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Research Article

Effects of Exogenous Amylase in Transition Dairy Cows Fed Low-Starch Diets: 2. Total Tract Digestibility and Blood Urea Nitrogen

Hıdır GENÇOĞLU [1,a] Çağdaş KARA [1,b] Mukaddes Merve EFİL [1,c] Hakan BİRİCİK [1,d]
İbrahim İsmet TÜRKMEN [1,e] Gülay DENİZ [1,f] Arda KOVANLIKAYA [1,g] Randy Duncan SHAVER [2,h]
Recep Tolga KIVANÇ [1,i] Ramazan YILDIRIM [1,j]

[1] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Bursa Uludag University, TR-16059 Bursa - TURKEY
[2] Department of Dairy Science, University of Wisconsin - Madison, 1675 Observatory Dr. Madison 53706, USA
[a] ORCID: 0000-0003-1067-2874; [b] ORCID: 0000-0003-2515-1211; [c] ORCID: 0000-0003-0646-9777; [d] ORCID: 0000-0001-7051-1349
[e] ORCID: 0000-0002-8111-7619; [f] ORCID: 0000-0003-3817-4359; [g] ORCID: 0000-0002-9246-7016; [h] ORCID: 0000-0002-7490-6177
[i] ORCID: 0000-0003-1659-7481; [j] ORCID: 0000-0002-2554-7032

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Abstract

The objective of this trial was to determine the effect of exogenous amylase during the transition period on total tract digestibility, rumen pH and blood urea nitrogen in lactating dairy cows. The effect of exogenous dietary amylase supplementation on lactation diets with low starch concentration (19.5% of dry matter) and dry period diets with moderate starch concentration was evaluated (15.5% of dry matter). A total of 30 multiparus Holstein cows were randomly assigned to two groups with amylase (n=15) or control (n=15). Three cows from each group were randomly selected and ruminally cannulated for digestibility trials. The research was conducted starting at 21 d prepartum until 84 d postpartum. Digestibility of dry matter, organic matter, neutral detergent fiber, starch, and crude protein remained unaffected by treatment in postpartum. Average pre- and postpartum rumen pH concentrations were 6.25 and 6.15, respectively, and did not differ between treatments. Blood urea nitrogen (BUN) concentrations were lower in cows fed amylase supplemented diet compared to those fed diet without amylase in both pre- and postpartum periods (P<0.001). In conclusion, the dietary supplementation of amylase did may not affect the digestibility of nutrients, however, it may decrease the BUN concentration in pre- and postpartum period for cows fed amylase. Therefore, it may offer potential for improving nitrogen efficiency in dairy cows.

Keywords: *Amylase, Starch, Total tract digestibility, Blood urea nitrogen, Dairy cows*

Düşük Nişastalı Rasyonlarla Beslenen Geçiş Dönemindeki İneklerde Amilaz Enziminin Etkisi: 2. Toplam Sindirilebilirlik ve Kan Üre Azotu

Öz

Bu araştırmanın amacı geçiş dönemindeki ineklerin rasyonlarına amilaz enzimi ilavesinin toplam sindirilebilirlik, rumen pH'sı ve kan üre azotu üzerine etkisini incelemektir. Rasyonların nişasta düzeyi kuru madde esasına göre kuru dönemdeki hayvanlar için %15.5, laktasyon dönemindekiler için ise %19.5 olarak tespit edildi. Araştırmada birden fazla doğum yapmış 30 baş siyah alaca ırkı inekler rastgele amilaz (15) ve kontrol (15) gruplarına dağıtıldı. Her bir grupta üçer inek rumen kanülü mevcuttu. Deneme doğumdan önceki 21 gün ile doğumdan sonraki 84. günler arasında yürütüldü. Doğum sonrası kuru madde, organik madde, nötral deterjan fiber, nişasta ve ham protein sindirilebilirlik düzeyleri bakımından gruplar arasında bir farklılık bulunmamıştır. Ortalama doğum öncesi ve sonrası rumen pH değerleri sırasıyla 6.25 ve 6.15 olarak tespit edilmiş ve gruplar arasında fark bulunamamıştır. Hem doğum öncesi hem de doğum sonrası amilaz ile beslenen ineklerde kan üre azotu değerleri kontrol grubuna göre daha düşük bulunmuştur (P<0.001). Sonuç olarak, süt ineği rasyonlarına amilaz enzim ilavesi besin maddesi sindirilebilirliklerini etkilemez iken diğer yandan hem doğum öncesi hem de sonrası kan üre azotu konsantrasyonlarını azaltmıştır. Böylece, amilaz ile beslenen gruplarda kan üre azotunun azalması; azotun kullanım etkinliğini iyileştirmesi için bir fırsat yaratabilir.

