



Full Length Article

Comparison of Blood Immunity, Antioxidant Capacity and Hormone Indexes in Finishing Bulls Fed Active Dry Yeast (*Saccharomyces cerevisiae*) and Yeast Culture

C.Y. Geng*, S. Ji, Y.H. Jin, C.Y. Li, G.J. Xia, Y.M. Li and M. Zhang

Agricultural University College, Yanbian University, Yanji 133000, China

*For correspondence: cygeng1011@163.com

Abstract

This study aims to compare the effect of active dry yeasts (ADY) and yeast cultures (YC) on blood immunity, antioxidant capacity and hormone indexes, and also to clarify the relationships between blood indexes and feed intake, blood indexes and beef tenderness in finishing bulls fed a high-concentrate diets. The results indicated that ADY supplementation decreased serum concentration of total antioxidant capacity (TAOC) ($p < 0.05$), superoxide dismutase (SOD) ($p < 0.05$) and catalase enzyme (CAT) ($p < 0.05$), but had no significant effect on concentration of malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) ($p > 0.05$). YC supplementation decreased activity of SOD ($p < 0.05$) and had no effect on other indexes above ($p > 0.05$). Both ADY and YC had no significant effect on serum concentration of IgA, IgG and IgM compared to CON ($p > 0.05$). Both ADY and YC elevated the plasma ghrelin concentration ($p < 0.05$), while have no effect on leptin, triiodothyronine (T_3) and thyroxine (T_4) ($p > 0.05$). There was a negative correlation between dry matter intake (DMI) and CAT ($r = -0.374$, $p < 0.05$), and a positive correlation between DMI and ghrelin ($r = 0.306$, $p = 0.027$). There was a negative correlation between shear force and ghrelin level ($r = -0.384$, $p < 0.05$). There are differences between effects of ADY and YC on antioxidant indexes and, CAT and ghrelin contributed to regulate DMI of finishing bulls fed high-concentrate diets, and the improved beef tenderness is related to elevated ghrelin concentration. © 2018 Friends Science Publishers

Keywords: Yeast preparations; Finishing bulls; Blood indexes; Feed intake; Beef tenderness; Relationship

Introduction

Yeast preparations based on *Saccharomyces cerevisiae* strains are used in ruminant animals to improve growth performance (Finck *et al.*, 2014; Geng *et al.*, 2016a), also ruminant production quality (Nikkhah *et al.*, 2004; Geng *et al.*, 2018). Nevertheless, published literature regarding active dry yeast (ADY) or yeast culture (YC), two typical yeast preparations in current markets, has not shown conclusive effect on animal performance at all times. Some studies indicated that animal performances were improved by supplementation of ADY preparations (Bontempo *et al.*, 2009; Finck *et al.*, 2014) or YC preparations (Hinman *et al.*, 1998). However, the other studies indicated that animal performances were not improved by ADY preparations (Castillo *et al.*, 2006), or declined by YC preparations (Swyers *et al.*, 2014). The clear effect of the two typical yeast products is very important for scientific application in ruminant production.

In previous study, we compared the effect of ADY (Levucell SC, *S. cerevisiae* CNCM1-1077), and YC (Diamond V XP, Cedar Rapids, IA) on growth performance, carcass traits, conventional biochemical blood indexes, ruminal fermentation parameters and beef quality

in finishing bulls fed high concentrate diets (Geng *et al.*, 2016a, 2018). The results indicated that ADY rather than YC significantly improved the growth performance including dry matter intake (DMI) [8.68 kg (CON) vs. 10.40 kg (ADY), 9.41 kg (YC)] and average daily gain of beef cattle compared to control (CON), and both ADY and YC significantly improved the tenderness of beef [8.44 kg (CON) vs. 6.53 kg (ADY) and 6.74 kg (YC)]. In addition, increased DMI was considered as the main cause of improvement of growth performance by ADY supplementation in finishing bulls (Geng *et al.*, 2016a). Until now, although several hypotheses have been proposed to explain why yeast products could stimulate DMI and productivity in ruminants (Throne *et al.*, 2009; Robinson, 2010; Montes de Oca *et al.*, 2016), the research on underlying action mechanism with endogenous appetite hormone changes is somewhat limited.

