

Evaluation of Corn Distillers Grains and Ruminally Protected Lysine and Methionine for Lactating Dairy Cows¹

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ABSTRACT

Twelve multiparous Holstein cows averaging 57 d (36 to 77 d) postpartum at the start of the experiment were utilized in a replicated 4 × 4 Latin square design. Dietary protein supplements were 1) soybean meal, 2) soybean meal plus ruminally protected Lys and Met, 3) corn distillers grains, and 4) corn distillers grains plus ruminally protected Lys and Met. Dry matter intakes were lower for cows fed diets containing soybean meal than for cows fed diets containing corn distillers grains. Milk yield increased with the corn distillers grains (34.3, 34.0, 35.3, and 36.7 kg/d for cows fed diets 1 through 4, respectively), especially when supplemented with ruminally protected Lys and Met. Milk protein yield and percentage were increased by amino acid supplementation. Milk fat yield and percentage were unaffected by diet. The only milk protein fraction affected was nonprotein N, which was lower in the milk of cows fed corn distillers grains. Lysine, Met, and Phe were indicated as the most limiting amino acids for all diets when using amino acid extraction efficiency and transfer efficiency to indicate limiting amino acids. When corn distillers grains were supplemented with ruminally protected Lys and Met, milk yield and milk protein yield and percentage increased because the diet containing corn distillers grains was probably deficient in Lys.

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

Abbreviation key: CDG = corn distillers grains, ECM = energy-corrected milk, RPLM = ruminally protected Lys and Met, SBM = soybean meal.

INTRODUCTION

The protein needs of a cow are met from ruminal microbial protein and dietary RUP, except for the occasional use of mobile body stores. The AA needs of high yielding dairy cows exceed the AA provided from ruminal microbes, emphasizing the necessity that AA be provided by RUP. Dietary adjustments are virtually ineffective in altering the AA composition of microbial protein in the rumen (8), thus RUP must more completely meet the AA requirements of cows (9).

Corn distillers grains (**CDG**) contain significant amounts of RUP (16) that is commonly viewed as a high quality protein source (10, 17, 18, 25). Schingoethe et al. (25) observed similar milk yields and compositions for cows fed diets containing soybean meal (**SBM**) and wet CDG. However, Palmquist and Conrad (18) observed a decreased milk protein content in milk from Jerseys fed CDG as the protein supplement but not in milk from Holsteins. Corn products such as CDG are typically low in Lys; thus, the supplementation of ruminally protected Lys to diets based on CDG may increase yields of milk and milk protein. In trials involving many universities (20, 22), yields of milk and milk protein were increased when cows were fed diets based on corn gluten meal that were supplemented with ruminally protected Lys and Met (**RPLM**), but milk yields never reached the amounts achieved when cows were fed diets based on SBM. We are not aware of similar studies that have been conducted with CDG and RPLM. Thus, this study was conducted to evaluate CDG as a protein supplement for lactating cows and to evaluate the role of supplemental RPLM on the improvement of yield responses to CDG.

MATERIALS AND METHODS

Twelve multiparous Holstein cows averaging 57 d (36 to 77 d) postpartum at the start of the experi-

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TABLE 1. Ingredient content of diets containing soybean meal (SBM) and corn distillers grains (CDG).

Ingredient	Diet	
	SBM	CDG
	(% of DM)	
Alfalfa hay, chopped	25.00	25.00
Corn silage	25.00	25.00
Concentrate mix		
Corn, shelled rolled	24.70	20.80
Barley, rolled	4.25	3.25
Molasses, liquid beet	2.50	2.50
Tallow	2.00	0.40
Soybean meal, 44% CP	14.00	...
Dried CDG	...	20.25
Urea	...	0.25
Dicalcium phosphate	0.625	0.625
Sodium bicarbonate	0.40	0.40
Trace-mineralized salt	0.25	0.25
Magnesium oxide	0.125	0.125
Limestone	1.00	1.00
Vitamin A, D, and E premix ¹	0.10	0.10
Vitamin E premix ²	0.05	0.05

¹Contained 4,409,000 IU of vitamin A, 881,834 IU of vitamin D, and 200 IU of vitamin E/kg.

²Contained 44,092 IU of vitamin E/kg.

ment were used in a factorial arrangement of treatments 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 through 4 were for data collection. Dietary protein supplements were: 1) SBM, 2) SBM plus RPLM, 3) CDG, and 4) CDG plus RPLM. The amounts of RPLM products fed supplied 20 g of Lys and 6 g of Met/d per cow (Smartamine™ ML; Rhône-Poulenc, Atlanta, GA).

