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Evaluation of the effects of live yeast on rumen parameters and in situ digestibility of dry matter and neutral detergent fiber in beef cattle fed growing and finishing diets

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ABSTRACT

Objective: This study evaluated the effects of live yeast (LY; *Saccharomyces cerevisiae*) on rumen parameters and in situ DM digestibility (DMD) and NDF digestibility (NDFD) during 3 consecutive feeding phases: grower (GRW) for 27 d, transition (TRANS) for 14 d, and finisher (FIN) for 14 d.

Material and Methods: Eight ruminally cannulated cattle (4 steers and 4 heifers) were blocked by sex into 2 pens containing Calan gate feeders and received a control diet (13.7% CP, 42.4% NDF, 88% DM) without LY for 10 d (−10 to −1). Animals were randomly assigned to treatments: control or LY fed every morning (0800 h) at 2.5, 5, or 10 g/d for 55 d. Digestibility was assessed on 9 collection days using in situ nylon bags containing 5 g of diet for GRW, TRANS, or FIN incubated for 48 h.

Results and Discussion: During the GRW-diet phase, treatment altered DMD and NDFD and tended to affect the rate of degradation and the acetate-to-propionate ratio. During the TRANS-diet phase, treatment affected total gas production, protozoa numbers, DMD, and NDFD. Throughout the FIN-diet phase, treatment affected rate of degradation, VFA concentrations, protozoa numbers, DMD, and NDFD and tended to affect methane and pH.

Implications and Applications: The LY affected rumen parameters and digestibility, but the dose-response pattern varied depending on the type of diet. The ideal dose of LY might be variable depending on the type of diet, and it might be greater than currently recommended for practical use in farms.

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Key words: bovine, dynamics, fermentation, *Saccharomyces cerevisiae*

INTRODUCTION

The beef production industry is a highly dynamic and evolving system that responds rapidly to increasing demand for animal-derived protein products, which is driven by an increasing population size and a degree of urbanization. In previous years, antimicrobial drugs has been used to increase the growth and productivity of production animals, promote feed conversion efficiency and growth, and reduce low-level infections in animals to improve the health and production efficiency of food animals (van den Bogaard and Stobberingh, 2000). About one-half of the antibiotics used are used as feed additives in the United States (Cromwell, 2002). Feeding antimicrobial feed additives for growth promotion is no longer an option.

Active dry yeast products such as *Saccharomyces cerevisiae* have been used as alternatives to antimicrobial feed additives (Lynch and Martin, 2002). Active dry yeasts are most commonly used in the making of bread and production of alcoholic beverages; however, the use of probiotic yeast in diets of ruminant animals has become a common practice for improving feed utilization efficiency of ruminants (Moallem et al., 2009). The ability of yeast to enhance the reducing environment in the rumen could assist in the growth of lactate-consuming and cellulolytic bacterial populations, sequentially aiding in the stabilization of the rumen and increasing the rumen's capacity to digest fiber (Marden et al., 2008). Given the complex composition of ruminant feeds, live yeast can alter the balance of rumen microbial communities and their activity (Fonty and Chaucheyras-Durand, 2006).

The supplementation of live yeast (**LY**) to ruminants has been shown to increase nutrient digestibility (Lascano et al., 2009), decrease methane production (Moallem et

al., 2009), and increase performance in dairy cows (Newbold and Rode, 2006), but few studies have determined their effect in the rumen of growing beef cattle receiving high concentrate feedlot-type rations. Due to an increasing emphasis on efficiency, performance, and feed digestibility of growing cattle and the increasing public awareness of methane production in agriculture livestock, the present study was designed to evaluate the effects of supplementing LY on ruminal parameters and nutrient digestibility when fed to growing beef cattle during the 3 feeding phases: grower (**GRW**), transition (**TRANS**), and finisher (**FIN**).

MATERIALS AND METHODS

The effects of the inclusion of LY (*S. cerevisiae* Sc47 CNCM I-4407, Actisaf, Phileo Lesaffre Animal Care, Milwaukee, WI, 1.10^{10} cfu/g) in diets of growing ruminants were examined in this study. Data in this study were collected from a 55-d in vitro and in situ trial and were analyzed to determine the total gas production as well as the fractional rate of degradation (**kd**) using the in vitro gas production technique (**IVGP**), total VFA concentration, acetate-to-propionate ratio (**A:P**), lactate concentration, methane production (CH_4), rumen pH fluctuation, protozoa counts, DM digestibility (**DMD**), and NDF digestibility (**NDFD**).

All experimental procedures were executed and animals were cared for according to the guidelines of the Texas A&M University Institutional Care and Use Committee (IACUC protocol #2016–0362). Eight ruminally cannulated mature cattle (n = 4 steers and n = 4 heifers that were 36 and 24 mo old, respectively) with BW of 550 ± 75 kg from Texas A&M McGregor Research Center and Angelo State University, respectively, were used in this experiment. Animals were blocked by sex and placed into 2 separate pens of 4 animals each. Each pen contained Calan gate feeders (American Calan, Northwood, NH), and water was available constantly. From d −10 to −1, animals went through an adaptation period when they received a standard diet (13.7% CP, 42.4% NDF, 88% DM; Table 1) without LY in the Calan gate feeders with the gates open so they would become acclimated to the bunks and Calan system. On d 0, they were fitted with the Calan sensor.

Treatments and Experimental Design

Because the main objective of this experiment was to study the effects of different dietary concentrations of LY on ruminal fermentation dynamics, not on animal performance, 2 animals of distinct sex and different sizes were selected to offer broad differences in feeding strategies, feed consumption, and rumination. Additionally, to further increase the ruminal fermentation variability, rumen fluid was collected temporally. On d 0, treatments were randomly assigned to animals using a randomized complete block design (2 pens; 4 treatments; 4 animals per pen). Each animal within a block was assigned to a treatment. This allowed for 2 animals, one of each sex, per treatment. Treatments were as follows: control (**CON**), **LY1** (2.5 g/d), **LY2** (5 g/d), and **LY3** (10 g/d) distributed by top dressing. Weighed amounts of fresh feed were provided twice daily at 0800 and 1700 h for 55 d at 2.5% (DM basis) of each animal's weekly BW. Each of the 3-phase diets was fed sequentially as follows: grower (GRW) for 27 d (10 d for adaptation) fed during wk 1 through 5, transition (TRANS) for 14 d fed during wk 6 and 7, and finishing (FIN) for 14 d during wk 8 and 9 of the study (Table 1). Baseline ruminal contents were collected on d −1, and

