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Determination of degradability of germinated and heated soybean seeds and its proteins fractions

ABSTRACT

A study on the sensitivity of watermelon variety Bojura to mutagenic agents was carried out in 2013-2014. The goal was to establish effective doses for mutagenic treatment of dry seeds with ^{60}Co gamma rays (80, 100, 200, 250, 350 and 450 Gy) and swollen seeds with water for 24 hours were treated with ethyl methanesulfonate (EMS) at a concentration of 2%. Dominant mutations were not observed in the M_1 generation. Morphological changes in 14 of 1395 M_2 plants were observed. Phenotypic variations changes were the colour of the seed coat, chlorophyll disorders of cotyledons, leaves, petals, and alterations of the location of the fruit set in the central stem. Visible changes of the morphological characteristics of the fruit were not observed. The doses induced certain morphological changes, however, higher doses or combined gamma rays ^{60}Co and EMS treatments would induce mutations more efficiently. Subsequent experiments are required to obtain mutants with changes that affect flowers and fruits. The results are important for increasing mutation efficiency in watermelon breeding.

Keywords: Soybean Seed, Germination, heat treatment, Degradability, SDS-Page

Introduction

Full-fat soybean, which contains 40% CP and 17% of fat, is interesting as a source of protein and energy in rations for cows in the first part of lactation (Grummer et al., 1994), but the protein is highly degradable by rumen microbes (Krishnamoorthy et al., 1982). Various chemical and physical processing has been suggested to decrease ruminal protein degradability. 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 (Harstad and Prestlokken, 2000; Sacakli, 2001; Nobar et al., 2009). Heat processing is the most commonly used physical method (Plegge et al., 1985; Pena et al., 1986). More recently, treatment of SBM by germination was successful in reducing degradation of soybean protein by rumen microorganisms.

Germination processes have been developed in some countries to overcome some of the disadvantages associated with ungerminated soybeans, such as undesirable flavour and odour and the presence of trypsin inhibitors (McKinney et al., 1958; Suberbie et al., 1981; Vanderstoep, 1981). Germination may also result in an increase in nutritive value relative to ungerminated seeds (Fordham et al., 1975). Germinated seeds were rated high in protein, appearance, flavor and texture and could be consumed uncooked in salad, boiled in water with suitable seasoning or fried in fat (Smith and Circle, 1978).

Also germination can increase amount of vitamin, mineral availability and decreased tannic acid and phytic acid in legumes (Ghavidel and Prakash, 2007; Hussein and Ghanem, 1999; Kaushik et al., 2010).

As mentioned earlier, various chemical and physical processing has been suggested to decrease ruminal protein degradability. Heat processing is the most commonly used physical method (Plegge et al., 1985; Pena et al., 1986). This improved production has been attributed primarily to an increase in escape of protein from ruminal degradation, thereby providing more amino acids (AA) for intestinal digestion and absorption (Nasri et al., 2008).

Use of SDS-PAGE allows direct visualization of protein degradation, isolation and quantification of feed proteins. Thus it can be used to predict ruminal degradation directly and to assess the type of proteins that will escape the rumen (Messman and Weiss, 1994). Moreover, this technique could detect differences in ruminal degradability of true proteins between untreated and treated protein supplements (Sadeghi et al., 2006).

The objectives of this study were to evaluate the effect of germination and heating treatments in the 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.

Material and Method

Sample preparation and treatment

In this study one of Iranian cultivars of soybeans (L17) was used. Thisis the most common cultivar used in ruminant diets in Iran.

For Germination soybean seeds were soaked in distilled water (1:5 w/v) for 6 h at room temperature. The water was drained off, and the seeds transferred to a moisture adherent flax cloth (tarts) to germinate for 4 days in the dark at 22 °C. Every 24 h, the seeds were moistened with distilled water and carefully shaken (Yasmin et al., 2008). For heat treatment, after 4 days of germination, a part of germinated grains placed in an autoclave at 121 °C with steam pressure (100 kPa). At the end, seeds were ground and dried in an air oven at 60 °C for analysis.

