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Estimation of Quality Indices of Iranian Alfalfa Varieties Using *in vivo* and *in situ* Methods

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Abstract: This study was conducted to evaluate quality indices in two common Iranian alfalfa varieties as a forage source in sheep nutrition. The studied alfalfa varieties were Hamedani (HAM) and Kareyonge (KAR). Chemical composition, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), leaf to stem ratio and predicted Dry Matter Intake (DMI), Dry Matter Digestibility (DMD) were determined and Total Digestible Nutrients (TDN), Net Energy for Lactation (NEL), Relative Feed Value (RFV), Relative Forage Quality (RFQ), of feeds were estimated. Three ruminal fistulated Gezel rams, were used for *in vivo* and *in situ* trials. The NDF and ADF contents of two alfalfa varieties were significantly different ($p<0.01$). No significant difference found between CP, ADL and leaf to stem ratio of two alfalfa hays ($p>0.05$). The TDN and NEL contents of two varieties and DMI (% of BW and g per kg $BW^{0.75}$) and DMD (%) had significant differences ($p<0.01$). The RFV and RFQ values for experimental varieties were significantly different ($p<0.01$). It can be concluded that HAM hay can have a higher nutritional quality than KAR hay because of lower cell wall and higher TDN contents and therefore, HAM hay may be evaluated more accurately when tested for RFQ instead of RFV.

Key words: Alfalfa, relative feed value, relative forage quality, *in vivo*, *in situ*

INTRODUCTION

Nutritive value is a term used to quantify the presence and availability of feed nutrients that are required by the animal and to predict the productive output from the animal to which it is fed. It depends on the following:

- The concentration of nutrients in the feed,
- The availability of these nutrients to the animal,
- The efficiency with which the absorbed nutrients are used by the animal
- The effect of feed composition on the voluntary intake of the feed (Freer and Dove, 2002; Mirzaei-Aghsaghali, 2006).

Nutritive value must be expressed in standard units that can be applied also to the nutrient requirements of the animal. Relative Feed Value (RFV) and Relative Forage Quality (RFQ) are indices used to measure the quality of forage and are determined by its content of ADF and NDF. The ADF is also used to calculate the energy (NE_m, NE_l and NE_g) content of forage and NDF is an evaluation of the total fiber content that includes hemicellulose in addition to the cellulose and lignin content. The NDF content is related to intake because it evaluates the bulkiness of forage. Nowadays, development of a new index provides the opportunity for flexibility in choice of equations for predicting DMI and TDN; these equations should be specific for different types of forage (Heydari *et al*., 2006). With the introduction of the new approaches to determining animal requirements in National Research Council (NRC, 2001), there is an opportunity to improve upon this quality index through use of newer analyses and equations.

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) but information about quality indices of Iranian hays specially for alfalfa varieties is very limited (Heydari *et al*., 2006; Maheri-Sis *et al*., 2007).

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The aim of this study, was to determine RFV, QI and RFQ and comparing DMD, DMI and RFV by three methods (fiber component, *in vivo* and *in situ*).

MATERIALS AND METHODS

Sample collection: Two alfalfa varieties including Hamedani (HAM) and Kareyonge (KAR) used in this study were randomly sampled from ten alfalfa farms at West Azerbaijan, Iran (located in the Urmia and Miandoab cities) in summer 2005. The samples were transported to the laboratories of Islamic Azad University - Shabestar Branch.

Both alfalfa, at harvested, were estimated to be at late maturity (mid to late bloom). Samples were collected, oven-dried at 60°C for 48 h, ground (5 mm screen) and prepared for chemical analysis.

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 8h and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude Protein (CP) was calculated as N× 6.25. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined by procedures outlined by Goering and Van Soest (1970) with modifications described by Van Soest *et al.* (1991); sulfite was omitted from NDF analysis (Table 1). Leaves (blades) and stems (true stem and seed heads plus leaf sheaths) were hand separated similar to Belanger and McQueen (1997) and Gustavsson and Martinsson (2004). Samples were collected, oven-dried at 60°C for 48 h, ground (5 mm screen), labeled and placed in glass screw-top jars. Leaf and stem dry weights were used to calculate leaf to stem ratio.

