

## Effects of Exogenous Amylase in Transition Dairy Cows Fed Low-Starch Diets: 1. Lactation Performance

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### Abstract

The objective of this trial was to determine the effect of exogenous amylase during the transition period in dairy cows on dry matter intake and lactation performance. The effect of exogenous amylase supplementation on lactation diets with low starch concentration (19.5% of dry matter) and dry period diets with moderate starch concentration (15.5% of dry matter) was evaluated. A total of 30 multiparous Holstein cows were randomly assigned to two groups fed diets with (n=15) or without amylase (n=15). Treatments were granular amylase (0.5 g of Ronozyme RumiStar per kg of total mixed ration dry matter) or control. The research was conducted starting at 21 d prepartum until 84 d postpartum. Starch and neutral detergent fiber concentrations averaged 15.5±0.5% and 15.7±0.9%, 42.6±1.1% and 43.4±1.2% in close up diets and 19.8±2.9% and 19.4±0.5%, 33.6±0.8% and 34.2±0.6% in lactation diets for control and amylase, respectively. Dry matter intake, milk yield and composition were evaluated for differences between treatments. Postpartum intakes of dry matter (DMI) and organic matter (OM), neutral detergent fiber (NDF), crude protein (CP), and starch intake were unaffected by treatment. Milk yield was not influenced by treatment, but numerically greater by 2.0 kg/d for cows fed amylase compared with control diet. The percentages of milk fat, protein and lactose were not impressed by treatment, however fat-, solid-, and energy-corrected milk were 2 kg/d greater for cows fed amylase diet than for cows fed control diet. Fat-, solid-, and energy-corrected milk feed conversions (kg/kg DMI) were 5 to 6% greater for cows fed amylase diet than for cows fed control diet (P<0.01). It was concluded that inclusion of amylase improved the feed efficiency of lactating cows fed a low starch diet, may offer for potential to increase milk yield; but the enzyme did not affect DMI.

**Keywords:** Amylase, Dry matter intake, Milk production, Feed efficiency, Dairy cows

## Düşük Nişastalı Rasyonlarla Beslenen Geçiş Dönemindeki İneklerde Amilaz Enziminin Etkisi: 1. Laktasyon Performansı

### Öz

Bu araştırmanın amacı geçiş dönemindeki ineklerin rasyonlarına amilaz enzimi ilavesinin kuru madde tüketimi ve laktasyon performansı 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 (n=15) ve kontrol (n=15) gruplarına dağıtıldı. Araştırma kontrol ve amilaz (0.5 g Ronozyme RumiStar/kg toplam karma rasyon kuru madde) grupları şeklinde oluşturuldu. Deneme doğumdan önceki 21 gün ile doğumdan sonraki 84. günler arasında yürütüldü. Kuruda bulunan kontrol ve amilaz grubundaki rasyonların nişasta ve nötral deterjan fiber (NDF) içerikleri sırasıyla %15.5±0.5 ve %15.7±0.9 ile %42.6±1.1 ve %43.4±1.2 iken laktasyondakilerde ise %19.8±2.9 ve %19.4±0.5 ile %33.6±0.8 and %34.2±0.6 arasında tespit edildi. Doğum sonrası kuru madde tüketimi, organik madde, NDF, ham protein, nişasta düzeyleri bakımından gruplar arasında bir farklılık bulunmamıştır. Süt verimi uygulamadan etkilenmedi ancak sayısal olarak amilaz ile beslenen ineklerde 2 kg/gün daha fazla süt elde edildi. Süt yağı, proteini ve laktöz düzeyleri denemeden etkilenmedi, ancak yağa, katı maddeye ve enerjiye göre düzeltilmiş süt verimleri amilaz ile beslenen ineklerde 2 kg/gün daha fazla süt üretildi. Yağa, katı maddeye ve enerjiye göre düzeltilmiş süt verimlerinin yeme dönüşüm oranları (kg/kg kuru madde tüketimi) amilaz ile beslenen ineklerde kontrol grubuna göre %5-6 daha yüksek bulundu. Sonuç olarak düşük nişasta içeriğine sahip rasyonlarla beslenen geçiş dönemindeki ineklerde amilaz enzimi ilavesinin yemden yararlanmayı iyileştirdiği, süt veriminde potansiyel bir artış sağlayabileceği; ancak kuru madde tüketimini etkilemediği tespiti yapılmıştır.

