Effects of *Saccharomyces cerevisiae*-based direct-fed microbial and exogenous enzyme products on enteric methane emission and productivity in lactating dairy cows

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ABSTRACT

The objective of this experiment was to investigate the effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product (SDM) and an exogenous enzyme product (ENZ) on enteric methane emission, milk yield and composition, total-tract digestibility of nutrients, ruminal fermentation, and nitrogen excretion and secretion in lactating dairy cows. Eighteen Holstein cows were used in a 3 × 3 Latin square design experiment with three 28-d periods. Treatments were (1) control (no additive), (2) 28 g of SDM/d per cow, or (3) 10 g of ENZ/d per cow. Treatments were top-dressed at the time of feeding. The basal diet consisted of (dry matter basis) 60% forage and 40% concentrates and contained 16.5% crude protein and 32.0% neutral detergent fiber. Treatments had no effect on enteric methane production, yield (methane per kg of dry matter intake, DMI), or intensity (methane per kg of energy-corrected milk yield). Carbon dioxide production was similar among treatments. Compared with control, SDM increased milk yield by 2 kg/d without affecting DMI or feed efficiency. Supplementation of the diet with ENZ did not affect DMI, milk yield, or feed efficiency. Concentrations and yields of milk fat, true protein, and lactose, and energy-corrected milk yield were not different among treatments. Neither SDM nor ENZ had an effect on total-tract digestibility of nutrients or nitrogen excretion and secretion. Concentration of total volatile fatty acids (VFA) in ruminal fluid was increased by both SDM and ENZ, and rumen pH was decreased by SDM compared with the control. At levels similar to the control DMI, the increased concentration of VFA in ruminal fluid of cows receiving SDM suggests an increased postruminal supply of energy and may partly explain the increased milk yield with that treatment. However, it is important to note that milk composition

and energy-corrected milk yield were not affected by treatment.

Key words: direct-fed microbial, exogenous enzyme, rumen fermentation, dairy cow

INTRODUCTION

Enteric methane produced during microbial fermentation in the rumen not only represents a loss of feed energy but it is also a major greenhouse gas contributing to climate change (Johnson and Johnson, 1995; IPCC, 2014). Enteric methane emissions from ruminants represent 2 to 12% of the animal's gross energy intake and approximately 4% of global anthropogenic greenhouse gas emissions (Johnson and Johnson, 1995; IPCC, 2014). Dietary interventions have been studied to mitigate enteric methane emission, with methane inhibitors (e.g., 3-nitrooxypropanol), electron receptors (e.g., nitrates), and dietary lipids being examples of successful mitigants (Hristov et al., 2013; Patra, 2016).

Direct-fed microbials (DFM) and exogenous enzymes (EXE) have different modes of action in the rumen. Direct-fed microbials are primarily yeast-based and their suggested mode of action is stabilization of ruminal fermentation by increasing ruminal pH, oxygen scavenging, and provision of soluble growth factors (Newbold et al., 1996; Callaway and Martin, 1997; Chaucheyras-Durand et al., 2008). Exogenous enzymes, on the other hand, are expected to contribute to degradation of carbohydrate (fiber and starch) and protein in the rumen according to their activities (Meale et al., 2014). Effects of DFM (Chaucheyras-Durand et al., 2008; Desnoyers et al., 2009) and EXE (Beauchemin and Holtshausen, 2011; Adesogan et al., 2014; Sujani and Seresinhe, 2015) on milk production in dairy cows have been extensively studied but responses are inconsistent. In a meta-analysis of EXE studies, Arriola et al. (2017) observed small trends for improved total-tract apparent fiber digestibility and milk production but also emphasized the large variability in the data. One apparent reason for these variable responses is that

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DFM and EXE largely differ in composition and activities and have been applied at various doses and using different application methods (Meale et al., 2014). New recombinant EXE are being developed and successfully tested in preliminary in vitro experiments (Ribeiro et al., 2018).

Through their effects on ruminal fermentation, DFM and EXE have been suggested as potential mitigants of enteric methane emission (Grainger and Beauchemin, 2011; McAllister et al., 2011; Hristov et al., 2013). An in vitro study demonstrated that *Saccharomyces cerevisiae* extract increased hydrogen utilization by acetogens and decreased methane production (Chaucheyras et al., 1995). Additionally, positive effects of DFM and EXE on milk yield and feed efficiency may decrease methane yield (per kg of DMI) and intensity (per kg of milk or ECM) in lactating cows (Hristov et al., 2013; Arriola et al., 2017). Although the effects of DFM and EXE on enteric methane emission have been studied in vitro (He et al., 2015; Elanthamil and Bandeswaran, 2017; Mohamed et al., 2017), experiments with lactating dairy cows are scarce.