As we know, blood immunity, antioxidant capacity and some hormone indexes are closely related to feed intake. Some latest reports indicated that these blood indexes above may also have a close relationship with meat quality (Foote *et al.*, 2014; Russell *et al.*, 2016). For example, oxidative damage induced by free radicals can damage the integrity of the myolemma and the

structure and morphology of skeletal muscle (Behrends *et al.*, 2009), and then may cause deterioration of beef tenderness. Furthermore, some appetite hormone such as ghrelin not only can regulate the ingestion (Wertz-Lutz *et al.*, 2006), but also intracellular Ca^{2+} concentration in skeletal muscle cells (Fang *et al.*, 2012) which plays an important role in improving the beef tenderness (Foote *et al.*, 2004; Rider *et al.*, 2004).

However, to our knowledge, there are few studies on the comparison of ADY and YC for blood immunity, antioxidant capacity and some appetite hormone indexes in bulls fed high concentration so far, and the study on the relationship between these blood indexes and feed intake, beef tenderness is limited. Therefore, based on our previous studies (Geng *et al.*, 2016a, 2018), this study was conducted to further compare the effect of ADY and YC on blood immunity, antioxidant capacity and hormone indexes, and also to clarify the relationship between these blood indexes and feed intake, blood indexes and beef tenderness in finishing bulls, which will lay the foundation for further action mechanism of ADY and YC for improved growth performance and beef quality.

Materials and Methods

The experiment was carried out from June to October of 2014, in Linyi Experiment Station of National Beef and Yaks Research System, Sishui County, Shandong Province, China. All procedures involving animal care were under the approval of the China Agricultural University Institutional Animal Care and Use Committee.

Animals Feeding and Management

Forty-five crossbred bulls (approximately 24 mo of age, mean body weight 505.4 ± 29.1 kg) were randomly assigned to three groups of 15 bulls each, and bulls in each group were randomly fed one of three treatment diets. The treatment diets respectively were basal diet (CON diet), basal diet+YC preparations (Diamond V XP, Cedar Rapids, IA) and basal diet+ADY preparations (Levucell SC, *S. cerevisiae* CNCM1-1077). More details for animals feeding and management can be found in our previous paper of Geng *et al.* (2016a). The trial lasted 112 d, and the final body weights (mean \pm s.d) of the three groups respectively were 576.8 ± 38.1 kg (CON group), 611.2 ± 47.2 kg (ADY group) and 587.0 ± 39.7 kg (YC group). The dietary dry matter intake (mean \pm s.d.) of the three groups respectively was 8.68 ± 0.68 kg (CON group), 10.40 ± 1.32 kg (ADY group) and 9.41 ± 1.75 kg (YC group). The shear force (mean \pm s.d) of the three groups respectively were 8.44 ± 1.84 kg (CON group), 6.53 ± 1.31 kg (ADY group) and 6.74 ± 1.18 kg (YC group). More details for growth performance and meat quality can be found in our previous paper of Geng *et al.* (2016a, 2018). The compositions of the basal diets are shown in Table 1.

Blood Sample Collection and Analysis

Blood of bulls were collected on the last day of the study, prior to supply of the rations in the early morning by venipuncture with 10 mL vacuum blood collection tubes containing anticoagulant and no anticoagulant for separation of plasma and serum, respectively. Tubes were centrifuged at $3200 \times g$ in a refrigerated centrifuge at 4°C for 15 min and the separated serum and plasma were saved -20°C for later analysis. The serum was used for measurement of concentrations of oxidative biomarkers [total antioxidant capacity (TAOC), malondialdehyde (MDA), superoxide dismutase (SOD), catalase enzyme (CAT), and glutathione peroxidase (GSH-Px)], content of immunoglobulin indexes (IgA, IgM and IgG). The plasma was used for measurement of concentration of hormone indexes [Ghrelin, insulin, leptin, triiodothyronine (T_3) and thyroxine (T_4)]. Briefly, Oxidative biomarkers (TAOC, MDA, SOD, CAT and GSH-Px) and immunoglobulin indexes (IgA, IgM and IgG) were analyzed by a Hitachi-7160 Auto-Biochemical Analyzer (Hitachi, Co. Ltd., Japan) with colorimetry methods. Plasma hormone indexes (Ghrelin, insulin, leptin, T_3 and T_4) were analyzed by a r-911 full automatic radio-immune counter (USTC, Co. Ltd., China) with radioimmunoassay methods. The blood indexes were examined in a service corporation (*Beijing Sino-Uk of Biological Technology, Beijing, China*).