**Anahtar sözcükler:** Amilaz, Kuru madde tüketimi, Süt verimi, Yemden yararlanma, Süt ineği



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## INTRODUCTION

Carbohydrates are the main source of energy, typically providing over half of the energy in ruminant diets <sup>[1]</sup>. Moreover, intensive production systems for lactating dairy cows are based on starch as the primary source of energy from carbohydrates. Starch contributes approximately 50 and 75% of the energy value of corn silage and grain, respectively <sup>[2]</sup>. High grain prices have enhanced the interest in feeding reduced-starch diets. There has been interest in feeding low-starch diets with improved starch digestibility because of high corn prices <sup>[3]</sup>. Therefore, improving starch utilization can reduce feed costs by reducing starch in diets or increase income by increasing milk production. In addition, there are some feed additives <sup>[4]</sup> and treatments <sup>[5]</sup> applied for providing improvements in performance of transition dairy cows.

The starch level of diets for lactating dairy cows is not well defined in dairy herds in Turkey. However, we estimated that dietary starch concentrations ranging from 19 to 26% in total mixed ration (DM basis) in Turkey. Total-tract digestibility of starch is variable ranging from 70 to 100% in dairy cows <sup>[6]</sup> and it was positively related to ruminal and post-ruminal starch digestibilities <sup>[7]</sup>. Various feed-related factors influence the digestibility of corn starch by dairy cows including: particle size, processing method, harvest/storage method and maturity, and endosperm type for grain, chop length, and kernel processing for corn silage <sup>[7,8]</sup>. However, increased ruminal starch digestion can undesirably induce ruminal acidosis, thereby leading to reduction in ruminal microbial synthesis, milk yield and composition.

Some exogenous amylases are resistant to ruminal degradation, and thus can increase diet digestibility in ruminants <sup>[9,10]</sup>. Several studies have reported that exogenous amylase resistant to ruminal degradation increased organic matter (OM) digestibility in dairy <sup>[3,10]</sup> and feedlot cattle <sup>[11]</sup>. The addition of exogenous amylase to the diets of lactating dairy cows has the potential to improve animal productivity. Exogenous amylase addition to TMR of dairy cows has increased milk yield by up to 3.9 kg/cow/d, and positive *in vitro* and *in vivo* digestibility responses to exogenous amylase were also reported <sup>[10]</sup>. Gencoglu et al. <sup>[3]</sup> found that adding exogenous amylase to low-starch diets (21% DM) increased feed efficiency when compared with a low-starch diet without amylase (1.77 vs 1.98 kg milk/kg DMI).

The effects of amylase supplementation on lactation performance, DMI, and feed efficiency of lactating cows have been inconsistent. The supplementation of amylase to reduced starch diet (21%) increased milk production, had no effect on DMI, and tended to increase feed efficiency <sup>[12]</sup>. Gencoglu et al. <sup>[3]</sup> observed a decreasing in DMI, no effect on milk production, an increase in feed efficiency when cows fed 21% starch diet was supplemented with amylase. Weiss et al. indicated that <sup>[13]</sup> supplemented amylase to

cows fed a 26% starch diet did not detect changes in milk yield, DMI, and feed efficiency.

Low starch diets may be an economic alternative when grain prices are high. The effect of amylase addition to diets with a much lower starch concentration (19.5%) has not been evaluated. Therefore, the objective of the trial was to determine the effect of exogenous amylase during the transition period on dry matter intake (DMI), and lactation performance in dairy cows fed low starch diet. The fact that changing of rumen fermentation with adding of exogenous amylase would improve feed efficiency and lactating performance of transition dairy cows was hypothesized.

## 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. Current lactation numbers for control and amylase cows were  $2 \pm 0.3$  and  $2 \pm 0.4$ , respectively. Previous lactation 305-d milk yields for control and amylase cows were  $8289 \pm 1322$  and  $8332 \pm 1779$  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 an 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. The orts samples were collected to determine dry matter intake (DMI) during the trial period (from d 21 prepartum to d 84 postpartum). Ingredient composition of the experimental diets is in *Table 1*. The all diets were formulated to comply with recommendations from NRC 2001 <sup>[2]</sup>.