Chemical analyses

Feed samples were analyzed for dry matter, crude protein, ether extract, crude fiber and crude ash content of soybean grains after treatment and rumen incubation by using the procedures of AOAC (2005).

In situ ruminal procedure

Three wethers fitted with rumen cannula were used to measure rumen degradability of feeds. The wethers were fed a diet composed of (on DM basis), 385g/day alfalfa hay, 280g/day barley grain, 35 g/day wheat bran and 1.5 g/day lime stone at maintenance (NRC, 1985). The wethers were kept in individual tie-stalls with individual feed bins in an animal house and had continuous access to water. Diets were given as total mixed ration with fresh feed offered twice each day (08:30 and 15:30 h). The nylon bag technique (Orskov and McDonald, 1979) was used to measure the DM degradation of feeds in the rumen. Nylon bags (4 cm×8 cm polyester bag; poor size 45-50 µm) containing 3 g of seeds ground through a 2 mm screen were incubated in the rumen for 2, 4, 8, 16, 24 and 48 h for seeds, immediately after the morning feeding. As a whole, there were six replicates for each feed sample and for each incubation time (3wethers × 2 bags). Immediately after removal from the rumen, the bags were washed in cold water and frozen at −18 °C. At the end of the collections, they were unfrozen and washed together with the zero time bags (not incubated in the rumen) for 20 min and then dried at 80°C for 24 h. The residues were weighed and submitted for analysis.

Determination of protein sub-units

Protein sub-units were fractionated by a SDS-PAGE discontinuous system (Laemmli, 1970). All ruminalundegradable fractions from each incubation period were dried, ground (0.25 mm particle size) and replicate samples pooled. Twenty micrograms of untreated or treated SBM 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 proteins was on 1 2.5% 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), RErease Bsp98I (25 kDa), β-Lactoglobulin (18.4 kDa) and Lysozyme (14.4 kDa) were used.

Calculations and statistical analysis

In situ Dry Matter (DM) rapidly degradable fraction (a), potentially degradable fraction (b), and rate of degradation of fraction b (c), were calculated according to model of Orskov and McDonald (1979) as $y=a+b(1-e^{-ct})$ that y is the actual degradation of DM after t, a is the intercept of the degradation curve at time zero, b is the potential degradability of the component of the insoluble but degradable DM, which will in time be degraded, c represents the constant of degradation rate of b at time, t is incubation time.

Data were subjected to one-way analysis of variance using the analysis of variation model (ANOVA) of SAS (1999). Multiple comparison tests used Duncan's multiple-range test (Snedecor and Cochran, 1989).

Results and Discussion

Soybean is used worldwide for animal nutrition and it is important sources of protein, carbohydrates and energy due to their high contents of oil. Germination and heating as separated processes have proved partially beneficial for the nutritional quality such as chemical composition and rate rumen degradability of soybean seeds. The study of the effect of combined germination and heating may provide useful information for optimization of use of soybean seeds as feed in ruminant rations.

Chemical composition

Table 1 shows the results of proximate analyses, as well as dry matter, crude protein, ether extract, crude fiber and crude ash. Germination increased DM and CP content of seeds ($p<0.05$) and had no effect on ether extract and crude ash. The heat treatment did not have a considerable effect on the DM, CP, EE and ash of the germinated soybean seeds.

The small variations observed were considered to be within the range of the method error.

Germination during 4 days modify the proximate composition of the soybean seeds on a dry matter basis such as DM, CP and ether extract, except for a small decrease crude fiber and ash. Increasing seed crude protein and change amino acid composition due to germination reported (Ghavidel and Prakash, 2007; Mostafa et al., 1987). A significant change in the oil content, due to the germination process, and slight decreases in the oil content of the beans amounting to 5.37% and 7.69%, respectively previously reported by Mostafa et al. (1987). This decrease could probably be ascribed to consumption of oil as energy and/or synthesis of certain structural constituents in the young seedling (Singh et al., 1968).

