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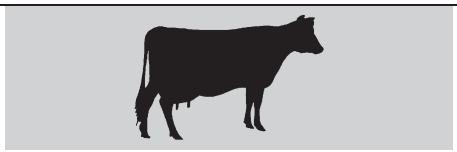

Case Study: Effects of a supplemental enhanced yeast product on digestion and milk production in dairy cows

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ABSTRACT

This research evaluated the effects of an enhanced yeast product (EY) on in sacco and total digestion of nutrients and milk production by lactating cows. For the in sacco study, 4 rumen-cannulated, lactating Holsteins received a common TMR for 1 wk. Thirty grams of EY per day was topdressed daily to 2 of the 4 cows. Corn silage, alfalfa silage, timothy hay, corn grain, and soybean meal were incubated in sacco. Digestion kinetics were calculated. The EY reduced lag to digestion for the corn silage ($P < 0.05$) and alfalfa silage ($P < 0.10$). Digestion rates of NDF were greater for corn grain and soybean meal ($P < 0.10$) with EY. Solubility of DM was greater with EY than control for corn grain and soybean meal. For the digestion trial, 8 lactating Holsteins were divided equally into 2 groups. Thirty grams of EY per day was topdressed after a.m. feeding for the test group, and this group was compared with the control. There were no differences ($P > 0.05$) in total-tract nutrient digestibility. Urinary N losses were reduced ($P < 0.10$) by EY. Fourteen on-farm feeding experiments were conducted. In 5 trials in which the control diet contained no yeast, 150-d milk increased in 4, fat percentage increased in 2, and protein percentage increased in 2 trials with EY ($P < 0.10$). In 9 trials in which EY replaced a yeast product, 150-d milk increased in 6, fat percentage increased in 2 and declined in 2, and protein percentage increased in 1 and declined in 1 study. The EY alters fermentation, which can positively but inconsistently influence milk production.

Key words: yeast, digestibility, milk production

INTRODUCTION

The use of yeast products based on live *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* cultures are common in dairy ration formulations and have been reported to provide a variety of advantages. Dann et al. (2000) and Ramsing et al. (2009) determined yeast culture improved feed intake in periparturient and early lactation cows. Robinson and Garrett (1999) found cows receiving yeast culture appeared to adapt to changes in the ingredient composition of diets more quickly than did cows receiving diets without yeast.

Yeast may alter fermentation. Miller-Webster et al. (2002) learned yeast cultures shifted the acetate-to-propionate ratio in favor of propionate. Shwartz et al. (2009) found the use of yeast culture did not alter DMI but did reduce rectal temperature in heat-stressed cows, suggesting heat of fermentation might have been reduced. In another study, Guedes et al. (2008) observed rumen lactate concentrations were reduced with yeast.

Responses to yeast products in diets for ruminants are generally positive (Rabiee et al., 2008; Desnoyers et al., 2009), subtle (Robinson and Garrett, 1999), and highly variable (Shaver and Garrett, 1997; Sales, 2011). If yeast can be mixed with other rumen-modifying ingredients, then perhaps the benefits of the yeast can be enhanced (**EY**). In this evaluation, a product containing live yeast, yeast culture media, fibrolytic enzymes, and monosodium glutamate was assessed. This study involves the quantification of the effects of the product on rumen and total-tract digestion, as well as milk production.

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MATERIALS AND METHODS

Digestion Studies

Animals and Treatments. These experiments were conducted at the Atlantic Dairy and Forage Institute, Fredericton Junction, NB, Canada. Animals participating in these studies were handled in accordance with guidelines outlined by FASS (2010).

In the first phase of the evaluation, 4 lactating, rumen-cannulated cows (2 test and 2 control) received a TMR formulated to meet guidelines established by NRC (2001) for 40 L of milk (Table 1). The ration was fed twice daily at levels estimated to provide 3 to 5% orts. Immediately after the a.m. feeding, 30 g of an EY product (Dairyman's Edge Pro; Papillon Agricultural Company, Easton, MD) was topdressed on the TMR of the 2 test cows. Test cows received the EY for 1 wk before the in sacco study began.