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

**Table 1.** Ingredient composition of the diets (As dry matter basis)

Ingredients	-21 to 0 DIM	1 to 84 DIM
Corn silage	27.7	28.3
Alfalfa Hay	7.2	19.1
Wheat Straw	31.7	2.6
Corn	5.3	10.5
Soybean meal-47%	7.4	11.3
Corn gluten meal-60%	0.0	1.8
Sunflower meal-30%	0.5	0.0
Full fat soybean	3.3	4.5
Wheat bran	9.5	11.2
Rice bran	3.5	5.3
By pass fat <sup>1</sup>	1.0	1.5
Molasses	1.7	2.5
Calcium carbonate	0.00	1.05
Salt	0.00	0.17
Vitamin-Mineral Premix <sup>2</sup>	0.36	0.18

<sup>1</sup> Minimum 99.5% total fat content (Ecolex, Malaysia)  
<sup>2</sup> Supplied per kilogram of vitamin-mineral premix : Vit. A 12.000.000 IU, Vit. D<sub>3</sub> 3.000.000 IU, Vit. E (dl- $\alpha$ -Tokoferol Asetat) 35 g, Mn 50 g, Fe 50 g, Zn 50 g, Cu 10 g, I 0.8 g, Co 0.15 g, Se 0.3 g, Antioxidant 10 g

provided by DSM Nutritional Products (Basel, Switzerland) was used for this study. The targeted dosage of 300 KNU/kg of the total mixed ration (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<sup>[14]</sup>. 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 Gencoglu et al.<sup>[3]</sup>, and Klingerman et al.<sup>[10]</sup>, Ferraretto et al.<sup>[12]</sup>.

Cows were milked 3 times daily at 0600, 1400, and 2200 h, and daily milk production was recorded at each milking. Milk samples were obtained from all cows weekly from all milkings on the same 2 consecutive days throughout the 12-wk lactation period of the trial and analyzed for fat, protein, and lactose concentrations by infrared analysis (Omer Matli Research Center, Karacabey, Turkey) using a Bentley 150 (near infrared spectroscopy; Bentley 150 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN) with average daily yields of fat, protein, and lactose calculated from these data for each week. Yields of 3.5%

fat-corrected milk (FCM), solid-corrected milk (SCM), and energy-corrected milk (ECM) were calculated according to NRC 2001<sup>[2]</sup> equations. Actual-milk, FCM, SCM, and ECM feed conversions were calculated by week using average daily yield and DMI data.

The TMR, concentrate mixtures, corn silage, and alfalfa hay were sampled weekly and composited by 3-week periods and analyzed for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), starch, sugar, ether extract, ash, and energy values were calculated from the analytical data using NRC 2001<sup>[2]</sup> equations. The composited samples were dried in a forced-air oven at 60°C for 48 h for measurement of DM content and then ground through a 1 mm diameter screen using a laboratory 3303 Mill (Hundenge, Sweden). Crude protein was determined by the Kjeldahl method<sup>[15]</sup>. Ash was determined by combustion at 550°C for 6 h. The NDF, acid detergent fiber (ADF), and lignin contents were determined using the methods described by Van Soest et al.<sup>[16]</sup> with heat-stable amylase (Sigma No: A-3306, Sigma Chemical Co., St Louis, MO, USA) and sodium sulfite used in the NDF procedure. Starch was measured on composited samples as described by Bal et al.<sup>[17]</sup>.

Data were analyzed as a completely randomized design using the Linear Mixed Model of SPSS (SPSS 13.0, 2004). The model included treatment, time, 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≤0.05 and P>0.05 to P<0.10, respectively.

## RESULTS

The nutrient composition of corn silage, alfalfa hay, wheat straw and concentrates mixtures during the study and the particle size of the corn silage are in Table 2. Corn silage and alfalfa hay were of good quality with 36.6% DM and 30.8% starch (DM basis) in corn silage and 16.4% CP and 50.1% NDF (DM basis) in alfalfa hay. Concentrate mixtures fed to control and amylase cows showed similar nutrient concentrations. The nutrient composition and particle size of diets (close up and lactating) are in Table 3. The nutrient concentrations and particle size of close up and lactating diets were similar for control and amylase.

