Performance of dairy cows fed normal- or reduced-starch diets supplemented with an exogenous enzyme preparation

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ABSTRACT

The objective of this study was to investigate the effects of supplementation of an exogenous enzyme preparation (EEP) on performance, total-tract digestibility of nutrients, plasma AA profile, and milk fatty acids composition in lactating dairy cows fed a reduced-starch diet compared with a normal-starch diet (i.e., positive control). Forty-eight Holstein cows (28 primiparous and 20 multiparous) were enrolled in a 10-wk randomized complete block design experiment with 16 cows per treatment. Treatments were as follows: (1) normal-starch diet (control) containing (% dry matter basis) 24.8% starch and 33.0% neutral detergent fiber (NDF), (2) reduced-starch diet (RSD) containing 18.4% starch and 39.1% NDF, or (3) RSD supplemented with 10 g/cow per day of an EEP (ENZ). The EEP contained amylolytic and fibrolytic activities and was top-dressed on the total mixed ration at the time of feeding. Compared with normal-starch diet, dry matter intake and milk and energy-corrected milk (ECM) yields were lower (on average by 7.1, 9.5, and 7.2%, respectively) for cows on the RSD treatments. Concentrations, but not yields, of milk fat and total solids were increased by RSD. Energy-corrected milk feed efficiency did not differ among treatments. Total-tract digestibility of NDF tended to increase by RSD treatments. Plasma AA concentrations were not affected by treatment, except that of 3-methylhistidine was increased by ENZ, compared with RSD. Blood glucose concentration tended to be lower in cows on the RSD treatments, but ENZ increased glucose and tended to increase insulin concentrations at 4 h after feeding when compared with RSD. Cows on the RSD treatments had decreased concentrations of de novo fatty acids and tended to have increased concentrations of preformed

fatty acids in milk. Overall, decreasing dietary starch concentration by 26% decreased dry matter intake, milk, and ECM yields, but ECM feed efficiency was not different among treatments. The negative effects of reducing dietary starch on production were not attenuated by the EEP.

Key words: dietary starch, exogenous enzyme, dairy cow, wheat straw

INTRODUCTION

The energy requirements of lactating dairy cows in the United States were established from experiments conducted in the 1970s (Moe et al., 1972; NRC, 2001). They are calculated based on animal characteristics such as BW, milk production, and stage of lactation (Weiss and Pinos-Rodriguez, 2009). To meet the NE_L requirements of a cow, several dietary interventions may be chosen by nutritionists, including provision of starch and digestible fiber in the diet (Oba and Allen, 1999). However, increasing the amount of fiber in the ration of dairy cows is limited by the inverse relationship between energy density and fiber content. For instance, higher proportion of dietary fiber can reduce feed intake (Allen, 1996), and the composition and physical characteristics of the fiber can influence the performance and ruminal parameters of cows (Dado and Allen, 1995). Decreased fiber inclusion in the diet, on the other hand, can result in digestive disturbances, ranging from altered ruminal fermentation pattern to severe acidosis (Mertens, 1997). The NRC (2001) reported that the ratio of forage to concentrate feeds in lactating dairy cow diets can affect DMI, and the mechanisms behind this effect are related to the amount and digestibility of forage fiber. In general, gut fill limits DMI at higher NDF concentrations, whereas energy demands regulate intake at lower NDF concentrations (Mertens, 1994).

As corn prices trend higher and are projected to continue to rise (USDA-ERS, 2021), investigating reduced-starch diets for dairy cattle is warranted (Gencoglu

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et al., 2010; Dann et al., 2015). Results from previous studies that partially replaced corn grain with forage or nonforage fiber sources reported similar FCM yields and minor changes in feed efficiency when dietary starch concentration was reduced by 15 to 20% (Weiss et al., 2011; Dann et al., 2015). In the Dann et al. (2015) study, however, a 18% decrease in dietary starch concentration tended to decrease DMI and decreased milk production of the cows. In addition, increasing fiber content in the diet can negatively affect ammonia uptake by the rumen microbes and, consequently, the efficiency of milk protein synthesis in lactating cows (Hristov et al., 2005).

Research has suggested that supplementing dairy cow diets with exogenous enzymes preparations (**EEP**) could be a nutritional strategy to increase fiber digestibility while maximizing feed efficiency when low-quality forage or decreased-starch diets are fed (Arriola et al., 2011). Suggested modes of action of EEP include provision of readily fermentable substrates for ruminal microbes (McAllister et al., 2001), thus enhancing fiber, starch, and protein degradation in the rumen (Meale et al., 2014). In a meta-analysis evaluating EEP supplementation, Arriola et al. (2017) reported statistical trends for improved total-tract fiber digestibility and lactational performance of dairy cows, even though a large variability and inconsistency of responses are present in the literature. Evaluation of EEP becomes more difficult because of the wide range of products with different composition, activity, and doses applied (Meale et al., 2014). Furthermore, the enzymatic effects may depend on interactions of EEP with the type of forage fed (Yang et al., 2019). It is not clear if EEP supplementation of a reduced-starch diet would be enough to attenuate the deficiency of rumen-available energy by enhancing fiber degradability, and thus resulting in increased performance of dairy cows.

Therefore, the objective of this study was to investigate the effects of an *Aspergillus* spp.-based EEP on lactational performance, total-tract digestibility of nutrients, plasma AA profile and metabolites, and milk fatty acids (**FA**) composition in mid-lactation dairy cows fed a reduced-starch diet compared with a normal-starch diet (i.e., positive control). We hypothesized that supplementation of EEP would benefit ruminal fermentation by enhancing structural carbohydrate degradation, thereby increasing rumen energy availability and improving cow productivity.

MATERIALS AND METHODS

All procedures involving animals carried out in the study were approved by The Pennsylvania State University's Animal Care and Use Committee.