Diets (Table 1) contained (DM basis) 25% coarsely chopped alfalfa hay, 25% corn silage, and 50% of the respective concentrate mix. Diets were formulated to meet nutrient requirements according to the NRC (16) for cows yielding 40 kg/d of milk. Dried CDG totally replaced SBM as the protein supplement in the CDG diet. Diets were fed individually as a TMR once daily (0700 h) to obtain 5% orts using the Calan-Broadbent feeding system (American Calan, Inc., Northwood, NH); 40 g/d of the RPLM product (Smartamine™ ML) were added to the top of the feed at the time of feeding and mixed in by hand. The amount of RPLM selected was intended to supply sufficient dietary Lys to cows consuming the CDG diet based on estimates from the literature (1, 20, 22) and on our estimates of the amount of Lys available in the various feeds. Amounts of the diet offered and orts were recorded daily. Body weights and body condition scores (28) were recorded for 3 consecutive d at the beginning of each treatment period and at

the end of period four. Cows were housed in a free-stall barn and milked twice daily (0600 and 1700 h) in a double-8 parallel parlor.

Milk samples from each cow were collected for two consecutive milkings (p.m. and a.m.) during each of the last 3 wk of each treatment period. The 24-h composite samples of the milk from each cow were split into two portions for analyses. One portion was refrigerated at 4°C and sent to a laboratory to be analyzed for fat, CP, lactose, and SNF by midinfrared spectrophotometry (Multispec; Foss Food Technology Corp., Eden Prairie, MN) (2) and for SCC using a Fossomatic 90 (Foss Food Technology Corp.) (2). During wk 3 and 4 of each period, another portion of the original milk sample was analyzed for milk nitrogen fractions (23).

Samples of concentrate mixes, alfalfa hay, corn silage, and TMR were taken weekly and frozen at -20°C until further processing for analyses. Samples were oven-dried at 55°C for 48 h and then ground through an ultracentrifuge mill with a 1-mm screen (Brinkman Instruments Co., Westbury, NY). Weekly feed samples were composited by period for analyses. Feed composites were corrected to a 100% DM basis after drying at 100°C for 48 h. Contents of CP, ether extract, ash, calcium, phosphorus, magnesium, and potassium in the composited samples were determined (2). The NDF content was determined by procedures of Van Soest et al. (27). Acid detergent fiber and permanganate lignin were determined by the methods of Robertson and Van Soest (21). Non-structural carbohydrate was calculated as 100 - (NDF + ether extract + CP + ash).

During the last week of each period, samples of ruminal fluid were obtained 2 to 4 h after feeding by applying vacuum pressure to an esophageal tube fitted with a suction strainer into a 250-ml bottle, which was placed immediately on ice until processed. A 10-ml aliquot of the sample was centrifuged at 475 × g for 10 min; the supernatant was decanted, acidified with 0.5 ml of 0.1N HCl, and frozen at -20°C until analyzed for ruminal ammonia (6). An additional 10-ml aliquot was acidified with 2 ml of 25% metaphosphoric acid, centrifuged at 475 × g for 10 min, decanted, and frozen until analyzed for VFA by GLC (5).

Blood was collected at the same time as ruminal fluid by venipuncture of the coccygeal artery, the jugular vein, and the subcutaneous abdominal vein into heparinized vacuum tubes (Becton Dickinson Vacutainer System, Rutherford, NJ). Blood was placed on ice until processed in the lab. The tubes were centrifuged for 20 min at 850 × g, and the

plasma was decanted and frozen at -20°C until analysis for urea in jugular plasma (6) and for AA (11, 15) in plasma from the coccygeal artery and subcutaneous abdominal vein. The AA uptake from the blood by the mammary gland was estimated using differences in AA concentration of arterial and venous blood; mammary blood flow was estimated using the regression equation of Kronfeld et al. (13). For mammary gland uptake to output ratios, the AA composition of milk protein was assumed to be similar to that reported by Jacobson et al. (12).

All data were averaged by week and subjected to least squares analysis of variance for a replicated Latin square design by the general linear models procedure of SAS (24). Sources of variance used were square; cows within square; period within square; protein source; RPLM; interaction of protein source and RPLM; interaction of protein source and square; interaction of RPLM and square; interaction of square, protein source, and RPLM; week (where applicable); and interaction of week, protein source, and RPLM (where applicable). No differences ($P > 0.10$) caused by week or the interaction of week, protein source, and RPLM were detected, which indicated that residual effects were not a concern by wk 2 of each period. Therefore, data were pooled by period for statistical comparisons (26). Differences were considered to be significant at $P < 0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Chemical composition of concentrate mixes, forages, and diets are shown in Table 2. Diets were formulated for 17% CP; both diets were higher in CP than anticipated, primarily because of higher protein in the alfalfa hay. Both the SBM and CDG diets contained sufficient amounts of RUP and RDP, according to the NRC (16). The SBM diet contained more CP than did the CDG diet, apparently because the SBM diet contained slightly more CP than expected. Analyses of the CDG prior to the start of this experiment indicated that it contained 33.6% CP, which was higher than NRC (16) values but typical of CDG currently available from ethanol plants in the midwest US. Total diets contained similar quantities of ADF, ash, and ether extract. Neutral detergent fiber was highest for the CDG concentrate mix and CDG diets.

