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Effect of active dry yeast on lactation performance, methane production, and ruminal fermentation patterns in early-lactating Holstein cows

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ABSTRACT

This study was conducted to examine the effect of active dry yeast (ADY) supplementation on lactation performance, ruminal fermentation patterns, and CH_4 emissions and to determine an optimal ADY dose. Sixty Holstein dairy cows in early lactation (52 ± 1.2 DIM) were used in a randomized complete design. Cows were blocked by parity (2.1 ± 0.2), milk production (35 ± 4.6 kg/d), and body weight (642 ± 53 kg) and assigned to 1 of 4 treatments. Cows were fed ADY at doses of 0, 10, 20, or 30 g/d per head for 91 d, with 84 d for adaptation and 7 d for sampling. Although dry matter intake was not affected by ADY supplementation, the yield of actual milk, 4% fat-corrected milk, milk fat yield, and feed efficiency increased quadratically with increasing ADY supplementation. Yields of milk protein and lactose increased linearly with increasing ADY doses, whereas milk urea nitrogen concentration and somatic cell count decreased quadratically. Ruminal pH and ammonia concentration were not affected by ADY supplementation, whereas ruminal concentration of total volatile fatty acid increased quadratically. Digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber, nonfiber carbohydrate, and crude protein increased quadratically with increasing ADY supplementation. Supplementation of ADY did not affect blood concentration of total protein, triglyceride, aspartate aminotransferase, and alanine aminotransferase, whereas blood urea nitrogen, cholesterol, and nonesterified fatty acid concentrations decreased quadratically with increasing ADY supplementation. Methane production was not affected by ADY supplementation when expressed as grams per day or per kilogram of actual milk yield, dry matter intake, digested organic matter, and digested nonfiber carbohydrate, whereas a trend of linear and quadratic decrease of CH_4 production was observed when expressed as grams per kilogram of fat-corrected milk and digested neutral detergent fiber. In conclusion, feeding ADY to early-lactating cows improved lactation performance by increasing nutrient digestibility. The optimal ADY dose should be 20 g/d per head.
Key words: yeast product, nutrient digestibility, milk production, methane emission

INTRODUCTION

Active dry yeast (**ADY**) is a widely used feed additive in the dairy industry. It is reported to increase milk production and feed efficiency and decrease liver abscess (Desnoyers et al., 2009; Moallem et al., 2009; Crossland et al., 2019), which are also the benefits of the ionophore antibiotics monensin and tylosin (Van der Werf et al., 1998; Phipps et al., 2000). However, antimicrobial resistance increases with the supplementation of ionophore antibiotics (Shen et al., 2019a), which may potentially reduce the effectiveness of antimicrobial drugs for treating human disease. Thus, the use of ionophore antimicrobial has been banned by many countries, and ADY has been suggested as an alternative (Jia et al., 2018; Ran et al., 2018).

The positive effect of ADY on milk production and feed efficiency may be explained by the improvement of the ruminal environment. It is reported that ADY can scavenge ruminal oxygen, thereby providing a strict an-

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aerobic environment, which is beneficial for the growth of anaerobic bacteria (Fonty and Chaucheyras-Durand, 2006). Moreover, ADY is believed to stabilize ruminal pH by inhibiting production and increasing the utilization of ruminal lactic acid (Chaucheyras-Durand et al., 2005; Fonty and Chaucheyras-Durand, 2006). Because most cellulolytic bacteria can survive in an anaerobic environment with a pH above 5.8 (Russell and Wilson, 1996), greater NDF and OM digestibility with ADY supplementation should be expected (Desnoyers et al., 2009; Jiang et al., 2017a). Additionally, as reviewed by Chaucheyras-Durand et al. (2008), the ADY supplementation was suggested to potentially decrease CH_4 emissions; however, the effect is inconsistent across studies (Lynch and Martin, 2002; Vyas et al., 2014; Muñoz et al., 2017),

