



## Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows

L. F. Ferraretto,\* R. D. Shaver,\*<sup>1</sup> M. Espineira,\* H. Gencoglu,† and S. J. Bertics\*

\*Department of Dairy Science, University of Wisconsin-Madison, 1675 Observatory Dr., Madison 53706

†Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Uludag, Bursa, Turkey, 16059

### ABSTRACT

The objective of this trial was to determine lactation performance responses in high-producing dairy cows to a reduced-starch versus a normal-starch diet and to the addition of exogenous amylase to the reduced-starch diet. Forty-five multiparous Holstein cows,  $68 \pm 29$  d in milk and  $696 \pm 62$  kg of body weight (BW) at trial initiation, were randomly assigned to 1 of 3 treatments in a completely randomized design; a 2-wk covariate adjustment period with cows fed the normal-starch diet was followed by a 10-wk treatment period with cows fed their assigned treatment diets. The normal-starch total mixed ration did not contain exogenous amylase (NS–). The reduced-starch diets, formulated by partially replacing corn grain and soybean meal with whole cottonseed and wheat middlings, were fed without (RS–) and with (RS+) exogenous amylase addition to the total mixed ration. All diets contained 50% forage and 19.8% forage neutral detergent fiber (dry matter basis). Starch and neutral detergent fiber concentrations averaged 27.0 and 30.9%, 22.1 and 35.0%, and 21.2 and 35.3% (dry matter basis) for the NS–, RS–, and RS+ diets, respectively. Expressed as a percentage of BW, dry matter intake was greater for cows fed RS– than for cows fed NS– or RS+. Intake of neutral detergent fiber ranged from 1.09 to 1.30% of BW among the treatments, with that of RS– being 21% greater than that of NS–. Milk yield tended to be greater for cows fed NS– compared with the RS diets. Milk fat content and yield were unaffected by treatment. Milk protein content and yield were greater for cows fed NS– compared with the RS diets. Concentrations of milk urea nitrogen were greater for cows fed RS diets compared with the NS– diet. Body weight, BW change, and body condition score were unaffected by treatment. Feed conversion (kg of milk/kg of dry matter intake) was 10% greater on average for cows fed NS– than for cows fed the RS diets, and tended to be 6% greater for

cows fed RS+ compared with RS–. Feeding a reduced-starch diet formulated by partially replacing corn grain and soybean meal with a wheat middlings and whole cottonseed mixture compared with a normal-starch diet without addition of exogenous amylase to either diet reduced milk and component-corrected feed conversions. Addition of exogenous amylase to a reduced-starch diet was of minimal benefit in this study.

**Key words:** amylase, byproduct feeds, lactating cow, starch

### INTRODUCTION

Increased corn prices have heightened the interest in feeding reduced-starch diets. Results from short-term dairy cattle feeding trials suggest that reduced-starch diets formulated by partially replacing corn grain with high-fiber, low-starch byproduct feedstuffs may be feasible (Shaver, 2008). In a longer term lactation trial with high-producing cows, Gencoglu et al. (2010) observed similar milk yield, greater FCM yield, and greater DMI for cows fed a reduced-starch diet formulated by partially replacing dry ground shelled corn (DGSC) with soy hulls.

Some exogenous enzymes are resistant to ruminal degradation (Hristov et al., 1998) and may offer potential for improving diet digestibility and animal performance. Klinger et al. (2009) reported that addition of exogenous amylase to a normal-starch diet (26% of DM) increased milk yield by dairy cows; positive *in vitro* and *in vivo* digestibility responses to exogenous amylase were also observed. In the trial of Gencoglu et al. (2010), addition of exogenous amylase to a reduced-starch diet (21% of DM) increased apparent total-tract digestibilities of OM and NDF, decreased DMI, and increased fat-, solids- and energy-corrected milk feed conversions (kg/kg of DMI). Greater conversion of feed to milk for dairy cows fed reduced-starch diets with inclusion of exogenous amylase may offer potential for improving economic performance.

Our hypotheses were that feeding a reduced-starch diet would result in greater DMI and reduced feed conversion compared with a normal-starch diet, and that

Received August 18, 2010.

Accepted November 29, 2010.

<sup>1</sup>Corresponding author: rdshaver@wisc.edu

the addition of exogenous amylase to the reduced-starch diet would improve feed conversions. The objectives of this trial were to determine DMI and lactation performance responses in high-producing dairy cows to (1) a reduced-starch versus a normal-starch diet formulated by partially replacing DGSC and soybean meal (**SBM**) with the moderate-protein, high-fiber, byproduct feed ingredients wheat middlings (**WM**) and whole cottonseed (**WCS**), and (2) addition of exogenous amylase to the reduced-starch diet.

## MATERIALS AND METHODS

Forty-five multiparous Holstein cows  $68 \pm 29$  DIM at trial initiation were randomly assigned to 1 of 3 treatments in a completely randomized design; a 2-wk covariate adjustment period with cows fed the normal-starch diet was followed by a 10-wk treatment period with cows fed their assigned treatment diets. Ingredient composition of the experimental diets is provided in Table 1. Diets contained 50% forage (DM basis). The concentrate mixes contained the WM, and the WCS was added to the TMR separately. The normal-starch TMR did not contain exogenous amylase (**NS-**). The reduced-starch diets were fed without (**RS-**) and with (**RS+**) exogenous amylase addition to the TMR. A granular amylase formulation, Ronozyme RumiStar CT, with an amylase activity of 600 Kilo Novo units (**KNU**) per g provided by DSM Nutritional Products (Basel, Switzerland) and Novozymes (Bagsvaerd, Denmark), was used for this study. One KNU is the amount of enzyme that releases in a 2-step reaction, Ronozyme RumiStar/ $\alpha$ -glucosidase, 6  $\mu$ mol of *p*-nitrophenol per minute from 1.86 mM ethylidene-G7-*p*-nitrophenyl-maltoheptaoside at pH 7.0 and 37°C. The Ronozyme RumiStar CT was provided in a premix with an amylase activity of 320 KNU/g by DSM Nutritional Products-USA (Ames, IA). This premix was mixed 50:50 with wheat red dog by Vita Plus Corp. (Madison, WI) to prepare the treatment premix. The targeted dosage of 300 KNU/kg of TMR DM in RS+ was achieved by adding 3.8 g of the treatment premix per kilogram of concentrate DM in the TMR mixer. For the NS- and RS- diets, a placebo premix composed of wheat red dog prepared by Vita Plus Corp. was added at the rate of 3.8 g/kg of concentrate DM in the TMR mixer. After mixing for 3 min, the forages were added and the TMR was allowed to mix for another 3 min. The RS+ TMR was prepared and delivered last with the mixer and then used to feed the remainder of the herd to avoid mixer clean-out concerns related to the amylase treatment. Samples of NS-, RS-, and RS+ concentrate mixes were obtained following mixing with-out or with amylase, respectively, every 3 wk, stored

