Effects of Saccharomyces cerevisiae on Nutrient Digestibility, Rumen Fermentation, and Plasma Biochemical Indices in Xiangzhong Black Cattle (Postprint)

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Abstract

This experiment aimed to investigate the effects of different supplementation levels of brewer’ s yeast on growth performance, nutrient digestibility, rumen fermentation, and plasma biochemical indices in Xiangzhong black cattle. Eighteen Xiangzhong black cattle [average body weight (330±30) kg] were selected and allocated to 3 groups using a single-factor completely randomized design, with 6 cattle per group. The control group was fed a basal diet, while the experimental groups were supplemented with 120 (Group I) and 240 g/kg brewer’ s yeast (Group II) in the basal diet. The pre-trial period was 10 days, and the formal trial period was 30 days. The results showed that: 1) Dietary supplementation of brewer’ s yeast had no significant effect on average daily gain and feed-to-gain ratio in Xiangzhong black cattle ($P>0.05$). 2) Dietary supplementation of brewer’ s yeast significantly reduced crude protein fecal excretion ($P<0.05$), but had no significant effect on dry matter, neutral detergent fiber, and crude protein digestibility ($P>0.05$). 3) There were no significant differences in ammonia nitrogen (NH3-N) and volatile fatty acid (VFA) concentrations in rumen fluid among all groups ($P>0.05$). 4) Dietary supplementation of 240 g/kg brewer’ s yeast significantly reduced plasma total cholesterol concentration in Xiangzhong black cattle ($P<0.05$). The results suggest that dietary supplementation of 240 g/kg brewer’ s yeast can reduce crude protein fecal excretion and plasma total cholesterol concentration in Xiangzhong black cattle, which is of great significance for reducing nitrogen emissions, improving the farming environment, and promoting healthy cattle production.

Full Text

Effects of Saccharomyces cerevisiae on Nutrient Digestibility, Rumen Fermentation and Plasma Biochemical Parameters of Xiangzhong Black Beef

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Abstract

This trial investigated the effects of different supplementation levels of *Saccharomyces cerevisiae* on growth performance, nutrient digestibility, rumen fermentation, and plasma biochemical parameters of Xiangzhong black beef cattle. Eighteen Xiangzhong black beef cattle [average body weight (330±30) kg] were allocated to three groups using a single-factor completely randomized design, with six cattle per group. The control group received a basal diet, while the experimental groups received the basal diet supplemented with 120 (Group I) and 240 g/kg (Group II) of *S. cerevisiae*, respectively. The pre-trial period lasted 10 days, followed by a 30-day formal trial period. The results showed that: (1) Dietary supplementation with *S. cerevisiae* had no significant effect on average daily gain or feed-to-gain ratio ($P>0.05$). (2) Supplementation significantly reduced fecal crude protein excretion ($P<0.05$) but did not significantly affect digestibility of dry matter, neutral detergent fiber, or crude protein ($P>0.05$). (3) No significant differences were observed among groups in ruminal ammonia nitrogen (NH -N) or volatile fatty acid (VFA) concentrations ($P>0.05$). (4) Dietary supplementation with 240 g/kg *S. cerevisiae* significantly decreased plasma total cholesterol concentration ($P<0.05$). These findings indicate that supplementation with 240 g/kg *S. cerevisiae* can reduce crude protein fecal excretion and plasma total cholesterol concentration in Xiangzhong black beef cattle, which is important for reducing nitrogen emissions, improving the

farming environment, and promoting healthy beef production.

Keywords: Xiangzhong black beef; total cholesterol; volatile fatty acid; feed-to-gain ratio; growth performance