Cows consumed more ($P < 0.01$) of the CDG diets than of the SBM diets (Table 3), but DMI were quite high (4.4 to 4.8% of BW) for cows fed all diets. The high DMI were partially because this experiment was conducted during the stage of lactation during which maximum DMI would be expected. Feed intake was not influenced by RPLM ($P = 0.72$) although there was a tendency ($P = 0.07$) for an interaction of protein source and RPLM. Higher DMI occurred when RPLM were added to the CDG diet than when RPLM were added to the SBM diet, but it is not known why

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

Item	Concentrate mixture ¹		Forage		Diet ²	
	SBM	CDG	Corn silage	Alfalfa hay	SBM	CDG
DM, %	87.5	87.8	40.7	80.7	66.9	67.0
	————— (% of DM) —————					
CP	21.3	19.6	8.4	23.9	18.7	17.9
RUP, ³ % of CP	37	39
ADF	6.5	9.5	27.0	34.6	19.4	20.9
NDF	15.2	29.9	44.9	46.0	30.3	37.6
NSC ⁴	49.3	36.3	39.6	19.1	39.3	32.8
Lignin	1.2	4.5	3.9	8.1	3.6	5.3
Ether extract	6.6	7.2	2.3	1.6	4.3	4.6
Ash	7.6	7.0	4.9	9.4	7.4	7.1
Ca	1.21	1.09	0.20	1.21	0.96	0.89
P	0.59	0.67	0.23	0.28	0.42	0.46
K	0.91	0.64	0.81	2.13	1.02	1.06
Mg	0.31	0.34	0.17	0.34	0.28	0.30
NE _L , ³ Mcal/kg	1.69	1.76

¹SBM = Soybean meal; CDG = corn distillers grains.

²Calculated at 50:25:25 of concentrate mix, corn silage, and alfalfa hay, respectively.

³Estimated from the NRC (16).

⁴Nonstructural carbohydrates.

this occurred. Other studies have also found RPLM to have no effect on DMI (1, 14, 25), although excessive dietary Met may result in a reduction in DMI (20). All cows likely consumed more Met and Lys than required, and the excess Met intake was greatest for cows consuming the CDG diets.

Milk yield, energy-corrected milk (ECM) yield, and SCM yield were higher ($P < 0.03$) for cows fed the CDG diets; 4% FCM also followed this trend ($P = 0.09$). No difference in milk yield caused by RPLM was detected, although there was a trend toward an interaction ($P = 0.08$). Milk yield increased for cows fed CDG plus RPLM ($P < 0.04$) but not for cows fed SBM plus RPLM. Similar trends occurred for FCM, ECM, and SCM. Differences in DMI accounted for approximately one-half of the differences in yield between cows fed SBM versus CDG diets. Fat yield and percentage were unaffected by diet. Milk protein percentages and yields were increased ($P < 0.05$) by RPLM; the greatest increase ($P < 0.02$) in protein yield occurred when RPLM were added to the CDG diet. When only data from cows fed diets containing no RPLM were considered, yields of milk, FCM, ECM, and SCM were similar for cows fed diets containing SBM and CDG. Lactose and SNF yields were in-

creased ($P < 0.01$) by CDG. Lactose and SNF yields showed an interaction ($P < 0.05$) of protein and RPLM, which corresponded with differences in milk yield.

Our data agree with previous research in which cows that were fed wet CDG (3, 25), or wet (17) or dried (17, 18) CDG plus solubles yielded similar amounts of milk as did cows fed diets based on SBM. Milk protein percentages and yields when cows were fed wet CDG were similar (25) or reduced (3) compared with milk protein percentages and yields when cows were fed SBM. Increased milk protein percentages and yields have occurred in several studies in which cows were fed RPLM (7, 19, 20, 22).

Body weight and body condition scores were unaffected by protein source or RPLM, which suggests that all diets were adequate for supporting the nutrient requirements of cows in this study. However, dietary effects on BW and body condition score may not be accurately reflected in experiments such as this with short-term experimental periods.