Therefore, we hypothesized that DFM and EXE, through their beneficial effects on ruminal fermentation and carbohydrate degradability, could decrease enteric methane production, yield, or intensity in lactating dairy cows. The objective of the experiment was to investigate the effects of a *Saccharomyces cerevisiae*-based DFM and an enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* on enteric methane emission, milk production and composition, total-tract digestibility of nutrients, ruminal fermentation, and N excretion and secretion in dairy cows.

MATERIALS AND METHODS

Animals and Treatments

The Pennsylvania State University Institutional Animal Care and Use Committee approved all procedures used in this experiment.

The experiment was conducted at The Pennsylvania State University's Dairy Teaching and Research Center. Eighteen lactating Holstein cows, including 6 rumen-cannulated (10-cm internal diameter cannulas; Bar Diamond Inc., Parma, ID) cows, were used in a 3 × 3 Latin square design experiment balanced for residual effects. Cows had average milk yield of 39 ± 10.2 kg/d, DIM of 115 ± 42.0 d, and BW of 609 ± 77.9 kg at the beginning of the experiment. Cows were housed in a tiestall barn and had free access to drinking water. The experiment consisted of 3 periods; each period lasted 28 d, including 21 d for adaptation to treatment and 7 d for data and sample collections. Feeds were offered as

TMR once daily in the morning at approximately 0800 h. All cows were fed the same basal TMR offered ad libitum, targeting 10% refusals. The basal diet (Table 1) was formulated to meet or exceed the nutrient requirements (NRC, 2001) of cows with BW of 680 kg and milk yield of 39.5 kg/d (with 3.80% fat and 3.20% true protein) at a DMI of 27.3 kg/d. Treatments were (1) control (basal diet with no additives), (2) 28 g/cow per day of a *S. cerevisiae*-based DFM product (FortiShield, PMI Nutritional Additives, Arden Hills, MN; **SDM**), and (3) 10 g/cow per day of an enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* (CelluloGest, PMI Nutritional Additives; **ENZ**). The DFM product contained 1.8×10^9 cfu/g of *S. cerevisiae* and 1.1×10^8 cfu/g of a mixture of *Lactococcus lactis*, *Bacillus subtilis*, *Enterococcus faecium*, and *Lactobacillus casei*. The ENZ product contained amylase (1.2×10^6 modified Wohlgemuth units/kg), hemicellulase (1.9×10^5 hemicellulase units/kg), cellulase (1.1×10^4 cellulase units/kg), glucanase (1.0×10^3 glucanase units/kg), and pectinase (1.0×10^3 pectinase units/kg). Doses of SDM and ENZ were based on the manufacturer's recommendation. Treatments were top-dressed daily and mixed with approximately 500 g of TMR. All cows were milked twice daily at 0600 and 1800 h.

Sampling and Analyses

Individual feed intake, milk yield, and BW (AfiFarm 3.04E scale system; S.A.E. Afikim, Rehovot, Israel) of the cows were recorded daily during the entire experiment. Samples were collected from TMR and refusals twice weekly and composited per experimental period for further analyses. Forages and concentrate feeds were sampled weekly and composited per experimental period for further analyses. Composite samples of the TMR, forages, and concentrate feeds were stored at −20°C, oven-dried (at 65°C) to constant weight, and ground through a 1-mm sieve (Wiley mill, Thomas Scientific, Swedesboro, NJ) for analyses of CP (AOAC International, 2000), NDF (Van Soest et al., 1991), ADF (AOAC International, 2000), ether extract (AOAC International, 2006), Ca (AOAC International, 2000), P (AOAC International, 2000), and estimated NFC (NRC, 2001) and NE_L (NRC, 2001) by Cumberland Valley Analytical Services (Waynesboro, PA). Analysis of OM was conducted by ashing the feed samples for 4 h at 600°C. Starch was analyzed as described in Hall (2009). The nutrient analyses of the individual feed ingredients and their inclusion rate were used to estimate nutrient content of the diets.

Milk samples were collected from p.m. and a.m. milkings on d 26 and 27 of each experimental period. Milk samples were preserved with 2-bromo-2-nitropro-

Table 1. Ingredient and chemical composition of diets fed in the experiment

Item	Diet
Ingredient, % of DM	
Corn silage ¹	44.5
Alfalfa haylage ²	10.5
Grass hay	4.95
Whole cottonseed	2.10
Corn grain, ground	7.92
Soybean seeds, whole heated ³	9.00
Canola meal ⁴	11.87
Soybean meal ⁵	2.47
Molasses ⁶	4.95
Vitamin and mineral premix ⁷	1.69
Composition, ⁸ % of DM (or as indicated)	
CP ⁸	16.9
RDP ⁹	11.5
RUP ⁹	5.4
NDF ⁸	29.3
ADF ⁸	18.5
Starch ⁸	26.7
NE _L , Mcal/kg ⁹	1.64
Ether extract ⁸	5.4
Ash ⁸	5.5
Ca ⁸	0.8
P ⁸	0.4
NFC ⁹	45.3
NE _L balance, ¹⁰ Mcal/d	(2.6, 2.5, 2.6)
MP balance, ¹⁰ g/d	(-92, -149, -125)

¹Corn silage was 46.8% DM and contained (DM basis) 7.4% CP, 33.2% NDF, and 38.4% starch.

