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ANIMAL PROTEIN BY-PRODUCTS AND LIQUID FEED SUPPLEMENTS IN RATIONS FOR HIGH-PRODUCING DAIRY COWS

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INDUSTRY SUMMARY

a) Non-technical summary

• Two experiments were conducted to measure milk yield, feed intake and nutrient digestibility responses to increasing dietary levels of supplemental bypass protein in high-producing dairy cows. In both trials, diets contained high levels (>50% of ration DM) of immature alfalfa silage and were supplemented with corn grain. In each study the forage and grain components of the diets were theoretically insufficient in bypass protein. The objectives of each study were to quantify intake and milk yield of cows when the basal diet was supplemented with various sources of supplemental bypass protein.

The first experiment was a 14-week study in which 52 mature cows were randomly assigned to one to four dietary treatments. All diets were formulated to meet mineral, energy, and fiber and total crude protein requirements of high-producing (>45 kg milk/day), early-lactation cows. Treatment (SMB) was a negative control diet (low bypass protein diet) in which 30% of the total protein fed was undegraded in the rumen. Source of supplemental protein in this diet was solvent-extracted soybean meal. Treatments LPS and DPS were diets supplemented with a blend of animal (meat and bone meal, blood meal) and poultry (hydrolyzed feather meal) protein. Diets LPS and DPS were formulated to provide 38% of the total protein as bypass protein. Treatment LPS contained liquid molasses while treatment DPS did not. Treatment FM was a diet supplemented with fish meal. Diet FM provided 35% of the total crude protein as bypass protein. Diet FM was used as a positive control because high-quality fish meal has been shown to be a high-quality protein that is very resistant to ruminal degradation.

All animals were fed a single diet during the dry period and were switched to experimental diets 3 to 4 days after calving. Milk production and feed intake were measured beginning one week after calving.

Level of bypass protein did not affect milk production. Feed intakes tended to be lower in cows fed diets containing animal and poultry proteins or fish meal than in cows fed the diet supplemented with SBM. Nutrient digestibilities were not affected by dietary level of bypass protein or source of the protein. There was also no benefit to feed intake or milk production associated with the addition of molasses to the animal-poultry protein supplement. The lack of milk production response to source and amount of supplemental bypass protein appears to be due to a depression in feed intake when the animal-poultry or fish meal supplements were used as sources of bypass protein.

In the second experiment, 60 cows were used to investigate the production response to protein supplementation of diets containing 60% of diet dry matter as alfalfa silage. Experimental period was from week 3 through 17 of lactation. Treatments were six isonitrogenous diets (19% CP) that contained from 5.0 to 6.8% of diet dry matter as bypass protein. The diets were formulated with either soybean meal, a blend of animal (meat and bone meal, blood meal) and poultry (hydrolyzed feather meal and poultry byproduct meal) proteins or both.

Intake of dry matter was 1.46 kg/day higher for cows fed diets supplemented with soybean meal than for cows fed diets containing animal-poultry proteins. There was no difference in daily milk yield due to source or level of protein supplement, but cows fed diets supplemented with soybean meal tended to produce more milk of a lower fat percentage than cows fed the blend of animal-poultry proteins. The lack of response to the increase in dietary bypass protein was, in part, due to high levels of intake by cows in this study. Cows fed diets supplemented with soybean meal ingested 26.3 kg dry matter per day (4% of body weight; 11% more than predicted by the National Research Council) which provided the 1.3 kg/d bypass protein required to produce 40 kg of milk (as predicted by the National Research Council). Addition of animal-poultry protein was effective in increasing the total supply of bypass protein to the cow to 1.6 kg/d but there was no increase in milk yield.

Results of both studies suggest that although animal-poultry blends can increase the concentration of bypass proteins in diets for dairy cattle, there may be significant declines in feed intake associated with these proteins, however, that could reduce the response to level of dietary bypass protein.

b) Objectives of proposal

1. Measure production response to increasing increments of ration UIP (bypass protein) in high-producing cows in early lactation.

2. Determine advantages (palatability, intake, milk production) of providing a protein supplement containing urea, meat and bone meal, blood meal and hydrolyzed feather meal as a liquid molasses supplement compared to providing the same as a dry supplement without molasses.

3. Determine whether a blend of animal protein byproducts is an effective, more economical source of bypass protein than high-quality fish meal for dairy cattle.

c) Results as they pertain to the objectives of the original proposal

Results objective 1: In neither trial were we able to measure an increase in milk yield as level of dietary bypass protein was increased with animal-poultry protein blends. In both experiments it appeared that cows fed proteins that contained either a blend of animal-poultry protein or fish meal had lower feed intakes than cows fed diets that contained supplemental protein from soybean meal.

Results objective 2: In experiment one, we observed no difference in feed intake or milk yield when cows were fed supplement containing urea, meat and bone meal, blood meal and hydrolyzed feather meal as a liquid molasses supplement compared to providing the same as a dry supplement without molasses. It appears that molasses had little to do with the palatability of the animal-poultry proteins in this experiment.

Results objective 3: These data suggest that a blend of animal protein byproducts is as effective and is a more economical source of bypass protein than high-quality fish meal for dairy cattle. However, results from both experiments indicates that the blends of animal protein by products used in these two experiments depress intake when compared to diets that contain lower levels of bypass protein, but contain protein from soybean meal. Both studies suggest that although animal-poultry blends can increase the concentration of bypass proteins in diets for dairy cattle, there may be significant declines in feed intake associated with these proteins.

d) Impacts of the Findings for the Industry

Results of this project demonstrate that expressing the bypass protein recommendation as a percentage of either dietary dry matter or dietary crude protein might lead to a misinterpretation of the adequacy of a diet to support high milk yield. In this project we were able to increase the concentration bypass protein in diets of lactating cows by using blends of animal and poultry proteins as dietary supplements, but we were not able to utilize these proteins without depressing feed intake, when compared to using soybean meal as a supplemental protein. Benefits of these supplements must be calculated on their potential to decrease the cost of the feeding dairy cattle rather than on their potential to increase milk production.

Experiment 1.

EFFECT OF FEEDING ANIMAL PROTEIN BYPRODUCTS ON INTAKE AND MILK YIELD BY DAIRY COWS

ABSTRACT

Effect of feeding animal protein byproduct blends or fish meal on milk yield were evaluated in early lactation cows. Fifty-two multiparous Holstein cows were blocked by calving date and fed one of four supplemental protein treatments for 100 d postpartum. Treatment diets were supplemented with either soybean meal (6.3%), liquid protein supplement (7.8%), dry protein supplement (3.9%) or fish meal (4.3% of dietary DM). The CP portion of liquid and dry protein supplements contained 22.4% blood meal, 44.8% hydrolyzed feather meal, and 32.8% meat and bone meal. Treatments were isonitrogenous at 16.5% CP, but varied in level of undegraded intake protein at 28.7, 36.2, 36.7, and 33.7% of CP for soybean meal, liquid protein supplement, dry protein supplement, and fish meal diets, respectively. Milk yield (kg/d) was not affected by protein supplement or level of undegraded intake protein. Intake of DM (kg/d) was reduced during weeks 1 through 7 postpartum when liquid protein supplement, dry protein supplement, or fish meal was fed. Cows fed fish meal versus soybean meal, liquid protein supplement, or dry protein supplement had higher milk protein percent (2.99 versus 2.82, 2.83, and 2.81%) and milk protein yields (1.11 versus 1.06, 1.00, and 1.03 kg/d). Results suggest that the animal protein byproduct blend was equal to soybean meal in ability to support milk yield.

MATERIALS AND METHODS

Fifty-two multiparous Holstein cows were used in a 14-week randomized complete block lactation trial with cows blocked by calving date and randomly assigned to one of four treatment rations at parturition. Intake, milk production and composition, and body weight were measured. Blood (BUN) and milk urea nitrogen (MUN) and apparent total tract nutrient digestibilities were measured on 32 cows (8 cows per treatment).

Beginning 14 d before expected calving date, cows were fed a dry cow diet containing on a DM basis 21% alfalfa silage, 21% corn silage, 40% wheat straw, 12% corn, 4% dry protein supplement (Table 1), 1% fish meal, .3% tallow, and .7% vitamins and minerals. The dry cow ration was formulated to contain 13% CP, 30% ADF, and 46% NDF.

Ingredients used to formulate experimental diets are presented in Table 2. Alfalfa silage was the primary forage used to formulate experimental diets comprising 43.0% of the dietary DM. Nutrient composition of the alfalfa silage averaged 60% DM, 19% CP, 33% ADF, and 39% NDF. Corn silage was included in the diets at 13.3% of DM. Shelled corn was added to meet NEl requirements (1.7 Mcal/kg) of a 600 kg cow producing 45 kg of milk in early lactation (NRC, 1989). Urea was added to the soybean meal (SBM), dry protein supplement (DPS) and fish meal (FM) diets to standardize for the urea contained in the liquid protein supplement. Tallow was added at 2.0, 1.9, 1.9 and 1.7% of DM to SBM, liquid protein supplement (LPS), DPS and FM diets, respectively, to equalize total fat content across diets.

Four experimental protein supplements formulated to vary UIP content of the diets are presented in Table 1. Treatment rations were formulated by supplementing CP with either soybean meal, a blend of animal byproducts in either liquid or dry forms, or fish meal at 6.3, 7.8, 3.9 and 4.2% of DM for SBM, LPS, DPS and FM diets, respectively. Resulting formulation levels for UIP as a % of CP, were 31, 36, 36 and 37% of CP for SBM, LPS, DPS and FM diets, respectively. Diets were formulated to contain 17% CP and meet or exceed ADF, NDF, mineral and vitamin requirements of dairy cows (>45 kg milk/d) in early lactation (NRC, 1989). Diets

were fed ad libitum as total mixed rations (TMR) at 0900 and 1500 h daily and amount offered was adjusted daily to ensure 10% refusal. All cows were fed a prepartum diet that contained 20.8% (DM basis) alfalfa silage, 20.8% corn silage, 40.2% wheat straw, 11.8% corn, .34% tallow, .34% urea, 4% dry protein supplement, 1% fish meal and .72% vitamins and minerals beginning 14 d before expected calving date.

Feed offered and refused was recorded daily. Dry matter content of alfalfa silage and corn silage was determined weekly by microwave (Dantoin and Rohweder, 1984) and diet formulations were adjusted accordingly to maintain the proper DM ratio of forage to grain. Alfalfa and corn silages were sampled weekly. Concentrate and protein supplements were sampled each time a new batch was mixed (10 batches/trial). Feed refusals were sampled for 14 days on the 32 cows used for digestibility measurements. Forage, concentrate, protein supplement and feed refusal samples were dried for 48 hours in a 60° C forced-air oven and ground to pass a 2-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Alfalfa and corn silages were composited by month, and feed refusals were composited by cow. Samples were analyzed for DM, OM, CP (AOAC, 1990), ADF (Goering and Van Soest, 1970), NDF (Van Soest, 1990), and long-chain fatty acids (C14-C18:3) (Sukhija and Palmquist, 1988).

All protein supplements were evaluated for ruminal CP degradation using the in situ procedures of Nocek (1988). Dacron bags with a finished area of 9 cm x 12 cm and a pore size of approximately 50 μm were filled with 2 grams dried, ground (2-mm Wiley mill screen) sample. Dacron bags were incubated ruminally in reverse order for 4, 8, 12, 16, 20, 24, 36, 48, 72 and 96 h. After incubation, bags were removed from the rumen, immediately soaked in ice water and transferred to a washing machine for rinsing (Cherney et al., 1990). Zero hour bags were soaked in warm water for 30 minutes to estimate the soluble "A" fraction. Bags were dried for 48 h at 60° C in a forced-air oven, weighed, and placed into a kjeldahl flask for CP analysis (AOAC, 1990). The undegradable "C" fraction was calculated by measuring the CP remaining in the bag after 96 h. Rumen passage rate (k_p) was assumed to be .06/h, which was similar to that reported (Hartnell and Satter, 1979; Eliman and Ørskov, 1984) for early lactation dairy cows. The fractional degradation rate (k_d) was determined as the slope of the regression of the natural logarithm of "B" versus time. The A, B, and C fractions, as described in the National Research Council (1985), and ruminal escape protein were calculated as follows:

A = rapidly disappearing or soluble fraction = CP disappearing at zero hour,
B = slowly degradable fraction = 100 - (A + C),
C = undegradable fraction = CP remaining in bags after 96 hours, and
Ruminal protein escape (% of CP) = $100 - [A + B (k_d/k_d + k_p)]$.

Cows were milked at 0300 and 1430 h, with milk yield recorded at each milking. Milk samples were taken from a.m. and p.m. milkings weekly, and were analyzed for fat and protein by infrared analysis (Wisconsin DHI Laboratory, Appleton, WI). Daily milk yield and composition was calculated by a weighted average of a.m. and p.m. milkings.

