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Use of Nylon Bag Technique to Determine Nutritive Value and Degradation Kinetics of Iranian Alfalfa Varieties

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Abstract: The aim of the study was to determine the chemical composition and ruminal degradation characteristics of two alfalfa varieties including Kareyonge (KAR) and Hamedani (HAM) grown in West Azerbaijan in Iran, using *in situ* technique. The nutritional parameters were Dry Matter (DM), Organic Matter (OM) and Crude Protein (CP). Nylon bags filled with 5 g of each forage were suspended in the rumen of three cannulated Gezel rams immediately before feeding and incubated for 7 different times (0, 4, 8, 16, 24, 48 and 72 h). Parameters for degradation kinetics included readily degraded fraction, slowly degraded fraction, lag time and fractional rate of passage. No significant difference found between DM, CP, Ash and Ether Extract (EE) of two alfalfa varieties although the difference for Crude Fiber (CF), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were significant ($p<0.01$).The degradability of DM, OM, CP and Effective Rumen Degradable Protein (ERDP) at a rate of 0.05/h for KAR and HAM varieties were 54.2, 52.16, 40.1%, 72.57 g kg^{-1} DM and 56.97, 54.9, 39.25%, 89.4 g kg^{-1} DM, respectively. Calculations based on *in situ* degradability indicate that HAM alfalfa can have a higher inclusion than KAR alfalfa in diets for ruminants because of lower NDF, greater cell contents and ERDP.

Key words: Chemical composition, degradation kinetics, nylon bag, ruminant, alfalfa

INTRODUCTION

Alfalfa, harvested as hay, is an important forage crop for ruminants because of its high protein concentration; however, research has indicated that the proteins in alfalfa are highly susceptible to degradation during field-wilting and ruminal fermentation (Coblentz *et al*., 1997).

Protein requirement of animals was used to be calculated based on crude protein concentrations of diets. However, studies have shown that addition of escape protein of fast growing ruminants and high producing dairy cows have resulted in an improvement in animal performance, indicating that crude protein system is lacking in term of meeting the protein requirements of animals. Therefore, metabolizible protein system was introduced to more accurately and preciously meet the protein requirements of ruminant animals. Forage protein serves as a source of metabolizable protein to the ruminants by providing both ruminally degradable protein for microbial growth and some ruminally undegradable protein for intestinal digestion. Due to rapid and extensive degradation of forages in the rumen, escape protein concentration of forages are usually low. Many factors such as maturity, forage species and preservation method influence the ruminal degradability of forage CP content. The analytical procedures for feedstuffs so as to characterize nutrient composition have been standardized.

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Recent improvements clarified specific constituents of macronutrients. For instance, the use of degradable and undegradable rumen fractions in place of crude protein is done to improve the utilization of feedstuffs. The nylon bag technique offers an easy, fast, economical and effective method to determine Crude Protein (CP) fractions (NRC, 1989; Hoffman *et al*., 1993; Broderick, 1995; Kaya *et al*., 2004).

In Lucerne, Broderick and Buxton (1991) found variation in protein degradability among 19 *M. sativa* accessions and 3 *M. falcata* accessions. Cherney *et al*. (1992) demonstrated that stage of maturity affects rate of ruminal NDF degradation so, degradation kinetics of forages vary. Bald *et al*. (1993) investigated degradation kinetics of alfalfa at early bud, early bloom and full bloom stages and showed that the digestibility of DM and CP linearly decreased with advancing stage of maturity. Skinner *et al*. (1994) found difference in degradation rates of proteins among nine Lucerne accessions. Kaya *et al*. (2004) also found cumulative disappearance and effective degradability for DM, OM, CP and CF linearly decreased with advancing maturity.

Alfalfa hay is an important forage crop for ruminants. There are two common varieties of Lucerne in Iran including Hamedani and Kareyonge. Inclusion of Hamedani in ruminant diets is higher than that of Kareyonge. A little study has been determined some nutritional characteristics of alfalfa varieties in Iran (Maheri-sis *et al*., 2007).

This study investigated the differences between chemical composition and *in situ* degradation kinetics of Hamedani (HAM) and Kareyonge (KAR) hays for total DM, OM and N.

MATERIALS AND METHODS

Forages

Two Iranian common alfalfa varieties (Hamedani and Kareyonge) were used in the experiment. Samples of two alfalfa hays were collected in 2005, from ten farms, near West Azerbaijan, Iran (located in the Urmia and Miandoab city) and evaluated at the laboratories of Islamic Azad University-Shabestar Branch. Both alfalfas, at harvest, were estimated to be at late maturity (both varieties in mid to late bloom). Samples were collected, oven-dried at 60°C for 48 h, ground (5 mm screen) and prepared for *in situ* analysis (Andrighetto *et al*., 1993).

