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Full Length Research Paper

Determination of degradability of treated soybean meal and its proteins fractions

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This study was carried out to determine the effects of xylose and moisture heating treatments (0, 5 and 10 g/kg DM) of soybean meal (SBM) on *in situ* rumen degradability characteristics in cow and its proteins sub-units fractions by a SDS-PAGE discontinues system. In the experiments, three ruminally cannulated male Taleshi cow were used to measure *in situ* rumen degradability characteristics of dry matter (DM) and crude protein (CP) of different treated SBM samples. SBM were treated with xylose (5 and 10 g/kg DM) and moisture heating. Xylose and moisture heated treatment significantly (P<0.05) reduced degradability values of DM and CP for SBM. Rumen effective protein degradability values of SBM (k = 0.02, 0.05 and 0.08 h^{-1}) were significantly decreased by all treatments (P<0.05). Effective degradability of DM (k = 0.05 h^{-1}) were 65.17, 64.90, 61.77 and 58.50 g/kg and CP was 63.77, 61.97, 60.83 and 60.23 g/kg for NTSBM (non treated SBM), TSBM (SBM + 30 min moisture heating treated), TSBM 5 g/kg (SBM + 30 min moisture heating treated + 5 g/kg xylose) and TSBM 10 g/kg (SBM + 30 min moisture heating treated + 10 g/kg xylose) treatments, respectively. Treatment of SBM with 5 and 10 g/kg of DM caused a reduction in the dry matter and effective crude protein degradability compared with NTSBM. Electrophoretic patterns of untreated and treated SBM protein residues revealed that β-conglycinin β, α and ά subunit were degraded completely within 4h of incubation in the rumen, whereas the acidic and basic subunits of glycinin were degraded after 48h incubation in treated SBM with Xylose. It is concluded that SBM proteins can be effectively protected from degradation in the rumen by xylose and moisture heating treatment.

Key words: Soybean meal, degradability, xylose, moisture heat, SDS-PAGE.

INTRODUCTION

Soybean is a major protein source for humans and other animals (Nielsen et al., 1989). Rapidly growing ruminants and lactating dairy cattle rely on both microbial protein and rumen-undegradable protein (escape protein) digested in the small intestine to meet their amino acid requirements. When good quality protein is fed to ruminants, it is subject to extensive microbial fermentation. During fermentation most protein is degraded to peptides, amino acids, and finally to ammonia (Chalupa, 1981). In these cases, the advantages of protein quality, in terms of balance of essential amino acid and digestibility, are lost. Attempts to decrease the rumen degradability of proteins have involved treatment with heat (Mir et al., 1984; Nakamura et al., 1994), formaldehyde (Nishimuta et al.,1974; Thomas et al., 1979; Cooker et al., 1983; Mir et al., 1984), acetic acid (Robinson et al., 1994), tannic acid (Driedger and Hatfield, 1972), lignosulfonate (Windschitl and Stern, 1988; McAllister et al., 1993) and xylose treatments (Windschitl and Stern, 1988; McAllister et al., 1993; Harstad and Prestlokken, 2000; Sacakli, 2001). More recently, treatment of SBM with xylose was successful in reducing degradation of soybean protein by rumen microorganisms (Cleale et al., 1987; Harstad and Prestlokken, 2000; Sacakli, 2001). Chalupa (1974) suggested that the Maillard reaction between sugar aldehyde groups and free amino groups can be controlled to decrease protein degradability in the rumen without adversely affecting intestinal protein digestibility. The objectives of this study were to evaluate the effect of different levels of xylose and microwave treatment in the

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in situ disappearance of the dry matter (DM) and crude protein (CP) fractions of soybean meal and their sub-unit fractions by a SDS-PAGE discontinues system.

MATERIALS AND METHODS

Sample preparation and treatment

The SBM samples (soybean meal imported from Brazil) were obtained from commercial sources in Iran. SBM was treated with 0, 5 and 10 g/kg xylose and moisture heating. The DM of a sample (3 g) of meal was determined by drying at 105°C for 24 h, and 500 g SBM mixtures of water and xylose were added to increase the moisture content of SBM to 25% and were subjected to autoclave heating at 1.2 bar pressure in 120°C for 30 min.

Animal and diets

Three ruminal cannulated Iranian Taleshi native male cow weighing approximately 450 kg were placed in individual 4.2×2.8 m pens with concrete floors that were cleaned regularly. Cows were fed 10 kg dry matter, a total mixed ration containing concentrate and alfalfa hay, diets twice daily at 09:00 and 16:00 h.