**Table 1.** Ingredient composition of the diets

Ingredient, % of DM	Normal starch	Reduced starch
Corn silage	33.3	33.3
Alfalfa silage	16.7	16.7
Dry ground shelled corn	22.5	11.8
Wheat middlings	2.2	10.9
Whole cottonseed	2.2	9.1
Soybean meal-48%	14.6	10.6
Distillers dried grains	5.5	5.5
Energy Booster 100 <sup>1</sup>	1.0	—
Calcium carbonate	1.18	1.18
Magnesium oxide	0.18	0.18
Mg-K-S <sup>2</sup>	0.11	0.11
Trace mineral salt <sup>3</sup>	0.45	0.45
Vitamin premix <sup>4</sup>	0.18	0.18

<sup>1</sup>Minimum 98% total fatty acids (MSC Company, Dundee, IL).

<sup>2</sup>Dynamate (11% Mg, 18% K, 22% S; The Mosaic Co., Plymouth, MN).

<sup>3</sup>88% NaCl, 0.002% Co, 0.2% Cu, 0.012% I, 0.18% Fe, 0.8% Mn, 0.006% Se, 1.4% Zn.

<sup>4</sup>Vitamin A 3,300,000 IU/kg; vitamin D 1,100,000 IU/kg; vitamin E 11,000 IU/kg.

at  $-20^{\circ}\text{C}$ , and then sent to DSM Nutritional Products Analytical Services Center (Basel, Switzerland) for analysis for amylase activity (Jung and Vogel, 2008). Determined amylase activities for NS-, RS-, and RS+ concentrates were 0,  $3.5 \pm 7$ , and  $583.8 \pm 53.4$  KNU/kg (as-fed basis), respectively. The RS+ TMR averaged 324 KNU/kg of DM, which was similar to the targeted dosage of 300 KNU/kg of DM recommended by DSM Nutritional Products (Basel, Switzerland) and the dosage used in the trials of Gencoglu et al. (2010) and Klingerman et al. (2009).

The animal research was conducted under an approved protocol by the Research Animal and Resource Center of the College of Agricultural and Life Sciences, University of Wisconsin, Madison. All cows were injected with bST (Posilac, Monsanto Co., St. Louis, MO) every 14 d commencing on d 1 of the covariate period. Cows were individually fed the TMR twice daily in tie-stalls for 10% refusals with daily DMI determined on individual cows throughout the 12-wk trial. Body weight and condition score (1 to 5 in 0.25 increments; Wildman et al., 1982) were recorded weekly throughout the 12-wk trial. Body weight change was determined by regression of the treatment period BW measurements over time. Milk yield was recorded daily on individual cows milked twice daily throughout the 12-wk trial. Milk samples were obtained from all cows weekly on the same 2 consecutive days from a.m. and p.m. milkings throughout the 12-wk trial and analyzed for fat, true protein, lactose, and MUN concentrations by infrared analysis (AgSource Milk Analysis Laboratory, Menomonie, WI) using a Foss FT6000 (Foss Electric, Hillerød,

Denmark) with average daily yields of fat, protein, and lactose calculated from these data for each week. Yields of 3.5% FCM, SCM, and ECM were calculated according to NRC (2001) equations. Actual-milk, FCM, SCM, and ECM feed conversions were calculated by week using average daily yield and DMI data. Estimated diet energy concentrations were calculated by summing the Mcal of  $NE_L$  from milk production, required for maintenance and in BW change (NRC, 2001), and then dividing the sum by DMI.

Samples of TMR, corn silage, alfalfa silage, concentrate mixes, DGSC, WM and WCS were obtained weekly and dried at 60°C for 48 h in a forced-air oven to determine DM content. Dried samples were ground to pass a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) screen, and then composited by 2-wk periods before sending to Dairyland Laboratories Inc. (Arcadia, WI) for analysis. The absolute DM was determined by oven-drying at 105°C for 72 h. All samples were analyzed for DM, OM (method 942.05; AOAC, 2006), CP (method 990.03; AOAC, 2006), NDF using  $\alpha$ -amylase and sodium sulfite (Van Soest et al., 1991), and ether extract (method 2003.05; AOAC, 2006). Starch content (Bach Knudsen, 1997; YSI Biochemistry Analyzer, YSI Inc., Yellow Springs, OH) and particle size were determined on all samples except for WCS. Sugar content (Hall et al., 1999) was determined only on TMR samples. Particle size of TMR, corn silage, and alfalfa silage samples was determined as described by Kononoff et al., (2003), and corn silage processing score (Ferreira and Mertens, 2005) was measured. Particle size of the concentrate mixtures, DGSC, and WM samples was determined by dry sieving using Tyler Ro-Tap Shaker model RX-29 (Mentor, OH) and sieves with 4,760-, 2,380-, 1,191-, 595-, 297-, 149-, and 63- $\mu$ m apertures plus bottom pan with mean particle size calculated using a log normal distribution (Baker and Herrman, 2002). Ruminal in vitro NDF digestibility (30 h) on TMR, alfalfa silage, corn silage, concentrate mix, and WM samples, and starch digestibility (7 h) on TMR, DGSC, corn silage, concentrate mix, and wheat middlings samples were determined at Cumberland Valley Analytical Services Inc. (Maugansville, MD) as described by Lopes et al. (2009).

