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Full Length Article

Effect of ADY and YC on Concentration of Ruminal Medium Chain Fatty Acid, Lactic Acid, Ethanol and Relative Abundance of Bacteria in Beef Cattle

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Abstract

The aim of this study was to evaluate the effect of two typical yeast preparation (ADY and YC) supplementation on the concentration of ruminal medium chain fatty acid, lactic acid, ethanol and the abundance of relative rumen bacteria in finishing beef cattle. The results showed that ADY supplementation significantly increased the concentration of caproate (C6:0) ($P < 0.05$) and tended to increase the content of total medium chain fatty acids ($P = 0.094$), while had no significant effect on concentration of caprylate (C8:0) and caprate (C10:0) ($P > 0.1$). YC supplementation did not show a significant effect on the content of total medium chain fatty acids and the concentration of individual volatile acids ($P > 0.1$); ADY supplementation significantly decreased the concentration of lactic acid ($P < 0.05$) and has a tendency to decrease the ethanol concentration ($P = 0.057$). YC did not affect significantly the concentration of lactic acid and ethanol ($P > 0.1$); Both ADY and YC supplementation significantly decreased relative abundance of *B. fibrisolvens* ($P < 0.05$) and increased relative abundance *S. ruminantium* ($P < 0.05$), and YC rather than ADY tended to increase relative abundance of *S. bovis* ($P= 0.053$). Furthermore, both ADY and YC did not show the significant effect on relative abundance of *M. elsdenii and C. kluyveri* ($P > 0.1$). These data suggested that there are significant differences between ADY and YC in the effects on rumen metabolites including MCFAs, ethanol and lactic acid, and increased concentration of caproate (C6:0) in rumen may be responsible for the increment of circulating ghrelin caused by ADY supplementation finishing bull.

Keywords: Yeast preparations; Medium chain fatty acid; Bacteria; Finishing bulls

Introduction

There are two typical yeast preparations, ADY (active dry yeasts) and YC (yeast cultures), in current markets and they hey have been widely used in ruminant to maintain health and improve growth performance and products quality (Chaucheyras-dur *et al*. 2008). Nevertheless, yeast preparations did not show a consistent conclusion for effect on animal growth performance and production quality when it was used in ruminant animals' production (Swyers *et al*. 2014; Geng *et al.* 2016). The results of variation are related to the strain of yeast, the basal diets, animal physiological state in studies and also related to the types of yeast preparations (Geng *et al.* 2016). At present, there are few studies comparing the effects of two types of yeast preparations, ADY and YC, on animal production performance under the same experimental conditions. We evaluated the effect of ADY (Levucell, *S. cerevisiae* CNCM1-1077) and YC (Diamond V XP, Cedar Rapids, IA, U.S.A.) on indexes of growth, carcass and beef quality in finishing beef cattle in a previous study, and we found that both ADY and YC improved the tenderness of beef since yeast preparations were added to the basal diets, but ADY rather than YC had more pronounced effect on improvement of feed intake and growth performance of beef cattle (Geng *et al.* 2016).

So far, the action mechanism of yeast preparations supplementation affect feed intake and beef tenderness has been reported (Geng *et al.* 2018a). The increased circulating ghrelin concentration caused by yeasts supplementation was a key factor for improvement of feed intake and beef tenderness (Geng *et al.* 2018a). However, the further mechanism for increment of circulating ghrelin concentration caused by yeasts supplementation is still unclear. Research showed that changes of ghrelin concentrations were related to the changes of ruminal fermentation production such as short chain fatty acids (SCFAs) (Fukumori *et al*. 2012) and medium chain fatty acids (MCFAs) (Fukumori *et al*. 2013). In addition, it was reported that some lactate-utilizing bacteria such as *S. ruminantium, M. elsdenii* can synthesize

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MCFAs with lactic acid as substrate (Zhu *et al.* 2015), and that *C. kluyveri* also can synthesize efficiently MCFAs with ethanol (Cavalcante *et al*. 2017).