Statistics and Analysis

Data were statistically analyzed according to a completely randomized design using the GLM model of SPSS 18.0 (SPSS Inc., Chicago, IL, USA), and multiple comparisons were performed by one-way with Duncan's method in the same line. Probability values of $p < 0.05$ were considered as significant. The correlations were determined by partial correlation analysis by the Pearson option (2-tailed).

Results

Blood Antioxidant Capacity Indexes

Effects of ADY and YC on blood antioxidant capacity indexes of finishing bulls are shown in Table 2. Compared to CON, ADY supplementation significantly decreased the serum concentrations of TAOC ($p = 0.03$), SOD ($p = 0.003$) and CAT ($p = 0.015$), but had no significant effect on concentrations of MDA and activity of GSH-Px ($p > 0.05$). YC supplementation significantly decreased activity of SOD ($p = 0.026$) and had no significant effect on other indexes above ($p > 0.05$).

Blood Immunoglobulin Indexes

Effects of ADY and YC on blood immunoglobulin indexes G, A, and M content in finishing bulls are shown in Table 3. Compared to CON, both ADY and YC supplementation had no significant effect on serum concentrations of IgA, IgG and IgM ($p > 0.05$).

Table 1: Ingredient and chemical composition of basal diets

Ingredient composition (%)		Chemical composition [†] (%)	
Corn silage	30.61	Dry matter, DM	71.45
Corn meal	51.14	Crude protein, CP	12.23
Cottonseed meal	7.79	Ether extract, EE	2.98
Soybean meal	5.79	Neutral detergent fibre, NDF	25.86
Salt	0.18	Acid detergent fibre, ADF	15.57
Sodium bicarbonate	1.04	Calcium, Ca	0.63
Compound premix [‡]	3.45	Phosphorus, P	0.37
Total	100.00	Sodium chloride	0.40
		NEg (Mcal/kg DM)	1.28

[†] Supplied per kilogram of product. Ca: 160g; P: 30 g; Cu: 450 mg; Zn: 1600 mg; Mn: 800 mg; I: 10 mg; Co: 10 mg; Se: 5 mg; vitamin A: 120 000 IU; vitamin D: 55000 IU; vitamin E :400 mg; vitamin B3:600 mg; vitamin B5: 200 mg; Monensin:1000 mg; Salt: 0.065 kg

[‡] The value reported for nutritional composition of diets was calculated based on the nutrient analysis from ingredient samples. NEg was estimated from CNCPS (6.0) values

Table 2: Effect of dietary supplementation active dry yeasts (ADY) and yeast cultures (YC) on blood antioxidant capacity indexes in finishing bulls

Items [†]	Treatments [‡]			SEM	P value
	CON (n=15)	ADY(n=15)	YC(n=15)		
T-AOC, U/mL	10.06	9.24*	9.55	0.16	0.09
MDA, nmol/mL	6.16	6.09	6.42	0.090	0.267
SOD ,U/mL	83.98	69.45**	72.94*	2.08	0.008
CAT, U/mL	43.36	38.58*	40.77ab	0.82	0.05
GSH-Px,U/mL	954.42	943.20	922.28	9.55	0.412

[†]TAOC, total antioxidant count; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase enzyme; GSH-Px, glutathione peroxidase

[‡] Treatments included a basal control diet (CON), a basal diet with supplemental active dry yeast (ADY, Levucell SC), a basal diet with supplemental yeast cultures (YC, Diamond V XP); Multiple comparison, * $p < 0.05$, ** $p < 0.01$

Table 3: Effect of dietary supplementation active dry yeasts (ADY) and yeast cultures (YC) on blood immunoglobulin G, A, and M content in finishing bulls

Items	Treatments [†]			SEM	P value
	CON(n=15)	ADY(n=15)	YC(n=15)		
IgA, g/L	0.83	0.77	0.80	0.016	0.409
IgM, g/L	2.63	2.61	2.68	0.045	0.801
IgG, g/L	10.73	10.26	10.31	0.15	0.376

[†] Treatments included a basal control diet (CON), a basal diet with supplemental active dry yeast (ADY, Levucell SC), a basal diet with supplemental yeast cultures (YC, Diamond V XP); Multiple comparison, * $p < 0.05$, ** $p < 0.01$

Blood Hormone Indexes

Effects of dietary supplementation ADY and YC on blood hormone indexes in finishing bulls are shown in Table 4. Compared to CON, both ADY and YC significantly elevated the plasma ghrelin concentration ($p < 0.05$), while have no significant effect on concentration of leptin, T₃ and T₄ ($p > 0.05$). Moreover, YC significantly increased the concentration of insulin compared to CON ($p = 0.004$).

