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Effect of Feeding of Various Types of Soybean Meal and Differently Processed Barley Grain on Performance of High Producing Lactating Holstein Dairy Cows

Research Article

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ABSTRACT

Twenty-four lactating Holstein dairy cows were used in a completely randomized design to investigate the effect of soybean meal (SM) and xylose protected soybean meal (XSM) in combination with ground (GB) or steam flaked (FB) barley on dry matter intake, milk production, rumen fermentation and blood responses. The experimental period lasted for 30 days. The diets consisted of the provision of 19.85% corn silage, 12.02% alfalfa hay, 0.72% wheat straw and 67.41% concentrate that averaged 17% crude proteins (DM basis) were offered two times daily. Dry matter intake (DMI) (P=0.051) and milk yield (P<0.0001) decreased by XSM in diets with GB or FB (P<0.05). The animals fed diets containing XSM had lower fat corrected milk (FCM 3.5%) and energy corrected milk (ECM) than diets containing SM (P<0.05). The milk fat and solids not fat percentage in XSM were significantly higher than SM (P<0.05) but milk protein percentage was lower (P<0.05). A comparison of GB versus FB and SM versus XSM diets indicated that the milk protein yield was higher for diets containing FB (P<0.05) and containing SM (P<0.001). Blood metabolites were significantly different between treatments (P<0.05). The diets containing XSM had lower levels of non-esterified fatty acids and higher levels blood urine nitrogen than those containing SM (P<0.05). The Acetate, propionate, valerate and butyrate concentrations differed significantly between the diets (P<0.05). In according to the results of our experiment, soybean meal in combination with ground barely may alter the performance of Holstein dairy cows.

KEY WORDS barley grain, Holstein cows, soybean meal, steam flaked, xylose protected soybean meal.

INTRODUCTION

Ruminants show less efficient use of nitrogen (about 25%) compared to other animals (Calsamiglia *et al.* 2010). Increasing levels of crude protein in the diet can have a negative effect on reproduction and the environment (Sinclair *et al.* 2014). Carbohydrates are major energy source for rumen microbiota. If carbohydrate and protein degradation does not occur in rumen synchronously, microbial protein synthesis may decrease (Bach *et al.* 2005). Therefore, the goal of protein nutrition in ruminants is to supply a sufficient amount ruminally degradable protein (RDP) for ruminal requirements to increase animal productivity (NRC, 2001). Traditionally, soybean meal (SM) has been widely used as the main protein source in dairy cow. Because soybean meal contains highly degradable protein in the rumen, therefore many attempts have been made to decrease the rate and extent of its rumen degradability, including the use of heat (Nakamura *et al.* 1992), formaldehyde (Mohamed *et al.* 2001), lignosulfonate (Windschitl and Stern, 1988; Ure *et al.* 2005), acetic acid (Atwal *et al.* 1995) and xylose (Harstad and Prestlokken, 2000). The processing of soybean meal with xylose decreases the degradability of protein in the rumen as a result of the Maillard reaction between sugar aldehyde and free amino groups (Nakamura *et al.* 1992).

The rate and extent of ruminal degradation of barley could also be affected by processing method used. The increase of digestibility of the barley grain through physical processing such as milling or rolling has been well studied (Mathison *et al.* 1991). However, in our knowledge, the appropriate temperature of processing of barley fed to dairy cattle is not known. It has been noted that steam flaking is more efficient because the heat used disrupts the protein matrix surrounding the starch granules in the grain and increases starch digestibility in animals (Huntington, 1997).

The NRC (2001) reports that, for diets deficient in ruminally-undegraded protein (RUP), milk yield and milk protein yield increase linearly with the increase in the dietary RUP level. Some studies have shown that xylose treated soybean meal in comparison with untreated soybean meal did not effect on dry matter intake and milk production (Reynal and Broderick, 2006).

