



## Performance and amino acid utilization of early lactation dairy cows fed regular or reduced-fat dried distillers grains with solubles

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### ABSTRACT

The objective of this study was to evaluate lactation response and AA utilization of early lactation cows fed 2 types of dried distillers grains with solubles (DG): regular (DDGS) or reduced-fat (RFDGS). Thirty-six Holstein cows  $19.7 \pm 2.6$  d in milk at the start of the experiment were used in a randomized complete block design for 14 wk including a 2-wk covariate period. Treatments consisted of the following diets: 1) control (CON) diet containing 0% DG; 2) diet containing 22% DDGS; and 3) diet containing 20% RFDGS. Distillers grains replaced soybean meal, expeller soybean meal, and soyhulls from the CON diet. Diets were formulated to be similar in crude protein, ether extract, neutral detergent fiber, and net energy for lactation concentrations. Dry matter intake (24.7 kg/d) and milk yield (39.3 kg/d) were similar for all diets. Milk fat and lactose percentages were unaffected by diets; however, protein percentage was greater for cows fed the DG diets compared with the CON diet. Consequently, milk protein yield was also greater for the DG diets compared with CON. Milk urea nitrogen decreased for cows fed DG diets and averaged 11.8, 10.9, and 10.1 mg/dL, respectively, for CON, DDGS, and RFDGS. Feed efficiency tended to be greater and N efficiency was greater for cows fed DG compared with CON. Body weight (711 kg), body weight change (+0.49 kg/d), and body condition score (3.36) were similar for all diets, but cows fed CON tended to gain more body condition (+0.14) than cows fed DG diets. Amino acid utilization was evaluated at the peak of milk production corresponding to wk 9 of lactation. Arterial Lys concentration was lower with DG diets (70.4, 58.6, and 55.8  $\mu\text{M}/\text{L}$ ). Cows fed DG had greater arterial Met concentration (21.3  $\mu\text{M}$ ) compared with CON (14.9  $\mu\text{M}$ ). Arterio-venous difference of Lys was similar across diets, whereas that of Met was greater for the DG diets compared with the CON diet (10.3 vs. 13.0  $\mu\text{M}/\text{L}$ ). Extraction efficiency of Lys by the mammary gland was greater for DG diets

than for CON (76.1 vs. 65.4%). Mammary uptake of Lys (2.56 g/kg of milk) was similar for all diets, and the uptake of Met tended to increase in cows fed DG diets. Plasma glucose, triglyceride, and total cholesterol were unaffected by treatment; however, cows fed DG diets had lower  $\beta$ -hydroxybutyrate and tended to have lower nonesterified fatty acid concentrations than cows fed the CON diet. Despite the apparent deficiency of Lys, milk protein percentage was increased in cows fed DG diets.

**Key words:** amino acid, plasma metabolite, early lactation dairy cow, dried distillers grains with solubles

### INTRODUCTION

During early lactation, the large increase in milk production causes a great metabolic demand for AA required for milk protein synthesis. Improving the supply of metabolizable AA can be provided through increased concentrations of protein in the diet or decreased protein degradability in the rumen. The most effective way to increase the supply of MP to the duodenum is to maximize microbial protein synthesis; however, dietary manipulations are virtually ineffective in altering the AA composition of microbial protein in the rumen (Clark et al., 1992). Therefore, the source of RUP and its AA profile are important for optimal supply of potentially limiting AA.

Dried distillers grains are a good source of digestible RUP (Kononoff et al., 2006; Boucher et al., 2009; Mjoun et al., 2010a), but Lys is often indicated as a potential limiting AA for milk production and protein synthesis. Recent studies (Anderson et al., 2006; Janicek et al., 2008) evaluating dried distillers grains with solubles (DG) as a source of protein have shown no negative effect on milk production and protein synthesis. Dried distillers grains with solubles produced by recent technology may be of higher protein quality than commonly thought. In fact, there are indications in the literature that both dietary Lys concentration (Spiels et al., 2002; Schingoethe et al., 2009; Mjoun et al., 2010a) and AA available for absorption (Boucher et al., 2009; Mjoun et al., 2010a) are greater than values used in most protein evaluation systems (NRC, 2001).

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Few studies have evaluated DG as a source of protein in early lactation (Palmquist and Conrad, 1982; Grings et al., 1992). In addition, to our knowledge, there are no published data on AA utilization by the mammary gland of early lactation cows fed DG as the primary protein supplement. We hypothesized that the high demand for metabolizable AA, and specifically for Lys, to synthesize milk in early lactation may be limited when feeding DG as the primary protein source in combination with other corn products. A study was conducted using early lactation cows entering peak of lactation to assess 2 different types of DG, regular dried distillers grains with solubles (**DDGS**) and reduced-fat dried distillers grains with solubles (**RFDGS**) compared with a soybean-based diet. The objectives were to evaluate milk production, milk composition, feed and N efficiency, plasma metabolites, and the mammary uptake of AA.

## MATERIALS AND METHODS

### Animals and Diets

Thirty-six Holstein cows (24 multiparous;  $726 \pm 65$  kg of BW and 12 primiparous;  $583 \pm 47$  kg of BW) were blocked by DIM and parity. Cows were individually introduced to the experiment at  $19 \pm 2$  DIM over a 6-mo period from April to September 2008. Treatments (Table 1) were randomly assigned to cows within a block. Dietary treatments were one of the following: 1) control containing 0% DG (**CON**); 2) 22% inclusion of DDGS; and 3) 20% inclusion of RFDGS. The rate of inclusion of the 2 types of DG (on a DM basis) was calculated to allow for similar contribution to the total dietary CP from either DG. Actual contributions were 39.6 and 38.6% of dietary CP, respectively, for the DDGS and RFDGS diets. In each diet, DDGS and RFDGS replaced soybean meal, expeller soybean meal, and soyhulls from the CON diet, whereas the proportion of forage and corn remained constant across diets. All diets were formulated to contain 25% corn silage, 25% alfalfa hay, and 50% concentrate (DM basis). Extruded soybeans were added to all diets at 3.5% on a DM basis to provide additional energy. A source of rumen inert fat (Energy Booster 100, Milk Specialties, Dundee, IL) was added to the CON and 20% RFDGS diets at 2% to balance for ether extract (**EE**). Diets were formulated (NRC, 2001) to be similar in nutrients (17.1% CP, 10.0% RDP, 7.1% RUP, 5.5% EE, 19% ADF, 30% NDF, and 1.63 Mcal/kg of  $NE_L$ ). During the covariate period (2 wk), cows were individually fed a common early lactation diet, followed by the experimental diets as TMR for 12 weeks starting at  $34 \pm 4$  DIM. Cows were fed for ad libitum intake once daily

**Table 1.** Ingredient composition of diets fed during the experiment

Ingredient	Treatment <sup>1</sup>		
	CON	DDGS	RFDGS
Corn silage	25.0	25.0	25.0
Alfalfa hay	25.0	25.0	25.0
Ground corn	22.5	22.5	22.5
DDGS	0	22.0	0
RFDGS	0	0	20.0
Soybean meal, 44%	6.4	0	0
Expeller soybean meal <sup>2</sup>	5.0	0	0
Soybean hulls	8.5	0	0
Extruded soybeans	3.5	3.5	3.5
Rumen inert fat <sup>3</sup>	2.0	0	2.0
Limestone	0.50	0.80	0.80
Dicalcium phosphate	0.37	0	0
Salt	0.40	0.40	0.40
Magnesium oxide	0.16	0.16	0.16
Sodium bicarbonate	0.47	0.47	0.47
Dairy premix <sup>4</sup>	0.17	0.17	0.17
Vitamin E (20,000 IU)	0.03	0.03	0.03

<sup>1</sup>CON = control diet containing a blend of soybean products; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>SoyPlus (West Central Soy, Ralston, IA).

<sup>3</sup>Energy Booster 100 (Milk Specialties, Dundee, IL).

<sup>4</sup>Contained: 10% Mg; 2.6% Zn; 1.7 mg/kg Mn; 4,640 mg/kg Fe; 4,712 mg/kg Cu; 396 mg/kg I; 119 mg/kg Co; 140 mg/kg Se; 2,640,000 IU/kg vitamin A; 528,000 IU/kg vitamin D<sub>3</sub>; and 10,560 IU/kg vitamin E (Land O'Lakes, St. Paul, MN).

at 0800 h. Variation in DM concentrations of forages (alfalfa and corn silage) was monitored weekly and as-fed diet composition was adjusted accordingly. Water was added daily to the forage mixes to achieve a dietary DM concentration of 60%. All procedures for this study were approved by the South Dakota State University Animal Care and Use Committee.