Correlations Among the Feed Intake, Beef Tenderness of Bulls and Blood Indexes

Correlations between blood immunity, antioxidant capacity, hormone indexes and DMI, beef tenderness in finishing bulls are shown in Table 5. As shown in Table 5, there were no significant correlations ($p > 0.05$) between the DMI and the concentration of blood indexes including TAOC, MDA, SOD, GSH-Px, IgA, IgG, IgM, insulin, leptin, T₃ and T₄ in finishing bulls fed high-concentrate diets. However, there was a negative correlation between DMI and blood activity of CAT ($r = -0.374$, $p = 0.009$), and a positive correlation between DMI and level of ghrelin ($r = 0.306$, $p = 0.027$). There was no significant correlation between shear force and TAOC, MDA, SOD, CAT, GSH-Px, IgA, IgG, IgM, insulin, leptin, T₃ and T₄, however, there was a significantly negative correlation between shear force and level of ghrelin ($r = -0.384$, $p = 0.023$).

Discussion

Blood Antioxidant Capacity Indexes

Changes in oxidative biomarkers (TAOC, MDA, SOD, CAT and GSH-Px) can be used as an indicator of the physiological and health status of an animal. Our findings showed that the dietary ADY supplementation decreased the activity of TAOC, SOD and CAT, but had no significant effect on concentrations of MDA and activity of GSH-Px compared to CON. YC supplementation decreased activity of SOD and had no significant effect on the indexes above. MDA is increasingly concerned because increased MDA concentration can cause further oxidative damage. It is worth noting that, as an indicator of lipid peroxidation and an oxidative stress marker, MDA concentration showed no difference among groups, which indicated that decreased antioxidant capacity induced by yeast preparations did not induce response to oxidative stress and lipid peroxidation injury in this study.

Until now, there were few reports on the effect of ADY (Levucell SC, CNCM1-1077, 0.8×10^9 CFU g⁻¹) supplementation on antioxidant capacity in finishing bulls. The serum TAOC concentration is a measurement of the reductant capacity or capability of the body and some of the prominent reductants or antioxidants involved in the antioxidant defense system include vitamin A, vitamin C, vitamin E, glutathione, glutathione peroxidase, superoxide dismutase and catalase. Moreover, some trace elements (such as Cu, Fe, Mn, Mo, Se, Zn) are involved in the active composition of the antioxidant enzyme, which may indirectly result in changes of TAOC activity. The mechanism why ADY significantly decreased the concentration of TAOC remains unclear in our study. A latest research investigated the association between the serum concentration of some trace elements (Br, Co, Cr, Cu, Fe, I, Mn, Mo, Ni, Se, Sr, V and Zn) with TAOC in dairy cattle, which indicated that Spearman correlation results (ρ)

Table 4: Effect of dietary supplementation active dry yeasts (ADY) and yeast cultures (YC) on blood hormone index in finishing bulls

Items [†]	Treatments [‡]			SEM	P value
	CON (n=15)	ADY(n=15)	YC(n=15)		
Ghrelin, ng/ml	83.69	101.07**	114.01**	3.27	0.001
Insulin, μ IU/ml	16.18	16.91	20.87*	0.66	0.009
Leptin, ng/ml	6.26	6.14	6.21	0.21	0.970
T ₃ , ng/ml	0.98	0.96	1.06	0.029	0.392
T ₄ , ng/ml	83.85	83.36	86.49	0.73	0.199

[†] T₃, triiodothyronine; T₄, thyroxine

[‡] Treatments included a basal control diet (CON), a basal diet with supplemental active dry yeast (ADY, Levucell SC), a basal diet with supplemental yeast cultures (YC, Diamond V XP); Multiple comparison, * $p < 0.05$, ** $p < 0.01$

Table 5: Correlations between blood immunity, antioxidant capacity, hormone indexes and feed intake, beef tenderness in finishing bulls

