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Estimation of Relationship Between In Situ and In Vitro Rumen Protein Degradability of Extruded Full Fat Soybean

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ABSTRACT

The objectives of this study were to estimate the protein degradability of extruded full fat soybean (ESB) by in situ (nylon bag) and in vitro enzymatic method and to develop an equation in order predict in situ degradability from in vitro values. In the study enzymatic technique; hydrolysis after 1 h (INV_1) and after 24 h (INV_{24}) by a purified protease extracted from *Streptomyces griseus* in a borate-phosphate buffer at pH 8 was used as in vitro method. Relationship between in situ effective protein degradability (INS_E) and in vitro degradability after 1 and 24 hours incubations (INV_1 and INV_{24}) were determined. In situ protein degradability was measured at 0, 2, 4, 8, 16, 24, and 48 and at 72 h incubations in the rumen of 3 Holstein cows. In the study INS_E, INV_1 and INV_{24} were determined as 58.05, 20.24 and 41.46% respectively. Despite there were differences between in situ and in vitro protein degradability values, correlation coefficients between in situ and in vitro protein degradability of ESB were high and regression equations for estimation of in situ from in vitro were found significant. As conclusion in vitro enzymatic protein degradability (INV_1 and INV_{24}) can be used for estimation of in situ effective protein degradability of extruded full fat soybean.

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Introduction

Ruminants differ significantly from other animals due to their unique digestive physiology. The microbial protein synthesized by rumen microorganisms is not sufficient to provide the high protein requirements of dairy cows in early lactation, early weaning calf and lambs especially (Broderick et al., 1988). Biologically high-protein value proteins that are enzymatically digested in abomasum and small intestines and not degradable in the rumen give better results when they are fed to high-yielding ruminants (Broderick et al., 1988). In order to provide the protein requirements of ruminants correctly, feed N compounds should be defined according to ruminal degradability characteristics such as rumen degradable protein (RDP) and rumen undegradable protein (RUP) (AFRC, 1987; Chamberlain and Wilkinson, 1998; Cömert and Sayan, 2000; McDonald et al., 2011).

There are a number of methods that involve different procedures used to determine the rumen degradability of feedstuffs. These methods can be classified as in vivo, in situ and in vitro methods. The in vivo method is usually the standard; but it is expensive and time consuming. Therefore, faster and cheaper methods have been developed which are in situ and in vitro methods. In situ method (nylon bag) is mostly accepted and used as a reference method to determine the RDP and RUP of feedstuffs. The exponential model developed by Orskov and McDonald (1979) has been widely used. Because in vivo and in situ methods require cannulated animals and the routine use of these techniques in the evaluation of feedstuffs are difficult. In vitro methods are needed which can be used in accurately estimating the ruminal degradability of feedstuffs. In these methods; buffer solutions purchased from commercial companies, buffer solutions extracted from rumen, chemical solutions, rumen liquid and enzymes were used. In contrast, for feed mixtures, the prediction very precise and much better with enzymatic method than the solubility method (Aufrere et al., 1991). Many researchers (Krishnamoorty et al., 1983; Poos Floyd et al., 1985; Susmel et al., 1989; Aufrere et al., 1991; Roe et al., 1991; Assoumani et al., 1992; Calsamiglia and Stern, 1995) have reported that there was a high correlation between in situ protein degradability values and protein degradability values obtained by enzymatic in vitro methods.

A number of enzymatic methods have been proposed by researchers to predict the digestibility of feedstuffs. In particular, methods using commercial proteases provide

advantages in terms of labour and time. Proteases with different origins have been tested by some researchers to determine ruminal protein degradability. The most commonly used protease enzyme was the enzyme which has been extracted from *Streptomyces griseus* (Krishnamoorthy et al., 1983; Chaudhry, 2005, 2007). Aufrere et al. (1991) reported that in the French protein system (digestible proteins in the intestine, PDI), the enzymatic method is used as a laboratory method for nitrogen evaluation.