Although the effect of ADY products on lactation performance and CH_4 emission has been largely evaluated, the dose effect has been rarely evaluated. According to our previous survey (our unpublished data), more than 70% of the investigated farms in Baoding, China, use yeast products that exceed the recommended dose by 50%. This led to the question, does a higher dose of ADY supplementation have additional benefits in early-lactating cows? The dose effect of ADY varies in different studies. Jiang et al. (2017a) carried out an experiment to evaluate ADY dose on production performance using dairy cows. A low dose of ADY supplementation increased milk yield significantly, whereas a high dose of ADY had no effect on milk production compared with control, demonstrating that more is not always better for ADY supplementation. However, Pinloche et al. (2013) found greater ruminal pH and lower ruminal lactic acid concentration when supplemented with a high dose of ADY, whereas no treatment effect was observed in the low ADY group. Ferraretto et al. (2012) also found that the NDF digestibility and milk fat percentage increased significantly with the supplementation of a high dose of ADY compared with a low dose. We hypothesize that a higher ADY dose would have additional benefits in dairy cows. Thus, the objective of this study was to examine the dose effect of ADY on lactation performance, ruminal fermentation patterns, and CH_4 emission to determine an optimal ADY dose.

MATERIALS AND METHODS

This study was conducted between November 2017 and February 2018 at Hongda Dairy Farm in Baoding, China, and the experimental protocol (JGL 1712) was approved by the Institutional of Animal Care and Use Committee at Hebei Agricultural University (Baoding, China).

Animals, Diet, and Experiment Design

Sixty Holstein dairy cows (15 head/treatment) in early lactation (52 ± 1.2 DIM) were used in a randomized complete design. Cows were blocked by parity (2.1 ± 0.2), milk production (35 ± 4.6 kg/d) and body weight (642 ± 53 kg) and assigned to 1 of 4 treatments. The treatments were a diet supplemented with 0 (control), 10, 20, or 30 g of ADY per day per head. The ADY in every diet was mixed with 72 g of ground corn and 18 g of molasses, split into 3 portions, and top-dressed 3 times daily at feeding. The ADY used was a strain of *Saccharomyces cerevisiae* purchased from Angel Yeast Co., Ltd. (Yichang, Hubei, China) with 2×10^9 cfu/g. The recommended dose for lactating cows is 20 g/d per head. The experimental period was 91 d, with 84 d for adaptation and 7 d for sampling. The sample collection was conducted only in the final week of the experimental period to prevent negative effects on animal welfare and to achieve response consistency. This was an intensive sample collection with a large number of lactating cows (40 cows in total), and samples were collected from multiple sites (rumen, rectum, and blood). Such a collection schedule was necessary for the experiment objective but stressful for lactating dairy cows, which are sensitive to any human intervention and environmental changes. CH_4 emissions, ruminal fermentation patterns, and blood indicators were measured before the experiment and did not differ among treatment groups; thus, these indicators were not used as covariates in statistical analysis.

Cows were housed individually in tiestalls with automatic drinking bowls. The diet contained 25% whole corn silage, 15% alfalfa hay, 2% oat hay, and 58% concentrate (DM basis; Table 1) and was formulated to meet the recommendation of NRC (2001). The cows were fed ad libitum (ensuring at least 5% refusals) at 0600, 1300, and 2000 h, and they had free access to fresh water throughout the experiment. Cows were milked 3 times daily at 0500, 1200, and 1900 h.

Sampling and Data Collection

Feed offered and refused for each cow was recorded daily during the experiment. The TMR, feed ingredients, and feed refusals were collected weekly, oven-dried at 55°C for 48 h to measure DM content, and then ground to pass through a 1-mm screen (stand model 4 Wiley Mill, Arthur H. Thomas, Philadelphia, PA) for chemical analyses. Daily DMI was calculated as the difference between DM offered and DM refusals. The DMI data were averaged weekly for statistical analysis.

Ten cows from each treatment were selected randomly for feces, blood, ruminal fluid, and CH_4 sampling. Fecal samples (approximately 50 g wet) were collected from the rectum every 6 h from d 85 to 87, pooled by cow, dried at 55°C for 48 h, and ground through a 1-mm screen (stand model 4 Wiley Mill; Arthur H. Thomas) for further analyses.

Blood samples were collected from the jugular vein on d 88 and 89. Before morning feed, approximately 40 mL of blood samples were collected into four 10-mL vacuum tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing Na heparin or no additive (2 tubes for each), and plasma and serum were prepared as described by Shen et al. (2019a). Plasma was used for the analysis of BUN, glucose, triglyceride, cholesterol, and NEFA, and serum was used for the analysis of total protein, aspartate aminotransferase (**AST**), and alanine aminotransferase (**ALT**). Both plasma and serum were stored at −20°C until analyzed.

