

Undegradable Protein Supplementation to Early-Lactation Dairy Cows in Grazing Conditions

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ABSTRACT

To determine the production responses to rumen undegradable protein (RUP) feeding in grazing conditions, we fed 18 multiparous Holstein cows concentrates containing either soybean meal (SBM) or blood meal (BM) during the first 8 wk of lactation. One cow from the SBM treatment was removed because of mastitis. Six additional dairy cows in late lactation fitted with ruminal cannula were used to evaluate the rumen environment and the in situ crude protein (CP) degradability of concentrates. On a dry matter (DM) basis, concentrates contained SBM (33%) or BM (13%), corn grain (64 and 84% for SBM and BM, respectively) and a mineral-vitamin complex (3%). Concentrates were offered at a rate of 6.6 kg/d per cow and herbage allowance averaged 31 kg/d of DM per cow. The BM reduced ruminal ammonia-N levels and had no effect on ruminal pH and molar volatile fatty acid concentration. The degradable fraction (63.59 vs. 22.46%) and the rate of disappearance of the CP (9.68 vs. 1.69%/h) were greater for the SBM compared with the BM concentrate. Cows fed the BM concentrate produced more milk (29.3 vs. 24.9 kg/d) and more milk protein (0.85 vs. 0.74 kg/d) than did those fed the SBM concentrate. Milk fat yield and percentages of milk fat, lactose and protein were not affected. Forage DMI was increased by BM (17.19 vs. 13.17 kg/d per cow). The in vivo responsiveness to lipolytic stimuli were increased by BM but enhanced body weight loss or higher plasma nonesterified fatty acids concentration were not observed. Results indicated that a concentrate with a high RUP content increased milk and milk protein yields when spring pasture was the sole forage. The highest milk yield was more likely caused by increased DM than by enhanced body lipid mobilization.

(Key words: grazing cows, early lactation, rumen-undegradable protein)

Abbreviation key: BM = blood meal, INS = insulin, ISO = isoproterenol, ISO-0 = sample taken before ISO challenge, ISO-15 = sample taken 15 min after ISO challenge, INS-0 = sample taken before INS challenge, INS-30 = sample taken 30 min after INS challenge, INS-60 = sample taken 60 min after INS challenge, INS-90 = sample taken 90 min after INS challenge, IVOMD = in vitro OM digestibility, J-M = jugular-mammary vein, ME = metabolizable energy, MP = metabolizable protein, SBM = soybean meal, NH₃-N = ammonia.

INTRODUCTION

In early lactation, the requirements of high producing dairy cows for metabolizable protein (MP) are greater than that which can be supplied by microbial and forage RUP, so body protein must be mobilized (Beever and Siddons, 1986). Dairy cows are able to mobilize between 5 to 13 kg of body protein during the first 2 to 4 wk postpartum, but Bauman and Elliot (1983) suggested that if more than 15 to 20 kg is mobilized, adverse effects on cow health might arise. The use of sources of RUP may be a way to increase the quantity of AA arriving to the small intestine to complement the microbial protein and to improve lactational responses of cows in early lactation. Santos et al. (1998) demonstrated that replacing RDP-like SBM with several protein supplements high in RUP does not consistently improve lactational performance, but most of the revised lactation trials have been conducted in confinement systems. In grazing systems, the cow consumes a forage with a high CP content, and ruminal CP is rapidly degraded (Hongherholt and Muller, 1998). In this situation, forage proteins are rapidly degraded in the rumen, leading to ammonia (NH₃-N) surplus and extensive preduodenal losses of N (Beever et al., 1986), and a shortage of absorbable protein may occur during early lactation. This observation could probably explain the lack of response to RDP when dairy cows are maintained on grazing pastures that contain more than 15% CP (Hamilton et al., 1992). Increasing RUP at the expense of RDP in the concentrate may be

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logical in diets in which RDP is excessive (Santos et al., 1998).

The effects of RUP supply on milk production under grazing conditions have not been extensively investigated with high producing cows; research with low-yielding dairy cows has reported (Flores et al., 1979; Stobbs et al., 1977) positive responses. In spring pastures, milk production (+6.4%), milk protein yield (+11%), and plasma NEFA were enhanced when a high quality source of RUP (fish meal) replaced sunflower meal in the concentrate of medium-yielding (27 kg/d) early-lactation cows (Schroeder and Gagliostro, 2000). A change in the responsiveness of the adipose tissue to lipolytic stimuli was not detected (Schroeder and Gagliostro, 2000). In high-yielding (39.8 kg/d of milk) dairy cows in early lactation (68 d of lactation), a supplemental grain mixture with a high RUP content tended to increase milk protein yield when a grass pasture was the sole forage source (Hongerhold and Muller, 1998).

Positive responses to RUP supplementation to the diets of cows on pasture above that observed with energy was suggested to most likely occur in early-lactation cows, when pasture quality was poor and when high concentrate grain is fed [Kellaway and Porta, 1993 cited by Hongerhold and Muller (1998)]. Two mechanisms were proposed to operate in protein-supplemented cows to maintain a constant metabolizable energy (ME) to protein ratio: increased DMI or depletion of adipose tissue. Both exogenous (feed) and endogenous (fat) energy may improve milk yield (Newbold, 1994). In addition, the ability of adipose tissue to mobilize fat may be involved because the responsiveness to epinephrine was increased by RUP in a grass silage-based diet (Cadórniga and López Díaz, 1995).

The objective of this experiment was to determine whether early-lactation dairy cows maintained under grazing conditions would respond to supplemental RUP feeding and to determine whether responses were linked to increased responsiveness of adipose tissue to adrenergic stimulation or to enhanced DMI.

MATERIALS AND METHODS

Cows and Treatments

The experiment was conducted during the spring at the Agricultural Research Station of Balcarce (INTA, 37°45'S; 58°18'W) in Argentina. Eighteen multiparous (561 ± 62 kg of BW) Holstein cows were assigned to the treatments according to their lactation number, milk production of the previous lactation, and live weight recorded d 30 before the expected calving date. Animals calved from September 13 to October 15. In the dry period, diets (12.5% CP of dietary DM) consisted (DM basis) on corn silage (63.5%), corn grain (8.2%), SBM

Table 1. Ingredient and nutrient composition of concentrates fed to grazing dairy cows in early lactation.