Treatment effects on least squares means for DM and nutrient intakes of lactation cows are presented in Table 4. Intakes of DM, OM, NDF, and CP were unaffected treatment (P>0.10). Least squares means by week on treatment for DMI are presented in Fig. 1; week (P>0.10) and week × treatment (P>0.10) interaction effects did not differ.

Treatment effects on lactation performance are in Table 5. Milk yield was unaffected by treatment (P>0.10), but

Item	Corn Silage	Alfalfa Hay	Wheat Straw	Concentrate Without Amylase	Concentrate With Amylase
DM, % as fed	36.6±1.4	91.8±0.6	92.0±0.2	90.6±0.4	90.3±0.3
<b>% DM</b>					
OM %	93.9±2.4	89.6±0.3	93.4±0.5	91.7±0.2	91.4±0.2
CP %	8.6±3.6	16.4±1.2	3.7±0.4	25.0±0.2	25.1±0.2
NDF %	46.3±2.0	50.1±0.9	83.7±6.6	21.9±0.4	22.0±0.3
Starch %	30.8±2.2	2.4±0.6	0.6±0.8	21.7±0.3	21.5±0.5
<b>Particle Size<sup>1</sup></b>	<b>% As Fed Retained</b>				
19 mm	3.2±1.0	-----	-----	-----	-----
8 mm	49.2±1.4	-----	-----	-----	-----
1.18 mm	44.7±1.4	-----	-----	-----	-----
Pan	2.9±0.5	-----	-----	-----	-----

<sup>1</sup> Determined as described by Kononoff et al.<sup>[18]</sup>

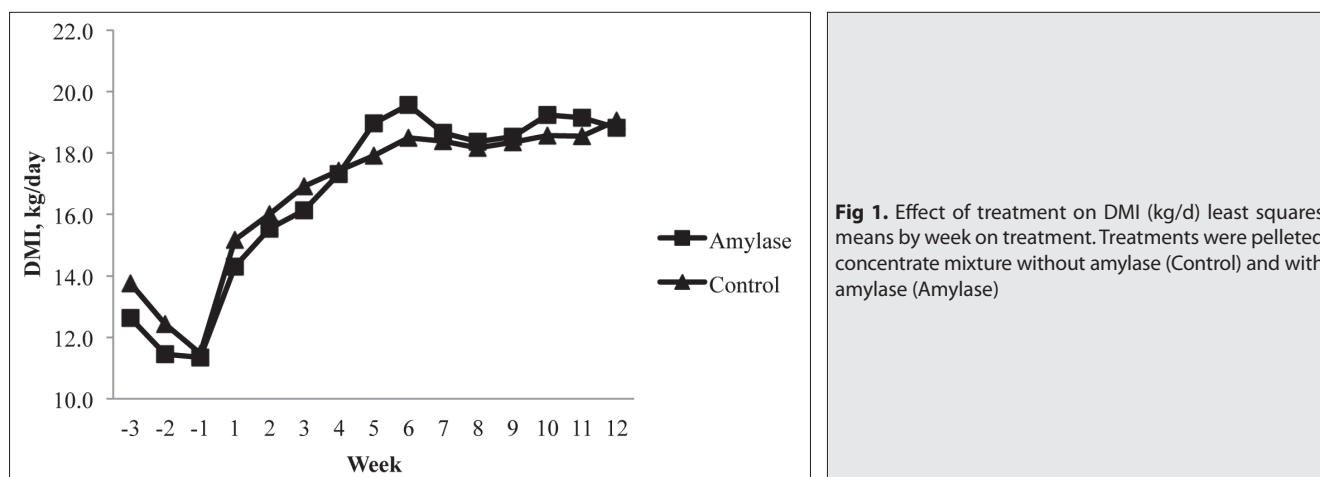
Item	Control	Amylase	Control	Amylase
<b>Nutrients</b>	<b>Close Up</b>		<b>Lactating</b>	
DM, % as fed	71.2±1.1	71.6±0.9	62.7±1.5	62.5±1.7
<b>% DM</b>				
CP %	14±0.4	13.9±0.8	17.2±0.9	17.3±0.9
Ether Extract %	4.5±0.3	4.6±0.4	6.6±0.6	6.5±0.6
NDF %	42.6±1.1	43.4±1.2	33.6±0.8	34.2±0.6
NFC %	30.3±0.8	30.1±0.9	34.5±1.8	34.1±2.2
Starch %	15.5±0.5	15.7±0.9	19.8±2.9	19.4±0.5
TDN <sub>1x</sub> <sup>3</sup>	67.4±0.8	67.7±0.5	-----	-----
NEL <sub>3x</sub> <sup>4</sup> Mcal/kg <sup>3</sup>	-----	-----	1.72±0.4	1.72±0.2
<b>Penn State Separator Sieves<sup>4</sup></b>	<b>% As Fed Retained</b>			
19 mm	41.8±3.2	43.0±4.2	10.9±2.5	9.5±4.7
8 mm	28.1±6.3	27.6±4.1	46.4±5.8	48.0±8.7
1.18 mm	24.1±3.2	24.2±2.5	31.0±5.0	31.6±3.9
Pan	6.0±0.3	5.3±0.3	11.7±0.9	10.9±2.1

