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Effect of *Saccharomyces cerevisiae* Fermentation Product on Ruminal Fermentation, Blood Metabolites, and Milk Production in Dairy Cows

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Abstract

The objective of this study was to evaluate the effects of supplementing a yeast culture (YC; *Saccharomyces cerevisiae*) on rumen fermentation, nutrient utilization, and milk production in dairy cows. Six Holstein cows were subjected to two dietary treatments in a crossover design. The treatments were control (no yeast culture) and yeast culture (fed at 30 g/head per day). Rumen pH and protozoal counts were similar between both treatments, although the concentration of rumen ammonia-N tended to be lower with YC treatment. Moreover, YC treatment did not affect the concentration of total short-chain fatty acids in the rumen, but tended to reduce the molar proportion of acetic acid (A), while significantly increasing that of propionic acid (P). Therefore, the A/P ratio decreased significantly after 5 hr of YC feeding as compared with the control. The treatments had no effect on the blood plasma concentrations of glucose, urea-N, and non-esterified fatty acids. However, YC treatment resulted in a significantly higher concentration of plasma free lysine, along with higher concentrations of isoleucine, arginine, and total free amino acids. Dry matter intake and milk yield were similar between both treatments, though the percentage of milk protein tended to be higher with YC treatment. In addition, YC treatment did not affect the milk fatty acid composition and somatic cell counts. Overall, the yeast culture had a subtle effect on ruminal fermentation. The higher plasma concentrations of some essential amino acids indicated the positive effect of YC supplementation on the availability of amino acids for milk protein synthesis in the mammary gland of dairy cows.

Discipline: Animal Science
Additional key words: amino acid, milk protein, rumen microorganisms, yeast culture

Introduction

The use of yeast culture (YC) based on *Saccharomyces cerevisiae* strains has been widely practiced to improve the ruminal environment and promote microbial growth in ruminants. Several studies have reported the positive effects of increasing feed intake (Dann et al. 2000) and milk production (Harrison et al. 1988, Williams et al. 1991, Zaworski et al. 2014) when cows were fed YC. Conversely, some studies showed that YC supplementation had no effect on feed intake or milk yield (Kobayashi et al.1995, Hristov et al. 2010).

The change in ruminal fermentation parameters with different supplemental diets is the primary indicator of the effect of physiological response by the host animal. YC supplementation has long been considered a useful tool to stabilize ruminal fermentation, including the ability to increase rumen

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pH, alter concentrations of short-chain fatty acids (SCFAs) and ammonia, and increase the cellulolytic bacteria that enhance the rate of fiber digestion (Williams et al. 1991, Callaway & Martin 1997).

Recent studies have revealed that the supplementation of YC and viable yeast cells attenuates the effect of subacute ruminal acidosis in lactating dairy cows. (AlZahal et al. 2014, Li et al. 2016). YC supplementation improves feed efficiency in maintaining strictly anaerobic conditions by scavenging oxygen within the rumen (Newbold et al.1996).

Different nutritional regimes aim to improve the production performance of high yielding dairy cows with the proper physiological condition (Poppy et al. 2012). Blood plasma metabolites such as glucose, urea, non-esterified fatty acids (NEFA), and free amino acids are indicators of the appropriate energy and protein supplement status in a healthy dairy management system. There have been a few reports on the effect of YC supplementation on nutrient utilization in dairy cows (Erasmus et al.1992, Hristov et al. 2010). But to the best of our knowledge, there is limited information on the effect of YC supplementation on the status of protein nutrition of dairy cows.

We hypothesized that YC supplementation increases milk production and changes milk composition with a favorable rumen fermentation pattern, while maintaining a proper balance of energy and protein in dairy cows. Therefore, the objective of this study was to investigate the effects of yeast culture supplementation on feed intake, ruminal fermentation characteristics, blood plasma metabolic parameters, milk production, and milk composition in lactating dairy cows.

