

Expeller Soybean Meal and Corn By-Products Versus Solvent Soybean Meal for Lactating Dairy Cows Fed Alfalfa Silage as Sole Forage¹

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ABSTRACT

Production responses obtained with supplemental protein from expeller soybean meal or corn by-products, relative to solvent soybean meal, were determined in three replicated 4 × 4 Latin square trials. Dietary forage (54 to 58% of DM) was solely alfalfa silage containing 30 to 55% DM and 21% CP (DM basis). Main concentrate ingredient was either ground shelled or high moisture corn; diets were fed as total mixed rations. In Trials 1 and 2, supplements were: control (0 CP), .6× (60% of the supplemental CP of the 1× treatment) solvent soybean meal, .6× expeller soybean meal, and 1× solvent soybean meal. In Trial 1 (DM intake = 24.4 kg/d), supplement had no effect on production but increased weight gain; expeller soybean meal increased production of milk and lactose relative to either amount of solvent meal. In Trial 2 (DM intake = 20.0 kg/d), supplement increased production of milk and milk components; milk production on .6× expeller soybean meal was greater than .6× solvent soybean meal. In Trial 3 (DM intake = 22.4 kg/d), distillers dried grains plus corn gluten meal replaced .6× solvent soybean meal; supplement increased production of milk, 3.5% FCM, protein, and fat with no dif-

ference among the three proteins. Across all three trials, response to supplemental protein appeared to decrease with increased DM intake. Results indicate that resistant protein from expeller soybean meal and corn by-products can replace greater amounts from solvent soybean meal, and suggest that, despite high dietary CP, absorbed protein supply may be inadequate when alfalfa silage is the sole forage.

(Key words: alfalfa silage, resistant proteins, expeller soybean meal)

INTRODUCTION

Most studies comparing protein supplements that are resistant to ruminal degradation have used corn silage as forage because its low CP allows formulation of diets with greater amounts of test protein. However, there is evidence that despite high CP, the protein in legume forages is poorly utilized by ruminants. This is particularly true for alfalfa silage, and resistant proteins also should be tested as supplements for that forage. Cows fed alfalfa silage or hay as sole forage produced less milk protein and milk with lower protein content than cows fed corn silage-based diets supplemented with soybean meal to equalize CP (4). Muck (21) reported that NPN accounted for 50 to 87% of total N in alfalfa silages ensiled at 35 to 85% moisture. Microbial protein synthesis in the rumen is required for utilization of the NPN in alfalfa silage, but this forage supplies only moderate amounts of digestible energy (22). Dehydrated alfalfa, a heat-treated protein source, increased milk production when it was fed to replace part of the high moisture alfalfa silage in the diet (26).

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¹Mention of commercial products in this paper does not constitute endorsement by the USDA or the ARS.

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TABLE 1. Composition of protein supplements.

| Component | Trials 1 and 2 | | Trial 3 | | | |
|--|--------------------------|--------------|-------------|--------------|-------------------|------------------|
| | Solvent SBM ¹ | Expeller SBM | Solvent SBM | Expeller SBM | DDGS ² | CGM ³ |
| CP, % DM | 47.4 | 43.7 | 47.8 | 46.6 | 26.2 | 71.2 |
| Total lysine, g/16g N | 6.44 | 5.92 | 6.05 | 5.57 | 1.45 | 1.56 |
| Available lysine, g/16g N | 6.18 | 5.35 | 5.67 | 5.06 | 1.01 | 1.50 |
| ADIN, % N | .83 | 1.23 | .55 | .75 | 22.92 | 4.34 |
| IV degradation rate (K_d), /h ⁴ | .140 | .024 | .096 | .038 | .026 | .017 |
| Intercept (B), % | 99.7 | 93.4 | 96.7 | 96.1 | 95.0 | 95.4 |
| Estimated escape, % ⁵ | 30 | 67 | 37 | 59 | 66 | 74 |

¹Soybean meal.

²Distillers dried grains plus solubles.

³Corn gluten meal.

⁴Ruminal degradation rate determined with an inhibitor in vitro system (6).

⁵Estimated ruminal escape, % = $\{B \times [K_p/(K_p + K_d)]\} \times 100$, where it is assumed that $K_p = .06/h$ (6).

Expeller soybean meal (ESBM), which is extensively heated during processing, had a ruminal protein escape value about two-thirds greater than solvent soybean meal (SSBM) in cows fed corn silage (5). In vitro experiments (6) and a lactation study in which cows were fed forage from both corn silage and alfalfa silages (5) demonstrated the greater ruminal protein escape for expeller than for SSBM. Distillers dried grains with solubles (DDGS) and corn gluten meal (CGM) are resistant to ruminal degradation (17), but a mixture of these proteins was ineffective in lactating cows fed corn silage-based diets, possibly because of low lysine content (31).

The objective of the present studies was to determine if ESBM, DDGS, and CGM would be used more efficiently than SSBM in lactating dairy cows fed diets based on alfalfa silage.