<sup>1</sup> Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase). <sup>2</sup> NFC: Nonfiber carbohydrate, %; calculated as: 100 - (NDF, % + CP, % + EE, % + ash, %)

<sup>3</sup> Calculated using NRC (2001) summative energy equation. <sup>4</sup> Determined as described by Kononoff et al.<sup>[18]</sup>

Item	Control	Amylase	SEM <sup>2</sup>	P
DMI, kg/d	17.7	17.9	0.2	NS <sup>3</sup>
OM Intake, kg/d	14.9	14.8	0.2	NS
NDF Intake, kg/d	5.5	5.5	0.1	NS
CP Intake, kg/d	2.8	2.8	0.03	NS
Starch Intake, kg/d	3.2	3.2	0.04	NS

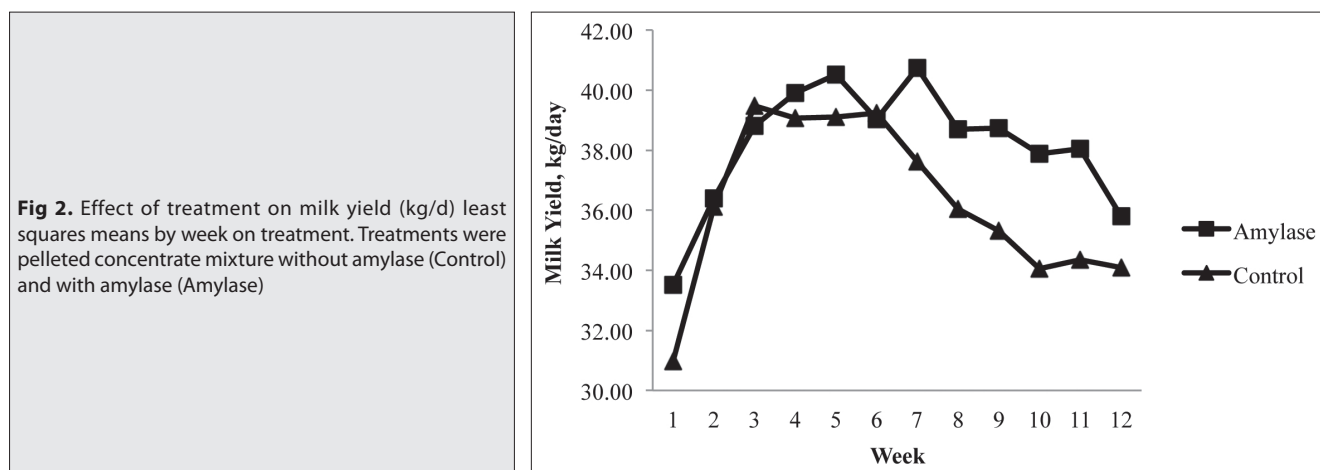
<sup>1</sup> Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase), <sup>2</sup> Standard error of the mean. <sup>3</sup> Non significant