Indexes [†]	Feed intake (dry matter intake)		Beef tenderness (shear force)	
	Correlation coefficient (r)	P value	Correlation coefficient (r)	P value
T-AOC	0.025	0.867	-0.041	0.831
MDA	-0.079	0.577	-0.104	0.558
SOD	0.193	0.190	0.201	0.295
CAT	-0.374**	0.009	-0.049	0.802
GSH-Px	0.197	0.179	-0.189	0.316
IgA	0.217	0.143	0.226	0.246
IgM	0.045	0.766	-0.153	0.394
IgG	0.070	0.622	-0.160	0.417
Ghrelin	0.306*	0.027	-0.384*	0.023
Insulin	-0.103	0.465	0.065	0.711
Leptin	-0.054	0.701	-0.178	0.307
T ₃	0.026	0.854	-0.062	0.730
T ₄	0.061	0.666	0.060	0.738

[†] T-AOC, total antioxidant count; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase enzyme; GSH-Px, glutathione peroxidase. T₃, triiodothyronine; T₄, thyroxine
Multiple comparison, * $p < 0.05$, ** $p < 0.01$

of metal ions with Co, Mn, Mo, Ni, Sr were greater than 0.50, and these metal ions were negatively correlated with TAOC except for Sr (Abuelo *et al.*, 2016). Live *Saccharomyces cerevisiae* yeast had the best performance for metal ions uptake, and the biotransformed metal ions by yeast has a higher absorption rate in intestinal tract (Cyert and Philpott, 2013). Based on above discussion, it is conceivable that decreased TAOC activity induced by ADY may be attributed to increased blood metal ions (such as Mn, Co and Mo) by live yeast uptake and bioconversion.

On the other hand, in human beings, TAOC has a significant negative relationship with daily calories intake (Barbosa *et al.*, 2014). Accordingly, the daily calories intake of bulls in ADY group was significantly greater than of those in CON (Geng *et al.*, 2016a), which may lead to decreased serum TAOC concentration.

Similarly, decreased SOD, CAT activity may be also due to changes of certain metal ions concentration induced by live preparations in our trial. Since SOD enzymes utilize Cu and Mn, and it is known that CATs are efficient with Mn as cofactors in their active sites, so the deficiency of Mn or

Cu would result in decreased antioxidant capacity. However, excess of Cu or Mn could induce oxidative stress-mediated damage (Garcia-Vaquero *et al.*, 2012; Apaydin *et al.*, 2016) and, therefore, result in decreased serum SOD concentration (Russo, 2010). On the other hand, total SOD activity was decreased in the highly efficient steers (Russell *et al.*, 2016). Similarly, a numerical improvement on feed efficiency was also observed in yeast preparations-feed bulls, which may be another factor caused decreased SOD activity.

However, in weaned piglets, live yeast (CCTCC, Y20007, 4.3×10^9 CFU g^{-1}) supplementation significantly increased the serum SOD activity, and decreased serum MDA concentration therefore contributing to improvement of antioxidant capacity (Zhu *et al.*, 2017). Differences in antioxidant activity were likely due to different kinds of animal and strains. There was a different metabolic mechanism between ruminants and non-ruminants and, different strains of yeast have different antioxidant activity (Hassan, 2011).

Although *Saccharomyces cerevisiae* fermentation products (YC) have been extensively used in the ruminant industry with beneficial effects on production parameters, the reports on effect of YC on antioxidant system of beef cattle are limited. In human beings, YC improved the capacity of serum antioxidant protection in healthy subjects (Jensen *et al.*, 2007). However, supplementation of YC (Diamond V XP, Cedar Rapids, IA) has no significant effect on the concentrations of oxidative biomarkers in the exception of decreased SOD activity compared to CON in this trial, and the similar results can also be observed in dairy calves (Alugongo *et al.*, 2017). In dairy goats, YC (Diamond V XP, Cedar Rapids, IA) supplementation decreased plasma MDA concentration, but did not increase plasma TAOC concentration during heat stress and lead to decreased plasma SOD concentration (Wang *et al.*, 2016). Differences in digestive systems between ruminants and human beings, physiological status of the animals, nature of the diets and modes of supplementation of feed may contribute to variable oxidative biomarkers results of YC.