Composition, % DM	Concentrates ¹	
	SBM	BM
Ingredient		
Corn grain	64	84
Soybean meal	33	...
Blood meal	...	13
Mineral-vitamin mix ²	3	3
Nutrient ³		
DM	91.2 ± 0.1	91.2 ± 0.1
OM, % DM	96.2 ± 0.3	98.0 ± 0.1
NDF, % DM	11.8 ± 1.0	19.2 ± 0.7
WSC, ⁴ % DM	18.1 ± 0.6	15.9 ± 1.1
CP, % DM	20.8 ± 1.0	22.9 ± 0.9
N Soluble, % DM	3.32 ± 0.24	1.18 ± 0.06
IVDMD, %	85.7 ± 1.6	76.1 ± 2.0
IVOMD, %	86.3 ± 1.6	76.8 ± 2.0
NEL, Mcal/kg DM ⁵	2.01	2.04

¹A flavoring agent was added to both concentrates (0.6%) to avoid reductions in feed intake. SBM = soybean meal (89.7% DM; 44% CP); BM = blood meal (93.1% DM, 85% CP; Protesan, Derisan Laboratories, Buenos Aires, Argentina).

²Contained 21% Ca₃(PO₄)₂, 4.6% P₂O₅, 6% MgO, 3% molasses, 0.15% FeSO₄, 0.4% CuSO₄, 0.3% ZnSO₄, 0.04% MnSO₄, 0.02% CoSO₄, 0.02% Na₂SeO₃, 0.01% I₂, vitamin A (6,500,000 IU/kg), vitamin D₃ (1,600,000 IU/kg), vitamin E (12,500 IU/kg), 64.46% excipient.

³Compositions are means ± standard error from weekly sampling over the experimental period.

⁴Water-soluble carbohydrates.

⁵Net energy for lactation was derived from INRA (1989). Corn grain = 2.159 Mcal NE_l/kg DM; SBM = 1.938 Mcal NE_l/kg DM; BM = 1.853 Mcal NE_l/kg DM.

(8.1%), and a mixed pasture (20.2%) composed of fresh winter oats and ryegrass. Two weeks before the expected calving date, the ration composition of cows was changed to adapt cows to the experimental diets. Corn silage was removed, and pasture was the sole forage offered in a system of rotational grazing in which cows were rotated to a new paddock every day. Cows grazed swards of perennial ryegrass (*Lolium perenne* L., 44%), red clover (*Trifolium pratense* L., 27%), white clover (*Trifolium repens* L., 11%), orchardgrass (*Dactylis glomerata* L., 8%) and dead material (10%). Nine of the cows were fed a concentrate formulated with a source of RDP and nine with another concentrate containing a source of RUP (Table 1).

The protein for the RUP concentrate was provided by batch-dried blood meal (BM) (Protesan, Derisan Laboratories, Buenos Aires, Argentina) that contained 93.1% DM, 85% CP (DM basis), 9.06% Lys, and 0.67% Met (as % of CP). The protein for the RDP concentrate was supplied by SBM that contained 89.7% DM; 44% CP; 7.6% Lys and 1.77% Met (as % of CP). Both concentrates were formulated to contain about 20% CP and were started at a rate of 1 kg/d per cow. The amount was gradually increased during 18 d, so by d 5 of lactation

all animals were well adapted to the full ration. Although diets differed in RUP content during about the last 15 d of the dry period, increasing dietary RUP for about 1 mo before parturition does not affect subsequent lactational performance of multiparous dairy cows (Santos et al., 2001; Wu et al., 1997). One cow from the SBM treatment was removed from the experiment before completion of 8 wk of lactation because of mastitis. After the adaptation period, concentrates (91.2% DM) were offered at a fixed rate of 6.6 kg/d per cow in two equal feeds during milking. A high dose of a flavoring agent (0.6 kg/100 kg) was used to avoid concentrate refusals in the pre- and postpartum periods. Concentrates were consumed well by cows in the two treatments. Average calving date was September 29 (± 16 d). The cows were milked between 0600 and 0700 h and between 1600 and 1700 h each day up to d 56 of lactation. The amounts of concentrate given and refused were measured individually every day. Concentrate was sampled weekly for nutrient composition.

Experimental Measures and Sample Analyses

In pasture, the animals were in a single herd under strip grazing management. The cows went to a new paddock every day after the morning milking. Front and back electric fences were moved every day at 0700 h. The areas necessary to provide a daily allowance of at least 31 kg of pasture DM/d per cow were based on estimates of the herbage mass present before grazing. After subtracting concentrate DMI (6 kg/d per cow) cows were offered an amount equivalent to 3 times the pasture DM requirement to obtain ad libitum intake (Meijs, 1981). Herbage mass was calculated from the herbage height using a disk meter and its relation to herbage mass as a nondestructive method (Spada and Cangiano, 1991). The refusals were cut with a forage harvester, and the residues were removed to obtain clean and uniform regrowth. The regression equation between sward height (X) and herbage mass (Y) was obtained by cutting to ground level two quadrats of low, medium, and high forage mass for correlating with the sward height. The regression equation obtained ($n = 6$) was Y (kg of DM/ha) = $1581 + 123 X$ (cm), $r^2 = 0.91$. Sixty measures of sward height were taken from each grazing strip to estimate herbage mass. To assess the quality of the grazed herbage, samples were obtained by hand plucking (about 2 kg of fresh forage) at the grazing height during intake measurements. Herbage samples were weighed fresh and dried at 60°C during 48 h. Pastures and concentrates were analyzed for OM, NDF (Goering and Van Soest, 1970), CP (Kjeldahl), buffer-soluble N, water soluble carbohydrates (Morris, 1948), in vitro DM digestibility and in vitro OM digestibility (IVOMD) (Tilley and Terry, 1963).