MATERIALS AND METHODS

Protein Supplements

Solvent soybean meal (purchased from local commercial sources) and ESBM (Soy-Plus, West Central Cooperative, Ralston, IA) were obtained in two separate batches (the first batch was fed in Trials 1 and 2 and the second in Trial 3). A single batch each of DDGS and CGM was fed only during Trial 3. Two subsamples from each batch were analyzed for DM and CP (1), total lysine (2), fluorodinitro-ben-

zene available lysine by a difference method (2), and ADIN (15). Each sample of protein supplement was assayed for fractional rate of ruminal protein degradation by an inhibitor in vitro system, and the proportion escaping the rumen was estimated (6). Results are in Table 1.

Trial 1

Twenty multiparous Holstein cows, averaging 635 kg BW, lactation number 3.5, 34 d in milk, and 34.2 kg/d milk were blocked according to production and stage of lactation into five groups of 4 cows each. Cows were randomly assigned to dietary treatment sequences within a balanced 4 × 4 Latin square. Diets contained (DM basis) about 54% alfalfa silage and 46% of a concentrate based on ground shelled corn (Table 2). The four treatments differed in source and amount of supplemental protein: 1) control (no supplement), 2) 1.93% dietary CP equivalent (CPE) from SSBM, 3) 1.87% CPE from ESBM, and 4) 3.21% CPE from SSBM. Supplemental CP in treatment 4) will be referred to as 1×; supplemental CP in treatments 2) and 3) as .6×. Diets were fed for periods of 3 wk before they were switched (total trial 12 wk); the 1st wk was considered transitional, and milk production data statistically analyzed were means from the last 2 wk of each period. Milk production was measured twice daily. Milk was sampled at both milkings 2 d each week, and proportional composites

TABLE 2. Composition of diets.

| Component | Supplemental protein (Trial 1) ¹ | | | | Supplemental protein (Trial 2) ¹ | | | | Supplemental protein (Trial 3) ¹ | | | |
|--|---|---------------|---------------|--------------|---|---------------|---------------|--------------|---|---------------------|---------------|--------------|
| | Control (0) | SSBM (.6x) | ESBM (.6x) | SSBM (1x) | Control (0) | SSBM (.6x) | ESBM (.6x) | SSBM (1x) | Control (0) | DDGS + CGM (.6x) | ESBM (.6x) | SSBM (1x) |
| | (%) of DM | | | | | | | | | | | |
| Alfalfa silage ² | 54.23 | 54.17 | 54.23 | 54.55 | 57.03 | 57.03 | 57.03 | 57.03 | 58.03 | 57.88 | 57.96 | 57.94 |
| Corn grain | 44.80 | 40.78 | 40.53 | 37.73 | .90 | ... | ... | ... | 7.10 | 1.62 | 2.61 | ... |
| High moisture corn | ... | ... | ... | ... | 41.16 | 38.21 | 38.01 | 35.66 | 33.89 | 33.80 | 33.85 | 33.83 |
| SSBM | ... | 4.08 | ... | 6.77 | ... | 3.85 | ... | 6.40 | ... | ... | ... | 7.23 |
| ESBM | ... | ... | 4.27 | ... | ... | ... | 4.05 | ... | ... | ... | 4.58 | ... |
| DDGS | ... | ... | ... | ... | ... | ... | ... | ... | ... | 4.19 | ... | ... |
| CGM | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1.51 | ... | ... |
| Dicalcium phosphate | .46 | .46 | .46 | .45 | .43 | .43 | .43 | .43 | ... | ... | ... | ... |
| Monosodium phosphate | ... | ... | ... | ... | ... | ... | ... | ... | .47 | .48 | .48 | .48 |
| Trace-mineral salt + Se | .46 | .46 | .46 | .45 | .43 | .43 | .43 | .43 | .46 | .47 | .47 | .47 |
| Vitamin premix ³ | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 | .05 |
| Chemical composition | | | | | | | | | | | | |
| CP | 18.0 | 19.1 | 19.1 | 19.5 | 16.7 | 17.9 | 17.6 | 18.8 | 17.0 | 18.1 | 18.1 | 18.9 |
| NDF | 27.6 | 27.9 | 28.0 | 28.1 | 41.4 | 40.9 | 40.9 | 40.3 | 29.6 | 31.0 | 29.9 | 30.0 |
| ADF | 20.9 | 21.0 | 21.2 | 21.3 | 24.7 | 24.8 | 24.9 | 24.8 | 19.9 | 20.5 | 20.2 | 20.1 |
| Estimated NE _l ⁴ Mcal/kg | 1.63 | 1.63 | 1.63 | 1.62 | 1.55 | 1.56 | 1.56 | 1.56 | 1.64 | 1.65 | 1.64 | 1.64 |

¹Values in parentheses are fractional amounts of supplemental CP, relative to the 1x of solvent soybean meal (SSBM) fed as SSBM, expeller soybean meal (ESBM), or a mixture containing equal N from distillers dried grains with solubles plus corn gluten meal (DDGS + CGM).

²Alfalfa silage compositions are in Table 3.

³Provided (per kg DM): 1600 IU vitamin A, 300 IU vitamin D, and .300 IU vitamin E.