Table 1. Diet formulation and chemical composition of period diets

Item	Grower	Transition	Finisher
Ingredient, % (DM basis)			
Cracked corn	40.0	52.5	65.0
Alfalfa pellets	28.0	21.75	15.5
Bermudagrass hay	8.00	9.00	10.0
Cottonseed hulls	15.0	7.50	0.00
Cow base mineral	1.50	1.25	1.00
Urea	1.00	1.00	1.00
Molasses	6.50	6.75	7.00
Limestone	0.00	0.25	0.50
Diet composition,[1] % DM unless otherwise noted			
DM, % as fed	88.0	87.8	87.4
CP	13.8	13.0	12.3
Soluble protein	56.4	54.0	47.1
ADIN	1.35	0.98	0.62
NDIN	1.66	1.48	1.48
ADF	25.5	19.1	13.4
NDF	36.9	31.7	27.3
Lignin	6.67	4.23	2.79
Sugar	6.90	6.40	6.20
Starch	29.6	37.3	45.5
Fat	2.88	2.85	2.77
Ash	6.09	5.35	4.49
Ca	0.84	0.80	0.74
P	0.37	0.37	0.35
Mg	0.21	0.19	0.16
K	1.57	1.34	1.10
S	0.24	0.23	0.23
Na	0.19	0.15	0.11
Fe	283	259	202
Mn	50.0	55.0	34.0
Zn	45.0	60.0	42.0
Cu	21.0	18.0	14.0
TDN	66.6	72.1	76.3
NE_m, Mcal/kg	1.50	1.68	1.81
NE_g, Mcal/kg	0.92	1.08	1.19

[1]Chemical analyses and the calculation of TDN, NE_m, and NE_g were conducted by Cumberland Valley Analytical Services (Waynesboro, PA). NDIN = neutral detergent insoluble nitrogen.

measurements were taken every 7-d following, for a total of 9 collection days.

Rumen Sampling and Analyses

During the sample collection process, for each treatment, whole rumen contents were extracted from the cranial, middle, and caudal compartments of the rumen approximately 4 h after the morning feeding time, for chemical analyses and to be used for IVGP and methane assays as described below. Chemical analyses of the diet were performed by Cumberland Valley Analytical Services (Waynesboro, PA), using the analytical methods discussed by Tedeschi and Fox (2018; chapter 18). A combined rumen content (approximately 500 mL) was strained through 8 layers of cheesecloth and placed into individual stainless-steel thermoses minimizing headspace to maintain both temperature and an anaerobic environment. Concurrently, in situ nylon bags were placed in each animal for a 48-h incubation. Rumen fluid was immediately transported to the laboratory and prepared for pH, CH_4, VFA, and lactate analyses; protozoa counts; and the IVGP technique.

The pH of each rumen fluid sample was recorded using a VWR sympHony benchtop meter (VWR International, Radnor, PA). Then, subsamples were taken for VFA and lactate analyses. Approximately 8 mL of rumen fluid from each sample was transferred into individual falcon tubes containing 2 mL of metaphosphoric acid (2 Falcon tubes per animal) for both VFA and lactate analyses, and then frozen at −20°C. The VFA and lactate concentrations were measured by gas chromatography (Hinton et al., 1990).

IVGP Measurements

Using a portion of the mixed rumen fluid, an in vitro anaerobic fermentation and gas production analysis (i.e., IVGP) was performed on a total of 288 samples (32 samples from each time point collection). Briefly, the IVGP technique uses an incubation chamber to mimic rumen temperature (39°C) with a multiplate stirrer that houses 32 fermentation flasks (125-mL Wheaton bottles; Tedeschi et al., 2009; Tedeschi and Fox, 2018, chapter 9). Flasks are attached to pressure sensors that measure and record gas pressure every 5 min for 48 h. A total of 200 mg of ground diet (GRW, TRANS, and FIN depending on the period) was weighed and transferred into each 125-mL Wheaton bottle containing Teflon-covered stir bars and dampened with 2.0 mL of distilled H_2O to prevent particle scattering during subsequent CO_2 flushing. Meanwhile, anoxic media (Goering and Van Soest, 1970) was continuously flushed with O_2-free CO_2. Anoxic media was sealed with lightly greased butyl rubber stoppers and closed with aluminum crimps (Bellco Industries, Vineland, NJ). Bottles were placed in a 39°C incubator and connected to their respective pressure sensors via needle insertion. Ruminal fluid from treated cattle was then again filtered through 4 layers of cheesecloth and glass wool, into a flask continually flushed with CO_2, and 4 mL of rumen inoculum was injected anoxically into each fermentation bottle via a needle and syringe. The pressure inside the bottles was equalized to atmospheric pressure at time zero by piercing rubber stoppers with a needle for approximately 5 s, before initiating recording. Once the pressure was equalized in all bottles, software recording was initialized, and atmospheric pressure was recorded. After 48 h of fermentation, software recording was terminated, and bottles were placed in the refrigerator to cease fermentation. Then, headspace gas samples (1 mL) were removed from each bottle and analyzed for methane concentration using the gas chromatography method (Allison et al., 1992). Final incubation pH was measured on the remaining rumen fluid, and 40 mL of neutral detergent solution (Van Soest et al., 1991) was added to each bottle, which was then resealed and autoclaved for 15 min at 120°C. The undegraded fiber was then filtered gravimetrically using Whatman 54 filter paper, oven dried at 60°C for 48 h, and weighed. The gas data were used to compute the asymptote (mL) and kd (%/h) for the exponential nonlinear function using the Gasfit software (http://www.nutritionmodels.com/gasfit.html; Tedeschi et al., 2009; Tedeschi and Fox, 2018).

Protozoa Count

Protozoa counts were determined by methods described by Dehority (1984) without staining. About 1 mL of original rumen fluid samples was added to 10 mL of formalin to achieve a 1:10 dilution of the original rumen contents. The counting technique was an adaptation of the procedure described by Purser and Moir (1959). A 1-mL aliquot of the ethanol-preserved sample was pipetted with a 1-mL-wide orifice (3 mm) into a Sedgewick Rafter Counting Chamber (Hausser Scientific, Horsham, PA). Protozoa were counted at a 100× magnification with a counting grid 0.5 mm square in the eyepiece; 25 evenly spaced grids from the entire chamber surface were counted for each sample.

In Situ Ruminal Incubations

There are many variants for the in situ incubation technique (Tedeschi and Fox, 2018, page 148). Small nylon bags, 5 × 10 cm, 50 μg micrometer porosity (Ankom Technologies, Macedon, NY) were weighed, filled with 5 g of ground sample (to pass a 2-mm screen), and sealed (Vanzant et al., 1998). Two sealed blank bags, 3 bags filled with the GRW diet, 3 bags filled with the TRANS diet, and 5 filled with the FIN diet (13 bags in total per animal) were incubated each week. The empty bags served as blanks to correct for feed particles and microorganisms that may adhere to the nylon. The small nylon bags were held together in a 32 × 42 cm polyester bag with a nylon zipper and weighted down with 2 sanitized, heavy bolts during the rumen fermentation period. After removal from the rumen, nylon bags were rinsed with distilled water to remove large particles of rumen contents off the bags and put through a series of washes until the water was colorless to remove

rumen fluid contamination. Upon completion of the washing process, the bags were placed in a forced-air oven and dried at 60°C for 48 h in preparation for analyses.

Digestibility Analyses

DMD. After nylon bags were removed from the forced-air oven, they were placed in a desiccator and dry weights were obtained from the samples. The residual weight of each sample was determined after drying to calculate in situ DMD by dividing the residue weight by the original sample weight before incubation.