Animals, Experimental Design, and Treatments

The experiment was conducted in the freestall barn of The Pennsylvania State University's Dairy Teaching and Research Center. Forty-eight Holstein cows (28 primiparous and 20 multiparous) averaging ($\pm$ SD) 151 ± 45 DIM and 593 ± 55 kg of BW at the beginning of the study were used in a randomized complete block design experiment. The experiment consisted of a 2-wk covariate period followed by an 8-wk experimental period, with the first 2 wk for adaptation to treatments and the last 6 wk for data and sample collection. Cows were blocked based on parity, DIM, and milk yield (**MY**) during the covariate period, and cows within a block were randomly assigned to 1 of 3 treatments as follows: (1) normal-starch, positive control diet (**NSD**), (2) reduced-starch, negative control diet (**RSD**), and (3) RSD supplemented with 10 g/cow per day of an exogenous enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* (Cellulogest, Purina Animal Nutrition; **ENZ**). According to the manufacturer specifications, the ENZ product contained the following activities: 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). Dose of ENZ followed the manufacturer's recommendations, and it was weighed individually to be top-dressed on the TMR daily during feeding. The EEP product was in a solid form and was mixed with approximately 500 g of the TMR after top-dressing. Based on visual observations by the research team, cows consumed the mixture of product and TMR immediately after they were fed. Based on the production data collected during the covariate period, the experimental diets were formulated to meet or exceed the NE_L and MP requirements (NRC, 2001) of a lactating Holstein cow with 611 kg of BW, 32.7 kg/d of MY, 4.00% milk fat, and 3.10% milk true protein at 23 kg/d of DMI. In the RSD, ground corn grain was replaced by straw (Table 1). The NSD was offered to all cows during the covariate period. Straw was processed with a Standard Roto Grind hammer mill, model 760 (Roto Grind Tub and Grain Grinders, Burrows Enterprises, LLC), equipped with 6 sets of hammers with 18 standard hammers and 6 surfaced lead hammers. Theoretical particle size of the processed straw was set to 2.5 cm. Cows were fed once daily at around 0800 h, and feed was offered ad libitum targeting 5 to 10% refusals. Cows were milked twice daily at 0600 and 1800 h and always had free access to drinking water sources. Individual DMI was monitored with a Calan Broadbent Feeding System (American Calan Inc.).

Table 1. Ingredients and chemical composition of the diets fed during the experiment

Item	Diet ¹	
	NSD	RSD and ENZ
Ingredient, % of DM or as indicated		
Corn silage ²	44.4	44.4
Alfalfa haylage ³	13.1	13.1
Wheat straw ⁴	2.52	11.1
Whole roasted soybeans ⁵	7.56	7.56
Whole cottonseed ⁶	4.09	4.09
Corn grain, ground ⁷	8.59	—
Canola meal ⁸	9.9	9.9
SoyPLUS ⁹	3.51	3.51
Molasses ¹⁰	4.55	4.55
Mineral and vitamin premix ¹¹	1.83	1.83
Composition, % of DM		
CP ¹²	16.3	15.9
RDP ¹³	9.5	9.3
RUP ¹³	7.2	7.0
NDF ¹²	33.1	39.1
ADF ¹²	20.4	25.1
Starch ¹²	24.8	18.4
Ether extract ¹³	4.9	4.7
NE _L , ¹³ Mcal/kg of DM	1.60	1.57
NE _L balance, ¹³ Mcal/d	2.80	1.40
MP balance, ¹³ g/d	−85	−96
NFC ¹³	34.6	29.1
Ash ¹²	5.05	5.25
Ca ¹²	1.01	1.03
P ¹²	0.42	0.41

¹NSD = control; RSD = reduced-starch; ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product.

²Corn silage was 53.0% DM and contained (DM basis) 8.3% CP, 36.6% NDF, 22.6% ADF, and 39.5% starch.

³Alfalfa haylage was 41.8% DM and contained (DM basis) 18.0% CP, 54.7% NDF, 29.8% ADF, and 0.7% starch.

⁴Wheat straw contained (DM basis) 3.6% CP, 80.1% NDF, 58.1% ADF, and 0.8% starch and was chopped with a Standard Roto Grind hammer mill, model 760 (Roto Grind Tub and Grain Grinders), equipped with 6 sets of hammers with 18 standard hammers and 6 surfaced lead hammers.

⁵Whole roasted soybeans contained 37.0% CP (DM basis).

⁶Whole cottonseed contained 23.7% CP (DM basis).

⁷Corn grain contained 8.3% CP (DM basis). Corn grain was ground using a hammer mill (Case IH model 1250) through a 500-μm screen.

⁸Canola meal contained 41.0% CP (DM basis).

⁹SoyPLUS (Landus Cooperative) contained 39.0% CP (DM basis).

¹⁰Molasses was from Westway Feed Products.

¹¹The premix (Cargill Animal Nutrition, Cargill Inc.) contained (% as-is basis) the following: trace mineral mix, 0.86; MgO (56% Mg), 8.0; NaCl, 6.4; vitamin ADE premix (Cargill Animal Nutrition, Cargill Inc.), 0.48; limestone, 37.2; selenium premix (Cargill Animal Nutrition, Cargill Inc.), 0.07; and dry corn distillers grains with solubles, 46.7; Ca, 14.1%; P, 0.39%; Mg, 4.59%; K, 0.44%; S, 0.39%; Se, 6.91 mg/kg; Cu, 362 mg/kg; Zn, 1,085 mg/kg; Fe, 186 mg/kg; vitamin A, 276,717 IU/kg; vitamin D, 75,000 IU/kg; and vitamin E, 1,983 IU/kg.

¹²Values calculated using the nutrient analysis of the individual feed ingredients (Cumberland Valley Analytical Services Inc.) and their inclusion in the diets.

¹³Estimated based on NRC (2001) using averaged DMI, milk yield, milk composition, and BW of the cows during the experiment.

Sample Collection

Feed and TMR Sampling. Weights of feed offered and refusals were recorded daily. Fresh TMR, refusals, and feed ingredient samples were collected twice weekly and stored frozen at −20°C until oven-dried at 55°C for 72 h for further analyses. Dried samples were ground using a Wiley mill (Thomas Scientific) through a 1-mm screen and composited by experimental week (TMR and forage samples) or for the entire experiment (concentrate feeds). Individual feed ingredients were analyzed at Cumberland Valley Analytical Services (Waynesboro, PA) by wet chemistry methods for CP (method 990.03; AOAC International, 2000), amylase-treated NDF (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), ether extract (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2000), minerals (method 985.01; AOAC International, 2000), and estimated NFC (NRC, 2001). Composite TMR samples were analyzed for starch according to Hall (2009) and indigestible NDF (**iNDF**) as described in Huhtanen et al. (1994) and as modified by Lee et al. (2012). Briefly, composited TMR samples (collected twice weekly) were dried at 55°C, ground through a 1-mm screen, weighted (0.5 g) into a 25-μm pore size filter bag (Ankom Technology Corp.), and incubated for 12 d in the rumen of a rumen-cannulated cow. Six filter bags were incubated per treatment (i.e., NSD, RSD, and ENZ). Nutrient composition of the diets was reconstituted from analyzed values of individual feed ingredients and their inclusion rate in the TMR (Table 1).