Ruminal samples (approximately 50 mL) were collected using an oral stomach tube before the morning feeding on d 90 and 91 (Shen et al., 2012). Ruminal pH was measured immediately after collection using a portable pH meter (Starter 300, Ohaus Instruments Co. Ltd., Shanghai, China). After being squeezed through 4 layers of cheesecloth, 2 subsamples of 5 mL of ruminal fluid were mixed with 1 mL of 25% (wt/vol) HPO_3 and 1 mL of 1% (wt/vol) H_2SO_4 and stored at −20°C until the determination of VFA and ammonia. Milk production (actual milk yield), milk fat, and milk protein concentration were recorded every day by cow and by milking time, using the Afikim milking system and averaged weekly for statistical analysis. Milk samples were collected 3 times daily from d 88 to 91 and stored at −20°C until the analysis of lactose, MUN, and SCC.

Methane emissions were measured from d 85 to 88, using the sulfur hexafluoride (**SF_6**) tracer gas technique as described by Chung et al. (2011). Briefly, a brass permeation tube (10.5 × 40 mm) containing 1,836 ± 56.6 mg (mean ± SD) SF_6 was used for CH_4 collection. The release rates of SF_6 were similar among treatments, ranging from 2.8 to 4.2 mg/d and averaging 3.45 ± 0.41 mg/d (mean ± SD). Halters were placed on the animals before 0600 h on d 85. The yoke canister was placed on the shelf above the cow at 0600 h on d 85, connected to the halter, and replaced every 12 h. The environmental concentration of SF_6 and CH_4 was monitored. The gas samples were collected from the yoke using syringes and analyzed immediately.

Sample Analyses

The content of DM, ash, ether extract, and CP (method 930.15, 942.05, 920.39 and 996.11, respectively; AOAC International, 2005) in TMR, feed refusal, and feces were determined according to AOAC International (2005). The OM content was calculated as OM% = 100% − ash%. The content of NDF in

Table 1. Ingredients and chemical composition of the experimental diets

Ingredient[1]	% DM	Chemical composition[2]	DM basis
Alfalfa hay	15.00	NE_L, Mcal/kg	1.64
Oat hay	2.00	CP, %	17.02
Whole corn silage	25.00	Ethanol extract, %	5.13
Cracked corn	13.58	NDF, %	34.48
Whole cotton seed	5.93	ADF, %	20.44
Steam-flaked corn	9.18	Calcium, %	0.68
Wheat bran	1.39	Phosphorus, %	0.37
Soybean meal	9.21		
Dried beet pellet	5.38		
Rapeseed meal	1.53		
Corn DDGS	1.65		
Premix	1.00		
Extruded soybean	4.10		
Molasses	1.00		
Fat powder	2.26		
Limestone	0.29		
Calcium phosphate	0.56		
MgO	0.14		
$NaHCO_3$	0.59		
NaCl	0.20		
Mycotoxin adsorbent	0.01		

[1]DDGS = distillers dried grains with solubles. Premix contained (per kg of DM): 800,000 IU of vitamin A, 180,000 IU of vitamin D_3, 7,000 mg of vitamin E, 45 mg of biotin, 300 mg of β-carotene, 600 mg of Cu, 1,000 mg of Fe, 2,200 mg of Zn, 1,800 mg of Mn, 20 mg of Co, 30 mg of Se, and 39 mg of I.

[2]Data were calculated according to NRC models (NRC, 2001).

TMR, feed refusals, and feces was measured using heat stable α-amylase and sodium sulfite (Van Soest et al., 1991). The ADF (method 973.18) contents in TMR, feed refusals, and feces were determined according to AOAC International (2005). Both NDF and ADF content were expressed including residual ash (Mertens, 2002). Acid detergent insoluble ash (**ADIA**) in TMR, feed refusals, and feces was used as an internal marker for apparent total-tract digestibility, and was determined as described by Van Keulen and Young (1977). Concentration of ruminal VFA was measured using gas chromatography (GC-14B, Shimadzu, Japan; 30 m × 0.32 mm × 0.25 mm; column temperature, 110°C; injector temperature, 180°C; and detector temperature, 180°C) as described by Shen et al. (2019b).