Cows were weighed on d 2 and 3 and on d 55 and 56 of lactation. Changes in BW gain were obtained as the difference between the mean values of the initial (d 2 and 3) and final (d 55 and 56) BW records. Individual milk yields were recorded twice daily. Milk samples were obtained in wk 4, 7, and 8 of lactation on two consecutive milkings, composited according to the corresponding volume measured at each milking time and analyzed for fat, protein, and lactose with an infrared milk analyzer (Foss 300 Milkoscan, Foss Electric, Hillerød, Denmark).

Pasture intake was estimated on each cow with Cr_2O_3 as an indigestible fecal marker only one time during wk 8 postpartum. One cow of the BM treatment was removed from the analysis because its fecal OM production was greater than the mean plus two times the standard deviation of the group. Cows were dosed twice daily for 14 d with gelatin capsules containing 5 g of Cr_2O_3 . Fecal grab samples were collected twice daily (at 0700 and at 1700 h) on d 10 to 14. At each time of fecal collection, pasture samples (0.46 ± 0.038 kg wet basis) were cut by hand plucking from 10 randomly chosen areas to obtain representative samples of the pasture eaten by the cows. Total fecal OM production (kg/d) of each cow was obtained by dividing the total Cr ingested (g/d) by the Cr concentration in fecal OM (g/d). Fecal OM production due to concentrate was measured as [concentrate intake / (1 - concentrate IVOMD)]. This quantity was subtracted from the total fecal OM production and the remaining fecal OM material was attributed to pasture. Pasture intake was calculated as the ratio between fecal OM yield due to pasture and pasture indigestibility (1 - IVOMD) (Hamilton et al., 1992).

Blood samples were collected from the jugular vein immediately after the morning milking and feeding. Samples were obtained at weekly intervals from wk 4 to 6 of lactation (1 d/wk). During wk 4, blood from the jugular and from the external mammary abdominal veins were drawn to determine jugular-mammary (J-M) differences in metabolites and estimate apparent mammary uptake (Schroeder and Gagliostro, 2000). During wk 4, blood samples were taken before (ISO-0) and 15 min after (ISO-15) an isoproterenol (ISO) challenge (4 nmol/kg of BW, Proterenal, Phoenix Laboratory, Buenos Aires, Argentina). During wk 5, blood samples were taken before (INS-0) and 30 (INS-30), 60 (INS-60), and 90 (INS-90) min after an intravenous bovine insulin (INS) challenge (0.12 U/kg of BW, Betasint, Beta Laboratory, Buenos Aires, Argentina; Schroeder and Gagliostro, 2000). During wk 6, blood samples were taken before and 5, 10, 15, 20, and 25 min after an intravenous glucose challenge (100 mg/kg of BW).

The fractional rate of glucose clearance (k) was calculated as the slope of the regression of time on $\ln C$ where C = glucose concentration (mg/dl) at times 5, 10, 15, 20,

and 25 minus basal glucose concentration (Gagliostro and Lavandera, 1997). The distribution volume for glucose and surfaces under the glucose curves were calculated as described in (Gagliostro and Lavandera, 1997). During wk 4, plasma-free AA were analyzed by HPLC (Shimadzu, model RF-530). Plasma (1 ml) was deproteinized with 0.15 ml of $C_2HCl_3O_2$ (12%), centrifuged ($1000 \times g$ for 10 min), and the supernatant was stored at $-20^\circ C$. Plasma was neutralized (KOH) and derivatized by the O-phthalaldehyde/N-acetyl-L cysteine method (Aswad, 1983). Fluorescence was measured at 470 nm after excitation at 344 nm. The free AA isoindols were fractionated in two reverse-phase columns (Hipersil-ODS) placed in continuous form. Samples were eluted performing a continuous gradient 0 to 80% methanol in 0.1 N sodium acetate, 0.25% tetrahydrofuran. In every sample, blood was immediately transferred to heparinized tubes (5 U/ml) and plasma was obtained ($2000 \times g$ for 10 min) and stored ($-24^\circ C$) until analysis. Commercial enzymatic kits were used for urea (Wiener Laboratory, Rosario, Argentina), NEFA (Wako Pure, Chemical Industries USA, Inc., Dallas, TX), glucose (Wiener Laboratory, Rosario, Argentina) and triglyceride (Wiener Laboratory, Rosario, Argentina).

Rumen Environment and in Situ Protein Degradability of Concentrates

Conditions in the rumen environment and the in situ protein degradability of concentrates were determined with six additional multiparous Holstein cows in midlactation (237 ± 7 d postpartum) producing about 16 kg/d of milk and fitted with ruminal cannulas in a two treatments, two periods crossover design. Fistulated cows received the same concentrates and feeding protocol as described previously for nonfistulated cows in the postpartum period. The experimental concentrates were thoroughly consumed by cows, and pasture intake was not measured. All cows (fistulated and nonfistulated) were in a single herd under strip grazing conditions as described earlier. Each experimental period lasted 14 d, with the first 10 d as the adjustment period followed by 3 d for data collection. During d 11 and 12 of each experimental period, samples of whole rumen contents were obtained from each cow every 3 h through the rumen cannula. These contents were strained through two layers of cheesecloth, and pH was immediately recorded in the collected fluid (Orion portable pH meter 250A, Orion Research Inc., Boston, MA). Then, the rumen fluid was acidified with sulfuric acid (1 ml of acid/100 ml of fluid) and frozen for later determination of NH_3 -N and VFA. After thawing, rumen fluid was centrifuged ($10,000 \times g$ for 10 min at 0 to $5^\circ C$) and NH_3 -N in the supernatant was determined colorimetrically with

phenol-hypochlorite with an automated analyzer (model 2, 1970; Technicon, Emeryville, CA). VFA concentrations were measured in a Gow-Mac P750 gas chromatograph (Bethlehem, PA) fitted with a glass column packed with 10% SD-1200, 1% H_3PO_4 on 80/100 Chromosorb WAW. Working temperatures for this determination were: column, $175^\circ C$; injector and detector, $200^\circ C$.

