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Solvent-extracted soybean meal top-dressed on a fresh cow diet increased milk production, but not milk components, and decreased plasma non-esterified fatty acids

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Abstract: Post-ruminally infused **c**asein has increased milk and milk protein yield in post-partum cows. We theorised top dressing (TD) higher amounts of soybean meal (SBM) might mimic these effects. Fifty-one multiparous Holstein cows 1 day after calving were assigned to 3 dietary treatments: a base total mixed ration (CON) with 196 g/kg crude protein and 329 g/kg neutral detergent fibre; 17 cows TD with l kg of SBM (SBM1); and 17 cows TD with 2 kg of SBM (SBM2) for 30 days. Milk and milk components were measured at days 9, 18, and 27. Rumen and urine samples were collected on day 27; blood samples were obtained on day 30. Statistical inference was by JMP software (Version 10.0.2, 2012) with production variables analysed as a repeated measures design. Cows fed SBM increased milk yield (P = 0.02; 35.4, 36.6, and 42.6 kg/day for CON, SBM1, and SBM2, respectively). Yield of milk true protein was not different among treatments. Cows fed SBM had lower serum non esterified fatty acids concentrations at day 30 (1.35, 1.13, and 0.59 mM/l; $P < 0.01$). We conclude that SBM TD beginning immediately after calving may increase milk yield rapidly and decrease dependence on fatty acids for energy.

Keywords: post-partum cows; NEFA; lactose yield; milk yield

The dairy cow in early lactation undergoes negative balances for amino acids (AA) and glucose due to depressed feed intake and increased nutrient demand for milk and milk protein production, resulting in body protein mobilisation in order to supply AA for milk protein production as well as for gluconeogenesis (Grummer 1995). It has been estimated (Bell 1995) that in the first days of lactation, approximately 500 g of glucose must be derived via gluconeogenesis from mobilisation of body proteins. Feeding increased amounts of protein has long been considered to increase milk production in very early lactation (Orskov et al. 1977), apparently by providing more AA for gluconeogenesis or through providing the AA necessary for lipoprotein synthesis, which may result in improved body condition mobilisation (Whitelaw et al. 1986). Since that time, numerous experiments have manipulated the amounts and types of proteins fed to early lactation cows with varying levels of success. Much of the variation in response may be due to differences in the days after parturition when the experiment began, differences in protein amounts, and differences in the balance of AA supplied by varying rumen undegradable protein. In addition complications

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in assessing responses to increasing protein arise due to different energy and protein levels in the basal diet. Thus, the true effect of feeding protein to early lactation cows is equivocal, and the practice has not been widely recommended.

Recent experiments (Larsen et al. 2014, 2015) reported large increases in milk production and milk protein were obtained when abomasal casein infusions were initiated immediately after calving and continued for 29 days. The same experimenters (Larsen et al. 2009) found negative milk protein responses to abomasally infused glucose during the same time after parturition. This suggests that supplementing protein immediately after calving might have a greater effect on early lactation production than increasing energy density of the diet. Previous studies with increased protein immediately after calving found positive results with soybean meal (SBM) supplementation (Cressman et al. 1980), casein infusion (Orskov et al. 1977; Whitelaw et al. 1986), supplementation with a mixture of cottonseed meal, corn gluten meal, and fish meal (Amanlou et al. 2017), but low or negative responses with corn gluten meal additions to the basal ration (Keery and Amos 1993). Taken together, these observations suggested that the essential AA pattern might be important in provoking a positive milk response.

Our theory was that a supplemental protein with an AA pattern much like casein and given at lactation initiation would result in increased production of milk and milk protein and would reduce mobilisation of body reserves resulting in lower plasma non-esterified fatty acids (NEFA) and blood ketones. Therefore, the primary objective of this experiment was to determine if supplementation of typical Iranian fresh cow diets with solvent extracted soybean meal (SBM) would increase milk and milk protein yield over the first 30 days of lactation. A secondary objective was to determine if protein addition reduced plasma NEFA and ketones within the same time frame.

MATERIAL AND METHODS

Selection of the test protein. The selection of a single test protein for this experiment was made difficult by lack of a wide variety of proteins with suitable characteristics which were widely available in Iran. All available protein sources were considered, including blood meal, canola meal, corn gluten meal, fish meal, poultry byproduct meal, and SBM. Determinations for similarity of AA distribution were conducted using AA values for casein and soybean meal from Bohlke et al. (2005). In order to assess AA pattern similarities, each AA was referenced to lysine (LYS) by dividing the percentage of each AA by the percentage of Lys within a protein. Differences between the AA distribution of each protein relative to casein were determined by subtracting the percentage AA in the test protein from the percentage AA distribution in casein. The differences were squared, summed, and a root mean square error (RMSE) determined. Desirability of the AA pattern of a protein was determined as the lowest RMSE for the Lys ratio. The AA amounts, AA distribution relative to Lys, and the RMSE for each protein are presented in Table 1.

Cows and treatments. All procedures were conducted under protocols approved by the Iranian Council on Animal Care (1995). To ensure that only healthy animals entered the experiment, after parturition cows were evaluated for lack of apparent metabolic disease, having good appetite, and for having released the placental membranes within 24 h after calving. After evaluation, the remaining 51 multiparous Holstein fresh cows (710 ± 65 kg body weight (BW), were assigned to one of three treatments: (1) basal diet which served as the control (CON), (2) basal diet plus 1 kg of top-dressed solvent soybean meal (SBM), (treatment SBM 1), and (3) basal diet plus 2 kg of top-dressed SBM (treatment SBM 2). Cows were assigned to treatment as they calved in the order as determined by lot with the first cow that calved assigned to diet SBM2, the second cow was assigned to the CON diet, and the third cow was assigned to the diet SBM1. This order was continued until all cows were assigned to treatment. Cows were fed the basal diets in the form of a total mixed ration (TMR) from parturition to 30 days postpartum at *ad libitum* intake twice daily at 7:00 and 15:00 h after orts had been collected at approximately 6:00 h. Amounts of feed offered were adjusted daily to allow refusals equal to 5–10% of intake. The SBM was top-dressed on the basal TMR in equal portions at the 2 feedings. Cows were housed in individual stalls throughout the experiment and were allowed to exercise daily in an outside lot for 3 h (12:00–15:00 h). Cows were