For the in sacco comparisons, locally grown corn silage (**CS**), alfalfa silage (**AS**), timothy grass hay (**GH**), corn grain (**CG**), and high protein soybean meal (**SM**) were dried and ground to pass through a 3-mm screen. Samples were weighed into nylon bags (20 × 20 cm) with pore sizes ranging from 30 to 50 μm. Twelve replicate 5-g samples were incubated per cow for each ingredient. Bags were removed in triplicate after 6, 12, 24, and 48 h of incubation. Residues were dried in a forced-air oven at 55°C for 48 h, weighed, pooled by time for each cow, and analyzed for CP, ADF, and NDF.

Analyses were provided by Livestock Feed Analysis (Department of Agriculture, Aquaculture and Fisheries, Fredericton, NB, Canada). Crude protein was determined by the Leco method (method 999.03; AOAC, 1997). Acid detergent fiber was determined according to AOAC (1997; method 973.18), and NDF was assessed using the procedure of Van Soest et al. (1991).

Eight lactating Holstein cows were employed to determine the effects of EY on total-tract digestibility. Cows were blocked by production and provided a TMR (Table 1) ad libitum (3–5% orts) for the duration of the 14-d feeding period. Fresh feed was provided twice a day. For the test group of 4 cows, EY was topdressed at a rate of 30 g/cow per day immediately after the a.m. feed was issued.

For the last 4 d of the feeding period, cows were placed in metabolic stalls, where urine and feces were collected separately. Feces were collected in boxes made to fit the manure gutter. Feces were weighed daily, and a 5% subsample was frozen for later analysis. At the end of the collection period, feces were mixed by cow and subsampled. Subsamples were dried in a forced-air oven at 55°C for 48 h and submitted to Livestock Feed Analysis for DM, CP, ADF, and NDF analysis. Urine was collected in 20-L sealed plastic containers placed behind the boxes used to collect feces. Urine was weighed twice daily and acidified, and a 3% subsample was frozen for analysis. Urine was analyses for CP using the Kjeldahl procedure (AOAC, 1990; method 978.02).

Milk production was measured and samples were collected from both the a.m. and p.m. milkings on the last 2 d of the experiment. Milk samples were submitted to the local Dairy Herd Improvement Association laboratory (Valacta, St. Anne-de-Bellevue, QC, Canada) for fat and true protein analysis via near infrared spectrometry.

Data Analysis. Pool sizes, rate functions, and digestion lag times were determined for DM, CP, ADF, and NDF as outlined by Dhanoa (1988). Differences between treatments were assigned using a 1-way ANOVA (Minitab 14, Minitab Inc., State College, PA). For the digestion study, results for the 2 groups of cows were compared using a GLM in which treatment was considered to be a fixed effect, and cows within each pair was assessed as a random effect (Minitab 14).

Table 1. Ingredient and nutrient composition of the diet used in both the in sacco and the digestibility study

Item	% of DM
Ingredient composition	
Grass silage	50.04
Ground corn grain	20.55
Ground barley grain	15.64
Soybean meal, 47.5% CP	9.84
Mineral vitamin mix[1]	2.44
Fat[2]	0.51
Urea	0.49
Limestone	0.40
Magnesium oxide	0.09
Nutrient composition	
DM	49.54
CP	17.02
ADF	17.76
NDF	32.38
Lignin	2.51
Ash	6.74
Fat	3.99

[1]Supplement provided calcium, 14.28%; phosphorus, 2.34%; magnesium, 6.60%; sulfur, 1.48%; sodium, 1.48%; iodine, 46 mg/kg; iron, 984 mg/kg; copper, 501 mg/kg; manganese, 1,784 mg/kg; zinc, 1,772 mg/kg; cobalt, 53 mg/kg; selenium, 14.3 mg/kg; vitamin A, 256,990 IU/kg; vitamin D, 77,100 IU/kg; vitamin E, 1,787 IU/kg.

[2]Megalac, Arm & Hammer Animal Nutrition, Princeton, NJ.

Feeding Trials

Description of Protocols. Fourteen on-farm feeding trials were conducted between 2006 and 2011. All

Table 2. Composition of ingredients evaluated in the in sacco study

Nutrient, % DM	Corn silage	Alfalfa silage	Timothy hay	Corn grain	Soybean meal
DM	29.93	33.15	85.97	90.08	88.27
CP	10.21	18.23	7.79	9.37	52.63
ADF	28.75	35.63	36.33	2.99	7.57
NDF	49.28	46.97	68.58	13.27	9.49

were high-producing herds located in Minnesota (2), New York (2), Ohio (2), Pennsylvania (4), and Wisconsin (4). Diets were formulated by either feed-company nutritionists or consulting nutritionists, and diets were not changed during the course of the trial. In 5 herds, EY was added at a feeding rate of 30 g/cow per day at the expense of corn grain. In the remaining herds, EY was substituted for any yeast product that was currently being used. If the amount of the EY added differed from the EY removed, an adjustment was made to the corn in the diet.

