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In situ and *In Vitro* Rumen Protein Degradability of Soybean Products: Relationship Estimation

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ABSTRACT: The objectives of this study were to estimate the protein degradability of Full Fat Soybean (FFS) and Soybean Meal (SM) by *in situ* (nylon bag) and *in vitro* enzymatic methods and to develop an equation in order to predict *in situ* degradability from *in vitro* values. In the study enzymatic technique; hydrolysis after 1 h (INV1) and after 24 h (INV24) 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 (INSE) and *in vitro* degradability after 1(INV1) and 24 (INV24) hours incubations 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 INSE, INV1 and INV24 of FFS were determined as 0.81, 0.60 and 0.76 respectively. The aforementioned parameters were determined as 0.59, 0,19 and 0.52 in SM, 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 FFS and SM were high and regression equations for estimation of *in situ* from *in vitro* were found significant. When a group was formed from FFS and SM, all equations were found to be important and the correlation coefficients increased. *In vitro* enzymatic protein degradability (INV1 and INV24) can be used for estimation of *in situ* effective protein degradability of FFS and SM. It has been concluded that the use of INV1 may be appropriate for the estimation of INSE in less labor, less cost and in a shorter time in the equations of the group formed from FFS and SM.

Keyword: *In situ*, *In vitro*, Enzymatic method, Streptomyces griseus, Protein degradability.

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INTRODUCTION

Ruminants differ significantly from monogastric animals due to their unique digestive physiology. Microbial protein synthesized by microorganisms in the rumen may not be a sufficient source for ruminants. Broderick et al. (1988) reported that the microbial protein source was not sufficient to meet the high protein needs of early lactation dairy cows, especially early weaned calves and lambs. Feeding proteins with high biological value, which are not digested in the rumen and enzymatically digested in the abomasum and small intestines, gives better results, especially in high-yield ruminants (Broderick et al., 1988). For these reasons, many researchers have reported that in order to accurately determine the protein needs of ruminants, feed N compounds should be defined according to rumen degradability characteristics such as rumen degradable protein (RDP) and rumen non-degradable protein (RUP) (AFRC, 1987; Chamberlain and Wilkinson, 1996; Cömert and Sayan, 2000; McDonald et al., 2011).

Methods used to determine the rumen degradability of feedstuffs and involving different procedures are listed under 3 headings. These methods are *in vivo*, *in situ*, and *in vitro* methods. Although the in vivo method is the first standard method developed, it is expensive and time consuming. Due to these drawbacks, alternative, faster, and cheaper methods have been developed, including *in situ* and *in vitro* methods. The *in situ* (nylon bag) method is mostly used to determine the RDP and RUP of feed ingredients. Since this method is performed directly in the rumen environment, it is accepted as the standard method by researchers. The exponential model developed by Orskov and McDonald (1979) is widely used. However, *in vivo* and *in situ* methods require cannulation of animals, and routine use of these techniques in feed evaluation is difficult. For this reason, new methods that do not require live animal material and are practical to use have been developed to accurately estimate the ruminal degradability of feedstuffs. These methods are called *in vitro* methods. In these methods; buffer solutions purchased from commercial companies, buffer solutions extracted from the rumen, chemical solutions, rumen fluid, and enzymes were used. As can be understood, many different *in vivo* methods have been developed by researchers, and the prediction by the enzymatic method for feed mixtures is very accurate and much better 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 investigated *in situ* protein They reported that there was a high correlation between 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 that 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. It has been stated that this enzyme, which is used to determine the rumen degradability of feeds, deserves more attention through standardization and verification of the results (Mohamed and Chaudhry, 2008).

This project was designed to determine the RDP amounts of FFS and soybean SK used in the nutrition of ruminant animals using *in situ* and *in vitro* enzymatic (Streptomyces griseus) methods and to develop regression equations that predict *in situ* protein degradability from *in vitro* protein degradability by taking advantage of the relationship between these methods. Thanks to these equations aimed at creating, it will be easier for feed industrialists and researchers to easily and quickly determine *in vitro* the amounts of RDP of FFS and SK to be used in the ration.

