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Rumen Fermentation Responses to Dairy Diets Differing in Protein Degradation Potential and Processed Barley Grain

Research Article

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ABSTRACT

This study was conducted to investigate the effect of dairy cow diets containing different protein sources [soybean meal, xylose protected soybean meal (Yasminomax®), and steam-flaked whole soybean] and various partially processed grain on *in vitro* gas production kinetics and the microbial nitrogen yield. Soybean meal (S) and xylose protected of soybean meal (XS) were provided as 0.56:0.44 (SXS_L) and 0.51:0.49 (SXS_H) ratios. The diets were ground barley (B) + S, B+ SXS_L, steam-flaked barley (FB) + S, FB + SXS_L, B + steam-flaked whole soybean (WS), B + SXS_H, FB + WS and FB + SXS_H. An *in vitro* gas production technique was used to define the differences in the microbial nitrogen production of the diets. Asymptotic gas volume (b) was higher than the others for diets containing steam-flaked whole soybean (P<0.01). The highest constant rate of gas production (c) belongs to diet with ground barley and soybean meal, the highest halftime of gas production ($t_{1/2}$) was obtained from diet with steam-flaked barley and soybean meal (P<0.01). The microbial nitrogen yield and consequently microbial nitrogen to diet nitrogen ratio were higher in diets containing xylose protected soybean meal than the others, especially, when compared with steam-flaked whole soybean diets (P<0.05). These results showed that WS, when compared with S and XS may not improve rumen fermentation and microbial nitrogen production with both ground and steam-flaked barley grain and the best performance in microbial nitrogen yield belongs to xylose protected soybean meal.

KEY WORDS fermentation, *in vitro*, microbial nitrogen, steam-flaked whole soybean.

INTRODUCTION

For several years, the amount of crude protein was used to balance the diet of dairy cows. Many researchers had assumed that the high quality of microbial protein synthesis in the rumen was complete as a result of the deficiencies of the quality of dietary protein which escaped from the rumen fermentation (Khorasani *et al.* 2001). Researches conducted in 1960 (Virtanen, 1966) reported that the rumen is able to provide all the amino acids needed by dairy cows to produce 4,500 kg of milk per lactation. More understanding of protein metabolism in ruminants can help to determine the amount and type of protein required in diets. For high-producing cows, microbial protein, synthesized in the rumen, may prepare a small amount of portion needed by the host animal; therefore, a considerable part be met the intestine from feed protein which escaped from rumen degradation (Hristov and Broderick, 1996). Both microbial protein and bypass protein make metabolize protein (MP) in ruminants. Therefore, the determination of MP efficiency in dairy cow nutrition is the key factor for any success in animal production (Krishnamoorthy *et al.* 2005). There are several factors which affect the MP quantity in ruminants. Crude protein (CP) and carbohydrates are the factors which stimulate rumen microbial production, hence have an impact on MP (Sinclair *et al.* 1995). It has been reported that carbohydrate and protein sources may alter rumen fermentation and had significant effects on MP production (Thirumalesh and Krishnamoorthy, 2013). The use of soybean and its by-products in the diet of dairy cows is a relatively common method. These are excellent sources of essential amino acids and any type of forage based diets are appropriate (Canbolat *et al.* 2005). Attempts have been made by researchers to alter the rumen degradable protein (RDP) using various processing methods (Faldet *et al.* 1995), to meet the high producing dairy cows' requirements (NRC, 2001), because soybean has a high RDP, which brings about its efficiency in dairy cows. Different methods have been adopted to change RDP and rumen undegradable protein content of soybean seed, such as physical processing (roasting, expeller and extrusion) or chemical applications such as lignosulfonate, xylose, and formaldehyde (Elwakeel *et al.* 2012). Treatment of soybean meal (S) with xylose reduces degradability of soybean protein by rumen microorganisms (Tuncer and Sacakli, 2003). It has been proposed the millard reaction between sugar aldehyde and free amino groups can decrease degradability of protein without any negative effect in post-ruminal absorption (Chalupa, 1974). However, there are conflicting results on the responses of animals feeding on heated soybeans. Increased milk production was obtained in dairy cows feeding on heated soybean when compared with cows consuming soybean meal (Fathi Nasri *et al.* 2007). However, there are some reports that did not show any improvement (Fathi Nasri *et al.* 2007). When dairy cows are fed with more nitrogen than the requirements, it may have effects on their productive and reproductive performance, economic efficiency, and dairy farms environment (NRC, 2001; Ipharragurre and Clark, 2005).

