Corn Distillers Grains versus a Blend of Protein Supplements with or without Ruminally Protected Amino Acids for Lactating Cows[1]

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ABSTRACT

In a replicated 4 × 4 Latin square design with 4-wk periods, we used 12 multiparous Holstein cows averaging 83 d postpartum to compare corn distillers grains (CDG) versus a blend (BLEND) of other protein sources with CDG (fish meal and soybean meal), and to determine the effectiveness of ruminally protected lysine and methionine (RPLM) in improving the utilization of CDG as a protein supplement for lactating cows. The 2 × 2 factorial arrangement of treatments was as follows: CDG diet, CDG diet plus RPLM, BLEND diet, and BLEND diet plus RPLM. All diets contained 30% corn silage, 20% alfalfa hay, and 50% the respective corn-based concentrate mixture. The array of amino acids available for absorption when cows were fed the BLEND diet was more desirable than for the CDG diet according to Milk Protein Score and Cornell Net Carbohydrate and Protein System. Dry matter intakes were similar among all diets. Milk yields (32.6, 31.7, 32.8, and 32.8 kg/d, respectively) were similar for cows fed all diets. Milk fat yields and percentages (3.72, 3.76, 3.67, and 3.63%) were unaffected by diet, but milk protein percentages (3.23, 3.26, 3.25, and 3.26%) tended to be higher when fed RPLM. Concentrations of most protein fractions in milk were similar for all diets, although β-lactoglobulin was increased slightly when cows were fed BLEND diets. Lysine, Met, and Phe were indicated as the most limiting amino acids for all diets according to extraction efficiency and transfer efficiency of amino acid from blood by the mammary gland. Methionine status was apparently improved by RPLM supplementation; Lys status was improved by the BLEND diets. Milk yield and composition when cows were fed CDG were not further improved by feeding blends of protein sources or RPLM; however, such dietary changes improved Lys and Met status of the cows.

(**Key words:** corn distillers grains, protein, ruminally protected amino acids, lactating cows)

Abbreviation key: BLEND = blend of protein supplements, **CDG** = corn distillers grains, **CNCPS** = Cornell Net Carbohydrate and Protein System, **ECM** = energy-corrected milk, **FM** = fish meal, **MPS** = milk protein score, **PEAA** = system of increasing Lys and Met to supply 15 and 5% of the predicted total essential AA in duodenal digesta, **RPLM** = ruminally protected Lys and Met, **SBM** = soybean meal.

INTRODUCTION

For maximizing milk and milk protein production, balancing dietary AA presented to the intestinal tract for absorption has become an important consideration in high producing dairy cows (22). The use of ruminally protected AA to supplement diets is one way to achieve this goal. Lysine and Met are often indicated to be the first- and second-limiting AA for milk protein synthesis (24, 25). Studies (16, 19, 30) have reported that ruminally protected Lys and Met (**RPLM**) increased yields of milk and milk protein, especially with substantial amounts of corn-based components in the ration. The blending of several protein supplements may offer another opportunity to improve the dietary array of AA provided to the intestine and is more commonly used in commercial feeding situations. When diets were equal in RUP, increased milk production was observed when corn distillers grains (**CDG**) (30) and corn gluten meal (6) were replaced with blends of several protein sources.

Like other corn by-products, CDG as a protein source is typically low in Lys (16, 22). Brouk (3) evaluated the AA composition of CDG in an in situ digestion study. Compared with the AA composition of milk, the AA profiles of RUP and total nitrogen mix presented to the intestine by CDG indicated that Lys was first-limiting AA for milk protein synthesis. Nichols et al. (16) observed that milk yield, milk protein yield, and milk protein percentage increased when CDG was supplemented with RPLM (20 g of Lys and 6 g of Met/d

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Table 1. Ingredient content of diets.

Ingredient	Diet	
	CDG[1]	BLEND
	(% of DM)	
Alfalfa hay	20.00	20.00
Corn silage	30.00	30.00
Concentrate mix		
Dried CDG	18.85	5.50
Fish meal, Menhaden	. . .	2.75
Soybean meal, 44% CP	. . .	7.45
Urea	0.50	. . .
Corn, shelled rolled	27.15	30.70
Fat (tallow)	1.50	2.00
Dicalcium phosphate	0.40	0.25
Limestone	1.00	0.75
Trace mineralized salt	0.30	0.30
Magnesium oxide	0.15	0.15
Vitamin A, D, and E premix[2]	0.10	0.10
Vitamin E premix[3]	0.05	0.50
Limiting AA		
MPS[4]	Lys (0.72)	His (0.77)
CNCPS[5]		
–Limiting AA, % of requirement	Lys (82)	Lys (103)
PEAA[5]		
–Lys, % of EAA	12.5	13.7
–Met, % of EAA	4.8	4.7

[1]Corn distillers grains.

[2]Contained 4,410,000 IU of vitamin A, 882,000 IU of vitamin D, and 441 IU of vitamin E per kg.

[3]Contained 44,100 IU of vitamin E/kg.

[4]Milk protein score (22).

[5]Cornell Net Carbohydrate and Protein System (17).

[6]System of increasing Lys and Met to supply 15 and 5% of the predicted total essential AA in duodenal digesta (25).

per cow); however, the CDG diet might still have been deficient in Lys even after RPLM supplementation because blood concentration of Lys was not elevated. We are not aware of any studies that evaluated production response to a blend of CDG with other protein sources. Therefore, the objectives of this research were to compare CDG versus a blend (**BLEND**) of other protein sources that included CDG, and to determine the effectiveness of RPLM in improving the utilization of CDG or BLEND as a protein supplement for lactating cows.