Production Data Collection and Milk Sampling. Milk yield (Afimilk system) and BW (AfiFarm 3.04E scale system; S.A.E. Afikim, Rehovot) of the cows were recorded throughout the experiment. Body condition score was evaluated (NRC, 2001) during the second week of the covariate period and experimental wk 8 by a single trained person. Milk samples were collected during the second week of the covariate period and every other week during the experiment (excluding the adaptation period) from 2 consecutive p.m. and a.m. milkings (i.e., a total of 4 samples per week) into 20-mL tubes containing bromo-2-nitropropane-1,3-diol. Samples were analyzed for milk fat, true protein, MUN, lactose, and total solids concentrations, and SCC by Dairy One laboratory using Milkoscan models 6000, FT+, or 7 and Fossomatic models 5000 or FC (Foss Electric A/S). Separate unpreserved samples were also collected as described above and stored frozen at −20°C. These samples were composited per cow and period (i.e., covariate and experimental, wk 3–8) and analyzed for FA profile as described in Rico and Harvatine (2013).

Fecal Sampling. During experimental wk 8, fecal samples were collected from each cow for estimation of total-tract digestibility of dietary nutrients. To obtain a representative sample of a 24-h feeding cycle, spot fecal samples (approximately 500 g per cow and per sampling) were collected 4 times over 2 d, at 0, 4, 8, and 12 h after feeding (i.e., 0800 and 1200 h on d 1, and 1600 and 2000 h on d 2). Fecal samples were obtained by rectal stimulation and dried at 55°C to constant weight. The dried samples were ground using a Wiley mill through a 1-mm sieve and composited by cow. Composited fecal samples were analyzed for CP ($N \times 6.25$) using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies Inc.), ash, iNDF, ADF, and NDF with Ankom 200 fiber analyzer (Ankom technology Corp.) and starch (Hall, 2009) to determine total-tract OM, CP, NDF, ADF, and starch digestibility. Indigestible NDF was used as a total-tract digestibility marker (Schneider and Flatt, 1975). Composited fecal samples collected on wk 8 were processed and analyzed for iNDF as described for the TMR samples above.

Blood Sampling. Blood samples were collected from the coccygeal vein or artery during experimental wk 8 at 0, 4, 8, and 12 h after feeding. Blood was collected into vacutainer tubes containing EDTA (Becton, Dickson and Company), placed on ice, and then the plasma was separated by centrifugation at $1,500 \times g$ at 4°C for 15 min within 30 min of collection. Plasma samples were stored at -20°C and composited by cow (on an equal volume basis). Composite samples of the 4 time points were analyzed for AA profile at the University of Missouri-Columbia's Agricultural Experiment Station Chemical Laboratory following the procedures of Deyl et al. (1986) and Fekkes (1996). Composite plasma samples were also analyzed for BHB (Autokit 3-HB Microliter Procedure; Wako Diagnostics), total FA (HR Series; Wako Diagnostics), BUN, and glucose (Idexx Laboratories Inc.). Insulin was analyzed using an ELISA kit (Bovine Insulin ELISA, Kit No. 10-1201-01, Mercodia AB). Plasma collected at 4 h after feeding was also analyzed for glucose. The inter- and intra-assay coefficients of variation for the insulin test were both <10%.

Statistical Analysis

One cow on the NSD treatment left the study during the first experimental week due to a toxemia caused by mycotoxins, and its data were not used for statistical analysis. All remaining data were analyzed using SAS (release 9.4, SAS Institute Inc.). Data were tested for normality using the UNIVARIATE procedure and processed for outliers based on the absolute studentized

residue value >3 using the REG procedure. Data that were not normally distributed (i.e., SCC) were log-transformed for the statistical analysis. Statistical analysis of all data was performed using PROC MIXED. Cow was the experimental unit. Production data (DMI, MY, BW, and feed efficiency) were averaged per experimental week, and the averaged weekly data were used for the statistical analysis. Data collected during the 2-wk adaptation period were not included in the statistical analysis; wk 3 data (i.e., Figure 1) represented the first experimental week. Milk composition data were weighted averages based on the MY of the morning and evening milkings. Milk composition, DMI, and MY during each experimental week were used to calculate component yields, ECM, and ECM feed efficiency. The model for the production data contained treatment, week, the interaction treatment $\times$ week as fixed effects, and a covariate measurement; most treatment $\times$ week interactions were nonsignificant ($P > 0.05$) and were removed from the final model. Where treatment $\times$ week interaction was significant, means separation was done using the PDIF procedure of SAS. Data were analyzed as repeated measures with week as the repeated term using the AR(1) covariance structure. Nutrient intake and total-tract digestibility, plasma AA and metabolites, milk FA, BCS, and BW change data were analyzed with treatment as the only fixed effect. Block and block $\times$ treatment effects were included in all models as random effects. Orthogonal contrasts were used to evaluate the effect of replacing ground corn grain by wheat straw (i.e., NSD vs. RSD and ENZ) and the effect of ENZ (i.e., RSD vs. ENZ). Statistical differences were considered at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$. Data are presented as LSM or covariate-adjusted LSM (production data).