Carbohydrates are always assumed as the limiting factor in the synthesis of microbial protein because nitrogen is often easily supplied, but this process is not easy for carbohydrates. If carbohydrates are fibrous then rumen microbial protein production can be altered, since a low amount of low available energy is provided for microbiota. Feeding on a high amount of non-fiber carbohydrates may alter rumen ecosystem condition and this can alter microbial activity (Md Jasim *et al.* 2015). Therefore, to obtain the maximum microbial yield in the rumen, dairy diets must provide sufficient nutrients with less negative effect on rumen environment. To achieve these goals, maximizing rumen microbial production and processing of grains as the main source of starch, have been proposed to change the rumen degradability accompanied the with better small intestine digestibility of starch. Barley grain has more rumen fermentation potential than corn (Tothi *et al.* 2003) and in some cases this leads to an increase in ruminal concentration of volatile fatty acids (Khorasani *et al.* 2001).

Physical processing method such as milling or rolling, increases the digestibility of the grain and the advantages and disadvantages of this method is well established. However, very little information exists about the appropriate degree of processing of barley fed to dairy cattle. Steam flaking process is more expensive than the other processing methods, since it needs heat to disrupt protein matrix surrounding starch granules in the grain and increases starch digestibility in rumen (Theurer *et al.* 1999). As stated earlier, the objective of this study was to evaluate the various diets with different sources of protein based on soybean seeds or meal and various physical processing methods used for barley grain preparation on *in vitro* kinetic parameters of gas and microbial nitrogen productions.

MATERIALS AND METHODS

Experimental diets

Experimental diets were provided and they included various sources of protein based on soybean seed or meal and physically processed barley grain. In these diets, barley grain was used as the ground (B) or steam-flaked (F) accompanied with soybean meal (S), xylose protected soybean meal (XS) and steam-flaked whole soybean (WS). Soybean meal (S) and xylose protected of soybean meal (XS) were provided as 0.56:0.44 (SXS_L) and 0.51:0.49 (SXS_H) ratios.

The diets were design as: B + S, B + SXS_L, steam-flaked barley (FB) + S, FB + SXS_L, B + steam-flaked whole soybean (WS), B + SXS_H, FB + WS, and FB + SXS_H. Ingredients and chemical composition of all the diets are in Table 1. All diets were formulated according to NRC (2001). The fat and CP contents of the diets were the same among the diets.

In vitro gas production technique

This method is similar to the procedure of Grins *et al.* (2005) who used 250 mg of feed and 20 mL of buffered rumen content (3 runs, 4 replicates per run). Rumen fluid was collected, just before the morning feeding, from two ruminally cannulated lactating Holstein dairy cows fed with 3.2 kg of dry matter (DM) corn silage, 5.1 kg DM alfalfa hay, and 12.8 kg DM concentrate (containing 24% corn grains, 20.5% barley grains, 27.1% soybean meal, 13.8% canola meal, 13.8% wheat bran, 0.3% calcium carbonate, 0.5% mineral, and vitamin premix).

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

Item	Diets[a]							
	BS	$BSXS_L$	FBS	$FBSXS_L$	BWS	$BSXS_H$	FBWS	$FBSXS_H$
Ingredient (% DM)								
Alfalfa hay	12.02	12.02	12.02	12.02	11.69	11.96	11.69	11.96
Corn silage	19.85	19.85	19.85	19.85	19.34	19.82	19.34	19.82
Wheat straw	0.72	0.72	0.72	0.72	0.7	0.72	0.7	0.72
Barley grain, ground	20.4	20.4	0	0	19.64	20.09	0	0
Barley (steam flaked)	0	0	20.4	20.4	0	0	19.64	20.09
Corn grain, ground, dry	14.41	14.41	14.41	14.41	14	14.32	14	14.32
Soybean meal	11.05	8.14	11.05	8.14	9.63	7.04	9.63	7.04
XS (Yasminomax®)[b]	0	6.48	0	6.48	0	6.96	0	6.96
Whole soybean (steam flaked)	0	0	0	0	8.26	0	8.26	0
Cottonseed, whole	4.44	5.26	4.44	5.26	6.46	6.96	6.46	6.96
Linseed	1.07	1.48	1.07	1.48	1.03	1.06	1.03	1.06
Full fat soybean	2.57	0	2.57	0	0	0	0	0
Fish meal	2.76	0	2.76	0	0	0	0	0
Fat powder	1.04	1.57	1.04	1.57	0.07	1.48	0.07	1.48
Sodium bicarbonate	0.95	0.95	0.95	0.95	0.93	0.93	0.93	0.93
Di-calcium phosphate	0.11	0.11	0.11	0.11	0.07	0.07	0.07	0.07
Calcium carbonate	0.54	0.54	0.54	0.54	1.04	0.55	1.04	0.55
Magnesium oxide	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Sugar beet pulp	5.65	5.65	5.65	5.65	4.77	5.63	4.77	5.63
Molasses	1.18	1.18	1.18	1.18	1.15	1.17	1.15	1.17
Salt	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Vitamin and mineral premix[c]	0.63	0.63	0.63	0.63	0.61	0.63	0.61	0.63
Crude protein (CP) (%)	17	17	17	17	17	17	17	17
Neutral detergent fiber (NDF) (%)	28.3	28.8	28.3	28.8	28.9	29.3	28.9	29.3
Acid detergent fiber (ADF) (%)	17.9	18.2	17.9	18.2	18.7	18.7	18.7	18.7
Ether extract (%)	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Nonfiber carbohydrates (NFC) (%)	44.7	46.2	44.7	46.2	44.2	45.9	44.2	45.9
Net energy for lactation (NE_L) (Mcal/kg)	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64