On d 10 of each experimental period, concentrates consumed by the early-lactation cows were ground to pass a 1-mm screen and suspended (5 g/bag) in the ventral sac of the rumen in Dacron bags (15.5×7.5 cm, $52\text{-}\mu m$ pore size, Ankom, Fairport, NY). Duplicate bags were removed at 0, 3, 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 42 and 48 h; manually rinsed in cold water for 5 min; and dried at $60^\circ C$ until constant weight. The residues from each bag were removed and analyzed for CP. The estimate of the extent of rumen protein degradability was based on the 48 h in situ protein residue. Kinetics of nitrogen disappearance were fitted to a first-order model described by Ørskov and McDonald (1979).

Statistical Analysis

Analysis of variance on milk and milk constituents yield, milk composition, and plasma metabolite concentration were analyzed by the following model:

$$Y_{ijk} = \mu + T_i + C_{(ij)} + W_k + T \times W_{ik} + e_{ijk},$$

where Y_{ijk} = the dependent variable, μ = the population mean, T_i = the effect of the i th source of concentrate protein, C = the random effect of the j th cow within the i th source of concentrate protein, W = the effect of the k th wk of lactation, e_{ijk} = the residual error associated with the ijk th observation. The $C_{(ij)}$ term was used as an error term to test the T_i effect.

Data for DMI, changes in BW gain, plasma-free AA concentration, J-M differences in metabolite data, and parameters associated to glucose metabolism were analyzed by the following model:

$$Y_{ijk} = \mu + T_i + e_{ijk},$$

where Y_{ijk} = the dependent variable, μ = the population mean, T_i = the effect of the i th source of concentrate protein, and e_{ijk} = the residual error associated with the ijk th observation.

To test whether the J-M differences were different from zero, Student's t -test (pairwise observations) was used.

Responses to ISO, INS, and glucose injections were analyzed by the following model:

$$Y_{ijk} = \mu + T_i + C_{(ij)} + H_k + T \times H_{ik} + e_{ijk}$$

Table 2. Chemical composition of spring pastures for grazing dairy cows in early lactation.¹

	Forage	
	Cut ²	Hand-plucking ³
DM	22.0 ± 1.9	27.4 ± 0.5
OM, % DM	90.0 ± 0.2	90.8 ± 0.2
NDF, % DM	44.9 ± 1.9	46.2 ± 1.6
WSC, ⁴ % DM	13.4 ± 0.9	12.3 ± 0.5
CP, % DM	13.7 ± 1.5	14.9 ± 0.6
N Soluble, % DM	0.93 ± 0.37	0.76 ± 0.14
IVDMD, %	70.9 ± 1.4	72.7 ± 0.5
IVOMD, %	70.6 ± 1.5	72.8 ± 0.4
NE _l , Mcal/kg DM ⁵	1.53	1.58

¹Values are expressed as means ± standard error.

² Values are the means for the whole experimental period.

³Values are the means for the period in which herbage intake was measured.

⁴Water-soluble carbohydrates.

⁵Net energy for lactation calculated as 4.4 × IVOMD × 0.82 × 0.6.

where Y_{ijk} = the dependent variable, μ = the population mean, T_i = the effect of the *i*th source of concentrate protein, $C_{(ij)}$ = the random effect of the *j*th cow within the *i*th source of concentrate protein and H_k = the effect of the *k*th hour. The $C_{(ij)}$ term was used as an error term to test the T_i effect.

Rumen associated variables (pH, NH₃-N, and VFA) were analyzed using the following model : $Y_{ijkl} = \mu + T_i + C_{(ij)} + P_k + H_l + T \times H_{il} + e_{ijkl}$, where P_k = the effect of the *k*th period, H_l = the effect of the *l*th hour; the other terms are as previously already defined. Kinetic parameters of concentrate protein degradation were tested using the model: $Y_{ijkl} = \mu + T_i + C_j + P_k + e_{ijkl}$. Probability values greater than 0.1 were considered to be nonsignificant. Data were analyzed using the GLM procedure of SAS (1985).

RESULTS

Average values of herbage mass, sward height, and herbage allowance were 4280 (±113) kg of DM/ha, 23.2 (±0.2) cm and 31 (±1) kg of DM/d per cow, respectively.

The pasture utilization coefficient $\{[(\text{herbage mass at the beginning of grazing} - \text{herbage mass at the end of grazing})/\text{herbage mass at the beginning of grazing}] \times 100\}$ averaged 50% for the whole experimental period. Values for the chemical composition of the forage consumed by cows are shown in Table 2.

The parameters of in situ disappearance of the concentrate CP are presented in Table 3. After 48 h of incubation, concentrate CP degradability was lower for the BM (52%) compared with the SBM (98%) supplement. The CP soluble fraction did not differ ($P > 0.1$) between SBM and BM concentrates. The fermentable CP fraction and the rate of disappearance were greater for the SBM compared with the BM supplement.

Neither treatment nor treatment × time interaction effects were observed for ruminal pH values, molar proportions of individual VFA, or branched-chain fatty acids measured in the late-lactation fistulated cows. Ruminal NH₃-N concentrations were greater ($P > 0.04$) in fistulated cows fed the SBM compared with the BM concentrate, and a significant treatment × time interaction was not detected. Average values obtained are presented in Table 4.

Weekly milk yields for the first 8 wk of lactation are presented in Figure 1.

The high RUP concentrate significantly increased milk yield throughout the trial, and a significant interaction between type of concentrate and week of lactation was not detected (Figure 1). Average values over the 8 wk of lactation for milk production, milk composition, changes in BW gain, and DMI of cows are shown in Table 5.

