 0.560 |
| IL-6 | 265.05 | 258.94 | 3.866 | 0.439 |
| IL-10 | 124.98 | 120.02 | 2.047 | 0.231 |
| TNF-α | 451.27 | 446.54 | 5.586 | 0.679 |
| Day 60 |   |   |   |
| IL-1β | 75.96 | 69.14 | 0.884 | <0.001 |
| IL-6 | 258.76 | 247.93 | 3.091 | 0.079 |
| IL-10 | 128.83 | 137.05 | 2.030 | 0.041 |
| TNF-α | 441.03 | 408.39 | 6.140 | 0.006 |

ADY $\equiv$ active dry yeast; SEM $\equiv$ standard error of mean; IL-1β $\equiv$ interleukin 1 beta; IL-6 $\equiv$ interleukin 6; IL-10 $\equiv$ interleukin 10; T NF-α $\equiv$ tumor necrosis factor alpha.

1 CON, control with no ADY and fed basal ration; ADY, fed basal ration and 5 g/d ADY (Angel Yeast Co., Ltd., Yichang, Hubei, China) per calf.

### 4. Discussion

#### 4.1. Effects of ADY on growth performance of weaned beef calves

At present, little research has been performed to assess the influence of ADY on the growth performance of weaned beef calves. ADY can adsorb pathogenic bacteria in the digestive tract of animals to inhibit them from adhering and colonizing the intestinal mucosa, which is helpful for reducing the occurrence of disease. In addition, ADY can competitively bind to the binding sites in the gastrointestinal tract with the toxins produced by pathogenic bacteria and contribute to eliminating the toxins, which is beneficial to promote growth performance of animals (Broadway et al., 2015). In the present study, no obvious difference of DMI was found between the 2 groups. Alugongo et al. (2017) reported that dietary supplementation of yeast products in calves’ diet had no significant influence on DMI, which was in accordance with our finding. However, inconsistent with DMI, we found that the ADG and feed efficiency were improved by ADY supplementation. A previous study reported that dietary supplementation of ADY improved the ADG and feed efficiency of finishing cattle (Geng 2015). In dairy calves, the addition of ADY increased the ADG of neonatal calves (Hassan et al., 2016). The improvements of ADG and feed efficiency were beneficial to attenuate the negative effects on growth performance of beef calves caused by weaning stress. Generally, better growth performance of ruminants is associated with improved rumen fermentation, nutrient digestibility, and immunity. Therefore, we performed the following study to investigate the effects of ADY on the rumen fermentation, nutrient digestibility, and serum parameters of weaned beef calves.

#### 4.2. Effects of ADY on rumen fermentation of weaned beef calves

Ruminal cannula and esophageal tube are 2 common methods adopted to collect ruminal fluid. The samples of ruminal fluid by esophageal tube could be subjected to saliva contamination; also, it is not easy to collect ruminal fluid from the same layer of the rumen when samplings are repeated several times. Compared with an esophageal tube, a ruminal cannula allows the collection of representative samples of ruminal fluid. However, a ruminal cannula is more expensive and invasive (the ruminal cannula is fixed by surgery). Previous studies have confirmed that when the experimental animal does not allow the application of surgery, an esophageal tube can be used to obtain the representative fluid samples in the rumen (Ramos-Morales et al., 2014; Xiao et al., 2016; Zhu et al., 2017). Thus, in the current study, we used an esophageal tube to collect ruminal fluid samples in order to ensure that the calves could grow healthily in the future. The ruminal pH, which normally ranges from 6 to 7, can be used to evaluate the health condition of the rumen. A previous study revealed that ADY could increase the ruminal pH of dairy cows (Malekkhahi et al., 2016). However, in the current study, the ruminal pH values of the 2 groups were within the normal range of 6.5 to 6.7, which suggested that ADY did not adversely influence the rumen fermentation. The difference in research results between previous studies and our study may be that the ratio of concentrate in the diet is distinct. Additionally, beef calves fed ADY decreased the concentration of ammonia-N in the rumen, indicating that ADY could increase the utilization of ruminal ammonia-N. In goats, Kamal et al. (2013) reported that live yeast could reduce the concentration of ammonia-N and increase the MCP content in the rumen, which was consistent with our results. Ammonia-N is the main raw material to synthesize MCP. Interestingly, the MCP concentration of the ADY group was higher than that of the CON group, which was in line with the ammonia-N result. The microbial community plays a critical role in the utilization of ruminal ammonia-N and synthesis of MCP. Yeast products can promote the growth and reproduction of microbes related to fiber degradation and protein utilization (Dias et al., 2018). The positive effects of ADY on MCP may be that ADY can promote the utilization of ammonia-N by ruminal microbiota and then synthesize more MCP.