MATERIALS AND METHODS

Feeds and Chemical Analyses

The experiment was conducted using ten extruded full fat soybeans (FFS) 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 FFS and SM were presented in Table 1 and Table 2 respectively.

Animals

Three Holstein dairy cows 5 years old and weighing 550±30 kg were fitted with permanent rumen cannula to measure the ruminal degradability of FFS and SM in the International Center for Livestock Research and Training. Cows were fed the cows a total mixed ration consisting of 70% barley straw and alfalfa hay and 30% concentrate, which was 1.25 times more than the maintenance requirements on a dry matter basis. The chemical composition of forage and concentrate offered to animals was presented in Table 3. During the *in situ* experiment, cows were kept in individual stalls, 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 5×10 cm polyester bags with 50 µm pore size (Ankom

Table 1. Chemical composition of FFS samples (Mean ± SEM, % of DM*)

Feeds	DM	Ash	CP	EE	NDF	ADF	ADL	CF
FFS_1	90.30	4.71	40.93	19.14	16.22	13.09	1.33	10.85
FFS_2	89.60	4.97	40.60	20.99	15.70	12.36	1.75	9.99
FFS_3	88.10	5.13	41.68	22.16	15.28	10.79	1.44	8.74
FFS_4	90.33	4.64	40.68	19.58	15.46	12.45	1.90	10.90
FFS_5	89.79	4.83	42.28	20.80	14.77	11.98	1.93	10.47
FFS_6	89.59	4.64	41.68	22.28	14.93	12.52	1.33	9.93
FFS_7	89.24	4.50	39.93	15.82	14.87	12.58	1.71	10.42
FFS_8	91.39	5.30	39.32	20.99	13.59	13.77	1.15	10.56
FFS_9	92.14	5.13	39.76	21.07	13.07	12.11	0.89	9.18
FFS_{10}	89.74	5.59	32.86	19.02	14.73	13.00	0.68	9.89
Mean	**90.02±0.32**	**4.94±0.10**	**39.97±0.76**	**20.19±0.55**	**14.86±0.27**	**12.46±0.23**	**1.41±0.12**	**10.09±0.20**

*FFS: 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 SM samples (Mean ± SEM, % of DM*)

Feeds	DM	CA	CP	EE	NDF	ADF	ADL	CF
SM_1	87.70	6.22	53.24	1.01	8.54	6.15	1.16	4.33
SM_2	89.26	6.29	52.21	0.53	7.65	6.44	1.64	3.75
SM_3	89.28	6.10	51.64	1.18	15.46	10.16	0.89	8.01
SM_4	87.26	6.15	54.71	1.04	9.54	5.82	0.65	5.04
SM_5	90.38	6.42	52.35	0.65	14.74	9.33	0.84	6.58
SM_6	90.47	6.20	52.40	0.60	8.82	6.28	0.57	4.09
SM_7	85.08	6.53	53.06	0.33	13.75	8.27	0.54	5.29
SM_8	89.46	6.24	50.84	0.98	9.29	6.02	0.52	3.69
SM_9	90.35	6.89	51.77	1.79	7.16	5.42	0.39	3.38
SM_{10}	89.16	7.14	52.82	1.37	10.05	7.73	0.41	5.10
Mean	**88.84±0.54**	**6.42±0.11**	**52.51±0.33**	**0.95±0.14**	**10.50±0.95**	**7.16±0.51**	**0.76±0.12**	**4.93±0.46**

*SM: Soybean meal; DM: Dry matter; CA: Crude ash; CP: Crude protein; EE: Ether extract; NDF: Insoluble fiber in neutral detergent fiber; ADF: Insoluble fiber in acid detergent fiber; ADL: Acid detergent lignin; CF: Crude fiber

Table 3. Chemical composition of animal's diet (g/kg DM basis)

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

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

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 the 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 explained in chemical analyses. *In situ* effective degradability of feed proteins (INSE) 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 Vérité et al., (1987).

