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Ruminal Dry Matter and Crude Protein Degradability of Some Tropical (Iranian) Feeds Used in Ruminant Diets Estimated Using the *in situ* and *in vitro* Techniques

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Abstract: The *in situ* ruminal dry matter (DM) and crude protein (CP) degradation and *in vitro* DM and CP disappearance of corn grain, soybean meal, wheat bran and alfalfa were compared. For *in situ* technique duplicate dacron bags were incubated for 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h in two wethers fitted with ruminal cannulas. The *in vitro* degradability of DM and CP calculated at 0, 2, 12, 36 and 48 h incubation time for test feeds. The model of Ørskov and McDonald as $y = a + b (1-e^{-ct})$ was used for determination of degradation characteristics for both of methods. Wheat bran had higher soluble DM (a) (30.3%), corn grain had higher insoluble potentially degradable DM (b) (59.9%) and soybean meal had higher degradation rate (9.67% h^{-1}) than other feeds, inverses corn grain (14%), alfalfa (40.1%) and alfalfa (3.40% h^{-1}) had lower soluble DM, insoluble potentially degradable DM and degradation rate than other feeds, respectively. Wheat bran had higher soluble CP (a) (33.3%), corn grain had higher insoluble potentially degradable CP (b) (54.5%) and soybean meal had higher degradation rate (9.78% h^{-1}) than other feeds, inverses soybean meal (5.7%), alfalfa (43.9%) and alfalfa (3.75% h^{-1}) had lower soluble CP, insoluble potentially degradable CP and degradation rate than other feeds, respectively. There were differences ($p<0.05$) among test feeds in dry matter and crude protein degradability after several incubation times. There was strong coefficient of correlation between extent of *in situ* dry matter and crude protein degradation and *in vitro* dry matter and crude protein disappearance. The key protein parameters in the proposed Metabolizable protein system, quick digestible protein, slowly digestible protein and digestible undegradable protein are derived from measurements of the rates of degradation of feed proteins suspended in a dacron bag in the rumen. The Metabolizable protein of soybean meal (381 g kg^{-1}) was numerically rather than the other feeds and for corn grain (86 g kg^{-1}) was numerically less than the other feeds.

Key words: *In situ*, *in vitro*, degradability, metabolizable protein

INTRODUCTION

The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion. Determining the digestibility of feeds *in vivo* is laborious, expensive, requires large quantities of feed and is largely unsuitable for single feedstuffs thereby making it unsuitable for routine feed evaluation (Getachew *et al*., 2004). There are a number of techniques available to evaluate the nutritive value of feeds at relatively low cost such as *in situ* and *in vitro* disappearance technique. The high correlation between extent of *in situ* dry matter disappearance and *in vitro* dry matter disappearance is consistent with Taghizadeh *et al*. (2006).

Ruminal degradability and small intestine digestibility are two important measurements to consider when determining the nutritive value of any feed (Woods *et al*., 2003a). The *in situ* nylon-bag technique is widely used to characterize the disappearance of feeds from the rumen (Woods *et al*., 2002). Nylon-bag technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuffs and feed constituents (Getachew *et al*., 1998). *In situ* incubation is a principle method for estimation of ruminal degradability of dry matter and crude protein, because it is simple, does not require specific equipment and could be applied in every research laboratory (Alexandrov, 1998). Dynamic models of carbohydrate and protein digestion rely on estimates of kinetics of ruminal degradation of feeds (Batajoo and Shaver, 1998).

The evaluation of the protein value of feeds for ruminants requires its division into quickly and slowly degradable crude protein (CP) and Undegradable CP (UDP; e.g., AFRC, 1993). Metabolizable protein is defined as the total digestible true protein (amino acids) available to the animal for metabolism after digestion and absorption of the feed in the animal's digestive tract

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(AFRC, 1993). In many protein evaluation systems for ruminants, the nylon bag technique is the standard method used for calculating the amount of protein escaping rumen fermentation (Cone *et al*., 2002). The key protein parameters in the proposed MP system, QDP, SDP and DUP, are derived from measurement of the rates of degradation of feed protein suspended in a dacron bag in the rumen for various periods of time (AFRC, 1993).

The objective of the present study was to determine *in situ* and *in vitro* DM and CP degradation characteristics and Metabolizable protein of some feedstuffs (corn grain, soybean meal, wheat bran and alfalfa).