Materials and methods

1. Animals, experimental design, and diets

Six multiparous Holstein cows (with an average body weight 660 kg) were blocked into two groups according to mean milk production (28.5 kg/day), mean number of days post-partum (142), and mean lactation number (2.3), and then randomly assigned to one of two treatment groups (control period and supplementation period). Treatments were applied as a two-period crossover design. Each of the periods lasted 14 days, with samples and data being collected during the final three days of each period. Cows were housed in a free-stall barn and individually fed twice daily, using Calan Broadbent feeding doors (American Calan Inc., Northwood, NH, USA). Cows were milked daily at 09:00 and 17:00 in an auto-tandem milking parlor, and individual milk yields were recorded electronically.

During the control period, the cows received a basal diet. The basal diet consisted of rice whole crop silage (RWCS), sudangrass hay, alfalfa hay cubes, and a concentrate mixture. Table 1 lists the ingredient and nutrient composition of the diet. RWCS and half of the other feedstuffs without sudangrass hay were offered every day at 09:30, and sudangrass hay and the remaining half of other feedstuffs without RWCS were

Table 1. Ingredient and nutrient composition of the basal diet fed in the study

Item	Amount
Ingredient (% of dry matter)	
Rice whole crop silage	23.2
Sudangrass hay	11.1
Alfalfa hay cube	18.1
Concentrate mixture-1 [1)]	37.3
Concentrate mixture-2 [2)]	10.3
Nutrient composition (% of dry matter) [3)]	
Total digestible nutrients	69.0
Crude protein	15.9
Ether extract	2.9
Neutral detergent fiber	34.4

[1)] Contained 49% grains (corn, wheat flour, milo, barley, wheat feed flour), 24% chaff and bran (wheat bran, corn gluten feed, rice bran), 22% oil seed meal (rapeseed meal, soybean meal, corn germ meal, copra meal), 5% others (molasses, silicic anhydride, $CaCO_3$, NaCl, vitamins (A, D) and trace mineral premix)
[2)] Contained 34% whole cottonseed, 28% wheat bran, 27% beet pulp, and 11% soybean meal
[3)] Calculated according to Standard Tables of Feed Composition in Japan (2009)

offered at 17:30. Diet was formulated according to the Japanese Feeding Standard for Dairy Cattle (2006). Drinking water and mineralized salt bricks were always available. During the supplementation period, 30 g of Yea-Sacc@ 1026 (*Saccharomyces cerevisiae* YC grown on media of yellow corn, diastatic malt and cane molasses, dried to preserve its fermenting action, 16% moisture, 25% crude protein; Alltech Inc., USA) was supplemented daily to each cow.

The experimental protocol was approved by the Guide for the Care and Use of Laboratory Animals prepared by the Tokyo University of Agriculture and Technology.

2. Sample collection and analyses

Milk yields were recorded from two consecutive milkings during the last three days of each period. Individual milk samples were collected from each milking and combined proportionally for each day. Milk samples were split into two portions for analysis. One portion was analyzed for fat, protein, and lactose contents by semiautomatic infrared spectroscopy (Milko-Scan 133N. Foss Electric, Hillerød, Denmark), and for somatic cell count by using the Fossomatic 90 instrument (Foss Electric, Denmark). The other portion was used for analyzing the composition of milk fatty acids. The extraction of milk fat and fatty acid methyl esterification (FAME) of extracted fat were analyzed as previously described (Sultana et al. 2011). FAME samples were analyzed by using a gas chromatograph (Model GC-14B, Shimadzu, Kyoto, Japan) fitted with a flame ionization detector and integrator with a fused silica capillary column (DB-23, 30 m × 0.25 mm i.d., J&W, Santa Clara, CA, USA). The gas chromatography conditions were as previously described (Sultana et al. 2011). The CLA standard was obtained from Rinoru Oil Mills (Tokyo, Japan).

Ruminal samples were obtained on the final day of each period at pre-feeding (0 h) and 2 h and 5 h after feeding. Ruminal samples were taken orally using a suction pump with a flexible stainless steel stomach tube (Fujihira Industries, Tokyo, Japan). Approximately 400 mL of fluid was separated from a particular matter by straining the digesta through two layers of surgical gauze, and then about 100 mL of the fluid was stored at −30°C for subsequent analysis. Concentrations of SCFAs, ammonia-nitrogen (N), and protozoal numbers were determined according to Mohammed et al. (2004).