The effective degradability (INSE), corresponding to the theoretical degradability in the French protein system proposed by Vérité et al., (1987) was calculated, weighted to account for rumen outflow rate, using the equation of Orskov and McDonald (1979): INSE = a + bc / (c+k)

Where INSE 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 the rumen outflow rate.

In Vitro Method

Enzymatic degradations of FFS and SM were measured by enzymatic hydrolysis for 1 h (INV1) and 24 h (INV24) using protease extracted from *Streptomyces griseus* in a boratephosphate buffer at pH 8 as described by Aufrere and Cartailler (1988). The INV1 and INV24 values were the percentage of the initial nitrogen contents of FFS and SM to the amounts of nitrogen degraded after 1 and 24 h hydrolysis, respectively. 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 ($Na_2B_4O10H2O$) in distilled water, and pH of the solution adjusted at 8 with 1 N NaOH and making up to 1000 mL. Each FFS and SM 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, FFS and SM 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. Full fat soybean and SM were 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 in 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 deionized water, and N on the 10 mL supernatant was analysed by micro Kjeldahl. The quantity of N degraded was calculated as the fraction of that present before incubation, after adjusting 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 (INSE) was determined by Neway. Correlations between the INSE and the enzymatic *in vitro* protein degradability for 1 and 24 h (INV1 and INV24) were tested by simple linear regression. Minitab (Ver-

sion 16) statistical program was used for regression analysis.

RESULTS AND DISCUSSIONS

Chemical Composition of Full Fat Soybean and Soybean Meal

The average DM, ash, CP, EE, NDF, ADF, ADL and CF values of FFS and SM samples as percent of DM were 90.02%- 88.84%, 4.94% - 6.42%, 39.97% - 52.5%, 20.19% - 0.95%, 14.86% - 10.50%, 12.46% - 7.16%, 1.41% - 0.76% and 10.09% - 4.93%, respectively (Table 1 and Table 2).

In situ and *In Vitro* Degradability for Full Fat Soybean

As given in Table 4; the average INSE, INV1 and INV24 values of 10 FFS samples used in the study were determined as 0.81, 0.60 ve 0.76, respectively. Looking at the average values, it was seen that the INV1 value of FFS was significantly lower than that of INSE and the difference between them was significant (P<0.001). Twenty-fourth hour *in vitro* protein degradability values (INV24) are seen to be closer to INSE, but the difference between them is not statistically significant (P >0.05).

Aufrère et al. (1991) determined the INV1 value of FFS as 0.79 and the value of INV24 as 0.86 in their *in vitro* study using the protease enzyme extracted from Streptomyces griseus bacteria. The difference between the results of this study and the results of the study by Aufrère et al. (1991) may be due to the different feed samples used. In an *in vitro* study by Roe et al (1991) using protease enzyme extracted from Streptomyces griseus bacteria, it was reported that the INV24 value of untreated FFS was 0.522. The results of the study conducted by Roe et al. (1991) were found to be lower than the results of this study. It is thought that the possible reason for this difference may be due to the method difference.

In situ and *In Vitro* Degradability for Soybean Meal

The mean INSE, INV1 and INV24 values of 10 SM samples were determined as 0.59, 0.19, and 0.52, respectively (Table 5). The difference between INV1, INV24 and INSE values of SM was found to be statistically significant (P <0.001). INSE value is higher than INV1 and INV24 values. The difference between INV1, INV24 and INSE values of SFK was found to be statistically significant (P<0.001).

INSE values of FFS and SFK (0.81, 0.59, respectively) were found to be higher than INV1 and INV24 values (0.60-0.76, 0.19-0.52, respectively).