²Alfalfa haylage was 40.1% DM and contained (DM basis) 21.3% CP and 39.9% NDF.

³Soybean seeds contained (DM basis) 37.4% CP.

⁴Canola meal contained (DM basis) 42.4% CP.

⁵Soybean meal contained (DM basis) 46.6% CP.

⁶Molasses (Westway Feed Products, Tomball, TX) contained (DM basis) 3.9% CP and 66% total sugar.

⁷The premix (Cargill Animal Nutrition, Cargill Inc., Roaring Spring, PA) contained (% as-is basis) trace mineral mix, 1.88; MgO (54% Mg), 8.0; NaCl, 24.9; vitamin ADE premix (Cargill Animal Nutrition, Cargill Inc.), 0.41; limestone, 36.8; selenium, 0.13; and dry corn distillers grains with solubles, 29.0. Ca, 14.4%; P, 0.75%; Mg, 2.48%; K, 0.28%; S, 0.50%; Se, 12.8 mg/kg; Cu, 651 mg/kg; Zn, 3,433 mg/kg; Fe, 798 mg/kg; vitamin A, 195,290 IU/kg; vitamin D, 62,500 IU/kg; and vitamin E, 1,863 IU/kg.

⁸Values calculated using the chemical analysis (Cumberland Valley Analytical Services Inc., Waynesboro, PA) of the individual feed ingredients and their inclusion rate in the diets.

⁹Estimated based on NRC (2001).

¹⁰Estimated based on NRC (2001) using actual DMI, milk yield, milk composition, and BW of the cows throughout the experiment for the control, SDM, and ENZ treatments, respectively. SDM = *Saccharomyces cerevisiae*-based direct-fed microbial product at 28 g/cow per day; ENZ = exogenous enzyme product at 10 g/cow per day.

pane-1,3 diol and submitted to Dairy One Cooperative Inc. (Ithaca, NY) for analysis of milk fat, true protein, lactose, SCC, MUN, other solids, and total solids using infrared spectroscopy (MilkoScan 4000; Foss Electric, Hillerød, Denmark). Milk composition data were weighted for the corresponding p.m. and a.m. milk yields.

Enteric methane and carbon dioxide emissions were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD) as described in Hristov et al. (2015). Gas measurements were collected 8 times on 3 consecutive days as follows: at 0900, 1500, and 2100 h (d 24), 0300, 1200, and 1700 h (d 25), and 0000 and 0500 h (d 26) during wk 4 of each experimental period. A cannula extension (C-Lock Inc.) was used for the rumen-cannulated cows to capture potential gas leaking through the cannula (Lopes et al., 2016). Enteric gas emissions were also calculated on DMI, milk yield, and ECM bases.

Ruminal samples were collected from the cannulated cows (2 per treatment in each period) on d 27 and 28 at 0 (before feeding), 3, 6, 12, 18, and 24 h after feeding. Whole-rumen contents were collected from 5 locations within the rumen (2 samples from the feed mat, 1 from the reticulum, and 2 from the ventral sac). Whole-rumen contents were filtered through 2 layers of cheesecloth to obtain approximately 300 mL of filtrate, and the pH of the filtrate was measured immediately. An aliquot of filtrate (5 mL) was mixed with a protozoal staining solution (MFS solution, Ogimoto and Imai, 1981), composited per cow and period, stored at 4°C, and processed for protozoal counts as described in Hristov et al. (2001). The remaining filtrate (45 mL) was centrifuged at low speed (500 × *g* for 5 min at 4°C) to remove protozoa and feed particles. Aliquots of the supernatant (6 mL) were mixed with 0.5 mL of chilled 65% trichloroacetic acid to acidify the sample and precipitate proteins. The acidified samples were stored frozen (−20°C) and later thawed and centrifuged at 20,000 × *g* for 15 min at 4°C; the supernatant was analyzed for ammonia according to Chaney and Marbach (1962). Another set of aliquots from the low-speed supernatant was combined with 1 mL of 25% metaphosphoric acid and 1 mL of 0.6% 2-ethyl-butyric acid (internal standard), kept frozen (−20°C), and later thawed and used for VFA analysis after centrifugation at 20,000 × *g* for 15 min at 4°C. Volatile fatty acids were analyzed using a GC (Agilent 7890A, Agilent, Santa Clara, CA). Separation was achieved by a custom packed column (1.8 m × 6.4 mm, Supelco Inc., Bellefonte, PA) operated at a flow rate of 15 mL/min and 125°C. Detection of VFA was conducted by a flame-ionization detector at 225°C.

