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The Effect of Partial Replacing Solvent Soybean Meal with Poultry Blood Meal on Performance and Metabolic Status of Fresh Holstein Dairy Cows

Research Article

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ABSTRACT

The objective of this study was to investigate the effect of replacing solvent soybean meal (SSBM) with poultry blood meal (PBM) on performance, metabolic status, and apparent digestibility of nutrients in Holstein fresh cows. Twenty-four Holstein cows (body weight (BW)±SD; 669.5±42.59 kg) were blocked by body condition score (BCS) at calving and previous lactation milk yield and randomly assigned to 1 of 3 experimental diets from calving until 21 days in milk (DIM): diet without PBM (0PBM), replacing 2.5% DMSSBM with PBM (2.5PBM), and replacing 5% DMSSBM with PBM (5PBM). There were no significant effects (P>0.10) of treatments on dry matter intake (DMI), milk yield, and milk composition, but cows fed the 2.5PBM diets had significantly higher milk urea nitrogen (MUN) levels than cows fed the 0PBM and 5PBM diets (P<0.01). Cows fed the 5PBM diet had significantly higher serum globulin, non-esterified fatty acids (NEFA), and β-hydroxybutyrate (BHB) and lower serum cholesterol concentrations than cows fed the 0PBM and 2.5PBM diets (P<0.05). Blood urea nitrogen (BUN) concentration in cows fed the 2.5PBM diet was significantly (P=0.01) higher than cows fed the 0PBM and 5PBM diets. The apparent digestibility of dry matter in the 5PBM diet was significantly (P=0.03) lower compared to the 0PBM diet. However, there was no significant difference between the 5PBM and 2.5PBM diets. Overall, the lack of significant differences in performance, apparent digestibility of nutrients and serum metabolites between the 0PBM and 2.5PBM shows that the SSBM could replace up to 2.5% of DM with the PBM. However, levels higher than 2.5% of DM are not recommended due to low palatability and negative effects on the metabolic status of cows.

KEY WORDS fresh cow, metabolic status, milk yield, poultry blood meal, solvent soybean meal.

INTRODUCTION

Immediately after calving, dairy cows experience negative nutrients balance, especially energy and protein due to rapidly increasing in milk yield along with slowly increasing DMI. High-producing dairy cows might mobilize as much as 1 kg of tissue protein per day from skeletal muscle during the first week of postpartum to supply amino acids (AAs) requirements (Bell *et al.* 2000). Microbial protein synthesized in the rumen and rumen undegradable protein (RUP) are the main sources of metabolizable protein (MP) and AAs requirements in dairy cows (Giallongo *et al.* 2015). Decreased DMI during early postpartum restricts fermentable energy intake, which prevents the maximal synthesis of microbial protein and, by that, reduces MP supply. Therefore, supplementing immediately after calving diets with RUP sources is one strategy to increase MP supply. Several studies on transition cows have been reported that additional MP supply through RUP supplementation or using abomasal casein infusion increased milk production

with (Amanlou *et al*. 2017; Farahani *et al*. 2019) or without (Larsen *et al*. 2014) positive effects on DMI. The PBM is a protein supplement with high crude protein (CP) (95.5% DM) and RUP contents (80% CP) and rich in Lys, Leu, and His (8.98, 12.82, and 6.36% CP, respectively; NRC, 2001), which these AAs have been known as limiting AAs in lactating cows (Schwab *et al*. 1976; Lee *et al*. 2012; Larsen *et al*. 2014). However, low palatability and AAs imbalances in the PBM have limited its consumption and reduced its biological value. Schor and Gagliostro (2001) fed early lactation dairy cows with concentrates containing either SSBM or blood meal (BM) in grazing conditions. They reported that milk yield increased by 17.7% and forage DMI by 25.5% in cows fed concentrate containing BM compared to concentrate containing SSBM. Reynal and Broderick (2003) found that DMI, milk yield, and NDF and ADF digestibility were similar between diets containing SSBM and BM, but DM digestibility in the BM diet decreased compared to the SSBM diet. In contrast, Pires *et al*. (1996) and Moss *et al*. (1995) reported that feeding BM to lactating cows decreased DMI compared to SSBM, with no effect on milk yield and composition. To the best of our knowledge, the effects of supplementing fresh diets with PBM as RUP source have not been investigated yet. Therefore, we hypothesized that partial replacement of SSBM with PBM in fresh dairy cows would improve MP supply and energy balance, and increase milk yield. Our objective for this study was to investigate the productive and metabolic responses and apparent total tract digestibility of nutrients from partial replacing SSBM with PBM in dairy cows during the first 21 DIM.