[a] BS: ground barley + soybean meal; $BSXS_L$: ground barley + xylose protected soybean; FBS: steam-flaked barley + soybean meal; $FBSXS_L$: steam-flaked barley + xylose protected soybean; BWS: ground barley + steam-flaked whole soybean; $BSXS_H$: ground barley + xylose protected soybean; FBWS: steam-flaked barley + steam-flaked whole soybean and $FBSXS_H$: steam-flaked barley + xylose protected soybean.

[b] XS: xylose protected soybean (Yasnamehr®) meal containing (DM: 93%, NDF: 12.7%, ADF: 13.4%, CP: 53.4%, Ash: 8.36%, EE: 8.16%) was provided from Iranian local company named Yasnamehr.

[c] 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.

Gas volume was measured manually in each bottle using a pressure gauge (Thirumalesh and Krishnamoorthy, 2013; Canbolat *et al*. 2005; Faldet *et al*. 1995) at 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 hours.

After the diminution of gas production from blank bottles, the data were fitted on an exponential model (Qrskov and McDonald, 1979):

$$Y = b\,(1 - e^{-ct})$$

Where:
y: cumulative volume of the gas produced at time t (h).
b: asymptotic gas volume (mL/250 mg DM).
c: fractional constant rate (mL/h) of gas produced over the incubation.

The halftime of gas production ($t_{1/2}$) for each substrate was calculated using $t_{1/2} = \ln 2/c$ equation (Goering and Van Soest, 1970).

The amount of microbial nitrogen produced was estimated indirectly using the following equation (Grins *et al*. 2005):

$$\text{Microbial N production at } t_{1/2} = \text{Diet N} + \Delta NH_3\text{-N} - \text{NDFN at } t_{1/2}$$

Where:
Diet N: nitrogen content of the diet.
ΔNH_3–N: changes in ammonia nitrogen level in incubation solution between time zero and $t_{1/2}$.
NDFN at $t_{1/2}$: NDF-bound nitrogen.

True substrate digestibility of the samples was calculated at $t_{1/2}$ (Goering and Van Soest, 1970). The conversion of dietary N to microbial nitrogen (MN/DN) was defined by microbial nitrogen yield divided by dietary nitrogen.

Chemical analyses

A chemical analysis was performed according to the standard methods described by AOAC (2000). Feed samples were ground (2 mm particle diameter) and analyzed for dry matter (135 ˚C for 24 hours as per method 930.15), ash (535 ˚C; method 942.05), CP (method 990.03), and extract (method 920.39) (AOAC, 2000).

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Van Soest *et al.* (1991). The non-fiber carbohydrates (NFC) content was calculated as 100 - (% CP+% ash+% EE+% NDF) (NRC, 2001).

Calculations and statistical analysis

The treatments were arranged as a completely randomized design containing two types of barley processed (ground and steam flaked) × three sources of proteins (S, SXS and WS) using the following statistical model:

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

Where:

Y_{ij}: dependent variable.

μ: overall mean.

a_i: effect of treatment.

e_{ij}: experimental error.

Data from gas production were analyzed in SAS (version 9.1) (SAS, 1991). The differences between the means were evaluated using Duncan test at $P \leq 0.05$. Predesigned differences were used to compare the groups of treatments. The result of the differences between the factors was significant and is in Table 2.

RESULTS AND DISCUSSION

The patterns of cumulative gas production of various diets are in Figure 1. The cumulative volume of gas production develops by increasing the time of incubation. The maximum gas volume production belongs to the FBWS and the minimum gas volume belongs to the $BSXS_H$. Gas production parameters including asymptotic gas volume (b), the constant rate of gas production (c), and halftime ($t_{1/2}$) of the experimental diets are in Table 2.