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Table 1. A comparison to casein for various proteins in terms of % AA in crude protein and in ratio of AA to Lys

Amino acid	Casein		Blood meal		Canola meal		Corn gluten meal		Fish meal		Poultry meal		Solvent soybean meal	
	g/kg CP	Lys ratio	g/kg CP	Lys ratio	g/kg CP	Lys ratio	g/kg CP	Lys ratio	g/kg CP	Lys ratio	g/kg CP	Lys ratio	g/kg CP	Lys ratio
Ala	28.5	0.37	79.5	0.99	45.2	0.80	82.4	5.28	66.6	0.77	68.0	1.07	44.6	0.68
Arg	35.7	0.46	39.2	0.49	62.1	1.10	28.4	1.82	79.1	0.92	71.2	1.12	73.4	1.11
Asp	66.3	0.86	103.6	1.30	70.6	1.25	59.7	3.83	94.3	1.10	87.0	1.37	115.2	1.75
Cys	3.5	0.05	14.1	0.18	31.0	0.55	19.9	1.28	11.1	0.13	15.8	0.25	16.1	0.24
Glu	202.2	2.63	94.6	1.18	189.1	3.35	207.5	13.30	138.7	1.61	144.0	2.27	179.5	2.72
Gly	17.4	0.23	41.5	0.52	53.6	0.95	22.7	1.46	69.3	0.81	93.4	1.47	42.5	0.64
His	29.1	0.38	56.3	0.70	28.2	0.50	15.6	1.00	31.9	0.37	26.9	0.42	27.1	0.41
Ile	50.9	0.66	9.1	0.11	48.0	0.85	42.6	2.73	48.5	0.56	45.9	0.72	45.4	0.69
Leu	91.6	1.19	120.7	1.51	76.2	1.35	160.6	10.29	77.7	0.90	74.4	1.17	80.9	1.23
Lys	77.5	1.00	80.5	1.00	56.5	1.00	15.6	1.00	86.0	1.00	63.3	1.00	66.5	1.00
Met	28.0	0.36	15.1	0.19	25.4	0.45	22.7	1.46	30.5	0.35	23.7	0.37	15.1	0.23
Phe	50.9	0.66	66.4	0.83	42.3	0.75	58.3	3.73	43.0	0.50	41.1	0.65	53.5	0.81
Pro	101.0	1.31	37.2	0.47	67.7	1.20	82.4	5.28	47.2	0.55	63.3	1.00	49.9	0.76
Ser	44.5	0.58	61.4	0.77	50.8	0.90	51.2	3.28	47.2	0.55	49.1	0.77	47.3	0.72
Thr	38.7	0.50	54.3	0.68	48.0	0.85	31.3	2.00	40.9	0.58	44.3	0.70	41.0	0.62
Trp	11.8	0.15	14.1	0.18	14.1	0.25	5.7	0.36	2.8	0.03	3.5	0.05	14.9	0.23
Tyr	56.4	0.73	30.2	0.38	31.0	0.55	48.3	3.10	33.3	0.39	33.2	0.52	40.1	0.61
Val	66.0	0.86	82.5	1.03	59.3	1.05	44.1	2.82	52.7	0.61	52.2	0.82	47.0	0.71
Total EAA[1]	480.2		538.2		460.1		424.9		493.1		446.5		464.8	
Total NEAA[2]	519.8		461.8		539.9		575.1		506.9		553.5		535.2	
RMSE[3]				0.49		0.39		3.98		0.39		0.42		0.33

[1]total essential amino acids as % of all amino acids

[2]total non-essential amino acids as % of all amino acids

[3]root mean square error of difference from casein, calculated as % of CP and Lys ratio

moved to individual stalls 2 days after parturition and immediately entered the trial with test diets being fed from day 2. Dry matter (DM) intake of each cow was measured daily. Also, in order to reduce the variation in dietary crude protein (CP) content, one batch of SBM was purchased before the trial began and was used throughout the trial.

Measurement and sampling. Samples of feed ingredients and TMR were taken weekly and analysed for DM content, CP (AOAC 2000; method 984.13), ether extract (EE) (AOAC 2000; method 920.39), ash (AOAC 2000; method 942.05), neutral detergent fibre (NDF), and acid detergent fibre (ADF) (Van Soest et al. 1991). NDF and ADF are expressed exclusive of residual ash. Alpha amylase and sodium sulfite were not used in the NDF assay. Rumen undegradable protein in top-dressed SBM was estimated by the *in situ* method as described by NRC (2001). The rumen undegradable protein (RUP) of the TMR was estimated using the NRC 2001 model (calculated by difference: RUP = CP – rumen degradable protein (RDP) based on kg/day or g/kg). Also the calculation of the non-fibrous carbohydrates (NFC) as 100 – (NDF + CP + EE + ash) was as recommended by NRC (2001). The BW and body condition score (BCS) were measured (1–5 system; Wildman et al. 1982) after the morning milking and before the morning feeding on two consecutive days on the first 2 and last 2 days of the experiment. The scores were averaged for each cow at each time of scoring. Cows were milked six times daily (3:00, 7:00, 11:00, 15:00,

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19:00, 23:00 h) and milk weights were recorded daily. Milk was sampled weekly from 6 consecutive milkings on days 9, 18, and 27 of lactation and composited in proportion to milk yield at each milking. Composite samples were analysed for fat, protein, lactose, urea N, and somatic cell count (SCC) using mid-infrared procedures (AOAC 2002) on an automated analyser (CombiFoss 5000; Foss Electric, Denmark) by Dairy Lab Services Inc. (Khorasan Razavi Jahad Keshavarzi Laboratory).