Contradictory data exist on the effect of increasing dietary RUP by replacing soybean meal with treated soybean meal. Broderick *et al.* (1990) reported an increase in milk production, but no difference in milk protein percentage or yield, when replacing soybean meal with treated soybean meal in diets with 19.1% crude protein (CP), where alfalfa silage is the primary forage source. All efforts are to increase efficiency of nitrogen in dairy cows and minimize its execration in the environment. This goal attained by increasing the synchronization of energy and protein in rumen (Malekjahani *et al.* 2017). The objective of the current study was to compare the effects of replacing a part of SM with xylose protected soybean meal (XSM, as a main source of RUP in diet) in combination with ground (GB) or steam flaked (FB) barley on rumen fermentation and the responses of lactating Holstein dairy cows.

MATERIALS AND METHODS

Animals and diets

This study was carried out using 24 multiparous high producing Holstein dairy cows (body weight (BW)= 684±31 kg; days in milk (DIM)= 133±5 d; milk yield= 51.2±2 kg/day) which were randomly assigned to 4 treatments (n=6) over 30-day period. Each experimental period consisted of 10 days of adaptation, then 10 days to feed experimental diets and followed by 10 days of data collection. Cows were kept in the separate stalls and were allowed *ad libitum* access to total mixed ration (TMR). All cows received diet GB + SM (Table 1) in adaptation period. The TMR was prepared freshly each feeding time per day (7 a.m. and 3 p.m.). Cows milked three times daily using a milking parlor at 5:00, 13:00, and 21:00 h. Prior to each milking, cows were checked for udder inflammation and existence of milk clots in the nipples to ensure that milk yield and composition did not affect by different forms of mastitis. Dietary ingredients, chemical composition and energy content of the experimental diets are shown in Table 1. The iso-nitrogenous and iso-caloric diets were provided to meet the animals' requirements as proposed by national research council (NRC, 2001). In the experimental diets, barley grain was used as GB or FB accompanied with SM or XSM (Yasminomax®). The dietary treatments were GB + SM, GB + XSM, FB + SM and FB + XSM. Full-fat soybean and fishmeal were not used in the two experimental diets containing XSM.

Sampling

Daily feed allotments to each cow were regulated to allow minimal (<5%) feed refusals in the feed bunk. The volumes of feed offered and feed refused were weighed daily. Daily DM intake was assigned by deducting the DM content of the orts from that of the TMR consumed every day. Feed samples were compound by period, weighed, were dried at 60 ˚C for 48 h using air-forced oven and milled in a Wiley mill (standard model 4; Arthur H. Thomas Co., Philadelphia, PA, USA) to passage a 1-mm screen. During the 10 d of sample collection, milk yield was specified for all cows. The volume of milk produced for each cow at each milking measured using special graduated jars (Agri and SD Co., Frankfurt, Germany). Milk sampled at each milking in prelabeled 50 mL plastic vials and milk samples combined on an individual cow basis. Three milk samples were taken from each cow per sampling period.

Blood samples were taken in tubes containing sodium heparin by jugular venipuncture on the first and last day of collection period at 4 h after the morning feeding. Blood samples were centrifuged (4000 g for 10 min at 5 ˚C) (Rotofix 32A, Hettich®, Germany); plasma was gathered by micropipette (Transferpette®S, Brand, Germany) and frozen at −20 ˚C until analysis. On the last day of collection period, 4 h after the morning feeding, a sample of rumen fluid was collected from each cow using a stomach tube. Fecal samples were taken over five continuous days from each cow daily and all were frozen for later analysis of acid insoluble ash (AIA).

Feeding behavior

The ruminating behavior of individual animals were visually observed and recorded at 10-min intervals for 24 h on day 18 of each period.

Table 1 Ingredients (% DM), chemical composition and energy content of the experimental diets