Additional a.m. and p.m. milk samples were taken for four consecutive days from 32 cows (8 cows per treatment averaging 72 days in milk), and proportionately composited by cow by day using milk yield at each milking as a weighting factor. Each milk composite was deproteinized by mixing equal volumes of composited milk and 25% (wt/vol) trichloroacetic acid solution. The mixture was vortexed for 30 seconds, allowed to sit for 30 minutes and spun at 15,000 x *g* for 30 minutes. The supernatants were frozen until analyzed for milk urea nitrogen (MUN) using procedure No. 535 from Sigma Diagnostics®. Blood was collected in heparinized vaccutainers at 1300 h for four days from 32 cows (8 cows/treatment) and spun at 827 x *g* for 15 minutes. Plasma was removed and frozen until analyzed for blood urea nitrogen (BUN) also using procedure No. 535 from Sigma Diagnostics®.

Body weight was measured at 1300 h on all cows for three consecutive days at the start of the trial, three consecutive days at the end of the trial, and every other week during the trial.

Ytterbium (Yb) was used as an external digesta marker to estimate apparent total tract nutrient digestibilities (Combs, 1985). Shelled corn sprayed with $YbCl \cdot 6H_2O$ was fed at 0900 and 1500 h daily for 14 days to 32 cows (8 cows per treatment) averaging 72 days in milk at the beginning of the digestibility trial. Feeding 227 g of the Yb-marked corn twice daily provided approximately 30 mg of Yb/kg of dietary DM. Feed refusals were sampled for 14 days and prepared for analysis as described. Fecal grab samples were taken the last 4 days during the feeding of Yb marked corn so that a sample was obtained every hour and a half over a 24-hour period. Fecal samples were composited on an equal volume basis for each cow, dried at 60° C in a forced-air oven for 72 hours, and ground to pass a 2-mm Wiley mill screen. Feed refusal and fecal samples were analyzed for DM, OM, CP, ADF and NDF as described previously. Composited feed refusal and fecal samples were prepared for analysis of Yb by the following dry ash method. One gram of sample (2 mm grind) was weighed in duplicate into tared 30 ml pyrex beakers. Blank (no Yb) samples of feed were included to be used as blank, pre-ash and post-ash high standards. One ml of 100 ppm Yb solution was added to the pre-ash sample. Beakers were placed in 105 °C oven for eight hours, desiccated for 3 hours and weighed for absolute DM determination. Beakers were then placed into a muffle furnace at 500 °C for a minimum of 16 hours. After desiccation, 15 ml of reagent grade HCl was added to each beaker. The beakers then stood for a minimum of one hour to convert oxides to soluble chlorides. One ml of 100 ppm Yb solution was added to the post-ash high standard sample. All beakers were diluted to 100 g with $LiOH \cdot H_2O$ (final concentration of 1000 ppm of Li). Samples and standards were then analyzed for Yb by direct current plasma spectroscopy as described by Combs (1985). Nutrient intakes along with Yb concentrations in the diet and feces were used to calculate apparent total tract nutrient digestibilities.

Data were examined by analysis of variance using the general linear models procedure of SAS (1985). using the following statistical model:

$$Y_{ijk} = \mu + B_i + T_j + (BT)_{ij} + W_k + (TW)_{jk} + d_{ijk}$$

where,
- Y = observed response,
- μ = overall mean of population,
- B_i = average effect of block i,
- T_j = average effect of treatment j,
- $(BT)_{ij}$ = interaction of block i with treatment j,
- W_k = average effect of week k,
- $(TW)_{jk}$ = interaction of treatment j with week k, and
- d_{ijk} = residual error assumed independent and normally distributed.

Dry matter intake, milk yield and composition, and body weight were analyzed using this model. The interaction of block i with treatment j was used as the error term to test the effect of block and treatment. The effect of week was tested using the interaction of treatment j with week k as the error term. Treatment differences of dependent variables with a significant F test ($P < .05$) were determined by least significant difference.

Blood and milk urea nitrogen were tested by the following statistical model:

$$Y_{ijk} = \mu + T_i + C_j(T)_i + D_k + (TD)_{ik}$$

where,
- Y = observed response,
- μ = overall mean of population,
- T_i = average effect of treatment i,
- $C_j(T)_i$ = average effect of cow j within treatment i,
- D_k = average effect of day k, and
- $(TD)_{ik}$ = interaction of treatment i with day k.

Treatment effects were tested with cow within treatment as the error term.

Nutrient digestibilities were tested by the model:

$$Y_{ijk} = \mu + R_i + T_j + E_{ij}$$

where,
- Y = observed response,
- μ = overall mean of population,
- R_i = average effect of run i,
- T_j = average effect of treatment j, and

E_{ij} = residual error assumed independent and normally distributed.

Treatment differences for all models were determined by least significant difference if a significant ($P < .05$) F test was found.

RESULTS AND DISCUSSION

Ingredient and nutrient composition of experimental protein supplements are presented in Table 1. The undegraded intake protein (UIP) content of soybean meal as determined by in situ methods was 28.6% of CP and was lower than the 35% of CP value listed in NRC (1989). However, Satter (1986) summarized ten experiments and found an average UIP content of soybean meal of 27.0% of CP, which is similar to our value. The liquid protein supplement was composed of molasses, hydrolyzed feather meal, meat and bone meal, blood meal, urea, propionic acid, and gum. In situ estimates of the soluble (A), slowly degraded (B), and undegraded (C) fractions, along with degradation rates (k_d) were 21.9, 17.4, 60.7% of CP, and .002/h for hydrolyzed feather meal, 39.6, 43.9, 16.5% of CP, and .01/h for meat and bone meal , and 13.4, 18.1, 68.5% of CP, and .002/h for blood meal. Estimated UIP contents (k_p = .06/h) of hydrolyzed feather meal, meat and bone meal, and blood meal were 77.4, 52.6 and 86.0% of CP, respectively. A UIP content of 51.0% of CP for liquid protein supplement was calculated by assigning UIP (% of CP) values to molasses (29.6), urea (0), propionic acid (0) and gum (0) in addition to our measured in situ values for hydrolyzed feather, meat and bone, and blood meals. The calculated UIP content for dry protein supplement was 75.1% and was calculated by assigning a UIP value of 55% of CP to corn gluten meal (NRC, 1989), which was included at 6.1 % of DM. Liquid and dry protein supplements each contained 2.0 units of hydrolyzed feather meal, 1.4 units of meat and bone meal, and 1.0 unit of blood meal. The FM diet utilized menhaden fish meal which had a similar nutrient profile to that listed in NRC (1989). The in situ UIP value of 52.9% of CP was similar to UIP content of fish meals as described by Zerbini (1989), Broderick (1992), and Windschitl (1991).

Nutrient composition of experimental diets are summarized in Table 3. Diets were formulated to be isonitrogenous, but varied slightly in CP from 16.5% to 17.1% in the SBM and FM diets, respectively. Acid detergent fiber, NDF, and LCFA content of experimental diets averaged 19.0, 25.0 and 3.7% of DM, respectively. Undegraded intake protein content of experimental diets was lowest for SBM at 4.7%, intermediate for FM at 5.8%, and highest for LPS and DPS at 6.0% and 6.2% of DM, respectively. Dietary UIP contents were 27.8, 36.2, 36.7, and 33.7 % of CP for SBM, LPS, DPS, and FM, respectively which met experimental objectives with the exception of FM which was intended to have a UIP content similar to LPS and DPS.

Influence of protein supplement on DM and nutrient intakes is presented in Table 4. Addition of liquid protein supplement, dry protein supplement or fish meal compared to soybean meal resulted in no significant reduction in DMI. Dry matter intakes were 22.8, 22.1, 21.3, and 21.0 kg/d for SBM, LPS, DPS, and FM diets, respectively. Dry matter intakes expressed as a percent of BW were also not different at 3.7, 3.5, 3.3, and 3.4%. This is similar to the results of Mantysaari et al. (1989) who fed soybean meal, fish meal, meat and bone meal and two animal protein byproduct blends to dairy heifers on corn silage based diets. Animal protein byproduct blends were a blend of meat and bone, meat, poultry, blood and feather meal. Soybean meal, fish meal, meat and bone meal, animal protein byproduct blend A and B were added to the diet of 210 to 314 day old Holstein replacement heifers at 9.1, 5.1, 6.2, 5.1, and 4.5% of DM, respectively. Diets were isonitrogenous at 14% CP with UIP contents of 31.4, 38.3, 35.3, 39.0, and 40.1% of CP. Average DMI were similar for heifers fed soybean meal, fish meal, meat and bone meal, and animal protein byproduct supplements A and B at 7.51, 7.17, 7.45, 7.44 and 7.52 kg/d, respectively. This study was similar to our study as soybean meal was compared directly to fish meal and animal protein blends. The animal protein blends were of a similar composition to those used in our experiment. Our data supports the findings of Mantysaari et al. (1989), however

caution is required in interpretation as Mantysaari utilized replacement heifers fed corn silage diets, while our study utilized lactating cows fed alfalfa silage diets.

Trials evaluating the effects of animal protein blends containing hydrolyzed feather, meat and bone, and blood meals on DMI by lactating cows are limited. Craig and Broderick (1983) saw no depression in DMI when meat meal replaced soybean meal as the primary protein supplement. Replacement of soybean meal with meat and bone meal has not reduced DMI in lactating cows (Higginbotham et al., 1989), lactating goats (Lu et al, 1990), sheep (Loerch et al., 1983), or steers (Loerch and Berger, 1981). The maximum inclusion rate of meat and bone meal in these studies was 13.6% of ration DM (Lu et al., 1990). No incremental inclusion rate studies have been conducted to determine whether DMI problems would exist if meat and bone meal inclusion rates were greater than 13.6% of ration DM.

Similar responses have been observed for blood meal. Waltz et al. (1989) saw no depression in DMI when replacing soybean meal with blood meal at 9.6% of ration DM. In studies (Goedeken et al., 1990; Nelson et al., 1985; Robinson et al., 1992; Loerch et al., 1983; Loerch and Berger, 1981) where blood meal addition ranged from .11% to 9.6% of DM, no depression in DMI was reported. As with meat and bone meal experiments, blood meal inclusion rate studies are nonexistent to determine at what level DMI may be depressed.

The addition of hydrolyzed feather meal to ruminant diets at 4% (Goedeken et al., 1990b), 4.3% (Goedeken et al., 1990a), 6% (Harris et al., 1992), or 9.8% of ration DM (Waltz et al., 1989) also has not conclusively demonstrated depressions in DMI. This is in contrast with field observations that in commercial dairy situations, animal protein byproducts reduce DMI. Stern and Mansfield (1989) suggested that palatability of animal protein byproducts is of concern, but cited no specific references that support this premise. The paradox may lie in defining DMI and palatability as similar animal responses. Problems of consumption of animal protein byproducts may be a singular acute event, whereby the animal simply refuses to consume the ration if animal protein byproducts are included at or above a certain critical level. Experiments designed to evaluate the nutritional effects of animal protein byproducts would not proceed under these circumstances.

Research evaluating DMI differences between soybean meal and fish meal fed to lactating dairy cows is well documented. Broderick (1992) compared soybean meal with fish meal based diets in three trials. In trial 1, soybean and fish meal were fed to early lactation cows at 4.3 and 2.9% of ration DM, respectively. In trial 2, soybean meal was compared to fish meal at 1.5, 3.0 and 4.5% of ration DM. Trial 3 compared soybean meal with high and low solubles fish meal at 3.7% of ration DM. In all three studies, fish meal did not significantly affect DMI. These observations support our data. Others (Windschitl, 1991; Blauwiekel et al., 1990) also found no depression in DMI when fish meal was fed.

Molasses and liquid suspensions of animal protein supplements have been formulated in an effort to help overcome palatability problems (Goedeken et al., 1988; Perry, 1988). The addition of liquid molasses to the animal protein byproducts had no effect on DMI as DMI of LPS and DPS diets were similar at 22.1 and 21.3 kg/d respectively. Also, we observed no differences in DMI between FM and animal protein byproduct blends in liquid or dry forms.

Comparison of DMI responses in a continuous lactation study with those observed in short-term switch back trials is difficult. Dry matter intake is known to vary with stage of lactation (NRC, 1989), and DMI changes over time is an important response variable. Split plot analysis for treatment by week interaction, demonstrated a significant ($P < .05$) difference in DMI between diets early in the experiment (Figure 1). Dry matter intake was lower for LPS, DPS, and FM diets than for the SBM diet during the first six weeks of the experiment. Analysis of DMI data in three separate time periods (weeks 1-5, 6-10 and 11-14) was conducted. In the first period (weeks 1-5) DMI of the SBM diet tended ($P < .08$) to be greater than LPS, DPS and FM diets at 20.6 versus 18.4, 18.7 and 18.6 kg/d, respectively. Inclusion of liquid protein supplement, dry protein supplement, or fish meal had a negative effect on DMI over weeks 1-5 of lactation. Reasons for this depression of DMI are unclear, and reductions in DMI in other experiments from feeding animal protein byproducts are not readily evident.