Blood was sampled into heparinized test tubes from the coccygeal vessels of each cow at 4 h after feeding on day 30 postpartum. The glucose concentration of the samples was measured immediately by a kit (Glucotrend; Roche, UK). The remaining heparinised blood was held on ice until returning to the laboratory where it was centrifuged at 3000 *g* for 15 min at 5°C. The plasma was then harvested and frozen at –20°C until analysis. After thawing, the plasma was analysed for concentrations of NEFA (Cat. No. FA 115, Randox Laboratories Ltd., UK), insulin (Cat. No. 2425-300A, Monobind Inc., USA) and beta-hydroxybutyric acid (BHBA) (kit number 310-A; Sigma, USA). For determination of total blood protein (Biuret method), and total blood cholesterol (CHOD-PAP enzymatic method), Pars Azmun kits (Pars Azmun Laboratory, Iran) were used. Absorbance was read using a spectrophotometer (PerkinElmer, USA). Plasma samples were also analysed for enzymes indicative of liver function at 30 days in milk (DIM) using an autoanalyser. These enzymes included alkaline phosphatase (AP), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT). Spot urine samples were obtained from all cows by mechanical stimulation of the vulva at about 4 h after feeding on day 27, and 15 ml of urine was pipetted into specimen containers holding 60 ml of 0.072 *N* H_2SO_4 and stored at –20°C until analysis. The pH was measured immediately after sampling by a pH meter (pH 340i/set, 2A30-1112; WTW Wissenschaftlich, Germany) in a separate 10 ml aliquot of urine. After thawing at room temperature, urine samples were analysed for creatinine using a picric acid assay adapted to flow-injection analysis (QuikChem 8000 FIA; Lachat Instruments, USA), and for urea with the colorimetric method used for milk urea nitrogen (MUN). On day 27 postpartum and 4 h after morning feeding, samples of rumen fluid were collected by stomach tube. The first 500 ml aspirated were discarded because of potential contamination with saliva. A portion was strained through cheesecloth, and 20 ml of it was acidified with 0.4 ml of 50% (vol/vol) sulfuric acid/water and stored at –20°C for later analysis of volatile fatty acids (VFA) and NH_3-N.

Calculations and statistical analysis. Diets, including ingredients, ingredient composition and measured DMI were entered into the NRC (2001) model to assess nutritional adequacy of diets, including energy and NDF consumption as well as to predict delivery of metabolisable protein (MP) and essential amino acids (EAA). Urine volume was determined from urinary creatinine using the method of Valadares et al. (1999) but adjusted for the increased loss of body protein in early lactation (Bell et al. 2000), and total elimination of uric acid and urea-N were calculated as concentration/l × urea volume/l. The N in milk was calculated as the g of milk true protein produced divided by 6.38, and fecal N was estimated as MP × 0.2 (Patton et al. 2009). Experimental data were analysed using JMP (Version 10.0.2, 2012) (SAS Institute Inc.) using a 3-day mean of daily milk production and DMI determinations centered on days 9, 18, and 27 of lactation coinciding with milk component determination. The following model was used in a repeated measure design for DMI, milk yield, milk components and DMI (SAS Institute Inc, 2012):

$$Y = SBM + DIM + (SBM \times DIM) + Cow\ (SBM) + error$$

where:

Y = observed response

SBM = fixed effects of SBM top-dress as 0, 1 or 2 kg

DIM = fixed effect of advancing lactation at day 9, 18, and 27

(SBM × DIM) = fixed effect of the interaction between SBM addition and DIM

Cow (SBM) = random effect of cow nested within SBM treatment

For variables which were measured once or were calculated from variables measured once, the model used was:

$$Y = SBM$$

where:

Y = observed value

SBM = fixed effect of SBM top-dress

Separation of treatment means was determined by pre-planned orthogonal contrast with the initial contrast of control (CON) (0 kg of top-dress SBM)

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compared to cows fed SBM (treatments SBM1 plus SBM2), and the remaining contrast tested SBM1 vs SBM2. Similarly, the significance of DIM was also determined by orthogonal contrasts as day 9 vs days 18 + 27, and day 18 vs day 27. Significance was declared when $P < 0.05$, and trends are discussed when $P < 0.10$.

RESULTS

Test protein and predicted energy, protein, and AA consumption. Soybean meal was found to have an AA pattern most closely resembling casein, based on the RMSE of the Lys ratio of 0.33; apparently methionine (Met) is the only EAA that is greatly deficient (Table 1) based on AA pattern. The ingredient and nutrient composition of the basal diet used in our study is presented in Table 2. The ingredients in the basal diet are typical of those commonly fed to milking cows in Iran. Although the actual CP content of the control diet was higher than anticipated (196 g/kg), fresh cows in Iran are normally fed a higher CP content in the diet (~180 g/kg) than cows in established lactation. The predicted net energy of lactation (NE_L) content for diets was similar, with CP, RDP, and RUP all increased in SBM1 and SBM2 due to the overall higher CP intake (Table 2). At observed levels of production, the NRC model predicted higher demands for energy and MP for SBM1 and SMB2 compared to CON, although the supplies of energy and protein were increased in SBM1 and SBM2 such that in general the predicted deficiencies of both were decreased relative to CON as SBM intake increased (Table 3). For SBM1, the NRC model predicted MP supplied to increase by 14.7% (299 g/day) relative to CON, with microbial protein increasing 13.5% and RUP increasing 22.3% compared to CON. Total MP supplied was estimated at 2031 g/day for CON and 2330 g/day for SBM1 (Table 3). Likewise, comparing CON vs SBM1 on a g/day basis, all EAA were predicted to increase at an average of 13.5% with the least increase of 12% for threonine (Thr) and the greatest increase of 14.7% for arginine (Arg). When CON and SBM1 comparisons were expressed as EAA g/kg MP, the EAA g/kg MP declined –1.13% on average with the largest decline for Met (–2.6%, from 1.89% to 1.84% MP), whereas both histidine (His) and phenylalanine (Phe) % MP remained constant.

Total MP increased by 20.9% (424 g/day) for SBM2 compared to CON with microbial protein increasing 17.9% and RUP increasing 32.1%. Total MP for SBM2 was calculated to be 2455 g/day, which indicates MP was estimated to have increased 299 g/day for the first kg of SBM, but only 125 g for the second kg of SBM addition. The EAA delivered to the small intestine in g/day increased by 31% for SBM2 compared to CON with leucine (Leu) and Phe increasing the most (33.3%) and Lys increasing by the smallest amount (27.9%). Conversely EAA g/kg MP decreased by –2.7% with His not decreasing at all, and Met decreasing by –5.3% (Table 3).