Ingredient (% DM)	Diets			
	GB + SM	GB + XSM	FB + SM	FB + XSM
Alfalfa hay	12.02	12.02	12.02	12.02
Corn silage	19.85	19.85	19.85	19.85
Wheat straw	0.72	0.72	0.72	0.72
Barley grain, ground	20.4	20.4	0	0
Barley grain (steam flaked)	0	0	20.4	20.4
Corn grain, ground	14.41	14.41	14.41	14.41
Soybean meal	11.05	8.14	11.05	8.14
Xylose protected soybean	0	6.48	0	6.48
Cottonseed	4.44	5.26	4.44	5.26
Linseed	1.07	1.48	1.07	1.48
Full-fat soybean	2.57	0	2.57	0
Fish meal	2.76	0	2.76	0
Fat prill[1]	1.04	1.57	1.04	1.57
Sugar beet pulp	5.65	5.65	5.65	5.65
Molasses	1.18	1.18	1.18	1.18
Calcium carbonate	0.54	0.54	0.54	0.54
Sodium bicarbonate	0.95	0.95	0.95	0.95
Di-calcium phosphate	0.11	0.11	0.11	0.11
Magnesium oxide	0.34	0.34	0.34	0.34
Salt	0.27	0.27	0.27	0.27
Vitamin and mineral premix[2]	0.63	0.63	0.63	0.63
Chemical composition[3], % of DM				
Crude protein (CP, %)	17	17	17	17
Ruminally degradable protein (RDP, % of CP)	63	71	63	71
Ruminally-undegraded protein (RUP, % of CP)	37	29	37	29
Neutral detergent fiber (NDF, %)	28.3	28.8	28.3	28.8
Acid detergent fiber (ADF, %)	17.9	18.2	17.9	18.2
Non structural carbohydrate (%)	44.7	46.2	44.7	46.2
Ether extract (EE, %)	4.9	4.9	4.9	4.9
NE_L (net energy for lactation, Mcal/kg)	1.64	1.64	1.64	1.64

[1] Fat prill: gracefill industries made by malaysia.
[2] Provided (per kg of DM): vitamin A: 1100000 IU; vitamin D_3: 300000 IU; vitamin E: 10000 IU; Ca: 130000 mg; P: 60000 mg; Mg: 20000 mg; Mn: 6000 mg; Zn: 15700 mg; Cu: 5000 mg; Se: 150 mg; I: 180 mg; Co: 180 mg and Antioxidant: 1000 mg.
[3] Crud protein, NDF, ADF and EE were analyzed and RDP, RUP, NFC and NE_L were calculated.
GB + SM: ground barley + soybean meal; GB + XSM: ground barley + xylose protected soybean; FB + SM: steam flaked barley + soybean meal; FB + XSM: steam flaked barley + xylose protected soybean and XSM: xylose protected soybean meal (Yasminimax®) containing (DM: 93%, NDF: 12.7%, ADF: 13.4%, CP: 53.4%, RUP (70% CP), Ash: 8.36%, EE: 8.16%) was provided from Iranian local company named Yasnamehr. All compositions were analyzed.

We presumed that a particular chewing incident persisted for the entire 10-min period between sequential visual observations. Chewing behavior was declared as the total minutes during a 24-h period.

Chemical analysis

All feed samples were analyzed for nitrogen (Kjeldahl procedure 988.05; AOAC, 2000), neutral detergent fiber (NDF) (Van Soest *et al.* 1991), acid detergent fiber (ADF) (973.18; AOAC, 2000), ether extract (920.39; AOAC, 2000), ash (942.05; AOAC, 2000) and acid insoluble ash (AIA; Van Keulen and Young, 1977).

Milk composition of each sample was determined using milkoscaner FT (4000). The DM and AIA of the fecal samples were also determined using the above procedures. Concentrations of plasma glucose (Trinder, 1969) and plasma urea nitrogen (PUN) (Talke and Schubert, 1965) were determined using commercial kits according to the manufacturer's guidelines (Zist-Shimi Co., Tehran, Iran). Commercial enzymatic kits were used to analyses plasma b-hydroxybutyrate (BHB) (D-3-hydroxybutyrate kit, AL1027, Randox Laboratories Ltd, Antrim, UK). Analysis was performed by an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy).

Ruminal pH was determined using a portable pH meter (Metrohm 744, Herisau, Switzerland) on fresh samples and the rumen fluid was then strained through four layers of cheesecloth and prepared for subsequent ammonia-N and volatile fatty acids (VFA) analysis. For ammonia-N determination, 5 mL of rumen fluid from each collection point (except 4.5, 5.5, 6.5 and 7.5 h) was acidified with 5 mL of 0.2 N HCl and then analyzed for ammonia-N concentration using the distillation method (Kjeltec Auto Analyzer, Model 1030, Tecator Co., Sweden).