Anahtar sözcükler: *Amilaz, Nişasta, Toplam sindirilebilirlik, Kan üre azotu, Süt ineği*

İletişim (Correspondence)
+90 224 2941363 Mobile: +90 532 2969034
gencoglu@uludag.edu.tr

INTRODUCTION

Dietary starch content is important to increase rumen microbial production [1]. Starch is fermented and increases propionate production in the rumen [2], and unfermented starch that escapes ruminal fermentation provides glucose that is absorbed or metabolized to lactate in the small intestine [3]. Starch level [4,5] and starch content [6,7] in ration was assessed in terms of performance in dairy cows.

There are studies in which some feed additives [8-10] and treatments [11] was used for transition dairy cows. In addition, exogenous amylase was evaluated in some trials to improve performance [12-14] and digestibility [15,16]. The supplementation of exogenous amylase in diets for dairy cows is designed to increase the utilization of starch in feeds. In some non ruminant animals, the salivary glands secrete amylase to begin breaking down starch when food enters the mouth. Ruminants do not have salivary amylase [17]; therefore the microbial population in the rumen is largely responsible for the degradation of starch.

The inclusion of exogenous amylase to the diet of the lactating cows can increase ruminal starch digestibility [18] and stability in ruminal fluid [19]. Increased ruminal starch availability may increase ruminal microbial yield and feed efficiency by intake regulation induced by increased liver oxidation of propionate [20]. However, some starch sources are rapidly fermented; excessive ruminal fermentability can decrease ruminal pH and alter ruminal biohydrogenation pathways, reducing milk fat concentration and yield.

Based on starch ruminal degradation rate, grains can be ranked from fastest to slowest degradations and, thus, it is possible to infer the respective potential for acidification in the following order: oats, wheat, barley, high-moisture corn, steam-flaked corn, dry-rolled corn, whole corn grain and whole sorghum grain [21-23].

Dietary alterations that increase ruminal digestibility have been demonstrated to affect the morphology of the rumen papillae [24] and may be valuable for providing more energy from the transition dairy cow's diet through increased volatile fatty acids (VFA) production and absorption. The VFA influence papillae growth in the rumen [24]. Increasing propionate concentration in the rumen favors elongation of papillae. Greater DMI increases passage rate through the gastrointestinal tract reducing the time for starch hydrolysis, thereby it limits starch digestibility [25] in rumen and intestine.

This paper is companion papers (1 of 2) [26] from an experiment designed to examine the effects of exogenous amylase in transition dairy cows fed low-starch diets: Lactation performance, total tract digestibility, rumen and blood parameters.

Low starch diets may be an economic alternative when grain prices are high. However, the effect of amylase addition to diets with a too low starch concentration (19.5%) has not been evaluated for total tract digestibility and blood urea nitrogen. Therefore, the objective of the trial was to determine the effect of exogenous amylase during the transition period on total tract digestibility, rumen pH, and blood urea nitrogen in dairy cows fed low starch diet. The energy supply supported by starch may affect negatively milk urea nitrogen by completing the deficiencies of protein metabolism [27] and milk urea nitrogen (MUN) is also positively correlated with blood urea nitrogen (BUN) [28,29].

MATERIAL and METHODS

The experiment was conducted from January 2011 through August 2011 at Omer Matli Research Center (Karacabey, Bursa Turkey). All the procedures were approved by the Bursa Uludag University, Animal Experiments Local Ethics Committee (Committee Number and Date: 2010-07/02 and 02.11.2010). Thirty (30) multiparous Holstein cows were randomly assigned to with or without (control) exogenous amylase groups in a completely randomized design. Three of the fifteen cows in each group were ruminally cannulated with soft plastic cannulae of 10 cm internal diameter (Ankom, pliable rumen cannula #29.4 inches, NY, USA) to determine rumen parameters. Current lactation numbers for control and amylase cows were 2±0.3 and 2±0.4, respectively. Previous lactation 305-d milk yields for control and amylase cows were 8289±1322 kg and 8332±1779 kg, respectively. Disease incidences for control and amylase cows, respectively, were retained placenta (1 vs. 0), milk fever (2 vs. 1), ketosis (4 vs. 5), dystocia (0 vs. 2), and mastitis (2 vs. 3).

