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Nutritional Value of Alfalfa Varieties for Ruminants with Emphasis on Different Measuring Methods: A Review

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Abstract: Alfalfa is highly valued for animal feed because of its high protein content, high intake potential anddigestibility. Alfalfa hay is valued by nutritionists for its relatively high energy value which supports milk production, its rapid ruminal digestion of structural fiber which stimulates feed intake, its coarse structural fiber that stimulates ruminative chewing and salivation, which results in rumen buffering, its structural fiber which has high buffering capacity, its high protein level which supports animal protein needs andthe relatively high proportion of its protein that escapes the rumen undegraded which minimizes dietary requirements for high cost protein supplements. This review evaluates Iranian hays especially common alfalfa varieties in regard to relative forage quality, nutrient composition, nutrient digestion andtheir impact on microbial protein synthesis. Different alfalfa species differ inherently in their rate of reproductive development. This results not only in changes in chemical and anatomical characteristics, but also in the proportion of plant parts, e.g. leaf, stem, pseudostem, potiole, inflorescence, which in turn differ significantly in their quality attributes. Management and environment can then play a significant role in affecting nutritive value, either by directly altering chemical and anatomical traits or by influencing the timing of changes in plant phenology.

Key words: Alfalfa, nutritional value, ruminant, microbial protein synthesis, feed intake

INTRODUCTION

Forages comprise 35-70% of the dry matter (DM) in diets for lactating dairy cows. Forage quality impacts DM intake, diet energy density, dietary grain and protein supplementation, feed costs andlactation performance (Undersander *et al*., 1993). Forages provide 83% of the protein requirements of beef cattle and 90% of the protein requirement of sheep (Maheri-Sis *et al*., 2007a). Alfalfa (*Medicago sativa*), also known as Lucerne, Purple Medic and Trefoil, called the Queen of the Forages, is a perennial flowering plant cultivated as an important forage crop. Alfalfa is one of the most important forage crops in Iran growing in various regions of the country, about 12 million tones (Table 1). Southwest Asia and possibly northern Iran are considered to be the place of its origin (Smith *et al*., 1991; FAO, 2007). Abbasi *et al*. (2007) showed that Alfalfa originated in Vavilov's Near Eastern Center Asia Minor, Transcaucasia, Iran and Turkistan. Alfalfa produces at different locations of Iran such as, West Azerbaijan, East Azerbaijan, Mashhad, Hamedan, Tehran, Ghom, Khash and Yazd.

Table 1: Yield (kg Ha^{-1}), Area Harvested (Ha) And Production Quantity (Tonnes) alfalfa for forage and silage in 2006

Country	Area Harvested (Ha)	Yield (Kg Ha^{-1})	Production Quantity (Tonnes)
Iran	505000	24158.42	12200000
Asian	1022590	25832.94	26416501
World	15213666	28663.73	436080348

Adapted from FAO (2007)

Legumes are a protein source in ruminant nutrition. These home-grown feeds make farmers less dependent from the purchase of other protein sources. This is an advantage for the farm economy and ecology, particularly because of restrictions concerning the environment. Moreover, plant proteins become increasingly important since the prohibition of the use of animal protein in livestock nutrition. The capacity of legumes to fix nitrogen (N) from the air results in high protein contents, particularly in Lucerne (Gosselink, 2004). Research at the USDA Dairy Forage Research Center has shown that dairy diets where at least ¼ of the forage is from alfalfa and ¼ is from corn silage produce the most milk (with the remaining half being either forage). While, there has been some interest in corn silage, many dairymen are realizing the benefits of maximizing the alfalfa in dairy rations.

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Alfalfa is high energy forage that provides fiber content and adequate particle size without wasting nutrient density. Inadequate fiber in lactation rations can cause erratic dry matter intakes, decreased milk yields andlowered milk fat production and health problems (acidosis, laminitis, ketosis, displaced abomasums).

Subacute acidosis can cause lowered production, health problems andhigher culling rates. Thirty five percent of cows in confinement operations are believed to have laminitis which is caused primarily by acidosis. Laminitis is a primary cause of lameness in dairy cattle and can cost the dairy producer in delayed reproduction, body weight loss anddecreased milk production. Alfalfa further reduces acidosis and associated health problems because it has a high buffering capacity. Thus many dairymen are realizing that maximizing alfalfa in dairy rations increases milk production and herd health resulting in more profit (USDA, 2007). In spite of these outstanding characteristics, critical factors remain that limit the increased utilization of alfalfa in sustainable dairy production systems. Factors limiting increasing use of alfalfa by dairy cattle are low digestion of plant cell wall, especially lignified cell wall, excessive protein if harvested as silage or harvested early to obtain high energy and excessive leaf loss from harvest or leaf drop during maturation or excessive leaf disease infestation. Alfalfa can be genetically redesigned into varieties that have greater cell wall digestibility, less protein degradation during ensiling, increased bypass protein andincreased yield without quality loss to fit the needs of the high-producing dairy cows (Torrent *et al*., 1994; Mirzaei-Aghsaghali, 2006). Ideal attributes for plant modification of alfalfa may include those that: Increase milk potential per acre and/or per ton; enhance digestible NDF; optimize protein content and amino acid balance; and improve agronomic traits for insect protection (safer forage supply), herbicide tolerance, virus resistance, drought tolerance, cold tolerance, improved mineral availability and enhanced yield (Martin *et al*., 2003; 2005).