For VFA analysis, 5 mL of rumen fluid (collected 4 h after the morning feeding) was mixed with 1 mL of 250 g/L meta-phosphoric acid. The concentrations of VFA were determined by gas chromatography (Chrompack, Model CP-9002, Chrompack, EA Middelburg, Netherlands) with a 50-m (0.32 mmID) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA, USA). Helium was used as carrier gas and oven initial and final temperatures were 55 and 195 ˚C, respectively; detector and injector temperatures were set at 250 ˚C. Crotonic acid (1:7 v/v) was used as internal standard.

Statistical analysis

Data for blood metabolites, nutrient digestibility, ruminal fluid measurements and rumination activity were analyzed using analysis of variance (ANOVA) procedure of SAS (SAS, 2003) for a randomized complete design with the following statistical model:

$$Y_{ij} = \mu + T_j + e_{ij}$$

Where:
Y_{ij}: dependent variable.
μ: overall mean.
T_i: effect of treatment.
e_{ij} : experimental error.

The ANOVA for averages of feed intakes, milk yield, energy corrected milk (FCM) (3.5%), energy corrected milk (ECM), milk composition, and feed efficiency were analyzed according to a completely randomized design with the repeated measures (days) using PROC MIXED procedure of SAS (SAS, 2003). The statistical model used was as follows:

$$Y_{ijk} = \mu + T_i + P_j + A_k + C_l + TP_{ij} + e_{ijkl}$$

Where:
Y_{ijk}: dependent variable.
μ: overall mean.
T_i: effect of treatment i.
P_j: effect of sampling period j.
A_k: random effect of animal.
C_l: covariate factor (milk yield of beginning experiment).
TP_{ij}: effect of contrast between time and treatment.
ε_{ijk}: effect of experimental error.

Data analyzed in SAS (SAS, 2003). The criteria of significant between treatments was differences more than two times standard error of the means (P<0.05) (Danesh Mesgaran and Stern, 2005).

The differences between the means were evaluated using Duncan test at P ≤ 0.05. Predesigned differences used to compare the groups of treatments. Treatment effects tested for the following orthogonal contrast: 1) GB *vs*. FB; 2) SM *vs*. XSM. Contrasts were considered significant when the P ≤ 0.05.

RESULTS AND DISCUSSION

Dry matter intake, milk yield and composition, and feed efficiency

Table 2 shows the data for dry matter intake, milk yield, FCM 3.5%, ECM, milk composition and yield of milk nutrients. DMI, milk yield, FCM 3.5% and ECM were affected by the treatments (P<0.05) with their highest values recorded for the FB + SM diet. The lowest values (DMI and milk yield) and (FCM 3.5% and ECM) were recorded for FB + XSM and GB + XSM, respectively. The diets containing XSM had the lower milk yield (P<0.0002), FCM 3.5% (P<0.05) and ECM (P<0.003) than diets containing SM. Milk fat, protein and solids not fat (SNF) concentrations were significantly different between diets (P<0.05). The maximum fat, protein and SNF percentage were recorded for FB + XSM, FB + SM and GB + XSM, respectively. The percentage of protein and SNF for GB *vs*. FB and the percentage of Fat, protein and SNF for SM *vs*. XSM differed (P<0.05). The milk composition yield (Fat, protein and SNF) were affected by the treatments (P<0.05). The highest yield of fat, protein and SNF were for FB + XSM, FB + SM and GB + XSM, respectively. Milk protein yield was highest in SM diets (P<0.0001). The ratio of ECM to DMI was different among treatments (P<0.05) and by using XSM in diets, the ratio of ECM to DMI was decreased.

Nutrient digestibility, rumen fluid and rumination activity

Table 3 shows the values for nutrient digestibility, ruminal pH and volatile fatty acids (VFAs) concentration and the data for rumination. DM and CP digestibility differed significantly between treatments (P<0.05). The highest and lowest DM and CP digestibility values belonged to GB + XSM and FB + XSM, respectively. The mounts of acetate, propionate, butyrate, valerate and the acetate/propionate ratio differed significantly among treatments (P<0.05). The maximum concentrations of acetate, propionate and valerate were recorded for GB + SM and of butyrate was for FB + XSM. The maximum and minimum acetate/propionate ratio was for the FB + SM and GB + SM diets, respectively. The acetate/propionate ratio for GB *vs*. FB was significant (P<0.05). The rumen pH was significantly highest in GB + XSM (P<0.05) compared with the other diets.