In the current study, dietary supplementation of ADY increased the concentrations of propionate and butyrate in the rumen of beef calves. GLU is the main energy substrate, which plays an important role in mammalian metabolism. In ruminants, GLU production is mainly derived from liver gluconeogenesis, and propionate is an important precursor of gluconeogenesis (Aschenbach et al., 2010). The positive abundances of Butyrivibrio and lactate-utilizing bacteria, which contributed to increased concentrations of propionate and butyrate in the rumen (Xiao et al., 2016). Butyrate and, to a lesser extent, propionate are the primary energy sources of the ruminal epithelium and have an important influence on the development of the ruminal epithelium (Plöger et al., 2012). Thus, the increased concentrations of propionate and butyrate in our study might have had a positive influence on ruminal development of beef calves. According to our results, it can be concluded that calves fed ADY increased the contents of MCP, propionate, and butyrate in the rumen. These positive effects are beneficial to improve the growth performance of weaned beef calves. In future research more...
attention should be paid to the effects of yeast on the ruminal development in weaned beef calves.

4.3. Effects of ADY on nutrient digestibility of weaned beef calves

Higher nutrient digestibility is beneficial for the improvement of growth performance of animals. In the current study, the ADY group showed higher CP and NDF digestibility when compared with the CON group. A previous study in dairy cows found that dietary supplementation with ADY could increase the CP and NDF digestibility (Dehghan-Banadaky et al., 2013), which was in line with our results. The positive effects of ADY on nutrient digestibility are associated with microbiota, and ADY can increase the proportion of cellulyotic bacteria in the digestive tract (Mosoni et al., 2007). In general, increased nutrient digestibility can lead to the corresponding improvement of ADG, which is consistent with the data mentioned earlier. In the rumen, feedstuffs can be used by microbes to produce MCP and small peptides, which are easy to absorb by ruminants to improve nutrient digestibility (Hao et al., 2018). According to the results of rumen fermentation, dietary supplementation of ADY increased the MCP content in the rumen of beef calves. This may explain why ADY causes an improvement in nutrient digestibility. In the future, more studies should be conducted to evaluate the effects of ADY on the gastrointestinal microbiota of weaned beef calves.

4.4. Effects of ADY on serum parameters of weaned beef calves

Blood biochemical indexes are closely related to the health of animals, which can not only reflect the metabolic status of the body, but also reflect changes in organ function. Thus, blood biochemical parameters can be used to monitor the health of animals (Vrankovi et al., 2018). The concentrations of TP, ALB, GLB, GLU, and UN in serum are important parameters of protein and energy metabolism, and TG can be used to reflect lipid metabolism. Additionally, ALT, AST, and ALP have important impacts on liver function, and they are the indicators of liver function (Vrankovi et al., 2018). In this study, no significant difference of TP, ALB, GLB, TG, ALT, AST, and ALP was found between the 2 groups except for GLU and UN, indicating that ADY did not have a negative influence on the hepatic metabolism of beef calves. In dairy calves, a previous study found that the yeast could increase the GLU content in the serum (Hassan et al., 2016). Another study reported that lambs fed live yeast decreased the UN content in serum (Issakowicz et al., 2013). Consistent with these studies, we found that dietary supplementation with ADY significantly increased the GLU content and slightly reduced the UN content in the serum of beef calves, suggesting that ADY could improve the energy and protein utilization. The possible reason is that ADY increases the precursor concentration (propionate) of glucogenesis in calves and promotes the glucogenesis process, which can lead to an increased GLU concentration in blood. But the specific molecular mechanism still needs elucidation.