MATERIALS AND METHODS

Twelve multiparous Holstein cows averaging 83 d postpartum were used in a replicated 4 × 4 Latin square design. Each period was 4 wk in length; wk 1 was for adjustment to diets and wk 2 to 4 were for data collection. Treatment diets were designated as: CDG, CDG plus RPLM, BLEND, and BLEND plus RPLM (Table 1). One hundred grams of RPLM product was fed to supply 50 g of Lys and 15 g of Met/d per cow (Smartmine ML; Rhône-Poulenc, Atlanta, CA). These amounts were selected on the basis of a previous trial (16) as amounts more than adequate to prevent Lys and Met deficiency.

Diets were composed of (DM basis) 30% corn silage, 20% alfalfa hay, and 50% of the respective concentrate mix. Diets were formulated to contain approximately equal amounts of CP and RUP, both of which met nutrient requirements according to the NRC (15). The supplemental protein in the BLEND diet was approximately 25% from CDG, 25% from fish meal (**FM**), and 50% from soybean meal (**SBM**). Fish meal is a good source of Lys and Met, while SBM is a relatively good source of Lys but deficient in Met, and CDG is deficient in Lys. We chose fish meal and SBM with the intention of compensating the AA array of CDG. The BLEND diet thus had more desirable AA array than the CDG diet (Table 1) according to milk protein score (**MPS**) (22) and Cornell Net Carbohydrate and Protein System (**CNCPS**) (17).

Cows were housed in a free-stall barn and individually fed a total mixed diet once daily ad libitum using Calan-Broadbent feeding system (American Calan, Inc., Northwood, NH). Amounts of diets fed and orts were recorded daily for each cow. For cows receiving RPLM, the RPLM was mixed into the TMR at the time of feeding. Body weights and BCS (29) were recorded for 3 consecutive d at the beginning of each treatment period and at the end of period four.

Concentrate mixes, alfalfa hay, and corn silage were sampled weekly and frozen at –20°C until processed for further analysis. Samples were dried in an oven at 55°C for 48 h and then ground through a standard Model No. 3 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) with a 2-mm screen. Weekly aliquots of each ingredient were composited by period for analysis. An aliquot of feed composites was dried at 105°C for 24 h to determine 100% DM. Contents of CP, ether extract, ash, Ca, P, and Mg were determined by AOAC methods (2). Fatty acids of feeds were analyzed as butyl esters on a capillary column [0.32 mm × 100 m (SP2830); Supelco, Inc., Bellefonte, PA] by gas chromatography (Hewlett Packard 6890 Series II, Hewlett Packard Co., Avondale, PA) by the modified (8) procedure of Sukhija and Palmquist (26). For the analysis of NDF, ADF, and acid detergent lignin, a portion of composited samples was reground through an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY) with a 1-mm screen. Neutral detergent fiber (27, procedure A), ADF (20), and acid detergent lignin (12) were determined by ANKOM fiber analyzer using the Filter Bag Technique (ANKOM Technology Corp., Fairport, NY). Nonstructural carbohydrate was calculated as 100 − (NDF + ether extract + CP + ash).

Milk samples from each cow were collected for two consecutive milkings (p.m. and a.m.) and composited by cow during each of the last 3 wk of each treatment period. Each week's composited milk samples were analyzed for protein, fat, lactose, and SNF (2) by mid-infrared spectrophotometry (Multispec, Foss Food Technology Corp., Eden Prairie, MN). Somatic cell counts (2) were determined with a Fossomatic 90 (Foss Food Technology Corp.). Additional portions of milk samples from the 3rd wk of each period were prepared for SDS-PAGE to separate and quantify milk protein fractions as described by Mistry and Hassan (14) and Verdi et al. (28).

Samples of ruminal fluid were collected 2 to 4 h after feeding during the last week of each period by applying vacuum pressure to an esophageal tube fitted with a suction strainer into a 250-ml bottle. Samples were cooled immediately on ice until processed. A 10-ml aliquot of sample was centrifuged at 475 × g for 10 min; the supernatant was decanted and acidified with 0.5 ml of 0.1 N HCl and frozen at –20°C until analyzed for ruminal ammonia (5). Another 10-ml aliquot was acidified with 2 ml of 25% metaphosphoric acid and then centrifuged at 475 × g for 10 min; the supernatant was decanted and frozen for VFA analysis (18).

At the time of ruminal fluid sampling, blood was collected into serum separation tubes by venipuncture of the jugular vein, coccygeal artery, and the subcutaneous abdominal vein. The serum was obtained by centrifuging and frozen at –20°C until analysis for urea in jugular serum (5) and for AA in serum from the coccygeal artery and the subcutaneous abdominal vein (9). The concentrations of arterial and venous blood were used to estimate the AA uptake of the mammary gland. Mammary blood flow was estimated from milk production by the regression equation reported by Kronfeld et al. (10).

All data were pooled by period and analyzed as replicated Latin square design by the MIXED procedure of SAS (11). Data were analyzed with the model

$$Y_{ijkl} = \mu + S_i + C_{j(i)} + P_k + T_l + e_{ijkl}$$

where:

μ = overall mean,
S_i = random effect of square (i = 1 to 3),
$C_{j(i)}$ = random effect of cow within square (j = 1 to 4),
P_k = fixed effect of period (k = 1 to 4),
T_l = fixed effect of treatment (l = 1 to 4),
e_{ijkl} = random residual error.