Table 2 Dry matter intake, milk production and composition of Holstein dairy cows fed diets differing in physically processed barley grain and types of soybean meal

Item	Diets				SEM	(P-value)	
	GB + SM	GB + XSM	FB + SM	FB + XSM		GB *vs.* FB	SM *vs.* XSM
Dry matter intake (kg/d)	26.07[b]	25.98[b]	27.86[a]	25.77[b]	0.56	0.16	0.051
Milk yield (kg/d)	48.88[ab]	47.46[bc]	49.75[a]	46.78[c]	0.78	0.61	< 0.0002
FCM 3.5% (kg/d)	42.00[a]	40.04[b]	42.14[a]	40.10[b]	0.78	0.90	0.011
ECM[1] (kg/d)	43.13[a]	40.93[b]	43.64[a]	40.98[b]	0.79	0.73	0.0024
Fat (%)	2.59[ab]	2.58[b]	2.49[c]	2.65[a]	0.03	0.61	0.033
Protein (%)	3.00[ab]	2.92[c]	3.04[a]	2.98[b]	0.02	0.014	0.0001
Solids not fat (%)	8.22[bc]	8.72[a]	8.11[c]	8.39[b]	0.09	0.012	< 0.0001
Fat (kg/d)	1.28	1.22	1.26	1.23	0.03	0.87	0.14
Protein (kg/d)	1.48[a]	1.39[b]	1.54[a]	1.38[b]	0.03	0.3	< 0.0001
Solids not fat (kg/d)	4.02[b]	4.14[a]	4.03[b]	3.92[b]	0.07	0.11	0.67
Feed efficiency							
Milk yield/DMI	1.89	1.84	1.84	1.84	0.04	0.50	0.43
ECM/DMI	1.65[a]	1.58[b]	1.57[b]	1.59[ab]	0.03	0.64	0.54

[1] Energy corrected milk (ECM)= [0.3×milk yield (kg)] + [12.95×fat yield (kg)] + [7.2×CP yield (kg)].
GB + SM: ground barley + soybean meal; GB + XSM: ground barley + xylose protected soybean; FB + SM: steam flaked barley + soybean meal and FB + XSM: steam flaked barley + xylose protected soybean.
DMI: dry matter intake.
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 Total tract apparent nutrient digestibility, ruminal fluid measurements and rumination activity in Holstein dairy cows fed diets differing in physically processed barley grain and types of soybean meal

Item	Diets				SEM	(P-value)	
	GB + SM	GB + XSM	FB + SM	FB + XSM		GB *vs.* FB	SM *vs.* XSM
Total tract apparent nutrient digestibility (%)							
Dry matter	67.95[b]	72.57[a]	71.09[ab]	66.04[b]	2.04	0.57	0.94
Crude protein	66.74[b]	72.52[a]	68.31[ab]	65.28[b]	1.77	0.30	0.60
VFA (mM)							
Acetate (A)	48.17[a]	43.87[b]	41[b]	42.57[b]	2.11	0.08	0.53
Propionate (P)	22.97[a]	17.37[b]	16.83[bc]	20.0[ab]	1.69	0.33	0.49
Butyrate	23.43[a]	18.6[b]	16.8[b]	24.4[a]	1.75	0.82	0.45
Valerate	1.37[a]	1.2[a]	1.17[a]	0.67[b]	0.17	0.06	0.34
Iso valerate	1.63	1.8	1.5	1.53	0.28	0.49	0.73
A/P ratio	2.07[b]	2.5[ab]	2.57[a]	2.16[ab]	0.23	0.04	0.95
pH	6.77[c]	7.4[a]	7.17[b]	6.87[c]	0.1	0.54	0.15
Rumination activity							
min/d	480[ab]	510[a]	432[b]	480[ab]	21.19	0.08	0.08
min/kg DM	18.43[ab]	19.55[a]	17.24[b]	18.79[ab]	0.92	0.29	0.15
min/kg NDF	63.48[b]	69.84[a]	62.93[b]	61.82[b]	2.28	0.08	0.26