During normal growth and metabolism of animals, reactive oxygen free radical will be produced. If they can not be removed in time, the accumulated free radicals in the body can damage the structure and function of cells, resulting in lipid peroxidation of unsaturated lipids in biofilms and the formation of lipid peroxides, whose final product is MDA (Akhalayya et al., 2006). The changes in oxidative biomarkers, including GSH-Px, SOD, MDA, and T-AOC, can be used to assess the physiological and health status of animals. GSH-Px can inhibit lipid peroxidation by scavenging excessive free radicals in the body. SOD is an important antioxidant enzyme in the body, which plays an important role in scavenging free radicals and preventing macromolecular damage. T-AOC represents the antioxidant capacity of the body’s defense system (Alugongo et al., 2017). As a source of stress, weaning can induce oxidative stress of calves (Hulbert and Moisa 2016). In the current study, the concentrations of oxidative biomarkers were similar between the 2 groups on d 0. However, beef calves fed ADY increased the concentrations of GSH-Px, SOD, and T-AOC, and decreased the concentration of MDA in the serum on d 60, indicating that dietary supplementation of ADY improved the antioxidant capacity of weaned beef calves. A recent study reported that yeast increased the activity of GSH-Px and SOD in the serum of fattening lambs (Jia et al., 2018), which was basically consistent with our results. The antioxidative effect of ADY may be related to the optimization of gastrointestinal microbial composition, but the specific mechanism needs further study.

Immunoglobulin can interact directly or indirectly with antigens or pathogens, and to a certain extent, the content of immunoglobulin can reflect the immune function of the body. Weaning stress can reduce the immunity of calves (Rezazadeh et al., 2019; Hulbert and Moisa 2016). In the current study, calves supplemented with ADY increased the concentrations of IgA, IgG, and IgM in the serum, indicating that ADY improved the immune function of weaned beef calves. Previously, a study found that live yeast could enhance the humoral immunity of lambs (Milewski et al., 2013). IgA, IgG, and IgM are mainly secreted by B lymphocytes and play vital roles in humoral immunity. Thus, the increased concentrations of IgA, IgG, and IgM were conducive to improving the immunity of weaned beef calves. At present, research showing that ADY can improve the immunity of ruminants is limited. The Saccharomyces supplementation could decrease the proportion of pathogenic bacteria, including E. coli, Salmonella, and Listeria, in the digestive tracts of goats (Beauchemin et al., 2006), which might explain the positive effects of ADY on immunity.

Weaning stress of calves often causes gastrointestinal dysfunction and triggers the onset of pro-inflammatory responses (Rezazadeh et al., 2019). IL-1β, IL-6, and TNF-α are important inflammatory mediators in the inflammatory response, and elevated levels suggest that inflammation exists in the body. IL-10 can selectively prevent the synthesis of pro-inflammatory cytokines and the binding between pro-inflammatory cytokines and receptors, then reduce the impairment of the inflammatory response in the body (Standiford 2000). In our study, the ADY group exhibited lower concentrations of IL-1β and TNF-α and higher concentration of IL-10 in the serum as compared to the CON group, suggesting that ADY supplementation attenuated the inflammatory response of weaned calves. In different kinds of animals (e.g. beef cattle, broiler, and pig), yeast has been confirmed to improve the health status and relieve the inflammatory response under conditions of stress (e.g. weaning, heat, and transport stress) (Broadway et al., 2015). Another study in Mannheimia haemolytica-infected beef cattle found that yeast supplementation increased the number of neutrophils and monocytes involved in nonspecific immunity (Kayser et al., 2019). The effects of ADY on inflammatory cytokines may be achieved by regulating the nonspecific immunity. However, more studies should focus on the potential mechanisms of ADY on the immunity of weaned calves. Based on our results, ADY supplementation improves the immune response of weaned beef calves, which is beneficial to promote growth of calves.

5. Conclusion

The results of current study provide evidence that the addition of ADY in the diet of weaned beef calves contributes in a number of ways to their growth performance, including improvement in the rumen fermentation, nutrient digestibility, and immune response. Therefore, ADY can be used as an effective feed additive to promote the healthy growth of weaned beef calves.
Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriate influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This research was supported by the National Key Research and Development Program (2017YFD0502005), Sichuan Science and Technology Program (2018NZ0002), and China Agriculture (Beef Cattle/Yak) Research System of MOF and MARA (CARS-37). We would like to thank the Zhangye Comprehensive Experimental Station of National Yak and Beef Industrial Technology System (Zhangye, Gansu, China) for providing the facilities. Moreover, gratitude is expressed to the Angel Yeast Co., Ltd., for supplying the ADY for the experiment.