Rumen degradability

Dry matter losses from the nylon bags incubated in the rumen and in situ DM degradability characteristics are presented in Table 2. There were differences ($p<0.05$) among treatment of soybean seeds in dry matter degradability after several incubation times. Total dry matter washing losses (zero time bags) represented 17.25 to 21.79% of DM in heated seeds and untreated seeds, respectively. Dry matter disappearance from nylon bags incubated in the rumen increased with increasing incubation time. The 48 h incubation time was sufficient for test seeds to be degraded. After 48 h incubation, untreated soybean seeds had highest DM rumen degradability. Results show that DM rumen degradability was reduced by heating treatment.

Crude protein rumen degradability and in situ CP degradability characteristics are presented in Table 3. There were differences ($p<0.05$) among treatment of soybean seeds in dry matter degradability after several incubation times. Crude protein washing losses (zero time bags) represented 9.87 to 20.55% of CP in heated seeds and untreated seeds, respectively. Dry matter disappearance from nylon bags incubated in the rumen increased with increasing incubation time. The 48 h incubation time was sufficient for test seeds to be degraded. After 48 h incubation, untreated and germinated soybean seeds had highest DM rumen degradability 86.77% and 83.14% respectively).

A large range of dry matter degradation characteristics was obtained: the 'a', 'b' and 'c' values ranged from 8.58 to 11.48% (for HGSS and GSS), 78.14 to 87.17% (for HGSS and NSS) and 0.046 to 0.052 $\%h^{-1}$ (for HGSS and GSS), respectively. Results show that heat treatment reduced CP effective degradability in rumen.

The relationship between the degradability parameters a, b and c and the chemical composition of 60 test feeds was reported by Woods et al. (2003). They reported that the slowly fermented structural carbohydrates are thought to play a dominant role in the degradation characteristics in the rumen. It seems that germination and heating can be changed mount and component of carbohydrate content of seeds.

Table 1.*Chemical composition of soybean seeds with and without germination and heat treatment (% DM) †*

	Soybean seeds		
Item	NSS	GSS	HGSS
Dry Matter	92.54^{b}	93.06^{a}	93.05^{a}
Crude Protein	35.32^{b}	38.66^{a}	39.32^{a}
Ether Extract	17^{b}	16.3^{a}	16.2^{a}
Crude Fiber	6.00	5.80	5.68
Ash	6.02	6.01	6.02

† Three samples analyzed for each feed
NSS: untreated soybean seeds, GSS: Germinated soybean seeds and HGSS: Heated and Germinated soybean seeds
^{a}, ^{b} Means within a column with different subscripts differ ($P<0.05$).

Table 2: *The in situ disappearance of dry matter (%) and DM degradation characteristics of test feeds in the rumen*

	DM degradability (%DM)							Degradation characteristics			
Feeds	0 h	2 h	4 h	8 h	16 h	24 h	48 h	a (%)	b (%)	*c* ($\%.h^{-1}$)	ED
NSS	21.7^{a}	22.7^{b}	27.1^{b}	34.8^{a}	53.0^{b}	79.5^{a}	83.9^{a}	16.77^{b}	80.99^{a}	0.042^{b}	72.9^{a}
GSS	21.0^{a}	26.8^{a}	31.6^{a}	37.3^{a}	58.6^{a}	69.8^{b}	80.4^{ab}	19.71^{a}	68.46^{b}	0.049^{a}	68.3^{b}
HGSS	17.2^{b}	22.5^{b}	25.1^{b}	30.6^{b}	45.1^{c}	70.3^{b}	79.4^{b}	15.37^{b}	82.19^{a}	0.034^{c}	67.8^{b}
SEM (n=6)	0.581	0.696	0.811	0.878	1.091	1.139	1.018	0.608	0.648	0.0006	1.22

NSS: untreated soybean seeds, GSS: Germinated soybean seeds and HGSS: Heated and Germinated soybean seeds.
a, b, c Means within a column with different subscripts differ ($P<0.05$).
ED = Effective degradability ($k=0.02$)

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Table 3: *The in situ disappearance of crude protein (%) and CP degradation characteristics of test feeds in the rumen*