GB + SM: ground barley + soybean meal; GB + XSM: ground barley + xylose protected soybean; FB + SM: steam flaked barley + soybean meal and FB + XSM: steam flaked barley + xylose protected soybean.
VFA: volatile fatty acids and NDF: neutral detergent fiber.
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 rumination values (min/d, min/kg DM and min/kg NDF) were high in animals fed GB + XSM (P<0.05).

Blood parameters

Table 4 lists the values for the blood metabolites of glucose, blood urine nitrogen (BUN), cholesterol, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA). All blood metabolites except BHBA were affected by the treatment (P<0.05). The maximum blood Glucose and cholesterol were recorded for the GB + SM diet. The minimum glucose, cholesterol and NEFA values were recorded for the FB + XSM diet.

The BUN concentration in animals fed FB + XSM was greater than for the other diets. The diets containing XSM had high levels BUN and low levels NEFA (P<0.01) in compare of others.

The results showed that DMI was affected by the treatments and cows fed FB + SM had the highest DMI. There was a significant difference between SM and XSM. It appears that the decrease in DMI in the GB + XSM and FB + XSM diets related to the limitation of RDP in the rumen (Mohamed *et al.* 2001).

When nitrogen is provided in insufficient quantities for microbial fermentation, the rate of digestion and DMI may decrease (Faverdin, 1999).

Researchers have reported contradictory results on nutrient intake for lignosulfonate-treated soybean meal versus ordinary soybean meal (Ure *et al*. 2005). In corn silage-based diets, cows fed diets containing higher levels of protein and RDP provided by soybean meal, recorded greater DMI (Mohamed *et al*. 2001).

Milk yield, FCM 3.5% and ECM were significantly influenced by the experimental diets. The difference in milk yield between the highest and the lowest groups was more than 2.5 kg, with a significant difference between SM and XSM. These results indicate the likelihood of deficient RDP in the diets containing XSM (Malekjahani *et al*. 2017). In a previous study, dairy cows fed diets containing a high level of RDP sustained greater microbial protein synthesis than diets with low RDP (Stokes *et al*. 1991) and consequently, had greater milk production.

Some reports showing no increases in milk yield compared lignosulfonate-treated soybean meal with non-treated soybean meal (Nakamura *et al*. 1992). The goal of the present study was to enhance the supply of protein to dairy cows by improving the balance between synthesis of microbial protein and RUP through the processing of barley and soybean meal. The present results indicate that diets containing SM had the highest fat, protein and SNF percentages. The highest protein and fat percentages in cows fed FB + SM could be a reflection of provision of additional RDP, which improved the supply of methionine and lysine to the animals (Brzoska, 2005; Mohamed *et al*. 2001).

Ipharraguerre and Clark (2005) stated that the increase in ruminal flow of methionine to the small intestine was more effective for corn by-products and fishmeal as RUP sources in comparison with soybean as a RUP source.

The low percentage of milk protein and fat in the GB + XSM and FB + XSM diets probably related to the low levels of RDP (Malekjahani *et al*. 2017), the resulting low production of microbial protein and the unsuitable combination of amino acids absorbed in the small intestine for synthesis of the protein and fat.

Generally, changes in milk compositions reflect differences in rumen fermentation patterns due to differences in diets (Shen *et al*. 2012). NRC (2001) reported that improved methionine and lysine nutrition increase the milk fat percentage, even though the results varied (Flis and Wattiaux, 2005).

Several studies have reported enhanced milk protein content from consumption of steam-flaked grains (Zhong *et al*. 2008), but other studies have not (Guyton *et al*. 2003). A meta-analysis (Ferraretto *et al*. 2013) reported that a 0.02 percentage unit increase in milk protein content when 1% of rumen-degradable starch was included in the diet. Cows fed XSM produced less milk fat and protein than those fed soybean meal. The requirements of the microbial protein and RUP may not be sufficient in the GB + XSM and FB + XSM diets. Feed efficiency (ECM/DMI) was affected by the dietary treatments; cows fed GB + SM had the highest ECM/DMI values. This suggests that milk production and the efficiency of nutrient conversion to milk can be improved with the combination of GB and SM in dairy cow diets.