Two possible reasons for decreased palatability at the initiation of the experiment exist. One, cows may require a palatability adaptation period to adjust to diets supplemented with animal protein sources. Quantification of such a period cannot be defined. Another possible explanation may be diet adaptation associated with the dynamics of rumen microbial populations. Cows assigned to LPS, DPS, or FM diets were introduced to animal protein byproducts for approximately 14 days prior to calving. This adaptation period may not have been adequate to allow rumen microbial populations to adjust to animal protein byproducts resulting in postpartum ruminal digestion DMI reductions for LPS, DPS and FM diets.

Intakes of OM, ADF and NDF averaged 19.9, 4.1 and 5.3 kg/d, and were not affected by treatment. Crude protein intakes were numerically higher for the SBM and LPS treatments at 3.8 and 3.9 kg/d, but were not significantly different from DPS and FM treatments (3.6 and 3.6 kg/d). Intake of UIP was highest for LPS (1.5 kg/d), intermediate for DPS (1.3 kg/d), and lowest for SBM (1.1 kg/d) and FM (1.2 kg/d). This was expected since diets were formulated to be higher in UIP for LPS, DPS and FM treatments. Increased UIP intakes were not achieved for FM versus SBM since DMI was slightly lower on the FM diet and the UIP content of FM was lower than expected.

Influence of protein supplements on milk yield and composition is presented in Table 5. Inclusion of liquid or dry protein supplements or fish meal did not affect milk yield. Ingredients comprising liquid and dry protein supplements (meat and bone, blood and hydrolyzed feather meal) have not been found to improve milk yield in other studies. Harris et al. (1992) examined the effects of 0, 3, or 6% of DM hydrolyzed feather meal supplementation on the performance of lactating dairy cows. Milk yield was not different at 26.2, 26.0, and 25.9 kg/d for 0, 3, and 6% hydrolyzed feather meal diets, respectively. In two lactation studies, Kellems et al. (1989) found similar responses to Harris et al. (1992). The studies of Kellems et al. (1989) compared milk yield potential of hydrolyzed feather meal and meat and bone meal. Milk yield was similar in cows supplemented either protein source. Higginbotham et al. (1989) found similar milk yield responses when meat and bone meal replaced soybean meal in the diet of lactating dairy cows. Meat and bone meal and soybean meal were supplemented to diets containing 32% corn silage, 15% alfalfa silage, and 10% whole cottonseed at high and low levels to yield diets of 18.5 and 15% CP. Cows responded to increasing dietary CP (15 versus 18.5% CP), but did not respond to protein source. Robinson et al. (1992) found similar results with blood meal when fed to eight late lactation cows. Milk yield was not significantly different between a blood meal or casein supplemented diet at 22.6 and 22.3 kg/d. Lu et al. (1990) found no milk yield differences in lactating goats fed soybean meal or meat and bone meal. Direct comparisons between these experiments and our study are difficult since only blood meal was used as the animal protein byproduct in their trials, and we used a blend of meat and bone, hydrolyzed feather, and blood meals.

Milk yield response to fish meal is well documented. Researchers (Oldham et al., 1985; Sloan et al., 1988; Blauwiekel et al., 1989; Spain et al., 1989) reported no change in milk yield when fish meal replaced soybean meal. These observations support our findings. Broderick (1992) however, observed an increase in daily milk yield when cows in early lactation were fed a diet of 70% alfalfa silage and 30% grain supplemented with fish meal. Milk yields were 36.0 and 37.1 kg/d for soybean meal and ruminant grade fish meal diets respectively.

Potential of treatment diets to support milk yield from dietary protein supply is presented in Table 6. Intake protein (IP) (g/d) was assumed to be CP intake, and supply of DIP and UIP were calculated from nutrient densities and DMI levels presented in Tables 3 and 4. Yield of bacterial crude protein (BCP) was evaluated by assessing whether dietary energy or supply of DIP was limiting BCP yield (NRC, 1989). The supply of DIP did not limit BCP for any treatment. All other protein units and calculations were from NRC (1989) and actual trial data were used as variables.

Absorbed protein available for milk yield (APMY) was calculated by subtracting maintenance (MPA), fecal (FPA), and retained (RPA) protein absorbed from absorbed protein (AP). Milk yield potential was back-calculated using the lactation protein absorbed (LPA) equation (NRC, 1989).

The SBM diet supplied 1226 g/d of APMY and potential milk yield was 37.6 kg/d which was similar to actual milk yield at 37.4 kg/d. Potential of FM to support milk yield was similar to SBM at 37.6 kg/d. These data suggest that minor differences in protein supply existed between SBM and FM and no difference in milk yield should be expected. Actual milk yield (37.4 versus 37.2 kg/d) from cows fed SBM or FM supports this conclusion. Milk yield potential based on protein supply for LPS and DPS was 40.7 and 40.5 kg/d. Calculated milk yield potentials over-estimated actual milk yield by approximately 4.5 kg/d.

Differences of dietary energy or DIP do not logically explain the discrepancy in actual versus potential milk yield. Dietary energy intake of LPS and DPS was approximately 36.2 Mcal/d, which is adequate to support a 600 kg cow milking 40 kg/d. Secondly, bacterial protein yield should not have been limiting as calculations (Table 6) suggest that DIP was adequate in both LPS and DPS diets.

The most plausible explanation of lower actual milk yield is inefficiency of UIP digestion in the liquid and dry protein supplements. In our first study of evaluating protein quality of animal byproducts, .2% pepsin-HCl analyses were conducted on the actual sub-components used in the liquid and dry protein supplements. Pepsin-HCl digestibilities of soybean, meat and bone, blood, fish, and hydrolyzed feather meals were 92.9, 76.8, 97.9, 95.0, and 8.5% of CP, respectively. This suggests that the hydrolyzed feather meal used in the liquid and dry protein supplements may have been of inferior quality. Hydrolyzed feather meal accounted for approximately 45% of the CP in the liquid and dry protein supplement and poor CP digestibility would have had a major impact on the CP digestibility of the entire supplement mixtures. Consequently, the additional UIP supplied from the liquid or dry protein supplement may not have been utilized at normal efficiencies resulting in the lower actual milk yield for the LPS and DPS diets.

Blood and milk urea nitrogen levels further support this argument (Table 7.). Milk urea nitrogen levels were similar at 9.2, 8.2, 6.2 and 7.2 mg/dl for SBM, FM, LPS, and DPS (Table 7). It has been suggested that milk urea concentrations greater than 5 mg/dl indicate adequate or excessive rumen ammonia (Oltner and Wiktorsson, 1983). These data would support that all treatment diets were adequate in DIP. Blood urea nitrogen levels were relative to milk urea nitrogen at 24.0, 21.5, 21.8, and 20.2 mg/dl for SBM, FM, LPS, and DPS, respectively.

Milk yield over time (Figure 2) was not significantly different between treatments. Milk yield was unexplainably variable between treatments over weeks 1-6 of lactation, but little variation in milk yield was apparent over weeks 6-14. This further suggests that addition of UIP via supplementation of animal protein byproducts or fish meal had no effect on milk yield at any stage of lactation.

Milk fat percentage was numerically lower for the FM at 3.26% versus SBM, LPS and DPS at 3.41, 3.39, and 3.42%, respectively, but was not significantly different (Table 5). Milk fat depression in this study was not expected as diets were formulated at equal NDF contents. Researchers (Sloan et al., 1988; Blauwiekel et al., 1989; Spain et al., 1989) have observed a reduction in milk fat percentage when fish meal replaced SBM in the diet. Storry (1981) reported that fatty acids contained in fish meal may reduce milk fat by altering rumen VFA ratios, inhibiting ruminal microbial fatty acid synthesis, or by reducing uptake of plasma fatty acids by the mammary gland. Hoover et al. (1989) also reported greatly reduced acetate to propionate ratio and impaired microbial CP production when fish meal containing 34.4% free fatty acids (FFA), 34.4% FFA with $CaCl_2$ added, or 65% FFA were added to rumen continuous cultures (pH = 6.2). These effects were not detected when defatted fish meal was added to the continuous culture. This suggests that these affects were due to the specific fatty acid content of the fish meal. Spain et al. (1989) however reported only small changes in ruminal VFA and theorized that it was an alteration in lipid metabolism post ruminally that most likely reduces milk fat secretion when fish meal is fed.

Milk protein yield was significantly higher ($P < .06$) for FM at 1.11 kg/d when compared to SBM, LPS, and DPS at 1.06, 1.00, and 1.03 kg/d, respectively (Table 5). Milk protein yield was increased due to a significantly higher milk protein composition for cows fed the FM diet. Milk protein percentage was higher ($P < .05$) for the FM diet at 2.99% versus SBM, LPS and DPS diets at 2.82, 2.83 and 2.81%, respectively. Broderick (1992) replaced SBM at 5.4% of dietary DM with either 3.7% high and low solubles fish meal and also demonstrated milk protein percent

and yield increases from fish meal supplementation. Our data as in agreement with the findings of Broderick (1992), but Broderick proposed no biological mechanism as to why fish meal enhances milk protein percentage. Others (Spain et al., (1990); Blauwiekel et al., (1990); Zerbini et al., (1987)) found no significant improvement in milk protein percentage due to inclusion of fish meal. The major differences between our study and the study of Broderick (1992), as compared to other studies where milk protein responses were not elicited, was the base forage fed. In these trials alfalfa silage was the primary forage, while the other studies used predominantly corn silage. It is unknown as to what factors in a corn silage-fish meal diet versus an alfalfa silage-fish meal diet would ellicit different responses in milk protein percentage.

The slight depression in DMI and equal milk yield for the LPS, DPS and FM diet as compared to SBM in weeks 1-7 of lactation should have resulted in increased body weight loss for cows fed LPS, DPS or FM diets. Average body weight of cows was 608, 638, 637, and 621 kg for SBM, LPS, DPS, and FM diets, respectively. Whole body weight change was not significantly different over time (Figure 3), cows on the LPS and DPS diets numerically did lose more of their original body weights over weeks 1-5. This would suggest that lower DMI was compensated for by increased body weight loss in early lactation. No explanation can be given for the lower DMI and comparative milk yield with no increased body weight loss for the FM diet.

Apparent total tract nutrient digestibilities are presented in Table 8. There were no significant differences in DM digestibility between SBM, LPS, DPS and FM diets which were 68.5, 68.3, 68.0 and 67.8% respectively. Similar results were found for OM digestibility. Apparent crude protein digestibilities were similar between SBM, LPS and DPS at 64.9, 64.7, and 63.9%, but lower ($P < .06$) for the FM diet at 59.6%. This data is inconsistent with actual performance parameters in this experiment. Milk yield, DMI and body weight change was similar for FM when compared with other treatments. Milk protein percentage was higher, indicating efficient absorption and utilization of protein from fish meal. From milk yield, DMI, body weight change, and milk composition data, it appears that no major problem existed with CP digestion of FM, and observed differences can not be explained. Acid and neutral detergent fiber digestibilities were not significantly different between treatments. No differences in fiber digestion were expected as alfalfa silage and corn silage were the primary sources of fiber and were fed in similar amounts across treatments. The relative ADF digestibilities across treatments were higher than NDF digestibilities. This is unexpected as the components of NDF should be more completely digested than ADF (Van Soest, 1982). Jerred et al. (1991) using nearly identical techniques also found an inversion of ADF and NDF digestibilities. This result is likely due to experimental technique error in the measurement of ADF and NDF in feed and feces. Pectins are not completely removed by ADF solution and therefore contaminate feeds if ADF analyses of feeds are not done on NDF residues (Van Soest et al., 1991). Since alfalfa is high in pectins, it is likely that the estimated ADF values of our diets were too high. Since pectin is rapidly and almost completely degraded in the rumen, little pectin contamination of fecal material would be expected. The difference in pectin contamination between feed and feces could account for the higher than expected apparent digestibility coefficients for ADF relative to NDF. One possible alleviation to this likely error is sequential analysis of NDF and ADF as opposed to nonsequential assays as was done in this study.

CONCLUSIONS

These studies evaluated increasing undegraded intake protein by replacing soybean meal with animal protein byproducts in diets of early lactation cows. In general, increasing dietary undegraded protein by supplementation of animal protein byproducts had no profound effect on lactation performance. While no benefits or detriments to productivity were demonstrated, the trials yielded valuable nutritional information on animal protein byproduct utilization by dairy cows.