Fecal samples (approximately 400 g per sample) were collected at 8 times in 3 d during each experimental period as follows: 1000, 1600, and 2200 h (d 24), 0400, 1300, and 1900 h (d 25), and 0100 and 0700 h (d 26). Samples were processed for DM, OM, CP, NDF, ADF, and starch analyses as described in Oh et al. (2013); starch was analyzed as for the feed samples. Apparent total-tract digestibility of DM, OM, CP, NDF, ADF, and starch was estimated using indigestible NDF (12-d rumen incubation) as an intrinsic marker [Huhtanen

et al., 1994; except that 25- μ m-pore-size filter bags (Ankom Technology, Macedon, NY) were used for the ruminal incubation]. Urine samples (approximately 300 mL per sample) were collected at the same time points as for fecal samples and processed as described in Oh et al. (2013). Urine was analyzed for total N, urea-N, allantoin, uric acid, and creatinine as described in Hristov et al. (2011). Purine derivatives (PD), allantoin and uric acid, were used as indicators of ruminal microbial synthesis. Daily urine volume and urinary N and urea-N excretions were estimated based on urinary creatinine concentration with a creatinine excretion rate of 29 mg/kg of BW, estimated from total urine collections (Hristov et al., 2011).

Statistical Analysis

All data were analyzed using SAS version 9.3 (2003; SAS Institute Inc., Cary, NC). Outliers were removed with the REG procedure based on an absolute studentized residual value >3 . Data were tested for normality using the UNIVARIATE procedure. Milk yield, DMI, and estimated feed efficiency data for the last 7 d of each experimental period were used in the statistical analysis. Averaged milk yield and milk composition data were used to calculate yields of milk fat, true protein, lactose, and ECM. Rumen fermentation data were averaged per day and the average data were used in the statistical analysis. All data were analyzed using the MIXED procedure.

Data for gas emissions, milk composition, digestibility, protozoa counts, and N excretion and secretion were analyzed by ANOVA Latin square. The model used was as follows:

$$Y_{ijkl} = \mu + S_i + C(S)_{ij} + P_k + T_l + e_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the square, $C(S)_{ij}$ is the cow within square, P_k is the kth period, T_l is the lth treatment, and e_{ijkl} is the error term. Square and cow within square were random effects and all others were fixed.

Dry matter intake, milk yield, feed efficiency, and rumen fermentation data (pH, VFA, and ammonia) were analyzed as repeated measures assuming a first-order autoregressive [AR(1)] covariance structure. The model used was as follows:

$$Y_{ijklm} = \mu + S_i + C(S)_{ij} + P_k + T_l + D_m + TD_{lm} + e_{ijklm},$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, S_i is the square, $C(S)_{ij}$ is the cow within square,

P_k is the kth period, T_l is the lth treatment, D_m is the time effect, TD_{lm} is the treatment $\times$ time interaction, and e_{ijklm} is the error term. Square and cow within square were random effects and all others were fixed.

Where the main effect of treatment was significant, means were separated by pairwise t -test (pdiff option of PROC MIXED). Statistical differences were declared at $P < 0.05$. Differences between treatments at $0.05 \leq P < 0.10$ were considered as a trend toward significance. Data are presented as least squares means.

RESULTS AND DISCUSSION

Enteric Methane Emission

Enteric methane and carbon dioxide emissions were not affected by treatment in the current experiment (Table 2). Methane emission was similar among treatments when calculated on the basis of DMI, milk yield, or ECM yield. Effects of DFM products on methane emission have been mainly studied in vitro. Lila et al. (2004) reported that a strain of *S. cerevisiae* (twin strain 8417 and 1026; 5×10^9 live organisms/g) linearly decreased methane emission after a 24-h incubation in a batch culture system where sudangrass hay and concentrates (60.5:39.5) were used as substrates. In a study by Lynch and Martin (2002), *S. cerevisiae* (0.35 g/L; 5×10^6 cfu) decreased methane emission by 20.2% after 48 h incubation of buffered rumen fluid with alfalfa hay as a substrate, although methane emission was not affected when the substrate was ground corn, soluble starch, or bermudagrass. Similarly, *S. cerevisiae* (0.1 to 2.0 g/L) linearly decreased methane emission when expressed per digested DM (mL/g), when perennial ryegrass was used as a substrate (O'Brien et al., 2014). The mode of action of yeast on methane emission is not well defined. According to Chaucheyras et al. (1995), *S. cerevisiae* stimulated hydrogen utilization of acetogenic bacteria and decreased methane emission in pure culture or in coculture. Gong et al. (2018) reported in a recent study that *S. cerevisiae* supplementation increased acetogen numbers and decreased methanogenesis in the large intestines of pigs. These promising effects of DFM from in vitro and nonruminant studies, however, have not been replicated in vivo with ruminants. Doreau and Jouany (1998) reported that dietary supplementation of *S. cerevisiae* (CNCM I-1077; 3×10^{10} cfu/d per head) did not affect enteric methane emission in early lactation dairy cows fed a corn silage-based diet. Only 4 cows were used in that experiment and carbon dioxide and hydrogen emissions were also not affected by treatment (Doreau and Jouany, 1998). In another experiment with nonlactating dairy cows, 2 different strains of *S. cerevisiae* (1×10^{10} cfu/d per head) had no effect