Blood concentrations of BUN, total protein, glucose, triglyceride, cholesterol, NEFA, AST, and ALT were determined using commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The interassay coefficients of variation were lower than 10%, and the intra-assay coefficients of variation were lower than 12%. Milk lactose was determined using a Milkoscan FT 120 (Foss Electric, Hillerød, Denmark), and SCC was determined using a Fossomatic cell counter (Foss Electric). The concentration of SF_6 and CH_4 were analyzed using a GC (GC-14B, Shimadzu, Kyoto, Japan; 1.8 m × 0.3 cm × 0.2 cm; injector temperature, 180°C; and detector temperature, 250°C) according to Chung et al. (2011).

Calculations and Statistical Analyses

Apparent total-tract nutrients digestibility was estimated by using ADIA (Rice et al., 2019), and the equation was:

$$100 - (100 \times (\%\ \text{ADIA in DM consumed}/\ \%\ \text{ADIA in feces}) \times (\%\ \text{nutrient in feces}/\ \%\ \text{nutrient in consumed DM})).$$

Data were analyzed using PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) for a randomized complete design. Treatments were the fixed effects, and cows were the random effects. Sampling day was considered as a repeated measurement for variables measured over time. Week of feeding was considered as a repeated measurement for DMI and milk production. The repeated measures statistical analysis of results was subjected to 5 covariance structures: AR, UN, CS, SP, and VC. The covariance structure that yielded the smallest Schwarz Bayesian criterion was chosen due to the most desirable and reliable analysis (Littell et al., 1998). The linear, quadratic, and cubic ADY dose responses were determined by using specific preplanned contrasts. Treatment effects were declared significant at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$.

RESULTS

Dry Matter Intake and Milk Production Characteristics

Supplementation of ADY in the diet of Holstein cows in early lactation had no effect on DMI, whereas actual milk yield ($P = 0.03$), 4% FCM ($P = 0.03$), and feed efficiency (4% FCM/DMI; $P = 0.05$) increased quadratically with an increasing dose of ADY supplementation (Table 2). The percentage of milk protein and lactose did not differ among treatments, whereas a linear increase ($P < 0.05$) in milk fat and lactose yield and a trend ($P < 0.10$) with a linear increase in milk fat percentage and milk protein yield were observed with increasing ADY amount. The concentration of MUN followed a trend ($P = 0.07$) to quadratically decrease with increasing ADY supplementation. Compared with control, SCC decreased quadratically ($P = 0.01$) by 21.1, 17.0, and 7.1% in cows supplemented with 10, 20, and 30 g of ADY per day per head, respectively.

Ruminal Fermentation Patterns and Nutrient Digestibility

Ruminal pH was not affected by treatments (Table 3), whereas total VFA concentration increased quadratically ($P = 0.04$) with increasing ADY supplementation. Molar proportion of acetate tended ($P = 0.08$) to increase linearly, whereas molar proportion of butyrate tended ($P = 0.06$) to decrease linearly with increasing ADY supplementation. Molar proportion of propionate, ratio of acetate to propionate and ratio of acetate plus butyrate to propionate were not affected by treatments. Ruminal ammonia concentration was not affected by ADY supplementation. As showed in Table 4, the digestibility of nutrients including DM, OM, NDF, ADF, NFC, and CP increased quadratically ($P = 0.01$) with increasing ADY supplementation.

Blood Metabolites

Concentration of BUN decreased quadratically ($P = 0.05$) with increasing ADY amount (Table 5), whereas no treatment effect was observed in total protein concentration. Blood concentration of glucose increased, whereas cholesterol decreased quadratically ($P = 0.01$)

Table 2. Effect of active dry yeast (ADY) supplementation on DMI, milk production, and milk composition in lactating Holstein cows