First, fish meal has demonstrated a potential to improve milk protein in alfalfa silage based diets. This effect has been observed in other research studies and these data support these observations. Although cows did not elicit improved milk production parameters when

supplemented with animal byproduct blends or fish meal, they did not perform inferior to cows supplemented with soybean meal.

Second, it is imperative that crude protein quality of animal protein byproducts used in experiments be defined. While speculative, the hydrolyzed feather meal used in these studies was of inferior crude protein quality. It is unjust to classify superior and poor quality manufactured animal protein byproducts within the same scientific framework.

Third, since limited information exists on nutritional aspects of specific animal protein byproducts, it may be more difficult to conduct research on animal protein byproduct blends. Experiments evaluating blends can never qualify the specific nutritional characteristics of a given animal protein byproduct.

Fourth, additional information is needed on palatability and intake potential of animal protein byproducts. Field observations of poor palatability and reduced DMI are not well supported by scientific literature. This does not suggest that field observations are incorrect, rather that limited studies are available to assess these effects. To address this issue, more titration studies of a given animal protein byproduct are needed. Titration studies would quantify animal responses to increasing levels of animal protein byproduct supplementation.

In conclusion, lactation studies utilizing animal protein byproducts require intensive material evaluation in the pre-trial period. This is generally not required when evaluating vegetable protein sources, but the vast variability of crude protein quality in animal protein byproducts requires a cautious approach.

Table 1. Ingredient and nutrient composition of experimental protein supplements.

Item	Soybean meal	Liquid protein supplement	Dry protein supplement	Fish meal
Ingredient	(% of DM)			
Molasses	-	48.7	-	-
Hydrolyzed feather meal	-	20.8	42.9	-
Meat and bone meal	-	15.1	30.5	-
Blood meal	-	9.9	20.5	-
Corn gluten meal	-	-	6.1	-
Urea	-	4.7	-	-
Propionic acid	-	.6	-	-
Gum	-	.2	-	-
Soybean meal	100.0	-	-	-
Fish meal	-	-	-	100.0
Nutrient [1]				
DM	89.9	53.7	91.0	87.8
CP	45.3	51.0	75.1	72.1
UIP [2]	28.6	26.6	54.7	52.9
LCFA [3]	1.8	2.6	5.2	4.1

[1] Values expressed on a DM basis, except DM was expressed on as-fed basis.

[2] UIP = undegraded intake protein. UIP values were calculated using in situ values for hydrolyzed feather, meat and bone, blood, soybean and fish meals. Other values for calculations were from NRC 1989.

[3] LCFA = long chain fatty acids; C14, C16, C18, C18:1, C18:2 and C18:3.

Table 2. Ingredients used to formulate experimental diets.

Item	Treatment [1]			
	SBM	LPS	DPS	FM
	(% of DM)			
Alfalfa silage	43.1	43.0	43.0	43.0
Corn silage	13.4	13.3	13.3	13.3
Shelled corn	32.3	31.9	35.4	35.7
Soybean meal	6.3	-	-	-
Liquid protein supplement	-	7.8	-	-
Dry protein supplement	-	-	3.9	-
Fish meal	-	-	-	4.2
Urea	.4	-	.4	.4
Tallow	2.0	1.9	1.9	1.7
Dicalcium phosphate	1.1	.8	.8	.5
Calcium carbonate	.4	.3	.3	.2
Magnesium oxide	.3	.3	.3	.3
Trace mineral salt [2]	.5	.5	.5	.5
Vitamin ADE premix [3]	.2	.2	.2	.2

[1] Experimental diets where supplemental CP was supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM = fish meal.

[2] Trace mineral salt contained 92.0 % NaCl, .345 % Fe, .002% Co, .140% Cu, .548% Mn, .548% Zn, .008% I, and .006% Se.

[3] Vitamin ADE premix contained 2,643 IU Vitamin A/g, 881 IU Vitamin D/g and 3.5 IU Vitamin E/g.

Table 3. Nutrient composition of experimental diets.

Item	Treatment [1]			
	SBM	LPS	DPS	FM
	(% of DM)			
OM	89.9	91.2	92.0	92.1
CP	16.5	16.6	16.9	17.1
UIP [2]	28.7	36.2	36.7	33.7
ADF	19.4	18.7	18.9	18.8
NDF	25.1	25.0	25.5	25.4
LCFA [3]	3.7	3.7	3.8	3.8
	(Mcal/kg)			
NEl	1.68	1.67	1.67	1.68

[1] Experimental diets where supplemental CP was supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM = fish meal.

[2] UIP = undegraded intake protein. UIP was calculated using in situ values for soybean and fish meals. UIP for liquid and dry protein supplements were derived by in situ evaluation of major subcomponents (hydrolyzed feather, meat and bone and blood meals). All other UIP values used in calculations were from NRC, 1989.

[3] LCFA = long chain fatty acids; C14, C16, C18, C18:1, C18:2 and C18:3.

Table 4. Influence of diet on dry matter and nutrient intakes [1].

Item	Treatment [2]					
	SBM	LPS	DPS	FM	Other[5]	SE
	(kg/d)					
DM	22.8	22.1	21.3	21.0	T x W	.63
OM	20.7	20.1	19.5	19.3		.58
CP	3.8	3.9	3.6	3.6		.11
UIP [3]	1.1[c]	1.5[a]	1.3[b]	1.2[c]		.04
ADF	4.3	4.1	3.9	3.9		.12
NDF	5.5	5.4	5.2	5.2		.17
LCFA [4]	.5	.5	.6	.5		.02

[1] Means within the same row with different superscripts are significantly different ($P < .05$).

[2] Experimental diets where supplemental CP was supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM = fish meal.

[3] UIP = undegraded intake protein. UIP was calculated using in situ values for soybean and fish meals. UIP for liquid and dry protein supplements were derived by in situ evaluation of major subcomponents (hydrolyzed feather, meat and bone and blood meals). All other UIP values used in calculations were from NRC, 1989.

[4] LCFA = long chain fatty acids; C14, C16, C18, C18:1, C18:2 and C18:3.

[5] Other significant ($P < .05$) effects where, T = treatment, W = week.

Table 5. Influence of diet on milk yield and composition [1].

	Treatment [2]				
Item	SBM	LPS	DPS	FM	SE
Yield	---------- (kg/d) ----------				
Milk	37.4	35.7	36.7	37.2	0.3
3.5% FCM	36.7	35.0	36.1	35.8	0.4
Fat	1.27	1.20	1.25	1.20	0.02
Protein	1.06^{ab}	1.00^{b}	1.03^{b}	1.11^{a}	0.01
Compostition	---------- (%) ----------				
Fat	3.41	3.39	3.42	3.26	0.04
Protein	2.82^{b}	2.83^{b}	2.81^{b}	2.99^{a}	0.01

[1] Means within the same row with different superscripts are significantly different ($P < .06$).

[2] Experimental diets where supplemental CP was supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM = fish meal.

Table 6. Potential of treatment diets to support milk yield from dietary protein supply.

Item [2]	Treatment [1]			
	SBM	LPS	DPS	FM
Supply	(g/d)			
IP	3762	3669	3600	3591
DIP	2682	2341	2279	2381
UIP	1080	1328	1321	1210
BCP	2548	2448	2352	2331
DBP	1630	1566	1506	1492
DUP	864	1062	1057	968
AP	2494	2628	2563	2460
Demand				
MPA	115	118	118	116
FPA	665	653	628	613
RPA	-38	-36	-74	-31
APMY	1226	1325	1324	1233
	(kg/d)			
MYP	37.6	40.7	40.5	38.5

[1] Experimental diets where supplemental CP was supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM = fish meal.

[2] IP = intake protein, DIP = degraded intake protein, UIP = undegraded intake protein, BCP = bacterial crude protein, DBP = digestible bacterial protein, DUP = digestible undegraded protein, AP = absorbed protein, MPA = maintenance protein absorbed, FPA = fecal protein absorbed, RPA = retained protein absorbed, APMY = absorbed protein available to support milk yield, MYP = milk yield potential.

Table 7. Influence of diet on milk and blood urea nitrogen.

Item	Treatment [1]				SE
	SBM	LPS	DPS	FM	
	(mg/dl)				
Blood urea nitrogen	24.0	21.8	20.2	21.5	1.3
Milk urea nitrogen	9.2	6.2	7.2	8.2	1.2

[1] Experimental diets where supplemental CP wass supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM = fish meal.

Table 8. Influence of diet on apparent total tract nutrient digestibilities [1].

Item	Treatment [2]				SE
	SBM	LPS	DPS	FM	
	(%)				
DM	68.5	68.3	68.0	67.8	1.3
OM	69.6	69.1	68.9	69.6	1.3
CP	64.9	64.7	63.9	59.6	1.5
ADF	52.7	49.4	51.8	53.0	2.4
NDF	39.2	40.1	41.7	41.9	2.7

[1] Means within the same row with different superscripts are significantly different ($P < .05$).

[2] Experimental diets where supplemental CP was supplied by SBM = soybean meal, LPS = liquid protein supplement, DPS = dry protein supplement, or FM – fish meal.

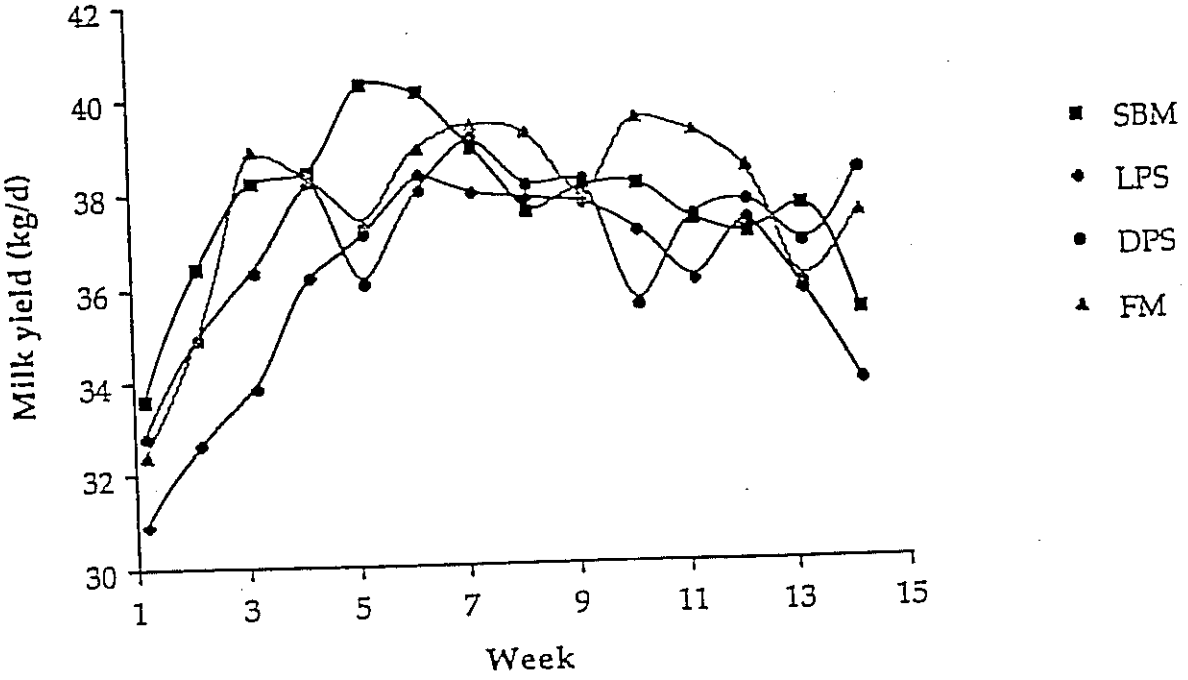

Figure 2. Effect of feeding soybean meal (SBM), liquid protein supplement (LPS), dry protein supplement (DPS), or fish meal (FM) on milk yield.