Introduction

Probiotics, a novel type of microbial additive, are live bacterial preparations that exert beneficial effects on the host, also known as microecological preparations. These primarily include lactic acid bacteria preparations, *Bacillus* preparations, yeast preparations, and mixed bacterial formulations [1]. Yeast has been applied as a valuable animal feed additive for many years, with *Saccharomyces cerevisiae* being the most commonly used species in feed additives, encompassing two major types: live yeast preparations and yeast culture preparations [2]. *S. cerevisiae* has been widely used to improve milk production performance in dairy cows, maintain cow health, and promote calf development [3]. In beef cattle production, extensive research has also been conducted on *S. cerevisiae*. Geng [4] reported that under high-concentrate dietary conditions, *S. cerevisiae* supplementation improved the growth performance of finishing cattle. Zhang [5] found that dietary supplementation with 0.1% active dry yeast maintained the rumen anaerobic environment and positively influenced rumen fermentation while promoting the growth of rumen fiber-degrading bacteria and lactate-utilizing bacteria. Ding [6] reported that dietary yeast supplementation had no significant effect on rumen fermentation patterns in beef cattle but improved lipid and phospholipid metabolism, which was beneficial for enhancing growth performance. Jing et al. [7] demonstrated that under high-concentrate dietary conditions, active dry yeast supplementation significantly increased the rapidly degradable fraction and effective degradation rate of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the rumen of beef cattle. However, few studies have reported the effects of dietary *S. cerevisiae* supplementation on growth performance, nutrient digestibility, rumen fermentation, and plasma biochemical parameters of Xiangzhong black beef cattle. Therefore, this experiment used Xiangzhong black beef cattle as experimental animals to investigate the effects of different supplementation levels of *S. cerevisiae* on these parameters, aiming to provide data support for healthy beef cattle production in practical farming operations.

Materials and Methods

1.1 Experimental Material The *S. cerevisiae* used in this experiment was an active yeast product manufactured by Lesaffre Group (France), with a viable count of 1.5×10^{1} CFU/g.

1.2 Experimental Design This experiment employed a single-factor completely randomized design. Eighteen Xiangzhong black beef cattle with an average body weight of (330±30) kg were randomly divided into three groups, with six cattle per group. The control group was fed a basal diet, while the experimental groups received the basal diet supplemented with 120 (Group I) and 240 g/kg (Group II) of *S. cerevisiae*, respectively. The pre-trial period lasted 10 days, followed by a 30-day formal trial period.

1.3 Experimental Diets and Management The basal diet for Xiangzhong black beef cattle was formulated according to NRC (2001) and consisted of rice straw and concentrate, with a concentrate-to-forage ratio of 55:45. The composition and nutrient levels of the basal diet are presented in Table 1 . Experimental cattle were housed individually in tie-stalls with *ad libitum* access to feed and water throughout the trial period. Ambient temperature was maintained at approximately 25 °C with good ventilation. Manure was removed twice daily, and pens were disinfected 2–3 times per week. Cattle were managed according to routine farm practices for immunization and deworming.

1.4 Sampling and Measurements

1.4.1 Growth Performance Body weight was measured on an empty stomach on days 1 and 30 of the formal trial period to record weight changes and calculate average daily gain. Feed intake was recorded from days 20–25 of the formal trial period, and average daily feed intake was calculated based on feed offered and refusals. The feed-to-gain ratio was then calculated from average daily gain and average daily feed intake using the following formulas:

Average daily gain = Total weight gain / (Number of animals × Trial days)
Average daily feed intake = Total feed intake / (Number of animals × Trial days)
Feed-to-gain ratio = Average daily feed intake / Average daily gain

1.4.2 Nutrient Digestibility Feces were collected from each group during days 25–30 of the formal trial period using the total collection method. Fecal samples were weighed, and 10% of the total weight was sampled. For every 100 g of sample, 20 mL of 10% H SO was added, and samples were stored at -20 °C for subsequent analysis of dry matter (DM), NDF, and crude protein (CP) content. Fecal samples from each animal collected over the 6-day period were pooled, and DM, NDF, and CP contents were determined using the feed analysis methods described by Yang [8]. Nutrient digestibility was calculated as follows:

DM digestibility = 100 × (Total DM intake - Fecal DM) / Total DM intake
NDF digestibility = 100 × (Total NDF intake - Fecal NDF) / Total NDF intake
CP digestibility = 100 × (Total CP intake - Fecal CP) / Total CP intake

1.4.3 Plasma Biochemical Parameters Blood samples were collected from the tail vein on day 30 of the formal trial period at three time points: pre-feeding (0 h) and 2 and 6 h post-feeding. Heparinized vacuum tubes were used for blood collection. Blood samples were immediately centrifuged at 5,000 r/min for 10 min at 4 °C to harvest plasma, which was stored at -20 °C. Plasma concentrations of alanine aminotransferase (ALT), lactate dehydrogenase (LDH), glucose (Glu), total cholesterol (TC), total protein (TP), triglycerides (TG), and urea nitrogen (UN) were measured using an automatic biochemical analyzer (Beckman Coulter CX4, USA) and commercial kits (Nanjing Jiancheng Bioengineering Institute).