MATERIALS AND METHODS

Experimental feeds: The samples of corn grain (CG), soybean meal (SBM), wheat bran (WB) and alfalfa (AA) were collected from dairy farm in northwestern of Iran. All samples were oven-dried at 80°C until constant weight. Samples of all test feeds for the *in situ* and *in vitro* incubation were milled through a 2.0 mm sieve and for chemical analyses they were milled through a 1.0 mm sieve.

Chemical analysis: Samples of feeds and feces were dried in an oven at 105°C for 24 h and the DM content calculated. Ground samples were analyzed for ash (AOAC, 2005). Determinations of N were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Neutral-detergent fiber and ADF were determined by the detergent procedures of Van Soest *et al*. (1991). Acid-detergent insoluble nitrogen (ADIN) was determined as nitrogen in Acid-detergent residue. Ether Extract (EE) was determined by extracting the sample with ether (AOAC, 2005).

***In situ* ruminal procedure:** Two wethers (38±1.5 kg BW) fitted with rumen cannulae were used to measure rumen degradability of feeds. The wethers were fed on a diet comprising (DM basis), 550 g kg^{-1} alfalfa hay, 400g kg^{-1} barely grain, 50 g kg^{-1} wheat bran and 2 g kg^{-1} lime stone at maintenance (NRC, 2001). 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 (Ørskov and Mc Donald, 1979) was used to measure the DM and CP degradation of feeds in the rumen. Nylon bags (6×12 cm polyester bag; poor size 45-50 μm) containing 5 g of feed ground through a 2 mm screen were incubated in the rumen for 2, 4, 6, 8, 12, 16, 24, 36 and 48 h for CG and SBM and 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h for AA and WB, immediately after the morning feed. As a whole, there were 4 replicates for each feed sample and for each incubation time (2 wethers ×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 weighted and submitted for analysis.

***In vitro* disappearance:** The rumen liquor was obtained from the same sheep used in the *in situ* trial and receiving the same diet to ensure similar conditions for both techniques. Equal volumes of ruminal fluid from each sheep collected 2 h after the morning feeding were combined and strained through four layers and mixed with Mc Dougall (1948) buffer prewarmed to 39°C. The inoculum was dispensed (20 mL) per vial into 100 mL serum vial (containing of 300 mg sample per vial) which had been warmed to 39°C and flushed with oxygen free CO_2. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc Melros dark, USA) set at (120 rpm) housed in an incubator. Vials for each time point, as well as blanks (containing no substrate), were prepared in triplicate. Triplicate vials were removed after 2, 12, 24 and 48 h of incubation. The residues were washed three times in phosphate buffer (pH = 7.4) followed by centrifugation (2500 rpm, 10 min, 4°C). The pellets were dried at 50°C the ground using a micro-mill. Total N and total dry matter in the residue were determined.

Calculations and statistical analysis: *In situ* and *in vitro* DM and CP rapidly degradable fraction, potentially degradable fraction and rate of degradation of fraction b, were calculated according model of Ørskov and McDonald (1979) as $y = a+b(1-e^{-ct})$ that p is the actual degradation of CP and DM after t, a is the intercept of the degradation curve at time zero, b is the potential degradability of the component of the slowly soluble CP and DM, which will in time be degraded, c represents the constant of degradation rate b at time, t is incubation time. The effective degradability of dry matter (EDDM) and Crude Protein (EDCP) from the outflow rates (k) were calculated by formula: $ED = A+ (b\times c)/(c+k)$ (AFRC, 1993).

The percent disappearance of the DM and CP at the different incubation time was calculated as the difference between the feed and the portion remaining after different incubation time. Difference between feedstuffs in rumen disappearance of DM and CP and gas production data were analyzed using the Analysis of Variation model (ANOVA). Procedure of SAS institute Inc (1999) with

Duncan multiple range tests used for the comparison of means. The test treatments were the only sources of the variation considers analytical variability was included in the error variance.

The quickly degradable protein (QDP), slowly degradable protein (SDP), effective rumen degradable protein (ERDP), rumen degradable protein (RDP), undegradable dietary protein (UDP), digestible undegradable protein (DUP) and metabolizable protein (MP) content of feeds was calculated using equations of AFRC (1993).

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of test feeds is presented in the Table 1. The CP content of feeds ranged from 11.8% in corn grain to 49.2% in soybean meal. The NDF content of feeds ranged from 10% in corn grain to 53.3% in wheat bran. Corn grain contained substantially higher OM level than the other feeds. Variation in their chemical composition has been observed in other studies with test feeds (NRC, 2001; AFRC, 1993; Milis *et al*., 2007).