This research was conducted to determine in situ and in vitro rumen protein degradability of ESB and to develop regression equations to estimate in situ effective protein degradability from in vitro protein degradability values. Thus, these equations will facilitate the determination of in situ effective protein degradability of ESB by using in vitro protein degradability values of ESB.

Materials and Methods

Feeds and Chemical Analyses

The experiment was conducted using ten extruded full fat soybeans (ESB) collected from feed plants located different provinces in Turkey. A sample of each ESB was ground to pass 1 mm sieve using Retsch ZM200 laboratory mill and analysed for dry matter (DM, method 930.15), crude ash (CA, method 942.05), ether extract (EE, method 920.39) according to procedures of AOAC (1995) and for Crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) by the procedure of Van Soest et al., (1991). Total N was assayed by Micro Kjeldhal (AOAC method, 984.13. 1995) and crude protein (CP) content was calculated by multiplying N by 6.25 in feeds and residues after incubations of in vitro and in situ. The chemical compositions of ESB were presented in Table 1.

Animals

Three Holstein dairy cows 5 years old and weighing 550±30 kg were fitted with permanent rumen cannula to measure ruminal degradability of ESB in International Center for Livestock Research and Training. Cows were fed with a total mixed ration containing 70 % mixture of barley straw and alfalfa hay and 30 % concentrate, 1.25 times above maintenance requirements on a dry matter basis. Chemical composition of forage and concentrate offered to animals was presented in Table 2. During in situ experiment cows were kept in individual stalls and allowed free access to water and fed twice daily at 08:00 and 17:00 h.

In Situ Method

In situ rumen protein degradability was measured using the nylon bag technique (Orskov and McDonald, 1979) adapted by Michalet-Doreau et al., (1987). Five grams air-dry samples ground in same manner as before chemical analyses were weighed into 5x10 cm polyester bags with 50 µm pore size (Ankom R510) and suspended in the rumen of three cows as three replicates for 2, 4, 8, 16, 48 and 72 h. The bags were simultaneously inserted in the rumen after the morning meal and removed sequentially at end of each incubation time. After incubation, the bags were immediately rinsed under tap water and washed in a commercial washing machine for 5 minutes. The 0 h bags were not incubated in the rumen but followed the same washing procedure. After washing the bags were dried in a forced-air oven at 65 °C for 48 h and the residues were analysed for crude protein content as it was explained in chemical analyses. In situ effective degradability of feed proteins (INS_E) was calculated from the kinetics of in situ degradation using the equation below from Orskov and McDonald (1979), assuming that theoretical ruminal passage rate (k) is 0.06/h for milking cows according to Verité et al., 1987.

Table 1 Chemical composition of ESB samples (Mean ± SEM, percent of DM*)

Feeds	DM	Ash	CP	EE	NDF	ADF	ADL	CF
ESB_1	90.6	4.8	41.7	22.3	10.1	7.9	0.2	7.8
ESB_2	88.4	4.6	41.4	21.4	14.2	10.9	0.5	9.5
ESB_3	93.4	7.6	36.8	16.7	13.5	10.4	1.0	8.9
ESB_4	89.7	4.7	41.7	21.3	10.4	8.3	0.8	8.0
ESB_5	89.8	5.6	42.0	22.7	9.3	7.4	0.8	6.3
ESB_6	94.4	5.5	38.7	18.9	10.2	7.9	0.8	6.9
ESB_7	89.0	5.0	39.7	26.1	9.5	8.3	0.8	7.9
ESB_8	94.5	6.4	34.5	20.4	9.7	6.7	0.7	5.6
ESB_9	89.2	5.0	37.7	21.3	10.1	8.2	0.6	7.2
ESB_{10}	90.3	5.0	38.6	17.9	9.9	8.1	0.7	5.4
Mean	90.9±0.72	5.4±0.29	39.3±0.80	20.9±0.84	10.7±0.54	8.4±0.40	0.7±0.07	7.4 ±0.42