NTSBM: SBM, non treated.
TSBM: SBM + 30 min moisture heating treated.
TSBM 5 g/kg: SBM + 30 min moisture heating + 5 g xylose.
TSBM 10 g/kg: SBM + 30 min moisture heating + 10 g xylose.

In situ evaluation of dry matter, organic matter and crude protein

Nylon bag technique was used to measure disappearance in the rumen of untreated and treated SBM. Nylon bags (45-m pore size; 9 cm×14 cm bag size) containing 5 g of SBM samples were incubated in the rumen of each cow. Two bags of each type of treated SBM were removed after 2, 4, 8, 16, 24 and 48 h of incubation in the rumen. Then individual bags with contents were washed in running tap water until the bags were free of rumen matter. Bags were then dried to a constant weight at 60°C for 48 h and weighed. The solubility or washing loss was determined by soaking samples of each material in water at 37–40°C for 1 h followed by the washing procedure above. Digestion kinetics of DM and CP were determined according to the equation of Orskov and McDonald (1979):

$$P = a + b(1 - e^{-ct})$$

where p is the amount degraded at a time, a the rapidly soluble fraction (g/kg), b the potentially degradable fraction (g/kg), c the constant rate of disappearance of b, and t the time of incubation (h). The effective rumen degradability of DM and CP was estimated using the equation of Orskov and McDonald (1979):

$$p_e = \frac{a + bc}{k + c}$$

Where P_e is the effective degradation, k the fractional ruminal outflow rate, a, b and c are as defined above. Effective degradability was calculated with an estimated solid outflow rate from the rumen (k) of 0.02, 0.05 and 0.08 h^{-1} (Bhargava and Orskov, 1987).

Chemical analyses

Feed samples were analyzed for DM and CP and DM and CP content of their residues after rumen incubation by using the procedures of AOAC (1984).

Determination of rumen degradability

In the procedure of ruminal incubation, the method of Mehrez and Orskov (1977) was followed. For this, 5 g of different samples of SBM were weighed in duplicate into nylon bags. Each group includes 42 samples (two replicates × seven incubation periods × three cows for each treatment) prepared into individual nylon bags for assay. Bags were incubated in the ventral sac of the rumen of three Iranian Taleshi native cows for 0, 2, 4, 8, 16, 24 and 48 h. Diet was offered at 20 g/kg of body weight daily in two equal portions (08:00 and 16:00 h). Immediately after removal from the rumen, bags were put in ice water to stop microbial fermentation, and washed under tap water until the rinsing water became colourless, then dried out and weighed.

Determination of protein sub-units

Protein sub-units were fractionated by a SDS-PAGE discontinuous system (Laemmli, 1970). All ruminal undegradable fractions from each incubation period were dried, ground (0.25 mm particle size) and replicate samples pooled. Twenty microgram of untreated or treated SBM was placed into 750 µl SDS-PAGE sample buffer. After 30 min of mixing (i.e., vortex and inverse), samples were immersed at 90°C for 3 min, and then centrifuged at 10 000 × g for 1 min. A 25 µl aliquot of each sample was loaded into the sample well. Electrophoresis of proteins was on 12.5% resolving gel (1.0 × 190 × 150 mm) with 3.75% acrylamide stacking gel. The gels were kept at a constant current of 30 mA until the bromophenol blue marker dye reached the bottom of the gel. Protein fixation and staining were completed simultaneously using a solution of Coomassie brilliant blue. Gel distaining was accomplished by using a 150 ml/l methanol and 100 ml/l acetic acid solution. One standard protein mixture including β-galactosidase (116 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), REease Bsp98I (25 kDa), β-Lactoglobulin (18.4 kDa) and Lysozyme (14.4 kDa) was used.

Statistics

Digestion kinetics of DM and CP were determined according to the equations of Orskov and McDonald (1979) as:

$P = a + b(1 - e^{-ct})$, $ERD = a + bc/(c + k)$

where 'P' is DM and CP disappearance (g/kg) at time t (h), 'a' the water soluble fraction (g/kg), 'b' the potentially degradable fraction (g/kg), 'c' the rate of degradation (h^{-1}) of 'b' fraction, ERD the effective rumen degradation, and 'k' the fractional ruminal out flow rate. Effective degradability was calculated with estimated solid outflow rates from the rumen of 0.02, 0.05 and 0.08 h^{-1}. The degradability parameters for the nylon bags were analyzed as a completely randomized design. Analysis was with the general linear means model of SAS (1996). When a significant difference occurred, means were separated using Tukey test (Steel and Torrie, 1980). Differences were considered to be significant if $P<0.05$.