RESULTS AND DISCUSSION

Production Data

Feeding cows with RSD and ENZ decreased ($P \leq 0.03$) MY and ECM compared with NSD (Table 2). There was a treatment $\times$ week interaction ($P < 0.001$) for DMI, where cows fed the NSD had greater ($P = 0.03$) DMI throughout the experiment, except during wk 3 and 5 (Figure 1). Examination of the raw DMI data did not indicate any reason for this discrepancy, and the interaction was considered an aberration. There was also a treatment $\times$ week interaction for feed efficiency ($P < 0.001$), which was caused by the differential effect of treatment on DMI during wk 5. For similar reasons, there was a treatment $\times$ week interaction ($P = 0.006$) for ECM feed efficiency. Milk fat,

milk solids, and MUN concentrations were increased ($P \leq 0.04$) by the reduced-starch diets, compared with NSD. The reduced-starch diets decreased ($P < 0.001$) milk lactose concentration and milk true protein, total solids, and lactose yields. There was a treatment $\times$ week interaction for lactose and MUN concentrations ($P \leq 0.04$). Examination of the data showed that lactose concentration was consistently greater ($P \leq 0.06$) for NSD than the reduced-starch diets, except during wk 3 ($P \geq 0.30$), which caused the interaction. In the case of MUN, the interaction was caused by a lack of effect of treatment ($P \geq 0.53$) during wk 6, whereas MUN concentration was greater ($P \leq 0.001$) for the reduced-starch diets compared with NSD during all other experimental weeks. Cows fed the reduced-starch diets had lower ($P = 0.02$) average BW and gained less weight ($P = 0.01$) than cows fed NSD; however, diet did not affect BCS or BCS change during the experiment.

In the present study, ground corn grain was replaced by wheat straw in the reduced-starch diets to allow us to test the hypothesis that ENZ would increase diet (straw) NDF digestibility and enhance nutrient availability for microbial fermentation in the rumen, thus partially or totally compensating for the lower starch content of RSD compared with NSD. Although some studies have demonstrated that performance and digestibility of nutrients in dairy cows can be sustained

when corn grain is replaced by brown midrib corn silage or nonforage fiber sources, the use of feed ingredients with more digestible NDF and other digestible nutrients was out of the scope of this study because the main idea was to challenge the capacity of the EEP to enhance availability of energy in RSD by replacing all the corn grain with a highly lignified NDF source (i.e., straw). Moreover, the use of other NDF sources would bring other digestible nutrients to the diet and be a confounding factor in the study. The beneficial effects of starch on overall rumen fermentation and microbial protein synthesis are well documented (Beckman and Weiss, 2005; Hristov et al., 2005; Salvati et al., 2021). Recent studies have demonstrated the possibility of maintaining cow performance even when lowering starch content in diets by replacing corn grain by nonforage fiber sources (e.g., beet pulp, wheat middlings; Dann et al., 2014, 2015) by increasing the amount of forage in the diet with the use of brown midrib silage hybrids (Ferraretto et al., 2015; Ferraretto and Shaver, 2015; Lim et al., 2015) or by supplementation of the diet with EEP (Arriola et al., 2017; Tirado-González et al., 2018). In the current study, however, milk production was decreased by RSD, primarily due to decreased DMI. Feed DMI is the dominant nutritional factor determining milk production in dairy cows (Hristov et al., 2004), and it was not surprising that the low DMI for RSD decreased MY, compared with NSD. The 18% higher NDF concentration of the RSD likely resulted in slower passage rate, which decreased DMI through rumen fill mechanisms (Dado and Allen, 1995; Allen, 2000; Miller et al., 2020).

Cows fed the reduced-starch diets had a 10% increase in milk fat content, but milk fat yield did not differ from that of NSD. Many factors such as breed, stage of lactation, parity, diet formulation, and environment can influence milk composition (Jenkins and McGuire 2006). Increased milk fat content in response to feeding higher NDF concentration diets has been extensively reported in the literature (Varga et al., 1984; Beauchemin, 1991). Dann et al. (2015) reported a tendency for increased milk fat content when the concentrate feeds in a normal-starch diet were replaced by alfalfa, corn silage, and nonforage fiber sources. The increased milk fat content of cows fed the reduced-starch diets in the current study was likely a result of their greater concentration of physically effective fiber compared with NSD. Although particle size analysis of the diet was not performed in the current study, it is safe to assume that replacing (8–9%) ground corn with chopped wheat straw would likely slightly increase overall TMR particle size for the reduced-starch diets. Mertens (1997) reported a positive relationship between physically effective fiber, milk fat content, and ruminal pH in

Figure 1. Dry matter intake of the cows during the experiment. Treatments were as follows: NSD = normal-starch diet (control); RSD = reduced-starch diet; and ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product. Weeks 3 to 8 are experimental weeks during which data were collected for the statistical analysis (following a 2-wk covariate period and 2 wk for adaptation to treatments). Data are arithmetic means and associated SE; $n = 18$ (number of independent observations used to derive each mean value). There was no effect ($P \geq 0.51$) of treatment on DMI during wk 3 and 5; DMI was greater ($P \leq 0.03$) for NSD vs. the reduced-starch diets during all other experimental weeks.

Table 2. Dry matter intake, milk yield and composition, BW, and BCS of dairy cows fed normal- or reduced-starch diets without or with an exogenous enzyme product

Item	Treatment ¹			SEM ²	P-value ³	
	NSD	RSD	ENZ		NSD vs. TRT	RSD vs. ENZ
DMI, kg/d	24.0	22.5	22.1	0.63	0.03	0.60
Milk yield, kg/d	35.2	32.5	31.2	0.73	<0.001	0.17
Feed efficiency, ⁴ kg/kg	1.50	1.44	1.42	0.587	0.24	0.80
Milk fat, %	3.75	4.15	4.08	0.087	0.001	0.58
Milk fat, kg/d	1.34	1.35	1.29	0.042	0.69	0.21
Milk true protein, %	3.11	3.10	3.11	0.036	0.82	0.78
Milk true protein, kg/d	1.14	0.99	0.99	0.027	<0.001	0.95
Milk lactose, %	4.86	4.77	4.77	0.019	<0.001	0.76
Milk lactose, kg/d	1.77	1.56	1.53	0.042	<0.001	0.56
Milk solids, %	12.7	12.9	12.9	0.095	0.04	0.73
Milk solids, kg/d	4.60	4.21	4.10	0.101	<0.001	0.40
MUN, mg/dL	11.4	13.6	13.2	0.31	<0.001	0.32
ECM, ⁵ kg/d	34.6	32.7	31.5	0.79	0.005	0.21
ECM feed efficiency, ⁶ kg/kg	1.46	1.46	1.46	0.045	0.96	0.88
SCC, ⁷ $\times 10^3$ cells/mL	1.68	1.80	1.80	0.046	0.05	0.98
BW, kg	617	605	610	3.6	0.02	0.26
BW change, ⁸ g/d	0.84	0.47	0.63	0.113	0.01	0.21
BCS	3.06	2.95	2.99	0.054	0.21	0.61
BSC change ⁹	0.06	0.02	0.05	0.061	0.67	0.71

¹Treatments were as follows: NSD = control; RSD = reduced-starch; ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product.