MATERIALS AND METHODS

Animals, diets, and experimental design

This study was conducted on a dairy farm (Barmayeh, Isfahan, Iran) from July to August 2021.Cows were cared according to the guidelines of the Iranian Council of Animal Care (1995). Twenty-four fresh Holstein cows (BW±SD; 669.5±42.59 kg) with eight replicates per treatment were blocked by body condition score (BCS) at calving and previous lactation milk yield and randomly assigned to 1 of 3 experimental diets from calving until 21 DIM: diet without PBM (0PBM), replacement of 2.5% DM SSBM with PBM (2.5PBM), and replacement of 5% DM SSBM with PBM (5PBM). At enrollment, cows averaged 3.35 ± 0.25 BCS, 10700 ± 879 kg previous lactation milk yield, and 3.04 ± 0.75 lactations. During the close-up period, cows were housed in a free stall barn and fed the same close-up diet (net energy for lactation (NE_L)=1.57 Mcal/kg DM and CP=14.3% DM) for *ad libitum* intake twice daily at 09:00 and 17:00 h to achieve 5 to 10% orts. After showing primary signs of calving, cows were moved to maternity pens. Immediately after calving, calf weight and first-milking colostrum yield were recorded by calving personnel. After calving, the cows were housed individually and assigned to their experimental diets from calving until 21 DIM, with free access to water. Before treating, cows suffering retained placenta, milk fever, mastitis, pneumonia, laminitis, dystocia, and rectal temperature (≥39.4 ˚C) were not enrolled in the experiment. Cows received the experimental diets (Table 1) *ad libitum* thrice a day at 09:00, 17:00, and 01:00 h. Cows were milked three times a day at 08:00, 16:00, and 00:00 h. The diets were formulated by NRC (2001) software. The ingredient and chemical composition of the diets fed close-up and fresh cows are illustrated in Tables 1 and 2. Protein supplies and AAs balances were estimated by actual individual cow DMI, BW, BCS, milk yield, and milk composition using NRC (2001). The BM used in this study was purchased from the Khazar factory (Gilan province, Iran), where the raw blood obtained from poultry slaughter houses was dried by drum drying method. Before the start of the experiment, the PBM samples were taken and sent to the Mabna veterinary laboratory (Alborz, Iran) to evaluate bacterial contamination using microbial culture and the residues of ruminants by polymerase chain reaction (PCR) test.

Sampling and data collection

The DMI of individual cows was determined daily from calving until 21 DIM. Samples of total mixed ration (TMR) and orts were taken twice a week for DM measurement, and were dried at 60 ˚C for 48 h, and then composited by week and treatment. Samples of TMR and orts were ground through a 1-mm screen and analyzed for nitrogen by Kjeldahl (AOAC, 1990; method 984.13), ether extract (AOAC, 1990; method 920.39), Ash (AOAC, 1990, method 942.05), acid detergent fiber (ADF) (AOAC, 1990; method 973.18), and neutral detergent fiber (NDF) (Van Soest *et al*. 1991). The composition of other components of experimental diets (i.e., NE_L, rumen degradable protein (RDP), rumen undegradable protein (RUP), AAs flow, and minerals) was estimated using NRC (2001) model. To determine the nitrogen fractionation in SSBM and PBM, samples of both feedstuffs were taken and sent to a nutrition laboratory (Vahdat Co., Isfahan, Iran) and five nitrogen fractions (A, B1, B2, B3, and C) were obtained according to Cornell Net Carbohydrate and Protein System (CNCPS) method. The A fraction is NPN, the B fraction is true protein, and C is unavailable protein. The B fraction is further subdivided into 3 fractions (B1, B2, and B3) with different digestion rates (Lanzas *et al*. 2007).

Table 1 The protein fractionation in solvent soybean meal and poultry blood meal based on cornell net carbohydrate and protein system (CNCPS) method

Nutrient	SSBM	PBM	Unit
Dry matter (DM)	92.90	90.90	%
Crude protein (CP)	47.00	64.50	% DM
True protein	46.91	55.05	%
Insoluble protein	45.09	53.12	%
A	27.50	8.45	% CP
B1	3.70	3.25	% CP
B2	58.65	36.45	% CP
B3	7.64	36.56	% CP
C	2.51	15.29	% CP
Ether extracts (EE)	1.08	7.50	% DM

A: non protein nitrogen (NPN); B: true protein and C: unavailable protein. The B fraction is further subdivided into 3 fractions (B1, B2, and B3) with different digestion rates.

SSBM: solvent soybean meal and PBM: poultry blood meal.

Table 2 Feed ingredients of the diets fed cows during close-up and fresh periods (% of DM)

Item	Close-up diet	Fresh diets		
		0PBM	2.5PBM	5PBM
Alfalfa hay, mature	24.10	15.37	15.37	15.37
Corn silage, normal	24.30	21.85	21.85	21.85
Wheat straw	7.52	-	-	-
Beet sugar pulp	-	5.54	5.54	5.54
Barley grain, rolled	8.85	13.70	13.70	13.70
Corn grain, ground, dry	15.42	14.73	15.80	16.86
Wheat grain, ground	-	2.50	2.50	2.50
Soybean meal, solvent extracted	8.66	12.67	9.00	5.28
Poultry blood meal	-	0	2.50	5.00
Canola meal, mechanical extraction	3.07	1.77	1.77	1.77
Poultry meat meal	3.00	5.24	5.24	5.24
Rice bran	-	3.23	3.23	3.23
$NaHCO_3$	-	0.84	0.94	1.10
Calcium carbonate	1.27	0.78	0.78	0.78
Magnesium oxide	0.16	0.28	0.28	0.28
Dicalcium phosphate	0.11	0.17	0.17	0.17
Salt	-	0.28	0.28	0.28
Bentonite	-	0.28	0.28	0.28
Calcium chloride	0.81	-	-	-
Magnesium sulfate	0.95	-	-	-
Ovarian cysts supplement[1]	0.89	0.21	0.21	0.21
Mineral vitamin premix[2]	0.89	0.56	0.56	0.56

[1] Supplement contained 140 mg of Co/kg, 12000 mg of Cu/kg, 22000 mg of Mn/kg, 340 mg of Se/kg, 62000 mg of Zn/kg.