Milk (+17.7%) and milk protein yields (+14.9%) were increased by the BM concentrate over the first 8 wk of lactation. Milk fat yield and percentages of milk fat and protein were not affected. Milk lactose content tended ($P > 0.09$) to be higher in BM. Although cows fed the BM concentrate gained less BW, differences in BW gain were not significant. Differences in DMI of concentrates were not significant. Forage DMI was increased in cows fed the BM concentrate compared with the cows that

Table 3. Parameters of in situ CP disappearance of the experimental concentrates.¹

Parameters	Concentrates ²		<i>P</i> <
	SBM	BM	
Protein degradability, % CP	98.25 ± 0.04	51.78 ± 0.37	0.001
Soluble fraction (a), % CP	35.27 ± 0.99	39.30 ± 0.76	0.514
Degradable fraction (b), % CP	63.59 ± 0.99	22.46 ± 0.58	0.001
Disappearance rate, % (b)/h	9.68 ± 1.6	1.69 ± 1.7	0.001
Effective degradability, ³ % CP	75.0	44.0	0.001

¹Values are expressed as means ± standard error.

²SBM = soybean meal; BM = blood meal.

³Assuming a rate of passage of 0.06/h.

Table 4. Parameters of rumen environment for lactating dairy cows¹ that received concentrates containing soybean meal (SBM) or blood meal (BM) in spring pastures.²

Item	Concentrates		<i>P</i> ³ <
	SBM	BM	
pH	5.7 ± 0.01	5.8 ± 0.01	NS
NH ₃ -N, mg/dl	25.3 ± 1.9	21.2 ± 1.8	0.04
VFA, mmol/L	119.0 ± 6.0	112.0 ± 5.0	NS
Acetate, molar %	57.6 ± 0.6	58.1 ± 0.5	NS
Propionate, molar %	24.8 ± 0.4	24.3 ± 0.3	NS
Butyrate, molar %	13.0 ± 0.2	13.1 ± 0.2	NS
BCFA, ⁴ molar %	2.8 ± 0.1	2.6 ± 0.1	NS
Acetate: propionate	2.40 ± 0.1	2.42 ± 0.01	NS

¹Fistulated cows on d 237 (±7) postpartum producing about 16 kg/d of milk.

²Values are expressed as means ± standard error.

³NS = *P* > 0.10. Treatment × h of sampling interaction was not detected for any parameter.

⁴BCFA = branched chain fatty acids (isobutyrate + 2 methylbutyrate + isovalerate).

consumed the SBM concentrate (Table 5). With estimates of pasture and concentrate intake (Table 5), pasture analyses (Table 2), and the effective degradability coefficients for each concentrate (Table 3), protein intake was calculated for the two treatments. Value for rumen effective CP degradability from pasture was that observed by Hongerholt and Muller (1998) (60.9%, *kp* = 0.06/h). Estimated total CP (3.66 vs. 3.04 kg/d) and total RUP (1.65 vs. 1.01 kg/d) intakes were higher in BM treatment. The estimated RUP/CP ratio of total diets was higher (0.453 vs. 0.334) in cows fed the high RUP concentrate (Table 5).

Plasma metabolite concentrations are given in Table 6.

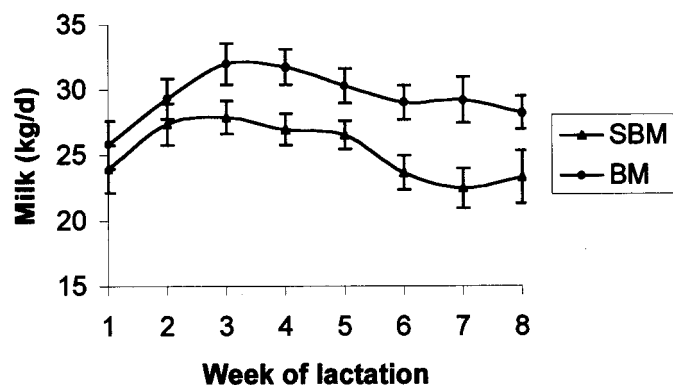


Figure 1. Milk yield in grazing dairy cows that received concentrates containing soybean meal (SBM) or blood meal (BM) during the first 8 wk of lactation. Each point represents the weekly average (± standard error) of eight cows in SBM and nine cows in BM. Treatment × week of lactation interaction was not detected. SBM versus BM = *P* < 0.016.

Table 5. Milk yield and composition, BW gain (BWG) and intake in grazing dairy cows that received concentrates containing soybean meal (SBM) or blood meal (BM) during the first 8 wk of lactation.¹

Item	Concentrates		<i>P</i> <
	SBM	BM	
Milk yield, kg/d	24.9 ± 0.60	29.3 ± 0.60	0.016
Fat, %	3.30 ± 0.14	3.26 ± 0.27	0.834
Protein, %	2.78 ± 0.13	2.85 ± 0.04	0.508
Lactose, %	4.63 ± 0.07	4.83 ± 0.05	0.089
Fat, kg/d	0.88 ± 0.05	0.90 ± 0.05	0.815
Protein, kg/d	0.74 ± 0.05	0.85 ± 0.03	0.040
BWG, kg/d	0.213 ± 0.567	0.039 ± 0.115	0.755
Intake, kg DM/d			
Concentrate	5.91 ± 0.04	5.68 ± 0.20	0.289
Pasture ²	13.7 ± 0.60	17.19 ± 1.83	0.056
Total CP, g/d	3035 ± 87	3657 ± 246	0.032
Total RUP ³ , g/d	1011 ± 33	1647 ± 96	0.001
RUP/CP, %	33.37 ± 0.18	45.25 ± 0.62	0.001

¹Values are expressed as means ± standard error. Significant treatment × wk of lactation interaction was not detected (*P* > 0.10).

²Based on eight cows per treatment group on wk 8 of lactation.

³Calculated from the effective degradability coefficients for each concentrate (Table 3) and the coefficient of 60.9 observed by Hongerholt and Muller (1998) for the effective pasture CP degradability (*kp* = 0.06/h).