The amount of asymptotic gas volume (b) of FBWS was significantly higher than the other experimental diets (P<0.01). The minimum parameter (b) belongs to $BSXS_H$.

Gas production constant rate (c) and halftime of gas production ($t_{1/2}$) were significantly the highest in BS and FBS, respectively (P<0.01). The differences between soybean meal versus xylose protected soybean meal and steam-flaked whole soybean versus xylose protected soybean meal were significant (P<0.01) for parameter (b). The difference between ground barley versus steam-flaked barley was significant (P<0.01) for parameter (c) and halftime of gas production ($t_{1/2}$).

The mean of microbial nitrogen (MN), true substrate digestibility (TSD), neutral detergent insoluble nitrogen (NDIN) and microbial nitrogen to dietary nitrogen ratio (MN/DN) in the experimental diets are in Table 3. The highest amount of MN belonging to $BSXS_L$ and $FBSXS_L$ diets belonging to SXS produced the highest MN regardless of the type of barley processed (P<0.05).

These two diets have the highest levels NFC (46.2%) among other diets. The difference between steam-flaked whole soybean and xylose protected soybean meal was significant (P<0.01) for MN. The difference between soybean meal and xylose protected soybean meal was significant (P<0.05) for MN/DN.

The difference between steam-flaked whole soybean and xylose protected soybean meal was significant (P<0.01) for MN/DN. MN/DN was the maximum in the $BSXS_L$ and $FBSXS_L$ diets and it was minimum in the BWS.

The main goal of the present study was to determine the effect of sources of protein based on soybean seed or meal and various physical processing methods of barley grain on ruminal fermentability and microbial nitrogen yield using gas production method. According to the results (Figure 1), the highest gas volume belongs to FBWS, which shows that steam-flaked whole soybean (WS) provided more rumen fermentable substrate (Aldrich and Merchen, 1995; Harris, 2003).

It is based on this principle that gas production is directly symmetrical to the quantification of the degraded substrate (Makkar, 2002). Minimum gas production was produced by $BSXS_H$; it seems that more proteins in xylose protected soybean meal (XS) escaped from rumen fermentation (Stanford *et al.* 1995; Calsamiglia *et al.* 1995).

Asymptotic gas volume (b) was influenced by the source of protein; (b) was the highest for FBWS which shows better substrate availability for rumen microorganisms. It seems that steam flaking processing on the whole soybean cannot increase the escape of protein from the rumen (Ure *et al.* 2005). The lowest of (b) was observed in $BSXS_H$. Smaller amounts of gas production and the asymptotic gas volume (b) in diets containing SXS can be shown as a fact that millard reaction might alter the level of ruminal microbial fermentation.

Table 2 *In vitro* gas production parameters of experimental diets

Item	BS	$BSXS_L$	FBS	$FBSXS_L$	BWS	$BSXS_H$	FBWS	$FBSXS_H$	SEM	Contrast (P-value)*		
										1	2	3
b	73.59^{ab}	70.46^{bcd}	69.62^{bcd}	71.03^{abc}	72.47^{ab}	65.94^{d}	75.71^{a}	66.21^{dc}	1.006	0.967	0.002	0.0001
c	0.058^{a}	0.055^{ab}	0.045^{c}	0.050^{bc}	0.054^{ab}	0.054^{ab}	0.050^{bc}	0.051^{bc}	0.001	< 0.0001	0.711	0.692
$t_{1/2}$	12.14^{b}	12.61^{b}	15.42^{a}	14.20^{ab}	13.12^{ab}	12.89^{b}	14.30^{ab}	13.74^{ab}	0.476	0.0001	0.321	0.410

BS: ground barley + soybean meal; $BSXS_L$: ground barley + xylose protected soybean; FBS: steam-flaked barley + soybean meal; $FBSXS_L$: steam-flaked barley + xylose protected soybean; BWS: ground barley + steam-flaked whole soybean; $BSXS_H$: ground barley + xylose protected soybean; FBWS: steam-flaked barley + steam-flaked whole soybean and $FBSXS_H$: steam-flaked barley + xylose protected soybean.
b: asymptotic gas volume (mL per 250 mg sample); c: the constant rate of gas production (mL/h) and $t_{1/2}$: halftime of gas production (h).
* Contrast 1: between B versus FB; 2: S versus SXS and 3: WS versus SXS.
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.