### Sample Collection and Analyses

Corn silage, alfalfa hay, and concentrate mixes were sampled weekly at the time of feeding, dried at 55°C for 48 h, and composited on a monthly basis. Individual ingredients were sampled during the preparation of the concentrate mixes. All feed samples were ground to pass through a 2-mm screen of a Wiley mill (model 3, Arthur H. Thomas Co., Philadelphia, PA) and then reground through a 1-mm screen of an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). Feed samples were analyzed for DM (105°C for 24 h). Concentrations of NDF (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were determined sequentially using an Ankom fiber analyzer (Ankom Technology Corp., Fairport, NY). Sodium sulfite and  $\alpha$ -amylase were added during NDF extraction. Crude protein was determined according to AOAC (2006);

method 968.06) using an automated N combustion analyzer (Elementar, Analysensysteme GmbH, Hanau, Germany). Ether extract was determined using an Ankom<sup>X110</sup> extractor with diethyl ether as the solvent (method 920.39; AOAC, 2006). Ash, Ca, P, K, Mg, and S were analyzed according to AOAC procedures (AOAC, 2006). Concentration of starch in concentrate mixes, forages, and concentrate ingredients were analyzed according to the procedure of McCleary et al. (1994) using a starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland; method 996.11; AOAC, 2006). Samples of corn silage, alfalfa hay, DDGS, RFDGS, and concentrate mixes were analyzed for AA composition according to AOAC (method 982.30 E [a,b,c]; AOAC, 2006). Chemical composition of diets was calculated based on analysis of individual forages and concentrate mixes and the dietary proportions of each ingredient in the ration. Particle size distributions were evaluated for all diets as TMR using the Penn State Particle Separator (**PSPS**) on both an as-fed and DM basis.

The amounts of feed offered and refused were measured daily. Cows were weighed on 2 consecutive days and evaluated for BCS by 3 individuals at wk 0, 4, 8, and 12 of the study (Wildman et al., 1982), approximately 3 h postfeeding. Cows were milked twice daily at 0600 and 1800 h, and individual yields were recorded at each milking. Milk samples were collected weekly from 4 consecutive milkings each week of the experimental period. Daily composites of milk samples based upon yield were analyzed by Heart of America DHIA Laboratory (Manhattan, KS) according to approved procedures of AOAC (2006). Milk true protein, fat, and lactose were determined by mid infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN). Concentration of MUN was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and somatic cells were counted using a flow cytometer laser (Somacount 500, Bentley Instruments).

Blood samples from the coccygeal artery and caudal superficial epigastric vein were collected by venipuncture 3 h postfeeding during 2 consecutive days at wk 0, 4, 8, and 12 of the experiment. Samples were collected into vacutainers (Becton Dickinson and Co., Franklin Lakes, NJ) containing either K-EDTA [for glucose, BHBA, NEFA, triglycerides (**TG**), total cholesterol] or lithium heparin [for AA, plasma urea N (**PUN**), 3-methylhistidine, and ornithine]. Plasma was obtained by centrifuging at  $2,400 \times g$  for 20 min and was then stored at  $-20^{\circ}\text{C}$ . Individual samples were composited into one pair of arterial and venous plasma for each cow.

Arterial concentrations of glucose, BHBA, NEFA, TG, total cholesterol, and PUN were determined using commercial enzymatic kits: Pointe Scientific Inc. (Canton, MI) for glucose, BHBA, TG, and total cholesterol; Wako Chemicals (Richmond, VA) for NEFA; and Stanbio Laboratory Inc. (Boerne, TX) for PUN. All determinations were made in triplicate using a microplate spectrophotometer (Varian Inc., Walnut Creek, CA). Colorimetric methods were used to determine the following: glucose using glucose oxidase (cat. no. G7521; Trinder, 1969); BHBA using  $\beta$ -hydroxybutyrate dehydrogenase and diaphorase (cat. no. H7587-58; Williamson et al., 1962); NEFA using NEFA-C kit (Johnson and Peters, 1993); TG using lipase and glycerophosphate oxidase (cat. no. T7532; Fossati and Lorenzo, 1982); total cholesterol using cholesterol esterase and oxidase (cat. no. C7510; Allain et al., 1974); and PUN using diacetylmonoxime (Stanbio Urea N Kit 580). Arterial and venous samples for each cow were analyzed for AA via HPLC (model 1100, Agilent Technologies Inc., Palo Alto, CA) with a PCX 5200 postcolumn derivatizer (Pickering Laboratories Inc., Mountain View, CA) using ninhydrin as described by Mondina et al. (1972), Pickering (1989), and Grunau and Swiader (1992).

### Parameter Calculations

Energy balance (**EB**) was calculated using the following equation (NRC, 2001):  $\text{EB} = (\text{DMI} \times \text{diet NE}_L) - [(0.08 \times \text{BW}^{0.75}) + (\text{milk NE}_L \times \text{milk yield})]$  with  $\text{NE}_L$  (Mcal/d) =  $\text{milk yield (kg/d)} \times [(0.0929 \times \% \text{ fat}) + (0.0563 \times \% \text{ protein}) + (0.0395 \times \% \text{ lactose})]$ .

The Fick principle was applied to estimate mammary plasma flow (**MPF**) using Phe and Tyr as internal markers (Mephram, 1982) with allowance for a 3.5% contribution from bloodborne proteins according to Cant et al. (1993) and with the exception that free milk Phe and Tyr values were neglected.

$$\text{MPF} = [(\text{milk Phe} + \text{Tyr}) \times 0.965 / [\text{arterio-venous (AV) difference of (Phe} + \text{Tyr)}]]$$

Estimates of Phe and Tyr concentrations in milk of 4.9 and 5.1 g/100 g of milk protein, respectively, were used (Jacobson et al., 1970). Extraction efficiency and mammary uptake of AA were calculated as follows:

$$\text{Extraction efficiency} = \frac{\text{AV difference}}{\text{arterial concentration}} \times 100;$$

$$\text{Mammary uptake} = \text{AV difference} \times \text{MPF}.$$

Amino acids were classified into essential amino acids (**EAA**) and nonessential amino acids (**NEAA**) based on their importance for milk protein synthesis (Clark et al., 1978). The EAA were His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; NEAA were Ala, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr; and branched-chain amino acids (**BCAA**) were Ile, Leu, and Val. Total amino acids (**TAA**) was calculated as the sum of EAA and NEAA.

### Statistical Analyses

All variables were analyzed using the MIXED procedure (SAS Institute, 2001) as a randomized complete block design. Week was the repeated measure for all production variables, while every fourth week was the repeated measure for all blood metabolites, BW, BCS, and EB values. Pretreatment values collected during the 2-wk covariate period for all measurements were used as covariates in the statistical model except for plasma AA, ornithine, citrulline, and 3-methylhistidine, where no covariate was used. Because plasma AA, ornithine, citrulline, and 3-methylhistidine were evaluated at a single time point (wk 4), data were analyzed using the same model except week and treatment  $\times$  week were not included in the model. The model included fixed effects of treatment, week, and interaction of treatment  $\times$  week and block as a random effect. The model was

$$Y_{ijk} = \text{Cov} + T_i + W_j + B_k + (T_i \times W_j) + e_{ijk},$$

where  $Y_{ijk}$  = dependent variable, Cov = effect of covariate,  $T_i$  = effect of treatment,  $W_j$  = effect of week,  $B_k$  = effect of block,  $T_i \times W_j$  = interaction between treatment and week, and  $e_{ijk}$  = residual error.

For each variable, the covariance structure corresponding to the lowest value given by the Akaike's information criterion was selected (Littell et al., 2006). Interactions with  $P > 0.15$  were sequentially dropped from the model. Significant interactions are identified in the results. Preplanned orthogonal contrasts were used to test the following comparisons: 1) CON vs. DG diets (DDGS and RFDGS) and 2) DDGS vs. RFDGS. Data were reported as least squares means. Statistical significance and tendencies were declared at  $P \leq 0.05$  and  $0.05 < P \leq 0.10$  respectively.

## RESULTS

### Diet Composition

Chemical composition of the concentrate mixes, forages, and DG sources are presented in Table 2 and chemical composition of diets is presented in Table 3.

Slight variations were observed in the nutrient compositions throughout the experimental period. Consistent with the formulation of experimental diets, all diets were similar in DM, CP, NDF, EE, starch, and mineral concentrations. Concentration of ADF was greater in the CON compared with DG diets. Replacing soybean products with either source of DG resulted in an increase in the concentrations of Leu, Met, and His, whereas the concentrations of Arg, Ile, Lys, and Trp decreased. Other EAA remained unchanged for all diets. Total EAA (**TEAA**) was similar for all diets ( $37.6 \pm 0.07\%$  of CP). Lysine as a percentage of TEAA decreased from  $11.9$  to  $9.4 \pm 0.18$ , respectively, for CON and DG diets. Conversely, Met as a percentage of TEAA increased from  $3.6$  to  $4.3 \pm 0.01$ , respectively, for CON and DG diets.

Particle size distribution was evaluated using PSPS and is presented on an as-fed and DM basis in Table 4. Because all diets contained similar proportion of forages, the proportion of particles with a diameter  $>19$  mm was similar for all treatments; however, as DG replaced soybean feedstuffs in the diet, the proportion of particles  $<1.18$  mm was increased in DG diets compared with CON diet. Particle size distributions of all diets followed recommendations for lactating cows (Heinrichs and Kononoff, 2002).