Table 2. Effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an exogenous enzyme product on enteric gas emissions in dairy cows

Item	Treatment ¹			SEM ²	P-value Treatment
	Control	SDM	ENZ		
Methane, g/d	337	351	359	16.2	0.42
Methane, g/kg of DMI	13.3	13.7	14.5	0.58	0.16
Methane, g/kg of milk yield	8.47	8.49	8.98	0.356	0.51
Methane, g/kg of ECM	9.26	9.23	10.1	0.417	0.13
Carbon dioxide, g/d	12,219	11,907	11,841	281.2	0.32

¹SDM = *Saccharomyces cerevisiae*-based direct-fed microbial product at 28 g/cow per day; ENZ = exogenous enzyme product at 10 g/cow per day.

²Highest SEM shown; n = 44 for all variables (n represents number of observations used in the statistical analysis).

on methane emission measured using the SF₆ tracer technique (Chung et al., 2011). Muñoz et al. (2017) also reported that *S. cerevisiae* (CNCM I-1077; 2×10^{10} cfu/d) did not affect methane emission or intensity in early lactation dairy cows fed a grass silage-based diet and 2 levels of concentrate (300 and 600 g/kg of DM). These reports are in agreement with methane emission data from the current study. Other experiments using beef cattle and sheep also showed no effects of *S. cerevisiae*-based DFM on methane emission (Mathieu et al., 1996; McGinn et al., 2004). Thus, there is an apparent discrepancy between in vitro and in vivo results when dietary interventions to mitigate enteric methane are studied. Limitations of in vitro systems to study rumen fermentation have been extensively discussed and include accumulation of fermentation end products, decreased concentration of VFA and fiber degradability, and protozoa disappearance (Hristov et al., 2012; Oh and Hristov, 2016).

Studies on the effects of EXE on enteric methane production are limited and results are equivocal, with a similar discrepancy between in vitro and in vivo responses. Tang et al. (2013) tested 3 fungal (*Neocalimastix patriciarum*, *Trichoderma longibrachiatum*, and *Trichoderma reesei*) cellulase products at different doses (0, 12, 37, and 62 IU/g of DM) in an in vitro batch culture system and reported that the EXE products linearly or quadratically decreased methane emission per unit of degraded DM. Dietary supplementation of cellulase and xylanase decreased or tended to decrease methane emission expressed per unit of ADF or NDF intake in growing goats, although total daily methane emission was similar among treatments (Lu et al., 2015). In a study with dairy cows, supplementation of an EXE product containing cellulase, xylanase, and esterase decreased estimated methane production (Arriola et al., 2011). In the latter study, however, methane emission was estimated based on VFA concentrations, which is not a reliable approach (Robinson et al., 2010). Other

studies reported that EXE did not decrease enteric methane emission. Mohamed et al. (2017), for example, reported in an in vitro study that an EXE mixture consisting of β -glucanase, xylanase, and cellulase did not affect methane emission. He et al. (2015) reported that xylanase increased methane emission in vitro with rice straw and grass as substrates, whereas no effect was observed with corn stover. In dairy cows, dietary supplementation of a fibrolytic EXE mixture containing endoglucanase and xylanase (1.0 mL/kg of DM) increased methane emission and methane yield and intensity (Chung et al., 2012). To our knowledge, the current study is the first report on the effect of an EXE containing both amylolytic and fibrolytic activities on methane emission in lactating dairy cows. Overall, it appears that responses in methane emission to EXE supplementation may depend on the type of enzyme activity, dose rate, and diet ingredient composition or type of substrate.

Milk Production and Total-Tract Digestibility

Feed intake, milk production and composition, and BW data are shown in Table 3. Treatments had no effects on DMI. Compared with the control, milk yield was increased ($P = 0.05$) by SDM but unaffected by ENZ. Feed efficiency was similar among treatments. Milk composition and BW were also not affected by treatment. Apparent total-tract digestibilities of DM, OM, CP, NDF, ADF, and starch were similar among treatments (Table 4). Positive effects of DFM on milk production have been reported in the past (Desnoyers et al., 2009; de Ondarza et al., 2010). Nocek and Kautz (2006) reported increased milk yield in dairy cows receiving 2 g/d of a DFM product containing 5×10^9 cfu of *S. cerevisiae* and 5×10^9 cfu of *Enterococcus faecium*. Ruminal infusion of a mixture of *S. cerevisiae* and *Enterococcus faecium* (80 mg/kg of DMI) alleviated the milk yield drop during a subacute ruminal acidosis

Table 3. Effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an exogenous enzyme product on feed intake and milk yield and composition in dairy cows

