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Effect of Non-enzymatic Browning Reaction on the Treated Soybean Meal Proteins Degradation

[1]R. Salamatdoust Nobar, [2]Mohammad Chamani, [2]Ali-Asghar Sadeghi, [1]A. Gorbani, [1]K. Nazeradl, [1]J. Giyasi Ghaleh Kandi and [3]V. Ebrahim Zadeh Attari

[1]Islamic Azad University, Shabestar Branch, Department of Animal Science, Shabestar, Iran
[2]Islamic Azad University- Science and Research Branch, Department of Animal Science, Tehran, Iran
[3]Tabriz University of Medical Science, Department of Biochemistry and Nutrition, Tabriz, Iran

Abstract: In order to study rumen degradation of crude protein of treating soybean meal with xylose and microwave radiation an experiment in nylon bag technique was performed. Samples of treating soybean meal in times of 0, 2, 4, 8, 16, 24 and 48 h in rumen of three Taleshi male cows were incubated. Soybean meal samples treated with xylose and microwave radiation decreased water soluble protein fraction (a) and increased slowly protein degradation fraction (b) and treatment with 10 mg kg^{-1} xylose and 4 min microwave radiation have suitable protection against the soybean meal crude protein degradability. SDS-PAGE results show that Glycinin acidic and basic sub-units in treated soybean meal is resisted until 24 hours in the rumen. Results suggest that non enzymatic reaction may be useful for increasing the amount of SBM which escapes ruminal degradation.

Key words: Soybean meal · Xylose · Microwave radiation · Nylon bags · SDS-PAGE

INTRODUCTION

Today, to provide protein requirements of lactating cows and fattening calves with high growth rate usage slowly degradable protein in the rumen, but digestible in the small intestine is essential. Decreased degradability of soybean meal crude protein with different methods including thermal treating method [1, 2] such as non-enzymatic browning to protect of feed protein degradability in rumen were performed. Proteins and carbohydrates at the suitable temperature cause non-enzymatic browning reactions. Briefly, microwave energy penetrates a food or feed material and produces a volumetrically distributed heat source, due to molecular friction, resulting from dipolar rotation of polar solvents and from conductive migration of dissolved ions. The dipolar rotation is caused by variations of the electrical and magnetic fields in the organic components [3]. Water, the major constituent of most food and feed products, is the main source for microwave interactions due to its dipolar nature. Heat is generated throughout the material, leading to faster heating rates and shorter processing times compared to conventional heating, where heat is usually transferred from the surface to the interior [4]. Other advantages include space savings and energy efficiency, since most of the electro-magnetic energy is converted into heat [5]. The purposes of this study were to evaluate effects of microwave processing on ruminal CP degradation of treated soybean meal and to monitor the fate of soybean meal true protein in the rumen using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Samples and Treatments: Soybean meal samples were obtained from commercial sources in Iran. Based upon this value, sufficient water with 10 g kg^{-1} xylose was added to increase the moisture content of 2 kg of soybean meal to 200 g kg^{-1}. Three samples (500 g each) were subjected to microwave heating at a power of 900 W for 2 and 4 min. The remainder of each sample was ground to pass a 2 mm screen for the ruminal in situ study and preserved as describe above.

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

Crresponding Author: R. Salamatdoust Nobar, Islamic Azad University, Shabestar Branch, Department of Animal Science, Shabestar, Iran, E-mail: salamatdoust@gmail.com.

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.