Two basic trial designs were used, based on the feeding and housing systems available at the farms. When the farm had multiple pens of animals, and similar pens receiving the same diet were available for use in the study, a side-by-side (S × S) experiment was used. Pens were paired by (in order of importance) lactation number, milk yield, and DIM. Periods were the length of time between Dairy Herd Improvement Association tests. During period 1, all cows received the control diet. During period 2, one pen from each pair was randomly assigned to the EY treatment. Only cows available for both periods were included in the data analysis.

When the farm did not have pens available, but fed cows a one-group TMR, a 3 period switchback experiment was conducted. The rations were fed in the following order: control, test control. Data from cows available for all 3 periods were used in the analyses.

Data Analysis. With respect to paired pen studies, the first period was used as a pretreatment covariate period. Data were analyzed as a randomized block, with a covariate. Treatment was a fixed effect, and pens within treatment was a random effect. Individual animals were used as the experimental unit (Robinson et al., 2006). Data were analyzed using Microsoft Excel (XLSTAT; AddInSoft, New York, NY). For the switchback studies, trials were analyzed as 2-tailed *t*-tests, again using Microsoft Excel.

RESULTS AND DISCUSSION

Table 2 provides the composition of the ingredients evaluated in sacco, and Table 3 shows the results from the incubations. There was insuf-

Table 3. Results of the in sacco incubations from rumens of cows fed the diet with or without an enhanced yeast (EY) product[1]

		Soluble fraction, %		Insoluble fraction, %		Digestion rate, %/h		Lag to digestion, h	
Test ingredient	Nutrient	Control	EY	Control	EY	Control	EY	Control	EY
Corn silage	DM	30.42	30.82	69.58	69.18	2.70	2.77	0	0
	CP	51.03	52.45	48.97	47.55	2.17	2.20	0	0
	ADF	0	0	100	100	2.35	2.48	5.97[a]	1.73[b]
	NDF	0	0	100	100	2.54	2.50	5.40[a]	4.32[b]
Alfalfa silage	DM	35.79	37.87	64.21	62.13	2.67[c]	2.48[d]	0	0
	CP	59.47	59.10	40.53	40.90	3.91	3.84	0	0
	ADF	0	3.54	100	96.46	1.20	1.83	1.14	0
	NDF	0	0.80	100	99.20	2.21	2.04	1.36[a]	0[b]
Grass hay	DM	14.15	18.38	85.85	81.62	2.16	2.00	0	0
	CP	29.44	24.37	75.63	70.56	2.25	2.77	0	0
	ADF	0	0	100	100	1.98[c]	2.35[d]	5.09	5.81
	NDF	0	0	100	100	2.16[a]	2.55[b]	4.68	4.69
Corn grain	DM	15.09[a]	30.51[b]	84.91[a]	69.49[b]	9.91	10.16	0	0
	CP	15.04	18.36	84.96	81.64	7.96	8.31	0	0
	ADF	0	0	100	100	1.98	2.35	5.09	5.35
	NDF	0	0	100	100	4.89[c]	7.15[d]	4.10	4.61
Soybean meal	DM	0.03[a]	14.54[b]	99.97[a]	85.46[b]	16.75	16.21	0.63	0
	CP	38.93	34.40	61.07	65.60	7.38[a]	5.78[b]	0	0
	ADF	0	0	100	100	8.46[c]	10.27[d]	2.78	3.40
	NDF	0	0	100	100	8.89[c]	10.57[d]	4.59	4.81

[a,b]Means within subheading differ by treatment ($P < 0.05$).

[c,d]Means within subheading differ by treatment ($P > 0.10$).

[1]Mean of 2 samples per treatment.