Item	Treatment ¹			SEM ²	P-value Treatment
	Control	SDM	ENZ		
DMI, kg/d	25.3	25.6	25.0	0.95	0.58
Milk yield, kg/d	39.8 ^b	41.8 ^a	41.0 ^{ab}	2.03	0.05
Feed efficiency, ³ kg/kg	1.59	1.62	1.63	0.041	0.31
Milk fat, %	3.57	3.45	3.46	0.178	0.66
Milk fat yield, kg/d	1.48	1.47	1.51	0.121	0.89
ECM ⁴ yield, kg/d	37.7	38.1	38.2	2.27	0.88
ECM feed efficiency, kg/kg	1.48	1.49	1.49	0.047	0.94
Milk true protein, %	3.04	3.04	2.98	0.062	0.49
Milk true protein yield, kg/d	1.23	1.29	1.27	0.076	0.51
Milk lactose, %	4.89	4.84	4.79	0.068	0.35
Milk lactose yield, kg/d	2.00	2.06	2.05	0.098	0.73
Other solids, %	5.26	5.23	5.25	0.043	0.43
Total solids, %	11.2	11.1	11.1	0.14	0.84
MUN, mg/100 mL	10.8	10.5	11.2	0.43	0.46
SCC, ⁵ ×10 ³ cells/mL	54.9	64.1	59.4	11.04	0.56
BW, kg	624	629	625	13.1	0.12

^{a,b}Means with different superscript letters differ at $P < 0.05$.

¹SDM = *Saccharomyces cerevisiae*-based direct-fed microbial product at 28 g/cow per day; ENZ = exogenous enzyme product at 10 g/cow per day.

²Highest SEM shown; n = 292 for DMI, n = 279 for milk yield, n = 277 for feed efficiency, n = 266 for BW, and n = 41 for milk composition data (n represents number of observations used in the statistical analysis).

³Milk yield/DMI.

⁴ECM (kg/d) = milk production (kg/d) × (383 × % fat + 242 × % true protein + 165.4 × % lactose + 20.7)/3,140 (Sjaunja et al., 1990).

⁵Statistical analysis was performed on log10-transformed data.

challenge in dairy cows (Chiquette et al., 2015). In a recent study with 6,700 dairy cows on a commercial farm, a yeast product (3 g/d per head providing 6×10^{11} cfu) increased milk yield by 0.7 kg/d (Rossow et al., 2018). A meta-analysis of 14 research trials (160 observations) concluded that dietary supplementation of *S. cerevisiae* increased milk yield by 1.2 kg/d (de Ondarza et al., 2010). It has been suggested that the response in milk production in some of these studies was due to increased ruminal fiber degradability by DFM (see the review of Chaucheyras-Durand et al., 2008). Live yeast reportedly increased NDF digestibility in situ and in vivo (Guedes et al., 2008; Dehghan-Banadaky et al., 2013). According to Newbold et al. (1996), some strains of *S. cerevisiae* scavenge oxygen in ruminal fluid, which may enhance fibrolytic activities, as evident by increased cellulolytic bacterial population. Additionally, yeast supplementation reportedly increased ruminal pH by stimulating lactate-utilizing bacteria, which is beneficial for the growth of cellulolytic bacteria (Chaucheyras-Durand et al., 2008). The increase in milk yield by SDM in the current experiment was apparently not a result of increased DMI or nutrient digestibilities. It is possible that the increase in ruminal total VFA concentration by SDM (assuming this corresponds to an increase in VFA production; Sutton et al., 2003) reflects increased energy supply

to the animal, which would explain the increased milk production at a similar DMI. Another possible explanation is that SDM may have stimulated the immune system of the cows (McAllister et al., 2011), which may have positively affected productivity in the current experiment. Dietary supplementation of *S. cerevisiae* reportedly regulated immune functions by decreasing bacterial colonization in pigs and increasing acute phase proteins in beef cattle (Emmanuel et al., 2007; Lessard et al., 2009). The milk production response in the current study, however, should be interpreted with caution because ECM and milk composition were not affected by SDM.

Milk production responses to EXE have not been consistent (Beauchemin and Holtshausen, 2011; Adesogan et al., 2014; Sujani and Seresinhe, 2015). In a production study with 28 lactating cows, for example, dietary supplementation of amylase (0.88 mL/kg of DM) increased milk production and total-tract apparent digestibility of DM, OM, and NDF (Klingerman et al., 2009). Interestingly, a higher dose (4.4 mL/kg of DM) did not affect milk yield and digestibility in the same study, which indicated that the responses to EXE could be dose-dependent. Andreazzi et al. (2018) recently reported that an amylase supplementation increased milk yield in cows fed a diet including 32% starch, which was higher than the dietary starch level in the current study

