

A Blend of Animal and Cereal Protein or Fish Meal as Partial Replacement for Soybean Meal in the Diets of Lactating Holstein Cows¹

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ABSTRACT

Six replications in Experiment 1 and four replications in Experiment 2 of a 3 × 3 Latin square arrangement of treatments were used to compare soybean meal or soybean meal partially replaced with fish meal or a protein blend for response in intake, milk yield and composition, ruminal NH₃ N, blood urea, and ruminal fermentation in lactating Holstein cows. The blend contained 30% corn gluten meal, 30% poultry by-products, 30% blood meal, and 10% feather meal. Periods were 28 d, and the first 7 d were used for adjustment. In addition to these protein sources, diets contained corn silage, alfalfa haylage, dried cracked corn, ground barley plus added fat, and a mineral and vitamin mixture.

In Experiment 1, mean DMI was 24.4 kg, mean milk yield was 36.7 kg, mean fat percentage was 3.48%, and mean milk protein percentage was 3.06%; there were no significant differences. In Experiment 2, DMI was different for soybeans (22.6 kg) versus other sources (21.4 kg), but milk yield (32.1 kg) and fat (3.39%) and protein (2.87%) percentages did not differ among diets. In Experiment 1, ruminal NH₃ N was greatest for cows consuming soybean diets (11.0 mg/dl) and lowest for cows consuming diets containing the protein blend (8.7 mg/dl). No differences in VFA were found. The lack of response to RUP can be explained by a rather high intake of a fermentable diet, which supplied sufficient absorbable AA according to the Cornell AA model.

(**Key words:** undegraded protein, lactating cows, protein sources, milk composition)

Abbreviation key: BPS = blend of protein sources, FM = Menhaden fish meal, MP = microbial protein, SBM = soybean meal.

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INTRODUCTION

Knowledge of protein nutrition of ruminants, especially of lactating cows, has advanced considerably over the past few years. Much of this knowledge has been the result of detailed studies of several factors that contribute to the availability and potential quality of protein for intestinal absorption and milk protein synthesis. Microbial protein (MP) production is the major protein source and is primarily dependent on the rate and extent of fermentation of dietary components (13, 16, 17). In all practical diets, more than 30% of dietary protein is RUP and potentially contributes to the absorbable AA pool at the duodenal site. The NRC (14) has indicated that at times of high milk yield, additional RUP, up to 39 to 42% of the dietary protein, is needed to meet protein demands. In many feeding trials, milk protein yield was increased by feeding greater amounts of RUP (2, 3, 4, 5, 6, 9); however, this result was not always consistent (3, 12, 21, 22). In surgically altered cows, MP flow to the small intestine was decreased slightly when RUP sources were fed relative to a soybean meal (SBM) control (7), but total protein flow increased. Flows of Met and Lys did not change significantly until RUP sources replaced at least 35% of the SBM protein (7). However, when sources of RUP were high in either Met or Lys, duodenal flow of Met and Lys increased accordingly.

If it is assumed that increased RUP is required to support higher yields of milk protein, then the AA contribution of the RUP source becomes important. The contribution of AA by MP and RUP sources can be estimated, as shown by Polan (19), Van Horn and Powers (22), and most recently by the Cornell program (17). The ultimate measure of response to diets with planned AA contributions is milk yield response.

Therefore, the following experiments were conducted to compare diets supplemented with SBM as the sole protein source or SBM substituted partially with Menhaden fish meal (FM) with a blend of protein sources (BPS) on intake, yield of milk and milk

TABLE 1. Dietary composition of Experiment 1.