[2] Supplied the following per kilogram of feed: vitamin A: 1000000 IU; vitamin D: 200000 IU; vitamin E: 8000 IU; Zn: 12000 mg; Mn: 10000 mg; Cu: 3500 mg; I: 100 mg; Se: 60 mg and Co: 40 mg.

0PBM: diet without poultry blood meal; 2.5PBM: replacing 2.5% DM solvent soybean meal with PBM and 5PBM: replacing 5% DM solvent soybean meal with PBM.

Milk yield of individual cows was recorded daily from 1 to 21 DIM. Milk samples from each cow were collected weekly from 3 consecutive milkings, composited in proportion to milk yield, and then analyzed for fat, true protein, lactose, and MUN using a MilkoScan (CombiFoss 78110; Foss Analytical A/S, Hillerød, Denmark). Feed efficiency was calculated by dividing 4% FCM by DMI.

Cows were weighed weekly before the morning feeding, and weekly weights were used to calculate NE_L and MP balance using the NRC (2001) model.

Furthermore, the BW at calving and at 21 DIM were used to calculate BW changes.

Body condition of cows were scored at calving and at 21 DIM by 2 trained investigators, using a 5-point scale (Edmonson *et al.* 1989; BCS 1=thin to 5=obese) and the average scores were used for analysis.

Blood samples were obtained 3 to 4 h after morning feeding from the coccygeal vein using an evacuated tube without anticoagulant (Vacumed no additive, FL Medical, Torreglia, Italy) at 0, 3, 7, 14, and 21 DIM.

Table 3 Chemical composition of the diets fed during close-up and fresh periods (% of DM, unless otherwise stated)

Composition[1]	Close-up diet	Fresh diets[2]		
		0PBM	2.5PBM	5PBM
NE_L (Mcal/kg DM)	1.57	1.67	1.67	1.68
CP	14.30	17.00	17.00	17.00
RDP	10.00	11.40	10.70	10.00
RUP	4.30	5.60	6.30	7.00
RDP:RUP	2.32	2.03	1.69	1.42
NDF	34.00	27.90	27.50	27.00
Forage NDF	27.00	17.50	17.50	17.50
Forage NDF (% of NDF)	79.40	62.70	63.60	64.80
ADF	23.40	17.70	17.40	17.00
NFC	41.10	44.90	45.20	45.50
EE	2.70	3.20	3.30	3.50
Ca	1.60	1.30	1.30	1.30
P	0.50	0.70	0.60	0.60
DCAD (mEq/kg)	-25	+242	+240	+243
Protein balance[3] (g/d)				
RDP	+10	+181	+55	-66
RUP	+390	-545	-390	-522
MP[4]	+172	-419	-298	-396
AA flow[3] (g/d)				
Met	28	40	45	39
Lys	100	144	168	152
Lys:Met	3.57	3.60	3.73	3.89
Leu	125	183	221	205
His	31	47	61	59

[1] NE_L: net energy for lactation; CP: crude protein; RDP: rumen degradable protein; RUP: rumen undegradable protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fiber carbohydrate; EE: ether extract; DCAD: dietary cation-anion difference; MP: metabolizable protein and AA: amino acid.
[2] 0PBM: diet without poultry blood meal; 2.5PBM: replacing 2.5% DM solvent soybean meal with PBM, and 5PBM: replacing 5% DM solvent soybean meal with PBM.
[3] All components were estimated using NRC (2001) model based on actual DMI, BW, BCS, milk yield, and milk composition in dietary treatments; protein balance= protein required – protein supplied.
[4] The MP requirement for close-up cows was NRC model plus 120 g of MP to account for mammary growth (Bell *et al*. 2000).

Serum samples were separated by centrifugation at 3000 × g for 15 min and stored in plastic tubes then frozen at −20 ˚C until analysis. Serum samples were analyzed for concentrations of glucose, albumin, total protein, BUN, cholesterol, and triacylglycerol by commercial kits (Farasamed Parsian Co, Tehran, Iran), using auto-analyzer (BT1500, Biotecnica instruments SPA, Italy). Serum concentrations of NEFA (colorimetric method) and BHB (enzymatic method; based on 3-hydroxybutyrate dehydrogenase) were measured by Randox kits (Randox Laboratories Ltd., Crumlin, UK), using a serum spectrophotometer (UNICCO, 2100, Zistchemi Co., Tehran, Iran). Globulin concentration was obtained as the difference between total protein and albumin.

Fecal samples were taken from the rectum (approximately 400 g) of all cows for 2 consecutive days every 8 h (3 times/d at 0700, 1500, 2300 h) from d 20 to 21 DIM, and dried at 65 ˚C for 48 h (Amanlou *et al*. 2017). Fecal samples were ground through a 1-mm screen, pooled by cow and sampling day, and analyzed for DM, CP, NDF, and ADF. To determine the apparent total-tract digestibility of nutrients, acid insoluble ash was considered as an internal digestibility marker (Van Keulen and Young, 1977).