Table 4. Milk production parameters for cows used in the digestion study receiving the control ration or a ration with an enhanced yeast product (EY)[1]

Item	Control	EY	SEM	*P*-value
Milk production pretrial, kg	36.75	33.00	1.81	0.22
Milk production end of test, kg	32.10	32.25	1.30	0.48
Change in milk, kg	−4.65	−0.74	1.37	0.13
Milk fat end of test, %	4.06	4.10	0.09	0.45
Milk protein end of test, %	3.00	3.09	0.052	0.31
DMI during test period	20.1	21.4	0.75	0.27

[1]There were 4 cows per treatment.

ficient sample remaining at 48 h to determine residual ADF or NDF for the SM.

The EY had no effect on the solubility or the rate of rumen digestion of any nutritive fractions for CS. The digestion lag time for ADF and NDF were reduced ($P < 0.05$) when EY was included in the diet of dairy cows with CS.

The addition of EY to the diet reduced ($P < 0.10$) the rate of digestion of DM in AS. There were no changes in distribution between soluble and insoluble fractions or rates of digestion of CP, ADF, or NDF ($P > 0.05$). The digestion lag times for ADF and NDF were low for this ingredient, but the value was reduced ($P < 0.05$) for the NDF fraction. Reduced lag time increases the extent of fiber digestion in the rumen (Grant, 1994).

When GH was examined in sacco, changes in digestion lag failed to reach significance ($P > 0.10$). Rates of digestion of the fiber fractions were greater when cows received the EY product for NDF ($P < 0.05$) and for ADF ($P < 0.10$). The expected outcome would again be improved rumen fiber digestion. No other variables measured were altered for GH when EY was supplied relative to results obtained for the control treatment.

Table 5. Whole-tract digestibility and nitrogen balance for diets with or without an enhanced yeast product (EY)[1]

Item	Control	EY	SEM	*P*-value
Digestibility, %				
DM	68.5	67.8	1.21	0.40
CP	70.9	69.0	1.24	0.37
ADF	42.4	40.6	1.87	0.36
NDF	47.1	45.8	2.07	0.23
N, g/d				
N intake	554	566	20.3	0.38
Fecal N	161	175	8.7	0.24
Urinary N	215	186	13.3	0.06
Milk N	156	161	8.9	0.91
Retained N	22	44	20.2	0.29
N balance, % of total				
Fecal N	29.1	31.0		
Urinary N	38.7	32.8		
Milk N	28.2	28.4		
Retained N	4.0	7.8		

[1]There were 4 cows per treatment.

The EY increased DM solubility for CG ($P < 0.05$). Solubility for CP, ADF, and NDF did not change, suggesting that the change occurred in the nonstructural carbohydrate fraction of this ingredient. Further research is required for verification. The EY did not alter rates of digestion or lag to digestion with this ingredient.

In similar fashion, EY increased DM solubility for SM ($P < 0.05$), with no effect on the CP, ADF, or NDF fractions. Again, the fraction most likely to have changed would have been the nonstructural carbohydrate fraction. Unlike any of the other ingredients, there was a decrease in the rate ($P < 0.05$) of digestion for the CP fraction of SM. The reason for this is not obvious and requires further study.

Both CG and CS contained significant amounts of nonstructural carbohydrates. Interestingly, solubility increased with CG but not with CS. Ensiling has been shown to alter the starch matrix in corn silage (Hoffman et al., 2011) and may have limited the likelihood that EY would produce any further changes.

Results from the digestion study are provided in Tables 4 and 5. Production levels were similar for both groups of cows (Table 4) and did not change during the feeding and collection period. Whole-tract digestibility of DM, CP, ADF, and NDF did not change because of the inclusion of EY in the diet. Urinary losses of N were lower with the EY treatment, resulting in improved ($P < 0.05$) levels of metabolizable protein.

With the lack of change in total-tract N digestion, the improvement in N retention would most likely be associated with changes in N metabolism in the rumen and thus changes in the N components presented in the duodenum. Yeast culture was found to reduce ammonia levels in several studies (Desnoyers et al., 2009; Lascano and Heinrichs, 2009), which might be associated with greater capture of ammonia N by rumen microbes. Miller-Webster et al. (2002) determined that yeast cultures can increase microbial yield and microbial efficiency. This would reduce the amount of ammo-

Table 6. Evaluation of the effects of enhanced yeast (EY) on milk yield, 150-d corrected milk yield, milk fat percentage, and milk protein percentage by herds in which the control diet contained no yeast product