Item[1]	ADY supplementation, g/d per head				SEM	*P*-value[2]		
	0	10	20	30		L	Q	C
DMI, kg/d	22.7	23.0	22.8	22.9	0.50	0.77	0.82	0.69
Milk yield, kg/d								
Actual	34.7	35.6	36.2	35.7	0.25	0.02	0.03	0.49
4% FCM, kg/d	31.8	33.0	34.6	34.2	0.37	0.01	0.03	0.15
Milk fat								
%	3.41	3.54	3.71	3.70	0.065	0.10	0.31	0.44
kg/d	1.18	1.26	1.34	1.32	0.025	0.01	0.06	0.21
Milk protein								
%	3.13	3.14	3.18	3.16	0.045	0.54	0.66	0.66
kg/d	1.09	1.12	1.15	1.13	0.018	0.06	0.12	0.19
Lactose								
%	4.76	4.71	4.72	4.78	0.028	0.67	0.04	0.97
kg/d	1.65	1.68	1.70	1.70	0.018	0.03	0.55	0.44
MUN, mg/dL	13.8	13.6	13.2	13.8	0.21	0.74	0.07	0.31
SCC, 10^5 cells/mL	5.78	4.56	4.80	5.37	0.277	0.43	0.01	0.37
Feed efficiency	1.40	1.44	1.52	1.49	0.030	0.03	0.05	0.28

[1]Feed efficiency = 4% FCM/DMI.

[2]L = linear, Q = quadratic, and C = cubic effects of ADY supplementation dose (0, 10, 20, 30 g/d per head).

with increasing ADY supplementation, and a trend (P = 0.09) of a quadratic increase was also observed in triglyceride concentration. Blood concentration of AST and ALT was not affected by ADY supplementation, whereas NEFA concentration in cows supplemented 10, 20, and 30 g of ADY per day per head decreased quadratically (P = 0.01) by 9.32, 10.17, and 9.32% compared with control.

Methane Production

The results of CH_4 production are shown in Table 6. Methane production was not affected by ADY supplementation when expressed as grams per day or per kilogram of actual milk yield, DMI, digested OM, and digested NFC. A trend of a linear and quadratic decrease respectively of CH_4 production was observed when expressed as grams per kilogram of FCM (P = 0.09) and digested NDF (P = 0.09).

DISCUSSION

As an active yeast product, ADY was reported to scavenge oxygen, increase redox potential, and reduce the accumulation of lactic acid to provide a better ruminal environment for ruminants (Chaucheyras-Durand and Fonty, 2002; Fonty and Chaucheyras-Durand, 2006; Chaucheyras-Durand et al., 2008). Moreover, in dairy cows, ADY was reported to increase DMI and nutrient digestibility, and thus be beneficial for milk production (Habeeb, 2017; Jiang et al., 2017a). In the present study, ADY was added in the diet of early-lactating dairy cows at the doses of 0, 10, 20, and 30 g/d, to investigate the dose effect of ADY on lactation performance and CH_4 emissions.

Though Habeeb (2017) reported an increase of DMI in dairy cows, the DMI was not affected by ADY supplementation in the present study, which was consistent with a previous study (Jiang et al., 2017a). The lack of

Table 3. Effect of active dry yeast (ADY) supplementation on ruminal fermentation patterns in lactating Holstein cows

Item	ADY supplementation, g/d per head				SEM	*P*-value[1]		
	0	10	20	30		L	Q	C
pH	6.36	6.27	6.22	6.25	0.294	0.56	0.67	0.93
Total VFA, m*M*	75.4	77.5	85.4	75.8	2.35	0.42	0.04	0.06
Acetate (A), %	57.8	58.8	61.4	59.5	0.85	0.08	0.12	0.15
Propionate (P), %	23.2	24.0	23.8	24.2	1.03	0.55	0.85	0.69
Butyrate (B), %	13.7	11.1	9.8	10.3	1.21	0.06	0.23	0.92
A/P	2.50	2.48	2.59	2.46	0.132	1.00	0.68	0.55
(A+B)/P	3.09	2.96	3.00	2.88	0.180	0.51	0.97	0.68
NH_3-N, mg/dL	13.6	12.9	10.9	12.0	1.26	0.28	0.49	0.66

[1]L = linear, Q = quadratic, and C = cubic effects of ADY supplementation dose (0, 10, 20, 30 g/d per head).