DMI and productivity. In this experiment, DMI was increased by SBM treatment ($P < 0.01$) but not by DIM (Table 4) and cows fed SBM2 tended to eat more than cows fed SBM1 ($P = 0.10$). As there were no significant SBM × DIM interactions for any production variables, interactions are not reported in Table 4. Milk production was greater for cows fed supplemental SBM compared

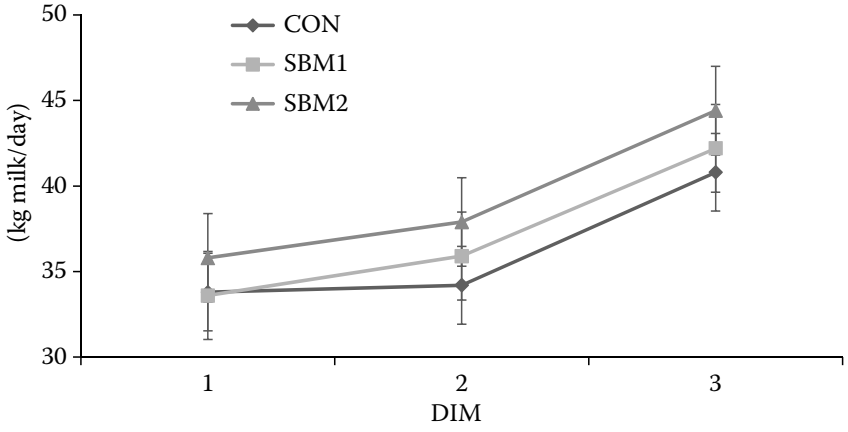

Figure 1. Milk production at 9, 18, and 27 days in milk (DIM) for cows top-dressed with solvent soybean meal

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Table 2. Ingredient composition of experimental total mixed ration and nutrient composition of solvent soybean meal, control diet total mixed ration, and treatment diets

Ingredients (g/kg DM)	TMR
Alfalfa hay	388
Barley grain	63
Triticale grain	63
Corn grain	130
Soybean meal (44% CP)	187
Canola meal	31
Whole cottonseed	31
Fish meal	18
Poultry byproduct meal	44
Bergafat[1]	18.1
Sodium bicarbonate	12.6
Calcium carbonate	7.7
Magnesium oxide	1.9
Salt	2.5
Trace mineral mix[2]	0.6
Vitamin mix[3]	0.6

Dietary chemical composition	Dietary treatments			
	SSBM[4]	Con-TMR[5]	SMB1	SBM2
NE_L (kJ/kg)	8.37	7.24	7.24	7.28
CP (g/kg DM)	520	196	212	228
RDP (g/kg DM)	302	139	148	157
RUP (g/kg DM)	218	57	64	71
ADF (g/kg DM)	76	239	200	194
NDF (g/kg DM)	127	329	320	310
NFC (g/kg DM)	251	340	336	332
Ash (g/kg DM)	80.7	80.6	80.6	80.7

TMR = total mixed ration, DM = dry matter, CP = crude protein, NE_L = net energy of lactation, RDP = rumen degradable protein calculated according to NRC 2001, RUP = rumen undegradable protein calculated according to NRC 2001, ADF = acid detergent fibre, NDF = neutral detergent fibre, NFC = non-fibrous carbohydrates, CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR + 2 kg of top-dressed soybean meal [1]fractionated palm fatty acids (Berg and Schmidt, Hamburg, Germany); [2]mineral mix contained 17.5% Ca, 7.5% Mg, 28.2 mg/kg Co, 2520 mg/kg Cu, 151 mg/kg I, 13 000 mg/kg Fe, 10 000 mg/kg Mn, 75 mg/kg Se, and 10 000 mg/kg Zn; [3]vitamin mix contained 1 500 000 IU/kg vitamin A, 400 000 IU/kg vitamin D, and 6000 IU/kg vitamin E; [4]soybean meal top-dress, solvent extracted; [5]control ration total mixed ration

to CON (P = 0.02) with SBM2 fed cows producing more milk than SBM1 cows (P < 0.01). However, as was the case for DMI, milk production did not significantly increase due to increasing DIM for the 30 days of this experiment (P = 0.17). This was the result of CON actually decreasing milk yield between days 9 and 18, although SBM1 and SBM2 fed cows increased production over the time course of this experiment (Figure 1). For this experiment milk fat (MF) g/kg was greater for CON cows compared to those fed SBM (P = 0.05) and was greater for SMB1 than SMB2 (P < 0.01) whereas there was a trend for MF g/kg to be greater at day 9 of lactation (P = 0.07) than for later DIM, but no difference between day 18 and day 27. Milk true protein (MTP) g/kg was unaffected by SBM treatment, but was greater for SBM1 cows compared to SBM2 cows (P = 0.04).

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Table 3. NRC model predicted energy and metabolisable protein requirements and supply as well as microbial CP, and essential amino acid flow for experimental diets at consumption

Item	Dietary treatments				
	Control	SBM1	SBM2	% increase[1] SBM1	% increase SBM2
Energy required (Mcal/day)	37.6	38.9	39.2	3.5	4.3
Energy supplied (Mcal/day)	31.9	34.5	37.2	8.1	16.8
Difference (Mcal/day)	–5.7	–4.4	–2.0		
MP required (g/day)	2410	2536	2745	5.2	14.3
MP supplied (g/day)	2031	2330	2455	14.7	20.9
Difference (g/day)	–379	–206	–290		
Microbial CP (g/day)	1630	1752	1921	7.5	17.9
RUP (g/day)	1049	1283	1362	22.3	32.1
Arg (g/day)	104	119	142	14.4	32.7
Arg (g/day MP)	51.4	51.1	51.1	–0.60	–1.20
His (g/day)	46	52	63	13.0	32.6
His (g/day MP)	22.4	22.4	22.5	0.0	0.0
Ile (g/day)	99	113	137	14.1	31.3
Ile (g/day MP)	48.9	48.4	48.2	–1.0	–2.0
Leu (g/day)	171	196	239	14.6	33.3
Leu (g/day MP)	84.2	84.1	84.3	–0.1	–0.2
Lys (g/day)	140	157	189	12.1	27.9
Lys (g/day MP)	69.0	67.4	66.3	–2.3	–4.5
Met (g/day)	38	43	5.1	13.2	28.9
Met (g/day MP)	18.9	18.4	18.0	–2.6	–5.3
Phe (g/day)	102	117	142	14.7	33.3
Phe (g/day MP)	50.2	50.2	50.3	0.0	0.0
Thr (g/day)	100	112	136	12.0	29.0
Thr (g/day MP)	49.2	48.2	47.6	–2.0	–3.9
Val (g/day)	111	126	153	13.5	30.6
Val (g/day MP)	54.8	54.0	53.5	–1.5	–2.7