(26.7% DM). Other production studies with dairy cows, however, reported no effect of amylase supplementation on milk yield although DM, CP, ADF, and NDF digestibilities were increased (Gencoglu et al., 2010; Weiss et al., 2011). Studies with fibrolytic EXE have also shown variable production effects in dairy cows. Fibrolytic EXE increased milk yield in some studies (Kung et al., 2000; El-Bordeny et al., 2015; Arriola et al., 2017), but had no effect in others (Elwakeel et al., 2007; Arriola et al., 2011; Peters et al., 2015). The meta-analysis by Arriola et al. (2017) concluded that EXE slightly increased total-tract DM and NDF digestibility (by 1.4 to 2.3%) and improved milk production (by 0.8 kg/d) and milk components in dairy cows, but the responses were largely variable. Gado et al. (2009) reported that an EXE mixture (7.1 units/g of cellulase, 2.3 units/g of xylanase, 61.5 units/g of α -amylase, and 29.2 units/g of proteases) increased milk yield in early lactating cows, likely because of increased DMI and digestibility. Variation in production responses to EXE could be attributed to differences in dose and enzyme properties, diet, and experimental design (Beauchemin et al., 2003; Adesogan et al., 2014).

Ruminal Fermentation

Compared with the control, both SDM and ENZ increased total VFA concentration in ruminal fluid and, in the case of SDM, this resulted in corresponding decrease in ruminal pH (Table 5). Treatments had no other effects on rumen fermentation. Effects of DFM on ruminal fermentation in dairy cows have been inconsistent (Chaucheyras-Durand et al., 2008). Chiquette et

al. (2015) and Malekkhahi et al. (2016) reported that dietary supplementation of DFM products containing *S. cerevisiae* (2×10^9 and 2×10^{10} cfu/d, respectively) increased or tended to increase ruminal pH in dairy cows during subacute ruminal acidosis challenge and had no effect on VFA concentrations. However, Ambriz-Vilchis et al. (2017) observed no effect of *S. cerevisiae* (1×10^{10} or 6×10^{10} cfu/d) on ruminal pH in cows fed a standard or acidogenic diet in a series of experiments. Chung et al. (2011) compared 2 strains of *S. cerevisiae* and reported that one strain (CNCM I-1077) decreased ruminal pH and increased propionate concentration compared with the control, which is in agreement with results in the current experiment, whereas the other strain (details were not provided) had no effects on ruminal fermentation in dairy cows.

Effects of EXE on ruminal fermentation have also been inconsistent. For example, Tricarico et al. (2005) reported that an *Aspergillus oryzae* extract containing α -amylase (0 to 720 dextrinizing units/kg of DM) tended to linearly increase acetate and decrease propionate molar proportions in lactating dairy cows. Hristov et al. (2008) reported no effect of EXE (amylases, xylanases, and an amylase/xylanase combination) on ruminal fermentation in dairy cows. Nozière et al. (2014), however, observed a decrease in acetate and an increase in propionate concentrations with amylase supplementation (10 g/d per cow; 300 kilo novo units) in dairy cows fed a 30% starch diet. The supplementation of amylase did not affect individual VFA when cows were fed a lower starch diet (20% DM) in the same study. In contrast, Andreazzi et al. (2018) reported no effects of amylase produced by *Bacillus licheniformis* (300 kilo

Table 4. Effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an exogenous enzyme product on apparent total-tract digestibility of nutrients in dairy cows

Item	Treatment ¹			SEM ²	P-value Treatment
	Control	SDM	ENZ		
Intake, kg/d					
DM	24.2	23.9	23.3	1.28	0.46
OM	22.5	22.2	21.6	1.19	0.46
CP	4.37	4.31	4.20	0.233	0.48
NDF	6.87	6.79	6.61	0.364	0.47
ADF	4.63	4.57	4.45	0.244	0.46
Starch	6.45	6.37	6.21	0.339	0.45
Apparent digestibility, %					
DM	71.9	71.5	71.7	0.30	0.51
OM	72.7	72.3	72.5	0.33	0.67
CP	71.0	70.7	70.8	0.42	0.83
NDF	47.1	46.8	46.9	0.51	0.87
ADF	39.4	39.9	40.0	0.98	0.85
Starch	99.0	99.0	99.1	0.09	0.44

¹SDM = *Saccharomyces cerevisiae*-based direct-fed microbial product at 28 g/cow per day; ENZ = exogenous enzyme product at 10 g/cow per day.

²Highest SEM shown; n = 51 for all variables (n represents number of observations used in the statistical analysis).

Table 5. Effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an exogenous enzyme product on ruminal fermentation in dairy cows

Item	Treatment ¹			SEM ²	P-value Treatment
	Control	SDM	ENZ		
Ruminal pH	6.19 ^a	6.08 ^b	6.14 ^{ab}	0.033	0.02
Ammonia, mM	3.22	3.26	3.40	0.301	0.87
Total VFA, ³ mM	94.7 ^c	101.2 ^a	97.9 ^b	2.69	0.002
VFA as % of total					
Acetate	62.0	62.0	61.1	1.46	0.19
Propionate	22.5	21.7	22.5	1.79	0.52
Butyrate	12.2	12.8	12.7	0.21	0.13
Isobutyrate	0.58	0.56	0.61	0.037	0.62
Valerate	1.73	1.78	1.79	0.035	0.43
Isovalerate	0.99	1.07	1.38	0.191	0.24
Acetate:propionate	2.83	2.91	2.85	0.298	0.80
Protozoa, ⁴ × 10 ⁴ /mL	88.9	82.4	104.6	11.09	0.18

^{a-c}Means with different superscript letters differ at $P < 0.05$.