Krishnamoorthy et al. (1983); In their *in vitro* study using protease enzyme extracted from Streptomyces griseus; The % total N dissolved in SM was determined as 0.813 after 1 hour incubation in 6 units/ml protease solution and 0.989 after 24 hours incubation. They found 0.204 after 1 hour incubation and 0.607 after 24 hours incubation in $22x10^{-3}$ units/ml protease solution. The results of the study using the protease solution as $22x10^{-3}$ units/ml are closer to the results of this study. Krishnamoorthy et al. (1983) stated that the coefficient of variation in the technique using $22x10^{-3}$ units/ml protease solution was less than 7% and showed good reproducibility.

Table 4. *In situ* and in vitro degradability of FFS

FEED	INS_E	INV_1	INV_{24}
FFS_1	0.84	0.60	0.76
FFS_2	0.86	0.62	0.83
FFS_3	0.83	0.54	0.73
FFS_4	0.73	0.59	0.68
FFS_5	0.79	0.60	0.74
FFS_6	0.82	0.54	0.73
FFS_7	0.80	0.56	0.75
FFS_8	0.80	0.53	0.77
FFS_9	0.80	0.63	0.74
FFS_{10}	0.85	0.79	0.87
Mean ± SEM	0.81 ± 0.01[a]	0.60 ± 0.02[b]	0.76 ± 0.02[a]

FFS: Full fat soybean; INSE: *In situ* effective degradability; INV1: *In vitro* degradability at 1 h; INV24: *In vitro* degradability at 24 h

Table 5. *In situ* and *in vitro* degradability of SM

FEED	INS_E	INV_1	INV_{24}
SM_1	0.63	0.20	0.55
SM_2	0.65	0.25	0.54
SM_3	0.62	0.17	0.50
SM_4	0.65	0.21	0.61
SM_5	0.53	0.16	0.46
SM_6	0.59	0.22	0.51
SM_7	0.53	0.14	0.42
SM_8	0.56	0.21	0.53
SM_9	0.60	0.24	0.58
SM_{10}	0.54	0.17	0.54
Mean±SEM	0.59 ± 0.02^a	0.19 ± 0.01^c	0.52 ± 0.02^b

SM: Soybean meal; INSE: *In situ* effective degradability; INV1: *In vitro* degradability at 1 h; INV24: *In vitro* degradability at 24 h

Aufrère et al. (1991) by using the protease enzyme extracted from *Streptomyces griseus* bacteria *in vitro* study; INV1 and INV24 of SM were determined 0.36 and 0.78, respectively. In another study by Roe et al. (1991), the amount of CP remaining without degradation after 24 hours of incubation in an *in vitro* study using the protease enzyme extracted from *Streptomyces griseus* bacteria was found to be 0.733 in Solvent SM and 0.659 in Expeller SM. It is thought that the reason why the results of the studies conducted by Aufrère et al (1991) and Roe et al (1991) are higher than the results of this study may be due to the feed raw materials used and the method differences.

Aufrère et al (1991) reported that the *in situ* method can give the most accurate result in the estimation of protein degradability in the rumen, and therefore the *in situ* method is used as a reference method. It has been stated that there may be two possible reasons for the differences between the *in situ* method and the *in vitro* method. The first; DM losses caused by the pore openings of nylon bags (Michalet-Doreau and Cerneau, 1991) and the second one; the fact that the N remaining in the bags is not only the remaining N from the undegraded feed samples, but also the N from the rumen microorganisms.

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) ve Calsaminga and Stern (1995) reported high correlations between *in situ* effective protein degradability values and protein degradability values obtained by enzymatic methods.

The relationship between *in situ* and *in vitro* protein degradability of FFS and SM

A correlation coefficient and regression were used to compare *in vitro* enzymatic protein degradability with *in situ* effective protein degradability for FFS and SM. The correlation values and prediction equations of *in situ* effective degradability of FFS and SM from enzymatic hydrolysis (INV1 and INV24) are presented in Table 6.