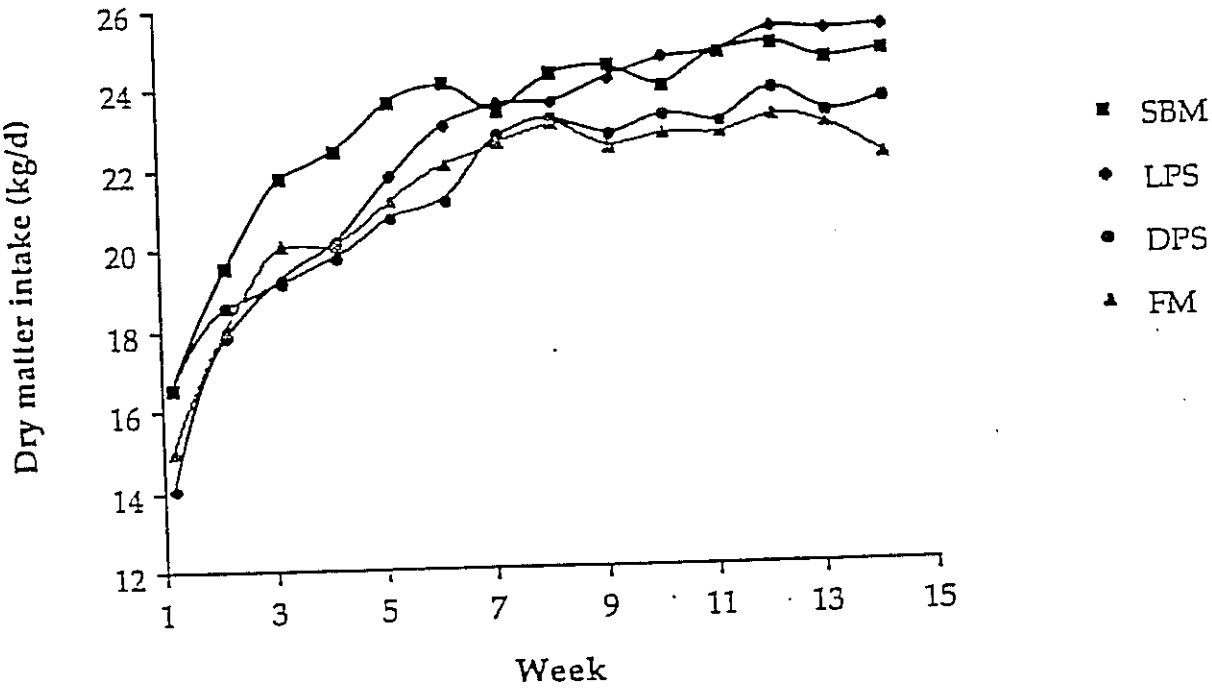

Figure 1. Effect of feeding soybean meal (SBM), liquid protein supplement (LPS), dry protein supplement (DPS), or fish meal (FM) on dry matter intake.

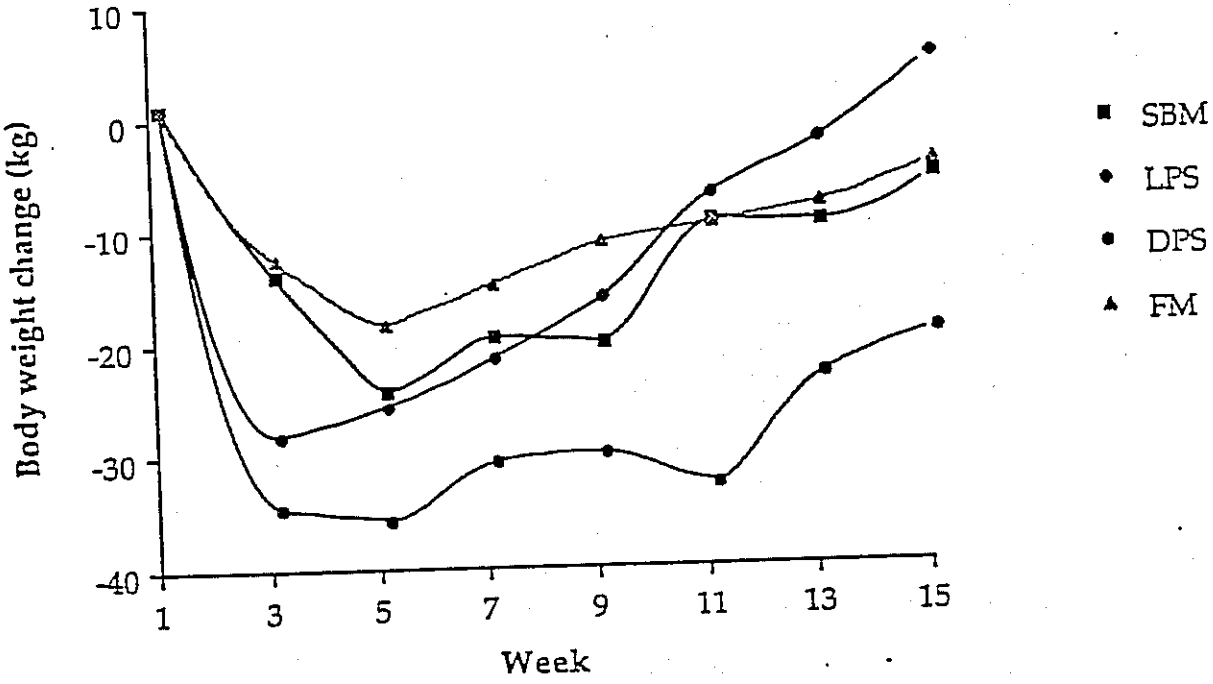

Figure 3. Effect of feeding soybean meal (SBM), liquid protein supplement (LPS), dry protein supplement (DPS), or fish meal (FM) on body weight change.

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Experiment 2.

LACTATIONAL RESPONSES TO RUMINALLY UNDEGRADABLE PROTEIN BY DAIRY COWS FED DIETS BASED ON ALFALFA SILAGE

ABSTRACT

Lactational responses to protein supplementation of diets containing 60% of DM as alfalfa silage were evaluated. Sixty multiparous Holstein cows were fed a covariant diet for the first 3 wk postpartum, blocked by calving date, and randomly assigned for 14 wk to one of six isonitrogenous (19.4 % CP) diets. Diets were formulated with soybean meal, a blend of animal by-products, or both, and contained 5.0, 5.6, 5.6, 6.2, 6.1, and 6.8% ruminally undegradable protein (DM basis). Percentage of ruminally undegradable protein or source of supplemental protein did not affect 3.5% FCM (39.4 kg/d), milk fat yield (1.38 kg/d), milk protein percentage (2.83%), milk urea (7.66 m*M*), and plasma urea (8.91 m*M*). However, cows fed diets supplemented with soybean meal had higher DMI (26.2 vs. 24.7 kg/d), milk yields (40.4 vs. 39.1 kg/d), and milk protein (1.15 vs. 1.09 kg/d) yields, but lower milk fat concentration (3.42 vs. 3.53%) and body condition score (2.85 vs. 2.93) than cows fed diets containing a blend of animal by-products. The lack of response to ruminally undegradable protein was partially caused by higher than predicted DMI (5 to 15% above NRC predictions); all diets provided at least 1.3 kg of ruminally undegradable protein, and there was no beneficial effect from ruminally undegradable protein intake increases to 1.6 kg/d.

Abbreviation key: **ABP** = animal by-products, **AP** = absorbed protein, **BCS** = body condition score, **CNCPS** = Cornell Net Carbohydrate and Protein System, **LPS** = liquid protein supplement, **RDP** = ruminally degradable protein, **RUP** = ruminally undegradable protein, **SSBM** = solvent soybean meal.

MATERIALS AND METHODS

Protein Supplements

Protein supplements were urea, SSBM, and a blend of ABP formulated with meat and bone meal, hydrolyzed feather meal, poultry by-product meal, and blood meal to contain at least 60% RUP in CP (Table 1). Most ABP were mixed commercially (Quality Liquid Feeds, Dodgeville, WI) with molasses, a suspension agent, and a preservative into a liquid protein supplement (**LPS**). Meat and bone meal also was used as a separate ingredient to enhance the RUP in diets further without increasing the percentage of molasses in ration DM above 5% (Table 2). Values for percentage of RUP in CP of meat and bone meal, hydrolyzed feather meal, and blood meal were those compiled by NRC (22). We assumed that CP in molasses (4% DM basis) was entirely degraded in the rumen (31). The RUP content of poultry by-product meal was determined by an in vitro gas production procedure (23). Samples of meat and bone meal and blood meal were used to validate the technique. Estimates of RUP for these two samples were nearly identical to NRC values and the RUP content of the poultry by-product meal averaged 46.6% of CP.

Diet Formulation

Six experimental diets were formulated to contain 60% alfalfa silage (DM basis) to meet recommended concentrations of calcium, phosphorus, and magnesium (22) and to contain 18.5% CP with either 5.0, 5.6, 6.2, or 6.8% RUP in dietary DM (Table 2). Urea and SSBM

were used to formulate diet 1, which contained 5% RUP. Urea was replaced by additional SSBM to obtain 5.6% RUP in diet 2. To avoid confounding between source of supplemental protein and dietary RUP, a second diet containing 5.6% RUP was formulated with LPS and urea (diet 3). Similarly, the increased RUP concentration in the dietary DM from 5.6 to 6.2% was achieved by increasing LPS fed in diet 3 and replacing urea with either SSBM (diet 4) or meat and bone meal (diet 5). Diet 6 was formulated with LPS and meat and bone meal to contain 6.8% RUP.

A covariant diet containing 5.6% RUP was also formulated with 57.4% alfalfa silage (DM basis) and all the ingredients used in the experimental diets, except poultry by-product meal (Table 2). Molasses and the ABP in the covariant diet were introduced as dry ingredients rather than as LPS.

Cows and Experimental Design

Sixty multiparous cows were blocked by calving date and randomly assigned to one of six experimental diets from wk 4 to wk 17 of lactation. For the first 3 wk of lactation, cows were fed the covariant diet. The experiment started in September 1991 and finished in early June 1992.

Sampling Procedures

Intake. The TMR were fed once daily for 5 to 10% orts. Concentrate mixtures for each diet and the LPS were sampled monthly for DM and CP analysis. Twice a week, samples of covariant and experimental silages were analyzed for DM by toluene distillation (2) to adjust the proportion of silage and concentrate mixture in the TMR. A subsample of the silage was dried in a 60°C forced-air oven for 48 h before DM, OM, CP (2), NDF, ADF, and acid detergent lignin determinations (11). Also, a second silage sample was stored frozen for measurement of in situ kinetics of N degradation. Orts were measured daily, and samples from each diet were dried for 48 h in a 60 °C oven and composited weekly. Monthly orts samples were composited for each diet and analyzed for NDF and CP.

Preparation of TMR. For diets containing the LPS (diets 3, 4, 5, and 6), the TMR were prepared by manually mixing the concentrates, minerals, and vitamins with the LPS before the prepared mixture was added to the mixer containing the silage. This procedure allowed for a more homogeneous distribution of the LPS within the TMR than did addition of LPS directly to the silage.

Milk Yield and Composition. Milk yield was recorded at each milking (0300 and 1430 h). The p.m. and following a.m. milk samples were collected three times on wk 3 and twice a week from wk 4 to 17 of lactation. Individual samples were analyzed for fat and protein content (Wisconsin DHIA, Madison).

BW and Body Condition Score. Body weights were recorded on 3 consecutive days at 1000 h on wk 4 and wk 17 of lactation and weekly from wk 5 to 16. For all cows on trial, body condition scores (BCS), on a scale from 1, emaciated, to 5, obese, (8), were determined by five individuals every 2 wk starting on wk 3.

Ruminal, Plasma, and Milk Parameters. On wk 9, 11, and 13 of lactation, blood samples were collected at 1000 h and 1400 h from the coccygeal vein or artery in tubes containing heparin. Samples were centrifuged immediately (3500 x *g*, 4 min, 4°C), and the plasma was stored in plastic containers at - 20°C. Also, samples from the p.m. and following day a.m. milkings were precipitated with trichloroacetic acid (25% wt/vol), centrifuged, and stored at - 20°C. After thawing, blood and milk plasma were analyzed for urea (5). Plasma glucose also was determined according to the method of Broderick et al. (5).

The day following blood sampling, ruminal fluids were sampled by stomach tube. Ruminal pH was recorded immediately after the collected fluid was strained through four layers of cheesecloth. Two 10-ml aliquots were collected and mixed with either .2 ml of sulfuric acid

(50% wt/wt) for colorimetric determination of ammonia (4) or 2 ml of formic acid for determination of VFA by GLC (AutoSystem gas chromatograph; Perkin Elmer, Norwalk, CN).

Silage In Situ Degradation

Kinetics of N degradation was determined on monthly composites of experimental silages using three ruminally cannulated, dry cows fed average quality alfalfa hay supplemented with minerals and vitamins. Duplicate samples of frozen silages (10 to 12 g) were placed into 12- x 15-cm dacron bags (nominal pore size 50 μm; Blumgardt and Co., Inc., New York, NY) and incubated for 4, 8, 12, 18, 24, and 36 h in the ventral rumen. Unincubated bags and incubated bags were washed with cold water for 10 min in a commercial washing machine. Residues were dried at 60°C, weighed, and assayed for CP as previously described. Residual CP at each incubation time were fitted with a nonlinear procedure (28) to a first-order kinetic model (20) assuming three N fractions and a digestion rate constant, but no lag times (15).