One RS- cow had truncated records in the later weeks of the trial due to an injury in the exercise lot that would not allow her continue on the trial, and 2 RS+ cows had truncated records at the later weeks of the trial because of toxic mastitis. Data were analyzed as a completely randomized design with the data from the preliminary period as a covariate using PROC MIXED (SAS Institute, 2004) with week of treatment as repeated measures using the first-order autoregressive covariance structure that provided the best fit

according to Sawa's Bayesian information criterion. A covariate was not used for analysis of BW change and estimated diet energy concentration data. The model included treatment, week, and treatment by week interaction as fixed effects, and cow within treatment as a random effect. Degrees of freedom were calculated using the Kenward-Roger option (SAS Institute, 2004). Means were determined using the least squares means statement, treatment means were compared using the PDIF option after a significant overall treatment  $F$  test, and interaction effects were partitioned using the SLICE option (SAS Institute, 2004). Statistical significance and trends were considered at  $P \leq 0.05$  and  $P \geq 0.06$  to  $P < 0.10$ , respectively.

## RESULTS AND DISCUSSION

Nutrient composition and particle size of forages and concentrates are presented in Table 2. The alfalfa and corn silages, WM, and WCS were of good quality (NRC, 2001). Diet nutrient composition and particle size are presented in Table 3. The RS diets on average contained 5.4 percentage units less starch and 4.3 percentage units more NDF than the NS- diet. This was related to the partial replacement of DGSC (approximately 11 percentage units less corn DM) with WM and WCS in the RS diets. The RS diets were 4.9 and 2.5 percentage units lower, on average, in calculated NFC and total digestible nutrients at a maintenance level of intake ( $TDN_{1x}$ ) contents, respectively, than the NS- diet. Measurements for other nutrient concentrations and particle size were similar across the 3 TMR. All diets contained 19.8% forage NDF (DM basis; data not provided in the table).

Treatment effects on covariate-adjusted least squares means for DM and nutrient intakes are presented in Table 4. Dry matter intake expressed as a percentage of BW was greater for cows fed RS- than for cows fed NS- ( $P < 0.01$ ). Bernard and McNeill (1991) reported no difference in DMI for lactating dairy cows fed WM replacing DGSC. In literature reviews, Coppock et al. (1987) reported no effect of WCS on DMI, whereas Arieli (1998) reported variable effects of WCS, with increased, decreased, and similar DMI noted across studies. Gencoglu et al. (2010) reported 9% greater DMI for an RS- diet than an NS- diet when DGSC was partially replaced with soy hulls. Although only a tendency ( $P < 0.07$ ) in the present study, numerically DMI (kg/d) was about 8% greater for RS- than NS-. Greater DMI for RS- than NS- may be related to reduced ruminal propionate concentration (Allen, 1997; Beckman and Weiss, 2005) leading to increased meal size and consequently greater DMI (Allen et al., 2009).

**Table 2.** Nutrient composition (mean  $\pm$  SD) and particle size (mean  $\pm$  SD) of corn silage, alfalfa silage, and concentrates

Item	Component <sup>1</sup>						
	CS <sup>2</sup>	AS	NSC	RSC	DGSC	WM	WCS
Nutrient							
DM, % as fed	35.5 $\pm$ 2.6	44.3 $\pm$ 9.4	90.0 $\pm$ 0.7	90.5 $\pm$ 0.7	89.3 $\pm$ 1.1	88.8 $\pm$ 0.8	90.3 $\pm$ 1.4
OM, % of DM	96.1 $\pm$ 0.3	90.0 $\pm$ 2.0	92.5 $\pm$ 0.5	89.8 $\pm$ 0.8	98.8 $\pm$ 0.3	94.3 $\pm$ 0.3	95.8 $\pm$ 0.2
CP, % of DM	6.6 $\pm$ 0.4	19.7 $\pm$ 0.5	22.6 $\pm$ 1.5	24.7 $\pm$ 1.5	9.8 $\pm$ 0.8	17.3 $\pm$ 0.5	22.3 $\pm$ 1.0
NDF, % of DM	39.5 $\pm$ 0.6	39.8 $\pm$ 1.5	13.1 $\pm$ 2.1	14.4 $\pm$ 1.0	9.5 $\pm$ 1.8	40.2 $\pm$ 2.4	43.4 $\pm$ 4.1
IVNDFD, <sup>3</sup> % of NDF	57.1 $\pm$ 0.7	52.5 $\pm$ 3.1	68.3 $\pm$ 1.5	74.4 $\pm$ 1.1	—	53.5 $\pm$ 0.8	—
Starch, % of DM	34.2 $\pm$ 0.9	2.2 $\pm$ 0.9	36.3 $\pm$ 1.2	30.4 $\pm$ 1.5	65.5 $\pm$ 3.1	19.3 $\pm$ 1.4	—
IVStarchD, <sup>4</sup> % of starch	84.0 $\pm$ 1.6	—	82.5 $\pm$ 1.1	83.3 $\pm$ 1.5	76.0 $\pm$ 1.5	88.7 $\pm$ 0.7	—
Ether extract, % of DM	3.5 $\pm$ 0.3	4.0 $\pm$ 0.3	6.2 $\pm$ 0.5	3.7 $\pm$ 0.5	4.2 $\pm$ 0.5	5.3 $\pm$ 0.3	17.0 $\pm$ 2.1
Particle size							
Tyler sieve							
GMPS, <sup>5</sup> $\mu$ m	—	—	845 $\pm$ 74	811 $\pm$ 61	844 $\pm$ 83	717 $\pm$ 36	—
Penn State sieves, % as fed retained on sieve							
19 mm	2.8 $\pm$ 1.1	8.7 $\pm$ 6.2	—	—	—	—	—
8 mm	52.3 $\pm$ 6.3	57.2 $\pm$ 10.0	—	—	—	—	—
1.18 mm	41.6 $\pm$ 6.3	29.3 $\pm$ 7.8	—	—	—	—	—
Processing score							
Starch passing 4,750- $\mu$ m sieve, %	75.7 $\pm$ 3.3	—	—	—	—	—	—

<sup>1</sup>CS = corn silage; AS = alfalfa silage; NSC = normal starch concentrate; RSC = reduced starch concentrate; DGSC = dry ground shelled corn; WM = wheat middlings; WCS = whole cottonseed.

<sup>2</sup>Gehl Model 1085 forage harvester (West Bend, WI) fitted with kernel processor (1.9-cm theoretical length of cut; 2-mm roll clearance).

<sup>3</sup>Ruminal in vitro NDF digestibility at 30 h.

<sup>4</sup>Ruminal in vitro starch digestibility at 7 h.

<sup>5</sup>Geometric mean particle size.