The research was conducted starting at 21 d prepartum until 84 d postpartum. Cows were housed in a free-stall barn and fed diets as a total mixed ration (TMR) with an automatic feeding door system. At 35 d prior to the expected calving date, cows were assigned to their respective diets and housed for adaptation to the automatic feeding door system 2 week before initiation of the experimental period. Cows were housed in individual maternity pens from parturition until 4 days in milk and then cows were moved to free-stall housing equipped with a automatic feeding door system. Cows were fed individually the TMR once daily (0800 h) to allow for *ad libitum* consumption and animals were allowed access to feed at all times, except during milking times. Ingredient composition of the experimental diets, nutrient composition and particle size of diets and all feedstuffs are shown in first companion paper [26].

The control diet did not contain exogenous amylase. The amylase diet was fed with exogenous amylase addition to the concentrate mixtures. A granular amylase formulation, Ronozyme RumiStar (Lot number: 600 (CT) AU360001) with an amylase activity of 600 Kilo Novo Units (KNU) per g provided by DSM Nutritional Products (Basel, Switzerland) was used for this study. The targeted dosage of 300

KNU/kg of the TMR dry matter (DM) in amylase diet was achieved by adding 1 g of Ronozyme RumiStar per kg of concentrate mixture (as-fed basis). The control and amylase concentrate mixtures were prepared as pelleted feed (pelleting temperature 65°C) by Matli Feed Co. (Karacabey, Turkey). The pelleted concentrates of control and amylase were sampled every 4 week, stored at -20°C, and then sent to DSM Nutritional Products Analytical Services Center (Basel, Switzerland) for analysis of amylase activity [30]. Determined amylase activities for control and amylase pelleted concentrates mixtures were 0±0, and 606.9±53.4 KNU/kg (as-fed basis), respectively. The treatment TMR for lactating cows averaged 303.4±27 KNU/kg of DM, which was similar to the targeted dosage of 300 KNU/kg of DM recommended by DSM Nutritional Products and the dosage used in the trials of Klingerman et al.[19], Ferraretto et al.[31], and Gencoglu et al.[32].

Ruminal fluid was collected from rumen-cannulated cows immediately before feeding (0 h) and at 2, 4, and 8 h after feeding. Target day and actual day of prepartum ruminal fluid sampling before calving were 21 and 22.3 (SD=2.6), 7 and 8.0 (SD=2.8) for control, 21 and 21.3 (SD=3.3), 7 and 7.1 (SD=1.6) for amylase. Postpartum ruminal fluid samples were taken at 1, 3, 5, 7, 10, and 12 weeks after calving. Samples were collected from multiple sites in the ventral rumen via the cannula using a metal filter probe. Samples were immediately squeezed through 2 layers of cheesecloth and pH was measured using a pH meter (Inolab pH, serial no: 00200018, pHElectrode SenTix 41, D-82362, Weilheim, Germany). The filtered duplicate rumen fluid samples of 1.5 mL were were acidified in 30 µL of 50% TCA solution and immediately frozen in microfuge tubes until prepared and analyzed for ammonia-N (NH_3-N) as described by Bal et al.[33].

Fecal grab samples were collected from each cow twice daily to cover 0400, 0800, 1200, 1600, 2000, and 2400 h time points over the 3-d sampling period in week 8 of the lactation period. The TMR and ort samples were collected from each cow daily during the 3-d sampling period. Fecal and ort samples were dried and ground as described previously and composited by cow within period; composite samples were analyzed for DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and starch. Total-tract nutrient digestibilities were determined using 120-h indigestible NDF as an internal marker. Composite fecal, ort, and TMR samples were incubated ruminally in Dacron bags in triplicate for 120-h at the end of experimental period. In situ Dacron bags were made from nitrogen-free polyester, with a pore size of 50 microns (Ankom, R1020-10×20 cm, forage bags, 14502, NY, USA). Composite fecal, ort, and TMR samples were dried at 60°C for 48 h and ground through 2 mm screen, the samples were weighed (5 g sample) into the bags, and incubated in the rumen of each cow for 120 h. After incubation, bags were withdrawn from the rumen, and plunged into cold water for 10 min to stop fermentation. Bags were then washed for approximately 120 min in cold water and dried in a forced-air oven at 60°C for 48 h, and then analyzed for NDF content. The NDF content of the bag residues was determined in triplicate using α-amylase and sodium sulfite [34]. Total-tract nutrient digestibilities were calculated from 120 h indigestible NDF and nutrient concentrations in the orts-adjusted diet and feces [35].