Up to now, the effect of ADY and YC on ruminal SCFAs has been compared in finishing cattle (Geng *et al.* 2018b), however, the effect on ruminal MCFAs has not been evaluated. Therefore, the purpose of this study is to lay a foundation for revealing the mechanism of improving beef quality by supplementation of yeast preparations by evaluating the effect of ADY and YC on the concentration of MCFAs, lactic acid, ethanol and the abundance of relative rumen bacteria in finishing cattle.

Materials and Methods

Animals and treatment diets

All animals were managed according to the Yanbian University of Health guidelines for the care of animal subjects. More detail about animal feeding and management have been reported in our companion paper (Geng *et al.* 2016). Briefly, forty-five bulls 24-month-old bulls with an average weight of 505 kg were randomly divided into three groups, and 15 bulls in each group. There are three treatment groups of diets in this study, which are CON group (basal diets), ADY group (basal diets plus Levucell *S. cerevisiae* CNCM1–1077) and YC group (basal diets plus Diamond V XP). The supplementation was 0.8 g/head/day for ADY and 50 g/head/day for YC. The trial lasted over 112 days. The basal diets were high-concentrate diets which the ratio of concentrate to forage based on a dry matter basis was 7:3. The composition and nutrient level for the basal diets are the same to our previous study (Geng *et al*. 2016).

Collection of rumen fluid

All the beef cattle were slaughtered after the trial, and the rumen fluid of bull was sampled at slaughter. The rumen fluid was used to determine the concentration of MCFAs, lactic acid and ethanol, and the relative abundance of bacteria. The MCFAs included caproate (C6:0), caprylate (C8:0), caprate (C10:0), and laurate (C12:0), and the relative bacteria included lactic acid-producing bacteria *(S. bovis, B. fibrisolvens, L. fermentum*), lactic acid-utilizing bacteria (*S. ruminantium, M. elsdenii*) and *C. kluyveri.*

Analyses of samples

Measurement of the concentration of MCFAs was performed on an Agilent 7890A GC-FID system equipped with a capillary column DB-225 (10 m × 0.1 mm × 0.1 μm film thickness) and the injector and detector temperatures were maintained at 250 and 230°C, respectively. The oven temperature was programmed at 55°C for 1 min and increased to 205°C at 30°C/min in 5 min, and at 205°C for 1 min and increased to 230°C at 5°C/min in 5 min and at 230°C for 1 min. The carried gas was helium, and split ratio was 15:1.

The measurement of lactic acid and ethanol were performed according to method of previous reports (Barker and Summerson 1941; Rahim and Geeso 1992). The measurement of relative abundance of bacteria was performed by real-time quantitative PCR (RT-qPCR). Briefly, 1 mL rumen fluid was centrifuged at 5 000 rpm/min for 3 min, then the supernatant was discarded and bacteria were collected. A lysozyme solution (10 mg/mL) was added to collected bacterial precipitates and incubated with rotation at 37°C for 5 min to break the cell wall of bacteria. Then, the DNA was abstracted using QIAamp DNA Stool Mini Kit (Qiagen, Germany).

RT-qPCR was performed in ABI Real time PCR (ABI 7500) and the gene fluorescence quantitative detection was performed with Qiagen fluorescent dye Kit (PN. 204054, Germany). The conditions of the RT-qPCR reactions were the same as described in previous reports (Stevenson and Weimer 2007; Chen *et al.* 2016) and the primers sequences for bacteria RT-qPCR are shown in Table 1. The total bacteria were used as the internal reference for fluorescence quantification, and the relative fold change was expressed using the $2^{-\Delta\Delta Ct}$ calculation (Schmittgen and Livak 2008).

Statistical Analyses

Data were analyzed in a GLM model of S.P.S.S. 18.0 (S.P.S.S. Inc., Chicago, IL, U.S.A.). The multiple comparisons were performed by Duncan method, and significance was determined at $P \leq 0.05$ and trends of significance were determined at $P > 0.05$ to $P \leq 0.10$.