### Intake, Milk Production, and Composition

Responses of DMI, milk production, and milk composition to feeding DG are presented in Table 5 and Figure 1. No treatment effect was observed for either DMI or CP intake. Averages of DMI and CPI were  $24.7$  and  $4.3$  kg/d, respectively. Intakes of Lys and Met (data not shown) averaged  $193$ ,  $153$ , and  $150$  g/d; and  $59$ ,  $69$ , and  $69$  g/d, for cows fed CON, DDGS, and RFDGS diets, respectively. Milk yield was not different among treatments and averaged  $39.3 \pm 0.46$  kg/d for the 12-wk experiment. Yields of milk fat and lactose were similar for all diets and averaged  $1.36$  and  $1.95$  kg/d, respectively, but yield of protein increased  $0.075$  kg/d ( $P = 0.05$ ) for cows fed DG diets compared with CON. Similarly, treatment had no effect on milk fat and lactose percentages, but milk protein percentage increased ( $P = 0.03$ ) for cows fed DG diets. Feed efficiency, expressed as ECM/DMI, tended to increase ( $P = 0.09$ ) in cows fed DG. Nitrogen efficiency calculated as milk N (kg/d)/N intake (kg/d) increased ( $P = 0.01$ ) with DG diets. A tendency ( $P = 0.07$ ) for an interaction effect of treatment  $\times$  week was observed for milk protein because cows fed CON produced a greater milk protein percentage during the first 3 wk but it was lower compared with cows fed DG diets during wk 4 to 12 of the experiment (Figure 1). Milk urea N decreased

**Table 2.** Chemical composition (% of DM unless otherwise noted) of concentrate mixes, dried distillers grains with solubles (DDGS and RFDGS), corn silage, and alfalfa hay (DM basis)

Item	Concentrate mix <sup>1</sup>					Corn silage	Alfalfa hay
	CON	DDGS	RFDGS	DDGS	RFDGS		
DM, % of diet	88.4	88.1	87.6	87.7	87.5	40.7	91.9
CP	21.0	20.4	20.9	31.3	34.0	7.9	20.5
NDF	20.1	17.9	20.7	31.2	42.8	45.9	41.5
ADF	10.5	4.9	5.7	9.2	12.5	22.9	29.1
Ether extract	8.5	8.4	8.6	10.8	3.5	3.3	2.4
Starch	30.6	34.7	33.8	8.9	5.6	25.2	2.3
Ash	7.7	6.8	6.6	4.5	5.3	4.5	10.3
Ca	1.10	0.82	0.83	0.05	0.06	0.31	1.33
P	0.49	0.51	0.52	0.79	0.84	0.26	0.27
Mg	0.40	0.41	0.42	0.31	0.34	0.27	0.31
K	1.06	0.75	0.74	0.94	1.01	0.85	2.32
S	0.20	0.37	0.42	0.60	0.74	0.70	0.23
AA, % of CP							
Ala	4.67	6.62	6.66	6.93	7.12	6.32	4.53
Arg	6.05	4.76	4.71	4.74	4.69	2.41	3.89
Asp	9.84	7.17	7.19	6.37	6.85	5.83	9.37
Cys	1.44	1.73	1.77	1.93	1.82	1.24	1.02
Glu	15.78	15.62	15.72	13.04	14.38	10.63	7.97
Gly	4.41	3.98	4.00	4.11	4.11	3.65	4.14
His	2.77	2.90	2.92	3.00	3.05	2.01	1.89
Ile	4.34	3.96	3.98	4.04	4.32	3.20	3.92
Leu	8.44	11.41	11.58	11.74	12.53	7.92	6.56
Lys	5.28	3.48	3.32	3.48	3.22	2.72	4.62
Met	1.33	1.82	1.84	2.04	1.99	1.47	1.30
Phe	4.65	4.59	4.67	4.52	4.73	3.61	4.14
Pro	5.17	6.97	6.89	8.63	7.43	5.03	4.75
Ser	3.75	3.68	3.79	4.07	4.32	2.62	2.73
Tau	0.23	0.17	0.16	0.00	0.03	0.40	0.23
Thr	3.39	3.42	3.48	3.78	3.80	2.80	3.28
Trp	0.95	0.74	0.77	0.81	0.75	0.53	0.81
Tyr	3.21	3.16	3.25	3.48	3.56	2.27	2.63
Val	4.76	4.91	5.00	5.30	5.34	4.22	4.69
TEAA <sup>2</sup>	42.0	42.0	42.3	43.4	44.4	30.9	35.1
AA N, % of total N	90.2	91.1	91.7	92.0	94.0	68.9	72.5
Lys, % of TEAA	12.58	8.28	7.86	8.01	7.25	8.79	13.16
Met, % of TEAA	3.16	4.34	4.36	4.69	4.47	4.75	3.71

<sup>1</sup>CON = control diet containing a blend of soybean products; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>TEAA = total essential amino acids.

in DG diets ( $P < 0.01$ ) and there was a tendency for a decrease ( $P = 0.09$ ) in MUN in cows fed RFDGS compared with DDGS diet. There were no treatment  $\times$  week interactions for the other production variables.

for milk production tended ( $P = 0.09$ ) to be greater for cows fed DG diets ( $67.5 \pm 0.85\%$ ) compared with CON diet (63.1%).

### BW, BCS, and EB

Body weight, BW change, and BCS were not affected by treatment (Table 6); however, there was a tendency ( $P = 0.06$ ) for cows fed the CON diet to gain more body condition (+0.14) by the end of the experimental period compared with cows fed DG diets. Negative energy balance was observed only at wk 0 for both DG diets, whereas EB was positive during subsequent weeks. Energy balance tended ( $P = 0.06$ ) to improve almost 2-fold for cows fed the CON diet compared with cows fed DG diets. Energy efficiency

### Plasma Metabolites

Plasma concentrations of glucose, BHBA, NEFA, TG, total cholesterol, and urea N are presented in Table 7. In general, changes in plasma metabolites over time reflected overall means of the experimental period. Circulating glucose concentrations were similar for all diets ( $62.0 \pm 1.85$  mg/dL). Cows fed either type of DG had lower ( $P = 0.04$ ) concentrations of BHBA than cows fed the CON diet. Similarly, there was a tendency ( $P = 0.08$ ) for CON cows to have greater NEFA concentrations. Total cholesterol concentration tended to increase ( $P = 0.10$ ) in cows fed the DG diets.

**Table 3.** Chemical composition (% of DM unless otherwise noted) and amino acid concentrations of experimental diets<sup>1</sup>

Item	Treatment <sup>2</sup>		
	CON	DDGS	RFDGS
DM, % of diet	61.2 (3.97) <sup>3</sup>	60.6 (3.62)	60.8 (3.60)
CP	17.6 (0.56)	17.4 (0.60)	17.6 (0.56)
RDP, <sup>4</sup> % of CP	58.5	56.6	56.1
RUP, <sup>4</sup> % of CP	41.5	43.4	43.9
Duodenal supply of Lys, <sup>4</sup> % of MP	6.32	5.69	5.60
Duodenal supply of Met, <sup>4</sup> % of MP	1.73	1.86	1.84
Lys:Met ratio	3.63:1	3.04:1	3.04:1
MP balance, <sup>4</sup> g/d	471	470	464
NDF	31.4 (0.91)	30.9 (0.62)	32.7 (1.20)
ADF	18.2 (0.61)	15.4 (0.45)	15.8 (0.71)
Ether extract	5.7 (0.27)	5.6 (0.28)	5.7 (0.12)
Starch	22.2 (1.77)	24.2 (2.46)	23.8 (1.53)
NFC <sup>5</sup>	37.8	38.8	37.0
NE <sub>L</sub> , <sup>4</sup> Mcal/kg	1.62	1.62	1.61
Ash	7.5 (0.44)	7.3 (0.80)	7.0 (0.52)
Ca	0.96 (0.06)	0.81 (0.01)	0.82 (0.04)
P	0.38 (0.01)	0.39 (0.02)	0.39 (0.01)
Mg	0.35 (0.02)	0.36 (0.02)	0.36 (0.01)
K	1.32 (0.09)	1.16 (0.06)	1.16 (0.07)
S	0.19 (0.01)	0.28 (0.01)	0.30 (0.01)
AA, % of CP			
Ala	5.06 (0.15)	6.02 (0.15)	6.04 (0.10)
Arg	4.63 (0.09)	3.95 (0.09)	3.93 (0.03)
Asp	8.77 (0.09)	7.39 (0.09)	7.39 (0.05)
Cys	1.27 (0.02)	1.43 (0.05)	1.45 (0.02)
Glu	12.6 (0.32)	12.5 (0.30)	12.5 (0.20)
Gly	4.16 (0.06)	3.94 (0.07)	3.94 (0.07)
His	2.35 (0.03)	2.42 (0.06)	2.43 (0.01)
Ile	3.94 (0.03)	3.76 (0.06)	3.77 (0.02)
Leu	7.81 (0.13)	9.33 (0.21)	9.41 (0.03)
Lys	4.48 (0.07)	3.57 (0.05)	3.49 (0.04)
Met	1.37 (0.04)	1.60 (0.02)	1.61 (0.02)
Phe	4.26 (0.06)	4.23 (0.09)	4.27 (0.01)
Pro	5.04 (0.10)	5.93 (0.08)	5.89 (0.06)
Ser	3.28 (0.12)	3.18 (0.08)	3.23 (0.10)
Tau	0.27 (0.00)	0.24 (0.02)	0.24 (0.02)
Thr	3.25 (0.07)	3.23 (0.04)	3.26 (0.02)
Trp	0.82 (0.02)	0.71 (0.04)	0.72 (0.02)
Tyr	2.85 (0.07)	2.80 (0.07)	2.85 (0.03)
Val	4.66 (0.11)	4.68 (0.07)	4.73 (0.07)
TEAA <sup>6</sup>	37.5 (0.57)	37.5 (0.64)	37.6 (0.10)
AA, % of total N	80.9 (1.57)	80.9 (1.66)	81.2 (0.46)
Lys, % of TEAA	11.93 (0.11)	9.53 (0.09)	9.28 (0.10)
Met, % of TEAA	3.62 (0.06)	4.28 (0.05)	4.29 (0.05)

<sup>1</sup>Calculated at 50:25:25 of concentrate mix, corn silage, and alfalfa hay, respectively.