Composition	Soybean meal	Fish meal	Blend ¹
	(% of DM)		
Ingredient			
Corn silage	40	40	40
Alfalfa haylage	10	10	10
Dried cracked corn	17	18.6	19.6
Ground barley	9	9	9
Soybean meal	18.6	13.2	12.2
Fish meal	...	3.8	...
Blend	3.8
Fat	2.4	2.4	2.4
Mineral and vitamin mix ²	3	3	3
Chemical			
DM, %	55.5	55.5	55.5
CP, % of DM	17.1	17.2	17.2
ADF, % of DM	15.9	16.0	16.0
NDF, % of DM	29.3	28.6	28.6
RUP, ³ %	33.4	39.1	38.8
NE _L , Mcal/kg of DM	1.69	1.68	1.68

¹Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

²Mineral and vitamin mix contained 13.5% Ca, 6.25% P, 2.25% Mg, 1.7% S, 25.0% NaHCO₃, 88,000 IU/kg of vitamin A, and 25,000 IU/kg of vitamin D.

³Percentage of dietary protein.

components, and certain biochemical parameters of cows that were fed these diets.

MATERIALS AND METHODS

Two experiments were conducted in virtually the same manner, except for numbers of cows, differences in DIM, forage differences, and some biochemical measurements. A 3 × 3 Latin square arrangement of treatments was used with 6 and 4 replications, respectively, in Experiments 1 and 2. The total mixed diets were based on corn silage and alfalfa haylage and contained dried cracked corn and ground barley (Tables 1 and 2). Soybean meal was the sole supplemental protein source in the control diet. Ruminant-grade FM (Zapata Protein USA, Inc., Mandeville, LA) was partially substituted for SBM in a second diet. In the third diet, SBM was partially substituted with a BPS containing 30% corn gluten meal (Corn Products, Winston-Salem, NC), 30% blood meal of swine origin (Smithfield Packing, Suffolk, VA), 30% poultry by-product meal, and 10% feather meal (both supplied by Valley Protein, Inc., Winchester, VA). Although the DM was similar for the sources, BPS was not adjusted for DM content of the sources. Fish meal contained 90.5% DM, 60% CP, and 7.75% fat (as-fed basis), and, as a percentage of

AA, 5.0% Lys and 2.1% Met. The FM was chosen because of its potential contribution of Lys and Met in the RUP (8). Protein blend was estimated to contain 92.4% DM, 78% CP, and 5.5% fat (as-fed basis) and, as a percentage of AA, 5.2% Lys and 1.9% Met. Both FM and BPS were estimated to supply potentially limiting AA to the more degradable SBM control diet offered at similar amounts (19).

In Experiment 1, the diets (Table 1) were lower in fiber and higher in energy than initially planned because body condition of the cows was less than desirable (initial DIM = 49 ± 12; parity = 2.8; no primiparous cows). In Experiment 2, diets (Table 2) contained more forage, especially alfalfa haylage and fiber, and were lower in energy. Cows averaged 96 ± 36 DIM (parity = 2.8; no primiparous cows) at the beginning of this study. In both experiments, diets were fed in equal portions twice daily (0600 and 1500 h) to support ad libitum intake (~10% orts).

The experimental feeding periods were 28 d; during the first 7 d, cows adjusted to the diet. A total mixed diet was fed twice daily, individually in Calan doors, in quantities of 5 to 10% in excess of intake. Orts were removed and weighed once daily for 4 d of each of the remaining 3 wk to estimate daily intake. Prior to the beginning of the study, cows were stratified into outcome groups according to milk yield; then, triplicates were randomly allotted to treatment with

TABLE 2. Dietary composition of Experiment 2.

Composition	Soybean meal	Fish meal	Blend ¹
	(% of DM)		
Ingredient			
Corn silage	34.3	35.3	35.7
Alfalfa haylage	30.9	31.1	31.2
Dried cracked corn	6.0	6.1	6.1
Ground barley	11.1	11.2	11.2
Soybean meal	12.5	8.4	7.8
Fish meal	...	2.7	...
Blend	2.8
Fat	2.2	2.2	2.2
Mineral and vitamin mix ²	3.0	3.0	3.0
Chemical			
DM, %	54.0	53.5	53.4
CP, % of DM	16.2	16.1	16.2
ADF, % of DM	19.0	18.9	19.1
NDF, % of DM	34.9	35.2	35.4
RUP, ³ %	30.7	34.6	35.1
NE _L , Mcal/kg of DM	1.57	1.56	1.55

¹Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

²Mineral and vitamin mix contained 13.5% Ca, 6.25% P, 2.25% Mg, 1.7% S, 25.0% NaHCO₃, 88,000 IU/kg of vitamin A, and 25,000 IU/kg of vitamin D.