The N efficiency was calculated by dividing milk N by N intake (Amanlou *et al*. 2017).

Statistical analysis

The data were analyzed using PROC MIXED of SAS, (2010) (version 9.3; SAS Institute Inc., Cary, NC). Dry matter intake, milk yield and composition, serum metabolites, and feed efficiency data were analyzed as repeated measures with the most suitable structure based on the lowest Akaike information criterion, corrected Akaike information criterion, and Bayesian information criterion values for each analysis (Littell *et al*. 1998). The following model was used:

$$y_{ijk} = \mu + Diet_i + Time_j + (Diet \times Time)_{ij} + Cow\ (block_k) + e_{ijk}$$

Where:

y_{ijk}: dependent variable.

μ: overall mean.

$Diet_i$: fixed effect of diet.

$Time_j$: fixed effect of time.

$(Diet \times Time)_{ij}$: fixed effect of diet × time interaction.

Cow ($block_k$): cows nested within blocks.

e_{ijk}: residual.

For analyzing serum metabolites, the concentrations of serum metabolites obtained at calving day were used as covariates if they were significant (P<0.10). To analyze BW and BCS changes and apparent total tract digestibility of nutrients, the fixed effects of time and treatment by time interaction were excluded from the above models. Data are reported as least square means (LSM) and statistical significances were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$ as trends toward significance using the Tukey's multiple comparison test.

RESULTS AND DISCUSSION

The chemical compositions of the SSBM and PBM are presented in Table 1. The SSBM contained 47.0% CP and 1.08% EE (as DM) and the PBM contained 64.5% CP and 7.5% EE (as DM), which were different from the expected values (NRC, 2001). Likewise, the different nitrogen fractions (A, B, and C) in both protein meals were different from the expected values (NRC, 2001). In this study, the ingredient profiles of the TMR across treatments were almost similar, except for an increase in levels of PBM, with a corresponding decrease in the SSBM levels. Other slight changes occurred, such as increase in corn grain levels and NaHCO3 in the diets to create a consistent nutrient profile across treatments (Tables 2 and 3). The experimental diets were isocaloric (NE_L=1.67 Mcal/kg DM) and isonitrogenous (CP=17% DM), varying in dietary RUP content at the expense dietary RDP. The RDP balances were +181, +55, and -66 g/d for the 0PBM, 2.5PBM, and 5PBM diets, respectively. The RUP balances were -545, -390, and -522 g/d for the 0PBM, 2.5PBM, and 5PBM diets, respectively. The MP balances were -419, -298, and -396 g/d for the 0PBM, 2.5PBM, and 5PBM diets, respectively. Overall, protein balances were improved in the 2.5PBM diet relative to the 0PBM and 5PBM diets (Table 3).

Main effects of treatment, time, and the interaction of treatment by time on DM and other nutrients are summarized in Table 4. Although, the DM, NE_L, and CP intakes were not affected by treatments (P>0.10), cows fed the 2.5PBM diet, consumed 1.58 and 2.36 kg/d higher DM than cows fed the 0PBM and 5PBM diets, respectively. Intakes of RDP (P=0.10) and RUP (P=0.08) in cows fed the 2.5PBM diet tended to increase compared to cows fed the 0PBM and 5PBMdiets. There was an effect of time (P<0.01) on all nutrient intakes but no time by treatment interaction (P>0.05; Table 4) on intakes. Over time, DM and other nutrients increased as lactation progressed from calving to 21 DIM (P<0.01).

Main effects of treatment, time, and their interactions on milk yield and composition, feed efficiency, N efficiency, and energy balance are presented in Table 5. Milk yield (P=0.79) and 4% FCM (P=0.84) were not significant across treatments, but cows fed the 2.5PBM diet produced 2.50 and 1.33 kg/d greater milk than cows fed the 0PBM and 5PBMdiets, respectively. With the exception of MUN (P<0.01), contents and yields of milk fat, protein, and lactose were not affected (P>0.10) by treatments. The concentrations of MUN were higher in cows fed the 2.5PBMdiet compared to cows fed the 0PBM and 5PBM diets (12.33 *vs.* 10.19 and 11.09 mg/dL, respectively). Time effect was significant (P<0.01) for the yields of milk and 4% FCM and composition, except for milk protein (P=0.60), lactose (P=0.30) contents, and MUN (P=0.35). The treatment by time interaction was not significant for milk yield and composition (P>0.10). Milk yield and 4% FCM increased and milk fat content decreased for all cows as lactation progressed from calving to 21 DIM (P<0.01).

Milk N efficiency was affected by treatments (P<0.01), as cows fed the 5PBM diet had higher milk N efficiency than cows fed the 0PBM and 2.5PBM diets (0.40 *vs.* 0.38 and 0.36, respectively). There was an effect of time (P<0.01), but no treatment by time (P=0.56) on milk N efficiency. Feed efficiency (FCM/DMI) was affected by treatments (P<0.01), as the lowest feed efficiency was for cows fed the 2.5PBM diet and the highest feed efficiency was for cows fed the 5PBM diet. Time effect (P<0.01) and no treatment by time interaction (P=0.25) were significant for feed efficiency.

