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Antioxidant Activity and Milk Fatty Acids Profile of Murciano-Granadina Dairy Goats Feeding Formaldehyde-Treated Sesame Meal at Different Levels of Dietary Crude Protein

Research Article

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ABSTRACT

We evaluated the impact of partial substitution of soybean meal (SBM) for sesame meal (SM) and formaldehyde-treated SM (FTSM) on milk yield and fatty acids, and blood and milk antioxidant parameters in goats. Forty mid-lactation Murciano-Granadina goats were randomly allocated to four groups: (1) diet with 16.5% crude protein (CP) containing SBM (CON); (2) diet with 16.5% CP containing untreated SM (USM); (3) diet with 16.5% CP containing FTSM (FT); (4) diet with 14.5% CP containing FTSM (LPFT; low protein formaldehyde-treated SM). FT goats had higher dry matter intake (DMI) than CON and LPFT, as observed for milk, fat-corrected (FCM), and energy-corrected milk (ECM) (P<0.05). LPFT reduced C6:0 and C10:0 FAs in milk comparison to CON and USM, while C8:0 and C12:0 were higher CON and/or USM as compared with LPFT (P<0.01). The results showed that FTSM goats had the lowest BUN level (P=0.004). As compared with SBM and FTSM fed groups, the concentration of blood glutathione peroxidase (GPX) was lower in USM goats (P<0.01). Partial substitution of SBM for FTSM in the diet of dairy goats can improve milk yield. Furthermore, reducing CP level along with FTSM inclusion in the diet may be considered as a proper tool in compensating the adverse effects of CP deficiency and to optimize the productive performance of dairy goats utilizing lower amounts of nitrogen sources.

KEY WORDS antioxidant, formaldehyde treatment, lactating goat, milk yield, sesame meal.

INTRODUCTION

Soybean meal (SBM) is one of the conventional protein sources in animal diets which increases the animal production costs due to either their imports from other regions outside the country or being considered a commodity (Mahmoud and Bendary, 2014). Therefore, finding an alternative protein feed ingredient especially from by-products which can provide nutrient requirements of animals may be a good practice in lowering production costs and economical production of livestock. Among the by-product, sesame meal (SM) as a by-product of sesame seed oil extraction, is a relatively good quality plant crude protein (CP; 44%) which can be replaced partially to conventional protein sources such as SBM (Obeidat *et al.* 2009). The SM contains fibers and chemical compounds such as phenolic antioxidants as well as proper balance of amino acids (AA), especially sulfur AA such as methionine which is more than that of SBM (Dosky, 2012). Few studies have investigated the impact of SM in ruminants' diets. Obeidat *et al.* (2019) reported that the inclusion of SM in the diet improved the milk production of Awassi ewes.

Consumption of feedstuffs with high rumen-degradable protein content leads to the rapid degradation of CP and the production of large amounts of ammonia in the rumen that is lost through urine after conversion to urea by liver. Furthermore, oilseeds meal protection against microbial degradation in the rumen can affect the rate and site of digestion (McKinnon *et al.* 1995), therefore being utilized more efficiently. Various methods were evaluated for protecting proteins against ruminal degradation and increasing the amount of feed-based protein reaching post-ruminal parts; however, due to the high cost of physical and mechanical methods, it seems necessary to seek the effective chemical methods to enhance the nutritional quality of meals for ruminant nutrition. Formaldehyde treatment is an effective chemical method that is environmentally safe and can reduce dietary protein degradation in the rumen (Mahima Kumar *et al.* 2015). Formaldehyde toxicity was studied in rats, rabbits, and dogs after oral feeding, and the LD_{50} was 800, 270, and 550 mg/kg of body weight, respectively (NCBI, 2023), indicating the low toxicity of formaldehyde. Formaldehyde significantly reduces the solubility of protein and makes it very resistant to microbial attack in the rumen without affecting its digestibility in the small intestine (Sanjukta and Rai, 2016).