⁴Computed from NE_l values of alfalfa silage (Table 3) and NRC tables (22).

were prepared and analyzed for fat and protein by infrared analysis (Wisconsin Dairy Herd Improvement Cooperative, 5301 Tokay Blvd., Madison 53711). Milk was deproteinized with TCA as described by Broderick (5). The high-speed ($31,000 \times g$, 15 min, 2°C) TCA supernatants were stored at -20°C until they were analyzed for lactose (28) and urea (30). Cows were weighed on 3 consecutive d at the start of the trial and at the end of each period.

Diets were fed as total mixed rations (TMR) for ad libitum intake. Silage was first-cutting alfalfa, which had been chopped to a theoretical length of 1.0 cm and stored in tower silos. Silage content of as-fed rations was adjusted at the beginning of each period based on DM determined at 60°C (48 h). A weekly composite of each TMR and the silage was collected from daily samples of about .5 kg and stored frozen. Feed refusals were determined daily, and subsamples of refusals from each diet were composited and stored frozen. Feed offered was adjusted to yield weighbacks of about 5% of amounts fed. The actual proportion of dietary DM from each component was computed from DM determined by toluene distillation (13) and at 105°C (1) for silage and concentrates, respectively. Diet ingredients also were analyzed for CP and ash (1), NDF, ADF, and ADIN by the method of Van Soest and coworkers (15, 25). Alfalfa silage also was analyzed for water-soluble N and NPN (12); ammonia and total amino acid (TAA) were determined colorimetrically (7) in the TCA-NPN extract. Proportions of total N as ammonia N and TAAN were computed using the TAA:N ratio in alfalfa protein without proline (3), because proline does not respond in the ninhydrin color assay used (7). Samples of TMR and feed refusals were analyzed for DM (60°C , 48 h), and DM intake (DMI) is reported on this basis. The NE_1 of alfalfa silage was computed from NDF using the legume equation of Mertens (20), which assumes intake at $3\times$ maintenance. The NE_1 content of the total ration was calculated using this NE_1 value for alfalfa silage and the NE_1 value reported in NRC tables (22). Compositions of rations and alfalfa silage fed in Trial 1 are in Tables 2 and 3, respectively.

Four hours after feeding on d 20 of each period, 5-ml blood samples were taken from each cow by venipuncture from the tail artery or vein. Blood was heparinized and stored at

TABLE 3. Composition of alfalfa silages.

| Component | Silage source | | |
|---|---------------|---------|---------|
| | Trial 1 | Trial 2 | Trial 3 |
| DM, % | 54.6 | 29.9 | 36.7 |
| Ash, % DM | 11.0 | 10.7 | 9.9 |
| CP, % DM | 20.9 | 20.5 | 20.5 |
| NDF, % DM | 43.5 | 47.1 | 39.9 |
| ADF, % DM | 36.0 | 38.5 | 30.5 |
| ADIN, % N | 5.6 | 6.3 | 4.7 |
| NE_1 , Mcal/kg DM ¹ | 1.38 | 1.31 | 1.46 |
| NPN, % N | 76.4 | 64.5 | 62.0 |
| NH_3N , % N | 6.0 | 14.9 | 5.8 |
| TAAN, % N ² | 41.9 | 45.3 | 39.3 |

¹Values of NE_1 computed from NDF using equation of Mertens (20).

²Total amino acid N (TAAN) computation based on 40.05 mmol TAA/g N for alfalfa protein (3).

-20°C until analyzed for glucose (29) and urea (30).

Data were analyzed as a 4×4 Latin square, replicated five times using the general linear model of SAS (27). The model included square, cow, period, and treatment, plus cow \times treatment and period \times treatment interactions; neither interaction was significant ($P > .19$), so both were removed from the final model. Single degree of freedom orthogonal contrasts compared: A) control vs. all three protein supplements, B) $.6\times$ SSBM vs. $.6\times$ ESBM, and c) $.6\times$ ESBM vs. $1\times$ SSBM.

Trial 2

Twenty multiparous Holstein cows averaging 588 kg BW, lactation number 3.2, 37 d in milk, and 35.6 kg/d milk were used. This experiment was also a replicated 4×4 Latin square; sources of supplemental protein and assignment of cows to treatments were the same as in Trial 1. The TMR differed in that 57% of the DM was from second-cutting alfalfa silage, and 43% of the DM was from a concentrate based on high moisture corn (Table 2). The four amounts of supplemental protein differed only slightly from Trial 1: 1) control (no supplement), 2) 1.82% dietary CPE from SSBM, 3) 1.77% CPE from ESBM, and 4) 3.03% CPE from SSBM. Designation of supplemental protein treatments in this trial, as well as length of periods, measurements of

milk production and composition, BW and feed intake, and feed sampling and analyses were as described for Trial 1. Blood samples were taken on d 19 or 20 of each period and analyzed for glucose and urea as described. Data were analyzed as described for Trial 1. As in Trial 1, neither cow \times treatment nor period \times treatment interaction was significant ($P>.21$).