NDFD. The NDFD was determined by methods described originally by Van Soest and Robertson (1980) using an Ankom 200 Fiber Analyzer (Ankom Technologies). After dry weights were retrieved on the in situ bag samples, they went through an additional wash procedure in the Ankom machine to determine the NDF residue. Bags were placed in the suspender (3 bags per level), and the machine was filled with approximately 1,900 to 2,000 mL of NDF solution. After the temperature reached 100°C, bags were placed into the solution, and the agitator was turned on for 70 min. Upon wash cessation, the bags went through a second and a third rinse with about 1,900 mL of preheated distilled H_2O (about 50°C) and were agitated for 10 min each time. The final rinse is approximately 1,900 mL of room temperature distilled H_2O with agitation for 10 min. Bags were then removed from the suspender, and excess water was manually removed. The bags were placed in a 100-mL beaker and covered with acetone for 3 to 5 min. Following this acetone bath, bags were removed, placed on a drying rack for 5 to 10 min, and then placed into a 55°C oven for 48 h. Once dry, they were immediately placed into a desiccator until final weights were able to be taken. The NDFD was calculated as follows:

$$\% \text{ NDFD} = (1 - \{[W_3 - (W_1 \times C_1)]/W_2\}) \times 100,$$

where W_1 is the bag tare weight, W_2 is the sample weight, W_3 is the dried weight of the bag after incubation, and C_1 is the blank bag correction or the running average of the final oven-dried weight divided by the original bag weight.

Statistical Analyses

The PROC MIXED of SAS (SAS Institute Inc., Cary, NC) was used to analyze the data (IVGP asymptote, IVGP kd, methane, total VFA concentration, A:P, lactate concentration, protozoa, DMD, and NDFD) as a complete randomized block design. Sex was the random effect, treatment was the fixed effect, and average DMI of each animal was used as a covariate. It was analyzed assuming a repeated measure design in which weeks (i.e., time) of rumen fluid collection were the repeated variable, using the REPEATED statement of PROC MIXED of SAS (SAS Institute Inc.), and animal within treatment was the subject. The effect of treatment was tested using the least squares means and orthogonal contrasts. The PROC IML was used to obtain the orthogonal coefficients for linear, quadratic, and cubic contrasts because treatments were not equally spaced (0, 2.5, 5, and 10 g/d). The same statistical model was used for pH except that the initial pH of the animals was also used as a covariate in addition to average DMI. The interaction between treatment and the covariate was removed from the statistical model if not significant at $P < 0.05$.

RESULTS AND DISCUSSION

In Vitro Fermentation and Rumen Parameters

Total Gas Production. The asymptote measurement or 48-h accumulated (total) gas production (mL) of IVGP is shown in Table 2 and Figure 1A. Though LY1 and LY3 had the least total gas production (21.6 and 21.3 mL, respectively), yeast treatments were not different while feeding the GRW diet ($P = 0.276$). Yeast treatment differed ($P = 0.002$) when cattle were fed the TRANS diet; LY3 had the least gas production (18.9 mL). Similar to the GRW diet, the FIN diet was not affected by yeast treatments ($P = 0.525$). Figure 1A shows a distinct pattern throughout all feeding phases in which the CON treatment produced the greatest amount of total gas. Mutsvangwa et al. (1992) stated that total gas production of a barley diet for beef cattle was on average less when supplemented with yeast culture (Yea-Sacc1026), but conversely, Tang et al. (2008) found that supplementation of a different type of yeast culture increased the total gas production of low-quality forages. The differences in the results of these 2 studies are likely attributed to the 2 products being of different strains and being supplemented to different diets. This shows relevance to the idea that product type, strain, and diets all can influence the results seen from any type of yeast product supplementation. Both studies supplemented with yeast culture, which is different from LY in the fact that LY contains high counts of viable active yeast cells and may be more proactive in the rumen. Wang et al. (2016) reported that when doses of LY (*S. cerevisiae*) were supplemented, total gas production was greater than the control. Although this is different than what was found in the present study, the study by Wang et al. (2016) also included supplementing different species of yeast: *Candida utiliz*, *Candida tropicalis*, and *S. cerevisiae*. Supplementation of these different strains of yeast to cattle receiving 2 different low-quality forages, maize stover, and rice straw resulted in *C. utiliz* yielding lower total gas production than control and the other 2 species of yeast across both diets (Wang et al., 2016). Discrepancies between all these studies suggest again that the selection of yeast species, strain, product type (e.g., LY vs. culture), and ration composition should be taken into consideration when supplementing LY to cattle rations as it could affect the variables of interest.

Fractional Rate of Degradation. Yeast treatments had no affect on the kd (Table 2, Figure 1B) for cattle con-

Table 2. Main effects of dry live yeast on rumen parameters of growing cattle for 3 types of diets

	Dietary treatment (TRT),[2] g/d					*P*-value[3]							
						Main effect			Contrast			Covariate	
Diet and substrate[1]	Con, 0	LY1, 2.5	LY2, 5	LY3, 10	SEM	TRT	Time (T)[4]	TRT × T	L	Q	C	Initial pH	DMI
Grower													
IVGP-a, mL	26.5	21.6	23.5	21.3	2.06	NS	***	NS	NS	NS	NS	—	—
IVGP-b, 1/h	0.10	0.17	0.148	0.136	0.021	NS	***	NS	NS	†	NS	—	—
Total VFA, m*M*	52.8^{b}	62.5^{a}	59.1^{ab}	54.7^{b}	2.99	NS	NS	†	NS	NS	NS	—	0.044
A:P	3.06	2.82	2.83	2.81	0.183	†	*	NS	NS	NS	*	—	0.021[5]
Lactate, µg/mL	2.51	2.67	2.50	2.54	0.100	NS	NS	NS	NS	NS	NS	—	—
Methane, m*M*	8.71^{a}	7.59^{ab}	8.30^{a}	5.58^{b}	0.689	NS	***	***	*	NS	NS	—	0.048
pH	5.71^{ab}	5.76^{a}	5.65^{b}	5.75^{a}	0.030	†	†	†	NS	†	NS	0.108	—
Protozoa, $\log_{10}$/mL	13.6	15.3	13.9	9.71	3.21	NS	*	*	NS	NS	NS	—	—
DMD, % DM	78.1^{a}	78.1^{a}	70.2^{b}	72.6^{b}	1.40	*	***	***	*	*	***	—	0.005[5]
NDFD, % DM	82.7^{a}	82.7^{a}	77.3^{b}	78.5^{b}	1.00	*	***	***	NS	NS	NS	—	0.004[5]
Transition													
IVGP-a, mL	30.2	19.8	20.4	18.9	1.69	**	**	NS	**	*	NS	—	—
IVGP-b, 1/h	0.137	0.192	0.138	0.206	0.016	*	†	NS	†	NS	**	—	—
Total VFA, m*M*	60.9	55.0	49.2	59.0	5.13	NS	*	NS	NS	NS	†	—	—
A:P	2.78	2.15	2.77	2.11	0.253	NS	*	NS	NS	NS	NS	—	—
Lactate, µg/mL	2.62	1.40	1.22	1.54	0.100	NS	NS	NS	NS	†	†	—	—
Methane, m*M*	14.2	15.2	13.2	12.5	1.12	NS	NS	*	NS	NS	NS	—	—
pH	5.79	5.85	5.73	5.65	0.088	NS	***	†	NS	†	NS	0.050	0.070
Protozoa, $\log_{10}$/mL	10.7^{ab}	13.7^{a}	6.30^{bc}	1.87^{c}	2.10	*	†	†	NS	NS	NS	—	0.045[5]
DMD, % DM	75.5	76.0	72.2	72.0	1.90	*	NS	NS	NS	NS	NS	—	5
NDFD, % DM	78.7	79.2	77.8	76.4	1.90	*	***	*	NS	NS	NS	—	5
Finisher													
IVGP-a, mL	26.9	20.1	27.6	29.0	4.48	NS	NS	NS	NS	NS	NS	—	—
IVGP-b, 1/h	0.146	0.233	0.176	0.215	0.061	NS	***	**	NS	NS	NS	—	—
Total VFA, m*M*	55.7^{b}	60.3^{ab}	73.1^{ab}	64.9^{a}	4.42	*	NS	NS	NS	*	NS	—	—
A:P	2.17	2.04	2.34	1.80	0.293	NS	NS	†	NS	†	†	—	—
Lactate, µg/mL	2.64	4.93	1.55	2.87	0.240	NS	†	NS	NS	NS	NS	—	—
Methane, m*M*	11.9^{b}	16.3^{ab}	18.9^{a}	15.3^{ab}	2.28	†	NS	NS	NS	NS	NS	—	—
pH	5.24	5.43	5.36	5.19	0.094	†	*	NS	*	*	NS	0.638	0.002[5]
Protozoa, $\log_{10}$/mL	5.67^{b}	10.2^{ab}	18.5^{a}	16.9^{a}	3.00	*	*	NS	NS	NS	NS	—	—
DMD, % DM	71.8^{a}	69.3^{a}	64.3^{b}	72.0^{a}	2.00	***	NS	NS	NS	NS	†	—	—
NDFD, % DM	75.7^{a}	72.3^{bc}	70.2^{c}	73.7^{ab}	1.60	***	***	*	NS	NS	NS	—	—