Furthermore, reducing the use of protein supplements in the diet by improving the efficiency of dietary protein utilization without adverse effects on productive performance are of high importance, which can also have environmental benefits (Rotz *et al.* 1999). Little studies have evaluated the effects of formaldehyde-treated SM in dairy goats feeding. Therefore, the purpose of this study was to investigate the impact of partial substitution of SBM by formaldehyde-treated SM (FTSM; 0.8%) at different levels of dietary CP on milk production and composition, and blood and ruminal parameters in Murciano-Granadina dairy goats.

MATERIALS AND METHODS

Animals, diets and experimental design

At week 16th of lactation, forty multiparous Murciano-Granadina dairy goats were randomly assigned to one of four treatments. The experimental diets were: (1) diet with 16.5% CP containing SBM (CON); (2) diet with 16.5% CP containing untreated SM (USM); (3) diet with 16.5% CP containing FTSM (0.8 g formaldehyde/100g CP) (FT); (4) diet with 14.5% CP containing FTSM (0.8 g formaldehyde/100g CP) (LPFT). Goats (CON, 43.4 ± 2.3 kg; USM, 43.7 ± 2.6 kg; FT, 42.8 ± 2.2 kg; LPFT, 43.5 ± 2.8 kg of BW and 1.95 ± 0.31 kg/d average initial milk production) were housed in individual pens (1.5 × 2 m) for 70 days including a 14 day of adaptation followed by a 56 day of data collection period. The experimental diets were isocaloric and formulated to meet the nutrient requirements of dairy goats according to (NRC, 2007) (Table 1). The diets concentrate: forage ratio were 62:38 (DM basis) and were served as TMR twice daily (at 08:00 and 16:00 h) for *ad libitum* access, free access to fresh water was provided throughout the experiment.

Sesame meal processing

In order for treating SM with formaldehyde, the formaldehyde was diluted with water and sprayed on the meal (0.8% of SM crude protein), mixed thoroughly for 15 minutes, then stored in nylon bags for 48 hours. Thereafter, the bags were opened and FTSM was dried in the shade for three days before being incorporated into experimental diets (Tajaddini *et al.* 2021).

Milk production and chemical analysis

During the experimental period, all goats were milked twice daily with a fully automatic machines and their milk production was recorded. In addition, daily milk fat, protein and lactose production was calculated by multiplying daily milk production by the concentrations of these contents. Another set of milk samples was stored at −20 °C for determination of milk fatty acids (FAs) profile. For this purpose, milk fat was extracted according to the method described by (Bouattour *et al.* 2008) and after methylation, milk FAs profile was determined using gas chromatography (GC; 3400 Varian Star; Varian Inc., Palo Alto, CA) equipped with capillary column (CP-SIL-88- 0.25 mm × 60 m).

Helium gas was used as the carrier gas. The temperature of the column was initially 50 °C for 1 minute and then gradually increased (4 °C per minute) to 190 °C. The injector temperature was 280 °C and the detector temperature was 300 °C.

Blood parameters

Blood samples were collected via jugular vein through vacuumed tubes containing lithium heparin anticoagulant on the last day of the experiment at 3 hours post-feeding. After that, the samples were placed on ice and centrifuged at 6000 rpm for 15 minutes and serum were collected, and stored at –20 °C until analysis for glucose, cholesterol, triglyceride, blood urea nitrogen (BUN), albumin, total protein, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), aspartate aminotransferase (AST) and alanine transaminase (ALT) using laboratory kits (Pars-Azmun Diagnostics, Tehran, Iran).

In order to determine the total antioxidant capacity (TAC) and malondialdehyde (MDA) in serum and milk, samples were stored at −70 °C until the analysis. The level of MDA in plasma and milk was determined using thiobarbituric acid method (Moore and Roberts, 1998).