<sup>2</sup>CON = control diet containing a blend of soybean products; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>3</sup>Numbers in parentheses represent standard deviation; n = 9 for CP, NDF, ADF, and ash; and n = 3 for ether extract, starch, and amino acids.

<sup>4</sup>Estimated from NRC (2001) using actual feed analysis, in situ data (Mjoun et al., 2010a), and average lactation performance for each treatment.

<sup>5</sup>NFC = 100 - (% NDF + % CP + % ether extract + % ash).

<sup>6</sup>TEAA = total essential amino acids.

Plasma urea N was decreased in cows fed DG diets ( $P < 0.01$ ).

Arterial concentrations of protein metabolism intermediates are shown in Table 8. Ornithine decreased

for cows fed DG diets ( $P = 0.01$ ) compared with cows fed CON. Mammary plasma flow, ammonia, citrulline, 3-methylhistidine, and carnosine concentrations were not affected by treatments.

**Table 4.** Particle distribution using the Penn State Particle Separator (PSPS) of diets containing regular or reduced-fat dried distillers grains with solubles

Item	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
PSPS distribution, % as fed						
>19 mm	10.3	10.0	9.8	0.38	0.40	0.65
8–19 mm	22.8	23.8	24.4	0.39	0.01	0.24
1.18–8 mm	44.3	41.5	37.7	0.48	<0.01	<0.01
Bottom pan	22.6	24.7	28.1	0.47	<0.01	<0.01
PSPS distribution, % as DM						
>19 mm	10.3	10.1	9.9	0.21	0.47	0.66
8–19 mm	18.8	20.0	20.9	0.20	<0.01	0.10
1.18–8 mm	45.8	42.9	38.8	0.32	<0.01	<0.01
Bottom pan	25.1	27.0	30.4	0.32	<0.01	<0.01

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

### Plasma Amino Acids

Arterial plasma AA concentrations are presented in Table 9. Arginine was omitted from the results because of a negative AV difference for some of the animals. As

expected, there were no differences between the 2 types of DG; however, clear differences were observed for all individual EAA except for Thr when comparing CON with DG diets. For DG diets, concentrations of Ile, Lys, and Val decreased, whereas concentrations of His,

**Table 5.** Dry matter intake, milk yield, and milk composition by early lactation cows fed regular or reduced-fat dried distillers grains with solubles

Item	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
Intake, kg/d						
DM <sup>2</sup>	24.8	24.7	24.6	0.51	0.85	0.86
CP <sup>2</sup>	4.3	4.3	4.3	0.09	0.98	0.97
Production, kg/d						
Milk <sup>2</sup>	39.2	38.9	39.8	0.83	0.84	0.45
ECM <sup>3</sup>	38.0	37.8	39.5	1.26	0.68	0.33
FCM <sup>4</sup>	35.7	35.3	37.1	1.33	0.77	0.35
Efficiency						
Feed <sup>5</sup>	1.50	1.57	1.61	0.04	0.09	0.47
N <sup>6</sup>	24.5	26.9	26.5	0.65	0.01	0.63
Milk composition						
Fat, %	3.63	3.24	3.57	0.15	0.24	0.14
Fat, kg/d	1.33	1.34	1.40	0.08	0.66	0.54
Protein, <sup>2,7</sup> %	2.82	2.88	2.89	0.03	0.03	0.73
Protein, <sup>2</sup> kg/d	1.07	1.15	1.14	0.03	0.05	0.82
Lactose, <sup>2</sup> %	4.90	4.99	4.96	0.04	0.10	0.61
Lactose, <sup>2</sup> kg/d	1.94	1.94	1.96	0.04	0.85	0.74
Total solids, <sup>2</sup> %	12.3	12.0	12.4	0.19	0.79	0.12
Total solids, <sup>2</sup> kg/d	4.73	4.70	4.90	0.14	0.66	0.26
MUN, <sup>2</sup> mg/dL	11.8	10.9	10.1	0.30	0.01	0.09
SCS <sup>8</sup>	3.38	3.91	3.83	0.23	0.09	0.79

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>Effect of week ( $P < 0.05$ ).

<sup>3</sup>ECM =  $[0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat yield (kg)}] + [7.2 \times \text{protein yield (kg)}]$ .

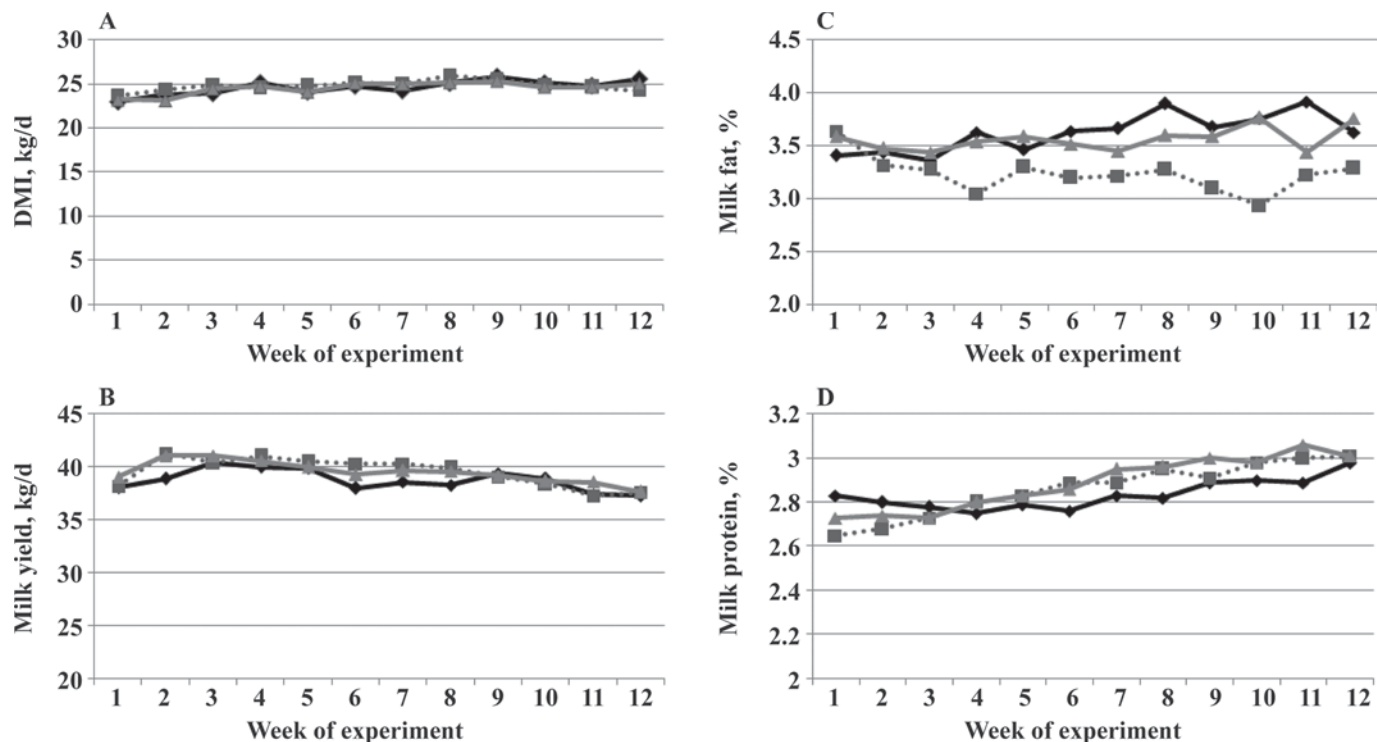
<sup>4</sup>FCM =  $[0.4 \times \text{milk yield (kg)}] + [15 \times \text{fat yield (kg)}]$ .

<sup>5</sup>Feed efficiency =  $(\text{ECM}/\text{DMI}) \times 100$ .