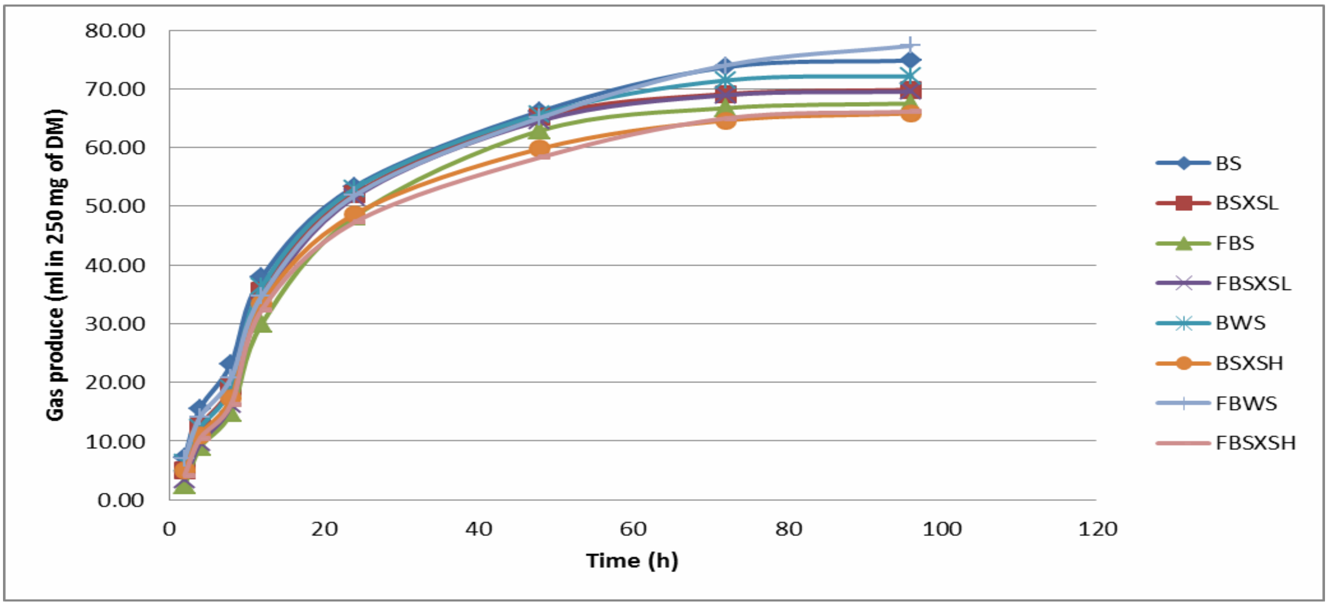

Figure 1 The charts of Gas production (ml in 250 mg of DM) in the experimental diets in different of times
BS: ground barley + soybean meal; $BSXS_L$: ground barley + xylose protected soybean; FBS: steam-flaked barley + soybean meal; $FBSXS_L$: steam-flaked barley + xylose protected soybean; BWS: ground barley + steam-flaked whole soybean; $BSXS_H$: ground barley + xylose protected soybean; FBWS: steam-flaked barley + steam-flaked whole soybean and $FBSXS_H$: steam-flaked barley + xylose protected soybean

Table 3 Microbial nitrogen (MN, mg), true substrate digestibility (TSD, mg/g), neutral detergent insoluble nitrogen (NDIN, mg per 250 mg sample) and microbial nitrogen to dietary nitrogen ratio (MN/ND) of experimental diets

Item	BS	$BSXS_L$	FBS	$FBSXS_L$	BWS	$BSXS_H$	FBWS	$FBSXS_H$	SEM	Contrast (P-value)*		
										1	2	3
MN	4.19^{ab}	4.93^{a}	3.23^{ab}	4.75^{a}	2.62^{b}	4.40^{ab}	3.44^{ab}	3.94^{ab}	0.447	0.541	0.051	0.0008
TSD	0.76^{a}	0.77^{a}	0.72^{a}	0.79^{a}	0.76^{a}	0.69^{a}	0.74^{a}	0.77^{a}	0.023	0.439	0.556	0.707
NDIN	1.09^{a}	0.96^{a}	1.38^{a}	1.13^{a}	1.31^{a}	0.85^{a}	0.92^{a}	1.69^{a}	0.239	0.195	0.691	0.837
MN/DN	0.6^{ab}	0.71^{a}	0.46^{ab}	0.68^{a}	0.37^{b}	0.63^{ab}	0.49^{ab}	0.56^{ab}	0.064	0.539	0.049	0.001

BS: ground barley + soybean meal; $BSXS_L$: ground barley + xylose protected soybean; FBS: steam-flaked barley + soybean meal; $FBSXS_L$: steam-flaked barley + xylose protected soybean; BWS: ground barley + steam-flaked whole soybean; $BSXS_H$: ground barley + xylose protected soybean; FBWS: steam-flaked barley + steam-flaked whole soybean and $FBSXS_H$: steam-flaked barley + xylose protected soybean.
* Contrast 1: between B versus FB; 2: S versus SXS and 3: WS versus SXS.
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.