Before determining the final testing model, we tested both square × treatment and square × period interactions in a fixed model with GLM procedure of SAS, and they did not show significant effects for almost all parameters. So in the final testing model, we assume that these interactions were not expected to be present and didn't list them in the model independently.

Orthogonal contrasts were conducted to determine the significance of differences among main effects (i.e., CDG vs. BLEND for protein sources and RPLM vs. no RPLM for RPLM supplementation) and interaction between protein source and RPLM supplementation. Model effects were considered significant at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

The chemical composition of concentrate mixes, forages, and total diets is listed in Table 2. Diets were formulated for 17% CP; both diets were slightly lower in CP than estimated, primarily because of lower protein in the corn silage than estimated. The CDG used in this study contained 32% CP, typical of CDG available from today's ethanol plants (3, 16) and higher than NRC values (15). The differences in NDF and ADF between the two diets were mainly due to the higher fiber content in CDG than the other protein sources (FM and SBM). Because we did not correct NDF values for any possible ash or CP, the NDF values for—especially the CDG diets—may have been slightly inflated. The ADF contents of both diets were slightly lower than recommended in NRC (15). Total diets contained similar quantities of lignin and ash. The ether extract and total fatty acid contents were higher for CDG diets, which was not expected. This could be attributed to a higher fat content of CDG or lower fat content of FM than anticipated. Total fatty acids were measured because they reflected a truer nutritional value of dietary fat than does ether extract. In these two diets, the amount of total fatty acids was correlated well with the ether extract.

Dry matter intakes (Table 3) were similar for all diets. Intakes were quite high for all diets, averaging 4.6 to 4.8% of BW, which could be expected because this experiment was conducted during the time of lactation when DMI would be highest (15). Similar high DMI were also observed in a previous study (16), which used CDG and SBM as protein supplements. Although some studies (23, 24, 30) showed that intake tended to increase with supplemental RPLM, other studies (1, 16) reported no effect of RPLM on DMI. Milk yields were similar for cows fed all diets; likewise, yields of energy-corrected milk (**ECM**), FCM, and SCM were similar. No increased milk production due to feeding the BLEND diet or RPLM may imply that intake of all AA was more than sufficient, so there was no AA

Table 2. Chemical composition of concentrate mixes, forages, and diets.

	Concentrate mixture[1]		Forage		Diet[2]	
Item	CDG	BLEND	Corn silage	Alfalfa hay	CDG	BLEND
DM, %	85.3	86.3	29.4	83.8	68.3	68.7
	(% of DM)					
CP	20.5	20.4	7.8	20.0	16.6	16.5
RUP,[3] % of CP	. . .	. . .	. . .	. . .	42.9	41.4
ADF	4.8	3.6	28.1	30.0	16.9	16.2
NDF	18.3	12.8	45.5	42.6	31.3	28.5
NSC	44.3	51.4	36.2	24.6	37.9	41.5
Lignin	0.1	0.2	4.9	8.4	3.2	3.2
Ether extract	10.7	8.6	3.1	2.7	6.8	5.7
Total fatty acids	8.7	6.6	2.1	1.2	5.2	4.2
Ash	6.2	7.0	5.5	10.1	6.7	7.1
Ca	1.25	1.31	0.29	1.52	1.02	1.04
P	0.54	0.63	0.27	0.24	0.40	0.44
Mg	0.37	0.35	0.21	0.35	0.32	0.31
NE_L[3], Mcal/kg	. . .	. . .	. . .	. . .	1.76	1.76

[1]CDG = corn distillers grains; BLEND = CDG, fish meal, and soybean meal.
[2]Calculated at 50:30:20 of concentrate mix, corn silage, and alfalfa hay, respectively.
[3]Estimated from the NRC (15).

limitation to milk production. Milk fat yields and percentages were unaffected by diet, but milk protein percentages tended ($P = 0.14$) to be higher when cows were fed RPLM. The milk protein yield was unaffected by diets. The marginal increase of milk protein content supported a hypothesis that milk protein percentage is

Table 3. Dry matter intake, milk yield, milk composition, BW, and BCS from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[1]	RPLM[2]	PROT × RPLM
						P		
DMI, kg/d	28.4	27.7	27.8	27.3	0.69	0.49	0.40	0.87
Milk, kg/d	32.6	31.7	32.8	32.8	0.57	0.27	0.45	0.43
3.5% FCM, kg/d	33.5	32.8	33.6	33.2	0.70	0.72	0.42	0.82
ECM,[3] kg/d	33.7	33.1	33.9	33.6	0.66	0.59	0.48	0.77
SCM, kg/d	31.4	30.7	31.4	31.2	0.63	0.69	0.49	0.71
Fat								
%	3.72	3.76	3.67	3.63	0.07	0.21	0.95	0.51
kg/d	1.20	1.16	1.20	1.18	0.03	0.97	0.46	0.94
Protein								
%	3.23	3.26	3.25	3.26	0.02	0.50	0.14	0.54
kg/d	1.05	1.02	1.06	1.07	0.02	0.23	0.84	0.59
Lactose								
%	4.96	4.93	4.89	4.91	0.02	0.07	0.78	0.09
kg/d	1.62	1.54	1.61	1.61	0.03	0.53	0.41	0.31
SNF								
%	8.91	8.92	8.87	8.91	0.03	0.40	0.39	0.55
kg/d	2.90	2.83	2.91	2.92	0.05	0.36	0.59	0.41
SCC, $\times 10^3$/ml	166	175	144	254	53.1	0.60	0.27	0.35
BW, kg	600	583	600	597	6.3	0.30	0.12	0.24
BCS[4]	3.0	2.9	2.9	3.0	0.1	0.89	0.89	0.22

[1]Protein source (CDG vs. BLEND).
[2]RPLM source (RPLM vs. no RPLM).
[3]Energy-corrected milk.
[4]Scored on a five-point scale where 1 = emaciated to 5 = overly fat (29).