³Percentage of dietary protein.

the restriction that an equal number of cows were on each treatment in each period.

Cow BW were measured at ~1400 h on 9 and 28 d of each period. Milk yield was recorded electronically twice daily (~0100 and 1330 h). Milk was sampled at two consecutive milkings on d 9 and 28 of each period. Milk fat, protein, lactose, and SNF content were determined by infrared analysis on a four-channel spectrophotometer (Multispec Mark I[®]; Foss Food Technology, Eden Prairie, MN).

Feed sources were sampled at wk 1 of each period for analysis and in situ RDP. Diet components were analyzed for DM, for CP by the Kjeldahl procedure (1) using a Tecator Kjeltex (Tecator, Inc., Herndon, VA), for ADF according to methods of Goering and Van Soest (11), and for NDF according to procedures of Van Soest et al. (23).

Degradability of the protein in feed sources was estimated by the in situ bag technique (15) using polyester dacron bags. The ratio of sample size to bag surface was 20 mg/cm². Forage samples were ground with dry ice through a 6-mm screen (Wiley Mill; Thomas Scientific, Swedensboro, NJ). Other feedstuffs were subjected to ruminal degradability in the same form as fed. Bags containing each feed were suspended for 0, 2, 6, 12, 24, 48, and 72 h through the ruminal cannulas of two lactating Holstein cows that were consuming a diet similar to the SBM control. Dried residuals of the two bags for each time interval were combined, ground through a 1-mm screen, and analyzed for N. The kinetics of CP degradation were computed using the first-order model proposed by Ørskov and McDonald (18). Equations were fitted using the Marquardt iterative method and PROC NLIN of SAS (20). Overall degradabilities were

predicted for the fractional passage rates of 5%/h.

Blood was taken by jugular puncture (2 to 4 h postfeeding) on d 20 of each period, and plasma was stored at -20°C until urea analysis. During the same period, ruminal fluid was aspirated by esophageal probe, and samples were stored for NH₃ and VFA determination by the addition of 2.5% metaphosphoric acid.

Plasma urea N was subjected to enzymatic release (Jackbean Urease, Type IX; Sigma Chemical Co., St. Louis, MO) of NH₃, which, as well as ruminal NH₃, was measured colorimetrically as described by Weatherburn (25).

Ruminal VFA were measured on a Varian Vista 6000[®] gas chromatograph (Varian, Sunnyvale, CA). The stationary column packing was 10% SP/200/1% H₃PO₄ on 80/100 Chromasorb W AW (Supelco, Bellefonte, PA). The carrier gas (N₂) flow was 30 ml/min, inlet temperature was 175°C, column temperature was 110°C, and detector temperature was 170°C. Detection was by flame ionization. A known solution of C₂ to C₅ volatile acids was used as a standard. 4-Methyl valeric acid (Pfaltz and Bauer, Waterbury, CT) was used as an internal standard for each unknown.

Data were summarized and analyzed using SAS (20). Values were means for the period. The model included diet, period, square, and cow nested within square. Cow nested within square was used to remove variance within cow. If the probability exceeded 0.10, the response was considered to be nonsignificant. Orthogonal contrasts were tested to compare dietary effects.

Experimental results were compared with the potential values obtained by the Cornell AA model

TABLE 3. Effect of protein source on DMI, milk yield, and yield and percentage of milk components in Experiment 1.