The genus *Medicago* has been reported to contain the following chemicals, relative toxicities of which are tabulated in Duke's Phytotoxin Tables (Duke, 1981): Choline, citric acid, hydrocyanic acid, limonene, malic acid, malonic acid, oxalic acid, pantothenic acid, pectin, quinic acid, saponin, shikimic acid, tannin, trigonelline andtryptophane. Four isoflavones are reported in alfalfa (daidzein, formononetin, genistein andbiochanin) and they, like coumestrol, produce an estrogen-like response, perhaps contributing to reproductive disturbances of cattle on high-estrogen forage. However, there are little information about the nutritive value of Iranian hays especially common alfalfa varieties.

NUTRITIVE VALUE

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 and) the effect of feed composition on the voluntary intake of the feed (Mirzaei-Aghsaghali, 2006; Mirzaei-Aghsaghali *et al*., 2007a).

The nutritive value of feeds should be ranked on the following characteristics (Leng, 1986):

- Voluntary consumption potential.
- Potential digestibility and ability to support high rates of fermentative digestion.
- High rates of microbial protein synthesis in the rumen relative to volatile fatty acids (VFA) produced (fermentation protein/energy (P/E) ratio).
- High rates of propionic acid synthesis (glucogenic) relative to total VFA synthesis (fermentation glucogenic/energy (G/E) ratio) and
- Ability to provide bypass nutrients (protein, starch and lipid) for absorption from the small intestine (absorbed P/E and G/E ratios).

WHAT INFLUENCES NUTRITIVE VALUE?

Different plant species differ inherently in their rate of reproductive development. This results not only in changes in chemical and anatomical characteristics, but also in the proportion of plant parts, e.g. leaf, stem, pseudostem, potiole, inflorescence, which in turn differ significantly in their quality attributes. Management and environment can then play a significant role in affecting nutritive value, either by directly altering chemical and anatomical traits or by influencing the timing of changes in plant phenology.

Plant maturity: Advancing plant maturity is associated with a lowering of nutritive value by virtue of a decrease in leafiness and an increase in the stem: Leaf ratio, change in the composition of the cell wall (Akin *et al*., 1977) and a loss of cell contents with maturity (Ballard *et al*., 1990).

Environment: Temperature and light are probably the most important environmental factors that effect nutritive value, both directly and indirectly. Higher temperatures usually promote the accumulation of structural material (i.e. cell-wall material) and also more rapid metabolic activity, which decrease the pool size of cell contents (Ford *et al*., 1979).

As a result of photosynthesis, there is diurnal variation in water soluble carbohydrates (WSC) levels, which rise to a peak during daylight hours (Ciavarella *et al*., 2000). A further advantage is that ruminants prefer afternoon-cut hay compared with morning-cut hay (Fisher *et al*., 1999), presumably because of the higher content of WSC (Ciavarella *et al*., 2000).

When considering the environment in which forages are grown, it is also pertinent to consider the effects of soil type and moisture availability. Plants grow on different soils have different mineral nutrients available to them, which will influence both their growth and their composition. Composition and nutritive value are affected by soil and weather, so that the results in the literature reflect the interaction between plant genetics and environment (Van Soest, 1994).

Management: In grazed system, the timing, frequency and intensity of grazing can all influence the botanical composition of the sward, the morphology and phenology of the plants present, the nutritive value of the regrowth and the spatial heterogeneity of quality. Selective herbicides may be used to manipulate botanical composition and thus improve the nutritive value of feed on offer; herbicides may also be used for spray topping to conserve high-quality spring pasture as summer/autumn feed for livestock (Gatford *et al*., 1999).

As has been appreciated for many years (McIlroy, 1967), of the many factors in plant nutrition, the application of N has a major effect on nutritive value, as distinct from forage yield. It influences botanical composition, particularly the legume: Grass ratio andincreases the CP level in the forage, sometimes at the expense of WSC.

Genetic variation: Plants have adapted to specific environments through evolution and those that have evolved under grazing have developed protective mechanisms against predatory attack (whether it be animal or insect). Some of these mechanisms include lignification, cutinization, silicification, secondary compounds, such as phenols and alkaloids andprostrate growth architecture. Fortunately, there is naturally occurring genetic variation that enables plant breeders to select and breed superior lines, whether they are superior in disease or pest resistance, agronomic traits or nutritive value. However, care must be taken that selection for high yield, quality or disease or pest resistance dose not inadvertently select against nutritive value. This has led some plant breeders to include more intensive nutritive evaluation as part of their genetic engineering (Tabe *et al*., 1993) and conventional breeding programmes (Ehlke *et al*., 1986).

The photosynthetic mechanism typical of a plant species can also influence nutritive value. C3 and C4 plant species are so called because their products of photosynthesis are, respectively, either three-carbon compounds or four-carbon compounds. C4 plants are photosynthetically more efficient andthey tend to exhibit high DM accumulations that are often of lower nutritive value (Minson, 1990).

Medicago sativa L. belongs in the order *Fabales*, family *Fabaceae*, *tribe Trifolieae* andgenus *Medicago*. The genus *Medicago* is very extensive, consisting of more than 60 different species; two thirds of the species are annuals and one third are perennials (Quiros and Bauchan, 1988).