$^{a–c}$Means within a row with different superscripts differ by $P \le 0.05$.

[1]IVGP = in vitro gas production; IVGP-a = the asymptote of the exponential nonlinear function (total gas production); IVGP-b = the fractional rate of gas production of the exponential nonlinear function; A:P = acetate-to-proprionate ratio; DMD = DM digestibility; NDFD = NDF digestibility.

[2]Treatment values are given as LSM.

[3]NS = $P \ge 0.10$; †$0.05 < P \le 0.10$; *$0.01 < P \le 0.05$; **$0.001 < P \le 0.01$; ***$P \le 0.001$. Contrasts: L = linear, Q = quadratic, and C = cubic.

[4]All animals were fed for 9 wk (1 wk of adaptation, 8 wk of observation). The DMD and NDFD samples were collected for 7 wk, protozoa and lactate for 8 wk, and all other variables were collected for 9 wk.

[5]There was a significant ($P < 0.01$) interaction between dietary treatment and DMI, so the dietary treatment means are reported for the average of DMI.

suming the GRW ($P = 0.152$) or the FIN ($P = 0.516$) diets. However, there was an effect of yeast treatment during the TRANS diet ($P = 0.049$). For the most part, the LY1 had the fastest kd throughout the feeding period (Figure 1B). When examining the TRANS diet (wk 6 and 7), yeast treatment affected kd in a linear fashion ($P = 0.072$) in which the LY3 had the fastest kd. Yeast-treated animals had a decreased amount of gas produced (Table 1, Figure

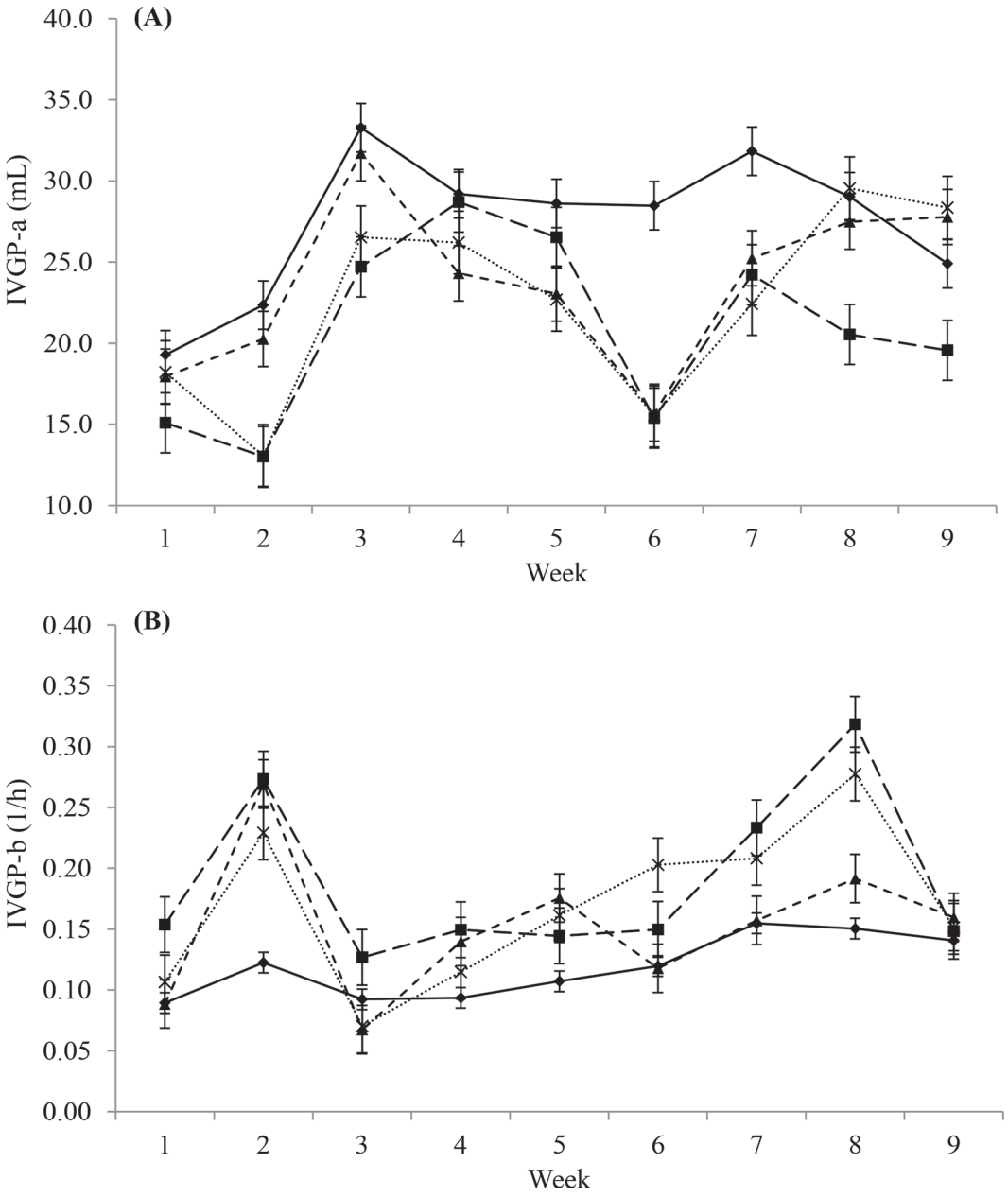

Figure 1. Effects of dry live yeast (LY) on the in vitro (A) total gas production (IVGP-a) and (B) fractional rate of fermentation (IVGP-b). ♦ = CON (control, 0 g of LY/d), ■ = LY1 (2.5 g of LY/d), ▲ = LY2 (5 g of LY/d), and × = LY3 (10 g of LY/d). The 3 diets were grower (wk 3 to 5), transition (wk 6 and 7), and finisher (wk 8 and 9). Vertical bars indicate the SE of the live yeast treatments.