No significant relationship was found in the relation equations of FFS between INSE and INV1 (P=0.350), the relation equation between INSE and INV24 was found to be statistically significant (P=0.009). The relationship equations between INSE and INV1- INV24 were statistically significant (P=0.018). Using INV1 and INV24 together in the equations increased the correlation coefficient (0.828) and the standard error of the regression equation decreased, thus increasing the reliability of the equation. It has been concluded that INV24 can be used alone in the estimation of INSE with less effort and less expense. The regression equations created to estimate the INSE value of FFS from INV have a lower standard error than the regression equations created by Roe et al. (1991) and the correlation coefficients are higher (R: 0.58, S: 1.22).

The regression equations that estimate the INSE value of SM from INV1 and from INV24 were found to be statistically significant (P = 0.034, 0.030, respectively) in Table 6. The regression equation, which estimates INSE from INV1-INV24, was not statistically significant (P = 0.070). In the estimation of INSE of SM, INV24 has a higher correlation

coefficient than INV1 (0.680>0.669). It has been concluded that INV1 can also be used effectively in the estimation of INSE in order to speed up the processes and save time. Aufrère et al (1991); In the regression equations they created for SM, they reported that the use of INV1 instead of INV24 in the estimation of INSE gave lower standard error of the regression equation (S) with higher correlation (r= 0.715; S=0.024 and r= 0.78-0.94 S=0.29-0.09, respectively).

In Table 7, the relationship equations of the INSE and INV protein degradability of the FFS and SM feed groups are given. According to Table 7, the equations providing the estimation of the INSE of the FFS-SM group from INV1, INV24 and INV1-INV24 were found to be significant (P<0.05). Regression equations that provide the estimation of INSE value from INV24 were found to be more reliable than INV1 and showed higher correlation. It caused the error to decrease, thus increasing the reliability of the equation. The combination of INV1 and INV24 in the regression equations caused an increase in the correlation coefficient and a slight decrease in the error. The regression equations that estimate the INSE value of the FFS, SM and FFS-SM feed group from INV24 were found to be more important than the estimates from INV1. These equations have higher correlation. In the equations of FFS-SM group, it is thought that the use of INV1 may be appropriate for the estimation of INSE in less labor, less expense and shorter time. These values are similar to the standard errors of the regression equations created by Aufrère et al. (1991) for 97 concentrate feeds and the regression equations created for 49 commercial feed mixes. The correlation coefficients of the feed groups are also very similar to each other. Researchers reported that the accuracy of the prediction increased when the regression equations were established for each feed type. They determined the standard error of the regression equation for SM as 0.024 and the correlation coefficient as 0.715. They reported that the estimation accuracy increased when the equation was established according to each feed type. They determined the standard error of the regression equations for oilseed meals as 0.026 and the correlation coefficient as 0.978.

The Rumen Degradation Characteristics of FFS and SM

In the Table8, the INSE value of FFS was determined as 81%. The INSE values found in this study

Table 6. Prediction equations for *in situ* protein degradability from *in vitro* enzymatic hydrolysis of FFS and SM

Feed	INSE	No of samples (n)	Constant	IN_V1	INV_{24}	r	S	P
	IN_SE	10	0.7136	0.1641		0.331	0.037	0.350
FFS	INS_E	10	0.4059		0.5344	0.772	0.025	0.009
	INS_E	10	0.375	- 0.209	0.740	0.828	0.024	0.018
	INS_E	10	0.4152	0.8872		0.669	0.038	0.034
SM	INS_E	10	0.2826		0.5867	0.680	0.037	0.030
	INS_E	10	0.305	0.496	0.357	0.729	0.037	0.070

FFS: Full fat soybean; INSE: *In situ* effective degradability; INSE: *In situ* effective degradability; INV1: *In vitro* degradability at 1 h; INV24: *In vitro* degradability at 24 h; r: Correlation coefficient; S: Standard error of the regression equation; P: Significance level