	No. of cows[2]		Start DIM		Milk, kg		150-d Milk, kg		Fat, %		Protein, %	
Trial[1]	Control	EY	Control	EY	Control	EY	Control	EY	Control	EY	Control	EY
1 (S × S)	40	41	172	176	38.84[c]	41.03[d]	41.91[c]	44.22[d]	3.41	3.44	2.89[d]	2.84[c]
2 (S × S)	383	377	53	53	39.46[a]	41.95[b]	36.65[a]	39.16[b]	3.91	3.92	2.85	2.89
3 (S × S)	55	46	55	46	41.84	44.21	39.97[c]	42.08[d]	3.65	3.88	3.01	3.08
4 (SB)	261	—	125	—	38.33	38.73	39.94[c]	40.84[d]	3.51[a]	3.57[b]	2.92[a]	2.97[b]
5 (SB)	131	—	136	—	38.38	38.88	39.74	40.09	3.37[a]	3.66[b]	3.04[a]	3.16[b]

[a,b]Means within subheading differ by treatment ($P < 0.05$).
[c,d]Means within subheading differ by treatment ($P > 0.10$).
[1]S × S = side-by-side trial design; SB = 3-period switchback trial design.
[2]The same cows were employed in both treatments with the switchback trial design.

nia absorbed and potentially reduce urinary N.

If, as the in sacco results suggest, more nonfiber carbohydrate is available, this would also support microbial yield from a greater energy supply. Yeast cultures alone have not been shown to increase carbohydrate solubility in the rumen (Miller Webster et al., 2002; Desnoyers et al., 2009). This change may, therefore, have been associated with other components of the EY product.

Results for the 5 trials in which EY was added to diets that contained no yeast are given in Table 6. Fat and protein percentages, rather than yields, were reported. Yields calculated from actual milk would be biased when DIM differ between the 2 treatment groups. Using adjusted milk to compute yields of components would likely be more accurate but is not a commonly accepted practice. Thus, percentage values are being reported along with actual milk and 150-d corrected milk.

Milk yield, adjusted to a common 150 DIM, was greater ($P < 0.10$) at 4 of the 5 herds when EY was provided to the cows. Milk fat percentages were significantly greater at 2 herds ($P < 0.05$) and numerically greater at all 5 herds with the EY treatment. This suggests that fat yields would have increased along with milk volume. Protein percentage declined in 1 herd but increased ($P < 0.10$) in 3 other herds.

The herd that saw no improvement in milk yield (herd 5) had a substantial increase in both milk fat and milk protein when cows received the EY additive. It would thus appear that the changes seen in sacco resulted in visible changes in yields.

Results were more variable from studies in which EY replaced yeast in the diet (Table 7). Milk volume, corrected to 150 DIM, was greater at 6 out of 9 ($P < 0.10$) of the feeding studies. Fat percentage increased at 4 of the trials but declined in 2 trials. Protein was largely unaffected, with changes failing to reach significance at 7 of the 9 trials, declining in 1, and increasing in 1. Two of the 9 herds

Table 7. Evaluation of the effects enhanced yeast (EY) on milk yield, 150-d corrected milk yield, milk fat percentage, and milk protein percentage by herds in which the control diet contained a yeast product

	No. of cows[2]		Start DIM		Milk, kg		150-d Milk, kg		Fat, %		Protein, %	
Trial[1]	Control	EY	Control	EY	Control	EY	Control	EY	Control	EY	Control	EY
1 (S × S)	171	171	161	202	36.36^{b}	34.63^{a}	37.85	37.75	3.59^{c}	3.78^{d}	3.01	3.02
2 (S × S)	62	59	53	52	39.46	38.14	36.99	35.74	3.84	4.11	2.93	2.97
3 (S × S)	155	242	198	175	44.01^{c}	47.39^{d}	46.22^{a}	49.56^{b}	3.63^{c}	3.77^{d}	3.12^{b}	3.06^{a}
4 (S × S)	216	82	164	181	39.01^{c}	39.68^{d}	39.30^{a}	42.17^{b}	3.36	3.38	2.66	2.69
5 (S × S)	88	89	141	140	41.75^{c}	43.30^{d}	41.48^{a}	44.32^{b}	3.71	3.61	3.06	3.09
6 (S × S)	193	200	51	46	41.61^{a}	43.60^{b}	29.63^{c}	30.46^{d}	4.00^{a}	4.11^{b}	2.92	2.92
7 (SB)	79	—	148	—	33.76^{c}	34.29^{d}	35.91^{c}	36.65^{d}	3.65^{b}	3.51^{a}	2.96	2.98
8 (SB)	485	—	154	—	40.75	40.47	40.81	40.53	3.56^{b}	3.44^{a}	3.17	3.16
9 (SB)	81	—	192	—	36.89	36.69	39.48^{a}	40.71^{b}	3.31^{a}	3.55^{b}	2.90^{a}	2.95^{b}