Target day and actual day of prepartum blood sampling before calving were 21 and 21.7 (SD=3.8), 7 and 6.9 (SD=2.5) for control, and 21 and 21.3 (SD=2.8), 7 and 6.5 (SD=2.0) for amylase. Postpartum blood samples were taken 1, 3, 5, 7, 10, and 12 weeks after calving. Before feeding, blood samples were taken from coccygeal vessel into evacuated tubes. Serum harvested for analyzed BUN, non-esterified fatty acids (NEFA), glucose, Ca, P, albumin, alkaline phosphatase (ALP), bilirubin, creatine, Na, K, total protein and globulin (Comprehensive profile kit, VetScan classic Abaxis, CA, USA). Approximately 4 h after feeding, blood was sampled from a coccygeal vessel into 1 evacuated tube and plasma was harvested and analyzed for beta-hydroxybutyric acid (BHBA) using KetoSite diagnostic kit (Stanbio Laboratory Boerne, Texas, 78006, USA) with STAT-Site Meter (GDS Diagnostic, 25235 Leer Drive Elkhart, 46514, IN).

Data were analyzed as a completely randomized design using the Linear Mixed Model of SPSS (SPSS 13.0, 2004). The model included treatment, time (week for prepartum and early lactation measurements and day for blood measurements), and treatment × time interaction as Fixed effects and cow within treatment as a Random effect. The REML (Restricted Maximum Likelihood) was the chosen estimation method. Means were determined using the least squares means statement. Statistical significance and trends were considered at $P \leq 0.05$ and $P > 0.05$ to $P < 0.10$, respectively.

RESULTS

Treatment effects on least squares means for apparent total-tract nutrient digestibilities are in *Table 1*. Dietary addition of exogenous amylase did not affect digestibility of DM, OM, CP, NDF, or starch ($P > 0.10$).

Treatment effects on least squares means for blood plasma parameters are presented in *Table 2*. The concentrations of serum NEFA, BHBA, albumin, bilirubin, Ca, P, creatinine, glucose, Na, K, protein and globulin unaffected by treatment in both prepartum and early-lactation cows. Dietary addition of exogenous amylase did not affect BHBA concentration, but was numerically 1 mg/dL greater for amylase than control during the postpartum period. BUN concentration ranged from 18.5 to 21.6 mg/dL across the treatments lactation period, and BUN concentration were reduced ($P < 0.001$) for cows fed amylase compared to control in both prepartum and lactation periods (Fig. 1).

Table 1. *Effect of treatment on least squares means for apparent total-tract nutrient digestibilities[1,2]*

	Item	Control	Amylase	SEM[3]	P
Digestibility, %	DM[4]	76.2	75.8	1.6	NS[5]
	OM[6]	76.9	77.1	1.6	NS
	CP[7]	82.9	82.0	1.3	NS
	NDF[8]	59.0	58.9	3.5	NS
	Starch	96.8	97.4	0.4	NS

[1] Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase); [2] Determined using 120 h indigestible NDF as an internal marker; [3] Standard error of the mean; [4] Dry matter; [5] Non significant; [6] Organic matter; [7] Crude protein; [8] Neutral detergent fiber

Table 2. *Effect of treatment on least squares means for plasma concentrations of BHBA, albumin, ALP, bilirubin, BUN, Ca, P, creatinine, glucose, Na, K, protein and globulin prepartum and early lactation cows[1]*