RESULTS AND DISCUSSION

The rumen degradation characteristics of DM and CP of different treated SBM are given in Tables 1 and 2, respectively. As seen in Tables 1 and 2, DM washing losses were 13.38, 10.14, 10.34 and 8.46% and for CP; 7.66, 8.27, 5.00 and 3.82% for NTSBM, TSBM, TSBM 5 g/kg and TSBM 10 g/kg, respectively. DM disappearance of

Table 1. The rumen degradation characteristics of dry matter in untreated and treated soybean meal.

Experimental feed	Washing loss (%)	Rumen degradation (g/kg) at different Incubation time						Degradation characteristics (g/kg)				Pe (g/kg)		
		2 h	4 h	8 h	16 h	24 h	48 h	a	b	a+b	*C* (h)	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
NTSBM	13.38^{a}	32.59^{a}	38.16^{a}	51.33^{a}	79.03^{a}	82.14^{a}	91.65^{a}	15.13^{a}	76.65^{b}	91.78^{a}	0.08^{a}	78.37^{a}	65.17^{a}	56.50^{a}
TSBM	10.14^{b}	23.46^{a}	35.24^{a}	56.49^{a}	76.12^{ab}	83.42^{a}	92.21^{a}	9.34^{ab}	83.37^{a}	92.70^{a}	0.08^{a}	78.90^{a}	64.90^{a}	55.63^{a}
TSBM 5 g/kg	10.34^{b}	18.76^{b}	29.35^{b}	52.04^{a}	74.80^{b}	84.54^{a}	89.35^{ab}	6.73^{b}	84.89^{a}	91.62^{a}	0.09^{a}	76.40^{a}	61.77^{b}	52.27^{a}
TSBM 10 g/kg	8.46^{b}	17.55^{b}	27.91^{b}	43.95^{ab}	74.84^{b}	80.00^{ab}	84.17^{ab}	5.17^{b}	82.95^{a}	88.12^{b}	0.09^{a}	72.97^{b}	58.50^{b}	49.17^{b}
SEM	0.4349	0.5538	0.6393	0.8440	1.3676	1.4548	1.5282	0.4347	1.2613	1.4877	0.0016	1.0081	0.8460	0.8099

Different superscripts in each column indicate significant difference at 0.05 level.

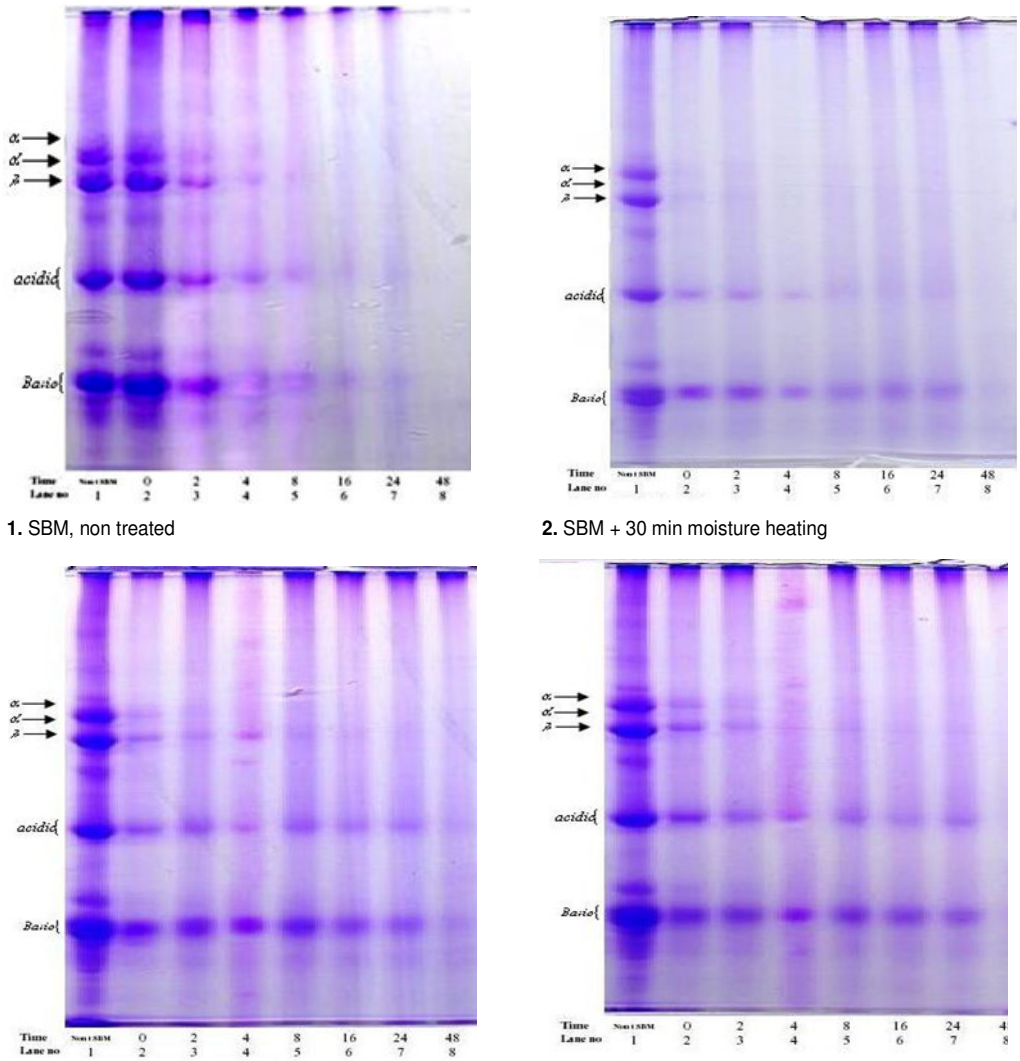
Table 2. The rumen degradation characteristics of crude protein in untreated and treated soybean meal.