1A) but increased fractional rate of fermentation (Table 1, Figure 1B) during the TRANS-diet phase, clearly suggesting an effect of LY during the transition phase.

Rumen Fluid Measurements

VFA. Total VFA concentration (m*M* of acetate + propionate + butyrate + isobutyrate + valeric acid + isovaleric acid) was not affected by yeast treatment while cattle were fed the GRW diet ($P = 0.115$) or the TRANS diet ($P = 0.301$). However, when cattle were fed the FIN diet, yeast treatment affected total VFA concentration in a quadratic fashion ($P = 0.033$) in which the LY2 rumen fluid had the greatest concentration of total VFA concentration (Table 2, Figure 2A). Similar results were found by Bakr et al. (2015) when they witnessed total VFA concentration was significantly greater in the yeast-fed animals compared with the controls throughout the study. Although our values did not reach significance for every diet, the common trend was present across treatments (Figure 2A).

When examining the effects of LY on the A:P ratio (Table 2, Figure 2B), the DMI effect was significant, and a cubic pattern ($P = 0.024$) was observed for the GRW diet. There was no significant interaction when cattle were fed the TRANS and FIN diets ($P = 0.174$ and $P = 0.562$; respectively, Table 2). The average A:P ratio decreased across all yeast treatments as well as the control as the

diet shifted to a ration with a greater percentage of concentrate. This follows in accordance with what Cho et al. (2014) reported when determining the effect of the energy level of the diet on the A:P ratio in the rumen of Hanwoo steers. As displayed in Table 2 and Figure 2B, LY3 repetitively had the least A:P ratio throughout each diet. In a previous study performed by Uyeno et al. (2017), similar results were observed when supplementing different inclusions (0, 5, 10 g/d) of the same LY product to Holstein cows. Although there was no significant effect of yeast treatment on the A:P ratio, 10 g/d of LY had a lower ratio during the study. This was attributed to the marginal decrease in the acetate concentration and an unchanged propionate concentration.

The lactic acid concentration, when represented as the average of each treatment per collection period, ranged from 0.76 to 10.0 μg/mL throughout the trial, which is within the acceptable range (Russell, 2002; Tedeschi and Fox, 2018). Nevertheless, there was no effect of yeast treatment on lactic acid concentration in the rumen when cattle were fed the GRW diet ($P = 0.996$), TRANS diet ($P = 0.168$), or the FIN diet ($P = 0.574$), but the LY2 treatment had the least concentration consistently throughout all diets (Table 2).

Methane. Yeast treatment did not significantly affect methane production ($P = 0.215$), but as expected, DMI did in a linear fashion ($P = 0.049$) when cattle were fed the GRW diet. The LY3 had the least methane production (Table 2, Figure 3A). Treatment was not different ($P = 0.265$) for the TRANS diet but tended to be during the FIN diet ($P = 0.052$), whereas the CON treatment produced the least amount of methane (Table 2, Figure