Table 1 Ingredients and chemical composition of the experimental diets fed to lactating goats

Item	Experimental diets			
	CON	USM	FT	LPFT
Ingredients (% DM)				
Alfalfa hay, chopped	16.00	16.00	16.00	16.00
Corn silage	16.30	16.30	16.30	16.30
Wheat straw, chopped	2.00	2.00	2.00	2.00
Corn grain, ground	16.00	16.00	16.00	17.00
Barley grain, ground	11.00	11.00	11.00	12.00
Soybean meal	18.80	7.50	7.50	2.00
Sesame meal	0.00	12.50	12.50	12.50
Beet pulp	4.00	4.00	4.00	4.00
Wheat bran	12.70	11.50	11.50	15.00
Sodium bicarbonate	1.40	1.40	1.40	1.40
Calcium carbonate ($CaCO_3$)	0.30	0.30	0.30	0.30
Mineral-vitamin premix[1]	1.20	1.20	1.20	1.20
Salt	0.30	0.30	0.30	0.30
Chemical composition (g/kg DM)				
Metabolizable energy (Mcal/kg DM)	2.61	2.61	2.61	2.61
Crude protein (CP)	166	167	167	146
Rumen undegradable protein (RUP) (% CP)	28.7	28.6	36.2	34.6
Rumen degradable protein (RDP) (% CP)	71.3	71.4	63.8	65.4
Dry matter (DM)	615	616	613	616
Organic matter (OM)	921	919	918	919
Ether extract (EE)	26.40	27.20	27.20	28.50
Neutral detergent fiber (NDF)	280	280	280	285
Acid detergent fiber (ADF)	200	200	200	200

CON: diet with 16.5% crude protein (CP) containing SBM; USM: diet with 16.5% CP containing untreated SM; FT: diet with 16.5% CP containing SM treated with 0.8 g formaldehyde/100g CP) and LPFT: diet with 14.5% CP containing SM treated with 0.8 g formaldehyde/100g CP.

[1] Each kg of the premix contained (DM basis): vitamin A: 500000 IU; vitamin D: 100000 IU; vitamin E: 2000 IU; Ca: 190000 mg; P: 25000 mg; Na: 40000 mg; Mg: 30000 m; Zn: 5000 mg; Mn: 3500 mg; Fe: 2500 mg; Cu: 400 mg; Co: 35 mg; I: 90 mg and Se: 40 mg.

Milk and blood TAC was determined by ferric reduction in antioxidant power (FRAP) method (Benzie and Strain, 1996). All measurements were taken in five replicates and in accordance with the manufacturer's instructions.

Statistical analysis

All data were analyzed as a completely randomized design using GLM procedure of SAS v. 9.1.3 (SAS, 2003). The statistical model used as follows:

$$Y_{ij} = \mu + T_i + A_j + e_{ij},$$

Where:

Y_{ij}, μ, T_i, A_j and e_{ij}: dependent variable, the overall mean, the fixed effect of treatment, the random effect of animal, and the random residual error, respectively.

Differences among treatment means were tested by the Tukey-Kramer test. The results were expressed as least square mean (LSM) ± standard error of means (SEM). Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Daily intake of dry matter was higher when feeding in the FT diet than that in the CON and LPFT diets, which was lower with LPFT followed by CON (P<0.05). Goats offered the FT diet had more daily milk, FCM, ECM and TSCM yields than those fed the CON and LPFT diets (P<0.01). The FT and USM diets had a similar effect on these variables (Table 2). Also, goats in the USM group (similar to FT) produced higher FCM and ECM than those in the CON and LPFT groups (P<0.001).

Feeding LPFT reduced C6:0 and C10:0 FAs in milk in comparison to CON and USM, while C8:0 and C12:0 were higher CON and/or USM as compared with LPFT (P<0.01) (Table 3). In addition, the milk contents of C18:1-cis and C18:3-cis increased significantly by feeding FTSM compared to CON (P<0.001).

Dietary treatments had no effects on blood biochemical parameters including glucose, triglycerides, cholesterol, HDL, LDL, VLDL, total protein, albumin, AST, ALT and hemoglobin except for BUN which was decreased when feeding FTSM compared to CON (P=0.004) with USM and FT being intermediate (Table 4).

Results from blood and milk antioxidant activity indices of experimental groups are shown in Table 5. As compared with SBM and FTSM fed groups, the concentration of blood glutathione peroxidase (GPX) was lower in USM goats (P<0.01). However, milk antioxidant indices were not affected by dietary treatments.