Chemical Analysis

Dry Matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude Protein (CP) was calculated as N×6.25. Neutral detergent fiber, ADF, acid-detergent sulfuric acid (ADL) and Acid-Insoluble Ash (AIA) were determined by procedures outlined by Goering and Van Soest (1970) with modifications described by VanSoest *et al*. (1991), sulfite was omitted from NDF analysis. Hemicellulose and cellulose were calculated as NDF-ADF and ADF-ADL-AIA, respectively (Andrighetto *et al*., 1993).

In situ Degradation Procedures

Three hundred fifty five kilogram ruminally cannulated Gezel rams were used to determine *in situ* degradation characteristics. Rams were housed in individual tie stalls bedded with sawdust. Rams fed ground alfalfa hay containing 14% CP and 45% NDF were used for incubation of samples in Dacron bags in this study. Alfalfa hay was offered 1.25 × maintenance levels of rams (Karsli *et al*., 2002).

In situ procedures were the same as those described previously (Coblentz *et al*., 1997), Dacron bags (18×9 cm; 520 mm pore size) were filled with 5 g samples of dried ground forage. Suspension of bags in the rumen was accomplished by tying of bags, into tygon tubing with nylon string. Sample in Dacron bags were placed in the rumen of rams and incubated for the periods of 0, 4, 8, 16, 24, 48, 72 h. After the removal of bags from the rumen, bags were washed in cold water until rinse were clear and dried at 60°C for 48 h (Karsli and Russell, 2002). Remaining residues were analyzed for DM, OM and N concentrations.

Dry matter, organic matter and N were divided into three fractions as follows: 1) The soluble DM, OM or N fraction (fraction a) determined as DM, OM or N loss during the washing process, 2) The potentially digestible DM, OM or N fraction (fraction b) determined as the differences between initial DM, OM or N content after washing and the amounts of DM, OM or N recovered after a 72 h incubation, 3) The indigestible fraction (fraction c) determined as the amount of DM, OM or N residue recovered after a 72 h incubation (Karsli and Russell, 2002).

Rumen degradation kinetics for DM, OM and CP were calculated using the nonlinear model proposed by Ørskov and McDonald (1979):

$$P = a + b*(1-e^{-c*t})$$

Where:
P = Percentage of degradability for response variables at t
t = Time relative to incubation (h)
a = Highly soluble and readily degradable fraction
b = Insoluble and slowly degradable fraction
c = Rate constant for degradation
e = 2.7182 (Natural logarithm base)

Following determination of these parameters, the effective degradability of nutrients in the two varieties was calculated using and equation described by Ørskov and McDonald (1979):

$$Pe = a + (b*c)/(c+k)$$

Where:
Pe = Effective degradability for response variables (%)
a = Highly soluble and readily degradable fraction
b = Insoluble and slowly degradable fraction
c = Rate constant for degradation
k = Rate constant of passage

When calculating effective degradability, rate constant of passage was assumed to be 0.02, 0.05 and 0.08% per hour (Bhargava and Ørskov, 1987) so that the results could be extrapolated to other ruminants that differ in rumen capacity.

Statistical Analysis

All of the data were analyzed by using software of SAS (1985) and means (obtained from three homogen samples) were separated by independent-samples t-test (Steel and Torrie, 1980). All of the means obtained from three homogen samples.

RESULTS AND DISCUSSION

Figure 1 and Table 1 show the differences in the composition between the two alfalfa varieties. There was considerable variation between alfalfa hays in term of chemical composition. Cell wall contents (NDF and ADF), which represent the most important fraction of dry matter for alfalfa hays,