*ESB: Extruded full fat soybean; DM: Dry matter; CP: Crude protein; EE: Ether extract; NDF: Insoluble fiber in neutral detergent solution; ADF: Insoluble fiber in acid detergent solution; ADL: Acid detergent lignin; CF: Crude fiber

Table 2 Chemical composition of animal's diet (g/Kg DM basis)

Concentrate	DM	CA	CP	EE	NDF	ADF	ADL	CF	ME (Mcal/Kg)
Feeds	890.3	105.5	194.9	16.6	339.7	288.5	58.5	219.5	2.18
Alfalfa hay	957.7	73.6	34.5	5.5	779.7	531.7	147.3	431.4	1.31
Barley straw	922.6	59.2	102.4	18.8	206.9	76.6	6.2	56.6	2.95

DM: Dry matter; CP: Crude protein; EE: Ether extract; NDF: Insoluble fiber in neutral detergent solution; ADF: Insoluble fiber in acid detergent solution; ADL: Acid detergent lignin; CF: Crude fiber; ME: Metabolizable energy

The effective degradability (INS_E), corresponding to the theoretical degradability in the French protein system proposed by Verité et al., (1987) was calculated, weighted to account for rumen outflow rate, using the equation of Orskov and McDonald (1979):

$$INS_E = a + bc / (c+k)$$

Where INS_E is the in situ effective degradability; a is the fraction of rapidly solubilized protein; b is the fraction of potentially degradable protein; c is the fractional rate constant for the disappearance of fraction b (/h); k is rumen outflow rate.

In Vitro Method

Enzymatic degradation of ESB was measured by enzymatic hydrolysis for 1 h (INV_1) and 24 h (INV_{24}) by protease extracted from *Streptomyces griseus* in a borate-phosphate buffer at pH 8 as described by Aufrere and Cartailler, (1988). The INV_1 and INV_{24} values were the percentage of the initial nitrogen content of ESB to the amounts of nitrogen degraded after 1 and 24 h hydrolysis. The enzyme solution was obtained by mixing 2 g of *S. griseus* protease (type XIV, Sigma no. P-5147; 3.5 titratable units/mg) with 1000 ml of phosphoborate buffer (pH 8.0, PBB), prepared by dissolving 12.20 g sodium dihydrogen phosphate ($NaH_2PO_42H_2O$) and 8.91 g sodium tetra borax ($Na_2B_40_710H_20$) in distilled water and pH of the solution adjusted at 8 with 1 N NaOH and making up to 1000 mL. Each ESB sample (0.5 g) was incubated in an 80 mL pyrex tube, with 0.5 ml enzyme solution and 0.5 ml tetracycline solution (Sigma no. T-3258, 10 mg/100 mL PBB). At the initiation of the incubation period, 0.5 mg nystatin (Sigma no N- 3503) and 50 ml PBB was added to each tube. Before incubation, ESB samples were milled to pass a 1 mm screen and 0.5 g samples were weighed in centrifugation tubes. Each sample was incubated in duplicate in 2 batches in a shaking water bath (Heto SBD 50) at 40°C for 1 and 24 h. Extracted soybean meal was used as a reference feed for 1 and 24 h in each batch. Duplicate blank tubes without samples were incubated for 24 h. In vitro enzyme assay was run as triplicate. Following incubations, the tubes were centrifuged for 5 min at 3000 rpm. Then the samples were filtered using filter paper (Whatman 54), residue was washed with deionize water, and N on the 10 mL supernatant was analysed by micro Kjeldhal. The quantity of N degraded was calculated as the fraction of that present before incubation, after adjustments for the relative blanks and the average change in 1 and 24 h solubility of the extracted soybean meal between the 2 batches.