1.4.4 Rumen Fermentation Parameters Rumen fluid was collected orally on day 30 of the formal trial period at 2 and 6 h post-feeding. Collected rumen fluid was placed in 10 mL centrifuge tubes and stored at -20 °C for NH -N concentration determination. The remaining rumen fluid was centrifuged at 10,000 r/min for 15 min at 4 °C, and 1.5 mL of supernatant was mixed with 0.15 mL of 25% metaphosphoric acid, then stored at -20 °C for volatile fatty acid (VFA) concentration determination. Rumen VFA and NH -N concentrations were measured using the methods described by Chen et al. [9].

1.5 Statistical Analysis Data were analyzed using the MIXED procedure of SAS 9.1 software. Statistical significance was defined as $P < 0.05$.

Results

2.1 Effects of Different Supplementation Levels of *S. cerevisiae* on Growth Performance of Xiangzhong Black Beef Cattle As shown in Table 2 , no significant differences were observed among the three groups in average daily gain or feed-to-gain ratio (P>0.05).

2.2 Effects of Different Supplementation Levels of *S. cerevisiae* on Nutrient Digestibility of Xiangzhong Black Beef Cattle Table 3 shows that compared with the control group, both experimental groups significantly reduced CP fecal excretion (P<0.05), with reductions of 19.35% and 16.13% for Groups I and II, respectively. CP digestibility in Groups I and II was 6.92% and 6.22% higher than in the control group, respectively, although the difference among the three groups was not significant (P>0.05). Different supplementation levels of *S. cerevisiae* had no significant effect on DM or NDF digestibility (P>0.05).

2.3 Effects of Different Supplementation Levels of *S. cerevisiae* on Rumen Fermentation Parameters of Xiangzhong Black Beef Cattle As shown in Table 4 , ruminal NH -N concentration tended to increase with increasing *S. cerevisiae* supplementation level, but the difference among groups was not significant (P>0.05). In Group I, ruminal acetate, propionate, valerate, butyrate, and total VFA concentrations were 3.43%, 1.45%, 8.22%, 23.10%, and 4.95% higher than in the control group, respectively. All VFA concentrations in Group I were higher than in Group II, with acetate, propionate, valerate, isovalerate, butyrate, isobutyrate, and total VFA concentrations being 8.37%, 9.67%, 9.72%, 7.35%, 15.24%, 6.19%, and 9.31% higher, respectively. However, different supplementation levels of *S. cerevisiae* had no significant effect on VFA concentrations (P>0.05).

2.4 Effects of Different Supplementation Levels of *S. cerevisiae* on Plasma Biochemical Parameters of Xiangzhong Black Beef Cattle Table 5 shows that no significant differences were observed among groups in plasma ALT and LDH activities or Glu, TG, TP, and UN concentrations (P>0.05). Plasma TC concentration in Group II was 15.61% and 28.11% lower than in the control group and Group I, respectively, with significant differences (P<0.05).

Discussion

3.1 Effects of Different Supplementation Levels of *S. cerevisiae* on Growth Performance of Xiangzhong Black Beef Cattle Geng [4] reported that yeast culture had no significant effect on growth performance of finishing cattle, and active dry yeast had no significant effect on feed-to-gain ratio. Similarly, Dong et al. [10] found that probiotics had no significant effect on growth performance indices such as average daily gain, average daily feed intake, or feed-to-gain ratio in suckling calves. These findings are consistent with the results of the present study. Sanchez et al. [11] also reported that dietary supplementation with probiotics [*Propionibacterium acidipropionici* P1691] had no significant effect on body weight of beef cattle.