Commercially cultivated alfalfa properly belongs to the *M. sativa* complex, a group of closely related subspecies that are interfertile and share the same karyotype. The most commonly cultivated alfalfa in the world is *M. sativa subsp. sativa*, but subspecies *falcata* is also cultivated on a limited basis, primarily under rangeland conditions and in colder regions. Other subspecies in the complex include *subsp. glutinosa, subsp. coerulea, subsp.* x *tunetana*, *subsp.* x *varia, subsp.* x *polychroa* and*subsp.* X *hemicycla* (Quiros and Bauchan, 1988). Two other closely related species, *M. prostrata* and *M. glomerata*, can be considered capable of limited natural hybridization with alfalfa (Quiros and Bauchan, 1988). There are two types of alfalfa landraces in Iran. The first is cold temperate alfalfa landraces such as Hamedani, Kareyonge and the second is sub-tropical alfalfa landraces such as Bami, Nikshahri and Yazdi (Abbasi *et al*., 2007). There are different cultivars of Lucerne in Iran including Yazdi, Khomin, Marandi, Hamedani, Khoei, Kareyonge, Miandoab, Afghani, Bami, Shahpor and etc., but there are two common cultivars of Lucerne including Hamedani and Kareyonge. A little study has been determined some nutritional characteristics of alfalfa varieties in Iran (Maheri-sis *et al*., 2007b). Kocheki *et al*. (1987) were evaluated 6 Iranian a long with seven imported cultivars. The highest yield was obtained by Hamedani and lowest yield by Bami cultivar.

A number of papers have evaluated alfalfa varieties for their composition and nutritive value. There is considerable genetic variation among varieties relative to alfalfa quality (Sheidai and Shafeineya, 2001). Fajri (2006) studied 3 cultivars (Sakoel, Hamedani and Kareyonge) at three culture time. Julier *et al*. (2003) also report the variation in 100 varieties grown in France. Kocheki and Riazi (1980) were compared the nutritional value of two Iranian (Yazdi and Hamedani) and four imported cultivars under the climatic condition on Mashhad state.

MORPHOLOGICAL CHARACTERISTICS OF IRANIAN ALFALFA VARIETIES

The general alfalfa plant morphology was considered by Teuber and Brick (1988) and Barnes and Sheaffer (1995). Hamedani Cultivar yields are 1497 kg ha^{-1} year

(with 4-5 cutting per year), but Kareyonge yields are 1364 kg ha^{-1} year. Yields vary due to region and with weather andwith stage of maturity when cut. Later cuttings improve yield but reduce nutritional content. Leaf to stem ratio, plant high, growth and protein percent of Hamedani are higher than that Kareyonge cultivar (Mirzaei-Aghsaghali, 2006; Mirzaei-Aghsaghali *et al*., 2007a).

CHEMICAL COMPOSITION

Much of the considerable information now available on the chemical composition of Iranian alfalfa cultivars (Maheri-Sis *et al*., 2007a; Tabatabaei *et al*., 2005) is from proximate analysis (total N. ether extract, crude fibre andnitrogen free extractives) and is of limited value as a predictor of nutritive value. Analyses based on detergent extraction are more useful since plant dry matter is separated into a completely digestible fraction (Neutral Detergent Solubles (NDS)) representing cell contents anda partially digestible fraction (Neutral Detergent Fibre (NDF)) representing plant cell walls. Table 2 shows differences in the Chemical composition, fiber components and energy contents of kraeyonge (KAR) and Hamedani (HAM) hays. KAR hay is low in cell contents and high in NDF compared with HAM hay. The energy values for HAM were higher than that of KAR. The DE, ADF and NDF contents of KAR were in line with those reported by Aghajanzadeh-Golshani *et al*. (2007).

Table 2: Chemical composition, fiber components and energy contents of Kraeyonge (KAR) and Hamedani (HAM) hays[a]

Item	HAM	KAR	SE
DM (%)	92.93	93.46	0.133
OM (%)	89.66	89.66	-
CP (%)	15.80	12.50	0.819
CF (%)	29.20	34	0.519
EE (%)	1.33	1.33	0.334
Ash (%)	10.33	10.33	0.493
NFC (%)	29.44	26.80	1.195
NFE (%)	43.30	41.84	1.195
CC (%)	56.90	51.00	0.450
NDF (%)	43.10	49.00	0.259
NDF_n (%)	40.10	45.60	0.241
NDFCP (%)	3.00	3.40	0.018
DNDF (%)	55.90	55.20	1.154
INDF (%)	44.10	44.80	1.010
ADF (%)	29.40	34.40	0.288
HEM (%)	13.70	14.80	0.080
CEL (%)	22.90	26.50	0.105
ADL (%)	6.30	7.30	0.231
AIA (%)	0.15	0.35	0.050
ADL/NDF	14.60	14.89	0.765
ADL/ADF	21.40	21.30	0.976
Leaf to stem ratio	0.62	0.55	0.025
OM of leaf (%)	88.70	90.60	0.251
OM of stem (%)	92.50	92.40	0.115
GE (Kcal kg^{-1})	4219	4250	-
DE (Mcal kg^{-1})	2.77	2.36	0.047
NEL (Mcal kg^{-1}	1.36	1.11	0.033
TDN (%)	65.67	61.87	0.231