Experimental feed	Washing loss (%)	Rumen degradation (g/kg) at different Incubation time						Degradation characteristics (g/kg)				Pe (g/kg)		
		2 h	4 h	8 h	16 h	24 h	48 h	a	b	a+b	C (h)	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
NTSBM	7.66^{a}	24.96^{a}	30.71^{a}	52.74^{a}	70.90^{a}	87.82^{a}	94.83^{a}	8.55^{a}	88.81^{b}	97.38^{a}	0.08^{a}	80.00^{a}	63.77^{a}	53.57^{a}
TSBM	8.27^{a}	14.85^{b}	29.31^{a}	49.27^{a}	73.28^{a}	85.15^{a}	93.91^{a}	4.84^{b}	92.21^{a}	93.43^{ab}	0.09^{a}	78.87^{a}	61.97^{a}	51.40^{a}
TSBM 5 g/kg	5.00^{ab}	14.06^{b}	30.22^{a}	47.99^{b}	72.17^{a}	84.93^{a}	90.55^{a}	2.53^{b}	90.90^{ab}	93.71^{ab}	0.09^{a}	76.83^{ab}	60.83^{b}	50.53^{a}
TSBM 10 g/kg	3.82^{b}	17.34^{a}	29.64^{a}	46.74^{b}	71.85^{a}	83.09^{a}	91.46^{a}	3.28^{b}	90.43^{ab}	95.86^{a}	0.08^{a}	76.40^{ab}	60.23^{b}	49.87^{ab}
SEM	0.2205	0.4803	0.5034	0.8444	1.4274	1.4732	1.1095	0.2631	1.1032	1.1725	0.0016	1.0054	0.8809	0.7850

Different superscripts in each column indicate significant difference at 0.05 level.

NTSBM, after 2, 4, 8, 16, 24 and 48 h of incubation in rumen were 32.59, 38.16, 51.33, 79.03, 82.14 and 91.65 g/kg, respectively, and for CP disappearance of NTSBM after the mentioned times were 24.96, 30.71, 52.74, 70.90, 87.82 and 94.83 g/kg, respectively. These values in NTSBM were significantly different from other treatment (Tables 1 and 2). The values for SBM are approximately similar to that reported by Deniz and Tuncer (1995) and similar to the results obtained in earlier studies (Mir et al., 1984; Windschitl and Stern, 1988; Harstad and Prestlokken, 2000). All discrepancies reported in *in situ* disappearance values can be attributed to varietal differences in the meal incubated, *in situ* technique, basal diet or variation in the extent of microbial contamination of the incubated samples (Freer and Dove, 1984; Nocek, 1988).