References

Akhalaya MY, Platonov AG, Baizhumanov AA. Short-term cold exposure improves performance and microbial protein synthesis in sheep consuming rations containing sea buckthorn pomace. J Anim Sci 2018;96:3412–9.

Makkar HPS, Sharma OP, Dawra RK, Negi SS. Simple determination of microbial protein synthesis, microbial protein yield and microbial protein efficiency of dietary feedstuffs as an alternative source of concentrates in dairy cows. Anim Biosci 2021;34:205–12.

Milewski S, Wojcik R, Zaleska B, Malczechewska J, Tanski Z, Siwicki AK. Effect of Saccharomyces cerevisiae dried yeast on the meat performance traits and selected indicators of humoral immunity in lambs. Acta Vet 2013;82:43–51.

Moallum U, Lehrer H, Livschitz L, Zachut M, Yakoob SY. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J Dairy Sci 2009;92:343–51.

Mosoni P, Chaucheyras-Durand F, Roger S, Stumpff F, Penner GB, Schulzke JD, García-Maillet C, Forano E. Quantiﬁcation of live yeast and yeast culture and dietary starch content on rumen fermentation and performance of lactating cows. J Dairy Sci 2014;97:756–68.

Ramos-Morales E, Arco-Pérez A, Martín-García AI, Yáñez-Ruiz DR, Frutos P, Hervás G. Use of stomach tubing as an alternative to rumen cannulation to measure the bacteria community composition in the forestomach of ruminants. Comp Biochem Physiol 2016;141:26–31.

Ramos-Morales E, Arco-Pérez A, Martín-García AI, Yáñez-Ruiz DR, Frutos P, Hervás G. Use of stomach tubing as an alternative to rumen cannulation to measure the bacteria community composition in the forestomach of ruminants. Comp Biochem Physiol 2016;141:26–31. 
Rezazadeh F, Kowsar R, Rafiee H, Riasi A. Fermentation of soybean meal improves growth performance and immune response of abruptly weaned Holstein calves during cold weather. Anim Feed Sci Technol 2019;254:114206.

Shen JS, Chai Z, Song LJ, Liu JX, Wu YM. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. J Dairy Sci 2012;95:5978–84.

Standiford T. Anti-inflammatory cytokines and cytokine antagonists. Curr Pharmaceut Des 2000;6:633–49.

Uyeno Y, Akiyama K, Hasunuma T, Yamamoto H, Yokokawa H, Yamaguchi T, et al. Effects of supplementing an active dry yeast product on rumen microbial community composition and on subsequent rumen fermentation of lactating cows in the mid-to-late lactation period. Anim Sci J 2016;88:119–24.

Van Keulen J, Young BA. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J Anim Sci 1977;44:282–7.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.

Vranković L, Aladrović J, Ljubić BB, Pipal I, Prvanović-Babić N, Mašek T, et al. Blood biochemical parameters of bone metabolism in cows and calves kept in a beef suckler system during the early postpartum period. Livest Sci 2018;211:8–13.

Xiao JX, Alugongo GM, Chung R, Dong SZ, Li SL, Yoon I, et al. Effects of Saccharomyces cerevisiae fermentation products on dairy calves: ruminal fermentation, gastrointestinal morphology, and microbial community. J Dairy Sci 2016;99:5401–12.

Zeng B, Sun JJ, Chen T, Sun BL, He Q, Chen XY, et al. Effects of Moringa oleifera silage on milk yield, nutrient digestibility and serum biochemical indexes of lactating dairy cows. J Anim Physiol Anim Nutr 2018;102:75–81.

Zhao XH, Chen ZD, Zhou S, Song XZ, Quyang KH, Pan K, et al. Effects of daidzein on performance, serum metabolites, nutrient digestibility, and fecal bacterial community in bull calves. Anim Feed Sci Technol 2017;225:87–96.

Zhu W, Wei Z, Xu N, Yang F, Yoon I, Chung Y, et al. Effects of Saccharomyces cerevisiae fermentation products on performance and rumen fermentation and microbiota in dairy cows fed a diet containing low quality forage. J Anim Sci Biotechnol 2017;8:36.