²Largest SEM published in table; n = 279 for all variables except DMI and milk yield, for which n = 280 (n represents number of observations used in the statistical analysis).

³Orthogonal contrasts: NSD vs. TRT = NSD vs. RSD and ENZ; RSD vs. ENZ = RSD vs. ENZ. There was experimental week $\times$ treatment interaction for DMI ($P < 0.001$), feed efficiency ($P < 0.001$), lactose and MUN concentrations ($P \leq 0.04$), and ECM feed efficiency ($P = 0.006$); experimental week $\times$ treatment interactions for all other variables, $P \geq 0.10$.

⁴Milk yield/DMI.

⁵Energy-corrected milk (kg/d) = kg of milk $\times$ [(38.3 $\times$ % fat $\times$ 10 + 24.2 $\times$ % true protein $\times$ 10 + 16.54 $\times$ % lactose $\times$ 10 + 20.7)/3,140] as per Sjaunja et al. (1990).

⁶ECM yield/DMI.

⁷Statistical analysis was performed on log-transformed data.

⁸BW change, g/d = [average BW (kg) during experimental wk 8 – average BW (kg) during second week of covariate period]/days on study.

⁹BCS change = (BCS during experimental wk 8 – BCS during second week of covariate period).

dairy cows. Rumen acetate and butyrate are the major precursors supporting milk fat synthesis (Jenkins and McGuire 2006), and increasing dietary NDF:starch ratio tends to increase the molar proportion of acetate, butyrate, and the acetate:propionate ratio (Beckman and Weiss, 2005).

The reduced milk lactose content and yield in cows fed the reduced-starch diets may have resulted from decreased rumen propionate concentration, which has often been reported for low-starch, high-fiber diets (Beauchemin, 1991; Dann et al., 2015). Propionate is the main gluconeogenesis precursor in ruminants, and the mammary gland is one of the tissues that have an obligatory requirement for glucose for lactose synthesis (Aschenbach et al., 2010). Therefore, milk lactose content and yield may have been compromised in the reduced-starch diets in our study by decreased ruminal propionate availability, also supported by the lower blood glucose concentration 4 h after feeding in RSD versus NSD (see later discussion).

Lower starch intake is the likely explanation for the higher MUN concentration associated with the reduced-starch diets (Gencoglu et al., 2010; NRC, 2001) compared with NSD. Greater intake of NFC, such as sugars and starch, increases the energy availability in the rumen and decreases ruminal production and flux of ammonia to the blood stream (Broderick, 2003). Consequently, dietary nitrogen use efficiency for milk protein synthesis is usually enhanced and the MUN concentration is decreased, as demonstrated by Hristov et al. (2005). Thus, it is possible to infer that NSD provided a greater energy availability for the rumen microbes to utilize ammonia for microbial protein synthesis, resulting in lower MUN (Nousiainen et al., 2004) compared with the reduced-starch diets. Oh et al. (2019) reported that MUN did not differ among cows fed with a basal normal-starch diet supplemented or not with the EEP used in the current study. In addition, the use of EEP in the Oh et al. (2019) study did not affect ruminal ammonia concentration and urine

N excretion, which agrees with data from the current study. Lower energy availability for microbial protein synthesis in the rumen can also be related to the lower milk protein yield in cows fed the reduced-starch diets. Microbial protein is the main source of MP for dairy cows and, consequently, the main source of AA supplied to the mammary gland for milk protein synthesis.

The performance variables evaluated in the current study were not affected by ENZ. Previous studies have also reported no effects of amylolytic and fibrolytic EEP on milk production or composition (Peters et al., 2015; Oh et al., 2019), and overall milk production responses to EEP have been inconsistent (Beauchemin and Holtshausen, 2011; McCarthy et al., 2013; Takiya et al., 2017).

Total-Tract Digestibility

Intake of NDF and ADF did not differ between NSD and the reduced-starch diets (Table 3) due to decreased DMI with the latter diets (during the digestibility data collection period). Also, there were no differences in NDF and ADF intake between RSD and ENZ. Intake of CP was higher ($P = 0.007$) for NSD compared with RSD, as a result of higher DMI with the former diet and higher CP concentration of corn grain versus wheat straw. As expected, starch intake was lower ($P < 0.001$) for the reduced-starch diets compared with NSD. Total-tract digestibility of DM and OM were decreased ($P \leq 0.003$) for the reduced-starch diets. Digestibility of

NDF tended to increase ($P = 0.09$) for the reduced-starch diets compared with NSD and was not affected by ENZ. There were no differences in ADF and starch total-tract digestibility among treatments.

The decreased total-tract apparent DM and OM digestibility in cows fed the reduced-starch diets can be attributed to replacing NFC (from corn grain) by a fibrous carbohydrate source (from straw), resulting in an overall decrease in nutrient digestibility. The tendency for increased NDF digestibility in reduced-starch diets can be associated with increased retention time and lower passage rate of the digesta in cows fed a higher NDF diet (Dado and Allen, 1995). The differences between total-tract digestibility of NDF in NSD and reduced-starch diets can be partially explained by the difference in starch content of the diets. A negative relationship between NDF digestibility and dietary starch has been described in meta-analyses by Nousiainen et al. (2009) and Ferraretto et al. (2013). In these studies, ruminal and total-tract digestibility of NDF were decreased from 0.48 to 1.03 percentage units, respectively, per each unit of dietary starch increase. Although we did not find any effects of ENZ in nutrient digestibility, Gencoglu et al. (2010) reported increased digestibility of fiber fractions by an amylase EEP supplemented to a reduced-starch diet. In another study with lactating cows, Weiss et al. (2011) also reported enhanced NDF digestibility of a low-starch diet supplemented with an amylase EEP. It should be noted, however, that unlike the above studies, the EEP used in the current

Table 3. Nutrient intake and apparent total-tract digestibility of dairy cows fed normal- or reduced-starch diets without or with an exogenous enzyme product