Statistical Methods

The in situ effective degradability of feed proteins (INS_E) was determined by Neway. Correlations between the INS_E and the enzymatic in vitro protein degradability for 1 and 24 h (INV_1 and INV24) were tested by simple linear regression. Minitab (Version 16) statistical program was used for regression analysis.

Results and Discussions

Chemical Composition of Extruded Soybean

The average DM, ash, CP, EE, NDF, ADF, ADL and CF values of ESB samples as percent of DM were 90.9%, 5.4%, 39.3%, 20.9%, 10.7%, 8.4%, 0.7% and 7.4% respectively (Table 1).

In situ and In Vitro Degradability for Extruded Soybean

The mean INS_E value of 10 ESB samples was found as 58.05 %; INS_E values of ESB samples are higher than INV_1 and INV_{24} values (Table 3). The values of INV_{24} were two times of the values of INV_1. The mean INV_1 value of ESB was 20.24 % and the mean INV_{24} value was 41.46 %.

The relationship between in situ and in vitro protein degradability of ESB

A correlation coefficient and regression were used to compare in vitro enzymatic protein degradability with in situ effective protein degradability for ESB. The correlation values and prediction equations of in situ effective degradability of ESB from enzymatic hydrolysis (INV_1 and INV_{24}) are presented in Table 4.

All the correlation coefficients and regression equations that predict INS_E from in vitro protein degradability at 1 h and 24 h (INV_1 and INV_{24}) and together INV_1-INV_{24} were found statistically significant ($P<0.05$). Prediction of in situ degradability from INV_{24} increased the correlation coefficient slightly ($P<0.05$) compare to in situ degradability from INV_1, although it did not cause any change in the residual standard error of regression equations (S). Also prediction of INS_E from both together in vitro 1h and 24 h (INV_1-INV_{24}) resulted slightly higher correlation coefficient (r= 0.837).

The Rumen Degradation Characteristics of Extruded Full Fat Soybean

The rumen degradation characteristics of ESB were shown in Table 5. The washing loss of CP in ESB was found higher than the rapidly degradable fraction of CP in ESB (33.65% versus 31.07%). The rate of CP disappearance and slowly degradable protein were 0.03 1/h and 79.21 % respectively.

The CP levels of ESB used in the study ranged from 36.8% to 42.0%. Bargalea et al. (1999) (39.55%, 39.76% and 39.54%), Demir and Şekeroğlu (2000) (36.62%), Griffiths (2004) (40.5%), Gonzalez et al. (2002) (41.4% to 42.5%), Nowak et al. (2005) (36.4%, 36.9% and 36.8%) had reported similar CP values to that of ESB in this study. In the present study CP levels of ESB are higher than the work done by Troegeler-Meynadier et al. 2006 (33.8%). Ether extract values of ESB ranged from 16.7% to 26.1%. These values are similar to EE reported by Bargalea et al. (1999) (21.42%, 22.13% and 20.63%), by Demir and Şekeroğlu (2000) (16.24%), by Gonzalez et al. (2002) (between 21.6% and 24.9%), by Griffiths (2004) (17.3%), by Nowak et al. (2005) (19.1%, 18.4% and 18.0%), by Troegeler-Meynader et al. (2006) (17.1%).

Table 3 In situ and in vitro degradability of ESB*

Feeds	INS_E	INV_1	INV_{24}
ESB_1	62.77	24.29	48.08
ESB_2	61.20	18.38	39.19
ESB_3	52.80	14.27	32.59
ESB_4	63.50	23.83	47.10
ESB_5	62.35	28.96	55.05
ESB_6	56.17	22.31	45.20
ESB_7	57.43	19.51	39.49
ESB_8	51.98	16.33	29.84
ESB_9	60.56	22.68	44.28
ESB_{10}	51.73	11.81	33.81
Mean	58.05±1.472	20.24±1.638	41.46±2.502

ESB: Extruded full fat soybean; INS_E: In situ effective degradability; INV_1: In vitro degradability at 1 h; INV_{24}: In vitro degradability at 24 h, *(%)