Jugular venous blood samples were collected 2 h after feeding on the final day of each period. Blood samples were collected into heparinized vacuum tubes, immediately placed on ice, and then centrifuged at 3,000 × *g* for 15 min. at 4°C. The plasma was removed and stored at −30°C. After thawing, glucose was determined by the *o*-toluidine boric acid method, urea-N was measured by the diacetyl mono-oxime method, and non-esterified fatty acid (NEFA) was determined by the Duncombe modified methods using a kit (Wako Pure Chemical Industries, Tokyo, Japan) as described by Mohammed et al. (2004). Plasma was deproteinized with an equal volume of 10% sulfosalicylic acid, and free amino acids were analyzed by a high-performance automatic amino acid analyzer (L-8800, Hitachi Industries, Tokyo, Japan).

3. Statistical analysis

Data are presented as mean values and standard errors of the means (unless otherwise stated). All data were subjected to analysis of variance using the General Linear Model procedure of SAS. Student's *t*-test was used to compare the mean values of ruminal parameters, blood plasma metabolites, and milk production data between the control and YC treatments. Differences among the treatments were considered significant at $P < 0.05$, and indication of a trend was declared at $P < 0.10$.

Results and discussion

Table 2 presents the means for rumen measurements. YC supplementation had no effect on rumen pH, which agreed with previous results (Erasmus et al. 1992, Lila et al. 2004). Williams et al. (1991) reported that YC supplementation caused small increases in rumen pH, although the response was inconsistent as the addition of YC lowered rumen pH in studies reported by Harrison et al. (1988). Total protozoa and individual genera counts (data not shown) were not different between the control and YC, which was similar to previous studies (Kobayashi et al. 1995, Lila et al. 2004, Hristov et al. 2010). In this study, *Entodinium* spp. represented approximately 92% of the total protozoa, and the others were *Epidinium* spp., *Eudiplodinium* spp. and holotrichs.

The concentration of rumen ammonia-N tended to decrease at 2 h after feeding by YC compared with the control. Previous investigations reported that the concentration of rumen-N decreased by 10% after the supplementation of YC (Harrison et al. 1988, Erasmus et al. 1992). These reduced concentrations of ammonia may be the result of stimulated ruminal microbial activity and increased incorporation of ammonia into microbial protein by YC (Williams et al. 1991, Hristov et al. 2010).

YC did not affect the concentration of total SCFAs, which disagreed with our previous *in vitro* study that

Table 2. Effect of yeast culture (YC) on ruminal characteristics in dairy cows

Item	h after feeding	Control	YC	SEM	*P*-value
pH	0	7.1	7.1	0.07	0.31
	2	6.6	6.7	0.05	0.25
	5	6.8	6.8	0.06	0.31
Total protozoa ($\times 10^4$/mL)	0	65.2	68.1	5.31	0.23
	2	37.5	34.8	7.23	0.63
	5	47.9	47.2	6.74	0.92
Ammonia-nitrogen (mg/100mL)	0	12.5	11.9	0.58	0.50
	2	19.2	18.1†	0.53	<0.10
	5	12.0	11.6	0.56	0.52
Total SCFA[1)] (mM)	0	65.1	65.8	1.97	0.79
	2	88.2	89.8	2.93	0.47
	5	80.0	82.5	2.37	0.51
SCFA composition (molar %)					
Acetic acid (A)	0	62.3	61.4	1.11	0.24
	2	61.8	60.5	0.39	0.15
	5	62.4	60.3	0.48	0.06
Propionic acid (P)	0	21.6	22.1	0.87	0.18
	2	21.5	23.7†	0.42	0.09
	5	22.1	24.3*	0.49	<0.05
Butyric acid	0	10.0	10.2	0.29	0.16
	2	10.8	10.0	0.21	0.96
	5	10.6	10.4	0.43	0.81
Valeric acid	0	2.0	2.2	0.32	0.89
	2	2.2	2.3	0.14	0.19
	5	1.7	1.9	0.17	0.68
iso-Valeric acid	0	2.1	2.2	0.13	0.32
	2	1.9	1.8	0.05	0.54
	5	1.7	1.6	0.12	0.51
Others	0	2.0	1.9	0.10	0.89
	2	1.8	1.8	0.11	0.56
	5	1.5	1.6	0.08	0.91
A/P ratio	0	2.9	2.8	0.25	0.26
	2	2.9	2.6†	0.09	0.08
	5	2.8	2.5*	0.11	0.04