	DM degradability (%DM)							Degradation characteristics			
Feeds	0 h	2 h	4 h	8 h	16 h	24 h	48 h	a (%)	b (%)	*c* (%.h^{-1})	ED
NSS	12.4^{a}	17.4^{b}	21.8^{b}	35.7^{b}	57.5^{b}	75.0^{a}	86.7^{a}	9.17^{b}	87.17^{a}	0.050^{b}	71.5^{a}
GSS	12.0^{a}	20.5^{a}	25.4^{a}	38.4^{a}	52.5^{b}	74.6^{a}	83.1^{a}	11.48^{a}	79.71^{b}	0.052^{a}	43.3^{a}
HGSS	9.8^{b}	17.2^{b}	20.2^{c}	31.5^{c}	40.4^{c}	72.8^{a}	75.2^{b}	8.58^{b}	78.14^{b}	0.046^{c}	37.1^{b}
SEM (n=6)	0.22	0.36	0.44	0.69	0.92	1.51	1.59	0.190	1.626	0.00004	1.34

NSS: untreated soybean seeds, GSS: Germinated soybean seeds and HGSS: Heated and Germinated soybean seeds.
a, b, c Means within a column with different subscripts differ (P<0.05).
ED = Effective degradability (k=0.02

The significant reduction in the rate of degradation(c) and hence ED of N by heat processing showed itappeared to increase by-pass protein from soybean andthereby increase the amount of available AA in the smallintestine, provided that the digestibility of by-passprotein does not decrease with heat treatment. Previousstudies also reported that heat processing reduced therate of disappearance of DM and N of whole soybean(Nasri et al., 2008; Faldet and Satter, 1991Hsu and Satter, 1995; Aldrich et al., 1997).

Heat facilitates the Maillard or nonenzymatic browreaction between sugar aldehyde groups and free amino acid groups of protein to yield an amino-sugar complex (Canbolat et al., 2005; Lin and Kung, 1999).

This complex is more resistant than normal peptides to enzymatic hydrolysis and reversibility of this reaction is dependent on temperature and duration of heat exposure (Lin and Kung, 1999). However, some precautions must be taken when heat treatments are employed because the Maillard reactions might render the protein and the carbohydrate unavailable in the small intestine if excess heat was employed