Heat facilitates the millard or non-enzymatic browning reaction between sugar aldehyde groups and free amino acid groups of protein to yield an amino-sugar complex (Canbolat *et al*. 2005) and free amino groups can be controlled to decrease protein degradability in the rumen without negatively affecting the intestinal protein digestibility (Chalupa, 1974).

Although, previous studies have shown that heat treated soybean increases rumen-undegradable protein (RUP) (Turner and McNiven, 2011).

The constant rate of gas production (c) and halftime of gas production ($t_{1/2}$) were influenced by the processing of barley grain. Parameter (c) in the steam-flaked barley (FB) diets was lower than ground barley (B) diets.

The highest and lowest of the constant rate of gas production (c) belong to BS and FBS, respectively. These results showed that steam flaking processing of barley leads to slow fermentation of carbohydrate in the rumen (Franco *et al.* 1995; Ljokjel *et al.* 2003). The rate of starch degradation in rumen is influenced by a number of interactions between kernel tissues and rumen microorganisms (McAllister and Cheng, 1996).

Most likely, heat treatment affects the extent of starch degradation by creating the protein matrix to be more resistant to proteolysis (Goelema *et al.* 1998). In addition, most of the protective effects of heat on the rate of starch degradation in rumen were already obtained at relatively low temperature of 100 ˚C, showing that the chemical reactions include maillard reactions and formation of disulphide bridges, which took temperature below 100 ˚C, contrary to the previous reports (Finley, 1989; Voragen *et al.* 1995; Ljokjel *et al.* 2003). The results of the present study were inconsistent with the conclusions of other experiments, which established that steams flaking of grains had a greater gas production when compared with an unprocessed grain (Calsamiglia *et al.* 1995; Depeters *et al.* 2003; Eyni *et al.* 2017).

The rate of gas production directly affects $t_{1/2}$ and therefore $t_{1/2}$ was the highest in the FBS diet. Microbial nitrogen (MN) and microbial nitrogen to dietary nitrogen ratio (MN/ND) were affected by protein sources used in the present study ($P<0.05$). Results of the present experiment showed that regardless of the kind of processing for barley, in diets containing xylose protected soybean meal (SXS), microbial nitrogen yield was higher than those of diets containing steam-flaked whole soybean.

This result was probably due to the synchronization between carbohydrate digestion and ruminal protein. Microbial protein synthesis was highly affected by the rate and extent of fermentation of carbohydrate and nitrogen in the rumen (Hristov *et al.* 2005). The lowest MN belongs to BWS diet and perhaps this is due to the imbalance between starch digestion in the rumen and the intestine to keep microbial protein synthesis.

We had diets differing in NDF and NFC contents at approximately 1 and 2 percentage units respectively and find only significance difference in MN between diets 2 and 4 with 5. This might be attributable to greater NFC content in the diets 2 and 4 compared with the diet 5 (46.2, 46.2, and 44.2%, respectively), which could contribute to more microbial nitrogen synthesis (Russell *et al.* 1992; Hristov *et al.* 2005; Eyni *et al.* 2017). According to the high value parameter (c) in the BWS diet, therefore steam-flaked whole soybean was rapidly degraded in the rumen and perhaps the microorganisms did not have enough time to use nitrogen to make their microbial cell. The ratio of MN/DN was significant between all the diets ($P<0.05$).

The maximum MN/DN was shown in the $BSXS_L$ and $FBSXS_L$ diets. It has been stated that a synchronous supply of energy and nitrogen to the rumen increases the efficiency of microbes in taking N and in utilizing ATP for microbial growth (Herrera-Saldana *et al.* 1990; Sinclair *et al.* 1993), which may mean that synchronization between energy and protein could increase microbial protein production in the rumen and boost rumen fermentation efficiency, thereby improving nutrient utilization and animal performance.

CONCLUSION

Conclusively, diets containing xylose protected soybean at a low level had the best performance in microbial protein production which probably increases synchronization between energy and N in rumen. The rate of fermentation of starch barley in rumen was reduced by steam flake processing. Perhaps, this result is in relation with the report that heat treatment at low moisture levels can decrease the digestibility of starch due to the formation of starch-protein complexes. Steam-flaked whole soybean rapidly degrades in the rumen and this process could not increase the escape of protein in the whole soybean.