Table 2 Milk yield in lactating goats fed experimental diets

Item	Experimental diets				SEM	P-value
	CON	USM	FT	LPFT		
Dry matter intake (g/day)	1629^{b}	1683^{ab}	1749^{a}	1641^{b}	19.1	0.005
Yield (g/day)						
Milk	1922^{bc}	2152^{ab}	2288^{a}	1878^{c}	85.5	0.006
FCM 4%	2139^{b}	2429^{a}	2523^{a}	2025^{b}	86.2	<0.001
ECM	2034^{b}	2298^{a}	2409^{a}	1936^{b}	83.3	<0.001
TSCM	1921^{bc}	2171^{ab}	2280^{a}	1669^{c}	93.9	0.001

CON: diet with 16.5% crude protein (CP) containing SBM; USM: diet with 16.5% CP containing untreated SM; FT: diet with 16.5% CP containing SM treated with 0.8 g formaldehyde/100g CP) and LPFT: diet with 14.5% CP containing SM treated with 0.8 g formaldehyde/100g CP.
FCM: fat corrected milk; ECM: Energy corrected milk and TSCM: total solids-corrected milk.
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.

Table 3 Milk fatty acids profile (g/100 g of total fatty acids) of lactating goats fed experimental diets

Fatty acids	Experimental diets				SEM	P-value
	CON	USM	FT	LPFT		
C4:0	1.48	1.45	1.41	1.42	0.02	0.090
C5:0	0.45	0.46	0.43	0.43	0.01	0.210
C6:0	2.48^{a}	2.53^{a}	2.27^{ab}	2.07^{b}	0.09	0.003
C8:0	0.45^{ab}	0.47^{a}	0.36^{c}	0.39^{bc}	0.02	0.009
C10:0	9.34^{a}	8.42^{ab}	8.03^{ab}	7.12^{c}	0.37	0.002
C10:1	0.34	0.35	0.33	0.34	0.02	0.690
C12:0	4.15^{a}	3.77^{ab}	3.81^{ab}	3.34^{b}	0.18	0.010
C12:1-cis	0.53	0.53	0.57	0.52	0.03	0.640
C12:1-trans	0.31	0.32	0.34	0.35	0.03	0.980
C14:0	11.8	11.1	10.6	10.5	0.44	0.070
C14:1-cis	0.82	0.80	0.78	0.85	0.02	0.230
C14:1-trans	0.17^{b}	0.21^{a}	0.20^{a}	0.22^{a}	0.01	0.040
C15:0	1.16	1.12	1.08	1.05	0.08	0.710
C15:1	0.39	0.41	0.40	0.44	0.03	0.730
C16:0	30.7	27.3	28.5	29.3	1.17	0.240
C16:1-cis	1.29	1.41	1.47	1.36	0.05	0.340
C16:1-trans	0.43	0.44	0.46	0.47	0.02	0.760
C17:0	0.77	0.80	0.79	0.79	0.04	0.960
C17:1	0.41	0.44	0.43	0.44	0.03	0.810
C18:0	9.33	10.34	9.90	9.67	0.48	0.520
C18:1-cis	15.2^{b}	19.1^{a}	19.7^{a}	19.9^{a}	0.55	<0.001
C18:1-trans	2.52	2.41	2.29	2.28	0.07	0.090
C18:2-cis	3.14	3.28	3.41	3.51	0.11	0.170
C18:2-trans	0.31	0.35	0.29	0.27	0.02	0.110
C18:3-cis	0.85^{b}	0.93^{b}	1.21^{a}	1.33^{a}	0.04	<0.001
C18:3-trans	0.01	0.02	0.01	0.01	0.001	0.110
C20:0	0.22	0.23	0.24	0.23	0.03	0.980
C20:1	0.26^{b}	0.27^{b}	0.32^{ab}	0.33^{a}	0.02	0.040
C22:0	0.14	0.14	0.15	0.16	0.02	0.750
C22:1	0.24	0.27	0.25	0.28	0.02	0.770
C24:0	0.12	0.15	0.15	0.15	0.02	0.490
C24:1	0.11	0.13	0.12	0.12	0.02	0.810

CON: diet with 16.5% crude protein (CP) containing SBM; USM: diet with 16.5% CP containing untreated SM; FT: diet with 16.5% CP containing SM treated with 0.8 g formaldehyde/100g CP) and LPFT: diet with 14.5% CP containing SM treated with 0.8 g formaldehyde/100g CP.
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.