Trial 3

Twenty-four Holstein cows averaging 567 kg BW, lactation number 2.2, 64 d in milk, and 32.8 kg/d milk were used. This experiment was also a 4 \times 4 Latin square, replicated six times (five squares of multiparous cows and one of primiparous cows). Method of assignment of cows to treatments was the same as in Trials 1 and 2. Dietary DM of TMR was 58% from alfalfa silage (second cutting, stored in a bunker silo) and 42% from a concentrate based on high moisture corn (Table 2); a supplement containing equal N from DDGS and CGM replaced the lower amount of SSBM fed in the previous trials. The four supplements were: 1) control (no supplement), 2) 2.17% CPE from DDGS plus CGM, 3) 2.13% dietary CPE from ESBM, and 4) 3.46% CPE from SSBM. Supplemental CP in treatment 4 will be referred to as 1 \times supplemental CP in treatments 2 and 3 as .6 \times . Length of periods, measurement of milk production and composition, BW, feeding, feed sampling, and analyses were as described for Trials 1 and 2.

Blood samples were taken on d 20 of each period; blood plasma was prepared, deproteinized, and stored as described previously (5). Deproteinized plasma was analyzed for glucose and urea as described for Trial 1. Also on d 20 of each period, rumen samples were taken from 4 nonlactating, ruminally cannulated cows fed the experimental diets in a single 4 \times 4 Latin square. Samples of strained rumen fluid (SRF), taken from the ventral sac at 0 (just prior to feeding), 1, 2, 3, 4, and 6 h after feeding, were prepared by straining about 250 g of whole rumen contents through two layers of cheese cloth; pH was measured immediately. The SRF was preserved by addition of 1 ml 50% (vol/vol) sulfuric acid per 50 ml SRF (14) and stored at -20°C . Samples were thawed, and high speed supernatants (30,000 \times g, 15 min, 2°C) were prepared and analyzed for ammonia,

TAA (7), and VFA. The VFA were determined (24) with α -ethyl-n-butyrate as internal standard (W. C. Ellis, personal communication). Total peptides were determined in high speed supernatants using a fluorescamine procedure (9). Peptide-bound amino acid (PBAA) were estimated as the difference in TAA concentration before and after hydrolysis of samples in 6 M HCl for 20 h at 105°C . The basis of this TAA analysis was a ninhydrin- CO_2 assay, adapted to an autoanalyzer (18), which responds to amino acids but not peptides (16). Average number of amino acid residues per peptide was computed by dividing PBAA by total (i.e., fluorescamine-reactive) peptides.

Data were analyzed as a 4 \times 4 Latin square as described for Trials 1 and 2 except replicated six times; observations on SRF from the four ruminally cannulated cows were analyzed as a single 4 \times 4 Latin square (27). Again, neither cow \times treatment nor period \times treatment interaction was significant ($P>.11$).

RESULTS

Trials 1 and 2

Trials 1 and 2 are discussed together because of similarity of design and supplements; the major dietary differences were in concentrate (Table 2) and DM content of alfalfa silage (Table 3). Also, protein content of the TMR averaged 6.6% higher in Trial 1 (Table 2). Dry matter intake was 22% greater on Trial 1 than Trial 2 (Table 4).

No effects ($P>.05$) of protein supplementation over control were observed in production of milk or milk components in Trial 1 (Table 4), although there were trends ($P<.10$) for increased DMI and milk protein concentration. Increased BW gains ($P<.01$) with supplemental protein (Table 4) likely were related to increased DMI (fill). Among the three proteins, ESBM increased ($P<.05$) production of milk and lactose in contrast to either amount of SSBM (Table 4). Gross efficiency (milk/DMI) with ESBM was greater ($P<.05$) than with 1 \times SSBM. Supplemental protein in Trial 2 (Table 4) increased ($P<.01$) DMI and production of milk, 3.5% FCM, protein, fat and lactose, and gross efficiency (Table 4). Actual milk secretion was greater with ESBM than .6 \times SSBM.

Milk and blood urea concentrations were

TABLE 4. Dry matter intake, body weight gain, and production of milk and milk components.