Table 4. Milk protein fractions quantified by SDS-PAGE from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[1]	RPLM[2]	PROT × RPLM
	(% of total milk protein)					*P*		
Casein								
α-Casein	39.56	38.01	37.54	37.67	0.62	0.07	0.26	0.19
β-Casein	28.89	29.23	28.53	27.39	0.69	0.13	0.57	0.30
κ-Casein	8.02	8.43	8.76	9.32	0.47	0.09	0.31	0.88
Total casein	76.46	75.67	74.83	74.38	0.80	0.08	0.44	0.83
Whey protein								
β-Lactoglobulin	17.15	17.78	18.52	18.63	0.52	0.04	0.49	0.62
α-Lactalbumin	4.09	4.17	4.51	4.74	0.29	0.09	0.60	0.78
Others	2.29	2.39	2.14	2.25	0.33	0.66	0.75	0.97
Total whey	23.54	24.33	25.17	25.62	0.80	0.08	0.44	0.83

[1]Protein source (CDG vs. BLEND).

[2]RPLM source (RPLM vs. no RPLM).

more sensitive to RPLM supplementation than either milk yield or milk protein yield in midlactation (21).

Milk protein score (22), CNCPS (17), and **PEAA** (system of increasing Lys and Met to supply 15 and 5% of the predicted essential AA in duodenal digesta, respectively; 23) were used to evaluate the AA adequacy of treatment diets (Table 1). Theoretically, blending CDG with FM and SBM or addition of RPLM to the CDG diet should have improved production according to all three evaluation systems. The BLEND diet also may have benefited somewhat from RPLM supplementation according to PEAA, but not according

Table 5. Amino acid concentrations in coccygeal arterial serum from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
AA	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[1]	RPLM[2]	PROT × RPLM
	(μmol/dl)					*P*		
EAA[3]								
Arg	15.5	17.1	16.9	17.7	0.78	0.56	0.04	0.91
His	5.6	5.7	5.3	5.8	0.31	0.81	0.39	0.49
Ile	10.7	11.4	11.8	11.8	0.89	0.43	0.71	0.69
Leu	19.2	20.4	16.8	16.7	1.36	0.03	0.68	0.65
Lys	6.9	8.0	8.7	8.9	0.64	0.06	0.24	0.54
Met	2.2	2.7	2.3	3.0	0.18	0.30	<0.01	0.88
Phe	4.6	5.0	4.5	4.3	0.30	0.13	0.70	0.43
Thr	9.6	9.6	9.8	9.7	0.62	0.84	0.99	0.98
Trp	3.0	3.0	3.3	3.1	0.24	0.40	0.55	0.69
Val	23.7	23.5	24.2	22.5	1.53	0.82	0.53	0.66
Total EAA	101.2	106.4	102.4	103.4	5.96	0.87	0.59	0.71
NEAA[4]								
Ala	24.1	22.7	22.7	23.6	1.23	0.74	0.96	0.30
Asp	0.8	0.9	0.8	0.8	0.08	0.65	0.61	0.28
Asn	4.1	4.1	3.8	4.1	0.34	0.62	0.64	0.84
Glu	5.1	5.4	5.1	5.5	0.30	0.70	0.54	0.80
Gln	23.2	21.9	21.3	22.8	1.14	0.62	0.85	0.21
Gly	26.7	24.6	26.9	26.1	1.23	0.47	0.25	0.61
Pro	9.7	9.5	8.3	8.5	0.62	0.06	0.95	0.71
Ser	8.7	9.9	9.1	9.0	0.62	0.61	0.31	0.35
Tyr	6.2	6.4	5.4	5.5	0.47	0.08	0.75	0.98
Total NEAA	108.6	105.6	103.2	106.0	4.50	0.58	0.98	0.53

[1]Protein source (CDG vs. BLEND).

[2]RPLM source (RPLM vs. no RPLM).

[3]Essential AA.

[4]Nonessential AA.

Table 6. Amino acid concentrations in subcutaneous abdominal venous serum from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected amino acids (RPLM).