Today's ongoing global challenge is to optimize the use of protein and fat supplements in various production systems, which is mostly focused on the quality of produced milk/meat in developed countries, rather than raising the quantity of production in developing countries (Gulati *et al.* 2005). To evaluate the hypothesis whether reducing level of dietary CP could be well compensated by providing more protein bypass, without adverse effects on animal performance, CP level of LPFT was reduced 2% by replacing FTSM for SBM, which increased RUP content 6-8 percent compared to CON diet. Incorporation of FTSM in diet with 16.5% CP enhanced DMI compared with other treatments.

Table 4 Blood plasma metabolites of lactating goats fed experimental diets

Item	Experimental diets				SEM	P-value
	CON	USM	FT	LPFT		
Glucose (mg/dL)	53.9	55.6	54.1	53.3	2.76	0.940
Triglyceride (mg/dL)	16.8	20.6	17.1	25.1	4.52	0.490
Cholesterol (mg/dL)	84.9	88.3	85.1	91.7	5.99	0.830
HDL (mg/dL)	41.1	40.3	41.1	37.5	4.24	0.920
LDL (mg/dL)	40.5	43.9	40.5	49.4	7.43	0.820
VLDL (mg/dL)	3.36	4.12	3.42	5.00	0.85	0.490
Total protein (g/dL)	7.36	6.87	7.89	7.31	0.26	0.070
Albumin (g/dL)	4.06	3.81	4.15	4.03	0.16	0.520
BUN (mg/dL)	22.21^{a}	19.18^{ab}	20.21^{ab}	16.90^{b}	1.14	0.004
AST (IU/L)	116	110.8	108.4	115.6	8.28	0.890
ALT (IU/L)	20.20	18.10	17.60	18.90	1.44	0.610
Hemoglobin (g/dL)	11.50	12.30	11.60	12.20	0.39	0.240

CON: diet with 16.5% crude protein (CP) containing SBM; USM: diet with 16.5% CP containing untreated SM; FT: diet with 16.5% CP containing SM treated with 0.8 g formaldehyde/100g CP) and LPFT: diet with 14.5% CP containing SM treated with 0.8 g formaldehyde/100g CP.
HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; BUN: blood urea nitrogen; AST: aspartate aminotransferase and ALT: alanine transaminase.
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.

Table 5 Antioxidant activity of milk and blood in lactating dairy goats fed experimental diets

Item	Experimental diets				SEM	P-value
	CON	USM	FT	LPFT		
Blood						
SOD (IU/gHb)	1547	1476	1494	1439	68.70	0.730
GP_X (IU/gHb)	69.70^{a}	60.60^{b}	68.30^{a}	68.80^{a}	2.49	0.010
MDA (nanomol/mL)	2.24	2.11	1.89	2.37	0.22	0.460
TAC (mmol Fe^{2+}/L)	0.40	0.46	0.43	0.39	0.02	0.090
Milk						
MDA (nanomol/mL)	0.78	0.74	0.74	0.77	0.03	0.850
TAC (mmol Fe^{2+}/L)	1.45	1.61	1.55	1.52	0.05	0.200

CON: diet with 16.5% crude protein (CP) containing SBM; USM: diet with 16.5% CP containing untreated SM; FT: diet with 16.5% CP containing SM treated with 0.8 g formaldehyde/100g CP) and LPFT: diet with 14.5% CP containing SM treated with 0.8 g formaldehyde/100g CP.
SOD: superoxide dismutase; GP_X: glutathione peroxidase; MDA: Malondialdehyde and TAC: Total antioxidant capacity.
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.

Treating oilseed meals in order to protect proteins from ruminal degradation by microorganisms increase the dietary bypass protein and enhances the flow of peptides and essential AA for absorption through the small intestine which helps the balance of absorbed AA and subsequently promotes feed intake and immune system (Baker *et al.* 1996).

Similar to our results, Tajaddini *et al.* (2021) reported that DMI increases when goats are fed with formaldehyde-treated canola meal, which can be mainly attributed to higher dietary rumen undegradable protein (RUP) content of diets by using formaldehyde-treated canola meal, leading to an improved balance of AA post-ruminally (Forbes, 1995).