Energy balance was affected by treatments (P<0.01) and negative energy balance in cows fed the 0PBM, 2.5PBM, and 5PBM diets were -5.99, -5.17, and -7.91 Mcal/d, respectively. Cows in the 5PBM group experienced a greater negative energy balance than cows in the 0PBM and 2.5PBM groups. Time effect (P<0.01) and treatment by time interaction (P<0.01) were significant for energy balance, as energy balance was more negative in cows fed the 5PBM diet than cows fed other diets from 1 to 3 week postpartum (Figure 1).

Main effects of treatment, time, and their interactions on serum metabolites are presented in Table 6. Among the indicators of protein metabolism such as total protein, albumin, globulin and BUN, only serum concentrations of globulin (P=0.01) and BUN (P=0.01) were affected by treatments, as cows fed the 5PBM diet had higher serum globulin concentrations than cows fed the 0PBM and 2.5PBM diets (3.69 *vs.* 3.03 and 3.32 g/dL). However, serum BUN concentrations in cows fed the 2.5PBM diet were higher than cows fed the 0PBM and 5PBMdiets (18.14 *vs.* 16.71 and 16.63 mg/dL). There was no effect of time (P>0.10) and treatment by time interaction (P>0.10) on protein metabolism indicators.

Among energy metabolism indicators only serum concentrations of NEFA (P=0.03), BHB (P=0.02) and cholesterol (P=0.01) were affected by treatments (Table 6).

Table 4 Effect of partial replacement of solvent soybean meal with poultry blood meal on nutrient intakes of Holstein fresh cows

Item[1]	Diets[2]			SEM	P-value		
	0PBM	2.5PBM	5PBM		Diet	Time[3]	Diet × Time
Intake							
DM (kg/d)	16.37	17.95	15.59	1.28	0.42	< 0.01	0.80
NE_L (Mcal/d)	27.35	29.97	26.20	1.63	0.45	< 0.01	0.89
CP (kg/d)	2.78	3.05	2.65	0.21	0.42	< 0.01	0.88
RDP (kg/d)	1.86	1.92	1.55	0.10	0.10	< 0.01	0.84
RUP (kg/d)	0.91	1.13	1.09	0.07	0.08	< 0.01	0.70

[1] DM: dry matter; NE_L: net energy for lactation; CP: crude protein; RDP: rumen degradable protein and RUP: rumen undegradable protein.
[2] 0PBM: diet without poultry blood meal; 2.5PBM: replacing 2.5% DM solvent soybean meal with PBM, and 5PBM: replacing 5% DM solvent soybean meal with PBM.
[3] From calving day to 21 dry matter intake (DIM).
SEM: standard error of the means.

Table 5 Effect of partial replacement of solvent soybean meal with poultry blood meal on milk yield and composition, feed efficiency, energy balance, and milk N efficiency of Holstein fresh cows

Item[1]	Diets[2]			SEM	P-value		
	0PBM	2.5PBM	5PBM		Diet	Time[3]	Diet × Time
Milk yield (kg/d)	34.64	37.14	35.81	2.90	0.79	< 0.01	0.72
4% FCM (kg/d)	31.52	33.49	32.63	2.74	0.84	< 0.01	0.86
Fat (%)	3.44	3.39	3.46	0.07	0.78	< 0.01	0.90
Fat (kg/d)	1.17	1.24	1.22	0.10	0.88	< 0.01	0.95
True protein (%)	3.15	3.12	3.12	0.05	0.85	0.60	0.23
True protein (kg/d)	1.09	1.15	1.12	0.06	0.86	< 0.01	0.72
Lactose (%)	4.73	4.74	4.77	0.05	0.88	0.30	0.16
Lactose (kg/d)	1.64	1.75	1.70	0.13	0.80	< 0.01	0.74
MUN (mg/dL)	10.19^b	12.33^a	11.09^b	0.33	< 0.01	0.35	0.12
NE_L balance (Mcal/d)	-5.99^a	-5.17^a	-7.91^b	0.31	< 0.01	< 0.01	< 0.01
FCM/DMI	1.91^b	1.85^c	2.07^a	0.015	< 0.01	< 0.01	0.25
Milk N (kg/d)	0.171	0.180	0.175	0.013	0.86	< 0.01	0.72
Milk N efficiency	0.38^b	0.36^b	0.40^a	0.008	< 0.01	< 0.01	0.56

[1] 4% fat corrected milk (FCM)= [0.4 × milk (kg)] + [15 × milk fat (kg)].
[2] 0PBM: diet without poultry blood meal; 2.5PBM: replacing 2.5% DM solvent soybean meal with PBM, and 5PBM: replacing 5% DM solvent soybean meal with PBM.
[3] From calving day to 21 dry matter intake (DIM).
MUN: milk urea nitrogen and NE_L: net energy for lactation.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