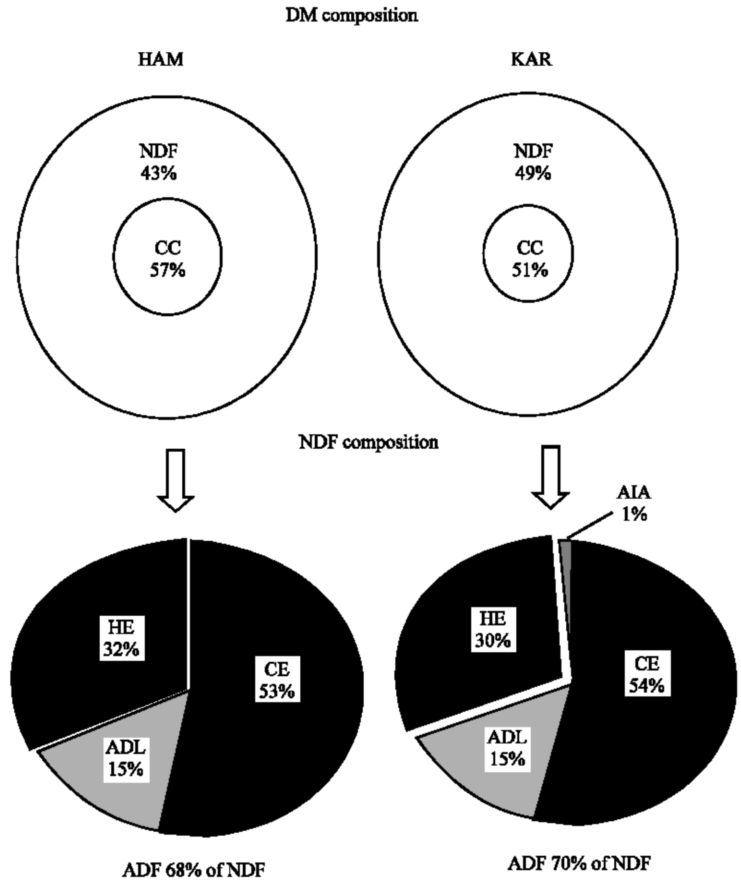

Fig. 1: Comparison of feed fractions of Hamedani (HAM) and Kareyonge (KAR) hays. ADL = Acid-Detergent Lignin, CC = Cell Contents, CE = Cellulose, HE = Hemicellulose and AIA = Acid-insoluble ash

Table 1: Chemical composition of Hamedani (HAM) and Kareyonge (KAR) hays

Components	HAM	KAR	SEM
DM (%)	92.93	93.46	0.133
(%DM)			
CP	15.80	12.50	0.819
Crude fiber	29.20	34.00**	0.519
Ether extract	1.33	1.33	0.334
Ash	10.33	10.33	0.493
Nitrogen-free extract	43.30	41.84	1.195
Cell contents	56.90***	51.00	0.450
NDF	43.10	49.00***	0.259
ADF	29.40	34.20***	0.288
Hemicellulose	13.70	14.80***	0.080
Cellulose	22.90	26.50***	0.105
Acid-Detergent Lignin (ADL)	6.30	7.30	0.231
Acid-Insoluble Ash (AIA)	0.15	0.35**	0.050
ADL/NDF[1]	14.60	14.89	0.765
ADL/ADF[2]	21.40	21.30	0.976

[1]: Lignification index based on NDF, [2]: Lignification index based on ADF, SEM: Standard Error of Means, **: p<0.01; ***: p<0.001

ranged from 43.3 to 49% and 29.4 to 34.2%, respectively. CP, EE and Ash concentrations for KAR and HAM hays were similar (p>0.05). As expected, the concentrations of neutral detergent fiber and ADF were significantly lower (p<0.001) in HAM hay than KAR hay (Table 1). The concentrations of NDF and ADF for HAM hay were in agreement with the results of Coblentz *et al*. (1998). The lignification index (Table 1), on either an NDF or an ADF basis (Van Soest, 1982), was similar in KAR and HAM hays (p>0.05). The concentrations of cellulose and hemicellulose were significantly higher (p<0.001) in KAR hay than HAM hay (Table 1).

This is the first study establishing the rumen degradation characteristics of these alfalfa varieties in around West Azerbaijan. Although there are some studies investigating the chemical composition of the KAR and HAM hays in the region, in none of these studies were degradation characteristics in rumen taken into account. However, studies have shown that vegetation, soil type and climate might dramatically affect the utilization of nutrients by animals (Kaya *et al*., 2004). The results of this study might therefore contribute to our current knowledge allow us to find strategies to improve animal nutrition in the region.