However, other studies have reported positive effects of yeast culture on calf growth. Francia et al. [12] demonstrated that dietary *S. cerevisiae* supplementation had positive effects on increasing average daily gain and feed conversion ratio in calves. Fu et al. [13] reported that dietary supplementation with *Bacillus licheniformis* alone increased average daily gain and body indices of calves (0–8 weeks of age), while composite probiotics (*B. licheniformis*, *Bacillus subtilis*, and *Lactobacillus plantarum*) improved body indices at 8 weeks of age. Chou et al. [14] found that dietary supplementation with probiotics (*Lactobacillus* and *Bifidobacterium*) significantly increased average daily gain in calves. Differences among studies may be primarily attributed to variations in probiotic species, supplementation levels, and experimental animals, as well as differences in dietary composition and cattle age.

3.2 Effects of Different Supplementation Levels of *S. cerevisiae* on Nutrient Digestibility of Xiangzhong Black Beef Cattle Vyas et al. [15] reported that probiotic supplementation (*Propionibacterium*) in beef cattle diets had no significant effect on ruminal digestibility of DM, organic matter (OM), NDF, ADF, or starch. Sanchez et al. [11] also found that probiotic supplementation (*Propionibacterium acidipropionici* P1691) in low-quality forage diets for beef cattle had no significant effect on OM or NDF digestibility and did not significantly alter body weight. Similarly, Wang et al. [16] reported that probiotic supplementation (*S. cerevisiae*) had no significant effect on in vitro DM or NDF digestibility of corn straw fermentation. These results are consistent with the findings of the present study. However, other studies have reported that dietary yeast supplementation improved fiber and DM digestibility [17–18]. This discrepancy may be due to differences in dietary composition. In the current study, *S. cerevisiae* supplementation significantly reduced CP fecal excretion and improved CP digestibility, possibly by promoting degradation of dietary protein by rumen microorganisms. This is indirectly supported by the ruminal NH -N concentrations observed across groups. Liu et al. [19] reported that dietary composite probiotics significantly increased ruminal NH -N concentration, which is consistent with our results.

3.3 Effects of Different Supplementation Levels of *S. cerevisiae* on Rumen Fermentation Parameters of Xiangzhong Black Beef Cattle

NH -N is the primary precursor for microbial protein synthesis in the rumen, primarily generated from degradation of dietary protein by rumen microorganisms. In this study, *S. cerevisiae* supplementation promoted protein degradation in the rumen, resulting in higher ruminal NH -N concentrations in the experimental groups compared with the control group. This finding is consistent with Liu et al. [19]. Volatile fatty acids in the rumen primarily originate from carbohydrate fermentation and represent an important energy source for ruminants while providing energy for microbial synthesis. In this study, *S. cerevisiae* supplementation had no significant effect on ruminal VFA concentrations, possibly because the supplementation did not significantly affect carbohydrate-degrading bacterial populations in the rumen. Liu et al. [19] reported that dietary composite probiotics had no significant effect on propionate or butyrate concentrations or acetate/propionate ratio but significantly increased total VFA and acetate concentrations. Other studies have reported that dietary active yeast or yeast culture altered ruminal VFA concentrations and proportions [20–22], while some found that *Propionibacterium* supplementation significantly modified ruminal VFA proportions [11,15]. Peng et al. [23] reported that dietary *Bacillus subtilis* natto supplementation significantly increased ruminal propionate concentration while decreasing acetate concentration. These discrepancies may be attributed to differences in probiotic species and supplementation levels, as well as dietary chemical composition.

3.4 Effects of Different Supplementation Levels of *S. cerevisiae* on Plasma Biochemical Parameters of Xiangzhong Black Beef Cattle