	Diet					Contrast		
AA	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[1]	RPLM[2]	PROT × RPLM
	(μmol/dl)					*P*		
EAA[3]								
Arg	10.8	12.4	11.4	12.6	0.70	0.75	0.03	0.97
His	4.4	4.2	3.7	4.3	0.25	0.25	0.39	0.19
Ile	6.0	6.2	6.6	6.7	0.73	0.44	0.92	0.93
Leu	11.6	12.0	9.5	9.4	1.10	0.03	0.84	0.83
Lys	2.5	2.7	3.2	3.5	0.35	0.07	0.34	0.76
Met	0.8	1.3	0.9	1.5	0.13	0.28	<0.01	0.44
Phe	2.3	2.3	2.1	1.8	0.24	0.16	0.77	0.52
Thr	6.8	6.4	6.8	6.7	0.49	0.80	0.73	0.63
Trp	2.7	2.6	2.7	2.5	0.21	0.82	0.65	0.96
Val	17.4	17.3	18.1	17.8	1.34	0.68	0.95	0.98
Total EAA	69.9	72.2	74.2	71.9	5.34	0.71	0.99	0.67
NEAA[4]								
Ala	20.8	18.9	19.2	19.6	1.20	0.62	0.63	0.29
Asp	0.5	0.5	0.5	0.6	0.06	0.82	0.72	0.59
Asn	2.7	2.6	2.5	2.7	0.22	0.71	0.78	0.67
Glu	2.8	2.4	2.4	2.7	0.21	0.89	0.64	0.14
Gln	16.7	15.9	14.8	17.0	1.05	0.68	0.53	0.16
Gly	26.1	23.8	25.3	25.3	1.22	0.74	0.33	0.34
Pro	7.5	6.7	5.8	6.4	0.48	0.04	0.86	0.14
Ser	4.3	3.9	3.8	3.7	0.25	0.18	0.30	0.50
Tyr	4.0	3.9	3.3	3.3	0.40	0.09	0.98	0.93
Total NEAA	85.5	78.7	82.0	81.3	3.78	0.90	0.33	0.44

[1]Protein source (CDG vs. BLEND).

[2]RPLM source (RPLM vs. no RPLM).

[3]Essential AA.

[4]Nonessential AA.

to MPS and CNCPS. However, the results of this research didn't match these expectations very well except for the small increase in milk protein percentages with the addition of RPLM. The MPS and PEAA systems do not include any feed intake information, and CNCPS usually estimates lower feed intake than often occurs. The high feed intakes in this study may be the cause of no production differences. Incorporation of more precise feed intake information or other improvements are needed to refine these protein evaluation systems, as suggested in another study (19).

The proportions of total caseins tended ($P = 0.08$) to be lower and whey proteins tended ($P = 0.08$) to be higher when cows were fed BLEND diets (Table 4). Only β-lactoglobulin was higher (P = 0.04) when cows were fed BLEND diet. The causes of these results were not clear.

Amino acid concentrations in coccygeal arterial serum and subcutaneous abdominal venous serum are presented in Tables and 6. Methionine contents were increased ($P < 0.01$) in both arterial and venous serum by RPLM. This may partially explain the slightly increased milk protein content by RPLM. Lysine tended ($P < 0.08$) to be increased by the BLEND diet but was not increased by RPLM. The higher Lys concentrations in both arterial and venous blood when cows were fed BLEND diets agreed with the corresponding higher Lys supply estimated by MPS and CNCPS from BLEND diets and would also explain the minimal response to supplemental Lys with the BLEND diet. The arterial and venous Leu concentrations were lower ($P < 0.05$) in BLEND diets. This reflected the higher Leu content in corn products than in SBM or FM. Proline and Tyr in arterial serum were slightly lower ($P < 0.10$) for the cows fed BLEND diets. The higher ($P < 0.05$) serum Arg associated with RPLM supplementation may be a result of either increased absorption or reduced tissue uptake.

Arteriovenous differences (Table 7) were nearly unchanged for all essential AA across the treatments. It appeared that the mammary gland had no further physiological need to increase its uptake of Met in response to the increased arterial Met content. The same phenomenon with Met was observed in a previous study (16) with RPLM supplementation. Arteriovenous differences of Lys across the mammary gland were slightly increased ($P < 0.12$) by the BLEND diets, which is consistent with the increased ($P < 0.07$) arterial Lys concentrations of cows fed those diets. Feeding RPLM caused a minor increase in the arteriovenous difference for Lys. The increased arteriovenous difference for Lys may imply the more limiting status of Lys for the need of mammary gland.

Amino acid extraction efficiencies, transfer efficiencies, and ratios of uptake to output are three common parameters that have been used in many studies (4, 7, 16, 19, 30) to evaluate the order of limiting AA and the AA status. Both extraction efficiencies (Table 8) and transfer efficiencies (Table 9) indicated that Lys, Met, and Phe were the three most limiting AA for all diets. Amino acid extraction efficiency indicated Lys as the first-limiting AA for all diets; Met was second-limiting for CDG-containing diets and for BLEND diet, and Phe was second-limiting for the diet containing BLEND plus RPLM. Amino acid transfer efficiencies showed that Lys, Met, and Phe were first-, second-, and third-limiting AA, respectively, for diets containing CDG. Methionine was first-limiting for the BLEND diet, Phe was first-limiting for the BLEND plus RPLM, and Lys was second-limiting for both BLEND-containing diets. If Tyr were considered as an essential AA, it would be first-, second-, and third-limiting AA for BLEND plus RPLM, BLEND and CDG containing diets, respectively. Methionine status, as indicated by lower extraction and transfer efficiencies, was apparently improved by RPLM supplementation ($P < 0.01$), but only transfer efficiency indicated a tendency toward improved Lys status with BLEND ($P < 0.07$) and RPLM ($P < 0.18$). The transfer efficiencies of Arg were lower ($P < 0.05$) for RPLM diets, which were related to higher arterial Arg contents in RPLM diets. In contrast, lower arterial Leu concentrations resulted in the higher ($P < 0.01$) transfer efficiencies for BLEND diets. The results of AA extraction efficiency and transfer efficiency were in good agreement with each other when used to evaluate the order of limiting AA. Amino acid extraction efficiency is a more accurate method of evaluating the AA status of diets because no errors from estimates of blood flow are involved (16, 19). The data from this study supported this conclusion.