Relative feed value: The RFV was calculated from the estimates of DMD and DMI (Rohweder *et al.*, 1978; Moore and Undersander, 2002a):

$$\text{DMI, \% of BW} = 120/(\text{NDF, \% of DM}) \quad (1)$$

$$\text{DMD, \% of DM} = 88.9- 0.779\ (\text{ADF, \% of DM}) \quad (2)$$

$$\text{RFV} = \text{DMI (\% of BW)} * \text{DMD (\% of DM)} / 1.29 \quad (3)$$

DMD = Dry Matter Digestibility
ADF = Acid-Detergent Fiber
DMI = Dry Matter Intake
RFV = Relative Feed Value

Quality index: QI was calculated from the estimates of TDN intake (g per $BW^{0.75}$). The QI value of forages usually ranged between 1-2.2 (Moore and Undersander, 2002a).

Table 1: Legume, grass and legume-grass mixture quality standards

Quality standard[a]	CP, % of DM	ADF, % of DM	NDF, % of DM	RFV
Prime	>19	<31	<40	>151
1	17-19	31-40	40-46	151-125
2	14-16	36-40	47-53	124-103
3	11-13	41-42	54-60	102-87
4	8-10	43-45	61-65	86-75
5	<8	>45	>65	<75

Adapted From Canbolat *et al.* (2006)

$$\text{QI} = \text{TDN intake, g per } BW^{0.75} / 29 \quad (4)$$

Relative forage quality: The RFQ was estimated by below equation by using DMI and TDN values (Moore and Undersander, 2002b):

$$\text{RFQ} = (\text{DMI, \% of BW}) * (\text{TDN, \% of DM})/1.23 \quad (5)$$

Digestibility trails (*in vivo*): Alfalfa was offered ad libitum to three Gezel rams (1.5 year old, average initial BW 55 kg) kept in metabolism cages to enable accurate determination of feed intake and allow easy collection of faeces. The forage was fed twice daily at 08:30 and 16:30h and fresh drinking water and mineral salt licks were freely available. The animals were adapted forage for 2 weeks, followed by balance trails of seven days (total period of digestibility trials were 42 days), in which daily measurement of food intake and fecal excretion were made. Sub-sample of forage was taken and data on their daily intake (g DM Kg^{-1} $BW^{0.75}$) and digestibility *in vivo* were obtained.

***in situ* degradation procedures:** Three 55-Kg 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.*, 2002b).

in situ procedures were the same as those described previously by 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, 72h. 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 *et al.*, 2002a). Remaining residues were analyzed for DM concentration.

Dry matter was divided into three fraction as follows: The soluble DM fraction (fraction a) determined as DM loss during the washing process, the potentially digestible DM fraction (fraction b) determined as the differences between initial DM content after washing and the amounts of DM recovered after a 72-h incubation, the indigestible fraction (fraction c) determined as the amount of DM residue recovered after a 72-h incubation (Karsli *et al*., 2002a).

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

$$P = a + b(1 - e^{ct}) \quad (6)$$

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.

The dry matter digestibility values of HAM and KAR hays samples were calculated using equation of Khazaal *et al*. (1995) as follows:

$$\text{DMI, g DM per kgW}^{0.75} = 9.9 + 0.4(a+b) + 408(c) \quad (7)$$

Equations: The net energy for lactation and total digestible nutrient values of feed samples were determined by equations below by Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) amounts.

$$\text{TDN, \%} = 88.9 - (\text{ADF\%} * 0.0123) \text{ (Schroeder, 2004)} \quad (8)$$

$$\text{TDN intake, g per BW}^{0.75} = 0.01 * \text{TDN, \%} * (120.7 - (0.83 * \text{NDF, \%})) \text{ (Zinn and Ware, 2007)} \quad (9)$$

$$\text{NEL, Mcal per kg DM} = 0.6(1+0.004(q-57)) \text{ ME (Seker, 2002)} \quad (10)$$

$$q = \text{ME} * 100 / \text{GE} \quad (11)$$

Where,

TDN = Total Digestible Nutrient

NEL = Net Energy for Lactation

ME = Mcal per kg

DM = Metabolizable Energy

GE = Gross Energy (Mcal per kg DM).

$$\text{NDF}_n = \text{NDF} * 0.93 \quad (12)$$

$$\text{NDFCP} = \text{NDF} - \text{NDF}_n \quad (13)$$

NDF_n, % = Nitrogen free NDF, NDFCP, % = CP of NDF (Heydari *et al*., 2006).