The differences among chemical composition test feeds can be resulted due to variation in variety, cultivate, environmental condition and cut stage of feeds. Some of the feeds used in the current study were by-product feeds (such as soybean meal and wheat bran), which are created as a result of processing to extract human foods and therefore their composition varies depending on the composition of original plant material, method of processing and type of components extracted or removed (Getachew *et al*., 2004).

***In situ* disappearance of DM and CP:** Dry matter and crude protein losses from the nylon bags incubated in the rumen are represented in Table 2. There were differences ($p<0.05$) among test feeds in dry matter and crude protein degradability after several incubation times. Total dry matter washing losses (zero time bags) represented 16.7-27.2% of DM in CG and WB, respectively. Dry matter and crude protein disappearance from nylon bags incubated in the rumen increased with increasing incubation time. The 96 h incubation time allowed the plateau of degradation to be achieved for all roughages such as alfalfa.

The *in situ* DM and CP degradation characteristics and effective degradation of test feeds presented in Table 3. A large range of dry matter degradation characteristics was obtained: the a, b and c values ranged from 14-30.3% (for CG and WB), 40.1-59.9% (for AA and CG) and 3.40-9.67% h^{-1} (for AA and SBM), respectively. A large range of crude protein degradation characteristics was obtained: the a, b and c values ranged from 5.7-33.3% (for SBM and WB), 43.9-54.5% (for AA and CG) and 3.75-9.78% h^{-1} (for AA and SBM), respectively.

Table 1: Chemical composition (%DM) of the test feeds

	Feedstuffs			
Item	CG	SBM	WB	AA
DM	92.5	94.0	92.9	94.9
CP	11.8	49.2	16.9	17.0
EE	6.1	4.2	5.4	3.3
NDF	10.0	44.9	53.3	49.9
ADF	6.2	13.0	15.8	40.3
Hemicellulose[1]	3.8	31.9	37.5	9.6
OM	95.4	90.1	90.6	86.9
ADIN[2]	0.41	0.55	0.07	0.12

[1]-Hemicellulose = NDF - ADF, [2]-Acid Detergent Insoluble Nitrogen

Table 2: The *in situ* disappearance of dry matter and crude protein (%)

	DM disappearance					CP disappearance				
Incubations time	CG	SBM	WB	AA	SEM[1] (n = 4)	CG	SBM	WB	AA	SEM (n = 4)
0	16.7^{b}	25.8^{a}	27.2^{a}	17.3^{b}	0.47	17.1^{b}	6.4^{d}	29.2^{a}	13.6^{c}	0.51
2	18.6^{d}	33.9^{a}	29.4^{b}	20.6^{c}	0.57	18.7^{b}	15.6^{c}	37.6^{a}	17.7^{bc}	0.77
4	28.9^{c}	37.2^{b}	40.2^{a}	24.0^{d}	0.70	29.4^{b}	17.8^{c}	42.9^{a}	20.3^{c}	0.82
6	35.3^{b}	44.9^{a}	43.2^{a}	26.3^{c}	0.72	39.8^{b}	27.1^{c}	51.6^{a}	22.9^{d}	0.84
8	40.9^{b}	48.5^{a}	48.2^{a}	28.3^{c}	0.62	42.7^{b}	32.0^{c}	56.4^{a}	26.0^{d}	0.74
12	51.0^{b}	56.4^{a}	52.3^{b}	31.2^{c}	0.53	50.9^{b}	42.6^{c}	58.2^{a}	31.8^{d}	0.78
16	55.8^{b}	58.5^{a}	53.5^{c}	36.4^{d}	0.61	55.8^{b}	44.7^{c}	60.6^{a}	37.5^{d}	0.98
24	64.1^{a}	62.0^{b}	55.3^{c}	40.2^{d}	0.66	63.4^{a}	47.7^{b}	63.8^{a}	40.4^{c}	1.19
36	67.2^{a}	65.3^{a}	58.3^{b}	45.2^{c}	0.62	64.1^{b}	51.2^{c}	68.2^{a}	45.6^{d}	0.82
48	73.6^{a}	69.0^{b}	65.5^{c}	50.4^{d}	0.63	69.6^{b}	55.6^{c}	75.9^{a}	48.9^{d}	0.90
72	-	-	69.2^{a}	55.4^{b}	0.45	-	-	80.4^{a}	55.9^{b}	0.96
96	-	-	76.5^{a}	57.0^{b}	0.56	-	-	83.2^{a}	58.5^{b}	0.96