Table 7. Relationship equations between *in situ* and *in vitro* protein degradability of FFS-SM feed groups

Feed	INSE	No of samples (n)	Constant	INV_1	INV_{24}	r	S	P
	INS_E	20	0.4887	0.5327		0.943	0.042	0.000
FFS-SM	INS_E	20	0.1364		0.8794	0.959	0.035	0.000
	INS_E	20	0.241	0.176	0.606	0.963	0.034	0.000

FFS: Full fat soybean; INSE: *In situ* effective degradability; INSE: *In situ* effective degradability; INV1: *In vitro* degradability at 1 h; INV24: *İn vitro* degradability at 24 h; r: Correlation coefficient; S: Standard error of the regression equation; P: Significance level

Table 8. Rumen degradation characteristics of FFS and SM*

Feed	Rumen Degradation Characteristics of FFS and SM					
	n	A (%)	a (%)	b (%)	c (1/h)	INS_E (k=0.06)
FFS	10	53.31±1.62[a]	53.32±1.61[a]	49.16±1.44[b]	0.08±0.0007[a]	0.81±0.12[a]
SM	10	27.22±0.95[b]	24.70±1.03[b]	84.99±2.14[a]	0.05±0.004[b]	0.59±0.15[b]

FFS: Full fat soybean SM: Soybean meal 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); INSE: The effective degradability of CP; k: rumen outflow rate *(Mean ± SEM)

were in agreement with the INSE values of Nowak et al. (2005) (83.10%), Griffiths (2004) (k=0.0625, 76.9%), NRC (2001) (78.5%) and Aufrere and Cartailler (1988) (86.7%). This value was found to be higher than Canbolat et al (2005) (k=0.02, 70.4%; k=0.05, 57.5%; k=0.08, 50.9%) and lower than Aufrère et al. (1991) (90%). The *in situ* protein degradability characteristics of whole-fat soybean a, b and c were determined as 53.32%, 49.16 and 0.08/h, respectively. In this study, Rapidly degradable fraction of FFS in the rumen (a) and the washing loss of FFS (53.31%) were found to be quite high. It is thought that the reason for this may be due to the fact that the feed samples used in the study were very finely ground (1mm) (Michalet-Doreau and Cerneau, 1991), the washing time of the nylon bags (Deniz et al., 2004) and the washing machine for 5 minutes (Cherney et al., 1990). The part that rapidly degradable fraction of CP (a) is in agreement with the results of Nowak et al. (2005) (50.76%) and Griffiths (2004) (57.3%). It is higher than Canbolat et al. (2005) (30.08%) and NRC (2001) (27.8%).

The value of the parameter b, which is the part that slowly degradable fraction of CP is in agreement with Nowak et al. (2005) (43.32%) but from Canbolatet al. (2005) (58.5%), Griffiths (2004) (60.2%) and NRC (2001) (70.2%) found low. The rate of CP disappearance (c) was found between 0.02/h and 0.1/h by Canbolat et al. (2005), Nowak et al. (2005), Griffiths (2004), NRC (2001) and the c value of this study is within these limits.

The INSE value of soybean meal, which was determined as 59%, was found to be consistent with the results reported by Nobar et al (2009) (k=0.05, 63.77%), Griffiths (2004) (57.3%), González et al. (2002) (46%-67%), Sacakli et al. (2011) (54.1%), Sarıçiçek (1999) (k=0.05, 65.80%; k=0.08, 57.15%), Madsen and Hvelplund (1994) (63%), Kirkpatrick and Kennelly (1987) (60.2%-72.8%), Orskov (1982) (k=0.05, 63%) and Orskov (1988) (k=0.05, 62.5%). It was found to be lower than Maxin et al (2013) (66%), Woods et al (2003) (k=0.06, 70.8%) and higher than Yörük et al. (2003) (49.05%).