**Table 3.** Diet nutrient composition and particle size (mean  $\pm$  SD)

Item	Treatment <sup>1</sup>		
	NS-	RS-	RS+
Nutrient			
DM, % as fed	52.2 $\pm$ 3.3	52.8 $\pm$ 3.1	52.9 $\pm$ 3.1
OM, % of DM	93.4 $\pm$ 0.6	93.1 $\pm$ 0.8	93.0 $\pm$ 0.7
CP, % of DM	15.9 $\pm$ 1.0	16.3 $\pm$ 0.4	16.5 $\pm$ 0.4
Ether extract, % of DM	5.3 $\pm$ 0.3	5.2 $\pm$ 0.1	5.0 $\pm$ 0.4
NDF, % of DM	30.9 $\pm$ 0.8	35.0 $\pm$ 0.5	35.3 $\pm$ 1.0
NFC, % of DM	42.1 $\pm$ 1.6	37.4 $\pm$ 1.1	37.0 $\pm$ 1.1
Starch, % of DM	27.0 $\pm$ 0.6	22.1 $\pm$ 0.4	21.2 $\pm$ 1.0
Sugars, % of DM	3.7 $\pm$ 0.6	3.3 $\pm$ 0.7	4.0 $\pm$ 0.8
TDN <sub>1x</sub> , <sup>2</sup> % of DM	72.0 $\pm$ 1.0	69.7 $\pm$ 0.9	69.2 $\pm$ 1.2
Particle size, <sup>3</sup> % as-fed retained			
19 mm	3.1 $\pm$ 1.4	2.7 $\pm$ 1.0	2.0 $\pm$ 0.5
8 mm	37.2 $\pm$ 4.9	38.9 $\pm$ 3.8	40.2 $\pm$ 4.2
1.18 mm	42.0 $\pm$ 3.5	40.8 $\pm$ 2.6	40.3 $\pm$ 3.2

<sup>1</sup>Treatments were normal-starch diet with no amylase added to TMR (NS-), reduced-starch diet with no amylase added to TMR (RS-), and reduced-starch diet with amylase added to TMR (RS+).

<sup>2</sup>Total digestible nutrients; calculated using NRC (2001) summative energy equation.

<sup>3</sup>Penn State Separator sieves.

W. P. Weiss (The Ohio State Univ., Wooster, OH, personal communication) reported that partially replacing DGSC with corn silage to formulate an RS- diet reduced DMI for RS- compared with NS-. Firkins (1997) suggested that increased digestibility and passage rate of nonforage or by-product NDF can allow for increased NDF intake relative to forage NDF. This could explain the difference in DMI response for RS- between the present trial and that of Gencoglu et al. (2010) compared with W. P. Weiss (The Ohio State Univ., Wooster, OH, personal communication). Intake of NDF was greater for RS- than NS- by 21% in the present trial and by 31% in Gencoglu et al. (2010). Likely DMI for the NS- diet in the trial of W. P. Weiss (The Ohio State Univ., Wooster, OH, personal communication) was limited by rumen fill (Allen, 2000),

whereas DMI for the NS- diets fed in the current study and in the trial of Gencoglu et al. (2010) was limited by ruminal propionate concentration (Allen, 1997; Beckman and Weiss, 2005), thereby resulting in different DMI responses to the feeding of reduced-starch diets.

The DMI expressed as a percentage of BW was greater for cows fed RS- than for cows fed RS+ ( $P < 0.05$ ). Addition of exogenous amylase to normal-starch diets has resulted in either similar (Tricarico et al., 2005) or increased (Klingerman et al., 2009) DMI. However, addition of exogenous amylase to a reduced-starch diet reduced DMI by 11% in the trial of Gencoglu et al. (2010). Although not significant ( $P > 0.10$ ) in the present study, numerically DMI (kg/d) was about 5% lower for RS+ than for RS-. W. P. Weiss (The Ohio State Univ., Wooster, OH, personal communication) reported

**Table 4.** Effect of treatment on covariate-adjusted least squares means for DM and nutrient intakes<sup>1</sup>

Intake <sup>2</sup>	Treatment <sup>3</sup>				Treatment	$P <$		
	NS-	RS-	RS+	SEM		NS- vs. RS-	NS- vs. RS+	RS- vs. RS+
DM, kg/d	25.7	27.6	26.3	0.7	0.14	—	—	—
DM, % of BW	3.48	3.86	3.60	0.09	0.02	0.01	0.39	0.05
OM, kg/d	24.0	25.7	24.5	0.6	0.16	—	—	—
NDF, kg/d	7.6	9.2	8.8	0.3	0.01	0.01	0.01	0.35
NDF, % of BW	1.09	1.30	1.29	0.04	0.01	0.01	0.01	0.84
Starch, kg/d	6.9	6.1	5.6	0.2	0.01	0.01	0.01	0.03
CP, kg/d	4.1	4.5	4.3	0.1	0.05	0.02	0.12	0.37

<sup>1</sup>Week effect ( $P < 0.01$ ) for all parameter estimates.

<sup>2</sup>Week  $\times$  treatment interaction effect ( $P < 0.02$ ) for all parameter estimates except NDF intake (% of BW).

<sup>3</sup>Treatments were normal-starch diet with no amylase added to TMR (NS-), reduced-starch diet with no amylase added to TMR (RS-), and reduced-starch diet with amylase added to TMR (RS+).



no differences in DMI between RS<sup>-</sup> and RS<sup>+</sup>. The DMI response to exogenous amylase addition is equivocal and merits further research.

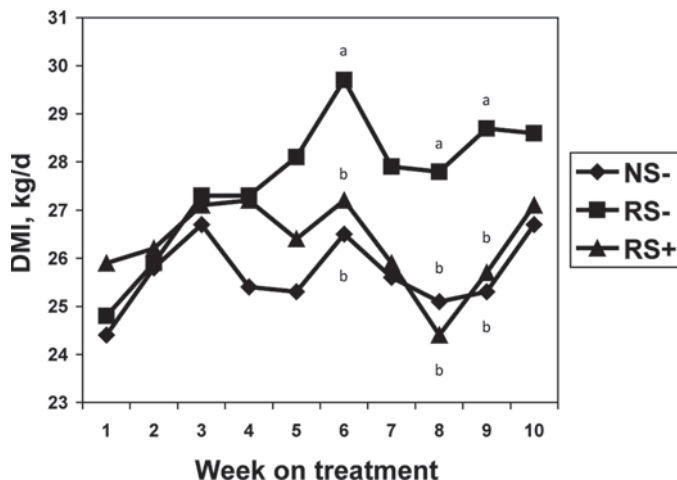
Least squares means by week on treatment for DMI (kg/d) are presented in Figure 1; week and week  $\times$  treatment interaction effects ( $P < 0.02$ ) were observed. The DMI was greater for RS<sup>-</sup> than for NS<sup>-</sup> and RS<sup>+</sup> for wk 6, 8, and 9 on treatment. Treatment differences for DMI were observed within 2 wk on treatment and continued thereafter in the trial of Gencoglu et al. (2010). We have no explanation for the difference between trials in the observed DMI response over time.