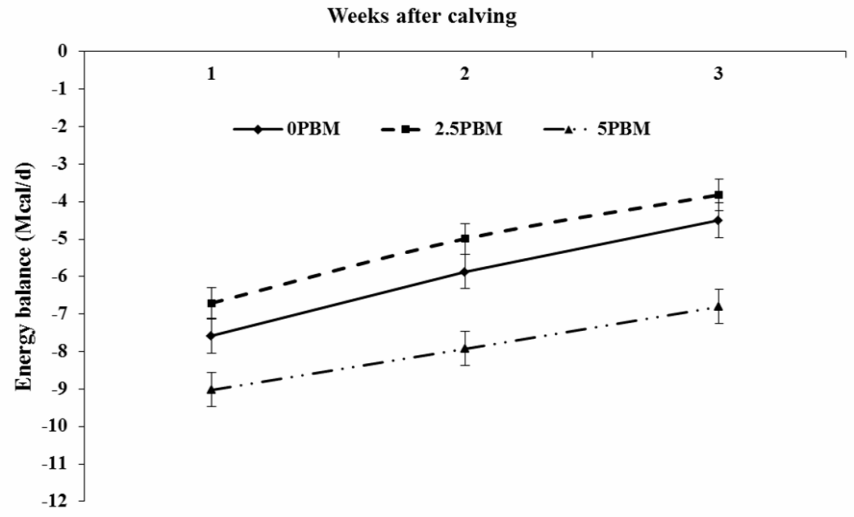

Figure 1 Effect of partial replacement of solvent soybean meal with poultry blood meal on energy balance in Holstein fresh cows. 0PBM: diets designated as diet without PBM; 2.5PBM: replacing 2.5% DM SSBM with PBM and 5PBM: replacing 5% DM SSBM with PBM. Data are presented as least squares means and standard error of the means (SEM). Diet, $P < 0.01$; time, $P < 0.01$; diet × time, $P < 0.01$

Table 6 Effect of partial replacement of solvent soybean meal with poultry blood meal on serum metabolites of Holstein fresh cows

Item[1]	Diets[2]			SEM	P-value		
	0PBM	2.5PBM	5PBM		Diet	Time[3]	Diet × Time
Total protein (g/dL)	5.69	5.94	6.18	0.14	0.16	0.27	0.47
Albumin (g/dL)	2.64	2.63	2.49	0.07	0.28	0.66	0.24
Globulin (g/dL)	3.03^{b}	3.32^{b}	3.69^{a}	0.11	0.01	0.33	0.94
BUN (mg/dL)	16.71^{b}	18.14^{a}	16.63^{b}	0.36	0.01	0.81	0.49
Glucose (mg/dL)	56.51	52.37	53.88	1.45	0.18	< 0.01	0.35
NEFA (mmol/L)	0.39^{b}	0.35^{b}	0.50^{a}	0.03	0.03	0.06	0.62
BHB (mmol/L)	0.60^{b}	0.55^{b}	0.85^{a}	0.07	0.02	0.10	0.67
Cholesterol (mg/dL)	107.39^{a}	97.45^{a}	75.29^{b}	6.50	0.01	0.02	0.25
Triglyceride (mg/dL)	23.49	19.76	20.67	1.91	0.34	0.90	0.47

[1] BUN: blood urea nitrogen; NEFA: non-esterified fatty acids and BHB: β-hydroxybutyrate.
[2] 0PBM: diet without poultry blood meal; 2.5PBM: replacing 2.5% DM solvent soybean meal with PBM, and 5PBM: replacing 5% DM solvent soybean meal with PBM.
[3] From calving day to 21 dry matter intake (DIM).
MUN: milk urea nitrogen and NE_L: net energy for lactation.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The cows fed the 5PBM diet had higher serum NEFA and BHB and lower serum cholesterol concentrations than cows fed the 0PBM and 2.5PBMdiets. Time effect was significant for serum cholesterol (P=0.02) and glucose (P<0.01) concentrations and tended to be significant for serum NEFA (P=0.06) and BHB (P=0.10) concentrations. However, no effects were found of treatment by time interactions (P>0.10) on energy metabolism indicators. Over time, the serum concentrations of cholesterol increased and glucose, NEFA, and BHB decreased as lactation progressed from calving to 21 DIM.

Main effects of treatment on apparent total tract digestibility of nutrients and the changes of BW and BCS are presented in Table 7. The apparent total tract digestibility of DM in cows fed the 5PBM diet decreased compared to cows fed the 0PBM diet (P=0.03), but there was no difference between cows fed the 5PBM and 2.5PBMdiets. Apparent digestibility of CP (P=0.41), NDF (P=0.78) and ADF (P=0.50) were similar among treatments. Changes in BW and BCS were not affected by treatments (P>0.10).

Replacing SSBM with PBM did not affect DM, NE_L, and CP intakes during the fresh period. In the present study, the DMI was numerically highest in the 2.5PBM diet and increasing PBM levels to 5% DM (5PBM diet) numerically decreased DMI.

Similar to our findings, Reynal and Broderick (2003) studied effect of using protein supplements differing ruminal degradability such as SSBM, expeller soybean meal, BM and corn gluten meal on DMI and milk production in early lactation cows.