Parameter		Control	Amylase	SEM[2]	P
NEFA[3] mEq/L	Prepartum	367.17	409.04	53.98	NS[4]
	Lactation	605.39	608.97	31.16	NS
BHBA[5] mg/dL	Prepartum	9.70	8.32	1.67	NS[4]
	Lactation	14.13	15.18	0.97	NS
Albumin g/dL	Prepartum	2.11	2.18	0.06	NS
	Lactation	2.44	2.40	0.04	NS
ALP[6] U/L	Prepartum	69.58	55.62	7.43	0.01
	Lactation	62.76	46.90	4.29	0.01
Bilirubin mg/dL	Prepartum	0.32	0.37	0.03	NS
	Lactation	0.37	0.35	0.02	NS
BUN[7] mg/dL	Prepartum	16.54	13.79	0.75	0.001
	Lactation	21.65	18.46	0.53	0.001
Ca mg/dL	Prepartum	9.46	9.49	0.14	NS
	Lactation	9.23	9.39	0.11	NS
P mg/dL	Prepartum	6.72	5.91	0.28	0.06
	Lactation	6.19	6.10	0.19	NS
Creatinin mg/dL	Prepartum	1.10	1.04	0.05	NS
	Lactation	0.93	0.85	0.05	NS
Glucose mg/dL	Prepartum	73.02	73.42	2.03	NS
	Lactation	63.99	64.92	1.62	NS
Na mmol/L	Prepartum	137.09	137.04	0.77	NS
	Lactation	133.03	133.25	0.47	NS
K mmol/L	Prepartum	4.87	4.90	0.07	NS
	Lactation	4.88	4.97	0.05	NS
Protein g/dL	Prepartum	7.03	7.04	0.15	NS
	Lactation	7.82	8.18	0.13	0.06
Globulin g/dL	Prepartum	4.92	4.87	0.18	NS
	Lactation	5.38	5.79	0.16	0.08

[1] Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase); [2] Standard error of the mean; [3] Non-esterified fatty acids; [4] Non significant; [5] Beta-hydroxybutyric acid; [6] Alkaline phosphatase; [7] Blood urea nitrogen

Least squares mean for rumen NH_3N and pH are presented in *Table 3*. The addition of exogenous amylases to the diet did not affect these parameters (P>0.10). Cows fed amylase compared with cows fed control in lactation tend to have lower average daily rumen pH (6.07 vs 6.22), but not significant as statistically.

Table 3. *Effect of treatment on least squares means for rumen NH_3N and pH in prepartum and postpartum cows[1]*

Parameter		Control	Amylase	SEM[2]	P
NH_3N[3] mg/dL	Prepartum	16.66	12.91	2.87	NS[4]
	Lactation	16.88	20.68	1.68	NS
pH	Prepartum	6.27	6.23	0.10	NS
	Lactation	6.22	6.07	0.60	NS

[1] Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase); [2] Standard error of the mean; [3] Ammonia-N; [4] Non significant

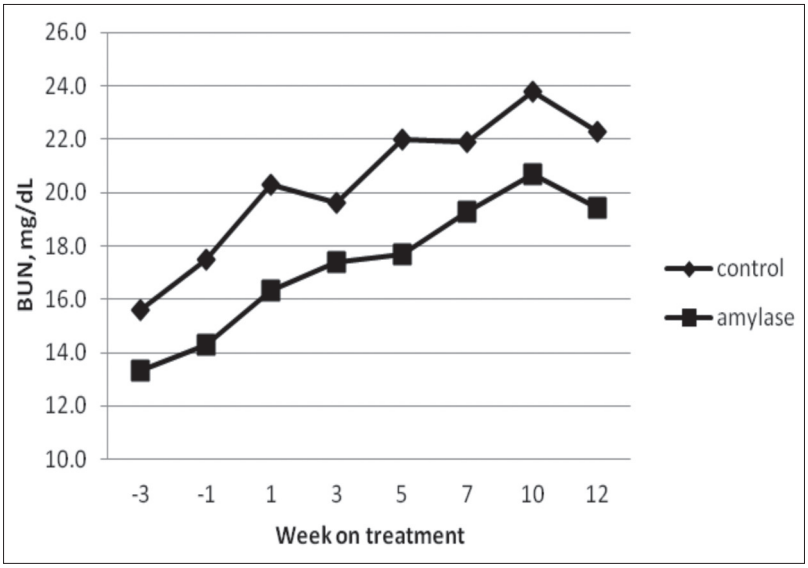

Fig 1. Effect of treatment on BUN (mg/dL) least squares means by week on treatment. Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase). Week and week x treatment interaction ($P<0.001$)

DISCUSSION

The results of the current study revealed that addition of exogenous amylase did not affect ruminal digestibility of nutrients. Gencoglu et al.[32] reported an increase in DM and OM digestibility for cows fed reduced starch with amylase compared to reduced starch without amylase. In contrast, Weiss et al.[36] reported no effect of exogenous amylase on total-tract digestibility of DM, OM, energy or starch. McCarthy et al.[37] reported no differences among treatments for apparent total-tract starch digestibility. Several researchers have reported increased NDF digestibility with addition of exogenous amylase [19,32,36]. Noziere et al.[18] reported that exogenous amylase supplementation increased the true ruminal digestibility of OM by an average of 4%, but the lower ruminal digestibility of OM and starch in the control diet without exogenous amylase addition was compensated for postruminally, and as result no differences were observed for total-tract digestibility measurements.