Treatment effects on OM intakes were similar to those observed for DMI. As discussed previously, NDF intakes were greater ( $P < 0.01$ ) for RS<sup>-</sup> than for NS<sup>-</sup>. This coincided with reduced ( $P < 0.01$ ) starch intakes and increased ( $P < 0.02$ ) CP intakes for RS<sup>-</sup> compared with NS<sup>-</sup>. No differences ( $P > 0.10$ ) in NDF intakes were observed between the RS diets, but starch intakes were 0.5 kg/d greater ( $P < 0.03$ ) for RS<sup>-</sup> than for RS<sup>+</sup> in relationship to the numerically greater DMI for RS<sup>-</sup> at a similar diet starch content.

Treatment effects on covariate-adjusted least squares means for lactation performance measurements are presented in Table 5. Milk yield tended to be 2.2 kg/d greater ( $P < 0.07$ ) for cows fed NS<sup>-</sup> than for cows fed RS<sup>-</sup> and RS<sup>+</sup>. Bernard and McNeill (1991) and Coppock et al. (1987) reported no differences in milk yield when cows were fed WM and WCS, respectively. Gencoglu et al. (2010) reported similar milk yield for cows fed NS<sup>-</sup> and RS<sup>-</sup> diets formulated by partially

replacing DGSC with soy hulls in cows producing 50 kg/d. In that study, the replacement was nearly direct because the CP content of soy hulls and DGSC is similar. In the present study, because WM and WCS are moderate-protein ingredients, they partially replaced both DGSC and SBM. Ruminal degradation of CP from WM and WCS is greater than the CP from DGSC and SBM (Bernard et al., 1988; Arieli, 1998; Batajoo and Shaver, 1998; NRC, 2001). The NRC (2001) RDP estimates (DMI of 4% of BW) were 76, 77, and 65% of CP for WM, WCS, and DGSC-SBM (calculated from NS<sup>-</sup> proportions in the present trial), respectively. Furthermore, the reduced starch intake for cows fed RS<sup>+</sup> and RS<sup>-</sup> diets may have reduced rumen microbial CP (MCP) production (Oba and Allen, 2003). In the present trial, decreased RUP and MCP, and thus MP flow, may partially explain the decrease in milk yield for RS<sup>+</sup> and RS<sup>-</sup> compared with NS<sup>-</sup>. The diet ingredient composition, feed ingredient nutrient composition, DMI, BW, DIM, and milk composition data were input into the NRC (2001) model, and the predicted MP-allowable milk was 96% of NE<sub>L</sub>-allowable milk for NS<sup>-</sup> compared with 88% for the RS diets, which supports the above premise. Addition of exogenous amylase increased milk yield with a normal-starch diet (Tricarico et al., 2005; Harrison and Tricarico, 2007; Klengerman et al., 2009), but did not affect milk yield with a reduced-starch diet (Gencoglu et al., 2010; W. P. Weiss, The Ohio State Univ., Wooster, OH, personal communication) in agreement with the present trial.

Treatment effects on FCM and ECM yields were similar to those observed for actual milk yield. The SCM yields tended to be 2.1 kg/d greater ( $P < 0.08$ ) for cows fed NS<sup>-</sup> than for cows fed RS<sup>-</sup>, and were 2.8 kg/d greater ( $P < 0.02$ ) for NS<sup>-</sup> than RS<sup>+</sup>. Gencoglu et al. (2010) reported greater yields of FCM, SCM, and ECM for RS diets than a NS<sup>-</sup> diet in response to greater milk fat percentage and hence yield for cows fed the RS diets. Milk fat at percentage and yield were unaffected ( $P > 0.10$ ) by treatment in the present trial, and averaged 3.30% and 1.66 kg/d, respectively, across the 3 treatments. Bernard and McNeill (1991) reported similar milk fat content for cows fed WM compared with DGSC. Arieli (1998) reported variable effects of dietary WCS inclusion, with increased, decreased, or similar milk fat content noted across studies. Mohamed et al. (1988) reported that the dietary addition of free cottonseed oil reduced milk fat content, while the addition of a similar amount of ether extract from whole cottonseed did not affect milk fat content. Addition of exogenous amylase did not affect milk fat content for normal-starch (Tricarico et al., 2005; Harrison and Tricarico, 2007; Klengerman et al., 2009) or reduced-starch (Gencoglu et al., 2010; W. P. Weiss, The Ohio



**Figure 1.** Effect of treatment on DMI (kg/d) covariate-adjusted least squares means by week on treatment. Treatments were normal-starch diet with no amylase added to TMR (NS<sup>-</sup>), reduced-starch diet with no amylase added to TMR (RS<sup>-</sup>), and reduced-starch diet with amylase added to TMR (RS<sup>+</sup>). Week and week  $\times$  treatment interaction effects ( $P < 0.02$ ); SEM = 0.7. Means within the same week with different letters differ ( $P < 0.02$ ).

**Table 5.** Effect of treatment on covariate-adjusted least squares means for lactation performance<sup>1</sup>

Item	Treatment <sup>2</sup>				<i>P</i> <			
	NS–	RS–	RS+	SEM	Treatment	NS– vs. RS–	NS– vs. RS+	RS– vs. RS+
Yield								
Milk, kg/d	52.1	49.9	49.5	0.8	0.07	—	—	—
3.5% FCM, kg/d	50.0	48.4	47.7	0.9	0.17	—	—	—
SCM, <sup>3</sup> kg/d	46.4	44.3	43.6	0.8	0.05	0.08	0.02	0.55
ECM, <sup>4</sup> kg/d	49.8	47.8	47.2	0.8	0.07	—	—	—
Milk components								
Fat, %	3.27	3.31	3.32	0.09	0.94	—	—	—
Fat, kg/d	1.70	1.65	1.62	0.04	0.47	—	—	—
Protein, %	3.03	2.95	2.93	0.03	0.03	0.04	0.01	0.60
Protein, kg/d	1.57	1.47	1.46	0.02	0.01	0.01	0.01	0.69
Lactose, %	5.00	4.94	4.94	0.02	0.06	—	—	—
Lactose, kg/d	2.60	2.47	2.43	0.05	0.04	0.06	0.02	0.60
MUN, <sup>5</sup> mg/dL	13.6	15.2	14.8	0.2	0.01	0.01	0.01	0.27

<sup>1</sup>Week effect for all parameter estimates ( $P < 0.01$ ) except for lactose % ( $P < 0.07$ ).