The only milk CP component significantly affected by diet was NPN, which was lower ($P = 0.01$) in milk from cows fed CDG (Table 4). This difference might have indicated improved efficiency of protein utiliza-

TABLE 3. Dry matter intake, milk yield, milk composition, BW, and body condition score (BCS) of cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM	SE	P ¹	RPLM	P × RPLM
DMI, kg/d	28.7	27.7	29.4	30.0	0.49	<0.01	0.72	0.07
Milk, kg/d	34.3	34.0	35.3	36.7	0.48	<0.01	0.27	0.08
4% FCM, kg/d	32.6	32.3	32.9	34.2	0.63	0.09	0.46	0.28
ECM, ² kg/d	34.9	34.8	35.5	36.9	0.63	0.03	0.31	0.24
SCM, kg/d	36.8	36.3	37.7	39.3	0.70	<0.01	0.40	0.12
Fat								
%	3.63	3.71	3.59	3.58	0.09	0.12	0.89	0.64
kg/d	1.26	1.25	1.26	1.30	0.03	0.52	0.61	0.50
Protein								
%	2.99	3.06	3.02	3.08	0.02	0.25	<0.01	0.69
kg/d	1.03	1.04	1.07	1.13	0.02	<0.01	0.05	0.17
Lactose								
%	4.78	4.72	4.74	4.78	0.03	0.19	0.20	0.26
kg/d	1.65	1.61	1.70	1.76	0.03	<0.01	0.69	0.04
SNF								
%	8.43	8.43	8.45	8.51	0.04	0.19	0.45	0.48
kg/d	2.88	2.81	2.93	3.11	0.05	<0.01	0.48	0.04
SCC, ×10 ³ /ml	106	182	109	160	26.6	0.55	0.15	0.66
BW, kg	624	624	626	629	16.2	0.85	0.91	0.91
BCS ³	2.2	2.2	2.3	2.3	0.1	0.82	0.85	0.90

¹Protein source (SBM vs. CDG).

²Energy-corrected milk.

³Scored on a five-point scale where 1 = emaciated to 5 = overly fat (28).

TABLE 4. Milk nitrogen constituents for cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

	Diet					Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM	SE	P ¹	RPLM	P × RPLM
Total CP, %	3.18	3.20	3.11	3.23	0.06	0.70	0.22	0.36
True protein, %	2.87	2.88	2.81	2.92	0.06	0.89	0.25	0.35
Casein, %	2.09	2.06	1.95	2.10	0.07	0.49	0.36	0.17
Whey protein, %	0.78	0.82	0.85	0.82	0.03	0.17	0.86	0.17
NPN, %	0.050	0.052	0.048	0.049	0.003	0.01	0.29	0.62
Fractions, % of total								
True protein	92.8	92.9	93.4	93.2	0.33	0.32	0.66	0.32
Casein	65.4	64.1	62.1	64.9	1.38	0.36	0.57	0.12
Whey	27.4	28.8	31.3	28.3	1.42	0.22	0.57	0.12
NPN	7.2	7.1	6.6	6.8	0.27	0.32	0.66	0.32
Fractions, % of true protein								
Casein	72.6	71.2	68.7	71.7	1.42	0.22	0.57	0.12
Whey protein	24.6	25.8	28.0	25.6	1.18	0.18	0.59	0.12

¹Protein source (SBM vs. CDG).

tion when cows were fed CDG but might have also indicated a statistical artifact of some very small differences in milk NPN content. Nonprotein N as a percentage of total N was not affected by diet ($P > 0.31$). Contents of CP, true protein, casein, and whey

proteins were not affected ($P > 0.10$) by dietary protein source or RPLM.

Arterial (Table 5) and venous (Table 6) concentrations of Met were increased ($P < 0.01$) by CDG and RPLM. Corn distillers grains increased concen-