Statistical analysis: All of the data were analyzed by using software of Statistical Analysis Systems (1985) and means were separated by independent-samples t-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There was variation between forages in terms of chemical composition, fiber components and some estimated parameters of HAM and KAR hays (Table 2). The CP content of HAM and KAR hays were similar ($p>0.05$). The ADL content was similar in both forages ($p>0.05$). The CP content of HAM hay was consistent with finding by Kamalak *et al*. (2005a). The CP content for HAM and KAR hays were lower than that reported by Coblentz *et al*. (1998) and Kamalak *et al*. (2005a) and similar with data of Abas *et al*. (2005). The ADF, NDF, NDF_n and NDFCP were significantly ($p<0.01$) higher in KAR hay. The cell wall (ADF and NDF) and ADL contents of HAM hay were similar than those reported by Coblentz *et al*. (1998), lower than that reported by Torrent *et al*. (1994) and higher than that reported by Kamalak *et al*. (2005a). The different result reported by several researchers about alfalfa hay cell wall content may be due to differences In maturity (Coblenze *et al*., 1998; Gulsen *et al*., 2004; Kamalak *et al*., 2005b,c) variety, environmental conditions and agronomic factors (Wechsler, 1981; Buxton, 1996) and leaves to stem ratio (Coblentz *et al*., 1998). Digestibility of forages is a function of its ADF content and intake of forages is a function of its NDF content (Allen, 2000). When using any fiber determination to estimate digestibility the assumption is made that there is a close relationship between fiber concentration and digestibility (Weiss, 1994). The NDF_n and NDFCP values of HAM hay were higher than that KAR of hay ($p<0.01$). These values are lower than those found in alfalfa by Elizalde *et al*. (1999). This difference may be due to the different drying procedures (forced air drying in our experiment vs. freezing dring in the experiment of Elizalde *et al*. (1999).

No significant differences were found between leaf to stem ratio of HAM and KAR hays. The leaf to stem ratio ranged from 0.55-0.62 for alfalfa varieties. The leaf OM concentration of KAR hay was significantly higher than that obtained at HAM hay ($p < 0.01$). The leaf OM concentration of HAM and KAR hays were 88.7 and 90.6%, respectively, whereas the stem OM content of them ranged from 92.4-92.5%. The leaf OM concentration of HAM and KAR hays were similar to those reported by Collins *et al*. (1988) and Putnam (2000). The TDN (%) and TDN intake (g per kg $BW^{0.75}$) of HAM hay were significantly ($p<0.01$) higher than that KAR hay. The

Table 2: The chemical composition, fiber components, energy contents and nutritive quality indices of Hamedani (HAM) and Kareyonge (KAR) hays

Parameters	HAM	KAR	SEM	Sig.
CP, %	15.8	12.5	0.819	NS
NDF, %	43.1	49	0.259	***
NDF_n, %	40.1	45.6	0.241	**
NDFCP, %	3	3.4	0.018	**
ADF, %	29.4	34.2	0.288	***
ADL, %	6.3	7.3	0.231	NS
Leaf to stem ratio	0.62	0.55	0.025	NS
% OM of Leaf	88.7	90.6	0.251	**
%OM of Stem	92.5	92.4	0.115	NS
TDN, %	65.67	61.87	0.231	**
TDN intake, g per $BW^{0.75}$	55.77	49.5	0.334	**
NEL, Mcal per kg DM	1.36	1.11	0.033	**
RFV	142.4	118.6	1.183	**
QI	1.92	1.7	0.009	**
RFQ	148.6	123.1	1.33	**

CP = Crude Protein; NDF = Neutral Detergent Fiber; NDF_n = Nitrogen free NDF, NDFCP = CP of NDF; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin; TDN = Total Digestible Nutrient (Schroeder, 2004); NEL = Net Energy of Lactation (Seker, 2002); RFV = Relative Feed Value; QI = Quality Index; RFQ = Relative Forage Quality. NS = Non Significant; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

TDN intake of KAR and HAM hays were 49.5 and 55.77 g per $BW^{0.75}$, respectively. The calculated TDN value (61.87%) of KAR hay was lower than that of reported by NRC (2001). The TDN content of HAM hay (65.67%) was in agreement with TDN value of alfalfa hay (late maturity; 63%) reported by NRC (2001). The NEL content of KAR hay (1.11, Mcal per kg DM) was lower than that HAM hay (1.36, Mcal per kg DM) and similar than those reported by Seker (2002).