Item	Treatment ¹			SEM ²	P-value ³	
	NSD	RSD	ENZ		NSD vs. TRT	RSD vs. ENZ
Intake, ⁴ kg/d						
DM	25.1	21.1	22.0	1.21	0.02	0.59
OM	23.5	19.7	20.5	1.13	0.02	0.59
NDF	8.32	8.24	8.59	0.444	0.85	0.55
ADF	5.13	5.29	5.52	0.283	0.41	0.53
CP	4.09	3.36	3.50	0.195	0.007	0.59
Starch	6.22	3.94	4.05	0.254	<0.001	0.73
Apparent total-tract digestibility, %						
DM	61.5	59.7	58.6	0.65	<0.001	0.11
OM	64.2	62.4	62.0	0.60	0.003	0.60
NDF	34.7	38.2	37.0	1.38	0.09	0.54
ADF	35.3	37.7	35.3	1.31	0.39	0.15
CP	64.1	65.3	64.3	1.33	0.60	0.55
Starch	96.7	96.6	96.4	0.20	0.49	0.33

¹Treatments were as follows: NSD = control; RSD = reduced-starch; ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product.

²Largest SEM published in table; n = 47 (n represents number of observations used in the statistical analysis).

³Orthogonal contrasts: NSD vs. TRT = NSD vs. RSD and ENZ; RSD vs. ENZ = RSD vs. ENZ.

⁴Intake during the digestibility data collection week (experimental wk 8).

experiment contained both amylolytic and fibrolytic activities.

The modes of action of EEP differ among preparations and can be classified according to their site of activity, such as pre-consumptive, ruminal, or postruminal effects (McAllister et al., 2001). Past research suggests that *Aspergillus oryzae*-based EEP can increase ruminal bacteria counts (Newbold et al., 1992a,b) and can work synergistically with extracts from ruminal microorganisms, thus enhancing the nutrient release from plant cell walls in the rumen (Newbold, 1995). Furthermore, EEP use has been associated with an increase in total enzymatic activity in the rumen, which

could increase the overall ruminal hydrolytic capacity, leading to enhanced digestibility of all dietary nutrients (Beauchemin et al., 2004).

Although we did not measure rumen fermentation variables in this study, the interpretation of the data allowed us to infer that replacing ground corn by wheat straw decreased energy availability to both the rumen microbes and the host animal, which led to decreased MY of cows fed the reduced-starch diets. Supporting current data, Oh et al. (2019) did not observe any differences in the total-tract digestibility of nutrients in cows fed ENZ supplemented to a normal-starch diet. As argued earlier, the variation in animal performance

Table 4. Blood plasma AA concentration (μM) in dairy cows fed normal- or reduced-starch diets without or with an exogenous enzyme product

Item	Treatment ¹			SEM ²	P-value ³	
	NSD	RSD	ENZ		NSD vs. TRT	RSD vs. ENZ
Arg	99.2	107	99.8	4.58	0.47	0.27
His	63.8	62.8	63.2	2.87	0.82	0.92
Ile	183	180	178	9.9	0.67	0.86
Leu	195	195	191	10.2	0.86	0.76
Lys	104	104	103	5.4	0.97	0.96
Met	23.5	23.6	22.8	1.06	0.78	0.60
Phe	55.0	53.4	52.3	1.93	0.32	0.65
Thr	127	116	120	6.70	0.29	0.69
Trp	63.7	64.4	62.3	2.70	0.92	0.60
Val	366	366	352	19.2	0.77	0.59
ΣEAA^4	1,279	1,272	1,244	56.1	0.74	0.71
Ala	324	326	331	17.3	0.83	0.86
Asn	52.1	49.7	48.6	2.72	0.39	0.76
Asp	7.64	8.13	7.55	0.370	0.67	0.28
Citrulline	88.4	86.6	87.0	4.18	0.75	0.94
Cys	1.77	1.69	1.60	0.087	0.24	0.47
Gln	265	276	266	11.6	0.70	0.56
Glu	67.9	68.9	72.4	2.01	0.20	0.15
Gly	332	295	320	14.4	0.19	0.23
Orn	59.5	63.8	60.8	3.30	0.49	0.53
Pro	114	106	104	5.38	0.16	0.84
Ser	98.2	90.4	88.1	4.14	0.10	0.70
Tau	69.2	66.6	72.0	4.75	0.98	0.35
Tyr	56.9	53.7	51.8	3.12	0.28	0.67
ΣNEAA^5	1,537	1,490	1,509	56.7	0.59	0.80
Carnosine ⁶	11.1	11.1	12.2	1.31	0.74	0.49
1-MH ⁷	22.6	24.1	23.5	1.58	0.48	0.75
3-MH ⁷	4.60	4.13	5.93	0.535	0.52	0.02
α -Aminoadipic acid	15.5	15.7	18.0	1.29	0.33	0.13
α -Aminobutyric acid	17.1	18.3	17.6	1.14	0.52	0.65
β -Alanine	1.84	1.76	1.85	0.143	0.80	0.63
Ethanolamine	2.42	2.13	2.04	0.149	0.06	0.64
GABA ⁸	3.53	4.12	4.73	0.443	0.09	0.30
Phosphoserine	32.7	31.8	32.7	3.60	0.90	0.82

¹Treatments were as follows: NSD = control; RSD = reduced-starch; ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product.

²Largest SEM published in table; n = 47 (n represents number of observations used in the statistical analysis).

³Orthogonal contrasts: NSD vs. TRT = NSD vs. RSD and ENZ; RSD vs. ENZ = RSD vs. ENZ.

⁴Sum of EAA (Arg, His, Ile, Leu, Lys, Phe, Thr, Trp, and Val).

⁵Sum of NEAA (Ala, Asn, Asp, Cys, Cit, Gln, Glu, Gly, Orn, Pro, Ser, Tau, and Tyr were considered as NEAA).

⁶Dipeptide (β -alanyl-L-His).

⁷Methylhistidine.

⁸ γ -Aminobutyric acid.

responses to EEP may be related to their properties, dose applied, and diet fed (Beauchemin et al., 2003; Meale et al., 2014). With the current heightened interest in direct-fed microbials, the dairy industry would benefit from future studies designed to evaluate how EEP products interact with dietary nutrients, feed ingredient composition, and different energy sources.

