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Use of SDS-PAGE to Measure Protein Degradation of Treated Soybean with *Pinus eldarica* Extract

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Abstract: Degradation proteins of treated soybean meal with pine needle methanolic extract was studied using SDS-PAGE. This technique facilitated the quantification of peptides of soybean proteins. Soybean meal samples treated with methanolic extract of pine needle. Incubation soy bean meal samples showed that, β-conglycinin [ά, α and - β subunits] were degraded completely within 2-4 h, while basic and acidic polypeptide components of glycinin were more resistant to degradation. Treating soybean meal with 8 and 10 percent pine needle extract and heating for 30 min could affect disappearance of soybean protein s subunit and decreased.

Key words: Soybean Meal · Pine Needle · Methanolic Extract · SDS-PAGE

INTRODUCTION

Soybean meal (SBM) is the most commonly used protein supplement in high producing ruminant includes dairy cattle and growing calves. Soybean meal is very palatable and has a good amino acid balance and high availability [1]. Relative to other commonly used feed proteins, Soybeans (SB) are rich in lysine but methionine, valine and isoleucine are the first, second and third limiting amino acids, respectively [2]. However, whole soybean (SB) and soybean meal (SBM) have relative low protein efficiency because of extensive ruminal degradation. It is estimated that only 25% to 34% of protein in SB and SBM escapes rumen fermentation, respectively [3]. Pinus eldarica is one of plant source of xylose and resin that decrease degradability of protein via stimulation of Millard reaction. The purpose of the following studies was to evaluate the degradation of soybean meal and treated SBM by pine needle methanolic extract and pressure heating using SDS-PAGE, a method that allows for direct visualization of protein degradation.

MATERIALS AND METHODS

Procedure of *Pinus eldarica* Extract Preparation: The *Pinus eldarica* methanolic extract were prepared with some modifications [4, 5].100 g of fresh ground leaves of *Pinus eldarica* were placed in 1000 ml of methanol solvent then agitated with a magnetic stirrer for 24 hrs at room temperature The solution was centrifuged at 3000 g for 10 min. The residue was re-extracted with 500 ml of methanol for 24 hrs stirring at room temperature and centrifuged again at 3000 g for 10 min then concentrated at approximately 65°C using a rotary-evaporator.

Sample Preparation and Treatment: The SBM samples were obtained from commercial sources in Iran. SBM was treated with 0, 6, 8 and 10 percent of pine needle methanolic extract and moisture heating at 1 bar pressure in 120°C for 30 min.

Animals: Three ruminally cannulated Ghezel rams were used for incubation of soybean meal samples, nylon bags (8cm × 16 cm) with a pore size of 46 mm were filled with approximately 5 g (sample size: bag surface area of 13 mg/cm) of the samples ground to pass from 2mm screen according to Nocek [6]. Duplicate bags filled with treated soybean meal were incubated in the rumen for periods of 0, 2, 4, 6, 8, 12, 16, 24 and 36 hrs. Two series of incubations were completed for each feed and sheep.

Treatments:

a: SBM, non treated
b: SBM + 30 min moisture heating + 6 percent pine needle extract
c: SBM + 30 min moisture heating + 8 percent pine needle extract
d: SBM + 30 min moisture heating + 10 percent pine needle extract

Determination of Protein Sub-Units: Samples were fractionated by SDS-PAGE discontinuous system [7].

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All ruminal undegradable fractions from each incubation period were dried, ground 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 10000 × 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 4% 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 to determine the molecular weight of subunit using UV-tech soft ware.

RESULTS AND DISCUSSION

Fig. 1 shows the SDS-PAGE profile of SBM protein subunits. Soybean meal proteins include β- conglycinin (ά:85.68 ; α:70.22 ; β:42.12 KDa) and glycinin (Acidic:28.91; Basic:19.84 KDa). The electrophoretic patterns of untreated soybean meal show that β- conglycinin subunits disappearance after 2 hours but glycinin subunits disappearance after 8 and 16 hours and represented a large proportion of the protein remaining in the nylon bags. This condition shown soybean meal protein can't escape from rumen and proteins extremely