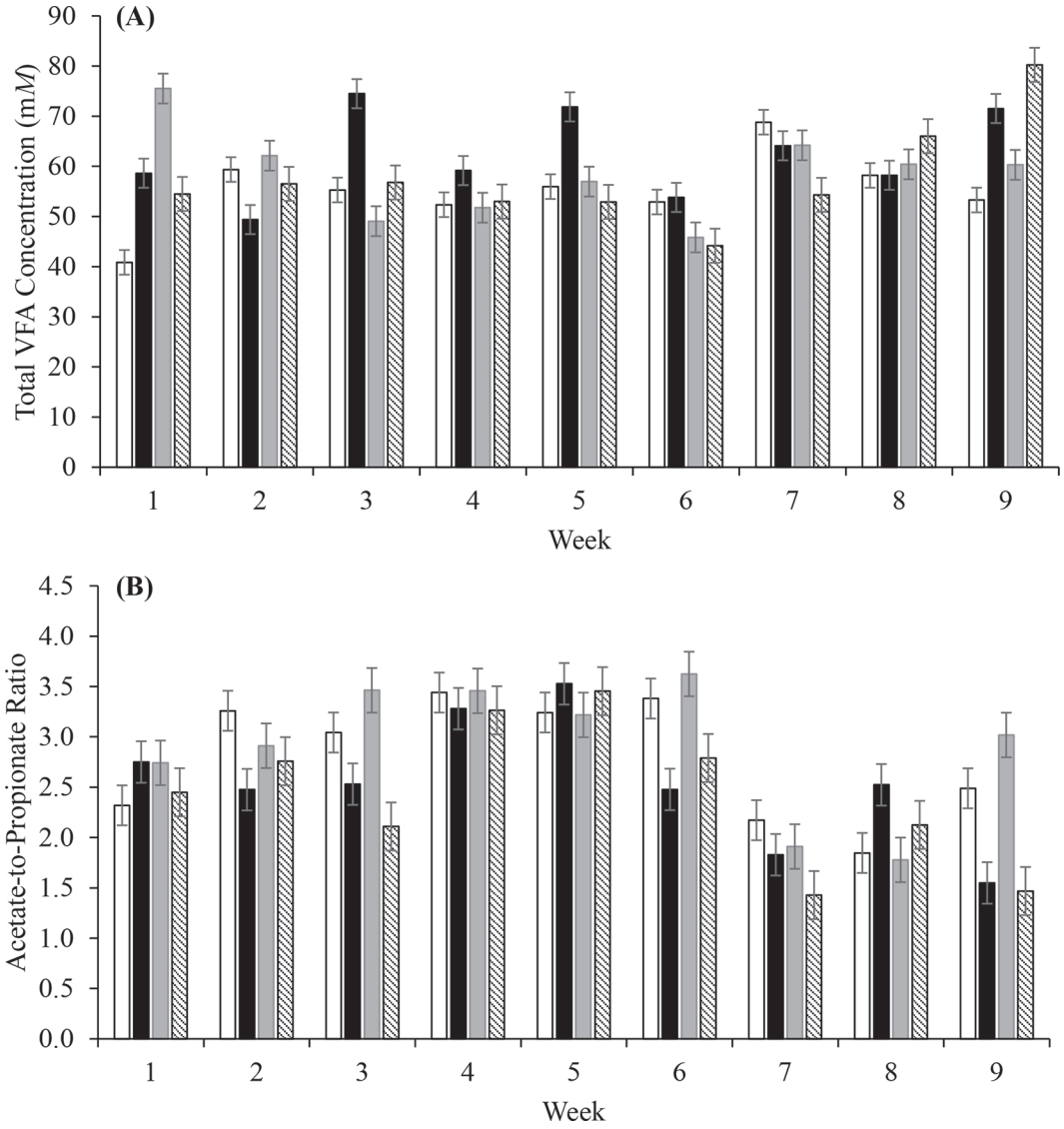

Figure 2. Effects of dry live yeast (LY; Actisaf hr+, Phileo Lesaffre Animal Care, Milwaukee, WI) on the (A) total VFA concentration in the rumen and (B) acetate-to-propionate ratio [white = CON (control, 0 g of LY/d), black = LY1 (2.5 g of LY/d), gray = LY2 (5 g of LY/d), diagonal stripes = LY3 (10 g of LY/d)]. The 3 diets were grower (wk 3 to 5), transition (wk 6 and 7), and finisher (wk 8 and 9). Vertical bars indicate the SE of the live yeast treatments.

3A). Although Carro et al. (1992) examined the effects of yeast culture, they reported the same patterns of methane production when cattle received 3 different levels of dietary concentrate in the ration. In our study, when cattle were fed low- and medium-concentrate rations, the treated animals produced less methane than did controls. When cattle were on the highest level of a concentrate ration, the control animals produced less methane than the treated animals. Similar responses of in vitro methane production have been observed when using a high-concentrate diet as a substrate by Dawson and Newman (1988) as well.

Protozoa Count. No treatment or DMI effects or their interactions were observed on the protozoa numbers in the GRW diet ($P > 0.05$; Table 2, Figure 3B); however, when cattle were fed the TRANS diet, effects of yeast treatment ($P = 0.049$), DMI ($P = 0.045$), and their interaction ($P = 0.049$) were observed. At the average DMI, LY1 had the greatest count of protozoa ($P = 0.029$). During the FIN diet, yeast treatment affected protozoa numbers ($P < 0.05$), where LY2 had the greatest count of protozoa. When high-concentrate diets are fed, and ruminal pH decreases below 6.0, protozoa populations decrease (Franzolin and Dehority, 1996). Small amounts of roughage are often included in high-grain finishing diets to reduce digestive and metabolic problems and may have a positive effect on maintaining the ruminal protozoa populations (Kreikemeier et al., 1990). Newbold et al. (1996) reported no difference in protozoa when supplementing LY in the form of *S. cerevisiae*. Conversely, the present study suggested that supplementation of LY when transitioning to

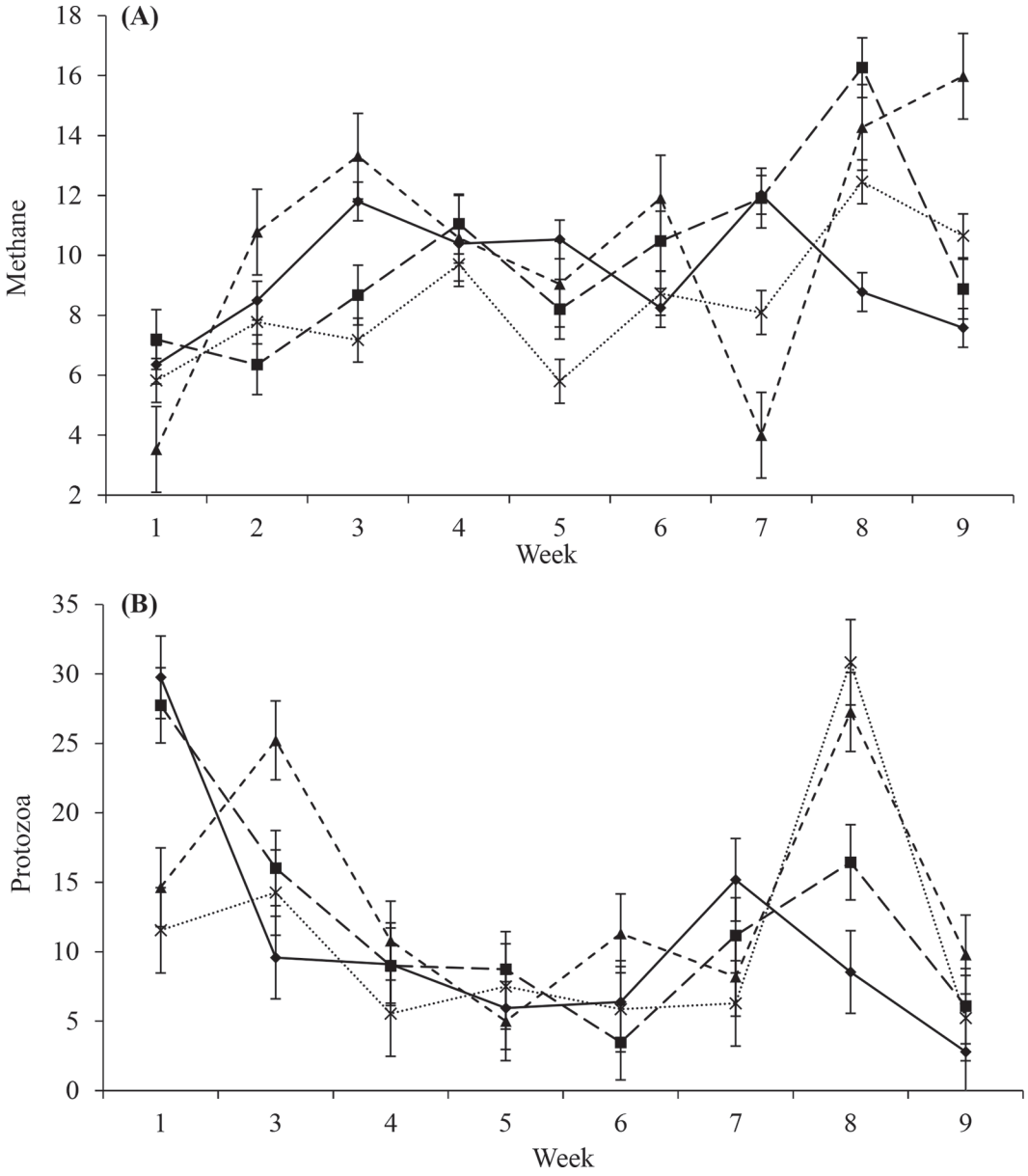

Figure 3. Effects of dry live yeast (LY) on (A) methane production and (B) protozoa. ♦ = CON (control, 0 g of LY/d), ■ = LY1 (2.5 g of LY/d), ▲ = LY2 (5 g of LY/d), and × = LY3 (10 g of LY/d). The 3 diets were grower (wk 3 to 5), transition (wk 6 and 7), and finisher (wk 8 and 9). Vertical bars indicate the SE of the live yeast treatments.

a grain-based diet may increase protozoa populations that can play a role in starch sequestration, which reduces the rate of starch fermentation, thereby reducing the risk of cattle developing ruminal acidosis. As previously documented (Ushida and Jouany, 1996), the increased protozoa count during the FIN diet is consistent with the tendency of increased methane production; thus, the inclusion of an ionophore might be beneficial to reduce methane when feeding LY. Further investigation is needed to fully understand the effects of LY on protozoa.