| Item | Supplemental protein ¹ | | | | SE | Contrasts ² |
|----------------------|-----------------------------------|---|---------------|--------------|-------|------------------------|
| | Control (0) | SSBM (Trials 1 & 2) or DDGS+CGM (Trial 3) (.6×) | ESBM (.6×) | SSBM (1×) | | |
| Trial 1 | | | | | | |
| Supplemental CP, g/d | 0 | 468 | 455 | 799 | . . . | |
| DM intake, kg/d | 24.0 | 24.2 | 24.4 | 24.9 | .3 | |
| Weight gain, kg/d | .05 | .56 | .39 | .33 | .11 | A** |
| Milk, kg/d | 36.7 | 36.5 | 37.5 | 36.5 | .3 | B*,C* |
| 3.5% FCM, kg/d | 35.5 | 35.6 | 36.3 | 35.5 | .3 | |
| Protein, % | 3.13 | 3.18 | 3.15 | 3.20 | .02 | |
| Protein, kg/d | 1.15 | 1.15 | 1.18 | 1.16 | .01 | |
| Fat, % | 3.32 | 3.37 | 3.33 | 3.35 | .04 | |
| Fat, kg/d | 1.21 | 1.22 | 1.24 | 1.22 | .02 | |
| Lactose, % | 4.89 | 4.90 | 4.95 | 4.94 | .04 | |
| Lactose, kg/d | 1.79 | 1.79 | 1.86 | 1.78 | .02 | B*,C* |
| Efficiency, Milk/DMI | 1.54 | 1.52 | 1.54 | 1.47 | .02 | C* |
| Trial 2 | | | | | | |
| Supplemental CP, g/d | 0 | 370 | 359 | 604 | . . . | |
| DM intake, kg/d | 19.5 | 20.3 | 20.3 | 19.9 | .2 | A** |
| Weight gain, kg/d | .36 | .35 | .20 | .09 | .10 | |
| Milk, kg/d | 29.0 | 30.2 | 31.1 | 30.7 | .4 | A***,B* |
| 3.5% FCM, kg/d | 31.0 | 32.1 | 32.7 | 32.4 | .4 | A*** |
| Protein, % | 2.95 | 3.04 | 3.04 | 3.02 | .02 | A*** |
| Protein, kg/d | .85 | .91 | .94 | .92 | .01 | A*** |
| Fat, % | 3.97 | 3.92 | 3.85 | 3.88 | .04 | A* |
| Fat, kg/d | 1.14 | 1.17 | 1.19 | 1.18 | .02 | A*** |
| Lactose, % | 4.80 | 4.83 | 4.76 | 4.79 | .04 | |
| Lactose, kg/d | 1.39 | 1.46 | 1.48 | 1.47 | .02 | A*** |
| Efficiency, Milk/DMI | 1.49 | 1.50 | 1.54 | 1.56 | .02 | A* |
| Trial 3 | | | | | | |
| Supplemental CP, g/d | 0 | 493 | 484 | 791 | . . . | |
| DM intake, kg/d | 22.4 | 22.7 | 22.7 | 22.9 | .2 | |
| Weight gain, kg/d | .41 | .61 | .54 | .58 | .11 | |
| Milk, kg/d | 30.1 | 31.0 | 31.0 | 31.1 | .3 | A* |
| 3.5% FCM, kg/d | 30.2 | 31.6 | 31.4 | 31.5 | .3 | A*** |
| Protein, % | 3.11 | 3.12 | 3.10 | 3.12 | .01 | |
| Protein, kg/d | .93 | .97 | .96 | .97 | .01 | A* |
| Fat, % | 3.55 | 3.64 | 3.59 | 3.58 | .03 | |
| Fat, kg/d | 1.06 | 1.12 | 1.11 | 1.11 | .01 | A*** |
| Lactose, % | 4.92 | 4.91 | 4.94 | 4.94 | .02 | |
| Lactose, kg/d | 1.48 | 1.53 | 1.53 | 1.54 | .02 | |
| Efficiency, Milk/DMI | 1.35 | 1.38 | 1.39 | 1.37 | .02 | |

¹Alfalfa silage fed in Trial 1, 2, and 3 contained 54.6, 29.9, and 36.7% DM (Table 3), and was fed at 54.2, 57.0, and 58.0% of the ration DM, respectively (Table 2). Values in parentheses are fractional amounts of supplemental CP relative to the 1× of solvent soybean meal (SSBM), fed as SSBM, expeller soybean meal (ESBM), or a mixture containing equal N from distiller dried grains with solubles plus corn gluten meal (DDGS + CGM).

²Single df orthogonal contrasts: A = control vs. .6× SSBM (or DDGS + CGM, Trial 3), .6× ESBM and 1× SSBM; B = .6× SSBM (or DDGS + CGM, Trial 3) vs. .6× ESBM; C = .6× ESBM vs. 1× SSBM.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

TABLE 5. Concentration of milk urea and blood urea and glucose.

| Item | Supplemental protein ¹ | | | | SE | Contrasts ² |
|------------------------------------|-----------------------------------|---|---------------|--------------|-----|------------------------|
| | Control (0) | SSBM (Trials 1 & 2) or DDGS+CGM (Trial 3) (.6×) | ESBM (.6×) | SSBM (1×) | | |
| | | | Trial 1 | | | |
| Milk urea, mM | 4.27 | 5.12 | 4.86 | 5.71 | .10 | A***,C*** |
| Blood ³ urea, mM | 4.28 | 5.32 | 5.10 | 6.16 | .11 | A***,C*** |
| Blood ³ glucose, mg/dl | 69.1 | 68.8 | 70.1 | 69.1 | .7 | |
| | | | Trial 2 | | | |
| Milk urea, mM | 5.07 | 6.00 | 5.77 | 6.67 | .10 | A***,C*** |
| Blood ³ urea, mM | 5.41 | 6.28 | 6.34 | 7.11 | .13 | A***,C*** |
| Blood ³ glucose, mg/dl | 68.1 | 69.4 | 66.6 | 70.1 | .9 | B*,C** |
| | | | Trial 3 | | | |
| Milk urea, mM | 4.79 | 5.80 | 5.89 | 6.75 | .08 | A***,C*** |
| Plasma ³ urea, mM | 3.95 | 4.18 | 4.86 | 5.35 | .21 | A**,B* |
| Plasma ³ glucose, mg/dl | 46.5 | 50.6 | 53.0 | 46.6 | 2.4 | |

¹Alfalfa silage fed in Trial 1, 2, and 3 contained 54.6, 29.9, and 36.7% DM (Table 3), and was fed at 54.2, 57.0, and 58.0% of the ration DM, respectively (Table 2). Values in parentheses are fractional amounts of supplemental CP, relative to the 1× of solvent soybean meal (SSBM), fed as SSBM, expeller soybean meal (ESBM), or a mixture containing equal N from distiller dried grains with solubles plus corn gluten meal (DDGS + CGM).