Statistical Analysis

The DMI, milk yield, milk component yields and percentages, BW, and BCS collected during the 3rd wk of lactation were used as covariates in the ANOVA performed with the general linear models procedure of SAS (28) with the following model:

$$Y_{ijk} = \mu + C + B_i + D_j + D_j \times B_i + W_k + D_j \times W_k + E_{ijk}$$

where Y_{ijk} is the dependent variable, C is the effect of the continuous covariant variable, B is the effect of block i (i = 1 to 10), D is the effect of the diet j (j = 1 to 6), D x B is the diet by block interaction (or cow effect; n = 60), W is the effect of week of experiment (k = 1 to 14), D x W is the week by diet interaction, and E_{ijk} is the residual variation. Diet by block interaction was used as an error term to construct an F test for the effects of block and diet. The change in DMI, milk yield, BW, and BCS between 2 weeks in the course of the trial (see Table 5) and the BW and BCS at the end of the trial were analyzed with a model including block and diet only. The effects of RUP concentration (n = 4) and the effects of source and concentration of supplemental protein (n = 6) were determined separately by 2 sets of orthogonal contrasts (Table 3). A test was declared significant at a $P \leq .109$.

The data for milk urea, plasma urea, and plasma glucose were analyzed with a model that included the effects previously mentioned plus the three-way interaction between week, block, and diet, sampling time, and sampling time by diet interaction. The three-way interaction between week, block, and diet was used as error term for testing the effects of week and week by diet interaction. After a significant week effect, differences among weeks were separated with the least significant difference option of the general linear models procedure of SAS (28). Pearson's correlation coefficient was computed between milk urea and plasma urea concentration after adjustment for cow and diet to avoid spurious correlation.

The data collected on three cows were not included in the statistical analysis because of health problems probably unrelated to treatments (1 cow for mastitis and 2 cows for displaced abomasum during the covariant period). All treatment means were estimated by the least squares method (28).

RESULTS

Alfalfa Silage and Diets Nutrient Composition

Alfalfa silage had a higher CP but a lower estimated RUP content than anticipated (Table 4). The concentration in NDF and CP of the orts were similar to the calculated dietary values, indicating that cows did not sort diet ingredients. Using the actual NDF, CP, and estimated

RUP for alfalfa silage and the CP of the concentrate mixtures, the actual NDF and RUP concentrations in the diets were calculated (Table 2). Compared with that in the formulated diets, the CP in the actual diets was about 1 percentage unit higher, but RUP concentrations agreed closely. Calculated values for nonstructural carbohydrates, ether extract, and NE_L varied little among dietary treatments.

DMI

Two cows had an acute reaction to the diet containing ABP in the form of LPS; they simply refused to consume the TMR. After the second day, these 2 cows were reassigned to one of the two dietary treatments without ABP.

Overall DMI from wk 4 to 17 of lactation was 25.2 ± .2 kg/d ($\bar{X}$ ± SE). With increased dietary RUP concentration, DMI decreased; however, this effect could be explained by a significant effect of protein source on intake (Table 5). Cows fed SSBM had a higher DMI than those fed ABP (diets 3, 4, 5, and 6). Detrimental effects of ABP relative to SSBM on DMI are illustrated also by the change in DMI when the covariant diet was replaced with the experimental diets (Table 5). When ABP were withdrawn and SSBM was increased (diets 1 and 2), DMI increased more rapidly than when SSBM was withdrawn and ABP was increased to the diets (diets 3, 4, 5, and 6). After wk 5, the difference in DMI between the diets supplemented with SSBM and ABP remained consistent (Figure 1), and DMI averaged 26.4 ± .7 and 25.3 ± .7 kg/d, respectively. The concentration of ABP in the diet contributed in part to the significant detrimental effect of RUP on DMI because the depression of DMI with ABP appeared to be proportional to dietary concentration. Although contrasts testing the effect of increasing ABP in the diet were not significant, DMI of cows fed diets 5 and 6, which contained more than 5% dietary DM as ABP, were depressed more than the DMI of cows fed diets 3 and 4 containing lower concentrations of ABP (24.9 ± .7 vs. 25.7 ± .7 kg/d, respectively).

Milk Yield and Milk Components

Mean milk yield from wk 4 to 17 of lactation was 39.7 ± .2 kg/d. Least squares estimates of milk yields were 1.35 kg/d higher for cows fed SSBM than for those fed ABP. Changes in milk yield between wk 3 and wk 4 and 5 were not affected by source of supplemental protein (Table 5); however, there were quadratic effects as RUP increased in the diet and as ABP increased in the diet. Concentration of dietary RUP did not affect milk yield, 3.5% FCM, percentage of milk protein, or milk fat yield (Table 5). Milk protein yield decreased as dietary RUP concentration increased; however, this relationship was confounded with the source of supplemental protein in the diets because cows fed SSBM produced more milk protein than cows fed ABP. Although no significant linear effect occurred as ABP increased in the diet, the lowest milk protein yields occurred with the highest dietary concentration of ABP (diets 5 and 6). Milk fat percentage increased linearly with increased dietary RUP concentration, but, again, this change was accompanied by differences between SSBM and ABP.

Changes in BW and BCS

Body weight at wk 3 did not differ among cows, and BW at wk 17 was not affected by treatments. From wk 3 to 17, mean BW gain was .22 ± .01 kg/d and was not affected by concentration of RUP or source of supplemental protein (Table 5). At wk 3, BCS did not differ among cows, and it tended to decrease for the cows fed both SSBM and ABP (Figure 2). However, BCS started to increase earlier for cows fed ABP than for cows fed SSBM, resulting in a week by diet interaction. From wk 7 to 17, BCS increased by more than .4 units for cows fed the ABP but less than .3 units for cows fed SSBM. At the end of the experiment, BCS was higher for cows fed ABP (2.93 ± .05) than for those fed SSBM (2.85 ± .06).

Ruminal pH, VFA, and Ammonia

Despite a possible increase in variability of measurements because of saliva contamination of ruminal samples collected with a stomach tube, ruminal pH and ruminal ammonia concentration were affected by dietary treatments (Table 6). Ruminal pH increased with an increased concentration of ABP in the diet, but it was not affected by dietary RUP concentration. As expected, ruminal ammonia concentration decreased as RUP in the diet increased. However, this effect was due primarily to the removal of urea accompanied by the addition of either SSBM (contrast D) or ABP (contrast E). Molar percentages of ruminal VFA were not affected by the concentration of RUP in the diet; however, the percentages of acetate was higher, the percentage of propionate was lower, and the percentage of isovalerate was higher when cows were fed SSBM rather than ABP. In addition, increased ABP in the diet lowered the percentage of propionate, increased the percentage of isobutyrate, and tended to increase the percentage of isovalerate ($P = .113$).

Blood Glucose and Urea in Milk and Blood

Concentration of urea in the plasma was not affected by dietary treatments (Table 6) or week of lactation (Table 7). The amount of urea excreted daily in the milk was higher for cows fed SSBM than for those fed ABP (Table 6). This result was due to the higher milk yield with the SSBM diets because milk urea concentrations remained similar among dietary treatments. Milk urea concentration was more than 1 m*M* higher in the p.m. milking samples than the a.m. milking samples. Plasma urea was significantly higher when collected at 4 h than at 2 h after feeding, but there was no diet by time of sampling interaction. Daily means for milk and blood urea were highly correlated (Figure 3). Diet did not affect the regression; however, after adjustment for the effect of cow ($P < .0009$), the slope of the regression was $.875 \pm .080$, and the correlation coefficient was .850 ($P < .0001$). Plasma glucose was depressed in wk 9 relative to wk 11 and 13 and was higher when collected at 2 h than at 4 h after feeding (Table 7).

DISCUSSION

DMI and RUP Intake

Across dietary treatments, DMI was 5 to 15% higher than that predicted by NRC (22). Other authors have reported higher than predicted intakes with diets based on alfalfa silage (10) or a mixture of hay and silage (13), as well as no effect of RUP level on DMI (10, 25). In this trial, supplemental protein sources were manipulated to increase the proportion of RUP and to decrease the excess RDP in isonitrogenous diets containing 60% alfalfa silage. Nutrient intakes recommended by NRC (22) and observed in this trial are compared in Table 8. Intakes of CP and RDP were in great excess. With increased RUP in diets, intake of RDP was reduced from 167% (diet 1) to 137% (diet 6) of the NRC recommendation. Despite a concentration of only 4.9% RUP (DM basis) in diet 1, the higher than expected DMI resulted in a RUP intake that was 96% of the NRC recommendation. In contrast, diet 6 contained 6.8% RUP (DM basis) and delivered an excess supply of RUP 22% above the NRC recommendation. However, comparison of diets 1 and 6, showed that the increased RUP intake of 347 g/d and the decreased RDP intake of 610 g/d (Table 8), did not affect milk yield (Table 5).

The absence of response to increased RUP intake from 1.3 to 1.6 kg/d (diet 1 and diet 6, respectively) contrasts with the prediction of a response of .6 to .7 kg of milk according to equations developed by Grings et al. (12, 13). These equations were developed with cows at similar DMI, milk yield, and fed a TMR based on alfalfa as the sole forage as in this trial. However, in those trials, increased RUP intake was confounded with increased CP intake

because alfalfa was supplemented with incremental amounts of cotton seed meal (12) or dried distillers grain (13) at the expense of corn or barley.

Supply of Absorbed Protein

Average BW, milk yield and BW gain for cows on each diet were used to compute the need for absorbed protein according to NRC (22). Also, the supply of absorbed protein (AP) was estimated as the sum of digestible RUP and digestible microbial protein reaching the small intestine calculated from NE_L intake (discounted for the NE_L from tallow) as proposed by NRC (22). In all diets, the supply of AP exceeded the need (Figure 4), suggesting that, even with 60% alfalfa silage in the dietary DM of early lactating cows, RUP was not limiting milk yield. Results from other experiments (6, 7) suggested that RUP intake may be more limiting than NE_L intake for early lactating cows fed diets with a high proportion of alfalfa silage (75% of dietary DM). These conclusions were drawn, in part, on experimental diets containing low amounts of nonstructural carbohydrates and for which nutrient intake was clearly limited by the fill effect of the diet (19). Under these conditions, microbial protein synthesis is likely to be reduced, which would result in a greater need for RUP in the diet to meet the requirements for AP.

The diets in our trial were formulated to make use of a maximum amount of alfalfa silage under a practical feeding situation. Cows ate 1.1 to 1.2% of BW as NDF (Table 8) and 9.6 to 10.7 kg of nonstructural carbohydrates, from which about 35, 50, and 15% were from alfalfa silage, shelled corn, and molasses, respectively. This wide range of fermentable carbohydrates might have enhanced the yield of bacterial protein from the rumen. For the DMI and the carbohydrate composition of the diets in this trial, the Cornell Net Carbohydrate and Protein System [**CNCPS**, (27)] predicts a production of digestible bacterial protein 278 g/d higher than the NRC (22) prediction based on NE_L intake (1939 g vs. 1661 g). The NRC (22) equation originates from a data set in which none of the cows were eating more than 20 kg of DM. Extrapolation of the equation beyond its data range should be done with caution. Response to RUP supplementation appears to decrease with increased DMI (5). When DMI is high, bacterial recycling is reduced, rumen turnover rates are increased, and bacterial protein yield is enhanced (27). These changes are accounted for in the CNCPS. Thus, according to this model, the 2750 g of AP required per day to produce 40 kg of milk would be met with about 1.0 kg RUP ((2750 - 1939)/.8) as opposed to 1.3 kg ((2750 - 1661)/.8) as predicted by NRC (22). Although none of the RUP intakes observed in this trial were below 1.3 kg/d, the calculation indicates that, under our feeding conditions, RUP intake of 1.0 kg/d might have met the AP requirement.

In addition to increasing bacterial protein yield, higher DMI can also improve the supply of AP to the cow by increasing the supply of RUP. Under the assumption of constant rumen fill, Robinson et al. (24, 25) illustrated, how an increased rate of digesta passage, which would occur at higher DMI, enhances the supply of RUP because less time is available for ruminal degradation of the protein. These authors also suggested that NRC requirements for RUP are excessive for cows in late (24) and midlactation (25). In our trial, milk yield of early lactation cows was not affected by RUP intakes ranging from 96 to 122% of that recommended by NRC (22).

ABP versus SSBM as a Supplemental Protein Source

In our trial, 2 cows refused to ingest a diet containing ABP in the form of LPS. Despite the attempt to enhance the palatability of ABP by mixing them with large amounts of molasses, some cows may exhibit an acute reaction after detecting the smell or taste of the ABP in the TMR. This type of observation may not be reported often (9) and may remain unaccountable statistically, because the animal is likely to be either removed from the trial or switched to a different treatment. Addition of a blend of ABP to a diet based on corn silage depressed DMI when fed with beet pulp, but did not depress intake when ground corn was a major ingredient

of the concentrate mix (16, 17). However, recent data (9) indicated that the rise in DMI of early lactating cows was depressed for a TMR containing a blend of ABP compared with the rise in DMI when SSBM was the source of supplemental protein. In our trial, DMI were higher than predicted by NRC for all treatments, but lower for the diets supplemented with ABP compared to SSBM.