*$P < 0.05$, $^{\dagger}P < 0.1$, SEM: standard error of mean
[1)] SCFA: short-chain fatty acids

showed increased SCFAs by YC (Lila et al. 2004). YC supplementation tended to decrease the proportion of acetic acid (A), and increased that of propionic acid (P) at 5 h after feeding. Accordingly, YC lowered the A/P ratio. This trend is in agreement with both *in vivo* and *in vitro* studies (Harrison et al. 1988, Williams et al. 1991, Ueno et al. 2017). Willams et al. (1991) suggested that this tendency is probably the result of an increased production of propionic acid rather than a reduced production of acetic acid by YC.

Table 3 shows the concentration of blood plasma components at 2 h after feeding. YC supplementation did not affect the plasma concentrations of glucose, urea nitrogen, and NEFA, which agreed with the results of previous studies (Kobayashi et al. 1995, Hristov et al. 2010, Shi et al. 2019). However, YC supplementation significantly increased the concentration of free lysine. Moreover, YC tended to result in higher concentrations of isoleucine, arginine, and total free amino acids as compared with the control. These trends were similar to our previous results during early lactation (Kobayashi et al.1995) and demonstrated that more amino acids were absorbed from the small intestine by YC supplementation. This confirmed the suggestion by Newbold et al. (1996) that YC might increase the flow of non-ammonia-N from the rumen and is likely related to changes in the number and activity of the rumen microbial population. Dawson et al. (1990) reported a significant increase in the counts of cellulolytic bacteria in the rumen by YC. Erasmus et al. (1992) found the apparent digestibilities of crude protein and acid detergent fiber were significantly increased with YC supplementation, and thus showed that YC stimulated rumen microbial activity, and that more feed N was incorporated into the microbial fraction and increased microbial N flow to the duodenum. Hristov et al. (2010) also reported that estimated duodenal microbial flow tended to increase by 9%. This increased flow of microbial N helps to explain the reduced rumen ammonia concentration and increased plasma amino

Table 3. Effect of yeast culture (YC) on concentrations of glucose, urea, NEFA, and free amino acids in blood plasma of dairy cows

Item	Control	YC	SEM	*P*-value
Glucose (mg/dL)	61.8	63.6	1.69	0.45
Urea nitrogen (mg/dL)	18.9	19.7	0.64	0.32
NEFA[1)] (μEq/L)	126.1	130.5	5.95	0.45
Free amino acid (μmol/dL)				
Aspartic acid	0.8	0.9	0.07	0.42
Serine	8.7	8.5	0.25	0.63
Proline	5.6	5.3	0.47	0.41
Glutamic acid	6.9	7.6	0.65	0.14
Glycine	27.6	28.8	2.25	0.32
Alanine	21.5	23.3	1.95	0.67
Tyrosine	6.2	7.3	0.41	0.28
Total non-essential amino acids	77.3	81.5	3.86	0.24
Threonine	9.5	9.4	0.86	0.37
Valine	25.8	24.3	2.13	0.43
Methionine	3.5	3.5	0.78	0.18
Iso-leucine	8.9	10.2†	0.85	0.06
Leucine	13.3	13.5	0.94	0.64
Phenylalanine	4.5	5.3	0.34	0.18
Histidine	4.6	4.4	0.25	0.34
Lysine	7.5	8.9*	0.42	0.03
Arginine	8.1	9.5†	0.36	0.08
Total essential amino acids	85.6	88.9	3.01	0.15
Total amino acids	162.9	170.5†	6.56	0.07

*$P < 0.05$, $^{\dagger}P < 0.1$, SEM: standard error of mean
[1)] NEFA: non-esterified fatty acids

acids concentration by YC. Ueno et al. (2017) recently reported that the population of *Bacteroidetes* tended to be less prominent with YC, while the fibrolytic bacterium *Fibrobacter* significantly increased. Rumen fibrolytic bacteria are known to have a high preference for ammonia as their N source (Bryant 1973). Therefore, the increase of *Fibrobacter* might contribute to enhancing the duodenal flow of non-ammonia-N and overall protein nutrition in cows fed YC.