²Single df orthogonal contrasts: A = control vs. .6× SSBM (or DDGS + CGM, Trial 3), .6× ESBM and 1× SSBM; B = .6× SSBM (or DDGS + CGM, Trial 3) vs. .6× ESBM; C = .6× ESBM vs. 1× SSBM.

³Urea and glucose were determined in whole blood in Trials 1 and 2 and in deproteinized plasma in Trial 3.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

increased ($P < .001$) by supplemental protein and had a similar pattern in both trials (Table 5): lowest on the control, intermediate on .6× ESBM and SSBM, and highest on 1× SSBM. Urea was lower ($P < .001$) on ESBM than 1× SSBM. Relative to Trial 1, urea concentrations on Trial 2 averaged .89 and 1.07 mM higher in milk and blood, respectively. Blood glucose was unaffected in Trial 1; however, it was lower on ESBM than either SSBM diet in Trial 2 (Table 5).

Trial 3

This study differed from Trials 1 and 2 in that 1) a mixture of DDGS plus CGM (with equal CP from each) replaced the .6× SSBM treatment, 2) high moisture corn was the principal concentrate ingredient (Table 2), and 3) alfalfa silage contained 37% DM. Protein supplementation increased production of milk and

protein ($P < .05$), and 3.5% FCM and fat ($P < .001$), but production was not different among protein treatments (Table 4).

Milk and plasma urea concentrations are in Table 5. Milk urea pattern was similar to Trials 1 and 2: increased ($P < .001$) with supplemental protein and lower on ESBM than 1× SSBM. Plasma urea also was increased ($P < .01$) by supplemental protein; however, plasma urea was lower ($P < .05$) on DDGS plus CGM than ESBM. Analysis of blood plasma yielded urea and glucose concentrations, which were much lower than those found in whole blood in the first two trials. Milk and blood urea concentrations were of similar magnitude in Trials 1 and 2. Milk urea averaged 5.88 and 5.81 mM in Trials 2 and 3, respectively. However, plasma urea was 4.59 mM in Trial 3, 73% of the blood urea concentration (6.29 mM) in Trial 2. Also, plasma glucose in Trial 3 averaged 72% of blood glucose values from Trial 2.

TABLE 6. Rumen pH and concentrations of N metabolites and VFA (Trial 3).

| | Supplemental protein ¹ | | | | SE |
|--|-----------------------------------|-------------------|---------------|--------------|------|
| | Control (0) | DDGS+CGM (.6×) | ESBM (.6×) | SSBM (1×) | |
| pH | 6.50 | 6.42 | 6.44 | 6.54 | .072 |
| Ammonia, mM | 17.6 | 17.5 | 20.2 | 20.5 | 1.12 |
| Total amino acids, mM | 4.26 | 5.15 | 5.84 | 5.33 | .853 |
| Total peptides, mM | .776 | .829 | .897 | .845 | .060 |
| Peptide-bound amino acids, mM | 2.70 | 3.10 | 3.24 | 3.35 | .253 |
| Amino acid:peptide | 3.70 | 3.88 | 3.86 | 4.10 | .102 |
| Total VFA, mM | 121.8 | 125.6 | 106.7 | 123.4 | 8.48 |
| Acetate, mol/100 mol | 64.0 | 65.0 | 64.2 | 64.0 | .43 |
| Propionate, mol/100 mol | 18.9 | 18.5 | 18.3 | 19.4 | .41 |
| Butyrate, mol/100 mol | 11.3 | 11.1 | 11.5 | 10.8 | .24 |
| Isobutyrate, mol/100 mol | 1.59 | 1.40 | 1.64 | 1.55 | .084 |
| Valerate, mol/100 mol | 1.96 | 1.93 | 2.01 | 1.99 | .044 |
| 2-Methylbutyrate + isovalerate, mol/100 mol | 2.28 | 2.09 | 2.39 | 2.29 | .090 |
| Acetate:propionate | 3.43 | 3.54 | 3.54 | 3.38 | .080 |

¹Values in parentheses are fractional amounts of supplemental CP, relative to the 1× level of solvent soybean meal (SSBM), fed as a mixture containing equal N from distillers dried grains with solubles plus corn gluten meal (DDGS + CGM), or expeller soybean meal (ESBM).

No significant effects ($P > .15$) were observed for pH or concentration of any rumen metabolite in Trial 3 (Table 6). However, only 4 cows were used in a single 4×4 Latin square for these measurements. Rumen pH, VFA concentrations, and acetate:propionate ratio all indicated normal rumen fermentation. Rumen N metabolites reflected the high dietary CP: ammonia, and TAA concentrations ranged from 17.5 to 20.5 mM and 4.3 to 5.8 mM, respectively. Total peptides, PBAA, and amino acids: peptide averaged .837 and 3.10 mM, and 3.9, respectively.