Yields of milk and milk protein were higher for SSBM than for ABP (Table 5). These results occurred despite the fact that on average, the RUP from SSBM contributed less to total RUP supply in diets 1 and 2 (16.9 and 28.9%, respectively) than did ABP in diets 3, 5, and 6 (25.0, 33.4, and 39.6%, respectively). It is difficult to discuss the effect of possible differences in AA profile between SSBM and ABP because, as previously mentioned, total supply of AP exceeded requirements. Although a blend of ABP is likely to contain a more balanced AA profile than any single ingredient (17), intestinal digestibility of some ABP may be lower than vegetal protein sources (32). The increased protein yield for cows fed SSBM may indicate a better AA profile than for the blend of ABP because high yielding cows appear to respond to an improved AA balance by increasing protein yield rather than milk yield (1, 3). However, others (10) attributed large milk yield responses to optimally heat-treated soybeans to an increased supply of postruminally available lysine.

Milk and Plasma Urea Concentration

In contrast to the results of this trial, Robinson et al. (25) reported a linear decrease in plasma urea with increased RUP in isonitrogenous diets based on alfalfa silage. In our trial, milk and plasma urea concentrations were not affected by treatment or week of lactation (Table 5), but plasma urea was affected by time of sampling relative to feeding and milk urea differed between a.m. and p.m. milk samples (Table 7). These results agree with recent work (14) showing the importance of time of the day and, in particular, time relative to feeding on the correlation between milk and plasma urea concentration. In our trial, the within-cow correlation ($r = .85$) between milk and plasma urea was obtained with cows fed isonitrogenous diets (19% CP). The slope of the regression (slope = .88, Figure 3) was identical to that reported by Roseler et al. (26) using cows eating diets ranging from 12 to 18% CP and having lower milk and plasma urea concentrations than the cows in our trial.

CONCLUSIONS

Results of this trial demonstrate that expressing the RUP recommendation as a percentage of either dietary DM or dietary CP might lead to a misinterpretation of the adequacy of a diet to support high milk yield. NRC (22) predicts that cows producing 40 kg of milk require 1.3 kg of RUP. In this trial, there was no production response to increasing RUP intake above 1.3 kg/d, and cows meet the requirement for AP without a source of supplemental protein resistant to ruminal degradation. Despite a high proportion of alfalfa silage in the diets, the carbohydrate status of the diets possibly played an important role in maximizing DMI and bacterial protein yield from the rumen, thereby reducing the need for supplemental RUP sources. More experimentation is required to test the concept that at high DMI the need for RUP supplementation might be lower than predicted by NRC because increased rumen turnover rate enhances microbial protein yield from the rumen and the RUP value of a diet by decreasing ruminal residence time.

TABLE 1. Content of CP (% of DM) and ruminally undegradable protein (RUP, % of CP) of the supplemental CP sources.

Supplemental CP sources	CP	RUP[1]	Percentage in LPS[2] DM
Urea	281.0	0	...
Solvent soybean meal	47.7	35.0	...
Meat and bone meal	55.7	49.0	...
Animal by-products in LPS	...	...	...
Hydrolyzed feather meal	81.2	71.0	9.7
Meat and bone meal	50.7	49.0	7.5
Poultry by-product meal	66.3	46.6	9.1
Blood meal	91.4	82.0	12.5
LPS[2,3]	32.6	60.2	...

[1] Estimates of ruminally undegradable protein (RUP) according to NRC (22), except for poultry by-product meal for which RUP was determined according to the method of Raab et al. (23).

[2] Liquid protein supplement.

[3] In addition to animal by-products, LPS also contained 60.2% molasses and 1% preservatives (propionic acid) and suspension agent (xanthan and gum).

TABLE 2. Ingredients and composition of experimental diets.

Item	Covariate	Diet 1	2	3	4	5	6
Expected diet composition, % of dietary DM							
CP	18.5	18.5	18.5	18.5	18.5	18.5	18.5
RUP[1]	5.6	5.0	5.6	5.6	6.2	6.2	6.8
Ingredient, % of dietary DM							
Alfalfa silage	57.36	60.0	60.0	60.0	60.0	60.0	60.0
Tallow[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Corn shelled[2]	33.02	27.33	23.58	29.47	25.85	28.04	26.63
Molasses[3]	3.00	5.00	5.00	4.92	4.90	4.90	4.90
Soybean meal[2]	2.00	4.91	9.40	...	3.97	...	...
Meat & bone meal[2]	1.64	...	...	...	...	1.71	3.87
Blood meal[2]	.26	...	...	...	...	...	...
Feather meal[2]	.72	...	...	...	...	...	...
Animal by-products from LPS[4]							
Meat & bone meal	...	...	...	.52	.61	.61	.61
Blood meal	...	...	...	.88	1.02	1.02	1.02
Feather meal	...	...	...	.68	.79	.79	.79
Poultry meal	...	...	...	.64	.74	.74	.74
Preservative in LPS[2,5]	...	...	...	.07	.08	.08	.08
Sodium monophosphate[2]	.55	.83	.75	.78	.69	.46	.08
Urea[2]	.20	.68	.02	.79	.10	.40	.03
Vitamin A, D, & E[2,6]	.10	.10	.10	.10	.10	.10	.10
Trace-mineralized salt[2,7]	.15	.15	.15	.15	.15	.15	.15
Total animal by-products	2.62	...	...	2.72	3.15	4.86	7.02
Actual diet composition, % of dietary DM							
CP	18.1	19.2	19.1	19.5	19.4	19.5	19.5
RUP	5.6	4.9	5.5	5.5	6.2	6.1	6.6
RDP[8]	12.4	14.2	13.6	13.9	13.2	13.4	12.9
NDF	27.3	29.2	29.6	28.7	29.0	28.6	28.4
NSC[9]	42.8	40.6	38.9	40.9	39.2	40.0	39.1
Ether extract[9]	4.5	4.3	4.3	4.2	4.3	4.4	4.5
Ca	1.1	.9	.9	1.0	1.0	1.2	1.5
P	.5	.4	.4	.4	.5	.5	.5
Nel[10], Mcal/kg DM	1.65	1.61	1.62	1.59	1.60	1.60	1.61
RUP,% of CP	31.1	25.7	28.8	28.4	32.0	31.3	33.9

[1] Ruminally undegradable protein.
[2] Prepared as a concentrate premix.
[3] Molasses was mixed with the concentrate premix in the covariate diet, diets 1 and 2; mixed with LPS in diets 3, 5, and 6; and mixed in part with the concentrate premix and in part with the LPS in diet 4.
[4] Liquid protein supplement.
[5] Preservative (propionic acid) and suspension agent (xanthan and gum) in LPS
[6] Contains 2645 IU of vitamin A/g, 882 IU of vitamin D_3/g, 2.2 IU of vitamin E/g.
[7] Contains 95% NaCl, .345% Fe, .548% Zn, .548% Mn, .14% Cu, .008% I, .002% Co, and .006% Se.
[8] Ruminally degradable protein.
[9] Nonstructural carbohydrate (NSC) = 100 - (CP + NDF + ether extract + ash).
[10] Estimated from NRC (22).

TABLE 3. Vector constants for calculation of sums of squares for the two sets of orthogonal comparisons to test the effect of ruminally undegradable protein (RUP) concentration and the effects of source and amount of supplemental CP in the diets.

Comparison		Diet 1 (5.0)[1]	2 (5.6)	3 (5.6)	4 (6.2)	5 (6.2)	6 (6.8)
		Effects of RUP					
A	linear effect of increasing RUP	-6	-1	-1	1	1	6
B	quadratic effect of increasing RUP	2	-1	-1	-1	-1	2
		Effects of the source and concentration of protein supplement[2]					
C[3]	ABP vs. no ABP (SSBM)	2	2	-1	-1	-1	-1
D	increasing SSBM and decreasing urea	1	-1	0	0	0	0
E	increasing ABP and decreasing urea	0	0	-1	0	0	1
F[4]	quadratic effect of increasing ABP	0	0	1	0	-2	1

[1] Expected percentage of RUP in the diet (DM basis).

[2] See Table 2 for the concentrations of protein supplement in the diets.

[3] Diets containing animal by-products (ABP, diets 3, 4, 5, and 6) versus diets with no ABP but solvent soybean meal (SSBM; diets 1 and 2).

[4] It was possible to determine the quadratic effect of increasing ABP in the diet because the difference in ABP between diet 3 and diet 5 (2.14% of dietary DM) was similar to the difference in ABP between diet 5 and diet 6 (2.16% of dietary DM).

TABLE 4. Average composition of the covariant (n = 6) and experimental (n = 9) alfalfa silages and the in situ kinetics of CP degradation of the exprimental silage (n = 10).

Item	Covariant silage		Experminental silage	
	$\bar{X}$	SEM	$\bar{X}$	SEM
	(% of DM)			
DM, % of the feed	53.0	1.0	45.9	.8
OM	90.1	.3	90.3	.4
CP	20.2	.2	20.2	.3
NDF	42.1	.6	43.5	1.0
ADF	36.9	.3	36.7	1.1
ADL	7.43	.07	7.58	.34
NE_l, Mcal/kg of DM[1]	1.41	.01	1.38	.02
ADIN, % of CP	5.16	.08	5.32	.28
	(% of CP)			
Pool size of A[2]	...	...	63.1	1.3
Pool size of B[3]	...	...	34.1	1.9
Disappearance rate of B, /h	...	...	.054	.005
Pool size of C[4]	...	...	2.8	.7
Estimated RUP[5]	...	...	22.4	1.0

[1] Values of NE_l computed from NDF using equation of Mertens (19).
[2] A = Nitrogen washed out of the dacron bags during the washing procedure.
[3] B = Nitrogen disappearing from the bag slowly over time.
[4] C = Asymptotic estimate of the nitrogen remaining in the bag.
[5] RUP = Ruminally undegradable protein calculated as follows:
Pool size of C + Pool size of B x $[k_p/(k_p+k_d)]$ x 100
where k_p, the passage rate from the rumen, is assumed to be .07/h.

TABLE 5. Least squares means for parameters relating to DMI, milk yield, milk components, BW, and body condition score (BCS) for each dietary treatment.

	Diet							Contrast ($P <$)[1]					
								RUP		Protein supplement			
Item	1	2	3	4	5	6	SEM	A	B	C	D	E	F
Cows, no.	9	10	10	10	8	10							
Change DMI, kg/d[2]	1.5	1.2	.5	.5	-.2	.3	.6	.106	...	.055	...	...	...
DMI, kg/d	26.3	26.1	25.2	25.1	24.0	24.6	.7	.065	...	.026	...	...	...
Change milk, kg[2]	3.6	2.3	2.2	3.1	1.1	3.6	.8	...	.061	--	...	...	.094
Milk, kg/d	40.2	40.7	38.7	39.3	38.7	39.7	.9	...	...	.098	...	...	...
3.5% FCM, kg/d	39.6	39.6	39.5	39.6	39.7	39.6	1.0	...	...	...	...	...	...
Milk protein, %	2.81	2.87	2.85	2.86	2.80	2.76	.04	...	...	...	...	...	...
Milk protein, kg/d	1.14	1.15	1.10	1.12	1.09	1.07	.02	.012	...	.004	...	...	...
Milk fat, %	3.39	3.45	3.45	3.49	3.56	3.61	.75	.042	...	.109	...	...	...
Milk fat, kg/d	1.37	1.40	1.34	1.36	1.39	1.39	.04	...	...	...	...	...	...
BW gain, kg/d	.18	.25	.19	.34	.10	.21	.08	...	...	...	...	...	...
Final BW, kg	652.0	665.1	631.6	672.3	660.5	641.4	16.0	...	...	...	...	...	...
Change in BCS[3]	.30	.27	.40	.47	.42	.40	.07	...	...	.044	...	...	...
Final BCS	2.81	2.86	2.78	3.04	3.12	2.88	.08	...	.103	.079	...	...	.003

[1] Contrast A, linear effect of increasing percentage of ruminally undegradable protein (RUP) in the diet;
B, quadratic effect of increasing percentage of RUP in the diet;
C, diets with animal by-products (ABP, diets 3, 4, 5, and 6) versus diets without ABP (diets 1 and 2);
D, effect of increasing solvent soybean meal and decreasing urea (diet 1 versus diet 2);
E, effect of increasing ABP and decreasing urea (diet 6 versus diet 3); and
F, quadratic effect of increasing ABP.