DM: Dry Matter; OM: Organic Matter; CP: Crude Protein; CF: Crude Fiber EE: Extract Ether; NFC: Non-Fibrous Carbohydrate, NFE: Nitrogen-Free Extract; CC: Cell Contents; NDF: Neutral Detergent Fiber; NDFn: Nitrogen free NDF; NDFCP: CP of NDF; DNDF: Digestible Neutral-Detergent Fiber; INDF: Indigestible Neutral-Detergent Fiber; ADF: Acid Detergent Fiber; HEM: Hemicellulose; CEL: Cellulose ADL: Acid-Detergent Lignin; AIA: Acid Insoluble Ash: ADL/NDF: Lignificaion index based on NDF; ADL/ADF: Lignificaion index based on ADF GE: Gross Energy; DE: Digestible Energy; NEL: Net Energy of Lactation; TDN: Total Digestible Nutrient; [a]Compiled from Mirzaei-Aghsaghali *et al*. (2006), Maheri-Sis *et al*. (2007b), Mirzaei-Aghsaghali *et al*. (2007a,b) and Mirza-Aghazadeh *et al*. (2007)

IN VIVO METHODS

Before 1860, fecal digestibility trials started at the Weende Experimental Station of the University of Goettingen in Germany. The measurement of the digestion in the different compartments of the digestive tract using intestinal cannulated ruminants become important about 30 years ago. However, to improve and to validate the feed evaluation systems and to evaluate new feeds, *in vivo* data are generally not available. *in vivo* experiments have disadvantages as they are laborious and expensive, they have complex methodologies resulting in variable values and they often investigate a ration and not a single feed (Gosselink, 2004).

Tables 3 include *in vivo* data of HAM and KAR hays with sheep. The reason why HAM hay had higher DMD and OMD than that of KAR probably is low cell wall content. DMD, OMD (%) and DMI (g kg^{-1} $W^{0.75}$) for KAR hay were similar with reported values (56.5, 58.4% and 56.8 g kg^{-1} $W^{0.75}$; respectively) in alfalfa (Torrent *et al*., 1994).

Table 3: A comparison of the DM, OM, CP and ME intake, apparent digestibility coefficients and digestible OM and CP contents in sheep

		HAM			
	KAR[(a)]	(b)	(c)	(d)	(e)
DMI					
Kg day^{-1}	1.2	1.64	-	-	-
g per $BW^{0.75}$	59.5	81.2	-	-	-
OMI					
Kg day^{-1}	1.1	1.5	-	-	-
g per $BW^{0.75}$	54.4	74.2	-	-	-
CPI					
Kg day^{-1}	0.145	0.182	-	-	-
g per $BW^{0.75}$	7.2	9	-	-	-
Digestibility coefficient					
DM	56.8	65	62.78	61.14	59.10
OM	58.25	66.7	65.86	62.86	61.57
CP	56.4	64	79.42	74.98	67.45
CF	-	-	53.02	57.81	56.93
DOMD[(f)] (g kg^{-1} DM)	520 ± 10.3	613.2	-	-	-
Predicted ME					
(MJ kg^{-1} DM)	8.14	9.6	-	-	-
ME intake (MJ day^{-1})	9.81	15.8	-	-	-
TDN (kg kg^{-1} DM)	-	-	61	59	58

[(a,b)]Both alfalfa were estimated to be at late maturity; c, d, e were estimated to be at pre-bud, bud and mature, respectively; [(f)] DOMD = Digestible Organic Matter in the Dry Matter; [(a,b)]Adapted from Maheri-Sis *et al*. (2007a); [(c,d,e)]Adapted from Tabatabaei *et al*. (2005)

But lowert han HAM hay. High DMD resulted in high DMI of forages. The often stated relationship that increased digestibility results in increased intake is influenced by the residence time of forage in rumen (Thornton *et al*., 1978). Analyzing data from 13 different legume silages, Wilkins *et al*. (1971) concluded there was a significant and positive correlation between voluntary intake and *in vivo* digestibility. DMD, DMI and CPI of HAM hay obtained in this review, were consisted with those reported by Vanzant *et al*. (1998), Martin *et al*. (2000) and Aghajanzadeh-Golshani *et al*. (2007). TDN contents for HAM hay were 61, 59 and 58 kg kg^{-1} DM at pre-bud, bud and mature stages, respectively (Table 3).

IN VITRO GAS PRODUCTION METHODS

The *in vitro* gas production technique also generates kinetic data but rather than measuring the disappearance of dietary components, it measures the appearance of fermentation gases notably CO2, CH4, H2. Compared to the *in situ* degradability technique, gas production methods are less animal dependent, more appropriate for characterizing soluble or small particulate feeds and they can be automated thus reducing the labor input. They can also be used to generate information on rates and extents of digestion, proportions of volatile fermentation products and microbial protein production. However, automated gas production methods are expensive and may or may not handle large numbers of samples. While manual methods are cheap, they are labor intensive, restricted in capacity and they often generate inadequate kinetic data for precise descriptions of fermentation rates. The results generated from both types of equipment are dependent on several procedural details and they are often misunderstood. Table 4 shows the effect of several factors on gas production. In addition to these factors, the results obtained vary with the type of system used whether closed or opened and the source, activity and consistency of the rumen fluid used (Schofield, 2000).