The increased milk, fat-corrected milk (FCM), energy-corrected milk (ECM) and Total solids-corrected milk (TSCM) yields by FTSM compared with diets containing SBM or LPFT diet might be attributed to the fact that treating protein sources with formaldehyde increases dietary metabolizable protein and essential AA bioavailability for intestinal absorption, especially through the duodenum (Yörük *et al.* 2016), thereby providing the mammary gland with higher levels of limiting AA for milk and milk components synthesis. Our findings are inconsistent with those of Tajaddini *et al.* (2021), who reported that goats fed formaldehyde-treated canola meal in low CP diet produced more milk compared to those fed control diets. However, LPFT was equal to the CON group in milk production and FCM, indicating that utilization of dietary CP was appropriately optimized.

All of the SCFA and about half of MCFA in ruminant milk are synthesized *de novo* primarily in the mammary gland by acetate and β-hydroxybutyrate as the main precursors (Kitessa *et al.* 2003). Moreover, the presence of LCFA has been reported to reduce the expression of genes involved in the *de novo* pathway which consequently inhibits *de novo* synthesis of milk short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) (Grummer, 1991) related to the high contents of PUFA in SM. On the other hand, since SM contains high amount of PUFA, the increase in milk unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in response to feeding SM containing diets might be explained by PUFA escaped ruminal biohydrogenation, absorbed and incorporated into milk FA profile. Given that MCFA makes up the hypercholesterolemic fraction of milk fat, lowering MCFA is suggested as a favorable alteration to enhance FA profile of milk from the human health aspects (Shingfield *et al.* 2013).

Experimental diets had no effect on blood glucose, triglycerides and cholesterol. The concentration of blood triglyceride and cholesterol is an indicator of the animal's energy status, and similarity of these parameters among various dietary groups of the present study indicate animals' suitable energy status. In accordance with our results, no impacts on blood glucose and cholesterol levels were found by substitution of SM for SBM in the diet of dairy cows (Ashjae *et al.* 2021). However, Ababakri *et al.* (2021) reported that feeding extruded flaxseed with high levels of RUP increased blood glucose and cholesterol concentrations of ewes. Unchanged liver enzymes such as ALT and AST in response to feeding dietary treatments suggests that either formaldehyde-treated or untreated SM consumption had no adverse effects on the liver. The nitrogen balance in ruminants is generally evidenced by BUN concentration and high BUN levels are associated with high levels of dietary RDP, which is degraded into ammonia in the rumen (Wanapat and Pimpa, 1999). The lower BUN content of the LPFT goats might be attributed to lower ruminal NH_3-N, also lower CP compared to other groups (Wright *et al.* 2005) reported decreased blood and milk urea nitrogen when dairy cows fed heat-treated canola meal.

Sesame seed contains many bioactive compounds including phytosterols, tocopherols and lignans such as sesamin, sesamolin and sesaminol which are considered to have notable antioxidant properties (Lee *et al.* 2007). Sesamin, one of the most important furfuran-type lignan compounds present in sesame seeds, oil, and meal serves a number of crucial functions including its antioxidant capacity (Jin *et al.* 2005), enhance the immune function, anti-cancer activity (Hristov and Giallongo, 2014). In comparison to USM, FTSM increased the level of blood GPx, indicating an effective method of protection. Our results corroborate with those obtained by (Tsiplakou *et al.* 2021), who observed that feeding goats a diet containing SM with Se and vitamin E improved their health and the oxidative status of their milk. In addition, sesame seed inclusion in the diet of goats increased the blood antioxidant activity and milk oxidative stability (Mitsiopoulou *et al.* 2021).

CONCLUSION

In conclusion, partial substitution of SBM for FTSM (about 65%) in the diet of lactating Murciano-Granadina goats improved DMI and milk yield. Moreover, regarding the same results for most of studied parameters in CON and LPFT goats, inclusion of FTSM in low CP diet might be used as a cost-effective strategy to reduce feed costs as well as environmental pollution resulting from greater nitrogen emissions in dairy farms rather than SBM.

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