Plasma biochemical parameters can reflect animal growth performance to some extent and are important indicators in animal production research. In this study, except for plasma TC concentration, *S. cerevisiae* supplementation had no significant effect on plasma biochemical parameters. Wang et al. [24] reported that dietary composite yeast culture significantly increased serum Glu concentration and significantly decreased serum UN concentration. Fu et al. [13] found that different probiotic combinations had no significant effect on serum biochemical parameters in calves, while Yan [25] reported that yeast culture supplementation had no significant effect on blood TP concentration in calves. Peng et al. [23] also found that dietary *B. subtilis* natto had no significant effect on blood biochemical parameters in dairy cows. These findings suggest that the effects of dietary probiotics on blood biochemical parameters may be related to animal species, growth stage, probiotic composition, and dietary factors. Total cholesterol is the main component of blood lipids, and its concentration can reflect lipid absorption and metabolism status [26]. Numerous studies have demonstrated that probiotics can reduce body cholesterol [27]. Interestingly, in this study, low-level probiotic supplementation significantly increased plasma TC concentration, possibly because low-level *S. cerevisiae* promoted lipid metabolism, leading to elevated plasma TC concentration. Ding [6] reported that yeast supplementation had positive effects on improving animal lipid and phospholipid metabolism. In contrast, high-level probiotic supplementation significantly decreased plasma TC concentration, possibly because high-level probiotics can absorb or adsorb cholesterol in the intestine, thereby reducing plasma TC concentration [28–29].

Conclusions

1. Dietary *S. cerevisiae* supplementation significantly reduced crude protein fecal excretion in Xiangzhong black beef cattle, which is important for reducing nitrogen emissions, improving the farming environment, and decreasing environmental pollution during beef cattle production.

2. Dietary supplementation with 120 g/kg *S. cerevisiae* significantly increased plasma total cholesterol concentration, whereas supplementation with 240 g/kg *S. cerevisiae* significantly decreased plasma total cholesterol concentration in Xiangzhong black beef cattle, which may have positive effects on improving meat quality and promoting healthy cattle production.

References

[1] Shi YH, Wang CZ, Han B, et al. Research progress on probiotics [J]. Journal of Henan Agricultural University, 2001, 35(4): 376–380.
[2] Geng CY, Ren LP, Zhou ZM, et al. Application effects and possible mechanisms of yeast preparations in ruminants [J]. Chinese Journal of Animal Nutrition, 2015, 27(4): 1011–1020.
[3] Chen L, Lu XY, Zhou CS, et al. Application of microecological preparations in adult dairy cow production [J]. Chinese Journal of Animal Science, 2013, 49(19): 97–100.
[4] Geng CY. Comparison of active yeast and yeast culture on growth performance, carcass traits and meat quality of finishing beef cattle [D]. PhD Dissertation. Beijing: China Agricultural University, 2015: 54–56.
[5] Zhang XF. Effects of active dry yeast on rumen fermentation, fiber degradation and microbial flora in beef cattle [D]. MS Thesis. Chengdu: Sichuan