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REFERENCES

Aldrich C.G. and Merchen N.R. (1995). Heat treatment of whole soybeans: influence on protein digestion by ruminants. *J. Anim. Sci.* **73(1),** 95-108.

AOAC. (2000). Official Methods of Analysis. Vol. I. 18th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Calsamiglia S., Stern M.D. and Firkins J.L. (1995). Effects of protein source on nitrogen metabolism in continuous culture and intestinal digestion *in vitro*. *J. Anim. Sci.* **73,** 1819-1827.

Canbolat O., Kamalak A., Efe E., Sahin M. and Ozkan C.O. (2005). Effect of heat treatment on *in situ* rumen degradebility and *in vitro* gas production of full-fat soyabeans and soyabean meal. *South African J. Anim. Sci.* **35(3),** 186-194.

Chalupa W. (1974). Nitrogen metabolism in ruminant animals. *J. Dairy Sci.* **58,** 1198-1218.

Depeters E.J., Getachew G., Fadel J.G., Zinn R.A., Tylor S.J., Pareas J.W., Hinders R.G. and Aseltine M.S. (2003). *In vitro* gas production as a method to compare fermentation characteristics of steam-flaked corn. *Anim. Feed Sci. Technol.* **105,** 109-122.

Elwakeel E.A., Titgemeyer E.C., Cheng Z.J., Nour A.M. and Nasser M.E. (2012). *In vitro* assessment of the nutritive value of expanded soybean meal for dairy cattle. *J. Anim. Sci. Biotechnol.* **3(1),** 1-7.

Eyni B., Mesgaran M.D., Vakili A. and Valizadeh R. (2017). *In vitro* yield of microbial-n from fermentation of glucogenic and lipogenic diets provided by different sources of rumen degradable amino acids. *J. Vet. Sci. Technol.* **8,** 4-10.

Faldet M.A., Satter L.D. and Broderick G.A. (1995). Determining optimal heat treatment of soybeans by measuring available lysine chemically and biologically with rats to maximize protein utilization by ruminants. *J. Nutr.* **122,** 151-160.

Fathi Nasri M.H., Mesgaran M.D., Kebreab E. and France J. (2007). Past peak lactational performance of Iranian Holstein cows fed raw or roasted whole soybeans. *Canadian J. Anim. Sci.* **87,** 441-447.

Finley J.W. (1989). Effects of processing on proteins: An overview. Pp. 1-7 in Protein Quality and the Effects of Processing. R.D. Phillips and J.W. Finley, Eds. Marcel Dekker, New York, USA.

Franco C.M.L., Ciacco C.F. and Tavares D.Q. (1995). Effect of the heat-moisture treatment on the enzymatic susceptibility of corn starch granules. *Starch. Stärke*. **47,** 223-228.

Goelema J.O., Spreeuwenberg M.A.M., Hof G., van der Poel A.F.B. and Tamminga S. (1998). Effect of pressure toasting on rumen degradability and intestinal digestibility of whole and broken peas, lupins, faba beans and a mixture of these feedstuffs. *Anim. Feed Sci. Technol.* **76,** 35-50.

Goering H.K. and Van Soest P.J. (1970). Forage Fiber Analysis (Apparatus, Reagents, Procedures, and some Applications).. USDA-ARS Agricultural Handbook No. 379. US Government Printing Office, Washington, D.C., USA.

Grins E.E., Blummel M. and Sudekum K.H. (2005). Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage-based diets. *Anim. Feed Sci. Technol.* **124,** 527-545.

Harris B.J.R. (2003). Feeding raw or heat-treated whole soybeans to dairy cattle. Ph D. Thesis. University of Florida, Gainesville, USA.

Herrera-Saldana R., Gomez-Alarcon R., Torabi M. and Huber J.T. (1990). Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. *J. Dairy Sci.* **73,** 142-148.

Hristov A.N. and Broderick G.A. (1996). Synthesis of microbial protein fed alfalfa silage, alfalfa hay, in ruminally cannulated cows or corn silage. *J. Dairy. Sci.* **79,** 1627-1637.

Hristov A.N., Ropp J.K., Grandeen K.L., Abedi S., Etter R.P., Melqar A. and Foley A.E. (2005). Effect of carbohydrate source on ammonia utilization in lactation dairy cows. *J. Anim. Sci.* **83,** 408-421.