Item	Soybean meal	Fish meal	Blend ¹	SE	P <
DMI, kg/d	24.5	24.4	24.2	0.40	0.89
Milk, kg/d	36.6	37.3	36.9	0.29	0.25
3.5% FCM, kg/d	36.6	36.9	36.6	0.36	0.80
Fat					
kg/d	1.28	1.28	1.28	0.02	0.97
%	3.52	3.45	3.48	0.05	0.54
Protein					
kg/d	1.12	1.14	1.12	0.01	0.19
%	3.07	3.07	3.05	0.01	0.57
SNF					
kg/d	3.15	3.19	3.15	0.03	0.58
%	8.68	8.66	8.69	0.02	0.51

¹Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

TABLE 4. Effect of protein source on DMI, milk yield, and yield and percentage of milk components in Experiment 2.

Item	SBM ¹	Fish meal	Blend ²	SE	P <	Contrast	
						SBM vs. Blend and fish meal	Blend vs. fish meal
DMI, kg/d	22.6	20.9	21.8	0.37	0.02	0.02	NS ³
Milk, kg/d	32.0	31.8	32.5	0.51	0.59	NS	NS
3.5% FCM, kg/d	2.6	1.8	2.4	0.44	0.22	NS	0.10
Fat							
kg/d	1.15	1.12	1.19	0.02	0.10	NS	0.04
%	3.63	3.53	3.61	0.05	0.30	NS	NS
Protein							
kg/d	0.88	0.89	0.90	0.02	0.73	NS	NS
%	2.79	2.80	2.75	0.02	0.08	NS	0.04
Lactose							
kg/d	1.61	1.62	1.67	0.03	0.35	NS	NS
%	5.07	5.08	5.06	0.02	0.64	NS	NS
SNF							
kg/d	2.74	2.74	2.83	0.05	0.42	NS	NS
%	8.61	8.63	8.56	0.01	0.01	NS	0.01

¹Soybean meal.

²Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

³P > 0.10.

(17), taking into account diet formulation and measured intake for data in Experiment 1.

RESULTS AND DISCUSSION

In Experiment 1, there were no differences in DMI, yield of milk or milk components, or milk composition (Table 3). The DMI exceeded predicted quantities and supported substantial milk yields. Milk fat and milk protein contents were near average for Holsteins in early lactation.

In Experiment 2, DMI and yields of milk and milk components were lower (Table 4) than those in Experiment 1, but again no differences were found for yield. However, DMI was greater for cows fed the

SBM diet than for those fed the diets with more RUP protein. More milk fat was secreted by cows fed BPS than by cows fed FM diets. Conversely, milk protein concentration was greater for cows fed FM versus that for cows fed BPS treatments.

Ruminal NH₃ N concentrations were in the expected range when dietary protein was supplied at 16 to 17% of the DM (Table 5). Orthogonal contrasts for Experiment 1 showed that NH₃ N was significantly greater for the SBM diets, but no differences were found in contrasts for Experiment 2 (Table 5). Plasma urea N did not differ in either experiment.

Ruminal VFA did not differ because of diet (Table 6). Concentrations and ratio of VFA were similar to

TABLE 5. Effect of protein source on ruminal NH₃ N and plasma urea of cows in Experiments 1 and 2.

Item	Soybean meal	Fish meal	Blend ¹	SE	P <
	(mg/dl)				
Ruminal NH ₃ N					
Experiment 1	11.0	9.8	8.7	0.64	0.07 ²
Experiment 2	10.8	12.5	12.3	1.83	0.63
Plasma urea N					
Experiment 1	16.2	15.6	15.8	0.53	0.63
Experiment 2	15.8	15.9	18.9	1.35	0.22

¹Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

²Contrast: soybean meal versus blend and fish meal (P < 0.04).

TABLE 6. Effect of protein source on VFA in Experiment 2.