Table 4 Prediction equations for in situ protein degradability from in vitro enzymatic hydrolysis of ESB

Parameters	ESB		
Y	INS_E	INS_E	INS_E
No of samples	10	10	10
Constant	0.4323	0.3752	0.3950
INV_1	0.7362		0.3320
INV_{24}		0.4971	0.2870
r	0.822	0.828	0.837
S	0.028	0,028	0.029
P	0.004	0.003	0.015

Table 5 Rumen degradation characteristics of ESB*

n	10
A (%)	33.65±1.00
a (%)	31.07±1.17
b (%)	79.21±2.17
c (1/h)	0.03±0.003
INS_E (k=0.06)	0.58±0.02

ESB: Extruded full fat soybean; n: No of samples; A: Washing loss (%); a: Rapidly degradable fraction of CP (%); b: Slowly degradable fraction of CP (%); c: Rate of CP disappearance (1/h); INS_E: The effective degradability of CP; k: rumen outflow rate *(Mean ± SEM)

DM values of ESB ranged from 88.4% to 94.5% with an average of 90.9%. These values are similar to those reported by Demir and Şekeroğlu (2000) (91.24%), by Nowak et al. (2005) (90.5%, 91.3% and 90.8%), and by Griffiths (2004) (92.5%) and they were lower than those reported by Bargalea et al. (1999) (96.25%, 96.27% and 96.28%). The CA values of ESB in present study ranged from 4.6% to 7.6% which are similar to the values reported by Bargalea et al. (1999) (4.65%, 4.72% and 4.67%), by Demir and Şekeroğlu (2000) (4.81%), by Gonzalez et al. (2002) (between 4.82% and 6.24%), by Griffiths (2004) (5.2%), by Nowak et al. (2005) (5.1%, 5.2% and 5.2%). In the study NDF and ADF values of ESB were 9.3% to 13.5% and 6.7% to 10.7% respectively and they are lower than those reported by Troegeler-Meynadıer et al. (2006) (NDF: 27.8% and ADF: 14.8%) and by Nowak et al. (2005) (NDF: 29.6%, 27.6%, 27.8%; ADF: 20.4%, 19.3% and 19.2%). NDF values of ESB were determined by Gonzalez et al., 2002 as between 12.3% and 19.6% which are also somewhat higher than the relevant values of this study but ADF values reported by same researchers (between 7.05% and 10.9%) were similar to ADF values in this study. CF values (5.6% to 9.5%) in the present study are similar to those reported by Demir and Şekeroğlu (2000) (5.48%), by Nowak et al. (2005) (6.8%, 6.4% and 6.0%), but they are lower than those written by Griffiths (2004) (11%), by Bargalea et al. (1999) (13.9%, 13.8% and 16.6%).

INS_E values of 10 ESB samples ranged from 51.98% to 63.50% and the mean INS_E value was 58.05%. Gonzales et al. (2002) have reported that protein values of soy products for ruminants increased with decreasing ruminal effective protein degradability by heat treatment. As reported by Nowak et al. (2005); INS_E values of ESB were found between 57.3% and 71.3% which were smaller than that of untreated soybean (INS_E 83.10%) and higher than those of ESB heat treated at 165°C (INS_E 44.03%), at 145°C (INS_E 50.08%) and 155°C (INS_E 50.26%) where k has been taken as 0.06. The rumen outflow rate was also effective on INS_E, therefore Griffiths (2004) found that INS_E values of same ESB 39.9% and 42.7% when rumen outflow rate (k) taken as 0.08 1/h and 0.0625 1/h respectively. Griffiths (2004) also indicated that protein sources were used in limited quantities in rations due to their high rumen degradability, extrusion allowed these sources to be used at higher levels. In situ effective protein degradability of ESB (INS_E) in this study is higher than those of found by Nowak et al. (2005) and by Griffiths (2004). The reason for the differences; could be caused by the chemical composition of feed samples (Gonzalez et al. 2002), the pore size of nylon bags, the particle size of the feed samples, differences between cannulated animals, differences in the processing of protein sources (Canbolat, 2005), differences in the rations consumed by animals (Yalçın et al. 1998; Yörük et al. 2003; Deniz et al. 2004). Kirkpatrick and Kennelly (1987) reported that the degradability of CP increases with increasing CP level in the ration.