They reported that compared to the basal diet (without protein supplement), diets supplemented with protein increased DMI and milk yield, but there was no difference in DMI and milk yield of cows fed diets supplemented with BM and SSBM. Likewise, Paula *et al*. (2018) fed mid lactation cows with extruded canola meal and canola meal, and found no difference in DMI than cows fed soybean meal, which is in agreement with our results on DMI. Pires *et al*. (1996) surveyed the effect of different protein sources on performance of dairy cows from 3 to 18 weeks of lactation. They reported that cows fed diets containing BM and whole roasted soybean consumed about 11% lower DM than cows fed diets containing soybean meal. Similarly, these researchers suggested that less than 2.7% BM may be recommendable for diets fed high-producing dairy cows to prevent reduced DMI.

Some previous studies (Schor and Gagliostro, 2001) observed an increase in DMI with replacing soybean meal with BM in early lactation dairy cows under grazing conditions. These contradictory findings could be due to differences in dietary MP supply and AAs content of MP, sources and levels of protein, lactation stage of cows, and basal diet composition (Farahani *et al*. 2017).

The yields of milk and 4% FCM were not significantly affected by treatments, but in accordance with the DMI findings, cows fed the 2.5PBM diet produced 2.50 and 1.33 kg/d greater milk than cows fed the 0PBM and 5PBMdiets, respectively.

Results on the effects of RUP supplementation on milk yield in dairy cows are inconsistent due to level and source of RUP used and lactation stage of cows. Giallongo *et al*. (2015) reported that substituting solvent soybean meal with extruded soybean meal in the diet of mid lactation cows increased DMI by 1.20 kg/d and milk yield by 3.20 kg/d without altering milk composition. In contrast, Paula *et al*. (2018) found no effect of replacing soybean meal with extruded canola meal on milk yield in high-yielding cows during mid-lactation period. Some previous studies reported that replacing soybean meal with blood meal in lactating cows had no effect (Pires *et al*. 1996; Reynal and Broderick, 2003) or even increased (Schor and Gagliostro, 2001) milk yield.

Table 7 Effect of partial replacement of solvent soybean meal with poultry blood meal on total-tract apparent digestibility of nutrients and BW and BCS changes of Holstein fresh cows

Item[1]	Diets[1]			SEM	P-value
	0PBM	2.5PBM	5PBM		Diet
Apparent digestibility (%)					
DM	74.03[a]	71.44[ab]	68.49[b]	1.51	0.03
CP	71.33	70.18	66.07	2.36	0.41
NDF	49.21	52.75	49.86	3.08	0.78
ADF	44.72	47.65	44.18	2.28	0.50
BW change (kg)	-27.62	-24.64	-32.21	8.31	0.76
BCS change	-0.37	-0.26	-0.46	0.10	0.46

[1] DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; BW: body weight and BCS: body condition score.
[2] 0PBM: diet without poultry blood meal; 2.5PBM: replacing 2.5% DM solvent soybean meal with PBM, and 5PBM: replacing 5% DM solvent soybean meal with PBM.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Grant and Haddad (1998) found an interaction between dietary CP content and the addition of animal protein sources on milk yield, as supplementation of mixture of blood and feather meals decreased milk yield for cows fed the 19.6% CP diet but increased milk yield for cows fed the 17.6% CP diet.

Milk fat, protein, and lactose contents were similar among treatments, except for MUN concentrations which were higher in cows fed the 2.5PBM diet compared to cows fed the 0PBM and 5PBM diets. The increase in MUN concentration is a function of CP intake, the ruminal degradability of protein, and the post-ruminal supply of protein (Jonker *et al*. 1998) and energy intake (Jordan *et al*. 1983; Oltner *et al*. 1985). In the present study, although the CP intake of cows was similar among diets, cows fed the 2.5PBM diet tended to consume greater RDP and RUP, thus increase in MUN concentrations in the 2.5PBM diet relative to other diets may be due to ruminal protein degradation or extra AAs catabolism for energetic purposes or both. Our results on MUN concentrations are in agreement with most studies (Larsen *et al*. 2014; Amanlou *et al*. 2017; Barros *et al*. 2017), which showed differences in MUN and BUN concentrations reflect changes in dietary RDP and RUP levels and their intake.

Milk fat content was expected to be greater in cows fed the 5PBM diet because of higher concentrations of serum NEFA and BHB during the fresh period when compared to cows fed the 0PBM and 2.5PBM diets. However, neither milkfat content nor other composition were affected by treatments in the present study. The lack of differences in milk fat content is difficult to interpret. In agreement to our results, others (Reynal and Broderick, 2003; Giallongo *et al*. 2015; Paula *et al*. 2018) reported lack of differences in milk fat and protein contents when RDP sources were replaced with RUP supplements.

Energy balance was affected by treatments, as cows fed the 5PBM diet experienced a severe negative energy balance than cows fed the 0PBM and 2.5PBM diets. These results are supported by lower DMI (numerically only) and higher serum NEFA and BHB concentrations (Table 6) during the first 21 DIM in cows fed the 5PBM diet.

Milk N efficiency was affected by treatments, as cows fed the 5PBM diet had higher milk N efficiency than cows fed the 0PBM and 2.5PBM diets, probably due to decreased RDP: RUP ratios. Similarly, Wang *et al*. (2008) reported improvement in the efficiency of N utilization in dairy cows when RDP: RUP ratios decreased from 2.25 to 1.40 in their diets. In contrast, Savari *et al*. (2018) reported higher N efficiency in cows fed high RDP: RUP ratio compared to cows fed low RDP: RUP ratio, probably due to higher milk yield and milk N excretion in this group.