Several models have been proposed for describing kinetic gas production data. Such models vary in complexity from single pool models digesting at a variable fractional rate models to empirical multipool models (France *et al*., 1993). Many of such models contain parameters that have little biological relevance and perhaps more importantly, many are often used with insufficient attention to their appropriateness for describing the fermentation profile of the feed being studied. Yet several reports have emphasized the inadequacy of some models for describing the fermentation of certain feeds (Beuvink and Kogut, 1993; Adesogan *et al*., 1998; Dhanoa *et al*., 2000). The results of gas production experiments are often misinterpreted and used to draw inappropriate deductions. Gas production is often assumed to be directly proportional to substrate digestion and hence nutritive value. This is inaccurate because gas production is dependent on substrate composition, microbial population and hexose utilization for microbial yield. Several authors have shown that less gas is produced from feeds high in propionate precursors relative to that in feeds high in acetate and butyrate precursors (Beuvink and Spoelstra, 1992; Beever and Mould, 2000; Williams, 2000). Others have shown that the ammonia in high protein feeds can decrease gas production by reaction with volatile fatty acids (Schofield, 2000). In spite of its' importance, very few reports have quantified the extent of hexose utilization for microbial biomass production during gas production experiments. All of these factors determine the quantity of gas produced during substrate fermentation. Consequently Beever and Mould (2000) stated that *in vitro* gas production values alone provide little direct information, apart from estimating fermentation rates. Therefore gas production data should be supplemented with measurements of substrate disappearance, volatile fatty acid profiles and microbial yield in order to give comprehensive nutritional information on the feed tested (Schofield, 2000). However, the additional labor and cost implications will continue to limit and perhaps prevent this suggestion from being implemented.

Gas production data for alfalfa varieties during the fermentation period are given in Table 5. The cumulative volume of gas production increased with increasing time of incubation. Gas production from the fermentation of

Table 4: Factors affecting the accuracy of *in vitro* fermentation gas production techniques (Adesogan *et al*., 1998)

Factor	Effect
Sample form	Wilting increases fermentation rate and freeze-drying and milling increases gas production relative to chopped/unchopped fresh forage.
Oven-drying samples	Eliminates volatile constituents from fermented substrates thus reducing the indirect gas produced from their reaction with the buffer.
Buffer composition	High phosphate buffers reduce gas production by utilizing protons that would have been used for CO2 production.
RF inoculum to buffer ratio	When greater than 1:2, blanks no longer truly represent the contribution of the inoculum to gas production.
Size of liquidga interface	Determines the potential for gas supersaturation and solubilisation, which reduces, gas production.
Prevailing pH and temperature	Decreases gas production if below optima for cellulolytic bacteria growth.
Atmospheric pressure	Determines actual gas volumes. Yet it is often omitted such that it is difficult to compare results from different labs.
Stirring	Reduces CO2 supersaturation which causes Erroneous volume/pressure readings.

Table 5: Organic matter digestibility, gas production (ml) and estimated parameters of HAM hay at different incubation times[a]

Time (h)	2	4	6	8	12	24	48	72	96
	18.4	27	45.02	50.3	54.8	64.4	69.8	72.2	73.1
Estimated Parameters									
	a	b	(a + b)		c	OMD		ME	
	0.9	68.7	69.6		0.137	71.2		10.96	

a = the gas production from the immediately soluble fraction (ml); b = the gas production from the immediately insoluble fraction (ml); c = the gas production rate constant for the insoluble fraction (mL h^{-1}); (a + b) = potential gas production (mL); OMD: Organic matter digestibility (% of DM); ME: Metabolisable energy (MJ kg^{-1} DM); [a]Adapted from Mirzaei-Aghsaghali *et al.* (2007b); Maheri-Sis *et al.* (2007b); Safaei *et al.* (2007)

HAM hay was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h *in vitro* gas tests adapted to describe the kinetics of fermentation on the modified exponential model y = a + b [(1-Exp(-ct)], Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) equation was chosen because of the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented (Blummel and Ørskov, 1993; Maheri-Sis *et al.*,2007b, 2008). Cumulative gas production and estimated parameters in HAM hay was comparable to those reported by Kamalak *et al.* (2005).

The OMD and ME contents of HAM were 71.2% and 10.96 MJ kg^{-1} DM, respectively. The decrease in digestibility is due to increase in concentration of cell wall contents (Wilson *et al.*, 1991), lignin content in mature plant (Morrison, 1980) and decrease in leaf/stem ratio (Coblentz *et al.*, 1998). Menk *et al.* (1979) suggested that gas volume at 24 h after incubation has been relationship with metabolisable energy in feedstuffs. Sommart *et al.* (2000) reported that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart *et al.*, 2000). Gas volumes also have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993).