VFA	Soybean meal	Fish meal	Blend ¹	SE	<i>P</i> <
Total, $\mu\text{mol/ml}$	98.14	95.52	99.10	4.09	0.82
	(mol/100 mol)				
Acetate (A)	69.05	68.27	69.42	0.83	0.62
Propionate (P)	17.10	18.21	16.78	0.83	0.46
Isobutyrate	0.78	0.75	0.78	0.03	0.60
Butyrate	10.08	9.88	10.09	0.28	0.85
Isovalerate	1.42	1.38	1.38	0.03	0.54
Valerate	1.56	1.51	1.55	0.03	0.43
A:P	4.12	3.88	4.16	0.19	0.55

¹Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

each other and in a normal range. In situ protein degradability was somewhat greater for cracked corn and SBM than the values used when the diets were originally calculated (Table 7). However, RUP was increased to values that were near those anticipated for diets with FM or BPS.

In several studies summarized by Van Horn and Harris (21), increases were observed in milk yield, but not always when undegraded sources of protein were included in the diet, often replacing SBM. Whether or not a positive response in milk yield occurred can probably be explained in part by the sources of bypass protein and the inherent AA composition. Van Horn and Harris (21) found that FM generated the most consistent response in milk yield, approximately 1 kg/d in 20 comparisons with SBM. Fish meal is a good source of Met and Lys; even after ruminal exposure, FM seemed to maintain an ade-

quate content of all essential AA (8); therefore, such a response was consistent with expectations. Inclusion of heat-treated SBM in the diet has given a similar response in milk yield (6, 21).

According to Van Horn and Powers (22), the response of milk yield to substitution of SBM with RUP sources was more positive for diets based on alfalfa than for diets based on corn silage. Van Horn and Harris (21) speculated that, because less protein supplementation is needed for alfalfa than for lower protein forages, small amounts of RUP substituted for SBM might be more effective. We suggest that MP contribution could be greater for corn silage than for alfalfa because of the fermentable starch content, possibly stimulated by available peptides from additional soy protein.

In Experiment 1, the forage was predominantly corn silage, but, in Experiment 2, contributions of

TABLE 7. Estimates of protein fractions and protein degradability in feedstuffs in Experiment 1 as determined by in situ incubation.

Feedstuff	DM	CP ²	Protein fraction ¹			K_d^3	RUP ⁴
			A	B	C		
			(%)			(%/h)	
Alfalfa haylage	56.3	17.9	35.6	39.2	25.2	4.2	46.7
Corn silage	36.5	7.2	44.1	29.6	26.3	5.2	40.9
Corn grain	88.0	10.8	39.6	58.8	1.6	7.2	25.7
Barley	91.7	10.1	73.3	17.5	9.2	4.5	18.4
Soybean meal	90.1	46.4	51.9	46.2	1.9	13.9	14.1
Fish meal	88.8	63.8	37.7	27.1	35.3	2.3	53.7
Protein blend ⁵	93.3	74.5	24.8	39.7	35.6	2.5	61.9

¹A = Water-soluble CP, B = CP degraded at a measurable rate, and C = residual CP after 72 h.

²Expressed on a DM basis.

³Degradation rate.

⁴Calculated at a ruminal turnover rate of 5%/h.

⁵Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, 10% feather meal; no adjustment was made for DM.

TABLE 8. Evaluation of experimental diets by the Cornell model (17) for predicting AA adequacy relative to mean group milk yield.

Item	Soybean meal		Fish meal		Blend ²	
	Actual ¹	Predicted	Actual	Predicted	Actual	Predicted
DMI, kg	24.5	20.3	24.4	20.5	24.4	20.4
MP ³ Allowable milk, kg	43.4	34.9	47.4	39.2	47.4	40.1
AA Allowable milk, kg	48.5	40.5	57.6	48.2	53.0	45.9
Metabolizable protein						
From bacteria, g	1663	1424	1630	1411	1640	1426
From RUP, g	1214	915	1434	1134	1407	1165
RDP, %	65	66	60	62	60	61
Excess Met						
g/d	10.0	3.4	18.9	11.0	13.7	7.7
%	122	108	140	125	130	117
Excess Lys						
g/d	40.0	16.0	54.3	30.1	47.3	27.3
%	127	112	136	121	132	120
Mean milk yield, kg/d	36.6		37.3		36.9	

¹Data in actual columns were derived using actual intake data; data in predicted columns were based on intake according to the Cornell model for a 600-kg cow yielding 41 kg of milk.