Serum concentrations of globulin and BUN were affected by treatments, as cows fed the 5PBM die thad higher serum globulin concentrations than cows fed the 0PBM and 2.5PBMdiets. However, serum BUN concentrations in cows fed the 2.5PBM diet were higher than cows fed the 0PBM and 5PBMdiets, which is in line with the MUN results.

Researchers who have studied different levels of crude protein (Farahani *et al*. 2017; Farahani *et al*. 2019) or different ratios of RDP to RUP with constant CP levels (Savari *et al*. 2018) on metabolic status of transition cows, have observed no effect on blood albumin or globulin concentrations. Globulin is known as a positive acute phase protein, and its hepatic synthesis typically increased during the inflammatory state (Bertoni *et al*. 2008). The data from this study suggest that higher globulin concentrations in the 5PBM group than in the 0PBM and 2.5PBM groups may be associated with some degree of inflammation. This finding is supported by the increase in serum NEFA levels in the 5PBM group (Table 6), which leads to impaired liver function and inflammation as a result of more processing of NEFA (Loor *et al*. 2007; Bertoni *et al*. 2008).

Cows fed the 5PBM diet had higher serum NEFA and BHB and lower serum cholesterol concentrations than cows fed the 0PBM and 2.5PBM diets. Pires *et al*. (1996) and Schor and Gagliostro (2001) found that supplementing diets with BM from 3 to 18 weeks of lactation and during the

first 8 weeks of lactation, had no effect on plasma NEFA concentrations. These contradictory results may be explained by differences in lactation period (fresh *vs.* early lactation) and nutritional demands of these cows.

High serum NEFA (>0.7 mmol/L) and BHB (>1.2 mmol/L) in immediately after calving cows is indicative of severe negative energy balance and greater risks of metabolic disorders incidence such as ketosis and displaced abomasum (McArt *et al*. 2013). In this study, although the average concentrations of NEFA and BHB among treatments were below 0.7 mmol/L and 1.2 mmol/L, respectively, in the first 14 d after calving, 4 out of 8 cows in the 5PBM diet exceeded 0.7 mmol/L NEFA and 1.2 mmol/L BHB suggesting that cow's energy requirements were not sufficiently met by the diet. However, cows fed the 0PBM and 2.5PBM diets might have mobilized less adipose tissue as indicated by lower negative energy balance and decreases in serum NEFA and BHB concentrations.

Cows fed the 5PBM diet had lower serum concentrations of cholesterol when compared to cows fed the 0PBM and 2.5PBM diets. Indeed, total plasma cholesterol indirectly measures the presence of very low density lipoprotein (VLDL) in the blood and thus the ability of the liver to produce VLDL (Kupczyński *et al*. 2011). Lower serum cholesterol in cows fed the 5PBMdietcompared to the 0PBM and 2.5PBM diets indicate conditions in which very-low-density lipoprotein (VLDL) production is likely limited by liver, which suggest impaired liver health and greater risk of fatty liver. Our findings are in accordance with earlier studies in which hepatic lipidos is was associated with lower serum cholesterol but higher NEFA concentrations (Holtenius, 1989; Kalaitzakis *et al*. 2010). Moreover, lower serum cholesterol in cows fed the 5PBM diet was supported by higher serum concentration of NEFA in postpartum.

The apparent total tract digestibility of DM in cows fed the 5PBM diet decreased compared to cows fed the 0PBM diet, but there was no difference between cows fed the 5PBM and 2.5PBMdiets. Apparent digestibility of CP, NDF, and ADF were similar among treatments. Similarly, Reynal and Broderick (2003) reported that digestibility of DM was lower in diets containing blood meal (5.4% DM) compared to diets containing SSBM (8.8% DM). However, digestibility of NDF and ADF was similar between PBM-diets and SSBM- diets. A factor that negatively affects rumen microbial activity is the reduction of DMI because fermentable energy stimulates microbial fermentation (NRC, 2001) and the lower DMI on the 5PBM diet might impair microbial activity and growth.

The body condition losses were expected to be higher in cows fed the 5PBM diet due to greater concentrations of serum NEFA and BHB during fresh period when compared to cows fed the 0PBM and 2.5PBM diets. However, neither BW nor BCS changes was affected by treatments in the present study, suggesting that BCS might not be sensitive enough during fresh period to detect losses of adipose depots that could alter metabolism and health of transition cows (Drackley *et al*. 2014). In agreement to our findings, Mantysaari *et al*. (1989) and Schor and Gagliostro (2001) reported lack of differences in BW and BCS changes when BM was supplemented into the diets.

CONCLUSION

Replacing 2.5% solvent soybean meal with poultry blood meal did not affect dry matter intake, milk yield, apparent digestibility of nutrients and blood metabolites in fresh Holstein cows. Thus, solvent soybean meal can replace up to 2.5% DM with poultry blood meal. However, levels higher than 2.5% DM poultry blood meal are not recommended due to low palatability and negative effects on the metabolic status of dairy cows. The utilization of livestock byproducts, apart from being an alternative feed ingredient, could also decrease environmental pollution.

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