Plasma AA Concentrations

Concentrations of most plasma AA were not different across treatments (Table 4). Concentration of 3-methylhistidine (**3-MH**) was greater ($P = 0.02$) for ENZ compared with RSD. Concentration of ethanolamine tended to be greater ($P = 0.06$) for NSD versus the reduced-starch diets, and that of γ -aminobutyric acid (**GABA**) tended to be greater ($P = 0.09$) for the reduced-starch diets compared with NSD.

Greater plasma 3-MH concentration for ENZ suggested increased muscle catabolism (compared with RSD). It is well documented that methylation of histidine in actin and myosin results in the formation of 3-MH, and its occurrence in plasma (or urine) is an indication of protein mobilization in dairy cows (Harris and Milne, 1981; Houweling et al., 2012). The tendency for decreased ethanolamine in cows fed the reduced-starch diets also supported the hypothesis that replacing ground corn with wheat straw decreased availability of nutrients for the animals and resulted in an energy-deficient diet. Milk ethanolamine has a positive correlation ($r = 0.52$) with the animal's energy balance (Xu et al., 2020), and its reduction, accompanied by 3-MH increase, suggests that cows fed the reduced-starch diets tried to compensate the lower energy supply by mobilizing muscle tissue. Our study also corroborates with that of Saleem et al. (2012), who

reported increased ethanolamine concentrations in the rumen fluid of cows fed a high-grain diet.

γ -Aminobutyric acid is a neurotransmitter that may regulate consumption through the inhibition of the vagus nerve. Therefore, higher GABA concentrations are expected to stimulate appetite (Forbes, 2007), and plasma concentration of GABA is reduced when the absorption of ruminal propionate is increased, triggering what is described as hepatic oxidation theory (Allen, 2000; Maldini and Allen, 2018). Our results demonstrated that GABA tended to increase in cows fed reduced-starch diets, which agreed with the lower DMI and possibly reduced output of propionate from the rumen.

Blood Metabolites and Insulin

Plasma total FA, BHB, BUN, and overall insulin concentrations did not differ among treatments (Table 5). The overall plasma glucose concentration tended to be lower ($P = 0.06$) in cows fed reduced-starch diets, compared with NSD, and glucose concentration at 4 h after feeding was decreased ($P < 0.001$) in cows fed the former diets. Cows fed ENZ had greater ($P = 0.01$) glucose and tended to have higher ($P = 0.07$) insulin concentration at 4 h after feeding when compared with cows fed RSD.

Circulating concentrations of total FA and BHB can be used as energy balance markers, with total FA being related to fat mobilization and BHB related to liver oxidation and ketone bodies formation (Duffield et al., 2009; Ospina et al., 2010). Holtshausen et al. (2011) and Dean et al. (2013) reported that cows receiving EEP had lower plasma BHB concentration compared with cows fed a control diet. To our knowledge, the present study was the first to report the effect of an EEP

Table 5. Blood plasma metabolites and insulin concentration in dairy cows fed a normal- or reduced-starch diets without or with an exogenous enzyme product

Item	Treatment ¹				P-value ³	
	NSD	RSD	ENZ	SEM ²	NSD vs. TRT	RSD vs. ENZ
Total fatty acids, μM	99.9	94.1	92.8	13.95	0.69	0.94
BHB, μM	1,027	1,086	932	90.6	0.86	0.18
BUN, mg/dL	7.43	8.89	9.66	1.165	0.22	0.65
Glucose, mg/dL	82.2	72.2	77.8	3.02	0.06	0.19
Insulin, $\mu g/L$	1.22	1.23	0.96	0.134	0.46	0.15
Glucose4h, $\mu g/dL$	97.4	55.0	74.3	5.34	<0.001	0.01
Insulin4h, $\mu g/L$	0.54	0.49	0.65	0.064	0.71	0.07

¹Treatments were as follows: NSD = control; RSD = reduced-starch; ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product.

²Largest SEM published in table; n = 47 (n represents number of observations used in the statistical analysis).

³Orthogonal contrasts: NSD vs. TRT = NSD vs. RSD and ENZ; RSD vs. ENZ = RSD vs. ENZ.

⁴Plasma glucose and insulin concentrations 4 h after feeding.

containing both amylolytic and fibrolytic activities on energy metabolism markers in dairy cows. Although the tendency for reduction in plasma ethanolamine (e.g., energy balance marker) and the increase in 3-MH (e.g., muscle mobilization marker) concentrations suggest greater tissue mobilization, plasma concentrations of total FA and BHB, and BCS changes did not support the hypothesis that cows fed the decreased-starch diets mobilized more tissue in response to an energy-deficient diet in our study. It is noted, however, that cows fed the reduced-starch diets were clearly not gaining as much BW as cows fed NSD, which reflected the lower dietary energy supply by RSD versus NSD.

Blood glucose is extensively produced through hepatic gluconeogenesis, and its concentration is also an indicator of the energy metabolism status of the lactating dairy cow (Drackley and Cardoso, 2014). The mechanisms that led to decreased glucose concentration in cows fed the reduced-starch diets were discussed in the above section. Increased insulin concentration postfeeding is a response to increased intake of nutrients and is positively correlated with blood glucose concentration (Livingstone and Borai, 2014). Thus, the 4-h postfeeding glucose and insulin data for ENZ suggested improved energy availability compared with RSD, although the improvement was not large enough

Table 6. Fatty acid composition of milk fat (g/100 g of total fatty acids) in dairy cows fed normal- or reduced-starch diets without or with an exogenous enzyme product

