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Original Research Article

Active dry yeast supplementation improves the growth performance, rumen fermentation, and immune response of weaned beef calves

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ABSTRACT

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.

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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

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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) (Rezazadeh 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 of lactate-utilizing bacteria in the rumen, and then promote the conversion from lactate to propionate so that calves can obtain more energy from their diets (Fomenky 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-Banadaky 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 (Gäbel 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 $\geq 2 \times 10^{10}$ 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 anticoagulant, 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 (Anscitech 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 (%, dry matter basis).

Ingredients	Content	Nutrient levels	Content
Alfalfa hay	50.00	NEg[2], 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	3.85	Ca	0.74
Fermented distiller's grains	3.50	P	0.41
NaCl	0.17		
Premix[1]	1.75		

NEg = 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] NEg 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 by 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 48 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.

2.4. Sample analysis and calculation

Before analysis, the serum samples were thawed and thoroughly mixed. Then serum samples were analyzed for biochemical indexes, including total protein (TP), albumin (ALB), globulin (GLB), GLU, urea nitrogen (UN), triglyceride (TG), alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), using a automatic biochemical analyzer (BS-280, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, Guangdong, China). The concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), TNF-α, IL-1β, interleukin 6 (IL-6), and interleukin 10 (IL-10) were analyzed using commercial kits (Solarbio Science & Technology Co., Ltd., Beijing, China) with reference to the instructions. Frozen ruminal fluid samples were thawed and centrifuged at 15,000 × *g* for 10 min at 4 °C, then the supernatant was analyzed for rumen fermentation parameters, including VFA (Erwin et al., 1961), ammonia-N (Broderick and Kang 1980), and MCP (Makkar et al., 1982).

The feces, diets, and orts were analyzed for dry matter (DM, 105 °C), ether extract (EE, No. 922.06), organic matter (OM, No. 942.02), and crude protein (CP, No. 988.05) according to the AOAC procedure (AOAC, 2006). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the methods described by Van Soest et al. (1991). Also, the contents of calcium (No. 977.29) and phosphorus (No. 995.11) in diets were analyzed with the AOAC methods (AOAC, 2006). The nutrient digestibility (D, %) was measured using the acid-insoluble ash (AIA) ratio technique (Ma et al., 2021). The AIA in the feces (Af, %) and diets (Ad, %) were determined according to Van Keulen and Young (1977). With the content of a nutrient in feces (Nf, %) and diet (Nd, %), the nutrient apparent digestibility was calculated via an equation as follows:

$$\text{Nutrient apparent digestibility (\%)} = [1 - (\text{Ad} \times \text{Nf})/(\text{Af} \times \text{Nd})] \times 100$$

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 $0.05 \leq P < 0.10$.

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 calves[1].

Item	Treatments		SEM	*P*-value
	CON	ADY		
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 calves[1].

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	0.020
Molar proportion, %				
Acetate	68.04	64.94	0.522	0.002
Propionate	17.29	18.40	0.326	0.089
Butyrate	10.02	11.14	0.211	0.006

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 calves[1].

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	12.90	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 calves[1].

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, nmol/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	0.044
MDA, nmol/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.

Table 7
Effects of ADY on serum immunoglobulin contents of weaned beef calves (μg/mL)[1].

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 = active dry yeast; SEM = standard error of mean; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = 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
Effects of ADY on serum cytokine contents of weaned beef calves (pg/mL)[1].

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	75.06	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 = active dry yeast; SEM = standard error of mean; IL-1β = interleukin 1 beta; IL-6 = interleukin 6; IL-10 = interleukin 10; TNF-α = 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). Thus, in this study, ruminal propionate type fermentation provided higher energy for the body, which was conducive to promoting the growth performance of beef calves. Moreover, a previous study in dairy calves found that the *Saccharomyces* could increase the relative 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 cellulolytic 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 (Vranković 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 (Vranković 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 gluconeogenesis in calves and promotes the gluconeogenesis 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 (Akhalaya 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 Moisá 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 Moisá 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.

Author contributions

Jian Ma: Study design, Animal trial, Sample collection, Laboratory analysis, Statistical analysis, Writing original manuscript, and Reviewing manuscript. **Cheng Wang:** Animal trial, Sample collection, Laboratory analysis, and Reviewing manuscript. **Zhisheng Wang:** Study design, Resources, Supervision, Funding acquisition, and Reviewing manuscript. **Guang Cao:** Animal trial, Sample collection, and Laboratory analysis. **Rui Hu:** Sample collection, Statistical analysis, and Reviewing manuscript. **Xueying Wang**: Sample collection and Laboratory analysis. **Huawei Zou:** Sample collection, Resources, and Supervision. **Kun Kang:** Sample collection, Resources, and Supervision. **Quanhui Peng:** Sample collection and Reviewing manuscript. **Bai Xue:** Sample collection and Reviewing manuscript. **Lizhi Wang:** Sample collection and Reviewing manuscript. **Yueming Zhu:** Study design and Resources. **Xiaopeng Zhu:** Study design and Resources. All authors approved the final manuscript.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately 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.

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