<sup>6</sup>Nitrogen efficiency =  $\text{milk N (kg/d)}/\text{N intake (kg/d)} \times 100$ .

<sup>7</sup>Effect of interaction of week by treatment ( $P = 0.07$ ).

<sup>8</sup>SCS =  $\log(\text{SCC})$ .



**Figure 1.** Dry matter intake, milk yield, and milk fat and milk protein by early lactation cows fed experimental diets: CON = control diet containing a blend of soybean products ( $\blacklozenge$ ); DDGS = diet containing 22% regular dried distillers grains with solubles ( $\blacktriangle$ ); and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles ( $\blacksquare$ ). Effect of week was significant ( $P < 0.05$ ) for DMI, milk, and milk protein. There was a tendency for treatment  $\times$  week for milk protein ( $P = 0.07$ ).

Leu, Met, Phe, and Trp increased. Of the NEAA, Gly, Pro, Ser, and Tyr were increased in DG diets and the remaining NEAA were unchanged. Tyrosine concentration was greater in RFDGS compared with the DDGS diet ( $P = 0.03$ ) and there was a trend for a decrease ( $P = 0.09$ ) in Tau concentration in RFDGS compared with DDGS. Because of these changes, plasma EAA and BCAA concentrations were similar for all diets, and TAA and NEAA increased ( $P < 0.05$ ) in DG diets.

Arterio-venous differences of AA are presented in Table 10. Except for Leu, Met, and Gly, the AV differences of all AA were unchanged for all treatments. The AV differences of both Leu and Met were increased ( $P < 0.05$ ) in DG diets, whereas the AV difference of Gly was decreased ( $P = 0.01$ ). The AV differences of EAA, NEAA, and TAA were similar for all diets; however, the AV difference of BCAA tended to increase ( $P = 0.09$ ) in DG diets mainly because of the increase in AV difference of Leu. No differences existed between the 2 DG treatments.

Mammary extractions of plasma AA are presented in Table 11. Of the EAA, the extraction efficiency of Ile, Lys, and Val increased ( $P \leq 0.01$ ) in DG diets compared with the CON diet. Opposite observations were noted for the NEAA; the extraction efficiency of

Gly, Ser, and Tyr decreased ( $P = 0.05$ ) in DG diets. Extraction efficiency of BCAA increased ( $P = 0.05$ ) and that of NEAA decreased ( $P = 0.05$ ) in cows fed the DG diets compared with CON. No differences existed between the 2 DG treatments.

Uptake of AA by the mammary gland is shown in Table 12. Uptakes of Leu, Phe, and Pro increased ( $P = 0.05$ ) in diets containing DG. Trends for an increase ( $P = 0.07$ ) were observed for Met and Asn. Mammary uptake of BCAA increased ( $P = 0.04$ ) in DG diets compared with CON. There were no differences in mammary uptake of AA between the 2 DG treatments.

## DISCUSSION

### Diet Composition

Chemical composition (Table 2) of corn silage and alfalfa hay was similar to reported values in NRC (2001) for CP, ADF, and NDF. The AA composition of forage showed a small deviation from values listed in NRC (2001) for a mid maturity hay (40 to 46% NDF) and a normal corn silage (32 to 38% DM). The chemical composition was different between the 2 types of DG. The DDGS had lower concentrations of CP, NDF, ADF,



**Table 6.** Body weight, BCS, and energy balance of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

Item	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
Initial BW, kg	693	682	660	26.5	0.31	0.39
Final BW, kg	734	722	704	25.4	0.34	0.47
BW, <sup>2</sup> kg	710	712	713	5.4	0.78	0.94
BW change, kg/d	0.47	0.47	0.53	0.09	0.80	0.66
BCS <sup>3</sup>	3.43	3.32	3.34	0.05	0.13	0.82
BCS change/d	0.14	0.02	0.00	0.07	0.06	0.79
NE <sub>I</sub> , <sup>4</sup> Mcal/d	41.3	40.1	40.3	0.98	0.38	0.86
NE <sub>M</sub> , <sup>2,5</sup> Mcal/d	11.0	11.0	11.0	0.06	0.76	0.91
NE <sub>L</sub> , <sup>6</sup> Mcal/d	26.4	26.5	27.4	1.17	0.60	0.49
EB, <sup>7</sup> Mcal/d	4.39	1.98	1.98	0.99	0.53	0.99
TRE, <sup>8</sup> Mcal/kg	20.7	20.0	20.1	0.36	0.08	0.74
Energy efficiency <sup>9</sup>	63.1	66.9	68.1	2.30	0.09	0.64

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>Effect of week ( $P < 0.05$ ).

<sup>3</sup>Body condition score: 1 = emaciated to 5 = obese (Wildman et al., 1982).

<sup>4</sup>Net energy intake = NE<sub>I</sub>(Mcal/kg) × DMI (kg/d).

<sup>5</sup>Net energy for maintenance = BW<sup>0.75</sup> × 0.08 (NRC, 2001).

<sup>6</sup>Net energy required for milk = milk yield (kg) × [(0.0929 × fat %) + (0.0563 × protein %) + (0.0395 × lactose %)] (NRC, 2001).

<sup>7</sup>Energy balance = NE<sub>I</sub> - (NE<sub>M</sub> + NE<sub>L</sub>).

<sup>8</sup>Total reserves energy = proportion empty body fat × 9.4 + proportion empty body protein × 5.55 (NRC, 2001).

<sup>9</sup>Energy efficiency = NE<sub>L</sub>/NE<sub>I</sub> × 100.

and ash. Starch concentration was slightly greater in DDGS compared with RFDGS. Ether extract was less in RFDGS (3.5%) compared with DDGS (10.8%). The AA concentration (as % of CP) was similar for both DG. Essential AA composition of DG used in this study is comparable to published data (Spiehs et al., 2002) regarding DG produced from “new generation” ethanol plants. Among the individual EAA, Lys concentration as percentage of CP was greater in DDGS (3.48%) and in RFDGS (3.22%) compared with that (2.24%) reported in NRC (2001). Total EAA, as a percentage of CP, was greater in DG (43.9%) compared with a value of 37.8% listed in NRC (2001). The nutrient profile of both DG used in this study reflects improved fermentation efficiency of new generation ethanol plants as described by Spiehs et al. (2002).

All diets were similar in CP, NDF, EE, NFC, and starch (Table 3). Because of greater ADF concentration in soybean feedstuffs, the CON diet contained more ADF (18.2%) than the DG diets (15.6%). Starch concentration was greater in DG diets; however, overall starch levels for all diets (23.4 ± 1.05%) were within the normal range recommended for lactating cows (Oba and Allen, 2003). Calculated dietary RDP and RUP based on in situ analysis of the feed ingredients (Mjoun

et al., 2010a) indicate that RDP slightly decreased following the substitution of soybean feedstuffs with either DG type, with a greater RUP value observed for the RFDGS diet. Amino acid composition of diets was similar for both DG diets. Comparing CON with both DG diets indicated that Lys concentration decreased by 21%, whereas that of Met increased by 17%. Estimated duodenal Lys supply using NRC (2001) ranged from 6.32 and 5.63, whereas duodenal Met supply ranged from 1.74 to 1.85 as a percentage of MP, respectively, for CON and DG diets. These concentrations were less than those suggested by the NRC (2001). Milk protein score, calculated as the concentration (percentage of CP) of first-limiting AA in the diet/AA concentration in milk protein (Schingoethe, 1996), was 0.53, 0.44, and 0.43, respectively, for CON, DDGS, and RFDGS diets. According to the milk protein score, Met was the first limiting AA in CON followed by Lys and Trp. In both DG diets, Lys was first limiting, followed by Met and Trp.

### Intake, Milk Production, and Composition

Similar DMI across diets (Table 5) is consistent with previous research in which DG were fed at approxi-

**Table 7.** Plasma metabolites of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

Item	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
Glucose, mg/dL						
Wk 4	61.8	57.3	60.9	2.32		
Wk 8	59.1	60.7	61.7	2.21		
Wk 12	69.3	61.6	65.4	2.21		
Overall <sup>2</sup>	63.4	59.9	62.7	1.30	0.17	0.13
BHBA, mg/dL						
Wk 4	8.21	7.04	6.81	0.62		
Wk 8	8.13	6.76	6.96	0.59		
Wk 12	8.73	8.28	7.70	0.59		
Overall	8.36	7.36	7.16	0.44	0.04	0.68
NEFA, mM						
Wk 4	0.17	0.17	0.17	0.02		
Wk 8	0.16	0.09	0.12	0.02		
Wk 12	0.15	0.11	0.09	0.02		
Overall <sup>2</sup>	0.16	0.12	0.13	0.01	0.08	0.72
Triglycerides, mg/dL						
Wk 4	9.92	9.26	10.94	0.96		
Wk 8	13.13	11.72	13.50	0.96		
Wk 12	9.93	10.16	9.82	0.96		
Overall <sup>2</sup>	11.00	10.38	11.42	0.57	0.89	0.18
Total cholesterol, mg/dL						
Wk 4	183.0	202.2	212.2	13.12		
Wk 8	220.4	240.7	243.9	12.54		
Wk 12	234.8	229.3	250.3	12.54		
Overall <sup>2</sup>	212.7	224.1	235.4	8.69	0.10	0.27
Urea N, mg/dL						
Wk 4	15.4	13.8	12.6	0.84		
Wk 8	13.8	11.3	11.3	0.80		
Wk 12	17.1	15.4	14.7	0.80		
Overall <sup>2</sup>	15.4	13.5	12.9	0.47	<0.01	0.33