Item	Treatment ¹			SEM ²	P-value ³	
	NSD	RSD	ENZ		NSD vs. TRT	RSD vs. ENZ
4:0	4.64	4.90	4.90	0.115	0.09	0.96
6:0	2.41	2.37	2.32	0.068	0.46	0.62
8:0	1.33	1.20	1.16	0.046	0.01	0.52
10:0	2.94	2.47	2.37	0.105	<0.001	0.50
12:0	3.31	2.69	2.60	0.106	<0.001	0.58
14:0	10.5	9.74	9.56	0.170	<0.001	0.50
<i>cis</i> -9 14:1	0.88	0.82	0.81	0.040	0.23	0.93
15:0	0.81	0.74	0.73	0.024	0.02	0.86
16:0	25.5	25.7	25.8	0.52	0.71	0.93
<i>cis</i> -9 16:1	1.13	1.22	1.19	0.056	0.25	0.69
17:0	0.45	0.46	0.47	0.010	0.32	0.52
18:0	11.9	12.6	12.9	0.32	0.04	0.47
<i>trans</i> -4 18:1	0.038	0.043	0.042	0.001	0.02	0.47
<i>trans</i> -5 18:1	0.03	0.03	0.03	0.001	0.72	0.57
<i>trans</i> -6,8 18:1	0.47	0.41	0.40	0.025	0.05	0.72
<i>trans</i> -9 18:1	0.36	0.32	0.31	0.015	0.04	0.64
<i>trans</i> -10 18:1	1.38	0.64	0.58	0.316	0.05	0.90
<i>trans</i> -11 18:1	1.31	1.30	1.26	0.070	0.72	0.67
<i>trans</i> -12 18:1	0.48	0.45	0.37	0.058	0.30	0.31
<i>cis</i> -9 18:1	20.3	22.7	23.0	0.46	<0.001	0.72
<i>cis</i> -11 18:1	0.82	0.73	0.72	0.037	0.05	0.78
<i>cis</i> -12 18:1	0.50	0.44	0.39	0.019	0.001	0.13
<i>cis</i> -9, <i>cis</i> -12 18:2	3.51	3.22	3.26	0.086	0.01	0.74
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 18:3	0.65	0.64	0.65	0.017	0.91	0.88
20:0	0.03	0.03	0.03	0.002	0.51	0.25
<i>cis</i> -9, <i>trans</i> -11 CLA	0.64	0.67	0.64	0.034	0.65	0.45
Others	0.40	0.17	0.20	0.045	<0.001	0.59
Total <i>trans</i> fatty acids	4.42	3.54	3.31	0.380	0.04	0.66
ΣSFA	65.2	64.4	64.4	0.75	0.37	0.99
ΣMUFA	28.4	29.7	29.7	0.67	0.13	0.93
ΣPUFA	5.85	5.54	5.58	0.138	0.09	0.82
ΣDe novo ⁴	26.3	24.4	24.0	0.51	0.003	0.53
ΣMixed ⁴	26.6	26.9	27.0	0.54	0.64	0.97
ΣPreformed ⁴	43.9	45.6	46.0	0.80	0.07	0.77
ΣOBCFA ⁵	2.85	2.85	2.89	0.049	0.82	0.61

¹Treatments were as follows: NSD = control; RSD = reduced-starch; ENZ = RSD supplemented with *Aspergillus* spp. exogenous enzyme product.

²Largest SEM published in table; n = 47 (n represents number of observations used in the statistical analysis).

³Orthogonal contrasts: NSD vs. TRT = NSD vs. RSD and ENZ; RSD vs. ENZ = RSD vs. ENZ.

⁴De novo FA (C4:0 to C15:0) are synthesized by the mammary gland; preformed FA (C17:0 to C20:0) originate primarily from extraction from plasma; and mixed fatty acids (sum of *iso* C16:0, C16:0, and C16:1 *cis*-9) originate from both sources.

⁵Odd- and branched-chain FA (sum of C11:0, *iso* C13:0, *anteiso* C13:0, C13:0, *iso* C14:0, *iso* C15:0, *anteiso* C15:0, C15:0, *iso* C16:0, *iso* C17:0, *anteiso* C17:0, C17:0, and C17:1 *cis*-9).

to result in an increased productive performance of the cows.

Milk FA Composition

Milk FA composition was only affected by dietary starch level (Table 6). Milk concentration of butyrate (4:0) tended to increase ($P = 0.09$), but concentrations of most de novo synthesized FA (8:0, 10:0, 12:0, and 14:0) and 15:0 were decreased ($P \leq 0.02$) by the reduced-starch diets compared with NSD. Concentrations of 18:0, *trans*-4 18:1, and *cis*-9 18:1 were higher ($P \leq 0.04$), and concentrations of preformed FA tended to be higher ($P \leq 0.07$) in cows fed RSD when compared with cows fed NSD. Conversely, concentrations of *trans*-6,8 18:1, *trans*-9 18:1, *trans*-10 18:1, *cis*-11 18:1, *cis*-12 18:1, *cis*-9, *cis*-12 18:2, and the sums of total *trans* and de novo FA were all decreased ($P \leq 0.05$) by the reduced-starch diets.

Milk fat depression is a syndrome generally characterized by a reduction in fat yield in response to altered rumen biohydrogenation when high-concentrate, low-forage diets or excessive dietary PUFA are fed to lactating cows (Bauman and Griinari, 2003). Studies report that cows experiencing biohydrogenation-induced milk fat depression have decreased concentrations of de novo FA and increased concentrations of *trans*-10 18:1 in milk (Shingfield et al., 2006; Gama et al., 2008; Rico and Harvatine, 2013). This, however, was not the case in the current study; cows fed the reduced-starch diets had lower concentration of *trans*-10 18:1, decreased concentration of de novo FA, and still had increased milk fat content, compared with NSD. It is possible that the lower fermentability of the reduced-starch diets affected the synthesis of de novo FA by reducing the availability of substrates in the mammary gland. Preformed FA in milk originate from the digestive tract or from adipose tissue mobilization (McGuire and Bauman, 2002; Harvatine et al., 2009), and the tendency for increased concentration of these FA in cows fed the reduced-starch diets was likely a consequence of increased body reserves mobilization (as a response to the lower energy balance, compared with NSD), which could help explain the difference in milk fat content between NSD and the reduced-starch diets.

CONCLUSIONS

A 26% reduction in dietary starch concentration (by substituting ground corn grain with wheat straw) decreased DMI and MY in mid-lactation dairy cows. Although both reduced-starch diets induced higher milk fat concentration, ECM yield was decreased by 7%, compared with the control diet. Our hypothesis that

an EEP containing amylolytic and fibrolytic activities would enhance structural carbohydrate degradation, and thus maintain the productivity of the cows, on a reduced-starch diet was not validated. The use of the enzyme additive increased postprandial blood glucose concentration, but this was not sufficient to compensate for the decreased dietary energy intake and to maintain production of the cows.

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