Results

Effect of ADY and YC on concentration of ruminal MCFAs, lactic acid and ethanol

Compared to the control, ADY supplementation significantly increased caproate (C6:0) concentration ($P < 0.05$) and significantly decreased lactic acid concentration ($P < 0.05$), and have a tendency to increase total MCFAs content ($P = 0.094$) and tended to reduce the concentration of laurate (C12:0) ($P = 0.072$) and ethanol ($P = 0.057$) in rumen fluid. Furthermore, ADY did not show a significant effect on caprylate (C8:0) and caprate (C10:0) concentration ($P > 0.1$) (Table 2). Compared to control, YC did not affect significantly the content of total MCFAs and the concentration of individual volatile acids (caproate, caprylate, caprate and laurate), lactic acid and ethanol ($P > 0.1$) (Table 2).

Effect of ADY and YC on relative abundance of ruminal lactic acid-producing, lactic acid-utilizing bacteria and *C. kluyveri*

Compared to the control, ADY supplementation

Table 1: Primers sequences for bacteria RT-qPCR

Items	Sequence of primers (5'-3')	Product size (bp)
Total bacteria	F: CGGCAACGAGCGCAACCC	130
	R: CCATTGTAGCACGTGTGTAGCC	
S. bovis	F: CGATACATAGCCGACCTGAG	235
	R: TAGTTAGCCGTCCCTTTCTG	
B. fibrisolvens	F: TAACATGAGTTTGATCCTGGCTC	136
	R: CGTTACTCACCCGTCCGC	
L. fermentum	F: AGCGAACAGGATTAGATACCC	233
	R: GATGGAACTAGATGTCAAGACC	
M. elsdenii	F: GACCGAAACTGCGATGCTAGA	129
	R: CGCCTCAGCGTCAGTTGTC	
S. ruminantium	F: GAGCGAACAGGATTAGATACCC	194
	R: TGCGTCGAATTAAACCACATAC	
C. kluyveri	F: GAGGAGCAAATCTCAAAAACTGC	400
	R:CCTCCTTGGTTAGACTACGGACTT	

Table 2: Effect of ADY (active dry yeast) and YC (yeast culture) on concentration of ruminal medium chain fatty acid, lactic acid and ethanol in finishing beef cattle

Items†	Treatment‡			SEM	*P* value
	CON	ADY	YC		
Caproate C6:0, μg/mL	80.23[b]	116.30[a]	71.30[b]	6.74	0.013
Caprylate C8:0, μg/mL	2.24	2.59	2.34	0.11	0.470
Caprate C10:0, μg/mL	3.10	2.38	2.71	0.22	0.452
Laurate C12:0, μg/mL	22.08	15.08	19.62	1.56	0.187
SUM, μg/mL	106.40[ab]	134.87[a]	95.47[b]	7.08	0.064
Lactic acid, μmol/mL	24.31[a]	18.91[b]	25.10[a]	0.96	0.014
Ethanol, μmol/mL	15.48	11.85	12.60	0.77	0.124

† SUM, the sum of caproate, caprylate, caprate and laurate.
‡ Treatments includes CON group (basal diets), ADY group (basal diets plus Levucell *S. cerevisiae* CNCM1–1077) and YC group (basal diets plus Diamond V XP)
[a, b] Differing superscript letters (a and b) denote significant difference ($P < 0.05$)

significantly decreased relative abundance of *B. fibrisolvens* ($P < 0.05$) and significantly increased relative abundance of *S. ruminantium* ($P < 0.05$) and has no significant effect on relative abundance of *S. bovis*, *L. fermentum* and *M. elsdenii* ($P > 0.1$) (Fig. 1). Compared to control, YC supplementation significantly decreased relative abundance of *B. fibrisolvens* ($P < 0.05$) and significantly increased relative abundance of *S. ruminantium* ($P < 0.05$) and have a tendency to increase relative abundance of *S. bovis* ($P = 0.053$), while did not affect significantly relative abundance of *L. fermentum* ($P > 0.1$) (Fig. 1). Compared to the control, neither ADY or YC supplementation had a significant influence on relative abundance of *C. kluyveri* ($P > 0.1$) (Fig. 2).