Ipharragurre I.R. and Clark J.H. (2005). Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. *J. Dairy Sci.* **88,** 2556-2570.

Khorasani G.R., Okine E.K. and Kennelly J.J. (2001). Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield, and milk composition of Holstein cows. *J. Dairy Sci.* **84,** 2760-2769.

Krishnamoorthy U., Chandrapal Singh K. and Kailas M.M. (2005). Evaluation of roughages for rumen microbial biomass synthesis. *Indian Vet. J.* **82,** 453-454.

Ljokjel K., Harstad O.M., Prestlokken E. and Skrede A. (2003). *In situ* digestibility of starch in barley grain (*Hordeum vulgare*) and peas (*Psium sativum*) in dairy cows: Influence of heat treatment and glucose additions. *Anim. Feed. Sci. Technol.* **107,** 105-116.

Makkar H.P.S. (2002). Applications of the *in vitro* gas method in the evaluation of feed resources, and enhancement of nutritional value of tannin-rich tree/browse leaves and agro-industrial by-products. Pp. 23-42 in Development Field Evaluation Animal Feed Supplementation Packages, International Atomic Energy Agency (IAEA)-TECDOC-1294, Vienna.

McAllister T.A. and Cheng K.J. (1996). Microbial strategies in the ruminal digestion of cereal grains. *Anim. Feed Sci.* **62,** 29-36.

Md Jasim U., Khandaker Z.H., Khan M.d.J. and Khan M. (2015). Dynamics of microbial protein synthesis in the rumen - A Review. *Ann. Vet. Anim.* Sci. **2(5),** 116-131.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Ed. National Academy Press, Washington, DC, USA.

Qrskov E.R. and McDonald I. (1979). Estimation of protein degradability in the rumen from Incubation measurements weighted according to rate of passage. *J. Agric. Sci.* **92,** 499-503.

Russell J.B., O'Connor J.D., Fox D.G., Van Soest P.J. and Sniffen C.J. (1992). A net carbohydrate and protein system for evaluating cattle diets. I. Ruminal fermentation. *J. Anim. Sci.* **70,** 3551-3561.

SAS Institute. (1991). SAS®/STAT Software, Release 9.1 SAS Institute, Inc., Cary, NC. USA.

Sinclair L.A., Garnsworthy P.C., Newbold J.R. and Buttery P.J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in the sheep. *J. Agric. Sci.* **120,** 251-263.

Sinclair L.A., Garnsworthy P.C., Newbold J.R. and Buttery P.J. (1995). Effect of synchronizing the rate of dietary energy and nitrogen in the diets with similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. *J. Agric. Sci.* **124,** 463-472.

Stanford K., MacAllister T.A. and Chang K.J. (1995). Comparison of lignosulfonate treated canola meal and soybean meal as rumen undegradable protein supplements for lambs. *Canadian J. Anim. Sci.* **75(3),** 371-377.

Theurer C.B., Huber J.T., Delgado-Elorduy A. and Wanderley R. (1999). Invited review: Summary of steam-flaking corn or sorghum grain for lactating dairy cows. *J. Dairy Sci.* **82,** 1950-1959.

Thirumalesh T. and Krishnamoorthy U. (2013). Rumen microbial biomass synthesis and its importance in ruminant production. *Int. J. Livest. Res.* **3(2),** 5-26.

Tothi R., Lund P., Weisbjerg M.R. and Hvelplund T. (2003). Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and *in situ* techniques. *Anim. Feed Sci. Technol.* **104,** 71-94.

Turner T.D. and McNiven M.A. (2011). *In vitro* degragability and N digestibility of raw, roasted or extruded canola, linseed and soybean. *J. Agric. Food Sci.* **20,** 298-304.

Tuncer S.D. and Sacakli P. (2003). Rumen degradability characteristics of xylose treated canola and soybean meals. *Anim. Feed Sci. Technol.* **107,** 211-218.

Ure A.L., Dhiman T.R., Stern M.D. and Olsan K.C. (2005). Treated extruded soybean meal as a source of fat and protein for dairy cows. *Asian-Australasian J. Anim. Sci.* **7,** 980-989.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods of dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74,** 3583-3597.

Virtanen A.I. (1966). Milk production of cows on protein-free feeds. *Science.* **153,** 1603-1614.

Voragen A.G.J., Gruppen H., Marsman G.J.P. and Mul A.J. (1995). Effect of some manufacturing technologies on chemical, physical and nutritional properties of feed. Pp. 93-126 in Recent Advances in Animal Nutrition. P.C. Garnsworthy. And D.J.A. Cole, Eds. Nottingham University Press, Nottingham. United Kingdom.