²Blend contained 30% corn gluten meal, 30% poultry by-product, 30% blood meal, and 10% feather meal; no adjustment was made for DM.

³Microbial protein.

corn silage and alfalfa haylage were about equal; in neither case was alfalfa the major contributor to the diet. Furthermore, in both of our studies, ground barley, which is more readily fermentable than corn, supplied either 9 or 11% of dietary DM. Soybean meal was the only supplemental protein in the control diet and was provided at 7.8% or greater in all other diets. Apparently, ample quantities of fermentable carbohydrates, soluble protein, and peptides were available for microbial growth in both experiments.

However, the lack of lactational response to RUP, even when large quantities of alfalfa were in the diet, could have been caused by high DMI. Wattiaux et al. (24) found no response when SBM was replaced with a blend of animal by-products in diets containing 60% of DM from alfalfa silage. Overall, DMI was 25.2 kg/d, 5 to 15% above NRC (14) predictions, giving a great excess of RDP across all dietary treatments.

During this study, the Cornell model (17) for predicting AA adequacy was published. Our diet composition, mean DMI, and an assumed milk yield of 41 kg/d were entered into this model.

Our DMI exceeded considerably that predicted by the model (Table 8); thus, larger quantities of metabolizable protein and RUP were predicted as being available to the cow. For cows fed diets containing FM and BPS, estimated metabolizable protein from bacteria was modestly reduced compared with SBM diets, based on actual intake. But metabolizable protein from RUP increased and total AA increased 5

to 7% above those in the SBM diet. According to the model, the SBM diet was adequate in first-limiting AA for 41 kg of milk. Because mean milk yield in Experiment 1 was approximately 37 kg, perhaps AA were adequate in the SBM diet. However, the experiment was conducted over a 12-wk period, so certainly some cows yielded more than 41 kg/d. Milk yields were similar across treatments; therefore, AA nutrition was apparently adequate. However, with the SBM diet, AA adequacy was probably due to higher intake than predicted. These data emphasize the importance of intake in nutritional models.

According to the Cornell model (17), diets containing FM and BPS increased the predicted amounts of Met and Lys available for milk yield. For both AA, the excess above the requirement for 41 kg of milk was greater than that predicted for the SBM diet (Table 8). Therefore, it is likely that either of the two RUP sources would allow higher milk yield than SBM would, even if DMI were less than that predicted by the Cornell model.

Cunningham et al. (10) found no differences in total flow of N or essential AA when feather meal plus blood meal replaced soluble sources of N in the diet; quantities of MP N declined numerically, but not significantly. The review by Clark et al. (7) also supported the theory that there would be no gain in intestinal proteins of RUP sources until the sources exceeded 35% of dietary supplemental N.

Beede et al. (3) introduced an animal-marine protein blend into 35 commercial herds in five different

regions of the US. Nineteen of the herds yielded significantly more milk. No detectable effects regarding whether a herd responded positively could be found for the amount of dietary fat, soluble CP, RDP, RUP, NE_L , or nonfiber carbohydrates. However, intake was not known but could probably explain the differences.

Until the last 4 to 5 yr, most studies that have focused on RUP have not placed much emphasis on predicted specific AA supplies reaching the small intestine. In several cases, positive responses in milk yield could be attributed to a particular protein source, but the response cannot be solely attributed to AA balance because often intake may increase. Unfortunately, intake has often been reduced compared with intake of the SBM control diets when RUP was included in the diet. Intake was not affected by diet in Experiment 1 of this study, but a modest decline was observed in Experiment 2. The amount of feed intake found in this study is probably quite common, but exceeded expectations of the Cornell model. Apparently, the diets in these experiments, which were highly fermentable, supplied abundant MP, negating the need for balanced RUP. Perhaps a more positive response in milk yield to our RUP sources would have been obtained if alfalfa instead of corn silage had served as the main source of forage.

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