The degradability characteristics of ESB; a, b and c are; 31.07%, 79.21% and 0.03 1/h, respectively. These results are similar with the Gonzales (2002) results (a, b and c; 14.9%-36.1%; 63.9%-85.1%; 0.04-0.06 1/h respectively) but higher than Griffiths (2004) relevant values (b and c; 54.1% and 0.022 1/h respectively) although the rapidly degradable fraction of CP (a) similar to that of (a) value reported by the latest mentioned author. In the present study, the slowly degradable fraction of CP (b) is similar to the (b) values (83.47%, 83.38%) of ESB treated at 145°C and 155°C reported by Nowak et al. (2005), but it is lower than the (b) value (96.08%) of ESB treated at 165°C reported by same researchers. The rumen rapidly degradable fraction (a) in this study is higher than the (a) values of the whole temperature treatments (6.51% to 11.56%) found by Nowak et al. (2005), however, the rate of disappearance of CP (c) is lower than that of (c) found in the mentioned study. In the present study, the washing loss (A) was found to be somewhat higher than the rapidly degradable fraction (a).

The INS_E value of ESB (58.05%) was higher than the INV_1 and INV_{24} values (20.24% and 41.46% respectively). As it was stated by Aufrere et al. (1991), in situ method is used as a reference method since it provides the most precise result for estimation of rumen protein degradability. It has been indicated that there were two possible causes of in situ and in vitro method differences; the loss of dry matter induced by the pore size of the nylon bags and the second; the residual N in the bags corresponds not only the non-degraded feed N, but also to that of microbes attached to particles remaining in the bags (Michalet Doreau, 1991). Also, it has been reported that the microbial contamination varies according to the nature of the feed (Nocek, 1988).

In this study, the regression equations that estimate INS_E from INV_1, INV_{24} and INV_1-INV_{24} were found that statistically significant ($P<0.05$). Although estimating in situ degradability from INV24 has increased the correlation coefficient slightly ($0.828>0.822$; $P<0.05$) compared to predicting it from INV1, it did not cause any change in the residual standard error of regression equations ($S=0.028$). The estimation of INS_E from INV_1-INV_{24} led to a higher correlation coefficient ($r=0.837$) than the estimation of INS_E from INV_1 and INV_{24}. However, the use of INV_1 for estimation of INS_E may be suitable with less effort, less expense and shorter time. Roe et al., (1991), found a similar correlation coefficient ($r=0.82$) in estimating the INS_E value of the heat-treated full fat soybean from the in vitro value but standard errors of the mean of regression equations were higher ($r=0.37$). Poos-Floyd et al. (1985) found that in the estimation of in situ rumen degradability from all proteolytic enzymes at 1 h and 4 h incubation times gave statistically significant correlations. (Nocek, 1988) reported that the in vitro protein solubility at 1h to 3 hours incubations was highly correlated with ruminal protein degradability. Aufrere et al. (1991) reported that in situ degradability can be estimated much more accurately by enzymatic method, even when only INV_1 is used for feed mixtures. They have noted that the degradability of many feed samples, except those that were very slowly degraded in the rumen, was highly dependent on the amount of protein degraded in the early times of incubation.

As a result; the relationship and regression equations found in this study provide in situ validity of the in vitro method, which is easier and less expensive than the in situ method in predicting the effective protein degradability of ESB. These equations can be used by feed manufacturers and researchers to estimate in situ protein degradability from in vitro values.

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