MP = metabolisable protein, CP = crude protein, RUP = rumen undegradable protein, CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR + 2 kg of top-dressed soybean meal
[1]% increase of treatment vs control

Similar to the situation for MF, the MTP g/kg was greater at day 9 compared to later DIM ($P < 0.01$), but was not different between day 18 and day 27. Milk lactose g/kg was not different between CON and SBM supplemented cows, although there was a trend for SMB1 cows to have a higher milk lactose g/kg than SBM2 cows ($P = 0.07$), and day 9 lactose g/kg was lower than later DIM ($P = 0.02$). Total milk solids (TS) g/kg were greater for CON compared to SBM supplemented cows ($P = 0.02$), but solids not fat (SNF) g/kg were not different. Both total milk solids (TS) and SNF g/kg were greater for SBM1 fed cows compared to those fed SBM2 ($P \leq 0.01$ and $P = 0.02$ for TS and SNF g/kg, respectively). Both TS and SNF g/kg were significantly greater at 9 DIM ($P < 0.01$ for both) with no difference between 18 and 27 DIM. Milk urea was lower in cows fed CON compared with those top dressed with SBM ($P = 0.05$), with no differences between levels of SBM. In this case, milk urea was less in cows at day 9 compared to later DIM, but there was no difference between 18 and 27 DIM.

Total production of MF, MTP, and TS was not different between CON and SBM fed cows, nor were there differences between SBM1 and SBM2, but milk lactose production was greater in cows fed

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Table 4. Production variables for post-partum cows top-dressed with soybean meal

Variable	Dietary treatment[1]			SE	Treatment contrasts		Sample DIM[2]			SE	DIM contrasts	
	CON	SBM1	SBM2		CON vs SBM	SBM1 vs SBM2	9	18	27		9 vs (18 + 27)	18 vs 27
DMI (kg/day)	18.6	20.0	22.2	1.0	< 0.01	0.10	19.5	20.7	20.5	0.7	0.16	0.87
Milk (kg/day)	35.4	36.6	42.6	1.4	0.02	< 0.01	36.6	38.1	39.8	1.4	0.17	0.37
Milk fat (g/kg milk)	43.9	43.7	37.9	1.3	0.05	< 0.01	43.8	42.3	39.5	1.3	0.07	0.11
Milk TP (g/kg milk)	33.8	32.9	28.2	1.7	1.2	0.4	38.7	29.0	27.2	1.7	< 0.01	0.45
Milk lactose (g/kg milk)	43.1	44.2	42.2	0.7	8.7	0.7	41.9	43.7	43.9	0.6	0.02	0.82
Total solids (g/kg milk)	127.9	127.0	115.4	2.3	0.2	< 0.1	130.9	121.7	117.6	2.3	< 0.01	0.21
Solids not fat (g/kg milk)	83.8	84.2	77.5	2.0	2.1	0.2	87.4	79.9	78.2	1.9	< 0.01	0.51
Urea (mM)	2.1	2.6	2.7	0.2	0.05	0.72	1.9	2.6	2.8	0.3	< 0.01	0.46
Milk fat (kg/day)	1.55	1.54	1.62	0.07	0.99	0.97	1.57	1.58	1.56	0.07	0.98	0.91
Milk TP (kg/day)	1.10	1.13	1.11	0.06	0.79	0.78	1.30	1.03	1.00	0.06	< 0.01	0.71
Milk lactose (kg/day)	1.52	1.62	1.80	0.07	0.02	0.07	1.53	1.66	1.75	0.06	0.02	0.32
Total solids (kg/day)	4.50	4.67	4.90	0.17	0.17	0.35	4.76	4.64	4.68	0.17	0.59	0.92
Solids not fat (kg/day)	2.94	3.09	3.26	0.13	0.09	0.25	3.12	3.04	3.10	0.11	0.45	0.67

DIM = days in milk, DMI = dry matter intake, TP = true protein

[1]CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR+ 2 kg of top-dressed soybean meal; [2]centered DIM when milk production and milk components were measured

SBM (P = 0.02) and there was a tendency for SBM2 fed cows to produce more lactose than in cows fed SBM1 (P = 0.07). The DIM did not affect the production of MF, but the production of MTP was the greatest at day 9 (P < 0.01) compared to 18 and 27 DIM, but MTP yield was not different between days 18 and 27. As a result of greater lactose production, SNF production, but not production of TS, tended to be greater for SBM supplemented compared to CON cows (P = 0.09), but with no difference between SBM2 and SBM1. Production of TS and SNF was not affected by increasing DIM, although milk lactose production was the lowest at day 9 (P = 0.02), but was not different between days 18 and 27.

Body weight changes. The initial BW of cows assigned to CON were found to be heavier than of those assigned to SBM1 + SBM2 (P = 0.04; Table 5), with a trend for cows on SBM1 treatment to be heavier than for those on SBM2 (P = 0.08). Mean BW followed a similar pattern, in CON being greater than in cows supplemented with SBM (P = 0.04) and with a trend for the mean BW of SBM1 cows to be heavier than in those fed SBM2 (P = 0.09). Loss of BW was not different between cows top-dressed with SBM and those that were not, but cows fed SBM2 lost less body condition than those fed SBM1. However, initial BCS only tended to be greater for the CON compared to SBM supplemented cows (P = 0.09) and was not different between the SBM treatments. Conversely mean BCS was not different between SBM supplemented and CON over the 30 days of study, although SBM1 cows had greater BCS than

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Table 5. Body weight (BW) variables for post-partum cows top-dressed with soybean meal

Variable	Treatment			SE	Contrasts (*P*-value)	
	CON	SBM1	SBM2		diet Contrast 1[1]	diet Contrast 2[2]
Initial BW (kg)	760	706	683	26	0.04	0.08
Mean BW (kg)	678	647	627	25	0.04	0.09
BW loss (kg)	63	66	50	10	0.54	0.02
Initial BCS	4.13	3.80	3.64	0.30	0.09	0.45
Mean BCS	3.57	3.42	3.34	0.26	0.87	0.03
BCS loss	0.54	0.38	0.32	0.10	0.02	0.94

BCS = body condition score, CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR + 2 kg of top-dressed soybean meal

[1]Contrast 1 = CON vs SBM1 + SBM2; [2]Contrast 2 = SBM1 vs SBM2

SBM2 cows during this time ($P = 0.03$). Additionally, CON fed cows lost more BCS compared to the SBM supplemented cows ($P = 0.02$) with no BCS loss difference between SBM1 and SBM2.