Table 4. Effect of active dry yeast (ADY) supplementation on nutrient digestibility in lactating Holstein cows

Item[1]	ADY supplementation, g/d per head				SEM	P-value[2]		
	0	10	20	30		L	Q	C
OM, %	68.8	73.8	73.2	71.2	1.16	0.24	0.01	0.43
DM, %	67.0	72.9	72.4	69.4	1.28	0.27	0.01	0.50
NDF, %	57.7	62.3	62.9	59.0	1.40	0.35	0.01	0.90
ADF, %	53.0	60.0	60.0	56.1	1.90	0.29	0.01	0.73
NFC, %	85.0	89.5	88.7	87.0	0.76	0.16	0.01	0.21
CP, %	71.4	76.4	76.6	73.4	1.03	0.21	0.01	0.78

[1]NFC = 100% – (% NDF + % CP + % EE + % ash). EE = ethanol extract.

[2]L = linear, Q = quadratic, and C = cubic effects of ADY supplementation dose (0, 10, 20, 30 g/d per head).

ADY effect on DMI was reported in both dairy cows (Malekkhahi et al., 2016) and beef cattle (Vyas et al., 2014) previously, using similar yeast products (*Saccharomyces cerevisiae*). As reported by Chaucheyras-Durand et al. (2012), the ADY supplementation is more relevant during challenges, such as a feed transition or periods of stress. In the present study, the diet was unchanged and the cows were not suffering from any stress; thus, the lack of ADY effect on DMI, which was consistent with previous studies, should be expected.

In the present study, the increased actual milk yield and 4% FCM yield in ADY-supplemented cows were consistent with previous studies (Desnoyers et al., 2009; Moallem et al., 2009; Jiang et al., 2017a). However, no treatment effect on milk yield was reported previously (Ferraretto et al., 2012; DeVries and Chevaux, 2014). The varied results among studies could be explained by the different ADY strains used. Furthermore, Chaucheyras-Durand et al. (2008) believed that ADY supplementation could be more relevant in a high concentrate diet; the effect of ADY supplementation on milk yield could also be affected by the concentrate content in different diets. The positive effect of ADY on FCM yield reported by Moallem et al. (2009) and Jiang et al. (2017a) used a high concentrate (68 and 58.3%, respectively) diet, whereas Ferraretto et al. (2012) and DeVries and Chevaux (2014), who did not find an ADY effect on FCM yield, used a low concentrate (32.7 and 42.3%, respectively) diet. The diet used in the present study is a high concentrate diet (58% concentrate); thus, the greater milk production in ADY-supplemented cows is expected. Jiang et al. (2017a) found greater milk yield when they supplemented with a low dose of ADY but not with a high dose, suggesting that higher levels of ADY supplementation may not be better. In the present study, the quadratically increased milk yield and FCM yield in ADY-supplemented cows illustrated that the supplementation of ADY at 20 g/d per head should be enough for increasing milk yield.

Milk fat production is greatly affected by ruminal acetate production in dairy cows, because acetate is an important precursor of milk fat (Popjak et al., 1951). In the present study, the linearly increased milk fat content and yield were consistent with the linearly increased acetate molar proportion. Similar increased milk fat with ADY supplementation was also reported previously (Ferraretto et al., 2012; Jiang et al., 2017a).

The concentration of MUN is usually used to represent nitrogen balance; less MUN suggested a better utilization of nitrogen in the mammary gland. The decreased MUN was also reported by Dehghan-Banadaky et al. (2013). They believe that the decreased MUN

Table 5. Effect of active dry yeast (ADY) supplementation on blood metabolites in lactating Holstein cows

Item[1]	ADY supplementation, g/d per head				SEM	P-value[2]		
	0	10	20	30		L	Q	C
BUN, mmol/L	5.27	4.12	4.14	4.16	0.279	0.02	0.05	0.37
Total protein, g/L	72.0	73.7	72.4	72.9	1.32	0.80	0.66	0.43
Glucose, mmol/L	3.25	3.49	3.65	3.28	0.107	0.54	0.01	0.28
Cholesterol, mmol/L	3.11	2.60	2.54	2.82	0.146	0.11	0.01	0.84
Triglyceride, mmol/L	0.19	0.24	0.23	0.21	0.021	0.50	0.09	0.55
AST, U/L	72.5	71.5	71.0	71.8	0.75	0.41	0.24	0.83
ALT, U/L	33.3	32.7	32.6	32.7	0.56	0.45	0.55	0.95
NEFA, μmol/L	472	428	424	428	7.5	0.01	0.01	0.45

[1]AST = aspartate aminotransferase; ALT = alanine aminotransferase; NEFA = nonesterified fatty acid.