<sup>2</sup>Treatments were normal-starch diet with no amylase added to TMR (NS–), reduced-starch diet with no amylase added to TMR (RS–), and reduced-starch diet with amylase added to TMR (RS+).

<sup>3</sup>SCM calculated according to NRC (2001) equations.

<sup>4</sup>ECM calculated according to NRC (2001) equations.

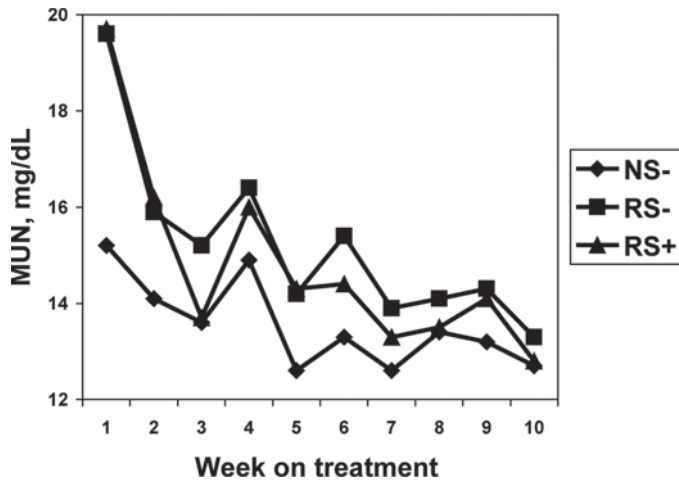
<sup>5</sup>Week  $\times$  treatment interaction ( $P < 0.01$ ).

State Univ., Wooster, OH, personal communication) diets. Tricarico et al. (2005) and Klingerman et al. (2009) reported greater FCM yields with amylase addition to normal-starch diets. However, Gencoglu et al. (2010) reported similar FCM, SCM, and ECM yields for reduced-starch diets with and without amylase addition, in agreement with the present trial.

Milk protein content was greater for NS– than for RS– ( $P < 0.04$ ) and RS+ ( $P < 0.01$ ). Greater milk protein content for normal-starch than for reduced-starch diets was also reported by Batajoo and Shaver (1994) and Gencoglu et al. (2010). Reduced milk protein content for cows fed reduced-starch compared with normal-starch diets may be related to a lower intake of starch reducing ruminal MCP production (Oba and Allen, 2003). Alternatively, reductions in starch digested postruminally for cows fed reduced-starch compared with normal-starch diets may have caused the observed reductions in milk protein content mediated through changes in arterial insulin concentrations (Rius et al., 2010). Furthermore, in the present trial, reduced RUP may have contributed to lower MP flow, and thus the decrease in milk protein content for the RS diets compared with the NS diet. Gencoglu et al. (2010) reported greater milk protein content for cows fed a RS+ than a RS– diet, possibly in response to greater ruminal starch digestibility increasing ruminal MCP production (Oba and Allen, 2003). In the present trial, exogenous amylase addition to the RS diet did not affect ( $P > 0.10$ ) milk protein percentage. We cannot explain the lack of response to exogenous amylase addition for milk

protein content in this trial compared with Gencoglu et al. (2010). Milk protein yield was greater for NS– than RS– and RS+ ( $P < 0.01$ ) in relationship to the greater milk yield and protein content for cows fed the NS– diets compared with cows fed the RS diets. Milk lactose content tended to be greater ( $P < 0.06$ ) for NS– than for RS– and RS+, but differences were small (5.00 vs. 4.94%) and likely not of much biological or economic significance.

The MUN concentrations were greater ( $P < 0.01$ ) for cows fed the RS diets compared with the NS– diet. Greater MUN for RS diets compared with the NS– diet may have been related to a lower intake of starch reducing ruminal MCP production (Oba and Allen, 2003) along with a greater RDP for the RS diets (NRC, 2001). Intraruminal dosing with starch decreased ruminal ammonia concentrations in lactating dairy cows more than dosing with NDF (Hristov et al., 2005). Gencoglu et al. (2010) reported a reduced MUN content for cows fed a RS+ compared with a RS– diet, possibly in response to greater ruminal starch digestibility increasing ruminal MCP production (Oba and Allen, 2003). In the present trial, exogenous amylase addition to the RS diet did not affect ( $P > 0.10$ ) MUN concentration. We cannot explain the lack of response to exogenous amylase addition for MUN concentration in this trial compared with Gencoglu et al. (2010). Least squares means by week on treatment for MUN (mg/dL) are presented in Figure 2; week and week  $\times$  treatment interaction effects ( $P < 0.01$ ) and treatment effects ( $P < 0.03$ ) were observed during wk 1 to 7. In agreement with Gencoglu et al.



**Figure 2.** Effect of treatment on MUN (mg/dL) covariate-adjusted least squares means by week on treatment. Treatments were normal-starch diet with no amylase added to TMR (NS-), reduced-starch diet with no amylase added to TMR (RS-), and reduced-starch diet with amylase added to TMR (RS+). Week and week  $\times$  treatment interaction effects ( $P < 0.01$ ); SEM = 0.2. Treatment effect ( $P < 0.03$ ) wk 1-7.