Ruminal pH. Yeast treatment tended to affect ruminal pH ($P = 0.104$) in a quadratic fashion ($P = 0.093$) when cattle were fed the GRW diet (Table 2). As shown in Figure 4, animals that received any inclusion of LY had greater pH than CON treatments after 21 d, suggesting an interaction between yeast treatment and time. The covariate DMI affected ruminal pH ($P = 0.070$) when cattle received the TRANS diet, but yeast treatment did not ($P = 0.308$). Treatments tended to behave in a quadratic pattern ($P = 0.089$) in which LY1 had the highest pH. In Figure 4, the increase in ruminal pH at wk 5 for CON treatments was unexpected and does not follow the general trend for this treatment. All other treatments had a similar pattern of ruminal pH decreasing over time. When we removed this anomaly from the data set, LY1 consistently produced greater ruminal pH than did CON. For the FIN diet, treatment also tended to affect ruminal pH ($P = 0.061$) in linear and quadratic fashions ($P = 0.010$ and $P = 0.033$, respectively) as did DMI ($P = 0.002$), and there was an interaction between treatment and DMI ($P = 0.056$; Table 2, Figure 4), suggesting that different levels of intake of DM might affect the LY effects on ruminal pH. Overall, in our study, the ruminal pH in animals fed LY1 was greater than that in CON animals, which is confirmed by previous studies (Erdman,1988; Fiems et al., 1993; Thrune et al., 2009). This finding is interesting considering the VFA concentration of LY1 throughout the study was greater than CON during the GRW and FIN diets, but according to Tedeschi and Fox (2018), VFA is not the only variable affecting ruminal pH. Although pH is sensitive to the acid load in the rumen, it also depends on the buffering capacity and the fractional rates of absorption of the fermentation acids through the rumen epithelium and their passage through the reticulum-omasum orifice. As long as the passage rate, buffering capacity, and absorption rate are greater than the rate of VFA production, ruminal pH may still be high (Tedeschi and Fox, 2018). This could explain why we see values of ruminal pH of certain treatments greater than one would think when comparing them to their VFA concentrations during the same period.

In Situ Digestibility

Williams et al. (1991) found that the inclusion of *S. cerevisiae* in ruminant diets increased DMD of hay incubated in the rumen of steers fed a mixed ration of hay and rolled barley after 12 h; however, after 24 h, degradation was similar across all treatment groups. On the other hand, Newbold et al. (1996) did not observe that degradation was affected significantly by treatment; however, they did see a trend toward an increase in the population of cellulolytic bacteria in the rumen with yeast present, which is favorable for increased degradation, but as stated previously, the effect did not reach significance in our study. Carro et al. (1992) discovered that yeast culture has no significant effect on DM and NDF degradability with me-

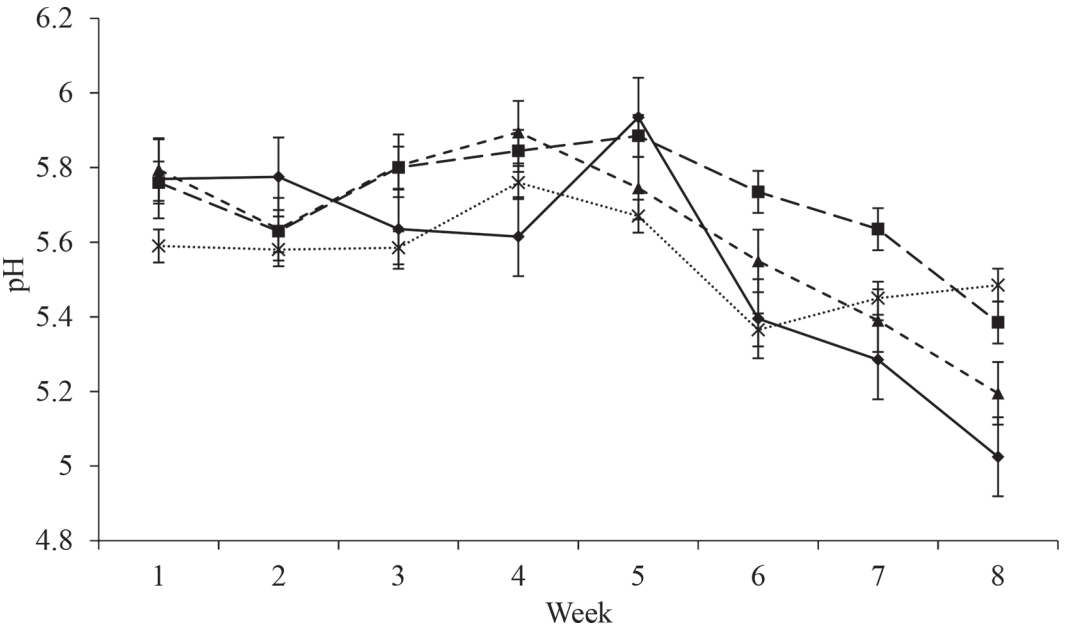

Figure 4. Effects of dry live yeast (LY) on rumen pH. ♦ = CON (control, 0 g of LY/d), ■ = LY1 (2.5 g of LY/d), ▲ = LY2 (5 g of LY/d), and × = LY3 (10 g of LY/d). The 3 diets were grower (wk 3 to 5), transition (wk 6 and 7), and finisher (wk 8 and 9). Vertical bars indicate the SE of the live yeast treatments.

dium- and low-concentrate diets, but when supplementing the high-concentrate diet, LY resulted in significantly greater DM and NDF degradation. Collectively, these studies suggest an advantage to feeding LY to aid with degradation, though results still vary widely.

DMD. There was an effect of DMI ($P = 0.005$), yeast treatment ($P = 0.003$), and an interaction between treatment and DMI ($P < 0.003$) on DMD (Table 2, Figure 5A) in a linear ($P = 0.047$) and quadratic fashion ($P < 0.007$) for the GRW diet. For the TRANS diet, yeast treatment also differed ($P < 0.007$), and there was an interaction observed between DMI and treatment ($P = 0.009$). When reporting treatment means for average DMI during the TRANS and GRW diets, LY2 resulted in greater DMD of the 3 yeast treatments ($P < 0.05$) but was not different from the control ($P > 0.05$). When cattle were fed the FIN diet, treatment affected the DMD ($P < 0.001$) in a cubic fashion ($P \leq 0.100$) in which LY3 had the highest DMD. Our results suggest that LY possibly increased the population of fiber-degrading bacteria or their activity, but they are contrary to the results of Carro et al. (1992), who found that LY resulted in greater DMD in low- to medium-concentrate diets but not with high-concentrate diets. This difference could also be because they were supplementing with a yeast culture, not live yeast. As mentioned before, the biological differences between the 2 probiotic