The blood glucose concentration was significantly different between animals ($P<0.05$). The maximum and minimum of glucose was in cows fed GB + SM and FB + XSM, respectively. These results are contrary to those of previous studies that did not report a difference between animals fed similar rations (Ure *et al*. 2005; Jahani-Moghadam *et al*. 2014). The blood glucose concentration is an important index of carbohydrate, protein and fat metabolism in the body of cows. Trace amounts of glucose represent the small amount of energy available in the diet for the animals (Brzoska, 2005). The minimum level of glucose in the FB + XSM diet showed that dairy cows fed with FB + XSM had the lowest milk yield and the lowest DMI, which is not an optimum level for energy.

The BUN concentration was significantly influenced by the protein source and the highest level of BUN was in cows fed FB + XSM that in contrast to the result of a previous study (Reynal and Broderick, 2003), which showed non-proper use of protein occurring in the rumen. As Malekjahani *et al*. (2017) proposed, using XSM as main source may provide asynchrony diets for dairy cow and it may impact on rumen microbial yield hence to decrease the use of ruminal $N\text{-}NH_3$.

Therefore, it has been assumed that a considerable proportion of $N\text{-}NH_3$ has been moved from the rumen to the liver and excreted as urea. BUN is an indicator of the concentration of ammonia in the rumen and the production of urea by the liver (Butler *et al*. 1996). Therefore, any inefficient use of dietary N will increase the levels of BUN. Usually, when dietary protein is degraded into NH_3 by ruminal bacteria, if it is not synchronized with energy to yield microbial protein immediately, the ammonia level will increase in the rumen, become diffused through the ruminal epithelial tissue into the blood and then transformed into urea nitrogen in the liver.

Blood cholesterol and NEFA concentration differed significantly between the treatments. These results are in contrast to the results of previous studies (Jahani-Moghadam *et al*. 2014). The maximum and minimum cholesterol levels were recorded for cows fed GB + SM and FB + XSM, respectively.

Table 4 Plasma blood metabolites of Holstein dairy cows fed diets differing in physically processed barley grain and types of soybean meal

Blood metabolite	Diets				SEM	(P-value)	
	GB + SM	GB + XSM	FB + SM	FB + XSM		GB *vs.* FB	SM *vs.* XSM
Glucose (mg/dL)	75.56[a]	72.08[ab]	71.11[ab]	67.25[b]	2.82	0.04	0.62
BUN (mg/dL)	24.71[b]	25.53[ab]	23.06[c]	26.22[a]	0.68	0.49	0.008
Cholesterol (mg/dL)	336.17[a]	322.5[a]	307.38[ab]	285.78[b]	16.04	0.054	0.28
NEFA (mg/dL)	0.30[ab]	0.28[bc]	0.32[a]	0.27[c]	0.011	0.83	0.01
BHBA (mg/dL)	0.46	0.40	0.47	0.48	0.05	0.48	0.72

GB + SM: ground barley + soybean meal; GB + XSM: ground barley + xylose protected soybean; FB + SM: steam flaked barley + soybean meal and FB + XSM: steam flaked barley + xylose protected soybean.
BUN: blood urea nitrogen; NEFA: non-esterified fatty acids; BHBA: β-hydroxybutyrate.
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 blood concentrations of NEFA and BHBA are indicators of energy balance in dairy cows (Ingvartsen, 2006). The mean NEFA in cows fed diets containing XSM was significantly lower than for those fed diets containing SM. The highest and lowest of NEFA values were for FB + SM and FB + XSM, respectively. An increase in NEFA and BHBA reflects an increased negative energy balance and mobilization of NEFA from adipose tissue to provide energy for lactogenesis (Janovick *et al.* 2011). Because NEFA is a substrate for ketogenesis, changes in ketone bodies would be expected in relation to differences observed in plasma NEFA (Mashek and Grummer, 2003). The current results showed that cows fed with diets containing XSM produced less milk and NEFA and BHBA levels than cows fed diets containing SM.