DISCUSSION

In Trial 2, supplemental protein increased production of milk and all milk components. Although milk production was much greater in Trial 1, production was not greater with supplemental protein than with control ($P > .10$). The greater resistance of ESBM protein to ruminal degradation (Table 1) resulted in milk production (Table 4), which was higher than on comparable or greater amounts of SSBM (Trial 1) and comparable amounts of SSBM (Trial 2). Absorbed protein (AP) needs were estimated by summing AP requirements for individual functions computed with the revised NRC protein system (22). Average BW over the course of

each trial was used to compute maintenance AP. Ration NE_1 values (Table 2) and maintenance NE_1 requirements (22) were used to estimate ration TDN with an adjustment downward 4% for each multiple of intake above maintenance (22). Indigestibility = $(1 - \text{adjusted TDN}) \times \text{DMI}$ was used to calculate fecal DM excretion and AP cost for metabolic fecal protein. Average BW and AP requirements for the controls in Trials 1 and 2 were 649 and 598 kg and 2565 and 2080 g/d, respectively. Supplies of AP were calculated from NE_1 values in Table 2 and protein undegradabilities of 23 and 52% for alfalfa silage and corn grain (22), and 42% for high moisture corn (average of corn grain and corn silage in 22). For the control diets, AP supplies were 2620 and 1929 g/d in Trials 1 and 2. These corresponded to an AP excess of 55 g/d in Trial 1, but an AP deficiency of 151 g/d in Trial 2, which may account for the lack of response to supplemental protein in Trial 1 despite 6.6 kg/d greater milk production.

The difference in response to protein between the two trials may be explained partly by a greater supply of microbial protein due to greater DMI in Trial 1. Average DMI was 4.4 kg/d greater on Trial 1 than Trial 2. Using the NRC (22) formula and a dietary NE_1 of 1.63

Mcal/kg DM (Table 2), each kilogram increase in DMI would give rise to 93.3 g/d more microbial true protein. If SSBM contains 47.4% CP (Table 1) and has a rumen undegradability of 35% (22), 1 kg increase in DMI would yield microbial true protein equivalent to the escaped protein content of $[(.0933)/(.474 \times .35)] = .56$ kg SSBM.

Small daily responses of 1.0 kg milk, 1.3 kg 3.5% FCM, 37 g protein, and 40 g fat were observed due to protein supplementation in Trial 3 (Table 4); DMI was intermediate between Trials 1 and 2. Average BW during Trial 3 was 590 kg; computations as described yielded AP requirement and supply (22) of 2278 and 2285 g/d on the Control diet. This suggests that total protein supply was adequate in this trial but specific essential amino acids may have been limiting. However, production with the corn by-products DDGS plus CGM was equal to that with ESBM and SSBM, despite lower total and available lysine (Table 1), suggesting that lysine was not limiting on this alfalfa silage-based diet. Voss et al. (31) reported that lysine deficiency may have accounted for reduced milk production observed when DDGS plus CGM replaced equal CP from SSBM in cows fed a corn grain and corn silage-based diet. The equal production with ESBM and corn by-products suggests these proteins have similar extents of ruminal escape.

Milk urea concentration (Table 5) has been suggested as an indicator of whether the diet has exceeded the need of ruminal microbes for degraded protein (5, 23). Urea readily equilibrates with body water, including milk (23), and milk urea and blood urea concentrations are highly correlated (8). Therefore, milk urea may represent an estimate, integrated over time, of urea concentration in body water that may be more reliable than blood urea measured at a single time point. Although 5 mM has been suggested (23) as the concentration of milk urea corresponding to adequate ruminal ammonia, the body urea pool originates from tissue N metabolism as well as ruminal ammonia absorption. Therefore, urea in body water may be only a crude guideline, and there may be no single optimal concentration. Milk urea concentration would be most valuable for within-experiment comparisons or when related to information on ruminal degradability and dietary protein supply.

The mean PBAA concentration of 3.1 mM observed in Trial 3 (Table 6) was used to estimate the proportion of protein flowing from the rumen as peptides (9). If it is assumed that the amino acid:N ratio of peptides is similar to casein (i.e., 54.2 μ mol/mg N; 3), rumen liquid volume and passage are 75 L and .16/h (4), and nonammonia N (NAN) flow is 80% (19) of the average total N intake of 654 g/d in Trial 3, then the proportion of protein N flowing out of the rumen as peptide N can be estimated: $\{[(3.10/54.2) \times 1000] \times (75 \times .16 \times 24)/(654 \times .8)\} \times 100 = 3.1\%$ of NAN flow. Earlier estimates (9) of 2.7 to 6.4% of NAN flow were made using the data of Chen et al. (11). Similar computations made using data from sheep fed lower protein, lower energy diets suggested that peptides contributed between 1.2 to 2.4% of NAN flow (9). The importance of peptides in ruminal protein degradation is being studied (10, 11).

SUMMARY

Protein supplementation of diets containing 54 to 58% DM from alfalfa silage and dietary CP of about 17 to 18% significantly increased milk production when DMI was 20 kg/d. Increase in production was smaller with DMI of 22.4 kg/d; there was no improvement due to protein when DMI was 24.4 kg/d. At the lowest DMI, production of milk and milk components was greater with ESBM than with an equal amount of CP from SSBM but was not different from that obtained with two-thirds more CP from SSBM. At the intermediate DMI, production of milk, 3.5% FCM, protein, and fat was similar with supplementation of SSBM, ESBM, and DDGS plus CGM, although the latter two protein sources provided only 60% as much CP. At DMI of 24.4 kg/d, milk and lactose production were greater with ESBM than with equal or greater CP from SSBM.