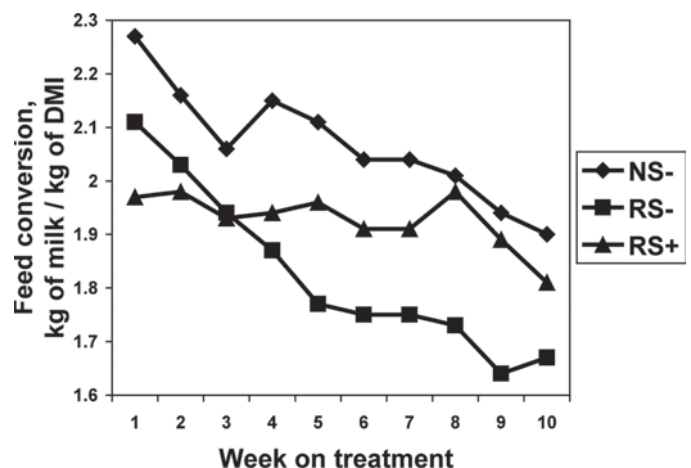
(2010), weekly MUN results suggest that 2 wk were required for adaptation to the RS diets.

Treatment effects on covariate-adjusted least squares means for BW, BCS, and feed conversion, and unadjusted means for BW change and estimated diet energy concentrations are presented in Table 6. Body weight, BW change, and BCS were unaffected ( $P > 0.10$ ) by treatment. Feed conversion (kg of milk/kg of DMI) was 10% greater on average for cows fed NS- than for those fed RS- and RS+ ( $P < 0.01$  and  $P < 0.03$ , respectively), and tended to be 6% greater for cows fed RS+ than RS- ( $P < 0.09$ ). Greater feed conversions for NS- compared with the RS diets were likely related to the greater energy concentration that was estimated for the NS diet. Least squares means by week on treatment for feed conversion (kg of milk/kg of DMI) are presented in Figure 3; week and week  $\times$  treatment ( $P < 0.02$ ) interaction effects and treatment effects ( $P < 0.02$ ) were observed during wk 1 and wk 4 to 10. The FCM, SCM, and ECM feed conversions for the NS- versus RS- comparison ( $P < 0.01$ ) were similar to the actual milk feed conversion results. However, milk component-corrected feed conversions were only numerically ( $P > 0.10$ ) greater for RS+ compared with RS-. Estimated diet energy content (Mcal of  $NE_L$ /kg of DM), calculated using ECM, BW, BW change, and DMI data, was greater for cows fed NS- than for cows fed RS- ( $P < 0.01$ ) and RS+ ( $P < 0.05$ ). The diet  $NE_L$  concentration calculated in this manner was decreased by 9% for RS- compared with NS- in the present trial versus a 4% reduction for the RS- diet in the trial of

Gencoglu et al. (2010). Inherently lower NDF digestibility (Firkins, 1997) and energy value (NRC, 2001) for WM than for soy hulls may explain this difference between trials. Gencoglu et al. (2010) reported that diet  $NE_L$  calculated in this manner was 12% greater for RS+ than for RS-, but in the present trial the numerical change for the RS+ diet was only +2%. We have no explanation for the difference in calculated diet  $NE_L$  response to exogenous amylase addition between the 2 trials; the nutrient composition and particle size of the corn silage and DGSC fed in both trials were similar. The main dietary difference between the 2 trials appears to be the sources of nonforage fiber (WM and WCS vs. soy hulls) that were used for replacement of starch. Research to determine possible interactions between source of nonforage fiber and exogenous amylase addition in reduced-starch diets appears warranted.

## CONCLUSIONS

Feeding a reduced-starch diet formulated by partially replacing corn grain and soybean meal with a wheat middlings and whole cottonseed mixture compared with a normal-starch diet without addition of exogenous amylase to either diet resulted in the following: greater intake of DM (as % of BW), NDF, and CP but lower starch intakes; trend for reduced milk yield and decreased protein percentage and yield; greater MUN; and decreased milk and component-corrected feed conversions. Addition of exogenous amylase to the reduced-starch diet resulted in the following: re-



**Figure 3.** Effect of treatment on feed conversion (kg of milk/kg of DMI) covariate-adjusted least squares means by week on treatment. Treatments were normal-starch diet with no amylase added to TMR (NS-), reduced-starch diet with no amylase added to TMR (RS-), and reduced-starch diet with amylase added to TMR (RS+). Week and week  $\times$  treatment interaction ( $P < 0.02$ ) effects; SEM = 0.04. Treatment effect ( $P < 0.02$ ) wk 1 and wk 4-10.



**Table 6.** Effect of treatment on covariate adjusted least squares means for BW, BCS, and feed conversion, and unadjusted means for BW change and estimated diet energy concentrations

Item	Treatment <sup>1</sup>				SEM	Treatment	P <		
	NS-	RS-	RS+	NS- vs. RS-			NS- vs. RS+	RS- vs. RS+	
BW, <sup>2</sup> kg	732	733	725	5	0.46	—	—	—	
BW change, <sup>3</sup> kg/d	0.02	0.12	-0.07	0.09	0.32	—	—	—	
BCS	2.67	2.73	2.71	0.05	0.73	—	—	—	
Feed conversion <sup>3</sup>									
kg of milk/kg of DMI <sup>4</sup>	2.07	1.83	1.93	0.04	0.01	0.01	0.03	0.09	
kg of 3.5% FCM/kg of DMI	1.99	1.78	1.84	0.04	0.01	0.01	0.02	0.26	
kg of SCM/kg of DMI <sup>5</sup>	1.84	1.63	1.68	0.03	0.01	0.01	0.01	0.31	
kg of ECM/kg of DMI <sup>6</sup>	1.98	1.75	1.82	0.04	0.01	0.01	0.01	0.20	
Estimated diet energy content, Mcal/kg of DM <sup>7</sup>	1.82	1.66	1.69	0.04	0.03	0.01	0.05	0.55	

<sup>1</sup>Treatments were normal-starch diet with no amylase added to TMR (NS-), reduced-starch diet with no amylase added to TMR (RS-), and reduced-starch diet with amylase added to TMR (RS+).

<sup>2</sup>Week effect ( $P < 0.01$ ).

<sup>3</sup>BW change was determined by regression of treatment period BW measurements over time.

<sup>4</sup>Week  $\times$  treatment interaction ( $P < 0.02$ ).

<sup>5</sup>SCM calculated according to NRC (2001) equations.

<sup>6</sup>ECM calculated according to NRC (2001) equations.

<sup>7</sup>Calculated by summing the Mcal of NE<sub>L</sub> from milk production, required for maintenance, and in BW change (NRC, 2001) and then dividing the sum by DMI.

duced intake of DM (as % of BW) and starch; trend for greater actual milk feed conversion. Reduced conversion of feed to milk and components for dairy cows fed reduced-starch diets creates an economic concern for nutritionists wanting to use this formulation strategy to reduce ration cost per unit of dry matter. Addition of exogenous amylase to a reduced-starch diet was of minimal benefit in this study.