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>Effect of week ( $P < 0.05$ ).

mately 20% of the diet DM (Liu et al., 2000; Kleinschmit et al., 2006). Milk production was not different among treatments. Most recent studies have shown an increase in milk production when DG were fed to dairy

cows in mid lactation (Powers et al., 1995; Anderson et al., 2006; Kleinschmit et al., 2006). In this experiment, dietary fat was balanced across diets and the ratio of metabolizable energy supply to requirement according

**Table 8.** Mammary plasma flow (MPF) and protein metabolism metabolites ( $\mu M/L$ ) of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

Item	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
MPF <sup>2</sup>						
L/d	14,909	16,978	15,479	1,028	0.24	0.24
L/kg of milk	374.3	416.2	418.3	33.6	0.30	0.96
Ammonia	176.8	170.2	175.2	7.7	0.52	0.48
Citrulline	93.5	88.0	93.8	5.6	0.66	0.41
3-Methylhistidine	3.41	3.17	3.31	0.34	0.65	0.74
Carnosine	9.45	9.07	9.17	1.14	0.81	0.94
Ornithine	42.85	35.82	34.12	2.58	0.01	0.61

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>Mammary plasma flow = [(milk Phe + Tyr) × 0.965/[arterial-venous difference of (Phe + Tyr)]] (Cant et al., 1993).

**Table 9.** Arterial plasma concentration of AA of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

AA, <sup>2</sup> $\mu\text{M/L}$	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
EAA						
His	46.7	52.1	55.2	2.35	0.02	0.34
Ile	130.4	99.7	101.2	6.25	<0.001	0.86
Leu	150.2	186.7	201.0	9.50	<0.001	0.29
Lys	70.4	58.6	55.8	3.55	<0.01	0.57
Met	14.9	21.2	21.4	1.11	<0.001	0.91
Phe	40.5	44.6	49.1	2.14	0.02	0.13
Thr	84.3	81.3	87.2	4.76	0.99	0.36
Trp	26.4	33.8	31.6	2.44	0.04	0.52
Val	272.4	234.2	244.3	12.46	0.03	0.55
NEAA						
Ala	220.3	227.7	244.2	11.76	0.26	0.31
Asn	42.6	43.3	44.1	1.91	0.61	0.75
Asp	8.4	7.0	7.2	0.72	0.13	0.81
Glu	46.0	41.2	42.3	2.72	0.17	0.74
Gln	260.8	260.0	245.5	9.11	0.40	0.20
Gly	305.0	372.0	357.4	22.6	0.03	0.62
Pro	66.2	87.2	96.6	5.99	<0.001	0.19
Ser	81.0	106.6	99.9	5.86	<0.01	0.41
Tau	70.9	77.5	67.6	4.65	0.74	0.09
Tyr	41.8	53.1	63.5	3.30	<0.001	0.03
EAA	836.3	812.2	841.9	30.44	0.81	0.49
NEAA	1,072.2	1,198.4	1,198.4	36.82	<0.001	1.00
BCAA	553.0	520.6	546.4	25.95	0.53	0.48
TAA	1,908.5	2,010.6	2,073.9	52.27	0.03	0.36

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>EAA = essential AA; NEAA = nonessential AA; BCAA = branched-chain AA (Val, Ile, and Leu); TAA = total AA = EAA + NEAA.

to the NRC (2001) was similar for all diets, averaging  $1.06 \pm 0.02$ . The increase in milk production in the studies previously cited may have been partially attributed to additional fat, which increased in diets containing DG. Another reason for the increase in milk production in those studies may have been the increase in RUP supply when DG replaced either soybean meal or corn in the CON diets. Although RDP and RUP were not perfectly balanced across diets, the slight increase in dietary RUP from the DG diets compared with CON was minimal in this experiment compared with greater RUP increases supplied by DG diets in other studies (Powers et al., 1995; Anderson et al., 2006; Kleinschmit et al., 2006). In addition, the MP balance was similar for all diets (+468 g/d), indicating similar supply of protein available for absorption.

Milk fat is usually not affected by feeding DG in diets containing adequate effective fiber (Anderson et al., 2006). Consistent with this observation, milk fat in this experiment was similar across diets; however, milk fat percentage was numerically lower (by 0.3 percentage units) in cows fed DDGS compared with those fed RFDGS and CON. In this experiment, all

diets contained similar proportions of forages (50% of diet DM) and physically effective fiber. This numerical decrease in milk fat percentage in cows fed DDGS diet may be attributed to differences in type of fat among treatments. Rumen inert fat containing 85% saturated fat (Energy Booster 100) was included in the CON and RFDGS diets to balance for dietary fat concentration. In previous experiments, the inclusion of saturated fats increased milk fat percentage (Elliott et al., 1996; Mjoun et al., 2010b).

Feeding DG at 20% or less of the diet of mid lactation cows usually has no effect on milk protein percentage (Anderson et al., 2006; Kleinschmit et al., 2007; Hubbard et al., 2009; Schingoethe et al., 2009). A few studies evaluated DG as a protein source in early lactation (Palmquist and Conrad, 1982; Grings et al., 1992). In the former study, milk production averaged only 18 kg/d. At such a level of production, a test of protein quality may not be relevant. The only study found in the literature that evaluated DG as a source of protein in early lactation cows producing high quantities of milk was that of Grings et al. (1992). In that study, the inclusion of DG up to 36% of the diet DM resulted

**Table 10.** Arterio-venous differences of amino acids of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

AA, <sup>2</sup> $\mu\text{M/L}$	Treatment <sup>1</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
EAA						
His	13.9	14.4	15.3	1.01	0.42	0.54
Ile	41.9	44.4	44.7	3.12	0.44	0.93
Leu	63.2	74.6	79.3	4.76	0.02	0.47
Lys	45.8	43.1	44.4	3.11	0.53	0.74
Met	10.3	12.7	13.3	1.06	0.03	0.70
Phe	19.8	20.7	23.5	1.27	0.13	0.13
Thr	26.6	24.9	26.6	1.94	0.71	0.53
Trp	1.9	7.9	6.6	2.80	0.11	0.74
Val	53.3	60.9	58.9	5.04	0.28	0.78
NEAA						
Ala	27.2	28.6	29.7	6.45	0.80	0.90
Asn	14.0	16.4	15.7	1.32	0.19	0.70
Asp	4.5	3.1	3.8	0.61	0.08	0.30
Glu	28.2	24.8	25.9	2.30	0.27	0.72
Gln	83.9	80.8	73.7	5.65	0.28	0.33
Gly	21.0	7.5	4.1	6.47	0.01	0.62
Pro	11.3	19.0	17.6	3.80	0.15	0.81
Ser	28.8	29.2	24.8	12.10	0.74	0.49
Tau	6.8	5.1	5.0	1.98	0.42	0.97
Tyr	20.2	19.5	19.5	1.67	0.73	0.99
EAA	283.5	305.0	312.1	21.5	0.32	0.81
NEAA	238.9	228.0	187.6	25.0	0.28	0.21
BCAA	158.5	179.9	179.9	11.4	0.09	0.84
TAA	522.4	533.8	517.0	34.3	0.93	0.71

<sup>1</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>2</sup>EAA = essential AA; NEAA = nonessential AA; BCAA = branched-chain AA (Val, Ile, and Leu); TAA = total AA = EAA + NEAA.

in a linear increase in both CP (13.9 to 20.3%) and RUP (26.1 to 36.3% of CP) in the diet. Milk production increased from 37.8 to 42.0 kg/d, and milk protein percentage increased from 2.63 to 2.80%. The increase in milk production and milk protein content was likely a result of increasing dietary protein per se rather than a direct effect of DG inclusion. In this experiment, the increase in milk protein percentage with DG diets may be partially related to the increase in the uptake of BCAA by the mammary gland. As observed in a previous experiment (Mjoun et al., 2010b), milk protein synthesis was not affected until 30% RFDGS was fed. A decrease in arterial concentration of Lys in cows fed DG is usually observed (Nichols et al., 1998; Kleinschmit et al., 2006; Mjoun et al., 2010b); however, this decrease does not imply a Lys deficiency because of the compensated increase in the extraction efficiency of Lys by the mammary gland that is usually sufficient to support milk production and milk protein synthesis.

Ruminal protein degradability affects MUN (Hristov et al., 2004). The estimated RUP of RFDGS and DDGS as ingredients were 60.4 and 52.3% (Mjoun et

al., 2010a). Replacing soybean meal and expeller soybean meal by DG products resulted in a slight increase in RUP that may explain the lower MUN in cows fed those diets compared with CON cows. The response in MUN in this study is in agreement with previous research evaluating DG (Kleinschmit et al., 2006; Janicek et al., 2008; Mjoun et al., 2010b).