The ratios of uptake to output (Table 10) showed that group 2 AA (Arg and branched-chain acids) had an excess uptake, which may be attributable to some other tissue metabolic needs for those AA as illustrated by Mepham (13). Tryptophan was the first-limiting AA for CDG diets, and BLEND diets tended to

Table 7. Arteriovenous differences in AA concentrations from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
AA	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[1]	RPLM[2]	PROT × RPLM
	(μmol/dl)					*P*		
EAA[3]								
Arg	4.7	4.7	4.9	5.0	0.35	0.49	0.70	0.89
His	1.3	1.4	1.6	1.5	0.13	0.09	0.69	0.34
Ile	4.7	5.2	5.2	5.1	0.33	0.62	0.47	0.36
Leu	7.7	8.4	7.3	7.3	0.52	0.20	0.52	0.48
Lys	4.4	5.3	5.5	5.4	0.37	0.11	0.26	0.20
Met	1.4	1.5	1.4	1.5	0.13	0.77	0.76	0.55
Phe	2.4	2.6	2.3	2.5	0.19	0.59	0.34	0.65
Thr	2.8	3.2	3.0	2.9	0.22	0.97	0.46	0.26
Trp	0.4	0.4	0.6	0.6	0.17	0.16	0.82	0.57
Val	6.4	6.1	6.2	4.6	0.79	0.27	0.27	0.43
Total EAA	36.6	40.1	36.5	38.0	2.86	0.69	0.38	0.73
NEAA[4]								
Ala	3.3	3.8	3.7	4.0	0.58	0.64	0.47	0.89
Asp	0.2	0.4	0.3	0.2	0.06	0.46	0.36	0.07
Asn	1.4	1.5	1.3	1.4	0.16	0.60	0.55	0.85
Glu	2.3	3.0	2.7	2.8	0.28	0.65	0.28	0.20
Gln	6.4	6.0	6.5	5.9	0.57	0.74	0.51	0.98
Gly	0.6	0.8	1.6	0.8	0.35	0.17	0.49	0.15
Pro	2.2	2.8	2.5	2.1	0.25	0.38	0.62	0.06
Ser	4.4	6.0	5.2	5.3	0.53	0.90	0.11	0.14
Tyr	2.2	2.4	2.1	2.3	0.20	0.49	0.45	0.89
Total NEAA	23.1	26.9	23.9	24.7	1.93	0.75	0.25	0.44

[1]Protein source (CDG vs. BLEND).
[2]RPLM source (RPLM vs. no RPLM).
[3]Essential AA.
[4]Nonessential AA.

Table 8. Amino acid extraction efficiency[1] of essential AA from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
AA	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[2]	RPLM[3]	PROT × RPLM
	%					P		
Arg	30.5 (6)[4]	28.0 (7)	30.1 (8)	28.6 (7)	1.85	0.84	0.24	0.89
His	22.1 (9)	25.1 (9)	31.0 (7)	26.5 (8)	1.97	0.02	0.70	0.07
Ile	44.6 (4)	45.9 (4)	45.3 (4)	43.3 (5)	2.44	0.61	0.97	0.52
Leu	40.7 (5)	41.2 (5)	45.0 (5)	43.9 (4)	2.60	0.18	0.90	0.74
Lys	64.6 (1)	66.9 (1)	65.1 (1)	60.7 (1)	2.28	0.28	0.53	0.11
Met	62.4 (2)	54.3 (2)	63.0 (2)	49.1 (3)	4.02	0.72	<0.01	0.30
Phe	52.8 (3)	53.1 (3)	52.8 (3)	57.3 (2)	3.68	0.47	0.61	0.66
Thr	29.2 (7)	33.9 (6)	32.0 (6)	30.7 (6)	1.68	0.96	0.38	0.07
Trp	12.9 (10)	11.9 (10)	17.9 (10)	16.6 (10)	2.40	0.25	0.66	0.81
Val	27.1 (8)	25.9 (8)	26.6 (9)	18.7 (9)	3.54	0.26	0.23	0.38
Tyr[5]	37.3 [6]	38.2 [6]	40.7 [6]	42.1 [6]	3.51	0.21	0.94	0.85

[1]Extraction efficiency = Arteriovenous difference of AA (μl/dl) × 100/arterial AA concentration (mmol/dl).

[2]Protein source (CDG vs. BLEND).

[3]RPLM source (RPLM vs. no RPLM).

[4]Numbers in parentheses indicate the apparent order of limiting AA.

[5]Numbers in brackets are ranking of Tyr if it were considered an essential AA.

improve Trp status ($P < 0.17$). Corn protein is low in Trp as well as Lys. Because of low concentrations in blood and analysis difficulties, results of evaluating Trp as the limiting AA for milk protein synthesis were not consistent among many studies (4, 7, 16, 19). The lower uptake than output of Trp for CDG diets in this study could be attributed to the inaccuracy of analysis, which is a common problem when analyzing for Trp. However, one still could make a reasonable inference that, like Lys, Trp would be deficient under certain situations especially when large amounts of corn products are included in the diet as in this research. For the other AA, there were no obvious differences between their ratios for ranking. Also, there were no significant effects ($P > 0.10$) of protein source, RPLM, or interactions on uptake to output ratios. It appeared that little information about AA status or limiting order could be inferred from these ratios.