Table 4 presents the dry matter intake (DMI) and milk production data. YC supplementation did not affect DMI, milk yield, and 4% FCM. Concentrations and yields of fat, lactose, and SNF were also not affected, whereas those of protein tended to increase by YC as compared with the control. This tendency for increased milk protein might be attributed to increased amino acid absorption, as described above. Somatic cell counts were not affected by YC. Until now, there is no report on the response of somatic cell counts to YC supplementation in dairy cows.

Milk fatty acid composition was not altered by YC supplementation (Table 5). Moreover, the proportions of milk fatty acids derived from *de novo* fatty acid synthesis and fatty acid uptake were not affected by YC. And no alteration in the concentration of CLA, which is a beneficial biohydrogenation intermediate for human health, was observed in this study. A recent study reported that YC reduced the palmitic acid content in milk fat (Hristov et al. 2010).

Recent comprehensive meta-analyses have reported an overall positive effect of various YC on milk yield (Robinson & Erasmus 2009, Poppy et al. 2012). These meta-analyses reported that YC increased milk yield by about 4% on average, and pointed out that the response depends on diet and the level of milk production. The conditions for obtaining the maximum response by YC remain unclear; however, the effects appear to be more pronounced in high concentrate diets and during early lactation (Williams et al. 1991). The level of milk yield in the current study was moderate. More studies are needed to investigate the effect of YC on milk production by using high yielding cows.

Overall, the YC tested in this study had subtle effects on ruminal fermentation, except that the ammonia concentration and A/P ratio tended to be lower, possibly due in part to the enhanced ruminal bacterial growth. More study is also needed to clarify the mechanism of the effect of YC from the standpoint of rumen microbes and fermentation.

Table 4. Effect of yeast culture (YC) on feed intake, milk yield and composition in dairy cows

Item	Control	YC	SEM	*P*-value
Feed intake (kg/day)				
Dry matter intake	21.9	22.3	1.57	0.45
Total digestible nutrients	14.5	14.6	1.68	0.57
Crude protein	3.45	3.49	0.40	0.41
Neutral detergent fiber	6.92	7.08	0.85	0.47
Milk yield (kg/day)				
Milk yield	26.1	27.0	2.36	0.34
4% fat-corrected milk yield	24.9	25.5	2.03	0.47
Milk composition (%)				
Fat	3.71	3.67	0.09	0.27
Protein	3.09	3.17†	0.06	0.09
Lactose	4.27	4.26	0.06	0.68
Total solids	12.0	12.0	0.18	0.88
Milk composition yield (kg/day)				
Fat	0.96	0.98	0.07	0.57
Protein	0.80	0.85†	0.06	<0.1
Lactose	1.11	1.14	0.09	0.28
Total solids	3.12	3.22	0.25	0.37
Somatic cell counts ($\times 10^3$/mL)	54.3	52.8	13.1	0.87

$^{\dagger}P < 0.1$, SEM: standard error of mean

Table 5. Effect of yeast culture (YC) on milk fatty acid composition in dairy cows

Variable	Control	YC	SEM	*P*-value
Summation of fatty acids[1)]				
< C16	27.8	27.2	0.62	0.75
C16	30.5	29.8	0.82	0.41
> C16	39.7	40.8	0.65	0.42
CLA, *cis*-9, *trans*-11	0.60	0.62	0.07	0.27

[1)] < C16: fatty acids with fewer than 16 carbons,
> C16: fatty acids with more than 16 carbons
CLA: conjugated fatty acid, SEM: standard error of mean

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