Other metabolites. There was a trend for NH_3-N concentration to be greater in the rumen of cows fed SBM compared to CON ($P = 0.07$; Table 6), and a trend for SBM2 fed cows to have greater NH_3-N than SBM1 fed cows ($P = 0.08$), as might be expected in a situation where greater amounts of RDP were fed. In addition, rumen butyric acid was significantly greater in cows fed SBM ($P < 0.01$), although there was no difference between SBM1 and SBM2 fed cows. *Iso*-valerate concentration was higher in the rumens of cows fed SBM2 than of those fed SBM1 ($P = 0.02$), but there was no difference between CON and SBM supplemented cows. Other VFA, including acetate, propionate, and valerate as well as total VFA, were not different among treatment groups.

The only significant change in the blood metabolites measured between CON and SBM supplemented cows (Table 7) was for NEFA which were lower in SBM treated diets than in CON ($P = 0.01$), and cows fed SBM2 had lower serum NEFA than those fed SBM1 ($P = 0.02$). Notably, plasma glucose and insulin were unaffected by treatment. However, BHBA was lower in the blood of cows fed SBM2 than in the blood of those fed SBM1 ($P = 0.04$), whereas there was no difference between CON fed cows and those top-dressed with SBM (Table 7). Also cows fed SBM tended to have lower plasma albumen ($P = 0.08$) and lower alkaline phosphatase (AP) ($P = 0.07$) than CON, but there were no differences between cows fed SBM1 and SBM2 diets. The AST in the blood of SBM1 fed cows was greater than of those fed SBM2 ($P = 0.02$), and total protein tended to be greater in SBM1 fed cows than in SBM2 fed cows ($P = 0.06$).

As planned, total N consumed (Table 8) was greater for cows top-dressed with SBM compared to CON

Table 6. Rumen metabolite variables for post-partum cows top-dressed with soybean meal

Variable	Dietary treatment			SE	Effect of diet (*P*-value)	
	CON	SBM1	SBM2		Contrast 1[1]	Contrast 2[2]
NH_3-N (mg/l)	1.97	2.00	2.66	0.25	0.07	0.08
Acetate (mM)	54.0	57.8	59.4	5.3	0.52	0.59
Propionate (mM)	21.3	20.5	22.0	2.5	0.98	0.79
Butyrate (mM)	4.8	5.8	5.8	0.9	< 0.01	0.80
Valerate (mM)	1.5	1.5	1.7	0.1	0.71	0.64
iso-Valerate (mM)	1.3	1.2	1.5	0.1	0.60	0.02
Total VFA (mM)	87.3	81.2	93.8	7.5	0.55	0.98

[1]CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR + 2 kg of top-dressed soybean meal, VFA = volatile fatty acids

[1]Contrast 1 = CON vs SBM1 + SBM2; [2]Contrast 2 = SBM1 vs SBM2

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Table 7. Blood metabolites for post-partum cows top-dressed with soybean meal

Variable	Dietary treatment			SE	Effect of diet (*P*-value)	
	CON	SBM1	SBM2		Contrast 1[1]	Contrast 2[2]
Glucose (mg/l)	443	506	515	53	0.41	0.87
BHBA (mmol/day)	2.05	1.94	1.08	0.12	0.12	0.04
NEFA (mmol/day)	1.35	1.13	0.59	0.15	0.01	0.02
Insulin (ng/ml)	3.5	3.5	3.3	0.8	0.49	0.56
Serum cholesterol (mg/l)	1290	1330	1370	90	0.49	0.99
Plasma albumin (g/l)	53	48	47	2	0.08	0.71
Total protein (g/l)	109	127	106	8	0.83	0.06
GGT (IU/l)	28.4	29.7	27.6	4.5	0.96	0.74
AST (IU/l)	71	86	63	6	0.64	0.02
AP (IU/l)	74	64	60	5	0.07	0.61

BHBA = beta-hydroxybutyric acid, NEFA = non-esterified fatty acids, GGT = γ-glutamyl transferase, AST = aspartate aminotransferase, AP = alkaline phosphatase, CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR + 2 kg of top-dressed soybean meal

[1]Contrast 1 = CON vs SBM1 + SBM2; [2]Contrast 2 = SBM1 vs SBM2

($P < 0.01$), and cows fed SBM2 consumed more N than cows fed SBM1 ($P < 0.01$). Both urine pH ($P = 0.02$) and urea N concentrations ($P = 0.01$) were greater in the cows fed SBM compared to CON (Table 8), and urine pH and urea N were lower for cows fed SBM1 than for those fed SBM2 ($P = 0.03$ and $P = 0.02$, respectively). Concentrations of uric acid and creatinine and calculated urine volume were not different among treatments. Although calculated milk N was not different among treatments, calculated fecal N and total urinary urea N excreted were greater for SBM supplemented cows compared to CON ($P < 0.01$ and $P = 0.03$, respectively). Fecal N was also greater in SBM2 fed cows compared to SBM1 ($P < 0.01$), whereas total urinary urea N only tended to be greater for SBM2 compared to SBM1 fed cows ($P = 0.10$).