IN SITU DIGESTIBILITY METHODS

The dacron bag technique (Ørskov *et al.*, 1980) for measuring the *in situ* rumen degradability of feeds has received widespread attention partly because it can be readily used in developing countries since it is not reliant on a steady electricity supply andmore importantly because it is one of the few techniques that describes the kinetics of feed degradation in the rumen. The technique has also provided relatively good predictions of forage intake and digestibility (Ørskov, 2000) and has greatly improved the understanding of nitrogen (N) supply to ruminants and their microbes. It now forms the basis of describing N requirements of ruminants in the feeding systems of several countries. Yet the technique is plagued by low reproducibility and repeatability (Noziere and Michalet-Doreau, 2000) and it is notoriously difficult to standardize despite repeated attempts (Madsen and Hvelplund, 1994). Several excellent reviews on the technique (Nocek, 1985; Noziere and Michalet-Doreau, 2000; Ørskov, 2000) indicate that the results obtained vary with sample preparation method, washing and drying procedure, extent and nature of particulate losses, incubation site and sequence, host animal species and diet, bag size, weave type and pore size andremoval of microbial contamination. The effects of some of these factors on degradability are shown in Table 6. These factors have hampered the comparison of results from different experiments. Some of the problems of the technique stem from the methods currently used to characterize incubated substrates. Noziere and Michalet-Doreau (2000) suggested that sample particle sizes should be stated instead of their grinding screen size because ground particles contain an array of particle sizes that differ in chemical composition and rate and extent of degradability.

In addition, the technique may not adequately account for effects of supplementation or antinutritive factors in feeds and it is not appropriate for characterizing soluble and small particulate feeds or single-celled proteins (Ørskov, 2000; Noziere and Michalet-Doreau, 2000). Although, there is widespread use of first order exponential models for characterizing degradability profiles, most of such models erroneously assume that a discrete lag phase occurs before the onset of degradation (Sauvant, 1997) and poorly describe the N degradability profiles of feeds high in soluble N (Givens, 1994). There has also been relatively little validation of the *in situ* degradability measurements with *in vivo* data, such that it is difficult to accept or refute the accuracy of the protein fractions derived from the technique (Beever and Mould, 2000). Attempts to characterize the degradability of starch and NDF with the technique have yielded variable and sometimes conflicting results (Beever and Mould, 2000). The shortcomings of the *in situ* degradability technique highlighted above reflect the need for caution in interpreting the results. However, the technique has advanced our knowledge of protein metabolism in ruminants significantly. In the absence of a valid alternative, it will continue to be a valuable tool for assessing the kinetic parameters of feed degradation.

Table 6: Factors affecting the accuracy of *in situ* rumen degradability techniques (Adesogan *et al*., 1998)

Factor	Effect
Oven drying	Reduces N degradability and solubility.
Freeze drying	Enhances particulate losses but is better than other drying methods for silages.
Grinding / pre-wetting samples	Underestimates the lag phase and overestimates degradation rates due to increased microbial colonization.
Particle size	The lag phase is prolonged with larger particles.
Washing procedure	Machine washing overestimates soluble and particulate losses but is less subjective than hand washing.
Particulate losses	Overestimates rumen soluble and the extent of degradation but can underestimate degradation rates if the particles lost would have degraded rapidly.
Incubation sequence	Reverse sequence incubation can reduce degradation rates due to interruptions and differences in rumen environment of samples incubated for different periods.
Incubation site	Substrate incubation in the dorsal rumen sac underestimates degradability due to lower colonization rates than those in the ventral sac.
Bag pore size	If < 15im can reduce degradation by restricting microbial colonization and diversity and trapping fermentation gases. If > 40 μm, causes losses of insoluble / undegradable particles.
Bag weave type	Unlike multifilamentous cloth, the pores of monofilamentous cloth are prone to stress-induced distortion that can enhance particulate losses.
Microbial contamination of residues	Underestimates N degradation in low N feeds. Removal methods can be expensive, laborious or inaccurate.

Table 7: *In situ* DM, OM and CP degradation characteristics of the alfalfa hays by sheep[(a)]

	(% of DM)				Effective degradability[3]				
Component an Alfalfa vareities[1]	A[2]	B[2]	C[2]	Extent[2]	Lag time (h)	2% h^{-1}	5% h^{-1}	8% h^{-1}	$K_{d(h)}$
DM									
KAR	32.2	34.3	33.5	66.5	1	60.53	54.2	50.13	0.091
HAM	34.43	38	27.5	72.43*	0.8	64	56.97	52.4	0.091
OM									
KAR	30.8	35	34.2	65.8	0.4	58.7	52.16	48.06	0.085
HAM	32.4	35.1	32.5	67.5	0.83	61.23	54.9	50.73	0.1
CP									
KAR	22.45	29.2	48.3	51.65	0.25	45.5	40.1	36.7	0.081
HAM	21.7	44.1	34.2	65.8	1.55	49.6	39.25	34.2	0.038

[1]KAR = Kareyonge, HAM= Hamedani; [2]A = immediately soluble fraction, B= Fraction degradable at a measurable rate, C= undegraded fraction andmaximum extent = 100-C; [3]Effective degradability at three ruminal passage rates; [(a)]Adapted from Mirzaei-Aghsaghali *et al*. (2008)

Table 8: Effective degradability (%), UDP and ERDP (g/kg DM) CP of KAR and HAM hays with different outflow rates[(a)]

	ED[1] (%)			UDP[2] (g kg^{-1} DM)			ERDP[3] (g kg^{-1} DM)		
	2% h^{-1}	5% h^{-1}	8% h^{-1}	2% h^{-1}	5% h^{-1}	8% h^{-1}	2% h^{-1}	5% h^{-1}	8% h^{-1}
KAR	45.5	40.1	36.7	40	46.82	51.07	79.4	72.57	68.32
HAM	49.6	39.25	34.2	45.3	61.7	69.7	105.8	89.4	81.4

[1]Effective degradability (ED), [2]Undegradable protein (UDP) and [3]Effective rumen degradable of protein (ERDP) are calculated using the equations of AFRC (1992). [(a)]Adapted from Mirzaei-Aghsaghali *et al*. (2008)

Studies have shown that vegetation, soil type and climate might dramatically affect the utilization of nutrients by animals (Kaya *et al*., 2004). Effective degradability values at 3 ruminal passage rates are in Table 7. Table 8 show Effective degradability (%), UDP and ERDP (g kg^{-1} DM) CP of KAR and HAM hays with different outflow rates.