The degradation kinetics of different feed fractions of HAM and KAR hays are described in Fig. 2 and the corresponding effective degradability values at three ruminal passage rates are in Table 2. In this study, proportions of fraction A and B were similar (p>0.05) for hays. Undegradabed fraction of DM and CP were significantly higher for KAR than (33.5 vs. 27.5 and 48.3 vs. 34.2, respectively). Maximum extents of DM and CP for HAM were greater (72.43 and 65.8, respectively; p<0.05). However, extents of degradation of OM for KAR (65.8%) and HAM (67.5%) were similar (p>0.05). Differences between lag time of DM and OM for HAM and KAR hays (0.8 vs. 1 and 0.83 vs. 0.4, respectively) were non-significant (p>0.05), but in HAM hay, CP had longer lag time (1.55 vs. 0.25). Effective DM degradability, at a ruminal turnover rate of 5%/h was 56.98% for HAM hay compared with 54.2% for KAR hay (p<0.01). Effective OM and CP degradability, at ruminal

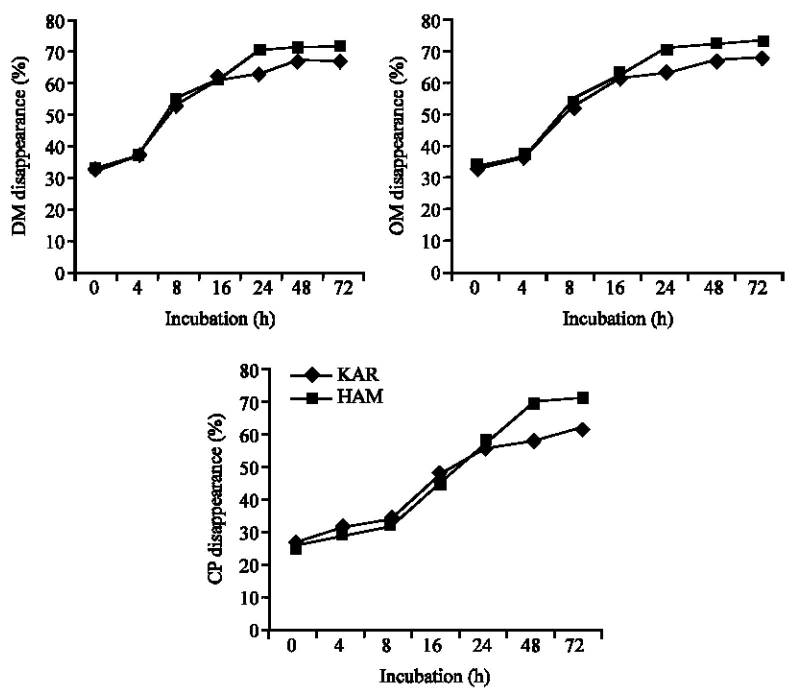

Fig. 2: Comparison of *in situ* degradation kinetics of alfalfa hays

Table 2: *In situ* DM, OM and CP degradation characteristics of the alfalfa hays by sheep

Component and forage type[1]	a[2]	b[2]	c[2]	Extent[2]	Lag time (h)	Effective degradability[3]			
	(% of DM)					2%/h	5%/h	8%/h	K_d[6] (h)
DM									
KAR	32.20	34.3	33.5*	66.5	1.00	60.53	54.20	50.13	0.091
SE[4]	0.98	1.7	0.92	ND[5]	0.28	0.31	0.41	0.57	0.050
HAM	34.43	38.0	27.5	72.43*	0.80	64.00**	56.97**	52.40*	0.091
SE	2.10	2.0	0.43	ND	0.10	0.35	0.40	0.50	0.004
OM									
KAR	30.80	35.0	34.2	65.8	0.40	58.70	52.16	48.06	0.085
SE	0.79	1.7	0.75	ND	0.28	0.24	0.54	0.70	0.010
HAM	32.40	35.1	32.5	67.5	0.83	61.23	54.90	50.73	0.100
SE	2.10	2.1	2.3	ND	0.14	1.60	1.10	1.01	0.005
CP									
KAR	22.45	29.2	48.3*	51.65	0.25	45.50	40.10	36.70	0.081
SE	1.30	1.9	ND	ND	0.39	1.00	1.00	0.79	0.010
HAM	21.70	44.1	34.2	65.8*	1.55*	49.60	39.25	34.20	0.038
SE	1.48	3.2	ND	ND	0.04	0.07	0.74	1.09	0.001

[1]: KAR = Kareyonge, HAM = Hamedani, [2] a = Immediately soluble fraction, b = Fraction degradable at a measurable rate, c = Undegraded fraction and maximum extent = 100 – c, [3]: Effective degradability at three ruminal passage rates, [4]: Standard Error, [5]: Non-determined, [6]: Rate of degradation, *: p<0.05; **: p<0.01

Table 3: Effective degradability (%), UDP and ERDP (g k^{-1}g DM) CP of KAR and HAM hays with different outflow rates