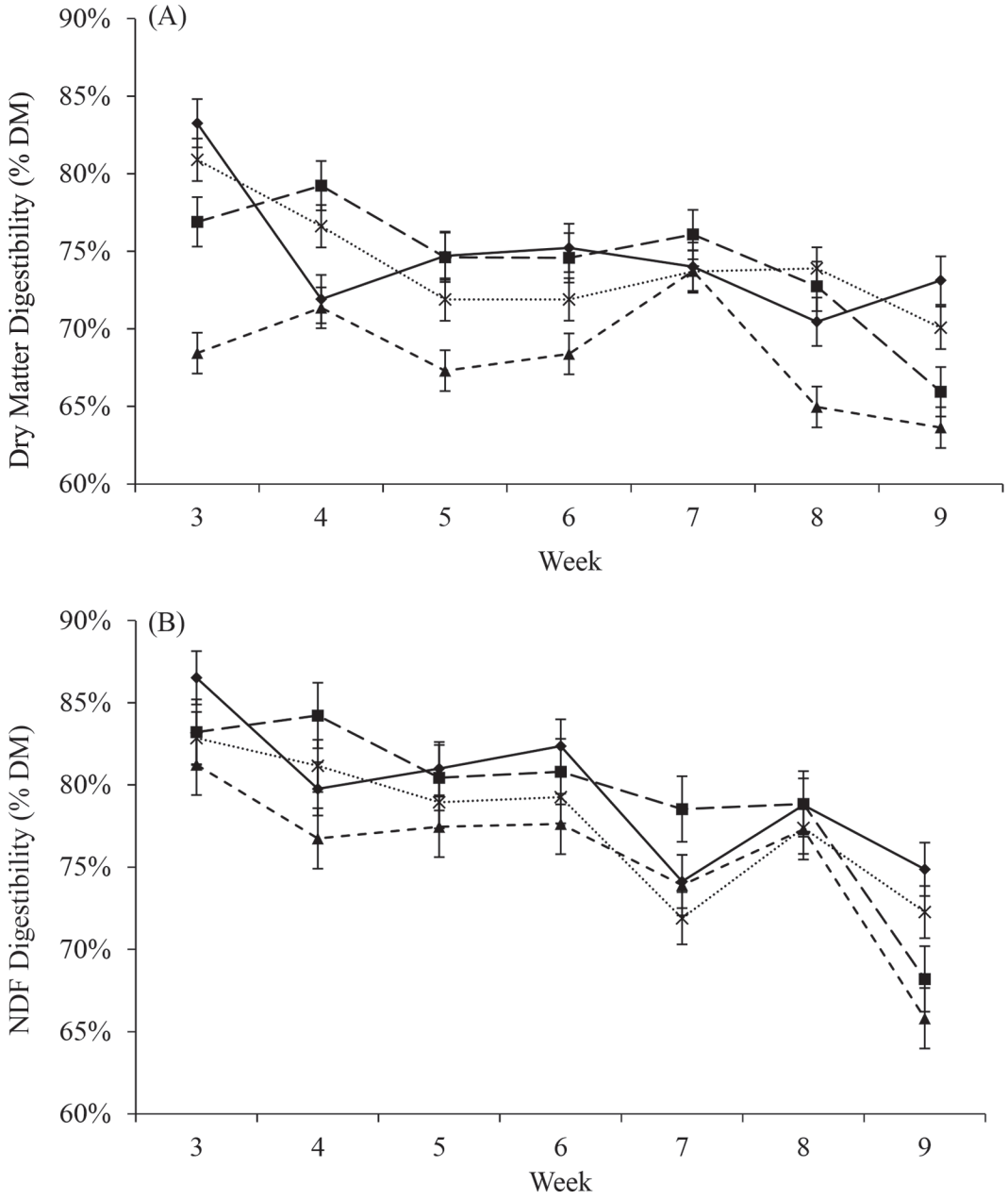

Figure 5. Effects of dry live yeast (LY) on (A) DM digestibility and (B) NDF digestibility. ♦ = CON (control, 0 g of LY/d), ■ = LY1 (2.5 g of LY/d), ▲ = LY2 (5 g of LY/d), and × = LY3 (10 g of LY/d). The 3 diets were grower (wk 3 to 5), transition (wk 6 and 7), and finisher (wk 8 and 9). Vertical bars indicate the SE of the live yeast treatments.

yeast products could influence what is observed, so additional data where LY products are the area of interest in determining the effects on DMD is needed to confirm our findings.

NDFD. For NDFD, as depicted in Table 2 and Figure 5B, treatment was different for all diets ($P < 0.05$), DMI affected the GRW diet ($P = 0.004$), and there was an interaction between DMI and yeast treatment ($P = 0.004$) during the TRANS diet. When the GRW diet was fed, LY1 and CON had the highest NDFD when treatment means were reported for the average of DMI. The LY1 had the highest NDFD for average DMI as well throughout the TRANS diet and while cattle were eating the FIN diet. The LY3 treatment and CON seemed to promote greater NDFD for unadjusted values. When examining values adjusted for DMI in low- and medium-concentrate diets, LY1 seems to have the greatest NDFD of the 3 yeast treatments, but in a high-concentrate diet comparing unadjusted values, LY3 provided the greatest NDFD. A study performed by Monnerat et al. (2013) found no significant changes in digestive parameters when supplementing 2 levels of yeast to beef cattle that were being fed diets containing different starch levels. The diverse results found throughout these studies solidify the need for additional studies to determine the correlation between yeast treatment and diet composition on NDFD.

APPLICATIONS

It is becoming critical to understand the nature of interactions among yeast probiotics, the ruminant gastrointestinal microbial population, and dietary components to predict the effect of probiotic supplementation on cattle nutrition. This acknowledgment is essential to select more targeted and reliable probiotics to capitalize on a promising antimicrobial alternative feed additive. The mechanisms and attributions of LY on targeted rumen microbial communities, animal growth, health, and overall productivity have been extensively studied, at least for some strains of *S. cerevisiae*. Although there remains some perception of probiotics as "magical additives," research investigating the effects of probiotics has restored credibility to probiotic use in ruminant nutrition; however, much of the effect of LY supplementation remains unknown. Indeed, field studies indicate that positive effects on milk or meat production can be obtained, but the animal response to such feed additives may be quite variable depending on various factors such as nature of the diet, level of productivity, animal physiological and genetic factors, dose, and strain of yeast used. Our study indicated that the daily supplementation of 10 g of LY/d yielded the fastest rate of fermentation in the transition phase (i.e., TRANS diet) but less total gas production. Supplementation with 2.5 g of LY/d yielded greater protozoa counts, greater DMD and NDFD in the GRW and TRANS diets, and a high ruminal pH in all diets, though not different from the 10 g of LY/d treatment. The rumen fluid from cattle supplemented with 5 g of LY/d had the least concentration of lactate throughout all diets and had the greatest protozoa numbers when cattle were fed the FIN diet. The supplementation of 10 g of LY/d provided the least A:P and, subsequently, least methane production during the feedings of all diets; least total gas production during the GRW and TRANS diets; and the greatest DMD and NDFD while cattle were fed the FIN diet. Results regarding yeast treatment effects on total VFA varied across all diets with no statistically significant difference between treatments.

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