In present study, the same to ADY, decreased SOD concentration by YC supplementation may also be due to numerical improvement of feed efficiency (Geng *et al.*, 2016a) and alterable blood metal ions concentration. Furthermore, there is a possibility that lower feed efficiency in bulls may induce a compensatory expression of SOD gene.

All in all, the lack of efficacy on some serum antioxidant indexes does not rule out the possibility that yeast preparations have an effect, as oxidative damage may occur only in a few tissues and at a particular time (Celi *et al.*, 2010). Alterations in serum antioxidants are not always consistent with that of the gut (Wittig and Zeitz, 2003). In the present study, although some serum antioxidant indexes were decreased by yeast preparations, there was no difference in MDA concentrations and has a

better growth performance (Geng *et al.*, 2016a). Thus, yeast preparations supplementation may be beneficial to alleviate oxidative damage in finishing bulls fed high concentrated diets, and further research is necessary for effect of yeast preparations on antioxidant capacity of other tissues (such as gut).

Blood Immunoglobulin Indexes

The primary immunoglobulins in blood are IgA, IgG and IgM. As we know, IgA are the most abundant antibody in intestinal mucosal secretions, and play an important role on protecting the intestinal epithelium from pathogens, toxins and antigens. In addition, IgA can modulate the sampling of antigens and the quality of the local immune response. The other 2 immunoglobulins can bind to the antigen and activate complement proteins for killing pathogens.

To our knowledge, few reports are available regarding dietary ADY (Levucell SC, CNCM1-1077, 0.8×10^9 CFU g^{-1}) and YC (Diamond V XP, Cedar Rapids, IA) supplementation on the blood IgA, IgG and IgM concentrations in finishing bulls fed by high-concentrate diets. Some previous studies indicated that administration of live yeast (*S. cerevisiae* CNCM I-4407) increased not only antibody levels in sows (Trckova *et al.*, 2014), but also IgA levels in the serum of weaned piglets (strain CCTCC, Y20007, Zhu *et al.*, 2017) and in the jejunum of weanling mice (Nagayama *et al.*, 2014). Moreover, results in dairy calves, pigs, and chickens suggest that feeding YC can improve immune function (Magalhães *et al.*, 2008; Gao *et al.*, 2009; Shen *et al.*, 2009) by activating the innate and adaptive immune response (Jensen *et al.*, 2008a).

Reversely, in the current study, both ADY (Levucell SC, CNCM1-1077, 0.8×10^9 CFU g^{-1}) and YC (Diamond V XP, Cedar Rapids, IA) feeding did not affect serums IgA, IgG and IgM concentrations of bulls fed high concentrated diets (Table 3). In consistent with our studies, Zaworski *et al.* (2014) also found YC (Diamond V XP, Cedar Rapids, IA) supplementation and dosage did not affect the serum concentrations of IgA, IgG and IgM in dairy cows. Similarly, YC consumption did not affect serum IgA and IgG concentrations in human beings (Jensen *et al.*, 2008b).

The efficacy of ADY and YC on immune function was related to the physiological status of animals. Previous experiments have shown that health effects of ADY and YC in animals are observed more apparently in animals under stress, such as weaning or poor health (Brewer *et al.*, 2014; Zhu *et al.*, 2017). Moreover, difference in strain of *Saccharomyces cerevisiae* possibly led to different results on immune function. Overall, lack of an immunoglobulin-induction protocol or yeast strain of itself in our study may explain why we did not observe an effect on immunoglobulin concentrations in finishing bulls. Alternatively, further work is needed to evaluate the effect of ADY and YC on immune system in beef cattle, especially during finishing phases.

Blood Hormone Indexes

Ghrelin which is a peptide mainly synthesized in the monogastric stomach and the abomasum of cattle (Hayashida *et al.*, 2001), can stimulate growth hormone release and appetite by binding to the growth hormone secretagogue receptor (GHSR1a), thereby, circulating ghrelin concentrations could potentially be used as a predictor of dry material intake (DMI) in cattle (Foote *et al.*, 2014). To our knowledge, however, there is no report for effect of ADY and YC on ghrelin in finishing bulls fed high concentration until now.