The washing loss (A) of SM was found to be slightly higher than rapidly degradable fraction of CP (a) in the rumen. As mentioned earlier, this height may be due to finely ground feed samples, the washing time of the nylon bags and the washing time of 5 minutes in the washing machine. The protein fraction that Rapidly degradable fraction of CP (a) was determined as 24.70%. This value is similar to the findings of Maxin et al (2013) (27.7%) and González et al. (2002) (17.8%-30.1%). It was found to be higher than the findings of Sacakli et al. (2011) (%14.3), Yörük et al. (2003) (%13.79), Woods et al. (2003) (%12.94), Sarıçiçek (1999) (%17.22), Nobar et al. (2009) (%8.55), Madsen and Hvelplund (1994) (%16). It is lower than the findings of Griffiths (2004) (31.1%). Rate of CP disappearance (c) was determined as 0.05/hr and this value is within the lower and upper limits of the literature reports (Madsen and Hvelplund, 1994; González et al., 2002; Woods et al., 2003; Yörük et al., 2003; Griffiths, 2004; Nobar et al., 2009; Sacakli et al, 2011; Maxin et al., 2013).

The differences between the published study results in the INSE and *in situ* degradability characteristics (a, b, and c) of FFS and SM are due to the chemical composition of the feed samples (González et al., 2002), the pore size of the nylon bags, the particle size of the feed samples, the used it may be due to differences between cannulated animals, differences in the processing process of protein sources (Canbolat et al., 2005), and differences in rations consumed by animals (Yalcin et al., 1998; Yörük et al., 2003; Deniz et al., 2004). Kirkpatrick and Kennelly (1987) reported that the degradability of HP increased with increasing HP level in the diet. According to the results of the study, the INSE value of TYS (0.81) was found to be considerably higher

than the SM (0.59), and this elevation was statistically significant (P<0.05).

As a result, the relationship and regression equations found as a result of the study ensure the validity of the *in situ* method (Streptomyces griseus), which is easier and more applicable than the *in situ* method, in the estimation of protein degradability in the rumen of FFS and SM, which are widely used in the nutrition of ruminant animals. When the feeds used in the study were grouped and evaluated, the correlation coefficients reached higher values. The regression equations established for the FFS-SM group were also found to be significant and their correlation coefficients were high. It is thought that the use of INV1 values in the estimation of INSE may be appropriate in order to save time by speeding up the operations with less effort and less expense. The regression equation, which is thought to be suitable for the use of INV1 in the estimation of INSE for the FFS-SM group, is given below.

$$INSE = 0{,}4887 + 0{,}5327\ INV1$$

The *in vitro* method, which will be used to determine the ratio of FFS and SM in the ration has been validated. Thus, these equations can be used to predict *in situ* protein degradability from *in vitro* protein degradability for feed manufacturers and researchers. It will be possible to detect protein degradability in the rumen easily and quickly. In future studies, it is thought that it may be appropriate to try to determine the INV1 and INV24 values in determining the INSE values of different feeds that are widely used in the nutrition of ruminant animals, apart from FFS and SM.

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Competing Interests
Author Arzu Erol Tunc, Yusuf Cufadar and Sema Yaman Fırıncıoğlu declare they have no financial interests.

Data Availability
This study was presented as part of a doctoral project. Archived in the Council of Higher Education National Thesis Center Database. The main text of the study and the necessary data can be accessed via the link "https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=E3IT-gO7Mvvuym1fR3rKuw&no=ygqIP6oGbQv1K7iLh0UfrQ" access to the main text is available.

Author Contributions
All authors contributed to the study conception and design. Material preparation, data collection and laboratuary analysis were performed by Arzu EROL TUNC. The first draft of the manuscript was written by Arzu EROL TUNC, Yusuf CUFADAR ve Sema YAMAN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval
This study was found to comply with the principles stated in the Working Directive of the Animal Experiments Local Ethics Committee of the Livestock Central Research Institute (03.12.2014).

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