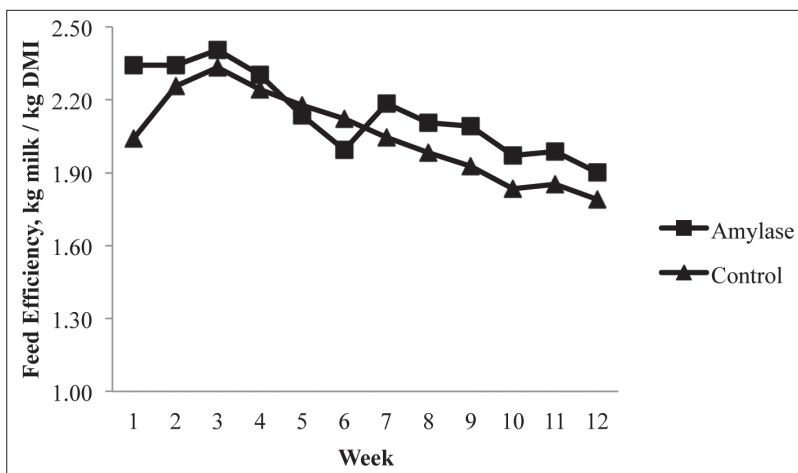


**Table 5.** Effect of treatment on least squares means for DMI of prepartum and early lactation cows and early-lactation lactation performance<sup>1</sup>

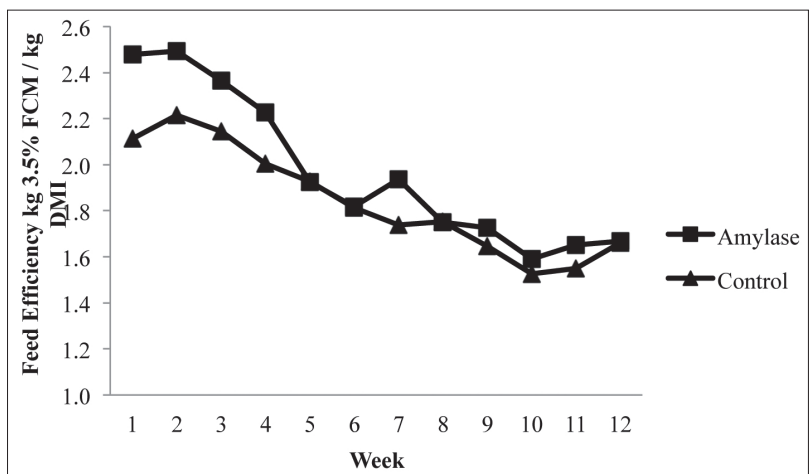
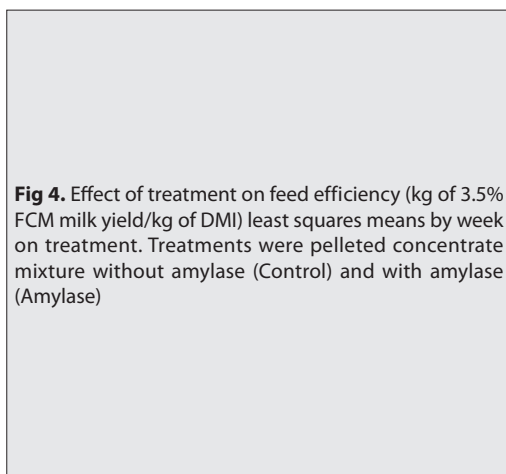
Item	Control	Amylase	SEM <sup>2</sup>	P	
DMI Prepartum (kg/day)	12.5	11.5	0.4	NS <sup>3</sup>	
DMI Postpartum (kg/day)	17.7	17.9	0.2	NS	
Milk Yield (kg/d)	36.3	38.2	0.7	NS	
3.5% FCM, kg/d <sup>4</sup>	32.7	34.7	0.4	0.001	
SCM, kg/d <sup>5</sup>	32.7	34.9	0.3	0.001	
ECM, kg/d <sup>6</sup>	35.4	37.7	0.3	0.001	
Fat,	%	3.31	3.42	0.06	NS
	kg/d	1.20	1.30	0.03	0.01
Protein	%	3.08	3.07	0.03	NS
	kg/d	1.12	1.17	0.01	0.01
Lactose	%	4.93	4.97	0.02	NS
	kg/d	1.79	1.90	0.02	0.01
kg Milk/kg DMI	2.05	2.15	0.03	0.01	
kg 3.5% FCM/kg DMI	1.84	1.97	0.04	0.005	
kg SCM/kg DMI	1.85	1.98	0.03	0.004	
kg ECM/kg DMI	2.01	2.14	0.04	0.004	