### **EB and Plasma Metabolites**

The positive energy status of cows is in agreement with Kendrick et al. (1999) who found that cows returned to positive energy balance between 21 and 49 d depending on the energy density of the diet. In this experiment, EB was evaluated between 35 and 120 DIM. The positive EB observed for all diets indicates that energy was not limiting in this experiment. Cows fed CON had twice the EB as cows fed the DG diets. Similarly, the tendency for CON cows to have greater total energy reserves and lesser energy efficiency for lactation indicates that CON cows may have preferentially partitioned metabolizable energy for body

**Table 11.** Extraction efficiency<sup>1</sup> of amino acids of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

AA, <sup>3</sup> %	Treatment <sup>2</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
EAA						
His	30.3	27.5	27.7	1.64	0.14	0.92
Ile	33.2	44.9	43.9	2.58	0.001	0.77
Leu	43.0	41.1	39.1	2.56	0.34	0.56
Lys	65.4	73.2	79.1	2.40	<0.001	0.06
Met	68.7	59.6	62.6	3.89	0.11	0.58
Phe	49.2	46.7	47.9	2.32	0.48	0.69
Thr	31.4	32.0	31.5	2.62	0.89	0.89
Trp	2.2	18.7	18.6	8.81	0.12	1.00
Val	19.9	25.9	24.0	1.67	0.01	0.42
NEAA						
Ala	13.7	12.5	12.1	2.71	0.66	0.91
Asn	33.3	37.4	35.1	2.27	0.25	0.23
Asp	53.8	41.2	51.2	6.70	0.28	0.45
Glu	60.1	59.9	60.9	2.72	0.94	0.77
Gln	32.0	31.2	30.1	1.88	0.49	0.63
Gly	6.9	1.7	1.2	1.92	<0.01	0.81
Pro	17.1	21.4	16.7	4.75	0.71	0.46
Ser	35.9	27.2	25.0	4.00	0.02	0.63
Tau	9.2	6.0	6.8	2.38	0.27	0.81
Tyr	48.6	37.6	31.0	2.64	<0.001	0.06
EAA	34.0	37.3	36.7	2.03	0.19	0.83
NEAA	22.4	18.9	15.6	1.96	0.03	0.23
BCAA	29.3	35.0	33.3	1.92	0.04	0.52
TAA	27.4	26.4	25.1	1.50	0.35	0.53

<sup>1</sup>Extraction efficiency = arterio-venous difference/arterial concentration × 100.

<sup>2</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>3</sup>EAA = essential AA; NEAA = nonessential AA; BCAA = branched-chain AA (Val, Ile, and Leu); TAA = total AA = EAA + NEAA.

reserve deposition rather than for synthesis of milk or milk components. Of the energy reserves, the proportion of body fat may have been greater in CON cows, therefore increasing their mobilization capability, as indicated by a tendency for greater NEFA and greater plasma BHBA concentrations. This conclusion is consistent with the gain in body condition for CON cows despite unchanged BW. An increase in plasma NEFA is associated with a negative energy balance (Pullen et al., 1989); however, in this experiment CON cows were in better EB than cows fed DG diets. Increased BHBA concentration in CON cows is consistent with the observation of Guretzky et al. (2006) that dairy cows in early lactation exhibit incomplete oxidation of fatty acids. The BHBA concentrations were below the threshold for subclinical ketosis for all treatments (Geishauser et al., 2000). The tendency for an increase in total cholesterol concentration in DG diets, and especially in the RFDGS diet, is consistent with our previous experiment (Mjoun et al., 2010b) where the increase was partially attributed to added dietary fat.

However, in this experiment, dietary fat concentration was similar across all diets. The increase in total cholesterol may be attributed to the fatty acids contained in the DG.

### Plasma AA

Mammary plasma flow per kilogram of milk output was unaffected by treatment, averaging 407 L/kg of milk. This estimate was less than values obtained in mid lactation cows (Mjoun et al., 2010b). Similar to this observation, Mabeesh et al. (2000) estimated MPF in dairy goats using a dilution technique by *p*-aminohippurate in the mammary vein and found that the ratio of blood flow to milk yield was much lower in early (80 DIM) compared with later (233 DIM) lactation (400:1 vs. 800:1). In this experiment, milk yield was 8 kg/d higher compared with cows in mid to late lactation (Mjoun et al., 2010b). Lower MPF obtained in early lactation cows is conflicted with the model developed by Cant and McBride (1995) in which MPF and

**Table 12.** Mammary uptake<sup>1</sup> of amino acids of early lactation cows fed regular or reduced-fat dried distillers grains with solubles

AA, <sup>3</sup> g/kg of milk	Treatment <sup>2</sup>				Contrast	
	CON	DDGS	RFDGS	SEM	CON vs. DG	DDGS vs. RFDGS
EAA						
His	0.80	0.91	0.98	0.10	0.15	0.56
Ile	2.07	2.40	2.45	0.28	0.15	0.85
Leu	3.09	4.03	4.38	0.45	0.01	0.48
Lys	2.52	2.49	2.68	0.26	0.78	0.51
Met	0.58	0.83	0.81	0.11	0.07	0.89
Phe	1.14	1.39	1.58	0.15	0.04	0.35
Thr	1.18	1.19	1.30	0.13	0.60	0.42
Trp	0.14	0.64	0.50	0.22	0.11	0.65
Val	2.35	2.87	2.87	0.33	0.11	0.99
NEAA						
Ala	0.84	1.05	1.04	0.24	0.48	0.98
Asn	0.70	0.86	0.87	0.10	0.07	0.93
Asp	0.23	0.15	0.20	0.03	0.22	0.31
Glu	1.48	1.50	1.60	0.18	0.72	0.66
Gln	4.54	4.91	4.40	0.55	0.83	0.44
Gly	0.59	0.36	0.21	0.23	0.15	0.55
Pro	0.30	0.91	0.92	0.24	0.04	0.98
Ser	1.15	1.35	1.06	0.29	0.87	0.41
Tau	0.30	0.31	0.29	0.12	0.99	0.89
Tyr	1.32	1.41	1.44	0.14	0.46	0.88
EAA	16.4	16.6	17.4	1.85	0.93	0.36
NEAA	11.1	12.5	11.7	1.44	0.49	0.63
BCAA	7.5	9.3	9.7	1.03	0.04	0.72
TAA	25.5	29.1	29.1	3.10	0.23	0.99

<sup>1</sup>Mammary uptake = arterio-venous difference × mammary plasma flow.

<sup>2</sup>CON = control diet containing a blend of soybean products; DG = distillers grains; DDGS = diet containing 22% regular dried distillers grains with solubles; and RFDGS = diet containing 20% reduced-fat dried distillers grains with solubles.

<sup>3</sup>EAA = essential AA; NEAA = nonessential AA; BCAA = branched-chain AA (Val, Ile, and Leu); TAA = total AA = EAA + NEAA.

nutrient uptake increase with milk production. Factors that may reduce MPF in early lactation remain to be elucidated.

The profile of plasma AA reflected the apparent difference in the AA composition of the 2 protein supplements, which is in agreement with previous research (Mjoun et al., 2010b). Similar plasma AA profile was observed for cows fed either type of DG. Changes in plasma AA concentrations were identical to those observed in mid lactation cows (Mjoun et al., 2010b).

Of interest, plasma Lys concentration was decreased and Met was increased with the feeding of the DG diets compared with CON. The AV difference of all EAA was constant except for Met and Leu, which increased for cows fed DG diets, indicating that more Leu and Met were available for absorption compared with CON cows. This was supported by increased uptake of these 2 AA by the mammary gland.

Despite the significant decrease in arterial Lys in cows fed DG diets, milk protein percentage increased. The mammary gland was able to sustain milk protein synthesis possibly because of the increase in the extrac-

tion efficiency of Lys. Mean arterial Lys concentration in this experiment appear to be sufficient to meet milk production and protein synthesis demands. Mjoun et al. (2010b) showed that when arterial Lys concentration decreased below 45  $\mu\text{M}/\text{L}$ , milk protein percentage was decreased. These results indicate that arterial concentration of AA alone may be a poor indicator of potential AA that are limiting milk production and milk protein synthesis.

Only minor differences in AA utilization were detected between the 2 types of DG. Extraction efficiency of Lys tended to be greater in RFDGS versus DDGS partially because of numerical differences in arterial concentration of Lys as the AV difference was constant. This difference may be explained by lower Lys digestibility in RFDGS vs. DDGS (Mjoun et al., 2010a). The increase in uptake of Leu and Phe, and the tendency for an increase in Met uptake by the mammary gland responded to the increase in their arterial concentrations. Enhanced protein synthesis in DG may be associated with the increase in plasma concentration of NEAA and increases in the uptakes of Met and BCAA. The

availability of NEAA may have spared the utilization of EAA for the synthesis of NEAA.