The ruminal parameters evaluated (Table 11) were not affected by treatments. Total VFA concentrations

Table 9. Amino acid transfer efficiency[1] of essential AA from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
AA	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[2]	RPLM[3]	PROT × RPLM
	%					P		
Arg	18.9 (8)[4]	16.6 (10)	17.6 (8)	16.7 (9)	0.91	0.85	0.04	0.74
His	22.3 (7)	21.5 (7)	24.3 (7)	22.2 (7)	1.29	0.26	0.22	0.53
Ile	30.6 (4)	28.0 (5)	28.2 (6)	28.4 (5)	2.12	0.74	0.46	0.61
Leu	27.9 (6)	25.5 (6)	32.3 (4)	32.9 (6)	2.23	<0.01	0.53	0.62
Lys	59.4 (1)	51.7 (1)	48.4 (2)	45.9 (2)	0.40	0.06	0.17	0.59
Met	58.0 (2)	45.5 (2)	54.5 (1)	43.4 (3)	3.22	0.43	<0.01	0.89
Phe	46.6 (3)	41.7 (3)	47.1 (3)	51.4 (1)	3.27	0.07	0.67	0.28
Thr	29.6 (5)	30.0 (4)	28.7 (5)	29.6 (4)	1.99	0.88	0.87	0.96
Trp	16.7 (10)	18.0 (8)	15.3 (10)	16.7 (10)	1.68	0.47	0.47	0.96
Val	17.1 (9)	16.8 (9)	17.1 (9)	19.0 (8)	1.22	0.27	0.65	0.50
Tyr[5]	47.8 [3]	45.1 [3]	54.3 [2]	57.7 [1]	4.88	0.04	0.85	0.71

[1]Transfer efficiency = AA output in milk (g/d) × 100/[arterial AA concentration (g/L) × mammary blood flow (L/d)].

[2]Protein source (CDG vs. BLEND).

[3]RPLM source (RPLM vs. no RPLM).

[4]Numbers in parentheses indicate the apparent order of limiting AA.

[5]Numbers in brackets are ranking of Tyr if it were considered an essential AA.

Table 10. Uptake to output ratios[1] of essential AA from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
AA	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[2]	RPLM[3]	PROT × RPLM
						P		
Arg	1.6 (5)[4]	1.7 (6)	1.7 (7)	1.7 (5)	0.12	0.71	0.58	0.97
His	1.0 (2)	1.2 (2)	1.3 (3)	1.2 (2)	0.10	0.13	0.59	0.24
Ile	1.5 (4)	1.7 (6)	1.6 (6)	1.6 (4)	0.11	0.86	0.36	0.29
Leu	1.5 (4)	1.6 (5)	1.4 (4)	1.4 (3)	0.10	0.11	0.38	0.39
Lys	1.1 (3)	1.4 (4)	1.4 (4)	1.4 (3)	0.09	0.16	0.20	0.16
Met	1.1 (3)	1.2 (2)	1.2 (2)	1.2 (2)	0.09	0.04	0.65	0.41
Phe	1.1 (3)	1.3 (3)	1.1 (1)	1.2 (2)	0.08	0.40	0.22	0.52
Thr	1.0 (2)	1.2 (2)	1.1 (1)	1.1 (1)	0.08	0.78	0.36	0.20
Trp	0.7 (1)	0.8 (1)	1.3 (3)	1.2 (2)	0.34	0.16	0.82	0.64
Val	1.6 (5)	1.6 (7)	1.5 (5)	1.2 (2)	0.19	0.24	0.32	0.39
Tyr[5]	0.8 [2]	0.9 [2]	0.7 [1]	0.8 [1]	0.05	0.35	0.36	0.75

[1]Uptake to output ratio = [arteriovenous difference (g/L) × mammary blood flow (L/d)]/AA output in milk (g/d).

[2]Protein source (CDG vs. BLEND).

[3]RPLM source (RPLM vs. RPLM).

[4]Numbers in parentheses indicate the apparent order of limiting AA.

[5]Numbers in brackets are ranking of Tyr if it were considered an essential AA.

Table 11. Ruminal VFA, ruminal ammonia, and blood serum urea N from cows fed corn distillers grains (CDG) and a blend (BLEND) of protein supplements with or without ruminally protected Met and Lys (RPLM).

	Diet					Contrast		
	CDG	CDG + RPLM	BLEND	BLEND + RPLM	SE	PROT[1]	RPLM[2]	PROT × RPLM
						P		
VFA, mol/100 mol								
Acetic (A)	70.8	69.5	69.9	69.4	1.41	0.64	0.48	0.76
Propionic (P)	17.9	17.8	17.5	19.1	0.74	0.52	0.32	0.28
Isobutyric	0.8	1.0	0.9	0.9	0.14	0.63	0.28	0.68
Butyric	9.3	8.9	9.3	8.8	0.58	0.91	0.40	0.90
Isovaleric	1.0	1.0	1.2	1.1	0.16	0.58	0.57	0.72
Valeric	0.7	0.7	0.7	0.6	0.09	0.37	0.51	0.35
Total VFA, μmol/ml	88.9	96.3	98.2	91.0	7.23	0.39	0.99	0.34
A:P	3.88	3.75	4.04	3.66	0.17	0.85	0.14	0.46
Ruminal NH_3, mg/dl	7.2	6.9	7.8	7.5	1.28	0.65	0.76	0.99
Serum urea N, mg/dl	15.5	15.1	15.7	15.5	0.54	0.54	0.62	0.79

[1]Protein source (CDG vs. BLEND).