[1]-SEM = Standard Errors of Means, The mean within a rows without common letter differ ($p<0.05$)

Table 3: The *in situ* dry matter and crude protein degradation characteristics and effective degradation of test feeds in the rumen

	DM degradation characteristics					CP degradation characteristics				
Feedstuffs	a (%)	b (%)	c (% h^{-1})	ED[1]	RSD[2]	a (%)	b (%)	c (% h^{-1})	ED	RSD
CG	14	59.9	7.38	61.2	2.12	14.2	54.5	9.07	59	2.63
SBM	25.8	41.9	9.67	60.6	1.49	5.7	48.5	9.78	46.1	2.28
WB	30.3	42.8	4.04	59	4.33	33.3	47.4	5.08	67.4	4.00
AA	18.2	40.1	3.40	43.6	0.9	14.5	43.9	3.75	43.2	1.69

[1]- ED = Effective degradability (k = 0.02), [2]- RSD = Residual standard deviation

Table 4: The *in vitro* disappearance of dry matter and crude protein (%)

	DM disappearance					CP disappearance				
Incubations time	CG	SBM	WB	AA	SEM[1] (n=3)	CG	SBM	WB	AA	SEM (n=3)
0	16.7^{b}	25.8^{a}	27.2^{a}	17.3^{b}	0.47	17.1^{b}	6.4^{d}	29.2^{a}	13.6^{c}	0.51
2	18.4^{b}	37.8^{a}	35.5^{a}	17.8^{b}	0.78	20.2^{b}	17.4^{c}	39.7^{a}	17.3^{c}	0.83
12	56.3^{a}	50.3^{b}	54.5^{a}	29.1^{c}	0.69	49.8^{b}	44.2^{c}	57.7^{a}	33.4^{d}	0.76
24	66.5^{a}	60.4^{b}	55.3^{c}	46.3^{d}	0.61	62.3^{b}	47.0^{e}	65.9^{a}	45.0^{e}	0.72
48	69.8^{a}	71.3^{a}	65.8^{b}	48.8^{c}	0.61	70.3^{b}	59.5^{c}	74.0^{a}	49.9^{d}	0.68

[1]- SEM = Standard Errors of Means, The mean within a rows without common letter differ ($p<0.05$)

Table 5: The *in vitro* dry matter and crude protein degradation characteristics of test feeds

	DM degradation characteristics				CP degradation characteristics			
Feedstuffs	a (%)	b (%)	c (% h^{-1})	RSD[1]	a (%)	b (%)	c (% h^{-1})	RSD
CG	13.08	58.25	10.11	4.88	15.20	56.91	7.41	2.54
SBM	29.14	44.88	5.40	3.89	7.61	49.45	9.79	4.78
WB	28.37	53.33	9.44	4.26	31.11	42.86	7.99	2.54
AA	15.53	38.57	4.85	4.30	13.11	39.15	6.42	1.20

[1]- RSD = Residual standard deviation

The relationship between the degradability parameters a, b and c and the chemical composition of the sum total of 60 test feeds was reported by Woods *et al.* (2003a). The slowly fermented structural carbohydrates, as the main components of these feeds, are thought to play a dominant role in the degradation characteristics in the rumen. The high level of wheat bran and soybean meal DM degradability in several incubation times can be assumed that rumen degradable nitrogen was not limiting microbial activity allowing the SBM and WB to be degraded according to their potential.

In comparison DM degradation characteristics of corn grain to Alexandrov (1998), the 'a' value of corn grain was lower; the 'b' value higher and 'c' value was same in this study. Where different degradability values are observed between this study and Alexandrov (1998), it is possible that differences in nutrient composition, pore size of nylon bag and milling screen size.

High degradability of DM for concentrates compared to the other test feeds was resulted due to low ADF and NDF, whereas due to high concentrate of ADF for roughage such as alfalfa, their degradability was lower than the other test feeds. The lower values for rate of DM degradation and the low ruminal DM degradation in roughages were associated with increases in NDF, ADF and ADL concentrations, which also affected the total potentially degradable fraction (Elizalde *et al.*, 1999; Griffin *et al.*, 1994; Hoffman *et al.*, 1993). Despite high NDF in WB, it's DM and CP degradability was higher than the other feeds. This result can be resulted from higher soluble containing fraction of DM and CP and high hemicellulose in NDF content that have high degradability.