The percentage of water soluble fraction (fraction a) and potentially digestible dry matter fraction (fraction b) were similar andindigestible dry matter fraction (fraction c) was greater in KAR hay compared with HAM hay (Table 7). The disappearance of DM, OM and CP increased with time of incubation in the rumen. 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).

Karsli *et al*. (2002) found that mean values of OM were 28.4, 43.3 and 28.3, respectively for alfalfa hay. The values obtained in the correct study for HAM and KAR hays were also in line with those of Karsli and Russell (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 Michalet-Dorean and Ould-Ban (1992) and Elizalde *et al*. (1999) might be due in chemical compositions, CP extent and harvested time.

Lag time (which indicates the time required for initiation of degradation) for degradation of CP was higher 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).

RELATIVE FEED VALUE, QUALITY INDEX, RELATIVE FORAGE QUALITY

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 hemicelluloses 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.

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

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

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

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

Table 9: Legume, grass and legume-grass mixture quality standards[a]

Quality standard	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

[a]Adapted From Canbolat *et al*., 2006

DMD = Dry matter digestibility, ADF = Acid-detergent fiber, DMI = Dry matter intake, RFV = Relative feed value.

Forages with values greater than 100 are of higher quality (Table 9). Dairy producer with high producing cows often require 150 or greater RFV value (Schroeder, 2004).

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).

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

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 9). 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, 2002b).

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

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

The effect of alfalfa varieties on forage quality are presented in Table 10. Undersander and Moore (2004) reported that RFQ will become the standard test for evaluating forages throughout the country and that it eventually will be 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).

Table 10: Effect of alfalfa varieties on RFV, RFQ, TDN and voluntary intake and forage quality[1]

Forage	RFV[a]	RFQ[b]	TDN intake[c] (% of BW)	Voluntary Intake[d] (% of BW)	Qi[e]
HAM	142.4	148.6	2.04	2.78	1.92
KAR	118.6	123.1	1.82	2.44	1.7

[a]Relative Feed Value; [b]Relative Forage Quality; [c]Total digestible nutrients; [d]Intake of dry matter expressed as percentage of body weight; [e]Voluntary TDN intake relative to maintenance requirement; 1.0 = maintenance; [1]Adapted from Mirzaei-Aghsaghali *et al.* (2007a)

MICROBIAL PROTEIN SYNTHESIS

It is well known that in ruminants, endogenous urea is partly recycled in the forestomech. This process is nutritionally advantageous for ruminants because ruminal bacteria are able to use urea nitrogen to synthesize proteins that will be absorbed in the small intestine (Cirio and Boivin, 1990). Microbial protein synthesis is important in ruminants because microbial protein synthesized in the rumen provides from 50% to nearly all amino acids required for beef cattle depending on the undegraded crude protein concentration of the diet. Synthesis of microbial protein and growth of ruminal microbes depends on adequate energy (ATP), resulting from fermentation of organic matter in the rumen and N resulting from degradation of non-protein and protein nitrogen sources. Other nutrients such as, sulfur, phosphorus andother minerals and vitamins are also required for microbial protein synthesis. It is estimated that between 40-80% of the total flow of protein reaching the intestine is of microbial origin (Sniffen *et al*., 1987; Clark *et al*., 1992; McDonald *et al*., 1995; NRC, 1996).

A widely used method for the estimation of microbial protein production requires ruminal and duodenal cannulas and microbial and digesta flow markers. However, the microbial protein entering the duodenum can be estimated by quantification of urinary allantoin. The nucleic acids synthesized by rumen micro-organisms are enzymatically degraded to purine and pyrimidine bases which are absorbed; their final products are excreted in the urine with allantoin being in the greatest proportion (Condon and Hatfield, 1970; Faichney *et al*., 1975; Macrae, 1975; Puchala and Kulasek 1992). Topps and Elliott (1965) were among the earliest investigators to suggest that urinary allantoin and uric acid excretion rates reflect the amount of microbial protein flowing into the small intestine. Later, several authors have confirmed that urinary purine derivatives (PD) can be an accurate index of rumen microbial protein flowing into the small intestine (McAllen and Smith, 1973; McAllen, 1980; Razzaque *et al*., 1981). Giesecke *et al.* (1984) suggested the use of purine derivatives as an effective measure for rumen microbial growth, when they found a significant relationship between the amounts of purine metabolites excreted in urine in sheep, maintained by intragastric infusions. Chen *et al.* (1990) have shown that purine derivatives (allantoin, xanthine, hypoxanthine and uric acid) could be used to estimate the supply of microbial protein from the rumen to the intestine.