TABLE 5. Amino acid concentrations in coccygeal arterial plasma of cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet					Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM	SE	P ¹	RPLM	P × RPLM
	(μmol/dl)							
Arg	17.9	18.9	18.2	17.7	0.73	0.57	0.76	0.32
His	4.8	4.7	5.1	5.3	0.14	0.01	0.77	0.25
Ile	13.3	13.4	13.0	14.6	0.49	0.36	0.13	0.15
Leu	18.4	19.5	27.2	29.4	1.01	<0.01	0.12	0.58
Lys	9.1	9.0	7.6	8.6	0.45	0.06	0.32	0.22
Met	2.0	2.2	2.2	2.8	0.11	<0.01	<0.01	0.28
Phe	4.8	4.7	5.4	5.5	0.23	<0.01	0.92	0.77
Thr	10.6	9.7	9.4	9.9	0.41	0.26	0.62	0.14
Trp	4.1	4.0	4.2	4.1	0.20	0.60	0.70	0.95
Val	30.9	30.8	32.1	35.0	1.09	0.03	0.24	0.20
Total EAA ²	115.9	116.9	124.5	132.9	3.26	<0.01	0.18	0.29
Ala	25.5	21.5	23.3	23.4	0.82	0.86	0.34	0.03
Asp	1.1	1.0	1.1	10.9	0.08	0.91	0.55	0.48
Asn	3.8	3.5	3.6	3.8	0.17	0.67	0.77	0.15
Glu	5.9	5.5	5.5	5.8	0.17	0.70	0.66	0.07
Gln	23.6	22.4	23.5	24.5	1.03	0.34	0.92	0.32
Gly	28.2	24.6	29.2	26.0	1.40	0.40	0.03	0.88
Hyp	4.2	4.2	4.9	4.7	0.22	0.02	0.59	0.68
Pro	7.2	6.0	9.2	10.5	0.81	<0.01	0.91	0.16
Ser	8.3	7.1	7.8	7.7	0.28	0.77	0.05	0.08
Tyr	5.8	5.8	6.6	7.0	0.35	0.01	0.51	0.65
Total NEAA ³	113.6	101.7	114.8	114.6	2.96	0.01	0.25	0.61

¹Protein source (SBM vs. CDG).

²Essential AA.

³Nonessential AA.

trations of Leu, Phe, Val, and total EAA in both arterial and venous plasma and increased concentrations of His in arterial plasma. Lysine was unaffected by RPLM ($P > 0.22$). Because plasma AA concentrations increase only when supply exceeds requirements, Lys might have still been limiting in this study. Another study (20) reported increased concentrations of Lys and Met in serum of cows fed RPLM.

Arteriovenous differences across the mammary gland (Table 7) were increased ($P < 0.05$) by CDG for His, Leu, and Pro. The arteriovenous differences were similar with or without RPLM, except for total NEAA and Gly, which decreased when RPLM were supplemented. Lysine uptake was increased ($P < 0.05$) with CDG plus RPLM but not with SBM plus RPLM, which might have contributed to the higher milk and milk protein yields when cows were fed that diet.

Extraction efficiency, transfer efficiency, and uptake to output ratios were calculated as a means of estimating AA that limited milk protein synthesis and whether or not AA status was improved by protein source or RPLM. Differences in concentrations of free AA in arterial and venous plasma combined with

measurements of mammary blood flow have been used to determine daily uptake of AA (4, 19).

Amino acid extraction efficiency (Table 8) showed that Met status was improved by the addition of RPLM ($P < 0.03$). Lysine was indicated as first-limiting for the diets containing CDG and for the diet containing SBM plus RPLM; Met was first-limiting for the SBM diet when using AA extraction efficiency to determine the apparent limiting order of AA. Phenylalanine was the third-limiting AA for all diets, and Tyr, if considered an essential AA at the mammary tissue level, would be the fourth-limiting AA for cows fed the diets containing SBM.

Mammary blood flow rates were estimated from the procedures of Cant et al. (4) and Kronfeld et al. (13) to estimate AA transfer efficiencies (Table 9) and uptake to output ratios (Table 10). For brevity, data obtained using the procedure of Cant et al. (4) are not reported because they paralleled the results using the procedure of Kronfeld et al. (13).

Amino acid transfer efficiencies (Table 9) of Leu, Met, and Phe were lower for cows fed the CDG diet but the transfer efficiency of Lys was higher, which indicated that the CDG diet provided a more ade-