[2] Average of wks 4 and 5 minus average of week 3 of lactation (covariant DMI).

[3] Change in BCS from wk 7 to 17 of lactation.

TABLE 6: Effects of dietary treatments on ruminal parameters, milk urea concentration and excretion, and plasma urea and glucose concentrations.

								Contrast ($P <$)[1]					
								RUP		Protein supplement			
	Diet												
Item	1	2	3	4	5	6	SEM	A	B	C	D	E	F
Ruminal parameters													
pH	6.96	6.88	6.76	6.90	6.88	6.99	.07	...	...	...	...	.059	...
Ammonia, m*M*	16.9	13.1	15.8	12.2	15.1	13.5	.9	.012	...	...	.007	.091	...
VFA, mol/100 mol													
Acetate (A)	66.6	66.4	64.7	65.6	65.8	66.0	.7	...	...	.093	...	...	...
Propionate (P)	16.0	15.4	17.4	16.8	16.2	15.9	.4	...	...	.011	...	.006	...
Butyrate	11.3	11.7	11.9	11.7	11.5	11.5	.2	...	...	...	...	...	...
Isobutyrate	1.45	1.57	1.20	1.28	1.64	1.57	.09	...	...	...	...	.071	...
Isovalerate	2.31	2.44	2.08	2.03	2.23	2.33	.09	...	...	.093	...	...	...
Valerate	2.85	2.81	2.66	2.65	2.67	2.76	.09	...	...	...	...	...	...
A:P	4.3	4.4	3.9	4.0	4.1	4.3	.1	...	.087	.015	...	.020	...
Milk urea													
Concentration, m*M*	7.92	7.68	7.97	6.83	7.62	7.94	.34	...	...	...	...	...	...
Excretion, m*M*/d	318.0	318.0	309.7	257.9	292.4	314.1	14.5	...	...	.062	...	...	...
Plasma													
Urea, m*M*	9.19	8.87	9.54	7.86	8.96	9.02	.32	...	...	...	...	...	...
Glucose, mg/dl	72.2	72.8	70.9	74.7	73.8	71.9	1.8	...	...	...	...	...	...

[1] Contrast A, linear effect of increasing percentage of ruminally undegradable protein (RUP) in the diet;
B, quadratic effect of increasing percentage of RUP in the diet;
C, diets with animal by-products (ABP, diets 3, 4, 5, and 6) versus diets without ABP (diets 1 and 2);
D, effect of increasing solvent soybean meal and decreasing urea (diet 1 versus diet 2);
E, effect of increasing ABP and decreasing urea (diet 6 versus diet 3); and
F, quadratic effect of increasing ABP.

TABLE 7. Effect of week of lactation and time of sampling on milk urea concentration and lasma concentration of urea and glucose.

Item	Lactation				Time of sampling[1]		
	wk 9	wk 11	wk 13	SE	a.m.	p.m.	SE
Milk urea, m*M*	7.55	7.64	7.76	.12	7.14 [b]	8.18 [a]	.10
Plasma urea, m*M*	8.84	8.97	8.92	.04	8.53 [b]	9.30 [a]	.03
Plasma glucose, mg/dl	70.72 [b]	73.56 [a]	73.64 [a]	1.86	73.57 [a]	71.71 [b]	1.52

[a,b] Means within a row with different superscripts differ ($P < .05$).

[1] For milk urea, times of sampling were the a.m. and p.m. milkings, respectively. For plasma urea and plasma glucose, times of sampling were 2 and 4 h after feeding (1000 and 1400 h, respectively).

TABLE 8. Estimated nutrient intake and NRC (22) recommendations of nutrients required for the level of performances observed in this trial.

	Nutrient Intake								
	Diet							Percentage of NRC (22)	
Nutrient	1	2	3	4	5	6	NRC[1]	Lowest	Highest
	..... (kg/d)						kg/d	..... (%)	
CP	5.07	4.98	4.84	4.86	4.68	4.80	3.85	122	132
RUP[2]	1.29	1.42	1.38	1.56	1.47	1.63	1.34	96	122
RDP[3]	3.78	3.56	3.47	3.30	3.21	3.17	2.31	137	164
NDF	7.73	7.71	7.14	7.26	6.86	7.01	...	...	...
NDF, % BW	1.20	1.18	1.14	1.10	1.05	1.11	...	...	...
NSC[4]	10.73	10.14	10.18	9.80	9.60	9.64	...	...	...

[1] NRC recommendations based on the average cow of this trial (i.e., BW = 646 kg; lactation number > 2, milk yield = 39.7 kg/d, milk fat percentage = 3.5%, and BW gain = .17 kg/d).
[2] Ruminally undegradable protein.
[3] Ruminally degradable protein.
[4] Nonstructural carbohydrate.

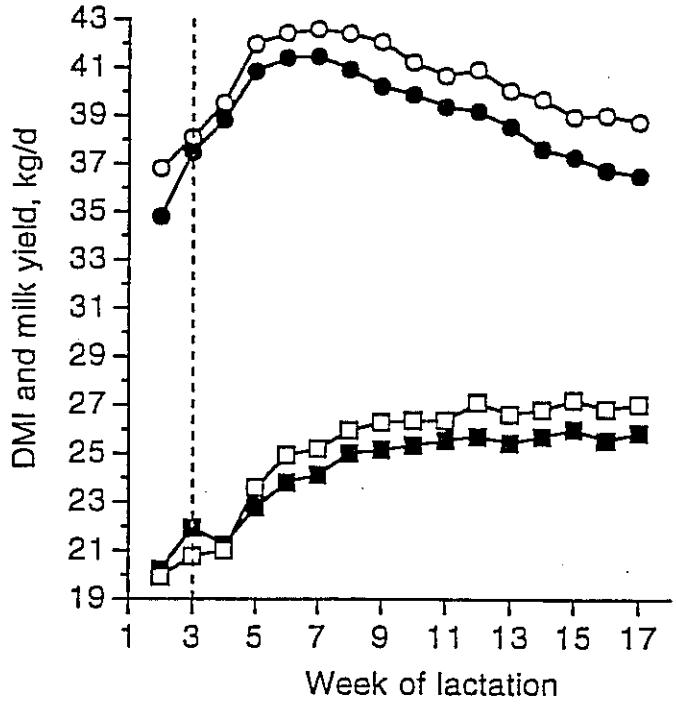
List of Figures

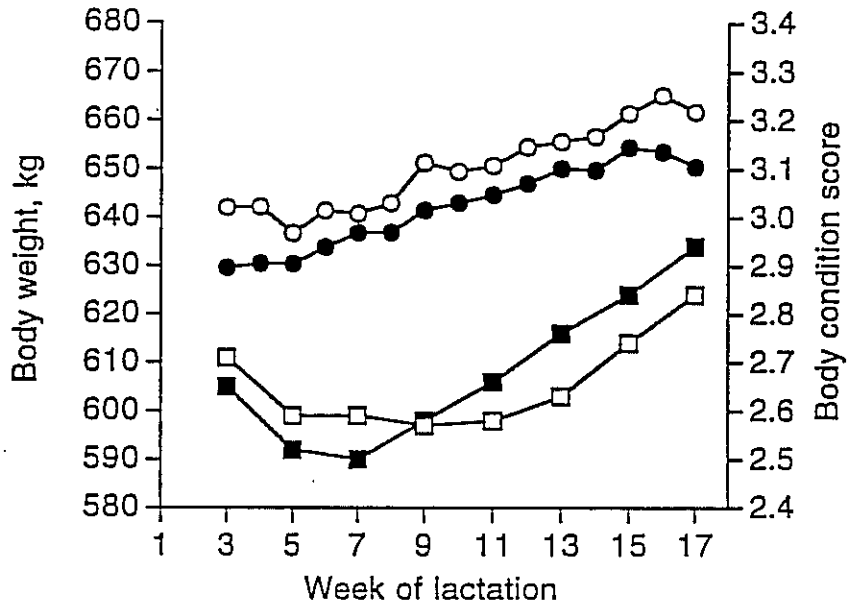
Figure 1. Effect of supplemental protein source on dietary DMI (□ = solvent soybean meal (SSBM), diets 1 and 2; ■ = animal by-products (ABP), diets 3, 4, 5, and 6) and milk yield (○ = SSBM, diets 1 and 2; ● = ABP, diets 3, 4, 5 and 6). The LSD for DMI and milk yield were 2.5 and 2.7 kg/d, respectively. The vertical dashed line indicates the transition from the covariant to the experimental period.

Figure 2. Effect of supplemental protein source on body condition score (□ = solvent soybean meal (SSBM), diets 1 and 2; ■ = animal by-products (ABP), diets 3, 4, 5, and 6) and BW (○ = SSBM, diets 1 and 2; ● = ABP, diets 3, 4, 5 and 6). The LSD for body condition score and BW were 0.8 unit and 6.8 kg, respectively.

Figure 3. Relationship between plasma urea concentration and milk urea concentration for each cow (n = 57) in the trial on diets 1 (○), 2 (△), 3(▲), 4 (◆), 5 (■), and 6 (●). The slope of the regression was .88 (r = .85).

Figure 4. Comparison of the NRC recommendations for supply of absorbed protein (□) and calculated supply of digestible bacterial protein (■) and digestible ruminally undegradable protein (▨) for the average cow on each experimental diet.


DMI and milk yield, kg/d
43
41
39
37
35
33
31
29
27
25
23
21
19
1
3
5
7
9
11
13
15
17
Week of lactation


Body weight, kg
680
670
660
650
640
630
620
610
600
590
580
Body condition score
3.4
3.3
3.2
3.1
3.0
2.9
2.8
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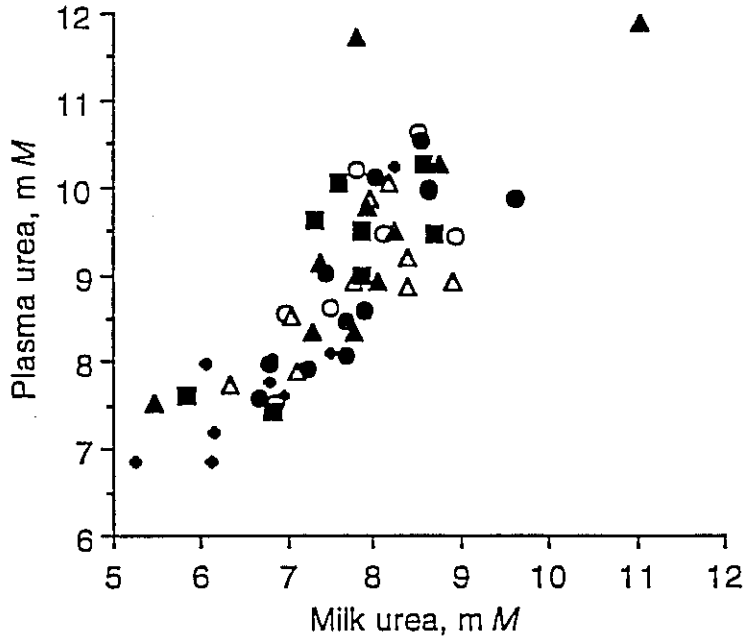
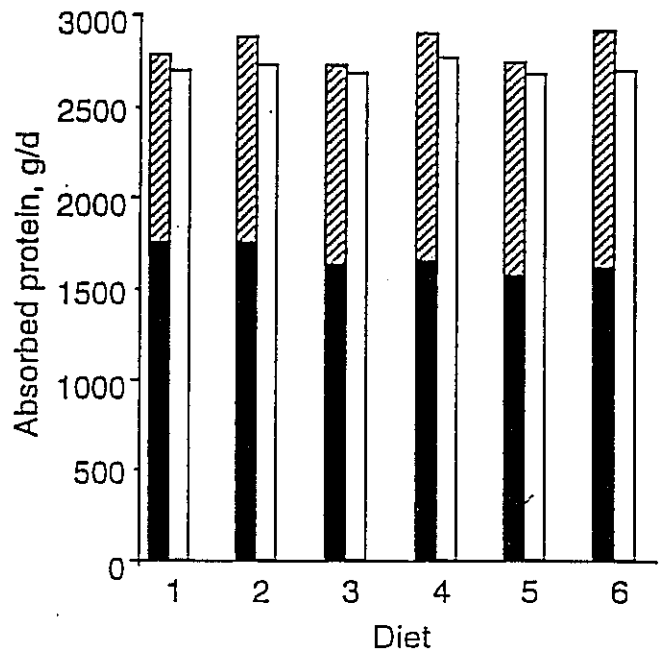
2.6
2.5
2.4
1
3
5
7
9
11
13
15
17
Week of lactation


Plasma urea, m M
Milk urea, m M
12
11
10
9
8
7
6
5
6
7
8
9
10
11
12


Absorbed protein, g/d
Diet
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6