When SBM were treated with 5 and 10 g/kg xylose , DM and CP disappearances in all of time especially in time 16 h significantly reduced compared with NTSBM ($P<0.05$). DM disappearance of SBM treated with 5 and 10 g/kg xylose, after rumen incubation of 48 h, was lower than that of NTSBM ($P<0.05$). The rapidly soluble fractions (a) of DM were 15.13, 9.34, 6.73 and 5.17 g/kg and CP is 8.55, 4.84, 2.53 and 3.28 g/kg for NTSBM, TSBM, TSBM 5 g/kg and TSBM 10 g/kg respectively. Similarly, xylose reduced this parameter in SBM at all tested levels ($P<0.05$). Rapidly soluble CP content of SBM was similar in the study of Mir et al. (1984). These results are in agreement with the other studies using SBM (McAllister et al., 1993; Stanford et al., 1995). The potential degradability (b) of DM were 76.65, 83.37, 84.89 and 82.95 g/kg and for CP were 88.81, 92.21, 90.90 and 90.43 g/kg for NTSBM, TSBM, TSBM 5 g/kg and TSBM 10 g/kg, respectively (Table 1 and 2). The maximum potential degradability (a+b) of DM were 91.78, 92.70, 91.62 and 88.12 g/kg and for CP were 97.38, 93.43, 93.71 and 95.86 g/kg for NTSBM, TSBM, TSBM 5 g/kg and TSBM 10 g/kg, respectively (Tables 1 and 2) These results show that maximum potential degradability (a+b) DM and CP in 5 and 10 g/kg xylose significantly decreased compared with other treatment. These values indicated c fraction did not alter in degra-



1. SBM, non treated

2. SBM + 30 min moisture heating

3. SBM + 30 min moisture heating + 5 g xylose.

4. SBM + 30 min moisture heating + 5 g xylose

Figure 1. A 12% SDS-PAGE slab gel analysis of different treated soybean meal proteins. α, α`, and β: sub-units of β-congylcinin, acidic and basic sub-units of glycinin.

dability of DM and CP. Treating SBM with xylose (5 and 10 g/kg) reduced maximum potential de-gradability of these parameters (Tables 1 and 2). The rate of DM and CP disappearances of SBM reduced as the level of xylose used in treatment increased. Effective degradability of DM (k = 0.05 h^{-1}) were 65.71, 64.90, 61.77 and 58.53 g/kg and for CP were 63.77, 61.97, 60.83 and 60.23 g/kg for NTSBM, TSBM, TSBM 5 g/kg and TSBM 10g/kg, respectively (Tables 1 and 2). Treatment of SBM with 5 and 10 g/kg xylose and moisture heating caused a significant reduction in the effective crude protein degradability (k = 0.05 h^{-1}) compared with NTSBM ($P<0.05$).

These results indicate that the most effective protection of SBM is obtained with 5 and 10 g/kg xylose levels, which are more effective at decreasing rumen degradability of SBM dry matter and protein. In this method for treating SBM, the adequate lysine content of SBM and the epsilon-amino groups of lysine are primarily reactive site for aldehydes in the Maillard reaction (Windschitl and Stern, 1988; Harstad and Prestlokken, 2000). In conclusion, the present study demonstrated that SBM proteins can be effectively protected from degradation in the rumen by xylose and moisture heating treatment. Further research is required to examine the effect of xylose treatment on the intestinal digestibility of SBM crude protein escaping rumen degradation, particularly its effect on the availability of lysine and other essential amino acids.

The SDS-PAGE analysis of different treated soybean meal protein is presented in Figure 1. Two major components were observed: β-congylcinin and glycinin. Three components of β-congylcinin α, α`, and β were separated with estimated molecular weights of 86.26, 70.59 and 43.41 kDa, respectively. Two main polypeptide bands

were identified as acidic and basic sub-units of glycinin with estimated molecular weights of 30.76 and 18.01 KDa, respectively. The estimated molecular weights for acidic and basic glycinin sub-units are in approximately agreement with those previously reported (Kella et al., 1989; Van der Aar et al., 1983). β-congylcinin is more susceptible to rumen degradation than were the glycinin sub-units (Figure 1). The resistance to ruminal degradation of glyci-nin compared with β-conglycinin is probably associated with its chemical and physical structure. Its acidic and basic subunits are associated through intermolecular disulfide bridges and most of the S-S links are buried in the interior part of the glycinin molecules (Langan, 1972). In addition, electrostatic and hydrophobic associations are involved in maintaining the tertiary structure of glycinin.

Conclusion

It is concluded that SBM proteins can be effectively protected from degradation in the rumen by both of xylose and moisture heating treatment. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis is useful in studying fractional protein degradation of soybean meal proteins in the rumen. This study may provide a useful description of sub fractional protein degradation of protein supplements occurring in the rumen.

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