The variation in the DM and CP effective degradability of the different test feeds sources in this study may be related to the chemical composition where variability in the concentration of CP, EE, NDF and ADF was noted previously. These results are in agreement with another study (Woods, 2003a) for test feeds.

***In vitro* disappearance of DM and CP:** Extents of DM and CP disappearance in test feeds are shown in Table 4. There were differences ($p<0.05$) among test feeds in dry matter and crude protein disappearance after several incubation times. After 48 h incubation soybean meal had high extent of DM disappearance (71.3%) between other test feeds and alfalfa had low extent of DM disappearance (48.8%). Wheat bran (74%) and alfalfa (49.9%) had highest and lowest CP disappearance between other feeds, respectively.

The *in vitro* DM and CP degradation characteristics of test feeds are presented in Table 5. The b value of DM and CP was lowest for alfalfa and highest for corn grain and the a value was lowest for corn grain and soybean meal (for DM and CP, respectively) and highest for soybean meal and wheat bran (for DM and CP, respectively).

Table 6: Equations to predict the degradability of dry matter

Feedstuffs	Equation for prediction of DM degradability	R^2
Corn grain	y = 0.9912x-0.349	0.9833
Soybean meal	y = 1.0262x-0.8576	0.9599
Wheat bran	y = 1.0484x-4.0489	0.9791
Alfalfa	y = 0.884x+3.785	0.9474

x, *in vitro* dry matter disappearance (DM%); *y*, *in situ* dry matter degradability (DM%)

Table 7: Equations to predict the degradability of crude protein

Feedstuffs	Equation for prediction of CP degradability	R^2
Corn grain	y = 1.0191x-0.8411	0.9982
Soybean meal	y = 0.9827x+0.0668	0.9878
Wheat bran	y = 1.032x-2.0767	0.9927
Alfalfa	y = 0.9148x+1.3611	0.9904

x, *in vitro* crude protein disappearance ; *y*, *in situ* crude protein degradability

Table 8: Evaluated parameters of Metabolizable protein by *in situ* results k = 0.02 (g kg^{-1}DM)

	Items						
Feedstuffs	QDP	SDP	RDP	ERDP	UDP	DUP	MP
Corn grain	16.66	51.25	67.91	46.58	50.68	45.37	86.55
Soybean meal	28.14	195.03	223.17	217.54	269.72	242.44	381.12
Wheat bran	54.38	59.03	113.41	102.54	55.78	50.16	115.53
Alfalfa	23.61	49.12	72.73	68.01	97.26	87.46	130.82

Relationship between DM and CP *in situ* rumen degradability and *in vitro* disappearance: The strong correlation between extent of *in situ* dry matter and crude protein degradation and *in vitro* dry matter and crude protein disappearance are shown in Table 6 and 7. These results are in agreement with Taghizadeh *et al.* (2006) results. The differences between *in vitro* and *in situ* data were attributed to small particle samples attached to vials and absence of *in vitro* fermentation environment in later times as same as initial time. The variation of DM and CP disappearance due to among test feeds can be expected regarding to differences between chemical compositions (Taghizadeh *et al.*, 2006).

Metabolizable protein: The parameters were evaluated for MP are indicated in Table 8. The key protein parameters in the proposed MP system, QDP, SDP and DUP, are derived from measurements of the rates of degradation of feed proteins suspended in a Dacron bag in the rumen for various periods of time, normaly up to 48 h for concentrate and 72 h for forages (AFRC, 1993; Mehrez and Ørskov, 1977). The amount of protein slowly degradable during the residence of the feed in the rumen is determined by the time spent in the rumen with the feed exposed to rumen bacterial digestion, which is a function of level of feeding (L) and outflow rate (AFRC, 1993). AFRC (1993) was reported that MP for CG, SBM and AA were 77, 323 and 115 g kg^{-1}DM, respectively. A difference between amounts of feed's MP can be resulted due to nutrient composition such as CP, ADIN, soluble protein and degradable protein.

CONCLUSION

There was strong positive correlation between *in vitro* and *in situ* disappearances of dry matter and crude protein so the *in vitro* technique can be suitable replacement for *in situ* dry matter and crude protein disappearance technique. As a whole, the wide variation in chemical composition of feedstuffs, *in situ* and *in vitro* DM and CP disappearances offer users flexibility in formulating ratios according to the productive performance of target animals.

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