No differences were detected in the mean values for glucose, urea, and NEFA, but triglyceride concentrations tended to be higher in SBM treatment. Jugular minus mammary differences in metabolites were not affected by treatments (*P* > 0.10). They were different from 0 (*P* > 0.01) for glucose (SBM = 16.5 mg/dl, BM = 16.9 mg/dl), NEFA (SBM = 96 μeq/L, BM = 123 μeq/L) and triglyceride (SBM = 33.4 mg/dl, BM = 52.2 mg/dl).

Percentages of plasma AA in jugular and mammary veins are presented in Table 7. Percentages of free Ile (3.39 vs. 4.48%, *P* < 0.05) and Lys (2.57 vs. 3.66%, *P* < 0.10) in jugular plasma were lower in cows fed the BM compared with SBM. The remaining plasma free AA did not show differences (*P* > 0.10) between treatments. Percentages of free Asn (0.6 vs. 0.8%, *P* < 0.10), Orn (1.19 vs. 1.76%, *P* < 0.10), Ile (2.60 vs. 3.42%, *P* < 0.10),

Table 6. Plasma metabolite concentration in grazing dairy cows that received concentrates containing soybean meal (SBM) or blood meal (BM) in early lactation.¹

Item	Concentrates		<i>P</i> ² <
	SBM	BM	
Glucose, mg/dl	62.8 ± 1.7	60.6 ± 1.7	0.464
Urea, mg/dl	25.1 ± 1.0	26.7 ± 1.5	0.547
Triglyceride, mg/dl	807 ± 36	649 ± 30	0.084
NEFA, μeq/L	674 ± 55	589 ± 44	0.497

¹Average values of samples obtained in wk 4, 5, and 6 of lactation. Values are expressed as means ± standard error.

²Significant treatment or treatment × wk of lactation effects were not detected (*P* > 0.10).

Table 7. Percentages of plasma free AA in jugular and mammary veins in grazing dairy cows (25 ± 6 d postpartum) that received concentrates containing soybean meal (SBM) or blood meal (BM).¹

	Jugular vein concentrate		<i>P</i> <	Mammary vein concentrate		<i>P</i> <
	SBM	BM		SBM	BM	
Asp	9.25 ± 1.13	10.42 ± 0.73	NS	7.97 ± 0.84	7.20 ± 1.04	NS
Asn	0.78 ± 0.07	0.77 ± 0.07	NS	0.80 ± 0.09	0.60 ± 0.08	0.10
Ser	2.91 ± 0.26	2.94 ± 0.37	NS	3.31 ± 0.33	2.93 ± 0.13	NS
Gln	5.85 ± 0.86	4.83 ± 0.54	NS	5.51 ± 0.34	4.98 ± 0.45	NS
His	3.95 ± 0.72	4.55 ± 1.18	NS	3.94 ± 0.85	4.59 ± 0.86	NS
Thr	6.87 ± 0.86	6.24 ± 1.00	NS	5.16 ± 0.06	5.27 ± 0.44	NS
Gly	14.79 ± 1.53	13.87 ± 0.70	NS	17.83 ± 0.73	16.52 ± 0.68	NS
Cit	8.22 ± 0.67	9.52 ± 1.00	NS	11.12 ± 0.62	13.45 ± 1.03	0.10
Arg	2.94 ± 0.32	3.03 ± 0.18	NS	2.59 ± 0.44	2.18 ± 0.19	NS
Tau	2.31 ± 0.23	2.25 ± 0.18	NS	2.62 ± 0.11	2.78 ± 0.23	NS
Orn	3.71 ± 0.60	3.78 ± 1.81	NS	1.76 ± 0.21	1.19 ± 0.17	0.10
Ala	10.80 ± 0.67	10.30 ± 0.92	NS	14.06 ± 1.31	12.76 ± 0.74	NS
Tyr	2.15 ± 0.18	2.10 ± 0.14	NS	1.85 ± 0.21	1.65 ± 0.09	NS
Val	9.94 ± 0.72	10.80 ± 0.58	NS	10.09 ± 0.82	12.58 ± 0.75	0.05
Met + Trp	0.76 ± 0.05	0.66 ± 0.08	NS	0.64 ± 0.12	0.49 ± 0.08	NS
Phe	1.77 ± 0.16	1.87 ± 0.39	NS	1.24 ± 0.17	1.39 ± 0.30	NS
Ile	4.48 ± 0.43	3.39 ± 0.20	0.05	3.42 ± 0.27	2.60 ± 0.35	0.10
Leu	6.34 ± 0.39	6.53 ± 0.32	NS	4.96 ± 0.42	5.74 ± 0.46	NS
Lys	3.66 ± 0.46	2.57 ± 0.33	0.10	2.18 ± 0.44	0.95 ± 0.08	0.05

¹Values are expressed in % of total amino acid (means ± standard error). NS = Nonsignificant difference.

and Lys (0.95 vs. 2.18%, *P* < 0.05) in mammary vein plasma were lower in cows offered the BM concentrate and those of Cit (13.45 vs. 11.12%, *P* < 0.10), and Val (12.58 vs. 10.09%, *P* < 0.05) were greater (Table 7).

Differences in plasma NEFA concentrations immediately before ISO challenge were not significant, averaging 750 and 629 μeq/L for SBM and BM, respectively. When the increase in plasma NEFA after the challenge was adjusted by subtracting the basal concentrations, the maximum increase resulted higher (*P* < 0.027) in BM (+347 μeq/L) than in SBM (+179 μeq/L).

Neither treatment nor interaction effects were detected for the decrease in plasma glucose or NEFA concentrations after a single intravenous INS injection (Table 8).

The hypoglycemic action of INS lasted up to 60 min after the hormone injection in SBM and up to 90 min in BM. The antilipolytic effect of INS lasted up to INS-30 samples in both treatments.

The calculated distribution volume for glucose (SBM = 46 ± 4 L, BM = 44 ± 3 L), the fractional rate of glucose clearance (SBM = -0.065 ln mg/dl per min ± 0.006, BM = -0.049 ln mg/dl per min ± 0.004), and the surfaces under the glucose curve (SBM = 2248 ± 47 mg/dl per min, BM = 2189 ± 33 mg/dl per min) did not show significant differences between treatments.