<sup>1</sup> Treatments were pelleted concentrate without amylase (Control) and with amylase (Amylase); <sup>2</sup> Standard error of the mean; <sup>3</sup> Non significant; <sup>4</sup> Fat-corrected milk calculated according to NRC (2001) equations; <sup>5</sup> Solids-corrected milk calculated according to NRC (2001) equations; <sup>6</sup> Energy-corrected milk calculated according to NRC (2001) equations





**Fig 3.** Effect of treatment on feed efficiency (kg of milk yield/kg of DMI) least squares means by week on treatment. Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase)



**Fig 4.** Effect of treatment on feed efficiency (kg of 3.5% FCM milk yield/kg of DMI) least squares means by week on treatment. Treatments were pelleted concentrate mixture without amylase (Control) and with amylase (Amylase)

was numerically greater by 2.0 kg/d for cows fed amylase compared to control.

Effect of treatment on milk yield (kg/d) least squares means by week are in Fig. 2. Cows fed amylase diet produced more milk than cows fed control in first week of lactation (31.0 vs 33.5 kg/d) but cows fed control and amylase produced a similar amount of milk from 2 to 6 week of lactation.

The FCM, SCM and ECM yields for cows fed amylase were 2.0 ( $P<0.001$ ), 2.2 ( $P<0.001$ ), and 2.3 ( $P<0.001$ ) kg/d greater than for cows fed control. Feed efficiency of the FCM, SCM and ECM was 6.5% greater for amylase than control in the current study. Least squares means by week on treatment for feed efficiency (kg of milk yield/kg of DMI,  $P<0.01$ ) and feed efficiency (kg of 3.5% FCM milk yield/kg of DMI,  $P<0.005$ ) are presented in Fig. 3 and Fig. 4, respectively.

The percentages of milk fat, protein and lactose were unaffected by treatment. However, yields of milk fat, protein and lactose for cows fed amylase were greater ( $P<0.001$ ) than for cows fed control.

## DISCUSSION

The DMI was unaffected by treatment in the present study.

Average prepartum DMI was not affected by treatment and averaged 12.0 kg/d. Prepartum decrease in DMI was greater for cows fed amylase relative to control cows (Fig. 1). On the other hand, postpartum DMI was not affected by the addition of amylase, averaging 17.8 kg/d during the 84 days in milk. A similar treatment effect was observed for DMI by <sup>[19,20]</sup> study, they observed no difference in short-term DMI in response to exogenous amylase. Although that is not significant, DMI tended to be lower by about 5% for reduced starch diet with amylase compared to reduced starch diet without amylase <sup>[12]</sup>. Weiss et al. <sup>[13]</sup> and McCarthy et al. <sup>[21]</sup> reported no differences in DMI between reduced starch diets with or without amylase. Exogenous amylase addition to normal starch diets (approximately 26% to 27% DM) has resulted in either similar <sup>[13,22]</sup> or increased <sup>[10]</sup> DMI. Similar DMI between control and amylase was consistent throughout the experimental period.

The supplementation of amylase to reduced-starch diets (21% on DM basis) has either not affected <sup>[12]</sup> or reduced <sup>[3,23]</sup> DMI. Gencoglu et al. <sup>[3]</sup> reported that DMI for cows fed by the reduced-starch (21% on DM basis) diet without amylase was 3.2 kg/d greater than for cows fed the reduced starch diet with amylase and thus both of NDF and starch intake was greater for cows fed without amylase reduced starch

diet. In addition, the expected increase in DMI might not be observed as the heat stress actualized in the last term of trial as shown *Fig. 1*.