^{a,b}Means within subheading differ by treatment ($P < 0.05$).
^{c,d}Means within subheading differ by treatment ($P > 0.10$).
[1]S × S = side-by-side trial design; SB = 3-period switchback trial design.
[2]The same cows were employed in both treatments with the switchback trial design.

saw no positive improvements in milk yield, fat percentage, or protein percentage.

These findings would indicate the enhancements to yeast resulted in improvements in performance at least equivalent to yeast and, in many cases, resulted in improvements beyond yeast alone. Economic advantages would depend on the cost of the product relative to other yeast products on the market.

IMPLICATIONS

In sacco results demonstrated EY positively influenced forage NDF digestion in the rumen, reducing NDF digestion lag for ensiled forages and increasing the rate of NDF digestion for GH. This would increase energy available in the rumen. The solubility of DM, but not NDF or CP, increased with CG and SM, suggesting that nonstructural carbohydrate was more readily available in the rumen for concentrate ingredients, also potentially improving rumen available energy. The EY did not change overall digestibility, indicating a shift in site but not extent of digestion. The metabolism study revealed reduced urine N output, possibly associated with greater conversion of N to microbial protein production. Feeding studies involving large numbers of cows indicated that milk production increased in most situations in which EY was fed for cows receiving yeast and those not receiving yeast.

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ERRATUM

Erratum to "Case study: Effects of a supplemental enhanced yeast product on digestion and milk production in dairy cows" (Prof. Anim. Sci. 28:682-688)

E. Evans, R. J. Patterson, and N. Clark

In the original article, data related to herd 8 shown in Table 7 was incorrect. The corrected table is provided below. The authors apologize for this error.

Table 7. Evaluation of the effects enhanced yeast (EY) on milk yield, 150-d corrected milk yield, milk fat percentage, and milk protein percentage by herds in which the control diet contained a yeast product

	No. of Cows[2]		Start DIM		Milk, kg		150-d milk yield		Fat, %		Protein, %	
Trial[1]	Control	EY	Control	EY	Control	EY	Control	EY	Control	EY	Control	EY
1 (S×S)	171	171	161	202	36.36^{b}	34.63^{a}	37.85	37.75	3.59^{c}	3.78^{d}	3.01	3.02
2 (S×S)	62	59	53	52	39.46	38.14	36.99	35.74	3.84	4.11	2.93	2.97
3 (S×S)	155	242	198	175	44.01^{c}	47.39^{d}	46.22^{a}	49.56^{b}	3.63^{c}	3.77^{d}	3.12^{b}	3.06^{a}
4 (S×S)	216	82	164	181	39.01^{c}	39.68^{d}	39.30^{a}	42.17^{b}	3.36	3.38	2.66	2.69
5 (S×S)	88	89	141	140	41.75^{c}	43.30^{d}	41.48^{a}	44.32^{b}	3.71	3.61	3.06	3.09
6 (S×S)	193	200	51	46	41.61^{a}	43.60^{b}	29.63^{c}	30.46^{d}	4.00^{a}	4.11^{b}	2.92	2.92
7(SB)	79	—	148	—	33.76^{c}	34.29^{d}	35.91^{c}	36.65^{d}	3.65^{b}	3.51^{a}	2.96	2.98
8(SB)	485	—	154	—	40.61^{a}	42.94^{b}	40.67^{a}	42.92^{b}	3.99	4.04	2.98^{a}	3.08^{b}
9(SB)	81	—	192	—	36.89	36.69	39.48^{a}	40.71^{b}	3.31^{a}	3.55^{b}	2.90^{a}	2.95^{b}

^{a,b}Means within subheading differ by treatment ($P < 0.05$).

^{c,d}Means with in subheading differ by treatment ($P > 0.10$).

[1]S×S = side-by-side trial design; SB = 3-period switchback trial design.

[2]The same cows were employed in both treatments with the switchback trial design.

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