The digestibility values of DM and CP were affected by the dietary treatments. The maximum and minimum DM and CP values for digestibility were for the GB + XSM and FB + XSM diets, respectively. The results of previous studies have shown that consumption of lignosulfonate-treated soybean meal or ordinary soybean meal produced no differences in digestibility of DM (Stanford *et al.* 1995). Our results were in agreement with the findings of Colmenero and Broderick (2006). Reynal and Broderick (2003) reported that when cows were fed diets containing heated soybean meal in place of non-heated soybean meal, the DM, NDF and ADF apparent total tract digestibility decreased. However, factors such as dietary composition, lactation stage or other factors could produce different responses related to nutrient digestibility.

The ruminal volatile fatty acids composition was affected by the treatments, except isovalerate and acetate/propionate ratio. The present results confirmed the findings of Windschitl and Stern (1988) that compared untreated soybean meal with treated soybean meal. In contrast, Ure *et al.* (2005) found no changes in the molar concentration of VFAs for a diet containing lignosulfonate soybean meal.

Diets containing GB, recorded lower acetate/propionate ratios than diets containing FB.

These results likely indicate that steam flake processing of barley slows fermentation of carbohydrates in the rumen (Khatibi shahri *et al.* 2018). A number of interactions between kernel tissue and rumen microorganisms (McAllister and Cheng, 1996) influence the rate of starch degradation in the rumen. Apparently, the protein matrix more resistant to proteolysis by heat treatment affects the extent of starch degradation (Goelema *et al.* 1998). In addition, most of the protective effects of heat on the rate of starch degradation in the rumen can be obtained at a relatively low temperature of 100 ˚C, including Maillard reactions and formation of disulfide bridges (Ljokjel *et al.* 2003). The maximum and minimum ruminal butyrate concentrations were for FB + XSM and FB + SM, respectively. Some studies (Ipharraguerre *et al.* 2005) have reported increased molar butyrate concentrations with an increase in dietary RUP.

The concentration of valerate was lower in cows fed FB + XSM, which is in agreement with results of studies by Windschitl and Stern (1988) and Ipharraguerre *et al.* (2005), who reported decrease in valerate with the feeding of XSM. These results relate to decreased protein degradation with treated SM (Windschitl and stern, 1988). Because valerate is an end product of the oxidative deamination and decarboxylation of branched-chain AA (Allison, 1978), it is likely that less feed protein was degraded in the rumen when SM was replaced with the other soy protein supplements in the diet.

The ruminal pH value was affected by the dietary treatments and the highest and lowest pH values were recorded for the GB + XSM and GB + SM diets, respectively. The lower pH in GB + SM could be explained by the higher total VFAs concentration found in the rumen of cows fed diets containing SM compared with other diets. Stern (1994) and Windschitl and Stern (1988) stated that, when treated soybean meal was used in dairy diets, the total VFAs flow from continuous cultures was lower.

Rumination values (min/d, min/ kg DM and min/kg NDF) differed significantly among cows fed diets of GB + XSM and FB + SM.

These results likely stemmed from the source of energy and protein, an increase in the number and activity of rumen microbial fermentation, a decrease in the large particle size of the digesta and its faster removal from the rumen (Warly *et al*. 1992).

CONCLUSION

The results of the present study indicate that the manner of processing of barley (ground *vs*. steam flaked) in combination with the type of protein resource (SM *vs*. XSM) alter the performance of lactating dairy cows. The highest milk yield, FCM 3.5% and ECM was in Holstein dairy cows fed diets including soybean meal. In addition, the highest feed efficiency (ECM/DMI) was in animals fed GB + SM. These results show some of the benefits of feeding a combination of processed barley grain and SM on the blood metabolic responses of the Holstein dairy cows. The lowest BUN levels observed in dairy cows fed diets containing soybean meal. It appears that the combination of GB and SM has more beneficial for dairy cow feeding regimes than those of the combination of FB grain and XSM.

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