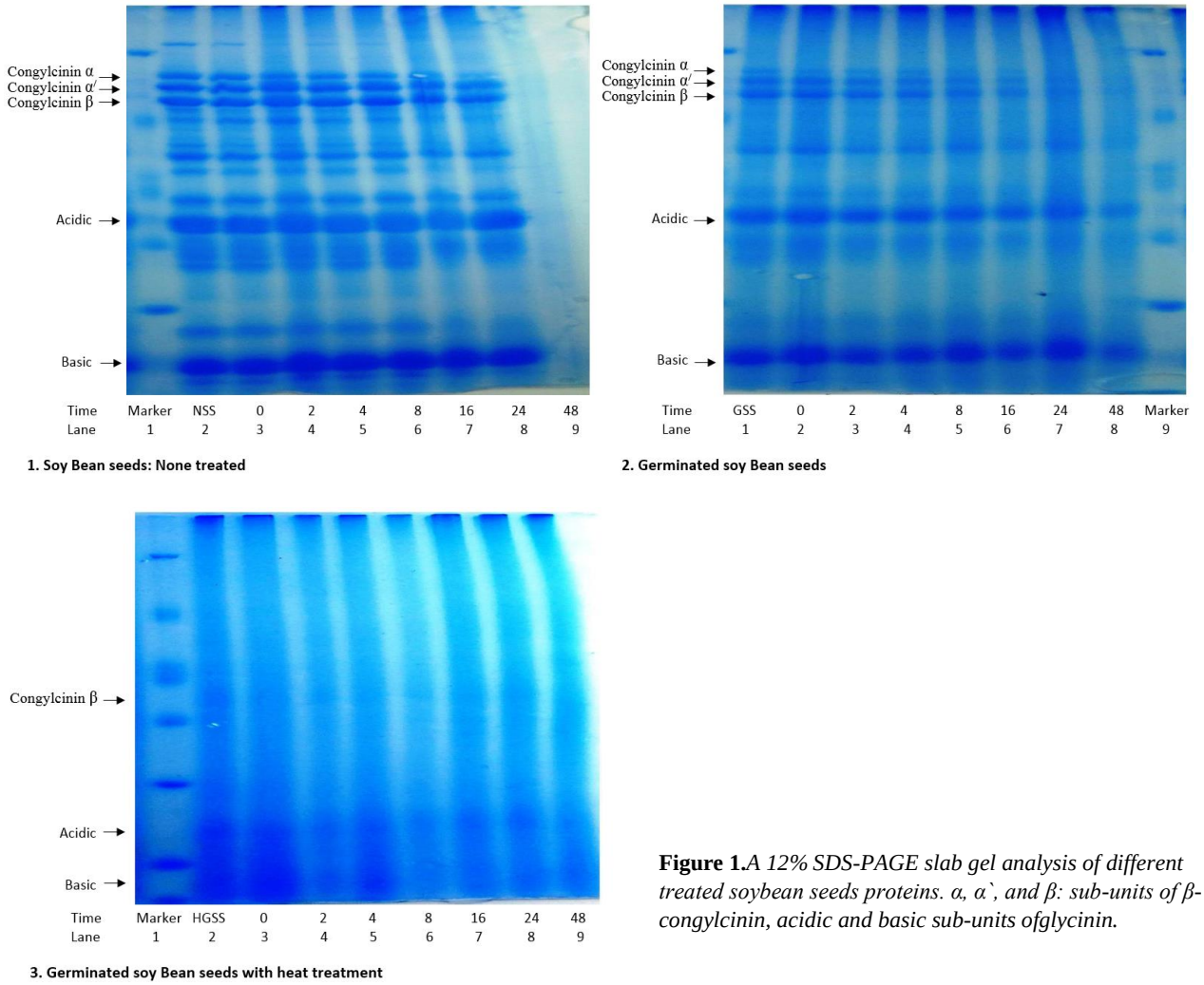

Figure 1.*A 12% SDS-PAGE slab gel analysis of different treated soybean seeds proteins. α, α`, and β: sub-units of β-congylcinin, acidic and basic sub-units ofglycinin.*

(Lin and Kung, 1999). In the Maillard reaction excess heat causes losses of sugar and amino acids, especially lysine, which can be 5 to 15 times greater than for the other amino acids (Andrian, 1974). Therefore, heat treatment should be kept to a minimum due to its cost and the possibility of destroying essential amino acids such as lysine and methionine and reducing the availability of other nutrients (Kratzer et al., 1990; Van der Poel et al., 1995; Qin et al., 1998). The effect of heat treatment on the rumen degradability of protein sources was not consistent.

SDS-Page

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 97.86, 88.86 and 78.74 kDa, respectively. Results show that all protein sub units such as α`-congylcinin in untreated seed, degraded in 48 h after incubation.Also, in germinated seed, glycinin sub units resistance to degradation in all incubation time.

Two main polypeptide bands were identified as acidic and basic sub-units of glycinin with estimated molecular weights of 26.58 and 14.40 KDa, respectively. The estimated molecular weights for acidic and basic glycinin sub-units are in approximately agreement with those previously reported (Nobar et al., 2005; Kella et al., 1989; Van der Aar et al., 1983). β-congylcinin is more susceptible to rumen degradation than the glycinin sub-units were (Figure 1) and this in agreement by Nobar et al. (2005) reporting. The resistance to ruminal degradation of glycinin 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 ofglycinin (Nobar et al., 2005).

References

Aldrich CG, Ingram S, Coldfelter JR.1997. Assessment of postruminal amino acid digestibility of roasted and extruded whole soybeans with the precision-fed rooster assay. J. Anim. Sci., 75:3046–3051.

Andrian J.1974.Nutritional and physiologic consequences of the Maillard reaction.World Rev.Nutr. Diet,19:71-88.

Association of Official Analytical Chemists 2005.Official Methods of Analysis, 16th ed. AOAC, Arlington, VA, USA.

Canbolat O, Kamalak A, Efe E, SahinM,Ozkan CO. 2005. Effect of heat treatment on in situ rumen degradability and in vitro gas production of full-fat soyabeans and soyabean meal. South African J. Anim. Sci.,35: 186-194.