Agricultural University, 2014: 56.
[6] Ding GZ. Effects of yeast supplementation on rumen fermentation, nutrient degradation and plasma metabolome in beef cattle fed diets with different concentrate-to-forage ratios [D]. PhD Dissertation. Beijing: China Agricultural University, 2014: 56.
[7] Jing XP, Peng QH, Zou HW, et al. Effects of active dry yeast on ruminal fiber degradation rate in beef cattle [J]. Chinese Journal of Animal Science, 2015, 51(S1): 57-60.
[8] Yang S. Feed Analysis and Feed Quality Detection Technology [M]. Beijing: Beijing Agricultural University Press, 1993: 58-64.
[9] Chen L, Ren A, Zhou CS, et al. Effects of *Lactobacillus acidophilus* supplementation for improving in vitro rumen fermentation characteristics of cereal straws [J]. Italian Journal of Animal Science, 2016, 16(1): 52-60.
[10] Dong XL. Screening and identification of probiotics and their effects on growth and gastrointestinal microorganisms of weaned piglets and calves [D]. PhD Dissertation. Beijing: Chinese Academy of Agricultural Sciences, 2013: 61.
[11] Sanchez PH, Tracey LN, Browne-Silva J, et al. *Propionibacterium acidipropionici* P1691 and glucogenic precursors improve rumen fermentation of low-quality forage in beef cattle [J]. Journal of Animal Science, 2014, 92(4): 1738-1746.
[12] Francia AD, Masucci F, De Rosa G, et al. Effects of *Aspergillus oryzae* extract and a *Saccharomyces cerevisiae* fermentation product on intake, body weight gain and digestibility in buffalo calves [J]. Animal Feed Science and Technology, 2008, 140(1/2): 67-77.
[13] Fu YQ, Diao QY, Tu Y, et al. Effects of different probiotic combinations on growth performance and serum biochemical parameters of 0–8 week-old calves [J]. Chinese Journal of Animal Nutrition, 2012, 24(4): 753-761.
[14] Chou YC, Ge M. Effects of compound *Ligustrum lucidum* and probiotics on growth performance and immune function of calves [D]. MS Thesis. Harbin: Northeast Agricultural University, 2012: 17.
[15] Vyas D, McGeough EJ, Mohammed R, et al. Effects of *Propionibacterium* strains on ruminal fermentation, nutrient digestibility and methane emissions in beef cattle fed a corn grain finishing diet [J]. Animal, 2014, 8(11): 1807-1815.
[16] Wang Z, Zhou CS, Tang SX, et al. Effects of two yeast strains on in vitro rumen fermentation characteristics in dairy cows [J]. Research of Agricultural Modernization, 2014, 35(2): 218-224.
[17] Tang SX, Tayo GO, Tan ZL, et al. Effects of yeast culture and fibrolytic enzyme supplementation on in vitro fermentation characteristics of low-quality cereal straws [J]. Journal of Animal Science, 2008, 86(5): 1164-1172.
[18] Guedes CM, Goncalves D, Rodrigues MAM, et al. Effects of a *Saccharomyces cerevisiae* on ruminal fermentation and fibre degradation of maize silages in cows [J]. Animal Feed Science and Technology, 2008, 145(1/2/3/4): 27-40.
[19] Liu CJ, Sun MJ, Sun JY, et al. Effects of dietary composite probiotics on rumen fermentation and cellulase activity in dairy cows [J]. Chinese Journal of

Animal Nutrition, 2011, 23(5): 821–827.
[20] Hucko B, Bampidis VA, Kodeš A, et al. Rumen fermentation characteristics in pre-weaning calves receiving yeast culture supplements [J]. Czech Journal of Animal Science, 2009, 54: 435–442.
[21] Helal FIS, Abdel-Rahman KA. Productive performance of lactating ewes fed diets supplementing with dry yeast and/or bentonite as feed additives [J]. World Journal of Agricultural Science, 2010, 6(5): 489–498.
[22] Vohra A, Syal P, Madan A. Probiotic yeasts in livestock sector [J]. Animal Feed Science and Technology, 2016, 219: 31–47.
[23] Peng H, Wang JQ, Kang HY, et al. Effect of feeding *Bacillus subtilis* natto fermentation product on milk production and composition, blood metabolites and rumen fermentation in early lactation dairy cows [J]. Journal of Animal Physiology and Animal Nutrition, 2012, 96(3): 506–512.
[24] Wang L, Lu YY, Cheng ZW, et al. Effects of compound yeast culture on milk performance, nitrogen emission and blood biochemical parameters in dairy cows [J]. Acta Prataculturae Sinica, 2015, 24(12): 121–130.
[25] Yan XG. Effects of yeast culture and pelleted concentrate on growth and development of Holstein calves [D]. MS Thesis. Changchun: Jilin Agricultural University, 2005: 40.
[26] Wang ZW, Mu XL, Yang GW, et al. Effects of dietary nutrient levels on growth performance and blood biochemical parameters of Northeast meat geese (1-28 days of age) [J]. Journal of Nuclear Agricultural Sciences, 2009, 23(5): 891–897.
[27] Wang W, Zou JH, Yuan JL. Research progress on probiotics with cholesterol-lowering effects [J]. Chinese Journal of Microecology, 2009, 21(2): 171–172.
[28] Guo CF, Zhang LW. Research progress on cholesterol-lowering functions of probiotics [J]. Acta Microbiologica Sinica, 2010, 50(12): 1590–1599.
[29] Wang SP, Dong XF, Tong JM. Research progress on regulation of cholesterol metabolism by probiotics in laying hens [J]. Chinese Journal of Animal Nutrition, 2013, 25(8): 1695–1702.

Note: Figure translations are in progress. See original paper for figures.