DISCUSSION

The initiation of lactation is critical in the productive life of a cow and is the result of the integration of many complex factors. It is clear that milk yield

Table 8. Urine excretion values of post-partum cows fed top-dressed soybean meal

Items	Diets			SE	*P*-value	
	CON	SBM1	SBM2		Contrast 1[1]	Contrast 2[2]
N consumed (g/day)	580	678	700	23	< 0.01	< 0.01
Urine pH	7.98	8.02	8.06	0.09	0.02	0.03
Uric acid (mg/l)	13.3	6.7	12.1	0.25	0.25	0.17
Urea N (g/l)	11.1	15.7	19.4	1.9	0.01	0.02
Creatinine (mol/l)	2.8	262	265	33	0.44	0.96
Urine volume (l/day)	52.3	43.6	44.5	8.3	0.43	0.94
Urinary uric acid excretion (mg/day)	643	299	435	144	0.11	0.50
Milk N (g/day)	183	186	188	16	0.88	0.97
Fecal N (g/day)	66	71	90	4	< 0.01	< 0.01
Urinary urea N excretion (g/day)	497	625	821	79	0.03	0.10

[1]CON = control total mixed ration (TMR), SBM1 = control TMR + 1 kg of top-dressed soybean meal, SBM2 = control TMR + 2 kg of top-dressed soybean meal, VFA = volatile fatty acids

[1]Contrast 1 = CON vs SBM1 + SBM2; [2]Contrast 2 = SBM1 vs SBM2

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increased in response to increasing SBM top-dress in this experiment, although the output of MF and MTP did not increase. Comparisons to the results of other studies feeding SBM in very early lactation are difficult. For example, Keery and Amos (1993) fed various types of SBM over the first 4 weeks of lactation in 2 trials and found no effects on milk yield. However, they fed 115 and 123 g/kg CP diets and changed only the form of SBM. Cressman et al. (1980) fed diets of 120, 150, and 180 g/kg CP based on SBM over the same time period as our experiment and found a milk response to 180 g/kg CP for multiparous cows, but no response in primiparous cows. In these same experiments, there was a quadratic response in MTP g/kg for cows fed 150 g/kg CP, but no difference for cows fed 120 or 180 g/kg CP. Komaragiri and Erdman (1997) fed a 160 g/kg CP ration based on soy protein compared to an 180 g/kg CP ration with greater RUP based on animal protein and found greater milk production with the soy-based ration over the early weeks of lactation (< 6 weeks). Canfield et al. (1990) fed diets of 165 and 192 g/kg CP for the first 20 days of lactation and found significantly higher production in both mature and primiparous cows. Their response was about 7.6 kg, which approximates the response we observed between CON and SBM2. Unfortunately neither Cressman et al. (1980) nor Canfield et al. (1990) provided data on milk component yield, but Keery and Amos (1993) found no effect on milk components as % or yield to changes in type of soybean products. From the data on Met flow to the intestine or Met g/kg MP, it does appear that in this study Met deficiency explains the lack of increased MTP yield and perhaps the lack of MF yield as well (NRC 2001).

Abomasal casein infusions in post-partum cows have been more consistent in producing an increase in both milk and MTP yield (Orskov et al. 1977; Whitelaw et al. 1986; Larsen et al. 2015), although post-partum infusion (Larsen et al. 2014) and feeding rumen protected casein (Rogers et al. 1980) resulted only in increased milk yield even if the numerical trends for higher MTP yield were evident. Thus the present study is within the productive bounds observed in other studies. Another feature of this experiment, as well as those of Larsen et al. (2014, 2015), was that milk production increased from the very earliest days of treatment (Figure 1). In fact for almost all variables measured, changes occurred before 18 DIM.

As stated, in addition to the AA balance of the test protein, the levels of CP in the control diet may also be a factor in the amount or lack of a response to increased protein. In this regard the studies of Rogers et al. (1980), who fed protected casein on top of a basal pasture diet of 175 g/kg CP and Schor and Gagliostro (2001) who fed a basal diet with 200 g/kg diet with SBM or blood meal, are more comparable to our study. Both studies found a response to increased protein in early lactation, with the SBM treatment of Schor and Gagliostro (2001) out performing the blood meal diet. In the Larsen's (2014, 2015) experiments casein was infused to maintain an estimated 2300 g of MP, which is in line with the estimated MP in SBM2 of our study. Rough calculation by the NRC model (2001) of the previously cited pasture studies indicated that both of these studies were above 2200 g of MP per day. Therefore, our study suggests that in addition to a balanced protein source, the protein needs to be given in amounts that will approach 2300 g/day, which may vary slightly depending on AA balance, in order to elicit a milk production response.

The implied increase in milk lactose output due to greater milk production and equal milk lactose concentration (Cressman et al. 1980) or the observed increase in lactose yield in the present study as well as those of others (Larsen et al. 2014; Galindo et al. 2015; Amanlou et al. 2017) suggest that the effect of feeding an AA balanced protein to post-partum cows has an effect on the glucose economy of the early lactation cow. This is further suggested by the observation that in the present study blood NEFA are reduced and BHBA are lower numerically, suggesting less dependence on the oxidation of body fat and a more positive dietary energy balance. In these aspects our study is different than that reported by Galindo et al. (2015), who reported both increased NEFA and increased BHBA with increased protein, and those by Whitelaw et al. (1986) and Larsen et al. (2014) who found no effect of infused casein on NEFA, but very much like the study of Amanlou et al. (2017) who found lower NEFA and lower BHBA in the blood of cows fed a mixture of corn gluten meal and fish meal. Taken together, this data indicates that as MP levels are increased, the relative levels of NEFA are decreased which further suggests less dependency on NEFA as energy sources in early lactation.

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The exact mechanism by which greater amounts of balanced protein might affect lactose synthesis, and hence the glucose economy of the early lactation cow, is not clear other than that it does not affect the blood levels of glucose or insulin. Because of the amount of protein calculated to be degraded in the rumen (2.58, 2.96, and 3.48 kg/day for CON, SBM1, and SBM2 respectively), it might be proposed that changes in VFA might be involved. However, in our study only butyrate was increased between CON and SBM supplemented cows. Generally supplementation with SBM does not change ruminal acetate, propionate or total VFA, but does increase valeric, *iso*-valeric, and *iso*-butyric acids (Broderick et al. 1990) with a variable effect on butyric acid (Mathis et al. 1999). Assuming that microbial protein = 0.13 × TDN (total digestible nutrients) (NRC 2001), then the increased energy from RDP degradation would not account for the estimated increase in rumen microbial protein. Therefore, it does not appear that energy balance or increased energy efficiency in the rumen would account for greater glucose production, although because of lower NEFA and higher milk output observed in SBM treated cows, energy must have been made more available. Therefore, focus appears be on the amount of AA digested by treated cows.