Cooker BA, Clark JH, Shanks RD.1983. Effect of formaldehyde treated soybean meal on milk yield, milk composition and nutrient digestibility in dairy cow. J. Dairy Sci.,66:492-504.

Driedger A, Hatfield EE. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. J. Anim. Sci.,34:465-468.

Faldet MA, Satter LD. 1991. Feeding heat-treated full fat soybeans to cows in early lactation. J. Dairy Sci.,74:3047–3054.

Fordham JR, Wells CE, Chen LH. 1975. Sprouting of seeds and nutrient composition of seeds and sprouts. J. Food Sci.,40:552-6.

Ghavidel RA, PrakashJ. 2007. The impact of germination and dehulling on nutrients, antinutrients,in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT, 40:1292-1299.

Grummer RR, Luck ML, Barmore JA. 1994. Lactational performance of dairy cows fed raw soybeans, with or without animal by-product proteins, or roasted soybeans. J. Dairy Sci.,77:1354–1359.

Harstad OM, Prestlokken E.2000. Effective rumen degradability and intestinal indigestibility of individual amino acids in solvent-extracted soybean meal (SBM) and Xylose -treated SBM (Soy Pass) determined in situ. Anim Feed Sci. Technol.,83:31 -47.

Hsu JT, SatterLD. Procedures for measuring the quality of heat-treated soybeans. J Dairy Sci., 78:1353–1361.

Hussein L, Ghanem KZ. 1999. Calciumbioavailability from selected Egyptian foodswith emphasis on the impact of germination and fermentation. International J. Food Sci. Nutrition,50:351–356.

Kaushik G, Satya S,Naik SN. 2010. Effect of domestic processing techniques on the nutritional quality of the soybean. Mediterranean J. Nutrition Metabolism,3:39-46.

Kella NKD, Barbean WE, Kinsella JE.1986.Effect of oxidative sulfitolysis of disulfide bonds of glycinin on soulubility, surface hydrophobicty, and in vitro digestibility. J. Agric. Food Chem.,34:251-260.

Kratzer FH, Bersch S, Vohra P.1990. Evaluation of heat-damage to protein by coomassie blue G dyebinding. Food Sci.,55:805-807.

KrishnamoorthyU, Muscato TV, Sniffen CJ, Van Soest PJ. Nitrogen fractions in selected feedstuffs. J. Dairy Sci.,65:217–229.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature,227:680-292.

Langan JL. 1972. Quantitive studies on nitrogen metabolism in the bovine rumen. Br. J. Nutrition, 27:261-283.

Lin C, Kung L.1999. Heat treated soybeans and soybean meal in ruminant nutrition. Technical Bull.American Soybean Assoc and United Soybean Board.

McAllister TA, Cheng KJ, Beauchemin KA, Bailey DRC, Pickaord MD, Gilbert RP. Use of lignosulfonate to decrease the rumen degradability of canola meal protein. Can. J. Anim. Sci., 73:211-215.

McKinney LL, Weakley FB, Campbell RE.Changes in the composition of soybean on sprouting. J. American Oil Chem. Soc.,35:364-366.

Messman MA, Weiss WP.1994. Use of electrophoresis to quantify ruminal degradability of protein from concentrate feeds. Anim. Feed Sci. Technol.,49:25–35.

Mir Z, MacLeod GK, Buchanan-Smith JG, Grieve DG, Grovum WL. 1984. Methods for protecting soybean and canola proteins from degradation in the rumen. Can. J. Anim. Sci.,64:853–865.

Mostafa MM,Rhama EH.1987. Chemical and nutritional changes in soybean during germination.Food Chem.,23:257-275.

Nakamura T, Klopfenstein TJ, Gibb DJ, Britton RA. 1994. Growth efficiency and digestibility of heated protein feed to growing ruminants. J. Anim. Sci.,72:774-782.

Nasri MHF, France J, DaneshMesgaran M, Kebreab E. 2008. Effect of heat processing on ruminal degradability and intestinal disappearance of nitrogen and amino acids in Iranian whole soybean. Livestock Sci.,113:43–51.