Similarly to Gencoglu et al.[32], we have several possible explanations for the lack of effect on starch digestibility when exogenous amylase was added to a reduced-starch diet: 1) starch digestibility was not affected ruminally or postruminally, 2) starch digestibility was increased ruminally [19] but small intestine compensatory starch digestion resulted in similar total-tract starch digestibilities for the treatments, or 3) starch digestibility was increased ruminally, but hindgut fermentation resulted in similar total-tract starch digestibilities for the treatments.

The increase in BHBA for cows fed amylase versus control could be related to the greater ruminal butyrate concentrations. Plasma samples for BHBA were collected 4 h post-feeding to capture peak BHBA concentrations [38]. Addition of exogenous amylases increased serum concentrations of BHBA in the trials of Tricarico et al.[39] and DeFrain et al.[40]. Duffield [41] and Oetzel [42] identified 14.4 mg/dL BHBA as the cut-point for significant subclinical ketosis. Excess amounts of butyric acid from ruminal production are easily converted to BHBA in the wall of the rumen. Huhtanen et al.[43] reported increases in blood BHBA and decreases in blood glucose concentrations. However, amylase supplementation did not change serum glucose concentrations in this study.

The results of the current study revealed that addition of exogenous amylase decreased the BUN concentration when compared with control cows in both prepartum and lactation periods. Greater ruminal starch digestibility could explain the reduced BUN for amylase compared to control [44]. In addition, the reduction in BUN for cows fed amylase versus control was likely related to greater

ruminal propionate concentrations [45]. The MUN is an indicator of protein intake and utilization in dairy cows [46,47], and MUN has a high correlation with BUN [48]. Gencoglu et al.[32] reported reduced MUN for cows fed reduced starch with amylase compared to reduced starch without amylase. Reduced MUN coupled with greater milk protein concentrations for reduced starch with amylase compared to reduced starch without amylase coincides with the suggestion of Voelker and Allen [49] that lower ruminal amylase activity for a reduced starch diet may reduce the rate of starch digestion ruminally and thus microbial protein production. This effect may be enhanced by addition of exogenous amylase to reduced starch diets. The NH_3-N is converted to microbial protein in rumen but a portion of NH_3-N is absorbed through the rumen wall. The NH_3-N that reaches the systemic circulation can be toxic, but in most physiological conditions it is converted in the liver to urea which is less toxic. The NH_3-N which enters the portal circulation may exceed the rate of hepatic metabolism. Murondoti et al.[50] reported that diets containing high concentrations of CP can increase the risk for fatty liver related to high concentrations of blood NH_3-N which are at toxic concentrations. Addition of exogenous amylase to normal-starch diets did not affect MUN in the trials of Klingerman et al.[19], Ferraretto et al.[31], and Weiss et al.[36] and Tricarico et al.[39]. The BUN is a useful indicator of protein status in dairy cows [47]. Ferguson et al.[51] reported that BUN exceeding 20 mg/dL was associated with reduced conception rates in lactating dairy cows. Least squares means by week on treatment for BUN are in *Fig. 1*; week and week × treatment interaction (P<0.001). The BUN values were greater than we expected, on average, based on dietary CP and increased throughout the trial for both treatment groups reaching 20 mg/dL or graeter by 3 week for control and 10 week for amylase. This could possibly be attributed to low starch intake related to the formulation of reduced-starch diets.

The addition of exogenous amylases to the diet did not affect rumen NH_3.N and pH parameters. Cows fed amylase compared with cows fed control in lactation tend to have lower average daily rumen pH (6.07 vs 6.22).

In conclusion, the supplementation of amylase did not affect the digestibility of nutrients. However, the BUN concentrations decreased in pre- and postpartum period for cows fed amylase, thus it may offer potential for improving nitrogen efficiency in dairy cows.

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