Milk yield was unaffected by treatment ( $P>0.10$ ), but was numerically greater by 2.0 kg/d for cows fed amylase compared to control. This observation is in agreement with the reports of Gencoglu et al.<sup>[3]</sup>, Ferraretto et al.<sup>[12]</sup>, Weiss et al.<sup>[13]</sup>, and DeFrain et al.<sup>[20]</sup> where exogenous amylase addition did not affect milk yields. McCarthy et al.<sup>[21]</sup> reported a tendency for 1.6 kg/d decreased milk production by cows supplemented with amylase (activity of 351 KNU/kg). On the other hand, exogenous amylase supplementation increased milk yield in experiments of Klingerman et al.<sup>[10]</sup>, Tricarico et al.<sup>[22]</sup>, Harrison and Tricarico<sup>[24]</sup>. In the current trial, the numerically increased milk yield for cows fed amylase versus control may be related to the greater ruminal total volatile fatty acid (VFA) concentrations. Seymour et al.<sup>[25]</sup> reported that milk yield was most highly related to ruminal concentrations of butyrate ( $r^2=0.47$ ) and propionate ( $r^2=0.23$ ).

Cows fed amylase diet produced more milk than cows fed control in first week of lactation (31.0 vs 33.5 kg/d, *Fig. 2*) but cows fed control and amylase produced a similar amount of milk from 2 to 6 week of lactation. Milk production decreased in all cows during 6 to 12 week of lactation. Decrease in milk production may attributed to the occurrence of heat stress in dairy cows<sup>[26]</sup>. In the current study, four cows in each group entered the trial the end of April, and heat stress could have affected these cows during the July and August. Orman and Ogan<sup>[27]</sup> observed that the seasons affect milk production in the Northwest of Turkey. As shown *Fig. 2*, milk production more sharply decreased for cows fed control than for cows fed amylase.

The FCM, SCM and ECM yields for cows fed amylase were 2.3, 2.2, and 2.3 kg/d greater than for cows fed control. The gain in feed efficiency has been driven by increased milk yield at same DMI. Klingerman et al.<sup>[10]</sup> and Tricarico et al.<sup>[22]</sup> and reported greater FCM yields with exogenous amylase addition to normal-starch diets (26% on dry matter basis). However, Gencoglu et al.<sup>[3]</sup>, Ferraretto et al.<sup>[12]</sup>, and Vargas-Rodriguez et al.<sup>[28]</sup> reported similar FCM, SCM and ECM yields for reduced-starch diets with and without exogenous amylase addition. However, McCarthy et al.<sup>[21]</sup>, reported less FCM for reduced starch diet with amylase than reduced starch diet without amylase.

Feed efficiency of the FCM, SCM and ECM was 6.5% greater for amylase than control in the current study. Klingerman et al.<sup>[10]</sup> reported greater feed efficiency with exogenous amylase supplementation in normal-starch diets, and other researchers<sup>[3,12,19]</sup> reported greater feed efficiency with exogenous amylase supplementation in low-starch diets. Weiss et al.<sup>[13]</sup> reported that addition of the exogenous amylase to a low-starch diet had no effect on feed efficiency.

The percentages of milk fat, protein and lactose were unaffected by treatment. However, yields of milk fat, protein and lactose for cows fed amylase were greater than for cows fed control. Addition of exogenous amylase did not affect milk fat percentage for normal-starch<sup>[10,22,24]</sup> or reduced-starch diets<sup>[3,13]</sup>. A tendency was found for milk protein percentage to be greater for cows fed reduced starch diet with amylase (3.06 versus 2.99%;  $P<0.10$ ) compared to increased milk protein percentage ( $P=0.006$ , respectively) cows fed reduced starch diet without amylase<sup>[3]</sup>. McCarthy et al.<sup>[21]</sup> reported greater milk protein percentage ( $P=0.006$ ) for cows fed reduced starch with amylase (22.9% as dry matter basis) compared to reduced starch without amylase (23.7% as dry matter basis). Addition of exogenous amylases to normal-starch diets did not affect milk protein percentage in the trials of Klingerman et al.<sup>[10]</sup>, Tricarico et al.<sup>[22]</sup>, Harrison and Tricarico<sup>[24]</sup>. Ferraretto et al.<sup>[12]</sup> reported that exogenous amylase addition to reduced-starch diets did not affect ( $P>0.10$ ) milk protein percentage. Milk lactose content was unaffected by treatment in other trials with exogenous amylase addition<sup>[3,12,21]</sup>.

Cows fed low starch diet with addition of exogenous amylase to either diet, resulted in similar intakes of DM, OM, NDF, and CP but greater FCM, SCM, and ECM yields. It was concluded that inclusion of amylase improved the feed efficiency of lactating cows fed low starch diet; but the enzyme did not affect DMI.

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