[2]L = linear, Q = quadratic, and C = cubic effects of ADY supplementation dose (0, 10, 20, 30 g/d per head).

Table 6. Effect of active dry yeast (ADY) supplementation on methane emission in lactating Holstein cows

Item[1]	ADY supplementation, g/d per head				SEM	*P*-value[2]		
	0	10	20	30		L	Q	C
CH_4, g/d	344	349	340	344	11.6	0.83	0.97	0.66
CH_4/actual MY, g/kg	9.77	9.83	9.23	9.37	0.31	0.20	0.89	0.32
CH_4/FCM, g/kg	10.7	10.6	10.1	9.9	0.38	0.09	0.96	0.56
CH_4/DMI, g/kg	15.3	15.4	15.0	15.0	0.51	0.59	0.93	0.66
CH_4/OMD, g/kg	21.7	21.2	20.4	20.4	0.79	0.18	0.72	0.75
CH_4/NDFD, g/kg	26.4	24.7	23.9	25.0	0.93	0.16	0.09	0.74
CH_4/NFCD, g/kg	17.8	17.3	16.9	16.9	0.61	0.29	0.65	0.89

[1]MY = milk yield; OMD = digested OM; NDFD = digested NDF; NFCD = digested NFC.

[2]L = linear, Q = quadratic, and C = cubic effects of ADY supplementation dose (0, 10, 20, 30 g/d per head).

should be explained by the greater microbial activity and greater incorporation of NH_3 into microbial protein in the rumen. In the present study, although the ruminal ammonia concentration was not affected, the greater NDF and ADF digestibility may approve this hypothesis. Somatic cell count is an important indicator to evaluate the inflammatory response in ruminants. Although the SCC was not affected in some studies (Moallem et al., 2009; De Ondarza et al., 2010), the decreased SCC in cows supplemented with ADY were reported by Degirmencioglu et al. (2013) and Szucs et al. (2013), which were consistent with the present study. Bobbo et al. (2017) found a significant association between SCC and serum total protein and globulin concentration. Although the reason was not very clear, it can be partly explained by better immune status with greater globulin in the serum. In the present study, the serum concentration was not measured, however, the less SCC in cows supplemented with ADY illustrated that ADY may potentially increase the immune status and decrease inflammatory response. The quadratic decrease of SCC and MUN concentration in cows supplemented ADY in different doses suggested 20 g/d per head should be the optimal dose for better nitrogen utilization and immune status.

Ruminal concentration of VFA is an important indicator to reflect feed degradation characteristics. Usually, greater VFA concentration is associated with greater OM digestibility. In the present study, the quadratic increase of ruminal VFA concentration was consistent with the quadratic increase of total-tract digestibility of OM. Both ruminal VFA concentration and OM digestibility peaked at ADY supplementation of 20 g/d per head, which illustrated that this should be the optimal dose for OM digestibility.

Fiber is mainly degraded in the rumen and fermented to produce acetate. Previous studies demonstrated that ADY has benefits in improving cellulolytic bacteria activity, which resulted in greater fiber digestibility and greater acetate proportion (Fonty and Chaucheyras-Durand, 2006). However, Vyas et al. (2014) and Jiang et al. (2017a) did not find an ADY effect on acetate production using a 3-wk experimental period. This differs from the linearly increasing acetate production with increasing ADY supplementation in the present study. Hasunuma et al. (2016) conducted a long-term experiment using ADY and monitored ruminal acetate for 15 wk. The increased ruminal acetate concentration in ADY-supplemented cows was observed until wk 15. Thus, the different results in acetate production between the present study and previous studies could be explained by the different durations of ADY supplementation. Moreover, according to (Chaucheyras-Durand et al., 2012), different ADY strains exhibit different effects on digestive microbiota. Thus, the different results of acetate production could be also due to the different ADY strains used in different studies.