TABLE 6. Amino acid concentrations in subcutaneous abdominal venous plasma of cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet				SE	Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM		P ¹	RPLM	P × RPLM
	(μmol/dl)					P		
Arg	12.4	12.4	11.6	10.9	0.43	0.02	0.51	0.46
His	3.4	3.4	3.5	3.6	0.14	0.26	0.85	0.91
Ile	8.2	8.5	8.1	9.0	0.44	0.68	0.19	0.50
Leu	10.9	12.4	18.7	19.9	0.97	<0.01	0.20	0.90
Lys	3.4	3.8	2.7	3.0	0.32	0.04	0.22	0.86
Met	0.7	0.9	0.9	1.3	0.08	<0.01	<0.01	0.34
Phe	2.4	2.3	2.9	2.9	0.17	<0.01	0.99	0.81
Thr	7.3	6.5	6.1	6.4	0.35	0.11	0.58	0.16
Trp	3.6	3.4	3.4	3.3	0.16	0.34	0.96	0.59
Val	24.3	24.8	25.3	28.0	0.98	0.03	0.20	0.38
Total EAA ²	76.5	78.4	83.7	88.5	2.76	<0.01	0.25	0.61
Ala	21.1	18.3	19.9	20.9	0.81	0.42	0.29	0.03
Asp	0.9	0.7	0.7	0.8	0.07	0.86	0.44	0.06
Asn	2.4	2.3	2.2	2.5	0.19	0.91	0.68	0.09
Glu	2.3	2.4	2.4	2.5	0.10	0.60	0.14	0.91
Gln	16.0	16.5	16.8	18.2	0.95	0.21	0.31	0.65
Gly	25.5	22.8	26.1	24.3	1.54	0.50	0.17	0.74
Hyp	4.2	3.6	4.6	4.6	0.31	0.04	0.44	0.36
Pro	4.5	5.0	6.6	4.6	0.41	0.45	0.31	0.25
Ser	5.3	4.4	4.6	4.6	0.41	0.45	0.31	0.25
Tyr	3.2	3.5	4.4	4.6	0.33	<0.01	0.44	0.92
Total NEAA ³	85.8	79.4	88.1	90.5	2.45	0.02	0.45	0.09

¹Protein source (SBM vs. CDG).

²Essential AA.

³Nonessential AA.

TABLE 7. Arteriovenous difference in AA concentrations of cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet					Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM	SE	P ¹	RPLM	P × RPLM
	(μmol/dl)					P		
Arg	5.6	6.5	6.7	6.8	0.58	0.23	0.37	0.45
His	1.5	1.3	1.5	1.7	0.09	0.02	0.88	0.06
Ile	5.1	4.8	4.9	5.6	0.27	0.31	0.48	0.11
Leu	7.5	7.1	8.5	9.5	0.43	<0.01	0.42	0.13
Lys	5.8	5.2	4.9	5.6	0.26	0.40	0.82	0.03
Met	1.3	1.3	1.4	1.5	0.10	0.21	0.60	0.67
Phe	2.4	2.4	2.5	2.6	0.15	0.37	0.89	0.87
Thr	3.3	3.2	3.3	3.4	0.18	0.48	0.97	0.55
Trp	0.5	0.7	0.9	0.9	0.28	0.35	0.79	0.78
Val	6.6	6.0	6.3	6.9	0.33	0.33	0.91	0.11
Total EAA ²	39.4	38.5	40.9	44.5	1.76	0.06	0.47	0.23
Ala	4.4	3.2	3.4	2.5	0.64	0.23	0.12	0.85
Asp	0.2	0.3	0.4	0.3	0.09	0.81	0.93	0.33
Asn	1.4	1.2	1.4	1.4	0.09	0.32	0.26	0.61
Glu	3.6	3.0	3.2	3.3	0.17	0.59	0.19	0.07
Gln	7.7	5.9	6.8	6.3	0.60	0.68	0.09	0.31
Gly	2.7	1.9	3.2	1.7	0.45	0.76	0.02	0.51
Hyp	<0.1	0.6	0.4	0.1	0.18	0.61	0.50	0.05
Pro	2.2	1.1	2.6	3.0	0.47	0.03	0.45	0.12
Ser	2.9	2.7	3.3	3.1	0.32	0.23	0.54	0.87
Tyr	2.5	2.4	2.2	2.3	0.12	0.22	0.81	0.34
Total NEAA ³	27.8	22.3	26.8	24.0	1.23	0.78	<0.01	0.30

¹Protein source (SBM vs. CDG).

²Essential AA.

³Nonessential AA.

TABLE 8. Amino acid extraction efficiency¹ of essential AA for cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet					Contrast		
	SBM	SBM +RPLM	CDG	CDG + RPLM	SE	P ²	RPLM	× RPLM
	(%)					P		
Arg	31.2 (6) ³	34.7 (6)	37.1 (5)	38.8 (5)	1.95	0.02	0.20	0.65
His	30.5 (8)	27.9 (8)	31.0 (8)	33.2 (7)	1.94	0.16	0.91	0.24
Ile	39.2 (5)	36.4 (5)	38.7 (4)	39.3 (4)	2.02	0.57	0.59	0.42
Leu	41.5 (4)	36.9 (4)	31.9 (7)	33.2 (7)	2.02	0.09	0.29	0.26
Lys	63.7 (2)	58.1 (1)	65.4 (1)	65.5 (1)	2.50	0.09	0.29	0.26
Met	65.7 (1)	57.4 (2)	61.2 (2)	54.3 (2)	3.00	0.23	0.03	0.82
Phe	51.3 (3)	51.9 (3)	46.8 (3)	46.8 (3)	2.24	0.05	0.91	0.89
Thr	32.1 (7)	34.4 (7)	36.4 (6)	36.4 (6)	1.53	0.07	0.48	0.47
Trp	11.5 (10)	15.7 (10)	20.2 (10)	20.5 (8)	1.02	0.28	0.72	0.71
Val	21.7 (9)	19.7 (9)	20.4 (9)	20.5 (8)	1.02	0.81	0.34	0.33
Tyr ⁴	45.8 [4]	41.8 [4]	35.3 [7]	36.2 [7]	2.70	0.01	0.57	0.39