Newer studies (Reynolds et al. 2003) present evidence that the direct contribution of AA to gluconeogenesis in early lactation is the neighbourhood of 9–20% of total liver glucose release and is dependent primarily on the quantity of the non-essential AA alanine (Ala), which supplies about half of the AA for liver gluconeogenesis with the other half from other gluconeogenic essential AA. Relative to casein on a per cent of AA basis, SBM protein provides about 2 × the amount of Ala, so it appears probable that the SBM protein is providing greater amounts of AA for gluconeogenesis. Earlier Lobley (1992) had suggested that branched chain EAA were efficiently used as energy fuels by muscle and fat tissue, thereby sparing glucose for use in lactose synthesis and perhaps milk protein formation. Galindo et al. (2015) suggested that very early in lactation extra glucose could be provided by increased gluconeogenesis from the kidneys or increased glycogenolysis from peripheral tissues as casein infusion did not increase net liver release of glucose. Finally, there is the possibility that specific AA might stimulate production of regulatory peptides which would increase the overall efficiency of fat use (Lys for carnitine and Met for s-adenosyl methionine, for example) as suggested by Whitelaw et al. (1986). However, our data would not support this concept as there was a clear deficiency of Met (Table 3) at each level of dietary treatment, and NEFA levels were lower for animals top-dressed with SBM as well as BHBA were not different among treatments. A similar situation was observed in Amanlou et al. (2017). In fact, assuming that Ala escapes ruminal degradation at the same rate as the intact protein, that Ala digestion is approximately 80%, and that Ala is converted to glucose with 100% efficiency, additional glucose from Ala can supply only 15 and 10% of the observed increases in lactose production for SBM1 and SBM2, respectively. Therefore, Ala conversion to glucose does not appear to be the sole source of the additional glucose noted in response to additional protein. It would require that the great majority of the absorbed AA be converted to glucose via gluconeogenesis to explain the amount of extra glucose produced.

While some studies have not shown a change in DMI in response to increases in a reasonably EAA balanced protein during the post-parturition period (Whitelaw et al. 1986; Larsen et al. 2014, 2015), some have shown an increase in DMI (Cressman et al. 1980; Canfield et al. 1990). But virtually all studies reviewed have shown that DMI increases in very early lactation as DIM increase, except this study. The reason that DMI did not increase in this study with advancing DIM probably has to do with the increasing heat as the spring season moved toward summer, but because no temperature/humidity numbers were taken, this remains speculation. However, the lack of a treatment × DMI effect suggests that treatment had no role in the lack of DMI changes over the time of this study, but the observation of no increase in DMI does have implications for this study because of the initial differences in BW and BCS (Table 5).

The initial BW and BCS differences were an unfortunate by-product of attempts to randomise those fresh cows that met the health criterion for entering the study, which under our protocol could not be determined until after calving. Because of the well-known tendency of fatter fresh cows to consume less dry matter, to lose more BCS, and to have increased BHBA and NEFA (Roche et al. 2009), the lack of BCS balance can affect the inter-

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pretation of our study with the thought that most of the effects on NEFA are due only to loss of BCS. In point of fact, all the cows had BCS which were higher than would be desired (Roche et al. 2009), but all maintained acceptable DMI and BCS loss.

Why the butyric acid in the rumen of cows fed SBM should be higher than in CON is a matter of speculation. Feeding increasing levels of SBM to mid-lactation cows has not produced changes in rumen butyrate in some studies (Spain et al. 1990), but did in others (Broderick 1986). Because the *iso*-valerate was also increased in this study, and *iso*-acids have long been known to be the result of protein breakdown (Allison et al. 1958), and because the level of RDP from SBM is relatively high, we suspect that the breakdown of AA in the soy protein is responsible. Blood variables are more consistent with the interpretation of an effect of SBM treatment on glucose economy in that NEFA levels are lower in SBM supplemented cows as we were hoping to find, and BHBA, although not significant, does show numeric agreement with changes in NEFA. However, although we do not view this as proof that this is a reflection of improved glucose status, we consider it strong supporting evidence. Also, although all the cows had high BCS (Roche et al. 2009), this was not reflected in the liver enzymes as no treatments indicated liver damage (Table 7).

While we expected that total urinary urea excretion for the SBM treated cows would be higher, for all treatments the numbers generated appear too high although probably not by a lot. The problem is likely to be in the prediction of urine volume which is very high because of an insufficient adjustment for tissue loss in early lactation, although these values are not outside the range of possibility (Dijkstra et al. 2013). Nonetheless, the calculated N excretions do point to a considerable environmental N loss when SBM is used at this rate. It may also be of interest that the urine pH is higher for cows fed SBM and may be reflective of more NH_3 formation. Thus both the source of protein supplement and the time for which it must be fed must be defined in order both to reduce environmental contamination and reduce the risk of potential adverse effects on future reproduction (Canfield et al. 1990).

In addition to use of a relatively balanced protein source, the other apparently important aspect is the necessity to begin feeding protein immediately after parturition. Many respected studies regarding protein supplementation in early lactation (Davidson et al. 2003) have been used to justify not increasing the protein in early lactation. However, these studies were initiated after several weeks in lactation. That experimental design makes it possible to substantially reduce variation by more uniform grouping, but that strategy may also miss the critical time that protein is required. It has been suggested that the need for gluconeogenesis from protein would be terminated by 11 DIM (Reynolds et al. 2003). Our study seems to indicate that early initiation of protein supplementation is necessary and that a balanced protein that supplies the total diet with 2300 g of MP is necessary as well. However, beyond these observations, this study leaves many more questions unanswered. Specifically, it would be important to try other protein supplements with greater RUP or with AA supplementation, especially of Met, to determine the best protein and AA balance as well as the length of time that increased protein would be needed to increase and maintain production. Finally, studies are needed to determine if the increase in milk production would be maintained after supplementation is discontinued and dietary protein returns to more traditional levels.

In conclusion, adding 1 or 2 kg of SBM to a relatively high protein diet increased milk yields without a concurrent increase in MP yield, which may be due to Met deficiency. Further, it appears that supplementation must be started as soon as possible after parturition and that the protein needs to have an AA balance as much like casein as possible.

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