Untreated Sbm
2 min irridation+10 g kg^{-1} xylose
4 min irridation+10 g kg^{-1} xylose

In Situ Evaluation of Crude Protein: Nylon bag technique was used to measure disappearance in the rumen of untreated and treated SBM. Nylon bags (45 µm pore size; 10 cm × 15 cm bag size) containing 5 g of SBM samples were incubated in the rumen of each cow. In a completely randomized design with three treatments and sex replications for each animal were performed, two bags of each type of treated SBM were removed after 2, 4, 8, 16, 24 and 48h of incubation in the rumen. Then individual bags with contents were washed in running tap water until the bags were free of rumen content. To reach constant weight, bags were dried at 60°C for 48h. The solubility or washing loss was determined by soaking samples of each material in water at 37-40°C for 1h followed by the washing procedure above. Digestion kinetics of CP was determined according to the equation of Ørskov and McDonald (1979):

$$P = \alpha + b(1-e^{-CT})$$

Where p is the amount degraded at a time, a the rapidly soluble fraction (g kg^{-1}), b the potentially degradable fraction (g kg^{-1}), c the constant rate of disappearance of b and t the time of incubation (h). The effective rumen degradability of CP was estimated using the equation of Orskov and McDonald (1979):

Determination of Protein Sub-units: Protein sub-units were fractionated by a SDS-PAGE discontinuous system [6]. All ruminal undegradable fractions from each incubation period were freeze dried, ground (0.25 mm particle size) and replicate samples pooled. Twenty microgram of untreated or treated corn were 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 10000×g for 1 min. A 25 µl aliquot of each sample was loaded into the sample well. Electrophoresis of proteinswas on 12.5% resolving gel (1.0mm×110mm×140 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 destaining was accomplished by using a 300 ml/l methanol and 70 ml/l acetic acid solution. The subunits of the gel were monitored by densitometric scanning at 580 nm. One standard protein mixture included β- galactosidase (116 kDa), bovine plasma albumin (66.0 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), Soybean trypsin inhibitor (21.5 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.4 kDa) was used. For statistical analysis of data, Neway and SAS softwares package was used. After analysis of variance, least squares means of each sample by the Tukey test were compared.

Statistical Analyses: Data were analyzed in a complete randomized design using the GLM procedure of SAS version 8.2 (SAS Inst. Inc. Cary, NC).

$$y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Whereas

y_{ij} = All dependent variable
μ = Overall mean
t_i = The fixed effect of oil levels (i=1,2,3)
ε_{ij} = The random effect of residual

RESULT AND DISCUSSION

The rumen degradation characteristics of crude protein in untreated and treated soybean meal are presented in Table 1 and SDS-PAGE result showed in Figure 1. The results show that soluble proteins in 0 time and 16h for experimental treatments had significant deference with control group ($p<0.05$). At other times of incubation (8, 24 and 48h), amount of degradation was reduced, but not statistically significant. Water soluble fraction (a), for 2 and 4min irradiation treatments significantly reduced and reached from 15.20 percent in the control group to 4.26 and 4.50 percent ($p<0.05$). Treating have good effect on slowly degradation fraction of protein (b) and decrease ruminal crude protein degradation so that from 82.54 percent in the control group significantly reached to 94.25 and 95.02 percent in treatments ($P<0.05$).