DISCUSSION

In our experiment, concentrates were formulated to be isoenergetic and isonitrogenous (Table 1); the effective

degradability of the CP was the main difference between them (Table 3). Coefficients for in vitro DM digestibility and IVOMD were lower with BM than with SBM concentrates. This could probably be explained by the lower CP degradability in BM (Table 3). Calculated parameters for the in situ disappearance of CP (Table 3) showed that the two concentrates differed in their effective degradability (SBM = 75% and BM = 44%). The high soluble fraction obtained in this experiment (Table 3) could be linked to an excessive grinding of the concentrates that introduced a bias.

Results obtained in this experiment showed that increasing dietary RUP from 33.4 to 45.3% in a 16% CP

Table 8. Plasma NEFA and glucose concentrations before (INS-0) and after insulin (INS) challenge in grazing dairy cows (38 ± 6 d postpartum) that received concentrates containing soybean meal (SBM) or blood meal (BM).¹

	Concentrates		<i>P</i> ² <
	SBM	BM	
NEFA, μeq/L			
INS-0	726 ± 105	618 ± 77	0.409
INS-30	514 ± 108	453 ± 76	0.652
INS-60	659 ± 73	559 ± 65	0.324
INS-90	717 ± 75	695 ± 72	0.839
Glucose, mg/dl			
INS-0	64.8 ± 3.2	64.3 ± 4.4	0.922
INS-30	28.4 ± 3.5	28.3 ± 2.5	0.990
INS-60	39.9 ± 3.6	40.3 ± 3.3	0.947
INS-90	61.8 ± 4.3	56.1 ± 4.3	0.368

¹Values are expressed as means ± standard error.

²Treatment × hour interaction was not detected (*P* > 0.10).

diet by BM feeding enhanced milk yield and milk protein output without changes in milk protein or fat contents (Figure 1; Table 5). The large difference detected in milk production suggested that an AA imbalance in the SBM treatment may have been occurring, but the AA profile that might have been delivered to the duodenum of cows on the two treatments is difficult to state. Amino acid composition in concentrates and pastures and microbial contribution to the AA flow to the gut (that could have been changed as corn intake was different between treatments) were not measured in the experiment. Because the proportions of free Asn, Orn, Ile, and Lys were lower in the mammary vein plasma of cows offered the BM concentrate (Table 7), whereas milk and milk protein yield were higher (Table 5), it is possible that these AA were the most limiting in our experiment. However, these results are only indicative and must be used with care because proportions of AA in venous blood may not be an accurate measure of limiting AA.

Positive responses on milk yield by supplementing protein sources resistant to ruminal degradation have been reported in early-lactation cows in grazing conditions (Hamilton et al., 1992; Roger et al., 1980; Schroeder and Gagliostro, 2000; Viglizzo et al., 1986). In low-yielding dairy cows (12 to 15 kg/d per cow), milk yield (+20%), milk fat yield (+13%), and milk protein (+27%) output were increased by formaldehyde-treated casein (1 kg/d), and the result was mainly attributed to a higher daily intake of forage (Stobbs et al., 1977). In another grazing study, formaldehyde-treated casein (0.25 kg/d) increased milk production by 5% over control cows without affecting pasture intake. The response was similar to that reported by Stobbs et al. (1977) after adjustment for the smaller quantity fed (Flores et al., 1979). In medium-yielding dairy cows (25 to 27 kg/d per cow) on spring pastures, fish meal feeding increased milk yield and plasma NEFA in early-lactation cows when measured at 59 to 80 DIM. The result suggested increased fat mobilization in response to RUP feeding, but pasture intake was not measured in this trial (Schroeder and Gagliostro, 2000). In high-yielding dairy cows (>35 kg of milk/d) on grass pasture, milk protein yield (but not milk yield) tended to be greater for cows fed RUP (animal protein blend) in early lactation (Hongherholt and Muller, 1998). No significant differences in total or pasture DMI or plasma concentrations of NEFA related to diets were detected (Hongherholt and Muller, 1998).

Two main mechanisms have been proposed to explain enhanced milk energy and milk protein output: increased DMI or increased body fat mobilization of supplemented cows (Newbold, 1994; Ørskov et al., 1987). The calculated energy deficit of early-lactation cows seemed to be increased when fish meal (80 g/kg of diet) was added to a basal diet composed of ryegrass silage (0.70

and concentrate (0.3) at 2 wk into lactation and also during wk 2 to 15 (Ørskov et al., 1987).

In the present experiment, the higher milk (+17.7%) and milk protein (+14.9%) yields observed in cows fed the BM concentrate (Table 5) could probably be explained by the higher response in forage DMI of cows because neither changes in BW gain of cows (Table 5) nor parameters associated to body lipid mobilization were affected by RUP feeding (Tables 6 and 7). Plasma NEFA concentrations resulted unchanged (Tables 6 and 7), but they were measured on wk 4 to 6 of lactation. It is possible that most dietary differences might have been detected earlier in lactation (i.e., less than 3 wk).

Why DMI increased in response to RUP feeding to dairy cows is not clear. In part, the supply of postruminal AA complements the microbial protein, improving the absorbed AA profile to the animal. Casein infusion to the duodenum of cows increased silage DMI, whereas infusion of soya protein fails to enhance DMI (Choung and Chamberlain, 1992).