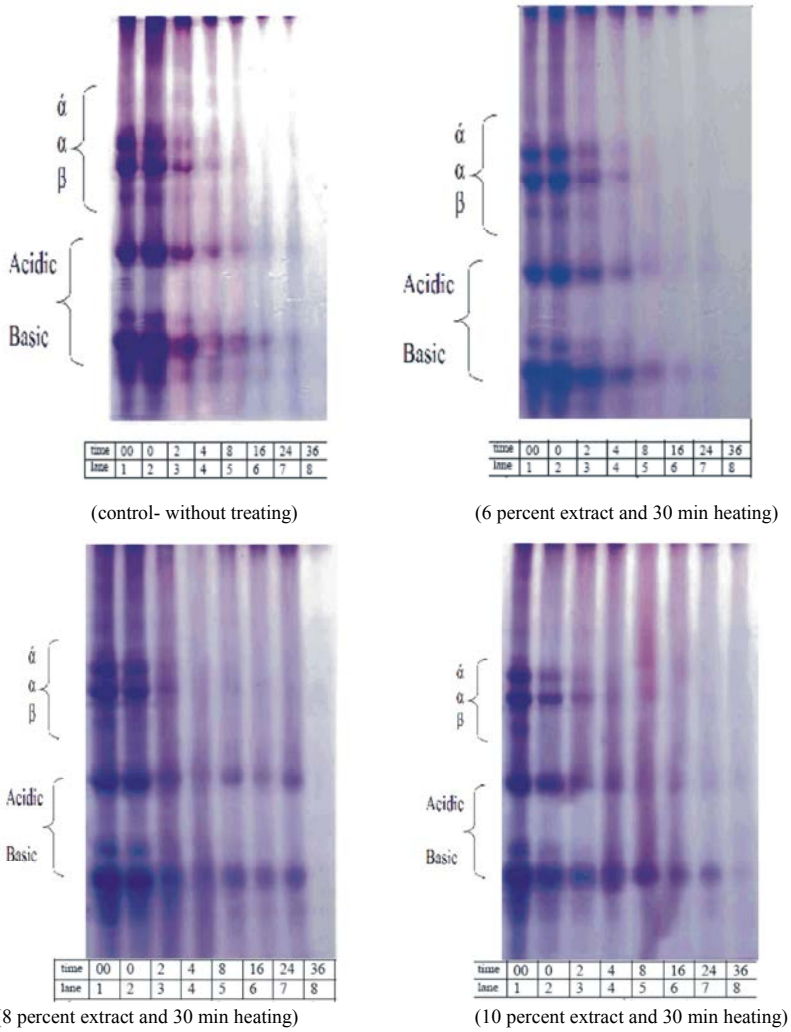

Fig. 1: 12% SDS-PAGE slab gels analysis of different treated soybean meal proteins ά, α and β sub-units of β-congylcinin, acidic and basic sub-units of glycinin.

Table 1: Molecular weight characterization of major subunits and polypeptides of soybean meal protein by SDS-PAGE

	Molecular Subunit, KDa	
Subunits	present study	Romagnolo *et al*. 1990
ß-Conglycinin		
ά	85.68	93.2
α	70.22	74.4
β	42.12	48.4
Glycinin		
Acidic	28.91	36.3
Basic	19.84	20.7

degradable. Usage 6 percent *Pinus eldarica* needle methanolic extracts and heating with pressure for 30 minute decreased β- conglycinin degradability and those subunits could until 8 hours resistance in the rumen the same conditions for glycinin subunits can be seen and resistance to 8 and 16 hours. Application of 8 and 10 percent of methanolic extracts has good effect on the decease subunit disappearance and β- conglycinin until 8 hours and acidic and basic subunits until 24 hours resistance. The resistance of glycinin to proteolytic attack can be attributed to higher intermolecular disulfide bonds that join its basic and acidic polypeptides compared with β- conglycinin [8]. The basic polypeptides of glycinin were more resistant to degradation than acidic polypeptides. This may be due to a difference in compactness of structure between the two type of polypeptides; basic polypeptides are more hydrophobic [9, 10] and thus more compact and less accessible to enzymatic attack. Also The cones and leafs contained large amounts of glucose (46%), mannose (25%), galactose and xylose. Maillard reaction is the catalyst for change, primarily by the addition of heat; and protein supplements with reducing sugar such as xylose, complex chemical reaction occurs on the surface. The pine needle contained xylose and combine with the amino acids of the soybean meal proteins. [11].

Molecular weights of the principal components of the soluble soybean protein extract are presented in Table 1. Acidic and basic polypeptides of glycinin and the ά, a and β subunits of β-conglycinin were shown.

Molecular weight estimates of the major soybean proteins also may be influenced by electrophoretic conditions (i.e., gel concentrations; percentage of cross linking) and molecular weight standards used [13]; however, interpretation of the results of this study was facilitated by amino acid sequence homology between major soybean proteins and peptides that appeared as products arising from partial hydrolysis of the soybean proteins.

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