¹Extraction efficiency = arteriovenous difference of AA (micromoles per deciliter) × 100/arterial AA concentration (micromoles per deciliter).

²Protein source (SBM vs. CDG).

³Numbers in parentheses indicate the apparent order of limiting AA.

⁴Numbers in brackets are rankings of Tyr if it were considered an essential AA.

TABLE 9. Amino acid transfer efficiencies¹ of essential AA for cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet					Contrast		
	SBM	SBM +RPLM	CDG	CDG + RPLM	SE	P ²	RPLM	P × RPLM
	(%)					P		
Arg	5.7 (10) ³	5.3 (10)	5.4 (10)	5.7 (10)	0.29	0.75	0.88	0.26
His	17.3 (6)	17.7 (6)	16.6 (6)	16.6 (5)	0.56	0.13	0.74	0.66
Ile	16.4 (7)	16.3 (7)	16.9 (5)	15.4 (6)	0.64	0.75	0.24	0.31
Leu	19.6 (4)	18.6 (5)	13.2 (7)	12.7 (7)	0.82	<0.01	0.37	0.75
Lys	29.2 (3)	29.6 (3)	35.9 (2)	31.9 (1)	1.95	0.04	0.37	0.29
Met	43.6 (1)	37.9 (1)	37.5 (1)	31.8 (2)	2.04	0.01	0.02	0.99
Phe	30.4 (2)	31.0 (2)	26.5 (3)	27.0 (3)	1.30	0.01	0.68	0.99
Thr	17.7 (5)	19.8 (4)	20.2 (4)	20.2 (4)	0.79	0.09	0.22	0.23
Trp	8.2 (9)	8.3 (9)	8.2 (9)	8.7 (8)	0.45	0.63	0.48	0.65
Val	8.9 (8)	8.9 (8)	8.6 (8)	8.1 (9)	0.35	0.15	0.55	0.44
Tyr ⁴	23.9 [4]	23.4 [4]	21.0 [4]	21.9 [4]	1.10	0.07	0.88	0.54

¹Transfer efficiency = AA output (grams per day) × 100/[arterial AA concentration (grams per liter) × mammary blood flow (liters per day)].

²Protein source (SBM vs. CDG).

³Numbers in parentheses indicate the apparent order of limiting AA.

⁴Numbers in brackets indicate rankings of Tyr if it were considered an essential AA.

quate supply of Leu, Met, and Phe than did the SBM diet but was more deficient in Lys. The Lys status of cows fed CDG tended ($P < 0.18$) to be improved by the addition of RPLM, but the Met status of cows was improved by RPLM ($P < 0.03$) with both protein sources. Lysine, Met, and Phe were indicated as the three most limiting AA for all diets, but the apparent order varied with diet.

Uptake to output ratios (Table 10) showed that the status of His and Leu was improved for cows fed the CDG diet. No RPLM effects or interactions of protein and RPLM were apparent. Tryptophan was first-limiting for the SBM diet when using uptake to output ratios to determine apparent limiting order, and Met was apparently the first-limiting AA for the other three diets.

TABLE 10. Uptake to output¹ ratios of essential AA by the mammary gland for cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet					Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM	SE	P ²	RPLM	P × RPLM
						P		
Arg	5.7 (9) ³	6.7 (7)	6.9 (7)	6.8 (6)	0.61	0.32	0.49	0.36
His	1.8 (4)	1.6 (1)	1.9 (3)	2.1 (3)	0.11	0.03	0.94	0.12
Ile	2.4 (7)	2.3 (6)	2.3 (4)	2.6 (4)	0.12	0.40	0.59	0.18
Leu	2.1 (5)	2.1 (5)	2.4 (5)	2.7 (5)	0.12	<0.01	0.51	0.21
Lys	2.2 (6)	2.0 (4)	1.9 (3)	2.1 (3)	0.11	0.31	0.99	0.08
Met	1.6 (3)	1.6 (1)	1.7 (1)	1.7 (1)	0.12	0.27	0.72	0.90
Phe	1.7 (2)	1.7 (2)	1.8 (2)	1.8 (2)	0.11	0.47	0.97	0.94
Thr	1.8 (5)	1.8 (3)	1.8 (2)	1.8 (2)	0.10	0.67	0.81	0.77
Trp	1.5 (1)	2.0 (4)	2.6 (6)	2.6 (4)	0.88	0.34	0.78	0.80
Val	2.5 (8)	2.3 (6)	2.4 (5)	2.6 (4)	0.12	0.40	0.94	0.17
Tyr ⁴	1.9 [6]	1.8 [3]	1.7 [1]	1.7 [1]	0.10	0.15	0.66	0.48