In current study, ghrelin concentration was significantly elevated by ADY supplementation compared to CON. The underlying action mechanism that increased ghrelin concentration by ADY supplementation remains unclear, which may be related to rumen metabolite changes induced by ADY supplementation. ADY (Levucell SC, CNCM1-1077) supplementation can significantly increase the number of the lactate-utilizing bacteria and decrease lactate concentration in rumen of cattle fed high concentrated diets (Ding *et al.*, 2014), and lactate-utilizing bacteria can use lactic acid as a fermentation substrate to synthesize medium chain fatty acids (Zhu *et al.*, 2015). It was reported that ingestion of medium chain fatty acids increased concentrations of plasma ghrelin and NEFA in lactating dairy cow (Fukumori *et al.*, 2013). Interestingly, the results of ghrelin and NEFA induced by ADY supplementation in our study were consistent with those by ingestion of medium chain fatty acids in study of Fukumori *et al.* (2013). Plausibly, increased ghrelin may be attributed to medium chain fatty acids induced by ADY supplementation in our trial. Therefore, further research into the action mechanism of ADY in ghrelin in cattle is warranted.

In the exception of ghrelin, other appetite hormones including insulin, leptin, T_3 and T_4 were not affected by ADY supplementation. Similarly, several previous studies also indicated that live yeast (P169, Agtech products Inc., Waukesha, WI) supplementation have no significant effect on insulin concentration in steers (Lehloenya *et al.*, 2008a) and in early-lactation dairy cows (Putnam *et al.*, 1997). In rabbits, Habeeb *et al.* (2006) reported that the levels of T_3 and T_4 hormones concentrations were significantly increased by adding ADY to the diet of growing rabbits. However, Ashour *et al.* (2009) found that treatment with yeast supplementation (Sc1026) did not affect concentrations of blood plasma thyroid hormones (T_3 , T_4) in Holstein cows, and the results were consistent with those of our current study with ADY (Levucell SC, CNCM1-1077) in finishing bulls.

In the present study, YC supplementation has no significant effect on concentration of leptin, T_3 and T_4 , however significantly elevated ghrelin and insulin concentration compared to CON. Similar to ADY, the action mechanism of YC may also be related to altered

medium chain fatty acid concentration in rumen which then stimulated the secretion of ghrelin (Fukumori *et al.*, 2013). It was reported that YC supplementation also can decrease the concentration of lactate by increasing number of lactate-utilizing bacteria in rumen which then use lactic acid as a fermentation substrate to synthesize medium chain fatty acids (Zhu *et al.*, 2015).

More interestingly, unlike ADY, YC stimulated the secretion of insulin in current study. It was reported that increased ratio of propionate in rumen can elevate insulin concentrations in cattle (Subiyatno *et al.*, 1996). However, the ratio of propionate of YC in rumen was not significantly different from those of CON and ADY (Geng *et al.*, 2016a), which indicated increased insulin levels are not directly mediated by propionate but rather through other mechanisms that require nutrients transiting in the gastrointestinal tract in current study. On the other hand, it's worth noting that ghrelin concentration of YC was not only higher than that of CON, but also tended to be higher than that of ADY ($P = 0.068$) in this study. In fact, both ghrelin and insulin are regulation hormones of energy balance of animal and there are complex interaction relationships which remain unclear (Chabot *et al.*, 2014; Geng *et al.*, 2016b). Nevertheless, increased insulin in YC group may be related to a much higher ghrelin level.

Other studies indicated that YC (Diamond V XP, Cedar Rapids, IA) supplementation have no significant effect on insulin in dairy cows (Lehloenya *et al.*, 2008b; Zaworski *et al.*, 2014). Moreover, there were few reports on effect of YC on leptin, T_3 and T_4 in ruminants. In weaning piglets, dietary supplementation with HKY (heat-killed whole yeast) significantly increased serum concentrations of T_3 and T_4 of piglets on day 21 (Jiang *et al.*, 2015). Variable results may depend on the basal diet fed, dose of YC given, and physiological condition of animals. In addition, fluctuation and response of some hormones are transient, thus only one-time collection in our study also may result in variable results. Consequently, frequent blood collections are necessary to detect effect of yeast preparations on blood hormones indexes in bulls.