In the early postpartum period, the physical capacity of the cow to consume DM is reduced. When DMI is under physical (bulk) rather than physiological control or when pasture availability is suboptimal, the intake response to RUP feeding would be low. In our experiment, the intake measurements were taken after the peak of lactation to better detect any putative effect of RUP on voluntary DMI of cows. In addition, the grazed forage was offered at a rate of about 5.5% (DM) BW with a high herbage allowance (4280 kg of DM/ha) and a relatively low pasture utilization coefficient was observed (50%) eliciting the ability of the cow to select high quality forages. The forage NDF content (Table 2) remained below the values proposed by Paterson et al. (1994) as critical for maximum intake and milk production (e.g., 500 to 550 g of NDF/kg of DM in grasses). It can be argued that forage was present in both, adequate quality and quantity that allowed the intake responses observed in the BM fed cows in their 8th wk of lactation. Average pasture CP content was below the range of 150 to 250 g of CP/kg of DM proposed by Minson (1990) to obtain high values of forage DM digestibility. Even so, the observed coefficients for pasture *in vitro* DM digestibility or IVOMD were high for both the cutting and the hand-plucking samples (Table 2). We calculated that concentrate intake increased CP content of total diets up to 15.9% in both treatments (Table 5). Forage protein represented about 60 and 64% of the dietary CP intake in SBM and BM treatments, respectively. The high protein degradability of fresh forage (Beever and Siddons, 1986; Hongherholt and Muller, 1998) may explain the elevated ruminal NH₃-N levels observed in both treatments in the fistulated late-lactation cows (Table 4). The replacement of a protein source of high ruminal degradability

(SBM) for another of low degradability (BM) did not cause an apparent shortage in $\text{NH}_3\text{-N}$ concentration because it was above the range proposed as critical (5 to 10 mg/dl) for appropriate ruminal microbial growth (Satter and Slyter, 1974). These results suggest a lack of benefit of feeding extra RDP in the concentrate when cows are grazing pastures containing at least 14% CP.

Ruminal $\text{NH}_3\text{-N}$ concentration is the main source of variation in plasma urea levels. Plasma urea levels did not decrease when BM replaced SBM in the concentrate (Table 6). A possible explanation could be the increase in forage (and hence RDP) intake in BM fed cows (Table 5). The pH of the ruminal fluid did not differ between the fistulated cows fed SBM or BM (Table 4), a result often obtained when protein sources of different ruminal degradability are compared (Erasmus et al., 1994; Robinson et al., 1991). In turn, the absence of differences in pH values between cows fed SBM or BM is consistent with the similar total VFA concentrations observed with both treatments (Table 4), because a close relationship exists between pH and VFA concentration.

It is not clear how absorbed protein may induce mobilization of body fat. Increased responsiveness of adipose tissue to lipolysis and a decreased response to antilipolytic stimuli (INS) has been proposed as possible mechanisms involved (Cadórniga and López Díaz, 1995). In our experiment, a greater NEFA response to β -adrenergic stimuli was observed in BM fed cows as reported by Cadórniga and López Díaz (1995), but changes in the antilipolytic or hypoglycemic effects of INS were not observed (Table 8). As changes in BW gain of cows (Table 5) or basal plasma NEFA concentrations (Table 6) were not detected, cows fed the BM concentrate appeared to be able to maintain an optimal protein:energy ratio at the metabolizable level by increasing DMI (and hence energy) after the peak of lactation without increasing body fat mobilization. The lack of an effect of increased RUP on lipid mobilization might have been due to a higher energy balance of cows. Data obtained during the period of herbage intake measurements allowed us to calculate that energy intake was higher in the BM (63 Mcal of ME/d per cow) compared with the SBM (52 Mcal of ME/d per cow) treatment ($P < 0.01$). Under adequate feeding conditions, lipid mobilization was not affected by changes in dietary RUP supply (Komaragiri and Erdman, 1998).

The BM concentrate failed to increase milk protein content (Table 5), and the lack of response could be linked to a dilution of the milk protein mass induced by the higher milk volume produced.

The lack of effect of BM on milk fat content (Table 5) was also observed in several experiments in which RUP was fed to lactating cows (Erasmus et al., 1994; Ørskov and McDonald, 1979; Rogers et al., 1980; Schroeder and

Gagliostro, 2000; Viglizzo et al., 1986; Voss et al., 1988). Variations in milk fat concentrations after AA delivery into the small intestine of cows are small, with an average response of +0.1 g/kg of milk (Rulquin and Verité, 1993). Variations in milk fat content are better explained by changes in the ruminal VFA proportions of cows. In this sense, no changes were observed in the ruminal acetate, propionate, butyrate, and branched-chain fatty acids concentrations measured in the fistulated cows (Table 4). Positive responses in milk fat concentration to duodenal casein infusions were observed only in early-lactation dairy cows in severe undernutrition (Ørskov et al., 1977).

In lactating dairy cows, the mammary gland may extract up to 85% of the total glucose available from all sources and glucose entry rate, and energy intake are closely related (Bickerstaffe et al., 1974). As J-M differences in glucose concentration were not lower in the BM treatment and blood flow did probably increase (owing to the higher milk yield) a higher mammary uptake of glucose may have been occurring to sustain lactose synthesis. Inflow of glucose into the glucose pool may occur from both gluconeogenesis and glycogenolysis. The indirect measures of glucose metabolism performed in this work showed no changes in the glucose disappearance rate, the calculated distribution volume, and surfaces under the glucose curve between cows fed the BM and the SBM supplements. Changes in the rate of glucose disposal after exogenous glucose administration would have reflected alterations in peripheral glucose utilization. The similar calculated distribution volume may have also been reflecting a paired ability of the injected glucose to reach and penetrate into the intracellular compartments. The extent in the plasma glucose concentration induced by ISO injection tended to be higher in the SBM treatment (+13.1% increase over basal concentration) compared with BM fed cows (+7.5%) (results not shown), suggesting that an enhanced hepatic glycogenolysis was not occurring in the BM supplemented cows. Alterations in glucose metabolism in cows fed the BM concentrate were not detected despite the higher milk yield and energy intake of cows.

In conclusion, supplementing early-lactation dairy cows with RUP in grazing conditions improved milk and milk protein yield. The responsiveness of the adipose tissue to lipolytic stimuli were increased by RUP feeding, but enhanced BW loss of cows or higher plasma NEFA concentrations were not observed. In this experiment, the highest milk yield was better explained by increased DMI rather than by enhanced body lipid mobilization.

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