Ranking EAA based on their extraction efficiency as an indicator of limiting AA shows that Phe was the third limiting AA for all diets, whereas the first and second limiting AA varied between Lys and Met, with Lys being first limiting AA in DG diets and Met being first limiting AA in the CON diet. However, these observations contrast with the milk production and milk protein results of this experiment, which suggest that Met was possibly limiting in the CON diet, but there is minimal evidence that Lys was deficient in DG diets because milk protein percentage increased in those diets. A similar ranking was reported for diets containing similar proportions of DG (Nichols et al., 1998; Liu et al., 2000; Kleinschmit et al., 2006).

The response of PUN is parallel to the response of urea cycle AA, especially that of arterial ornithine. Increased PUN in CON cows may be related to greater RDP or increased oxidation of AA for metabolic energy. Both PUN and MUN were lower in this experiment compared with cows in mid lactation fed diets with similar CP concentrations (Mjoun et al., 2010b). This discrepancy may be explained by an increase in the utilization efficiency of AA through reduced oxidation and an increase in their utilization in milk protein synthesis.

## CONCLUSIONS

Similar DMI and milk production were obtained when early lactation cows were fed a soybean-based diet or either of 2 types of DG, a regular or a reduced-fat product. Milk composition was similar for all diets except that milk protein percentage was greater in DG diets compared with CON diet. In addition, milk protein yield was increased in DG diets. Feed and N efficiencies were improved in cows fed DG diets. Plasma AA profile was similar for cows fed either type of DG. Cows fed DG diets had low arterial Lys concentration, which was compensated for by an increase in Lys extraction efficiency, allowing these cows to produce milk with greater protein percentage than CON cows. Results from this study strongly confirm that DG is a good source of metabolizable AA and that, at 20% of the diet, Lys does not present a limitation to milk production or protein synthesis for cows producing 40 kg/d of milk. Moreover, feed and N efficiencies were improved in DG diets, which can represent an economic and environmental advantage over traditional sources of protein such as soybean meal.

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## REFERENCES

- Allain, C. C., L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20:470-475.
- Anderson, J. L., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. *J. Dairy Sci.* 89:3133-3142.
- AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.
- Boucher, S. E., S. Calsamiglia, C. M. Parsons, M. D. Stern, M. Ruiz Moreno, M. Vázquez-Anón, and C. G. Schwab. 2009. In vitro digestibility of individual amino acids in rumen-undegraded protein: The modified three-step procedure and the immobilized digestive enzyme assay. *J. Dairy Sci.* 92:3939-3950.
- Cant, J. P., E. J. DePeters, and R. L. Baldwin. 1993. Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. *J. Dairy Sci.* 76:762-774.
- Cant, J. P., and B. W. McBride. 1995. Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. *J. Dairy Res.* 62:405-422.
- Clark, J. H., T. K. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *J. Dairy Sci.* 75:2304-2323.
- Clark, J. H., H. R. Spires, and C. L. Davis. 1978. Uptake and metabolism of nitrogenous components by the lactating mammary gland. *Fed. Proc.* 37:1233-1238.
- Elliott, J. P., J. K. Drackley, and D. J. Weigel. 1996. Digestibility and effects of hydrogenated palm fatty acid distillate in lactating dairy cows. *J. Dairy Sci.* 79:1031-1039.
- Fossati, P., and P. Lorenzo. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.* 28:2077-2080.
- Geishauser, T., K. Leslie, J. Tenhag, and A. Bashiri. 2000. Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows. *J. Dairy Sci.* 83:296-299.
- Grings, E. E., R. E. Roffler, and D. P. Deitelhoff. 1992. Responses of dairy cows to additions of distillers dried grains with solubles in alfalfa-based diets. *J. Dairy Sci.* 75:1946-1953.
- Grunau, J. A., and J. M. Swiader. 1992. Chromatography of 99 amino acids and other ninhydrin-reactive compounds in the Pickering lithium gradient system. *J. Chromatogr. A* 594:165-171.
- Guretzky, N. A., D. B. Carlson, J. E. Garrett, and J. K. Drackley. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *J. Dairy Sci.* 89:188-200.
- Heinrichs, J., and P. Kononoff. 2002. Evaluating particle size of forages and TMRs using the new Penn State forage particle separator. Pennsylvania State University, Cooperative Extension. DAS 02-42. <http://dairyanimalscience.psu.edu/pdf/das0242.pdf>.
- Hristov, A. N., R. P. Etter, J. K. Ropp, and K. L. Grandeen. 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. *J. Anim. Sci.* 82:3219-3229.

- Hubbard, K. J., P. J. Kononoff, A. M. Gehman, J. M. Kelzer, K. Karges, and M. L. Gibson. 2009. The effect of feeding high protein distillers dried grains on milk production of Holstein cows no effect on milk protein percentage. *J. Dairy Sci.* 92:2911–2914.
- Jacobson, D. R., H. H. Van Horn, and C. J. Sniffen. 1970. Lactating ruminants. *Fed. Proc.* 29:35–40.
- Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. *J. Dairy Sci.* 91:3544–3553.
- Johnson, M. M., and J. P. Peters. 1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. *J. Anim. Sci.* 71:753–756.
- Kendrick, K. W., T. L. Bailey, A. S. Garst, A. W. Pryor, A. Ahmadzadeh, R. M. Akers, W. E. Eyestone, R. E. Pearson, and F. C. Gwazdauskas. 1999. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. *J. Dairy Sci.* 82:1731–1741.
- Kleinschmit, D. H., D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur. 2007. Dried distillers grains plus solubles with corn silage or alfalfa hay as the primary forage source in dairy cow diets. *J. Dairy Sci.* 90:5587–5599.
- Kleinschmit, D. H., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of various sources of corn dried distillers grains plus solubles for lactating dairy cattle. *J. Dairy Sci.* 89:4784–4794.
- Kononoff, P. J., S. K. Ivan, and T. J. Klopfenstein. 2006. Estimation of the proportion of feed protein digested in the small intestine of cattle consuming wet corn gluten feed. *J. Dairy Sci.* 89:3968–3971.
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models. 2nd ed. SAS Institute, Cary, NC.
- Liu, C., D. J. Schingoethe, and G. A. Stegeman. 2000. Corn distillers grains versus a blend of protein supplements with or without ruminally protected amino acids for lactating cows. *J. Dairy Sci.* 83:2075–2084.
- Mabjeesh, S. J., C. E. Kyle, J. C. MacRae, and B. J. Bequette. 2000. Lysine metabolism by the mammary gland of lactating goats at two stages of lactation. *J. Dairy Sci.* 83:996–1003.
- McCleary, B. V., V. Solah, and T. S. Gibson. 1994. Quantitative measurement of total starch in cereal flours and products. *J. Cereal Sci.* 20:51–58.
- Mephum, T. B. 1982. Amino acid utilization by lactating mammary gland. *J. Dairy Sci.* 65:287–298.
- Mjoun, K., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2010a. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains with solubles products. *J. Dairy Sci.* doi:10.3168/jds.2009-2883
- Mjoun, K., K. F. Kalscheur, A. R. Hippen, D. J. Schingoethe, and D. E. Little. 2010b. Lactation performance and amino acid utilization of cows fed increasing concentrations of reduced-fat dried distillers grains with solubles. *J. Dairy Sci.* 93:288–303.
- Mondina, A., G. Bongiovanni, S. Fumero, and L. Rossi. 1972. An improved method of plasma deproteination with sulphosalicylic acid for determining amino acids and related compounds. *J. Chromatogr. A* 74:255–263.
- Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows. *J. Dairy Sci.* 81:482–491.
- 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.
- Palmquist, D. L., and H. R. Conrad. 1982. Utilization of distillers dried grains plus solubles by dairy cows in early lactation. *J. Dairy Sci.* 65:1729–1733.
- Pickering, M. 1989. Ion-exchange chromatography of free amino acids. *LC GC* 7:484–490.
- Powers, W. J., H. H. Van Horn, B. Harris Jr., and C. J. Wilcox. 1995. Effects of variable sources of distiller dried grain plus solubles on milk yield and composition. *J. Dairy Sci.* 78:388–396.
- Pullen, D. L., D. L. Palmquist, and R. S. Emery. 1989. Effect on days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. *J. Dairy Sci.* 72:49–58.
- Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in *The Analysis of Dietary Fiber in Food*. W. P. T. James and O. Theander, ed. Marcel Dekker Inc., New York, NY.
- SAS Institute. 2001. SAS User's Guide. Statistics, Version 9.01 ed. SAS Institute Inc., Cary, NC.
- Schingoethe, D. J. 1996. Balancing the dairy cow amino acid needs. *Anim. Feed Sci. Technol.* 60:153–160.
- Schingoethe, D. J., K. F. Kalscheur, A. R. Hippen, and A. D. Garcia. 2009. The use of distillers products in dairy cattle diets. *J. Dairy Sci.* 92:5802–5813.
- Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J. Anim. Sci.* 80:2639–2645.
- Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.* 6:24–27.
- 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 Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to standard production characteristics. *J. Dairy Sci.* 65:495–501.
- Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymatic determination of D(-)- $\beta$ -hydroxybutyrate and acetoacetic acid in blood. *Biochem. J.* 82:90–96.