An interesting finding in the present study is that NDF digestibility increased quadratically with increasing ADY supplementation. On the contrary, Ferraretto et al. (2012) conducted a study using 2 ADY doses and found that NDF digestibility improved by using a high ADY dose but not a low dose. Jiang et al. (2017a) also evaluated the dose effect using 2 different ADY doses. The researchers found that both low and high doses of ADY had a positive effect on NDF digestibility compared with control, but no difference was observed between these 2 doses. Ruminal microbiota of cows supplemented with a different dose of ADY was analyzed by Jiang et al. (2017b), who noted that the abundance of *Butyrivibrio fibrisolvens*, an important hemicellulolytic species, decreased in cows supplemented with a high dose of ADY. Although it is not clear why *Butyrivibrio fibrisolvens* decreased the high dose, the decreased cellulolytic bacteria abundance could partly explain the quadratic effect of ADY supplementation on NDF digestibility in dairy cows.

In the present study, the NDF digestibility ranged from 53.7 to 64.8%, which was greater than the value (range of 45.0–54.5%) reported by Jiang et al. (2017a).

The forage used in the present diet consists of 25% corn silage, 15% alfalfa hay, and 2% oat hay (DM basis), whereas in Jiang et al. (2017a), the forage was 41.7% corn silage. As it is known that NDF in alfalfa hay is more digestible than corn silage (Eun and Beauchemin, 2007), the greater NDF digestibility in the present study should be expected. According to the meta-analysis published by Weld and Armentano (2017), NDF digestibility ranging from 28.8 to 66.8% were previously reported; therefore, the NDF digestibility value in the present study should be acceptable.

In the present study, although the blood concentration of total protein was not affected, BUN decreased with ADY supplementation. Together with greater CP digestibility and lower MUN concentration, nitrogen utilization might be improved in ADY-supplemented cows; thus, the greater milk protein yield in cows supplemented with ADY in the present should be explained. Similarly, Dehghan-Banadaky et al. (2013) also reported a decrease of BUN concentration in ADY-supplemented cows, illustrating that ADY may have some potential benefit in improving nitrogen utilization.

The concentration of blood glucose and NEFA are widely used to evaluate energy status in dairy cows. In the present study, lower NEFA concentration in ADY-supplemented cows illustrated that body fat mobilization might be decreased by ADY supplementation. Concentration of glucose is mainly affected by NFC digestibility. In the present study, glucose concentration increased quadratically with increasing ADY supplementation, which was consistent with the quadratically increased NFC digestibility. Greater glucose concentration in ADY-supplemented cows was also reported by Dehghan-Banadaky et al. (2013). In the present study, the lower NEFA and higher glucose concentration in ADY-supplemented cows demonstrated that ADY supplementation may improve the energy status by increasing NFC digestibility in dairy cows.

The CH_4 production did not differ when expressed as grams per day, which was consistent with a previous study that found CH_4 production was not affected by ADY supplementation (Bayat et al., 2015). As it is known that CH_4 production is mainly modified by propionate fermentation in ruminants (Fukuzaki et al., 1990), the lack of ADY effect on CH_4 production should be expected. In the present study, the CH_4 production tended to decrease when expressed as grams per kilogram of FCM and digested NDF, although the tendency is quite weak. The ADY effect on CH_4 production varies by different studies. Mwenya et al. (2004) reported a decrease in CH_4 emissions by ADY supplementation. Bayat et al. (2015) found no effect of ADY on CH_4 emissions, and Muñoz et al. (2017) even reported an increase in CH_4 production when supplemented with ADY in lactating dairy cows. The different effects of ADY supplementation on CH_4 production could be due to the different ADY strains, doses, or diets used by the different studies.

CONCLUSIONS

Supplementation of ADY in early-lactating cows at different doses had no influence on DMI and ruminal pH. Conversely, with certain doses, the milk yield and milk fat production increased by increasing nutrient digestibility. However, because bacteria populations in the digestive tract were not analyzed, the mechanism by which ADY affects nutrient digestibility is not clear and needs further exploration. Considering the relative greater milk yield, milk fat, and nutrient digestibility, and lower SCC and MUN concentration, 20 g of ADY per day per head should be the optimal supplementation dose in early-lactating dairy cows.

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