## ACKNOWLEDGMENTS

Appreciation is extended to Dutch State Mines (Heerlen, the Netherlands) for partial funding for this project; Mike Peters and Valerie Schutzkus and the staff at the University of Wisconsin Madison Campus Dairy Cattle Center for animal care and trial management; Peter Crump of the University of Wisconsin CALS Computer Consulting Lab for assistance with statistical analysis of the data; and Al Schultz of Vita Plus Corp. (Madison, WI) for premix formulation.

## REFERENCES

- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447-1462.
- Allen, M. S. 2000. Effects of short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598-1624.
- Allen, M. S., B. J. Bradford, and M. Oba. 2009. The hepatic oxidation theory of the control of feed intake and its application to ruminants. *J. Anim. Sci.* 87:3317-3334.
- AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem. Arlington, VA.
- Arieli, A. 1998. Whole cottonseed in dairy cattle feeding: A review. *Anim. Feed Sci. Technol.* 72:97-110.
- Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim. Feed Sci. Technol.* 67:319-338.
- Baker, S., and T. Herrman. 2002. Evaluating particle size. MF-2051. Kansas State Univ. Coop Ext. Serv., Manhattan, KS.
- Batajoo, K. K., and R. D. Shaver. 1994. Impact on nonfiber carbohydrate on intake, digestion, and milk production by dairy cows. *J. Dairy Sci.* 77:1580-1588.
- Batajoo, K. K., and R. D. Shaver. 1998. In situ dry matter, crude protein, and starch degradabilities of selected grains and by-product feeds. *Anim. Feed Sci. Technol.* 71:165-176.
- Beckman, J. L., and W. P. Weiss. 2005. Nutrient digestibility of diets with different fiber to starch ratios when fed to lactating dairy cows. *J. Dairy Sci.* 88:1015-1023.
- Bernard, J. K., H. E. Amos, M. A. Froetschel, and J. J. Evans. 1988. Influence of supplemental energy and protein on protein synthesis and crude protein reaching the abomasum. *J. Dairy Sci.* 71:2658-2669.
- Bernard, J. K., and W. W. McNeill. 1991. Effect of high fiber energy supplements on nutrient digestibility and milk production of lactating dairy cows. *J. Dairy Sci.* 74:991-995.
- Coppock, C. E., J. K. Lanham, and J. L. Horner. 1987. A review of the nutritive value and utilization of whole cottonseed, cottonseed meal and associated by-products by dairy cattle. *Anim. Feed Sci. Technol.* 18:89-129.
- Ferreira, G., and D. R. Mertens. 2005. Chemical and physical characteristics of corn silages and their effects on in vitro disappearance. *J. Dairy Sci.* 88:4414-4425.
- Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. *J. Dairy Sci.* 80:1426-1437.
- Gencoglu, H., R. D. Shaver, W. Steinberg, J. Ensink, L. F. Ferraretto, S. J. Bertics, J. C. Lopes, and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. *J. Dairy Sci.* 93:723-732.
- Hall, M. B., W. H. Hoover, J. P. Jennings, and T. K. M. Webster. 1999. A method for partitioning neutral detergent-soluble carbohydrates. *J. Sci. Food Agric.* 79:2079-2086.

- Harrison, G. A., and J. M. Tricarico. 2007. Case study: Effects of an *Aspergillus oryzae* containing alpha amylase on lactational performance in commercial dairy herds. *Prof. Anim. Sci.* 23:291–294.
- Hristov, A. N., T. A. McAllister, and K. J. Cheng. 1998. Stability of exogenous polysaccharide-degrading enzymes in the rumen. *Anim. Feed Sci. Technol.* 76:161–168.
- Hristov, A. N., J. K. Ropp, K. L. Grandeen, S. Abedi, R. P. Etter, A. Melgar, and A. E. Foley. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *J. Anim. Sci.* 83:408–421.
- Jung, S., and K. Vogel. 2008. Determination of Ronozyme RumiStar Alpha-Amylase Activity in Feed and Per Se Samples. DSM Nutritional Products Ltd. Regulatory Report No 2500706. DSM Nutritional Products Ltd., Basel, Switzerland.
- Klingerman, C. M., W. Hu, E. E. McDonell, M. C. Der Bedrosian, and L. Kung Jr. 2009. An evaluation of exogenous enzymes with amylase activity for dairy cows. *J. Dairy Sci.* 92:1050–1059.
- Kononoff, P. J., A. J. Heinrichs, and D. R. Buckmaster. 2003. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. *J. Dairy Sci.* 86:1858–1863.
- Lopes, J. C., R. D. Shaver, P. C. Hoffman, M. S. Akins, S. J. Bertics, H. Gencoglu, and J. G. Coors. 2009. Type of corn endosperm influences nutrient digestibility in lactating dairy cows. *J. Dairy Sci.* 92:4541–4548.
- Mohamed, O. E., L. D. Satter, R. R. Grummer, and F. R. Ehle. 1988. Influence of dietary cottonseed and soybean on milk production and composition. *J. Dairy Sci.* 71:2677–2688.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Oba, M., and M. S. Allen. 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *J. Dairy Sci.* 86:195–207.
- Rius, A. G., J. A. D. R. N. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M. L. McGilliard, B. J. Bequette, R. M. Akers, and M. D. Hanigan. 2010. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. *J. Dairy Sci.* 93:3114–3127.
- SAS Institute. 2004. SAS/STAT 9.1 User's Guide. Version 9.1 ed. SAS Institute Inc., Cary, NC.
- Shaver, R. D. 2008. Coping with high corn prices: Low starch diets and lactation performance by dairy cows. Pages 128–133 in Proc. 6th Mid-Atlantic Nutr. Conf. Timonium, MD. Accessed June 8, 2009. <http://www.manc.umd.edu/documents/Proceedings%20of%20the%206th%20Mid-Atlantic%20Nutrition%20Conference%20-%202008.pdf>.
- Tricarico, J. M., J. D. Johnston, K. A. Dawson, K. C. Hanson, K. R. McLeod, and D. L. Harmon. 2005. The effects of an *Aspergillus oryzae* extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. *Anim. Sci.* 81:365–374.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy-cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501.