[2]RPLM source (RPLM vs. no RPLM).

were similar for all diets, which indicated that dietary factors evaluated in this experiment did not influence ruminal microbial fermentation. Although concentrations of all VFA and ratios of acetate to propionate were not different across the treatments, a confirmed conclusion was hard to reach since there was large variation associated with these values, as indicated by relatively large standard errors compared with other studies (16, 19). The accuracy of sampling rumen fluid with an esophageal tube rather than from a ruminal fistula may be questioned. The ruminal ammonia concentration was constant among all diets, which was anticipated because RUP was similar in all diets. Serum urea N was not affected by treatments.

CONCLUSIONS

Milk yield and composition were not further increased by blending CDG with other protein sources or by supplementation with RPLM in this experiment. However, according to blood concentrations and AA extraction and transfer efficiencies, the Met status was improved by RPLM supplementation, and Lys status was improved by BLEND diets. Lysine status was not significantly improved by RPLM and Lys was still the first-limiting AA for CDG diets, which implies that CDG diets might be deficient in Lys even after RPLM supplementation. Factors more limiting than AA may have been limitations in this study because no obvious increases of milk production were associated with the improved AA array. However, the high feed intake occurring in this experiment possibly caused excess AA intake and concealed the quality difference between protein sources evaluated.

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REFERENCES

1 Armentano, L. E., S. M. Swain, and G. A. Ducharme. 1993. Lactation response to ruminally protected methionine and lysine at two amounts of ruminally available nitrogen. J. Dairy Sci. 76:2963–2969.
2 Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
3 Brouk, M. J. 1994. Net energy of lactation and ruminal degradability of wet corn distillers grains. Ph.D. Diss., South Dakota State Univ., Brookings, SD.
4 Casper, D. P., D. J. Schingoethe, C.M.J. Yang, and C. R. Mueller. 1987. Protected methionine supplementation with extruded blend of soybeans and soybean meal for dairy cows. J. Dairy Sci. 70:321–330.
5 Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130–132.
6 Chen, K. H., J. T. Huber, C. B. Theurer, D. V. Armstrong, R. C. Wanderley, J. M. Simas, S. C. Chan, and J. L. Sullivan. 1993. Effect of protein quality and evaporative cooling on lactational performance of Holstein cows in hot weather. J. Dairy Sci. 76:819–825.
7 Drackley, J. K., and D. J. Schingoethe. 1986. Extruded blend of soybean meal and sunflower seeds for dairy cattle in early lactation. J. Dairy Sci. 69:371–384.
8 Hippen, A. R. 1996. Influence of saturation of dietary fat supplements on production and composition of milk from dairy cows. M.S. Thesis, Iowa State Univ., Ames, IA.
9 Grunan, J. A., and J. M. Swiager. 1992. Chromatography of 99 amino acids and other ninhydrin-reactive compounds in the pickering lithium gradient system. J. Chromatogr. 594:165–171.
10 Kronfeld, D. S., F. Raggi, and C. F. Ramberg, Jr. 1968. Mammary blood flow and ketone body metabolism in normal, fasted, and ketotic cows. Am. J. Physiol. 215:218–227.
11 Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Inc., Cary, NC.
12 Lowry, J. B., L. L. Conlan, A. C. Schlink, and C. S. McSweeney 1994. Acid detergent dispersible lignin in tropical grasses. J. Sci. Food. Agric. 65:41–50.
13 Mepham, T. B. 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65:287–298.
14 Mistry, V. V., and H. N. Hassan. 1991. Delactosed, high milk protein powder. 1. Manufacture and composition. J. Dairy Sci. 74:1163–1169.
15 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sc., Washington, DC.
16 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.
17 O'Connor, J. D., C. J. Sniffen, D. J. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. J. Anim. Sci. 71:1298–1311.
18 Ottenstein, D. M., and D. A. Bartley. 1971. Separation of free acids C2–C5 in dilute aqueous solution column technology. J. Chromatogr. Sci. 9:673–681.
19 Piepenbrink, M. S., D. J. Schingoethe, M. J. Brouk, and G. A. Stegeman. 1998. Systems to evaluate protein quality of diets fed to lactating cows. J. Dairy Sci. 81:1046–1061.
20 Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Page 123 *in* The Analysis of Dietary Fiber. W.P.T. James and O. Theander, ed. Marcel Dekker, New York, NY.
21 Rulquin, H., and R. Verite. 1993. Amino acid nutrition of dairy cows: productive effects and animal requirements. Pages 71–94 *in* Recent Developments in Ruminant Nutrition 3. Nottingham University Press, Loughborough, England.
22 Schingoethe, D. J. 1996. Balancing the amino acid needs of the dairy cow. Anim. Feed Sci. Technol. 60:153–160.
23 Schwab, C. G. 1994. Optimizing amino acid nutrition for optimum yields of milk and milk protein. Pages 114–132 *in* Proc. Southwest Nutr. Mgt. Conf., Phoenix, AZ. Univ. Arizona, Tucson.
24 Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 2. Extent of lysine and methionine limitation. J. Dairy Sci. 75:3503–3518.
25 Schwab, C. G., C. K. Bozak, and N. L. Whitehouse. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. J. Dairy Sci. 75:3486–3502.
26 Sukhija, P. S., and D. L. Palmquist. 1991. A rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202–1206.
27 Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.
28 Verdi, R. J., D. M. Barbano, M. E. Dellavalle, and G. F. Senyk. 1987. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milk. J. Dairy Sci. 70:230–242.
29 Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Trout, Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and it relationship to selected production characteristics. J. Dairy Sci. 65:495–501.
30 Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, K. Watanabe, T. Ueda, and H. Suzuki. 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. J. Dairy Sci. 81:1062–1077.