Forage type[1]	ED[2] (%)			UDP[3] (g kg^{-1} DM) (%)			ERDP[4] (g kg^{-1} DM) (%)		
	2	5	8	2	5	8	2	5	8
KAR	45.50	40.10	36.70	40.00	46.82	51.07	79.40	72.57	68.32
SE	1.00	1.00	0.79	0.37	0.43	0.68	0.03	0.09	0.34
HAM	49.60	39.25	34.20	45.30	61.70	69.70*	105.80**	89.40*	81.40
SE	0.07	0.07	1.09	2.20	3.50	4.10	1.70	3.04	3.60

[1]: KAR = Kareyonge, HAM = Hamedani, [2]: Effective degradability (ED), [3]: Undegradable protein (UDP) and [4]: Effective Rumen Degradable of Protein (ERDP) are calculated using the equations of AFRC (1993), *: p<0.05; **: p<0.01

turnover rates of 2, 5 and 8%/h were similar for HAM and KAR hays (p>0.05). CP undegradability, at a ruminal turn over rate of 8%/h was 69.7 g kg^{-1} DM for HAM hay compared with 51.07 g kg^{-1} MD for KAR hay (Table 3, p<0.05). Effective rumen degradability protein, at ruminal turnover rates of 2, 5%/h were significantly higher (p<0.05) in HAM hay than KAR hay (Table 3).

The percentage of water soluble fraction (fraction a) and potentially digestible dry matter fraction (fraction b) were similar (p>0.05) and indigestible dry matter fraction (fraction c) was greater in KAR hay compared with HAM hay (p<0.05; Table 3). The disappearance of DM, OM and CP increased with time of incubation in the rumen (4-48 h, Fig. 2). These values were in line with those of Komprda *et al*. (1993). They incubated Lucerne (*Medicago sativa*), harvested at different stages of maturity, for 48 h and showed that the disappearance of OM decreased linearly by up to advancing maturity. They also observed a 22% reduction in CP disappearance with advancing stage of maturity (Komprda *et al*., 1993). Decreases in degradation could be attributed to an increased lignification process in the cell wall, because lignified tissues limit feed intake and occupy space in the rumen, which may in turn reduce the attachment of bacteria to substrates (Kaya *et al*., 2004).

Overall, the cumulative disappearance pattern for nutrients appears to decrease linearly with advancing maturity, but slight differences in cumulative disappearance reported in the literature could be due to differences in forage sources, stage of maturity and environmental conditions (Kaya *et al*., 2004).

In the current study, rapidly degradable fraction (a) and slowly degradable fraction (b) of DM were similar in alfalfa hays (Table 2). These values were in line with those of Seker (2002). The mean values obtained for DM potential degradability in the HAM hay (72.43%) is similar to that obtained by Bald *et al*. (1993) for alfalfa (77.2%). The slight differences between the current study and those of reported by Andrighetto *et al*. (1993) and Kamalak *et al*. (2005) might be due in part to the different plant species used.

Rapidly (a) and slowly (b) degradable fractions of OM were similar in two varieties. Karsli *et al*. (2002) found that mean (a), (b) and (c) values of OM were 28.4, 43.3 and 28.3, respectively for alfalfa hay. The values obtained in the current study for HAM and KAR hays were also in line with those of Karsli *et al*. (2002).

Microbial protein contributes a large amount to the CP that passes to the intestine of the dairy cow, because microbial CP synthesis in the rumen is highly dependent on the amount of rumen-degradable OM (Kamalak *et al*., 2005).

Rapidly (a) and slowly (b) degradable fractions of CP observed in the study for HAM hay were in range of result reported for alfalfa by Coblentz *et al*. (1998). The difference between the current study and those of reported by Michalet-Doreau and Ould-Ban (1992) and Elizalde *et al*. (1999) might be due in chemical compositions, CP extent and harvesting time.

Lag time (which indicates the time required for initiation of degradation) for degradation of CP was significantly higher ($p<0.05$) in HAM hay. Lag time may be caused by the need for chemical or physical alteration of fiber before bacterial attachment and enzymatic digestion can occur, or by a need for bacterial growth and increases in enzyme content. Physical factors such as wettability of the substrate, rate of solution and nutrient limitations also could influence lag time (Olubobokun *et al*., 1990).

CONCLUSION

In an overall conclusion, it seems that regarding chemical composition and degradation kinetics, nutritive value of Hamedani hay was higher than that of Kareyonge and we can recommended that Hamedani hay may be used more than Kareyonge hay in ruminant diets.

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