The ruminal OM digestion of Lucerne was limited as a result of the high rumen outflow rates. Probably two other mechanisms were also used to deliver energy for the yield of microbial N. At first N was not only used as protein source but also at energy source. Secondly the high outflow rates were favorable for the escape of microbes from the rumen low ruminal retention time of microbes decreases the intra-ruminal recycling of microbes by reducing bacterial breakdown and protozoal engulfment (Leng and Nolan, 1984).

The microbial nitrogen (MN) supplies as calculated from Digestible Organic Matter Fermented in the Rumen (DOMR) were from 15.1-22.8 g N d^{-1}. The value of MN in HAM hay (22.8) was close to the value (18.2) obtained from the Gosselink (2004) study. Moreover, EMNS values were 32 g N kg^{-1} DOMR for both forages and lower than that reported by Gosselink (2004). This difference may be due to the maturity (late maturity in our experiment vs. first cut and beginning of flowering in the experiment of Gosselink) and technique used in experiment.

In literature, similar trends in the efficiency of microbial N synthesis in the rumen with Lucerne with different presentation forms are found (Gosselink, 2004). High efficiency (>45 g microbial N kg^{-1} OM apparently digested in the rumen) were found with Lucerne hay in steers (Elizalde *et al*., 1999) andwith fresh Lucerne in lambs in combination with high outflow rates of Ru and Cr, respectively 12.0 and 17.7% h^{-1} (Cruickshank *et al*., 1992). Merchen and Satter (1983) found a higher efficiency for Lucerne hay than for Lucerne silage.

Numerous studies have been conducted to determine microbial protein synthesis in the rumen under various conditions (Clark *et al*., 1992; Beever and Cottrill, 1994). The efficiency of microbial protein synthesis varies significantly among studies. Some of these variations were attributed to the techniques used in these experiments. But there are other factors that caused differences in microbial protein synthesis among the studies. These factors include nitrogen concentrations, nitrogen sources, rates of nitrogen and carbohydrate degradation, carbohydrate sources, the ratio of forage to concentrate in the diets, dry matter intake andsynchronization of nitrogen and simultaneous release of energy. Other factors such as rates of solid and liquid passage and dietary sulfur concentrations must also be considered (Rode *et al*., 1985; Hoover *et al*., 1991).

Table 11: Estimated digestible organic matter fermented in the rumen, microbial N yield, efficiency of microbial N supply and urinary excretion of purine derivatives in sheep fed at ad libitum[1]

	HAM	KAR
[2]DOMD (g kg^{-1} DM)	613.2	520
DOMR (kg d^{-1})	0.713	0.473
MN (g d^{-1})	22.8	15.1
EMNS (g N kg^{-1} DOMR)	32	32
P_a (mmol d^{-1})	31.6	20.8
PD_e (mmol d^{-1})	28.5	19.5
A_e (mmol d^{-1})	24.2	16.5
UA_e (mmol d^{-1})	4.27	2.93

[1]Mirza-Aghazadeh *et al.* (2007); [2]Adapted from Maheri-sis *et al.* (2007a).\; DOMD = Digestible Organic Matter in Dry matter; DOMR = Digestible Organic Matter Fermented in the Rumen; MN = Microbial N yield; EMNS = Efficiency of Microbial Protein Supply; P_a = Purine absorbed; PD_e = Purine Derivatives excretion; A_e = Allantoin excretion; UA_e = Uric Acid excretionMay 30, 2008

The individual values for daily absorption and excretion of purine derivatives (A_e and UA_e) and Chemical structures and the enzymes involved in the conversion of the derivatives are shown in Table 11. Purine absorbed (P_a) ranged from 20.8-31.6. The Pd_e ranged from 19.5-28.5 mmol d^{-1}. The PD_e concentrations in the present experiment were in a greet agreement with PD_e values of alfalfa hay reported by Belenguer *et al.* (2002). This difference may be due to the feeding level and animal species (ad libitum vs. maintenance level and sheep vs. goat in our experiment and in the experiment of Belenguer; respectively). A close relationship was found between urinary excretion of purine derivatives and duodenal supply of purines, as previously in other species as sheep (Chen *et al.*, 1990; Balcells *et al.*, 1993) or cattle (Colucci *et al.*, 1984). The A_e and UA_e contens of Ham hay were higher than that KAR hay (Table 11). Allantoin and other purine derivatives are degraded by rumen bacteria (McAllen and Smith, 1973) and N could be used for microbial synthesis (Belasco, 1954).

CONCLUSION

This review has emphasized nutritional value of alfalfa in Iran. In overall conclusion, results of this review showed that Legumes are a protein source in ruminant nutrition. These home-grown feeds make farmers less dependent from the purchase of other protein sources. This is an advantage for the farm economy and ecology, particularly because of restrictions concerning the environment. Alfalfa hay is valued by nutritionists for its relatively high energy value which supports milk production, its rapid ruminal digestion of structural fiber which stimulates feed intake, its coarse structural fiber that stimulates ruminative chewing and salivation, which results in rumen buffering, its structural fiber which has high buffering capacity, its high protein level which supports animal protein needs andthe relatively high proportion of its protein that escapes the rumen undegraded which minimizes dietary requirements for high cost protein supplements (Martin *et al.*, 2003). Alfalfa use by dairy cattle has decreased in recent years because of excessive non-protein nitrogen and low fiber digestibility.

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