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REFERENCES

- 1 Association of Official Analytical Chemists. 1980. Official methods of analysis. 13th ed. Assoc. Offic. Anal. Chem. Washington, DC.
- 2 Association of Official Analytical Chemists. 1984. Official methods of analysis. 14th ed. Assoc. Offic. Anal. Chem. Washington, DC.
- 3 Block, R. J., and K. W. Weiss. 1956. Amino acid handbook. Charles C. Thomas, Springfield, IL.
- 4 Broderick, G. A. 1985. Alfalfa silage or hay versus corn silage as the sole forage for lactating dairy cows. *J. Dairy Sci.* 68:3262.
- 5 Broderick, G. A. 1986. Relative value of solvent and expeller soybean meal for lactating dairy cows. *J. Dairy Sci.* 69:2948.
- 6 Broderick, G. A. 1987. Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. *Br. J. Nutr.* 58:463.
- 7 Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64.
- 8 Broderick, G. A., and G. T. Lane. 1978. Lactational, in vitro and chemical evaluation of untreated and formaldehyde-treated casein supplements. *J. Dairy Sci.* 61:932.
- 9 Broderick, G. A., and R. J. Wallace. 1988. Effects of dietary nitrogen source on concentration of ammonia, free amino acids and fluorescamine-reactive peptides in the sheep rumen. *J. Anim. Sci.* 66:2233.
- 10 Broderick, G. A., R. J. Wallace, and N. McKain. 1988. Uptake of small neutral peptides by mixed rumen microorganisms in vitro. *J. Sci. Food Agric.* 42:109.
- 11 Chen, G., C. J. Sniffen, and J. B. Russell. 1987. Concentration and estimated flow of peptides from the rumen of dairy cattle: effects of protein quantity, protein solubility, and feeding frequency. *J. Dairy Sci.* 70:983.
- 12 Craig, W. M., and G. A. Broderick. 1981. Comparison of nitrogen solubility in three solvents to in vitro protein degradation of heat-treated cottonseed meal. *J. Dairy Sci.* 64:769.
- 13 Dewar, W. A., and P. McDonald. 1961. Determination of dry matter in silage by distillation with toluene. *J. Sci. Food Agric.* 12:790.
- 14 Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768.
- 15 Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). USDA Handbook No. 379, USDA, Washington, DC.
- 16 Greenstein, J., and M. Winitz. 1961. Chapter 12. Page 1318 in: *Chemistry of the amino acids*. Vol. III. Academic Press, New York, NY.
- 17 Klopfenstein, T., R. Britton, and R. Stock. 1982. Nebraska growth system. Page 323 in *Protein requirements for cattle: Symposium*. F. N. Owens, ed. Oklahoma State Univ. Press, Stillwater.
- 18 Lacy, W. W., and O. B. Crofford. 1964. Automated determination of free plasma α -amino acids by the ninhydrin-carbon dioxide method: normal sex difference in human plasma. *J. Lab. Clin. Med.* 64:828.
- 19 Merchen, N. R., and L. D. Satter. 1983. Changes in nitrogenous compounds and sites of digestion of alfalfa harvested at different moisture contents. *J. Dairy Sci.* 66:789.
- 20 Mertens, D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. *J. Anim. Sci.* 64:1548.
- 21 Muck, R. E. 1987. Dry matter level effects on alfalfa silage quality. I. Nitrogen transformations. *Trans. Am. Soc. Agric. Eng.* 30:7.
- 22 National Research Council. 1988. Nutrient requirements of domestic animals. No. 3. Nutrient requirements of dairy cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.
- 23 Oltner, R., and H. Wiktorsson. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. *Livest. Prod. Sci.* 10:457.
- 24 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.
- 25 Robertson, J. B., and P. J. Van Soest. 1981. Chapter 8. Page 123 in *The analysis of dietary fiber in foods*. W.P.T. James and O. Theander, ed. Marcel Dekker, New York, NY.
- 26 Roffler, R. E., and L. D. Satter. 1986. Evaluation of alfalfa preserved as high-moisture silage, low-moisture silage, or dehydrated pellets. *J. Dairy Sci.* 69(Suppl. 1):142. (Abstr.)
- 27 SAS Institute, Inc. 1985. SAS User's guide: statistics. 5th ed. Cary, NC.
- 28 Technicon. 1973. Lactose in milk. Technicon Industrial Method No. 120-71A. Technicon Ind. Syst., Tarrytown, NY.
- 29 Technicon. 1976. Glucose in fermentation broth. Technicon Industrial Method No. 389-76P. Technicon Ind. Syst., Tarrytown, NY.
- 30 Technicon. 1977. Urea nitrogen. Technicon Industrial Method No. 339-01. Technicon Ind. Syst., Tarrytown, NY.
- 31 Voss, V. L., D. Stehr, L. D. Satter, and G. A. Broderick. 1988. Feeding lactating dairy cows proteins resistant to ruminal degradation. *J. Dairy Sci.* 71:2428.