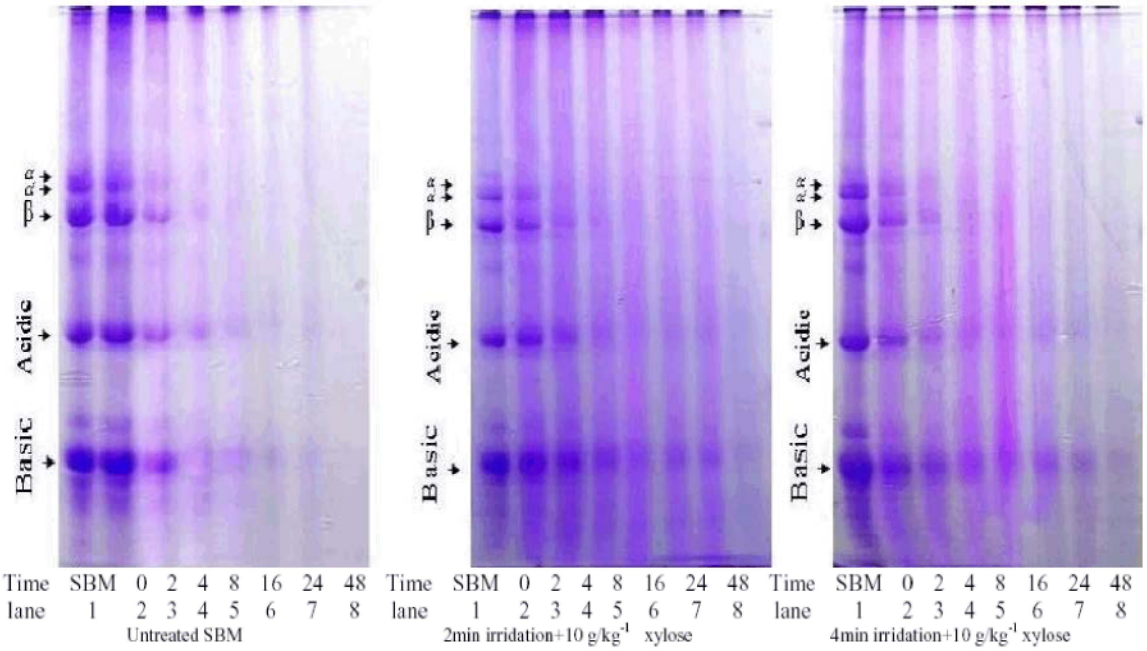

Fig. 1: A 12% SDS-PAGE slab gel analysis of different treated soybean meal proteins. α, α' and β sub-units of β-congylcinin, acidic and basic sub-units of glycinin

Table 1: The rumen degradation characteristics of crude protein in untreated and treated soybean meal

	Rumen degradation (g kg^{-1}) at different Incubation time							Degradation c haracteristics (g kg^{-1})	
	0	2	4	8	16	24	48	A	b
Untreated SBM	22.05^{a}	23.70^{a}	31.30^{a}	47.88^{a}	88.08^{a}	91.68^{a}	99.59^{a}	15.20^{a}	82.54^{b}
2min irridation+10 g/kg^{-1} xylose	10.18^{b}	14.67^{ab}	27.71^{ab}	49.33^{a}	75.96^{b}	87.65^{ab}	96.76^{a}	4.26^{b}	94.25^{a}
4min irridation+10 g/kg^{-1} xylose	9.96^{b}	13.64^{b}	25.26^{ab}	46.78^{a}	74.01^{b}	86.41^{ab}	94.97^{a}	4.50^{b}	95.02^{a}
SEM	1.0320	1.4395	1.7532	1.3776	1.6996	1.8524	1.0831	0.9729	1.5605
P value	<0.0001	<0.0001	0.0310	0.5421	0.0238	0.0253	0.5904	<0.0001	0.0304

The SDS-PAGE analysis of different treated soybean meal protein is presented in Figure 1. Two major components were observed: β-congylcinin and glycinin. 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. In addition, electrostatic and hydrophobic associations are involved in maintaining the tertiary structure of glycinin. SDS-PAGE results show that soybean meal ά, α sub-units of β-congylcinin proteins completely degradation after 4 hours and β subunit of protein after 8 h incubation degraded by rumen microorganisms. Glycinin acidic and basic subunits compared to β-congylcinin have slower breakdown rate and in control group until to 16h their subunits degraded, while in the experimental group affected protein subunit and resistance to 24 hours. Electrophoresis results show that microwave radiation for 2 and 4 minutes with 10 kg kg^{-1} xylose for resistance acid and basic subunits up to 24 hours of incubation was effective. Moisture is necessary for non-enzymatic browning reactions to occur because water serves as the medium through which reactants interact. However, excessive moisture content in reaction mixtures can slow the rate of non-enzymatic browning through simple dilution of reactants and, because a molecule of water is produced for each amino sugar formed, In conclusion, non-enzymatic browning reduced treated Soybean protein degradability. Results suggest that non enzymatic reaction may be useful for increasing the amount of SBM which escapes ruminal

degradation. Other chemical methods of protecting proteins from ruminal degradation include application of formalde- hyde [7, 8, 9], tannins [10, 11, 12], alcohols [13], sodium hydroxide [14] and divalent cations such as zinc [15].

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