The values of RFV, QI and RFQ were significantly different for HAM and KAR (Table 2). The RFV value of HAM and KAR hays higher than that reported by Dunham (1998). Forages with values greater than 100 are of higher quality (Table 1). Dairy producer with high producing cows often require 150 or greater RFV value (Schroeder, 2004). The QI value is high in HAM hay (1.92) compared to KAR hay (1.7). The major difference between QI and RFV is that, for QI, the reference base is a defined animal requirement for energy rather than the quality of particular forage chosen arbitrarily. The base QI was set 1.0 rather than 100 in order to avoid confusion with RFV (Table 1). Forage quality is affected most by variations in genotype, maturity, season and management. Other anti-quality factors may be encountered occasionally (Moore and Undersander, 2002a, b). The effect of alfalfa varieties on forage quality are presented in Table 3. The TDN intake (% of BW) and voluntary intake (% of BW) values of HAM hay were higher than that of KAR hay ($p<0.01$). The RFQ value of KAR and HAM hays were 123.1-148.6, respectively. Undersander and Moore (2004) reported that RFQ will become the standard test for evaluating forages throughout the country and that it eventually will be

Table 3: Effect of alfalfa varieties on TDN and voluntary intake and forage quality

Forage	TDN intake[a] (% of BW)	Voluntary Intake[b] (% of BW)	Qi[c]
HAM	2.04	2.78	1.92
KAR	1.82	2.44	1.7
SEM	0.0129	0.0159	0.009
Sig.	**	**	**

[a]TDN = total digestible nutrients, [b]Intake of dry matter expressed as percentage of body weight, [c]Voluntary TDN intake relative to maintenance requirement; 1.0 = maintenance

Table 4: Comparison of Dry Matter Intake (DMI), Dry Matter Digestibility (DMD) and Relative Feed Value (RFV) in HAM and KAR hays determined by Fiber components, *in vivo* and *in situ*

Nutrient contents	Method	HAM	KAR
DMI, % of BW	Fiber components	2.783	2.448
	in vivo	2.981	2.216
	in situ	2.781	2.717
DMI, g DM per kg $BW^{0.75}$	Fiber components	75.78	66.6
	in vivo	81.2	59.5
	in situ	75.71	74
DMD, %	Fiber components	66	62.25
	in vivo	65	56.8
	in situ	64	60.5
RFV	Fiber components	142.4	118.6
	in vivo	193.8	124.8
	in situ	179.3	164.5

used even more widely than RFV is today. RFQ has the following advantages:

- RFQ may be translated into energy requirements for maintenance and production,
- Development of a new index provides the opportunity for flexibility in choice of equations for predicating DMI and TDN,
- Associative effects between forages and concentrates that influence forage intake and digestibility can predicted from estimates of forage TDN intake when fed alone.

On other advantage of the RFQ prediction is that it differentiates legumes from grasses (Moore and Undersander, 2002a).

Comparison of the DMI, DMD and RFV obtained by fiber component, *in vivo* and *in situ* methods (Table 4) indicated that fiber component technique was a good method to estimate *in vivo* DMI (% of BW and g DM per kg $BW^{0.75}$) and DMD. The fiber component and *in situ* methods were similar method to estimate *in vivo* DMI and DMD. However, in the fiber component, the values of RFV in forage hays were close to the value obtained from the *in vivo* study.

CONCLUSION

In overall conclusion, results of this study showed that the NEL, TDN, DMI, DMD of Hamedani hay was higher than that of Kareyonge and the fiber components (NDF, NDF_n, NDFCP and ADF) were lower than it. The quality indices (RFU, QI and RFQ) of Hamedani hay were also greater than Kareyonge. Therefore, Hamedani hay has better nutritional quality than Kareyonge and we can recommend that Hamedani hay may be used more than Kareyonge hay in ruminant diets.

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