¹SDM = *Saccharomyces cerevisiae*-based direct-fed microbial product at 28 g/cow per day; ENZ = exogenous enzyme product at 10 g/cow per day.

²Highest SEM shown; n = 15 for protozoa and n = 25 for all other variables (n represents number of observations used in the statistical analysis).

³Treatment × time interaction, $P = 0.06$.

⁴Statistical analysis performed on log₁₀-transformed data.

novo units) on ruminal VFA production in dairy cows fed 32% starch. Responses to dietary supplementation of fibrolytic EXE have also varied among studies. A fibrolytic EXE product containing xylanase, endoglucanase, and exoglucanase activities increased total VFA concentration and tended to decrease acetate proportion in the rumen of dairy cows (Arriola et al., 2011). Similar products containing xylanase and endoglucanase activities, however, did not affect ruminal fermentation in other studies with dairy cows (Peters et al., 2010; Chung et al., 2012). The observed increase in total VFA concentration with both SDM and ENZ in the current experiment suggests enhanced fermentation in the rumen and potentially greater postruminal supply of energy for productive purposes, which could explain the observed response in milk yield with SDM.

Excretion of Nitrogen and Purine Derivatives

Excretion of urinary total-, urea-, and fecal-N and secretion of milk-N were not different among treatments (Table 6). Treatments also did not affect urine output or urinary PD excretion in the current experiment. Studies that investigated the effects of DFM or EXE on excretion of urinary- or fecal-N, or both, in dairy cows are limited. Dietary supplementation of a strain of *S. cerevisiae* (5×10^{10} cfu/d; CNCM I-4407) increased N digestibility and decreased fecal-N excretion in dairy cows fed a diet containing a low level of RDP (Julien et al., 2015). Fecal-N excretion and digestibility, however, were not affected by treatment when an adequate RDP

diet was fed (Julien et al., 2015), which is in agreement with results from the study by Muñoz et al. (2017) and the current experiment. Knowlton et al. (2007) reported a decrease in fecal-N excretion with an EXE product containing cellulase and phytase activities, which the authors attributed to improved apparent digestibility of dietary N. The lack of effect of treatment on PD excretion in the current experiment is consistent with other studies of DFM (Julien et al., 2015) and EXE (Gado et al., 2009; Andreazzi et al., 2018) in dairy cows.

CONCLUSIONS

In this experiment, dietary supplementation of a *Saccharomyces cerevisiae*-based DFM and an EXE extract from *Aspergillus oryzae* and *Aspergillus niger* had no effect on enteric methane emission, total-tract digestibility of nutrients, or N excretion and secretion. Both SDM and ENZ increased total VFA concentration in ruminal fluid, which, in the case of SDM, may help explain the increased milk yield with that treatment compared with the control. The milk production response with SDM, however, should be interpreted with caution because ECM and milk composition were not different from the control.

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Table 6. Effects of a *Saccharomyces cerevisiae*-based direct-fed microbial product and an exogenous enzyme product on nitrogen excretion and secretion and urinary excretion purine derivatives in dairy cows

Item	Treatment ¹			SEM ²	P-value Treatment
	Control	SDM	ENZ		
N intake, g/d	719	706	692	38.3	0.56
N secretion and excretion, g/d					
Urine N	192	190	191	12.8	0.98
Urea-N	129	139	140	9.3	0.19
Fecal N	208	205	203	13.1	0.85
Total excreta N	400	396	394	24.3	0.92
Milk N	197	207	203	12.1	0.51
As proportion of N intake, %					
Urine N	27.1	27.4	28.0	1.27	0.91
Fecal N	28.9	29.2	29.2	0.47	0.89
Total excreta N	56.0	56.5	57.2	1.39	0.87
Milk N	27.6	28.5	29.3	1.16	0.38
Urine output, kg/d	20.9	21.9	20.7	1.17	0.41
Urinary PD ³ excretion					
Allantoin, mmol/d	408	389	422	29.0	0.55
Uric acid, mmol/d	53.7	51.8	48.2	3.64	0.15
Total PD, mmol/d	462	441	470	31.9	0.64

¹SDM = *Saccharomyces cerevisiae*-based direct-fed microbial product at 28 g/cow per day; ENZ = exogenous enzyme product at 10 g/cow per day.

²Highest SEM shown; n = 45 for all variables (n represents number of observations used in the statistical analysis).

³Purine derivatives.

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