Discussion

In this study, we first revealed the effect of two typical yeast preparation (ADY and YC) supplementation on the concentration of ruminal MCFAs of finishing cattle fed high-concentrate diets, and compared the effects of ADY and YC on the concentration of ruminal lactic acid, ethanol and the relative abundance of related bacteria under the same experimental conditions. We found that ADY supplementation significantly increased the concentration of caproate (C6:0), and significantly decreased the

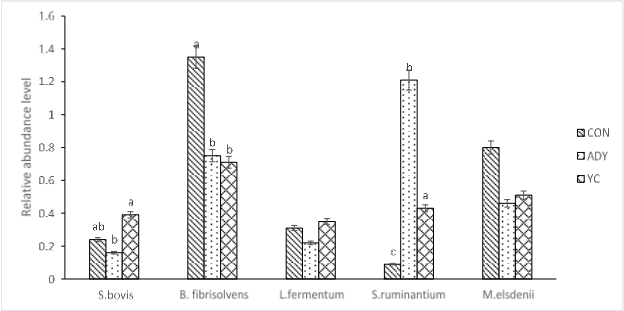

Fig. 1: Effect of active dry yeast (ADY) and yeast culture (YC) on relative abundance of ruminal lactic acid-producing, lactic acid-utilizing bacteria in finishing beef cattle
[a, b] For the same strain, differing letters denote significant difference ($P < 0.05$)

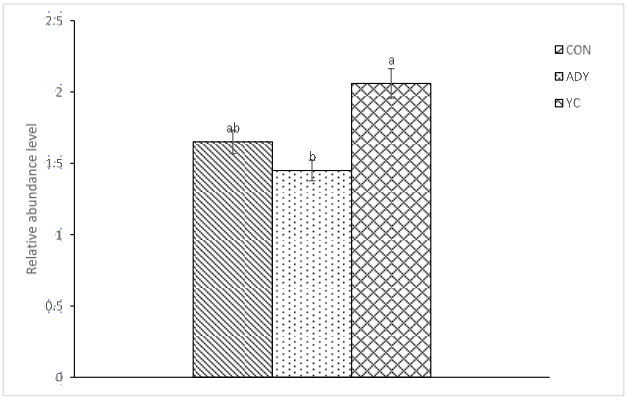

Fig. 2: Effect of active dry yeast (ADY) and yeast culture (YC) on relative abundance of ruminal *C. kluyveri* bacteria in finishing beef cattle
[a, b] Differing letters denote significant difference ($P < 0.05$)

concentration of lactic acid and the relative abundance of *B. fibrisolvens*, and significantly increased the relative abundance of *S. ruminantium*. At the same time, it also tended to decrease the concentration of laurate (C12:0), ethanol and tended to increase the content of total MCFAs. However, supplementation of YC had no significant effect on the concentration of lactic acid, ethanol, total medium chain fatty acids and individual volatile acids including caproate (C6:0), caprylate (C8:0), caprate (C10:0), and laurate (C12:0).

It has been reported that long-term high-concentrate diets can significantly increase the content of lactic acid in the rumen of beef cattle, while active yeast preparation can reduce the concentration of lactic acid by increasing the number of lactic acid utilizing bacteria and inhibiting lactic acid producing bacteria (Mao *et al*. 2016). In this study, the effect of ADY supplementation on lactic acid concentration was consistent with previous reports (Mao *et al*. 2016), and decreased lactic acid concentration attributed to decrease of the relative abundance of *B. fibrisolvens* and increase of the relative abundance of *S. ruminantium*. Lynch and Martin (2002) compared the effects of active yeast cells and their

cultures on lactic acid by rumen fermentation in *vitro*, and the results showed that active yeast cells rather than yeast culture decreased significantly the concentration of lactic acid (Lynch and Martin 2002). In this study, although YC also significantly reduced the abundance of *B. fibrisolvens* and increased the abundance of *S. ruminantium*, it also increased the abundance of *S. bovis*, which may be a reason why YC supplementation had no a significant influence on ruminal lactic acid.