National Research Council. 1985. Nutrient Requirements of sheep. NatlAcadSci, Washington, DC.

Nishimuta JF, Ely DG, Boling JA. 1974. Ruminal bypass of dietary soybean protein treated with heat, formalin and tannic acid. J. Anim. Sci.,39:952-957.

Nobar RS, Chamani M, SadeghiAA,MirzaAghazadeh A. 2009. Determination of degradability of treated soybean meal and its proteins fractions. African J. Biotechnology,8:98-102.

OrskovER, McDonald Y. 1979. The estimation of protein degradability in the rumen from determining the digestibility of feeds in the rumen.J.Agri. Sci.92:499-503.

PenaF, Tagari H, Satter LD.1986. The effect of heat treatment of whole cottonseed on site and extent of protein digestion in dairy cows. J. Anim. Sci.,62:1423–1432.

Plegge SD, Berger LL, Fahey GC.1985. Effect of roasting temperature on the proportion of soybean meal nitrogen escaping degradation in the rumen.J. Anim. Sci.,61:1211–1218.

Qin GX, Verstegen MWA, Van der Poel AFB.1998. Effect of temperature and time during steam treatment on the protein quality of full-fat soybean from different origins. J. Sci. Food Agri.77:399-398.

Robinson PH, Khorasani GR, Kennelly JJ.1994. Forestomach and whole tract digestion in lactating dairy cows fed canola meal treated with variable levels of acetic acid. J. Dairy Sci.77:552-559.

SacakliP.2001. Bazıküspelerinksilozilemuameleedilmesininsindirilmederecesiv e rumen parçalanabilirliközellikleriüzerineetkisi.Doktoratezi, Ankara ÜniversitesiSaglikBilimleriEnstitüsü, Ankara.

Sadeghi AA, Nikkhah A, ShawrangP,Shahrebabak MM. 2006. Protein degradation kinetics of untreated and treated soybean meal using SDS-PAGE.Anim.Feed Sci.Technology.126:121–133.

SAS.1999. SAS user's guide Statistical analyses systems institute. Cary, USA.

Singh BB, Hadley HH, Collins RI. 1968. Distribution of fatty acids in germinating soybean seeds. Crop Sci.,8:171-173.

Smith AK, Circle SJ. 1978. Soybeans: Chemistry and technology. AVI Publishing Company, Inc., Westport, Connecticut.

Snedecor GW, Cochran WG.1980. Statistical Methods.Iowa State University Press, Ames, IA, USA.

Suberbie F, MendizabalD,Mendizabal C.1981. Germination of soybeans and its modifying effects on the quality of full fat soy flour. J. Amer. Oil Chem. Soc.,58:192-194.

Thomas E, Trenetle A, Burroughs W.1979. Evaluation of protective agents applied to soybean meal and fed to cattle. Part I. Laboratory measurements. J. Anim. Sci., 49:1337-1345.

Van der Aar PJ, Berger LI, Wujek KM, Mastenbroek I, Fahey GC.1983. Relationship between electrophoresis band patten and in vitro ammonia relase of soluble soybean meal proterin. J. Dairy Sci.,66:1272-1276.

Van der Poel AFB, Verstegen MWA,Tamminga S.1995. Chemical physical and nutritional effects of feed processing technology.Proc. 16th Western Nutrition Conference, Saskatoon, Canada.

Vanderstoep J. 1981. Effect of germination on the nutritive value of legumes. Food Tech.,3: 83-85.

Windschitl PM, Stern MD.1988. Evaluation of calcium lingo sulfonate treated soybean meal as a source of rumen protected protein for dairy cattle. J. Dairy Sci.,71:3310-3322.

Woods VB, O'Mara FPO,MoloneyAP.2003. The nutritive value of concentrate feedstuffs for ruminant animals. Part I. In situ rumen degradability of dry matter and organic matter. Anim. Feed Sci. Technol.,110:111-130.

Yasmin A, Zeb A, Khalil AW, ParachaGM,Khattak AB. 2008. Effect of processing on antinutritional factors of red kidney bean (Phaseolus vulgaris) grains. Food Bioprocess Technol.,1:415-419