¹Uptake to output ratio = [arteriovenous difference (grams per liter) × mammary blood flow (liters per day)]/AA output in milk (grams per day).

²Protein source (SBM vs. CDG).

³Numbers in parentheses indicate the apparent order of limiting AA.

⁴Numbers in brackets indicate rankings of Tyr if it were considered an essential AA.

TABLE 11. Ruminal VFA, ruminal ammonia, and blood plasma urea N of cows fed diets containing soybean meal (SBM) and corn distillers grains (CDG) with or without ruminally protected Lys and Met (RPLM).

AA	Diet					Contrast		
	SBM	SBM + RPLM	CDG	CDG + RPLM	SE	P ²	RPLM	P × RPLM
VFA, mol/100 mol								
Acetic (A)	66.2	65.6	67.0	68.0	0.50	<0.01	0.70	0.13
Propionic (P)	18.4	18.6	18.0	17.5	0.44	0.10	0.77	0.36
Isobutyric	1.2	1.3	1.2	1.1	0.06	0.07	0.68	0.57
Butyric	10.8	11.0	10.9	10.5	0.28	0.56	0.78	0.27
Isovaleric	2.0	2.0	1.7	1.7	0.09	<0.01	0.95	0.57
Valeric	1.5	1.5	1.2	1.3	0.06	<0.01	0.58	0.96
Total VFA, μ mol/ml	92.6	92.8	82.3	79.0	3.75	<0.01	0.60	0.57
A:P	3.64	3.53	3.78	3.94	0.09	0.02	0.61	0.29
Ruminal ammonia, mg/dl	18.1	18.3	9.3	11.1	3.73	0.05	0.77	0.65
Plasma urea, mg/dl	22.7	23.2	22.0	22.1	1.22	0.28	0.85	0.81

¹Protein source (SBM vs. CDG).

Amino acid extraction and transfer efficiencies were fairly consistent with each other when used as a means to determine the apparent order of limiting AA and were consistent with expected results based on the AA composition of diets. Amino acid extraction efficiency is likely the most accurate method because it uses only direct numbers, unlike the other procedures that involve estimates of mammary blood flow, which are not always estimated accurately. Therefore, AA extraction efficiency would be our method of choice when evaluating the AA status of diets.

Concentrations of ruminal VFA (Table 11) were highest ($P < 0.05$) for cows fed the diets containing SBM. Ruminal fermentation might not have been maximized with the CDG diets because those diets contained more NDF and less nonstructural carbohydrates than did the SBM diets. However, sampling errors might also be more of a problem with sampling from an esophageal tube as in this experiment versus sampling from a ruminal fistula. The ratio of acetate to propionate was higher ($P < 0.05$) for the CDG diets primarily because of higher ($P < 0.01$) acetate concentrations. Isovaleric and valeric were lower ($P < 0.01$) for the CDG diets. No differences in VFA concentrations caused by RPLM supplementation were detected. Palmquist and Conrad (18) suggested that the increase in the ratio of acetate to propionate for the CDG diet was due to the relatively high fat content and low fermentable starch of CDG.

Ruminal ammonia was higher ($P < 0.05$) with the SBM diets. This result was expected because the protein in CDG is less degradable in the rumen than is the protein in SBM (3, 16). Ruminal ammonia concentrations were more variable than desired, which

probably reflected problems in sampling. Plasma urea concentrations were similar across all diets.

CONCLUSIONS

Lactating cows fed CDG as the protein supplement yielded as much or more milk than did cows fed diets supplemented with SBM. The supplementation of RPLM to CDG diets fed to cows increased milk yield and milk protein yield and percentage, but a similar response to RPLM did not occur when SBM was the protein supplement. The CDG diet might still have been deficient in Lys even after RPLM supplementation because blood concentrations of Lys were not elevated. Therefore, additional supplementation of Lys may further improve milk yield.

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