Correlations Among the Feed Intake, Beef Tenderness of Bulls and Blood Indexes

A large number of data have indicated feeding on feed mixture contained yeast preparations can improve the appetite of animals which lead to increase the feed intake and consequently increased the daily weight gain of the treated animals (Bontempo *et al.*, 2009; Finck *et al.*, 2014; Geng *et al.*, 2016a). In our recent studies, we found that yeast preparations supplementations not only increased feed intake but also improved the beef tenderness in bulls fed high concentration diets (Geng *et al.*, 2016a, 2018). Until now, although several hypothesis have been proposed to explain why yeast products could stimulate DMI and productivity in ruminants (Throne *et al.*, 2009;

Robinson, 2010; Montes de Oca *et al.*, 2016), the research on underlying action mechanism with endogenous appetite hormone changes is somewhat limited. It was reported that the antioxidant capacity, immune and some hormones such as ghrelin not only are related to animal performance, but also ruminant production quality (Foote *et al.*, 2014; Russell *et al.*, 2016). Thus, it is necessary to reveal the relationship between these indexes and feed intake and beef tenderness, and it is meaningful to more clearly understand underlying action mechanism of yeast preparations function in ruminants.

In the present study, we found that there were significantly negative correlations between DMI and blood CAT activity, and significantly positive correlations between DMI and blood ghrelin level. CAT is an important enzyme that can remove the hydrogen peroxide generated by oxidases involved in β -oxidation of fatty acids and purine catabolism. A negative relationship between feed intake and CAT indicated that increased feed intake may induce greater oxidant stress and then lead to decreased CAT activity.

Ghrelin is a 28 amino acid peptide, and in the circulation, ghrelin is present as acylated (AG) and unacylated (UAG) forms which paly opposing action on appetite (Asakawa *et al.*, 2005). AG was considered as the bioactive form of ghrelin on appetite, and UAG can be transformed into AG by the enzyme ghrelin o-acyltransferase (GOAT) (Yang *et al.*, 2008). Research on beef cattle has indicated that exogenous AG can increase DMI (Wertz-Lutz *et al.*, 2006), and AG rather than total ghrelin (AG and UAG) has a positive association with DMI (Foote *et al.*, 2014). Difference between this study and previous study of Foote *et al.* (2014) in relationship of ghrelin and feed intake may be caused by varied ratio of AG to UAG induced by supplementation of yeast preparations. As mentioned above, increased ghrelin by supplementation of yeast preparations may be attributed to increased medium chain fatty acids produced by lactate-utilizing bacteria and lactate in rumen. Moreover, medium chain fatty acids can be directly used for the acyl-modification of ghrelin *in vivo* (Nishi *et al.*, 2005), which could lead to an increased ratio of AG to total ghrelin. The ratio of AG to total ghrelin has a better indicator of DMI than AG in finishing cattle (Foote *et al.*, 2014). Therefore, increased AG ratio plausibly explained the result that Ghrelin has a positive correlation with DMI in present study. Unfortunately, we did not detect changes of AG and UAG concentration in this study. In the future, it is necessary to further research into the changes of AG and UAG concentration in finishing bulls fed high concentrated diets.

In addition, we found that there was a significantly negative correlation between shear force and the level of ghrelin, which indicated that ghrelin may contribute to regulation of beef tenderness induced by yeast preparations in finishing bulls fed concentrated diets. Many factors such as the amount of water, pH, content of intermuscular fat of beef are related to beef tenderness (Chraki *et al.*, 2013), and

degradability of connective tissue protein is a key factor (Koochmaraie and Geesink, 2006). However, the pH, drip loss, cooking loss, marbling grade, intermuscular fat content and protein content were not all affected by yeast preparations in finishing bulls (Geng *et al.*, 2016a). Available evidences have indicated that increased intracellular Ca²⁺ concentration plays an important role in improving the beef tenderness by regulating degradability of connective tissue protein (Foote *et al.*, 2004; Rider *et al.*, 2004; Mohrhauser *et al.*, 2015), and increased endogenous ghrelin concentration can increase intracellular Ca²⁺ concentration in skeletal muscle cells (Fang *et al.*, 2012). These results suggest that ghrelin may potentially contribute to regulation of beef tenderness by increasing Ca²⁺ concentration in skeletal muscle.

Conclusion

All in all, there are differences in effect of ADY and YC on antioxidant indexes and, the antioxidant status index CAT activity and appetite index ghrelin concentration may contribute to regulation of feed intake of finishing bulls fed high concentrated diet. Furthermore, improved beef tenderness was related to elevated ghrelin concentration.

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