The effect of ADY supplementation on the concentration of caproate (C6:0) may be related to the change of relative abundance of ruminal lactic acid utilizing bacteria. It was reported that lactic acid utilizing bacteria could synthesize caproate (C6:0) using lactic acid as fermentation substrate (Fukumori *et al*. 2013). In this study, that ADY supplementation significantly increased the relative abundance of lactic acid utilizing bacteria *S. ruminantium* and significantly decreased the concentration of lactic acid suggested that caproate (C6:0) may be synthesized from lactic acid by *S. ruminantium*. In addition, it was found that *C. clarkii* in rumen could produce caproate (C6:0) using ethanol (Weimer and Stevenson 2012) and its coculture with active *Saccharomyces cerevisiae* significantly increased the production of caproate (C6:0) (Weimer *et al.* 2015). In this study, although ADY supplementation did not affect significantly the relative abundance of *C. clarkii* in rumen, the concentration of ethanol in rumen was decreased, which indicated that the increase of caproate (C6:0) concentration might be also related to the enhancement of the utilization of ethanol by *Clostridium clarkii*. Moreover, laurate (C12:0) is the main precursor of lauroylcarnitine synthesis. Ogunade *et al*. (2019) found that the rumen lauroylcarnitine concentration decreased significantly after ADY supplementation (Ogunade *et al*. 2019), which was consistent with the conclusion that laurate (C12:0) concentration was decreased after ADY supplementation in this study.

Ghrelin plays an important role in animal feeding and meat tenderness regulation, which also explains part of the reasons for the improvement of beef cattle performance by supplementary yeast preparation (Geng *et al.* 2018a). However, the mechanism for increment of ghrelin concentration caused by yeast preparations supplementation is still unclear. It was found that the changes of SCFAs and MCFAs in rumen could cause the changes of ghrelin concentration in blood of dairy cows (Fukumori *et al.* 2012, 2013). Rumen perfusion of short chain fatty acids significantly reduced the blood ghrelin level of calves (Fukumori *et al.* 2012), while supplementation with medium chain fatty acid calcium significantly increased the blood ghrelin level of dairy cows (Fukumori *et al*. 2013). Our previous study found that both ADY and YC significantly increased the blood ghrelin level of beef cattle (Geng *et al*. 2018a), but there were significant differences in the effects of ADY and YC on rumen short chain fatty acids (Kowalik *et al*. 2012; Geng *et al*. 2018b). ADY supplementation had no significant effect on ruminal SCFAs, but YC significantly increased the concentration of acetic acid and the ratio of acetic acid to propionic acid, and significantly decreased the concentration of valeric acid (Geng *et al*. 2018b). Presumably, the increase of rumen caproate (C6:0) may be a key for the increase of ghrelin caused by ADY, and the increase of ghrelin concentration caused by YC may be related to the change of rumen SCFAs. Further comprehensive research is required to determine the correlation of ghrelin with ruminal fatty acid including the types and ratio of MCFAs and SCFAs.

Conclusion

In this study, we first revealed that ADY rather than YC supplementation significantly increased MCFA concentration (caproate, C6:0), and the increment of caproate (C6:0) concentration may be related to increment of the relative abundance of *S. ruminantium*. Moreover, the increased concentration of caproate (C6:0) may be responsible for the increment of circulating ghrelin caused by ADY in finishing bull. The data lay a foundation for the further study on the mechanism of improving ghrelin secretion by yeast preparation supplementation in finishing bulls fed high-concentrate diets.

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Author Contributions

CY and MZ design the study. CY, SJ and YJ performed the experiments. CY and SJ analyzed the data. CY and LY wrote the manuscript. All authors have read and approved the manuscript.

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