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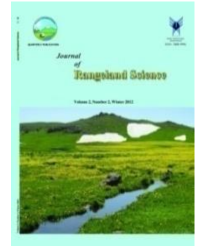

Full Length Article:

Determination of Nutritional Value in Three Forage Species in Three Phonological Stages in Sabalan Rangelands, Ardebil, Iran

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Abstract. In order to estimate the nutrient value of three dominant range species; *Festuca ovina*, *Trifolium montanum* and *Alopecurus textilis* via proximate analyses and gas production technique, samples were collected in three stages during grazing period from 26^{th} May to 1^{th} September in six areas of Sabalan Mountain in North West of Iran. Results showed that *Trifolium montanum* as a legume had higher values for Crude Protein (CP), total ASH, Organic Matter Digestibility (OMD), Metabolizable Energy (ME) and gas production at 24 hours and showed lower values for ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) as compared to two other species that were considered as gramineae. Variation of quality traits in *Trifolium montanum* was lower than two other species. The obtained values of CP, NDF, ADF, ASH, EE, OMD, gas production and Dry Matter (DM) for *Trifolium montanum* were 18.65%, 41.83%, 26.97%, 8.95%, 0.26%, 64.50% g/kg and 50.96 ml/200mg, respectively. For *Festuca ovina*, they were 9.37%, 68.63%, 36.87%, 6.77%, 0.58%, 53.82% and 40.04 ml/200mg DM and for *Alopecurus textilis*, they obtained 5.01%, 73.03%, 42.18%, 5.97%, 0.56%, 55.63 % and 35.46 ml/200mg DM, respectively. The values of ME prediction that were calculated via Menke equation for *Trifolium montanum*, *Festuca ovina* and *Alopecurus textilis* were 2.31, 1.93 and 1.75 Mcal/kg DM, respectively. The mean level of predicted CP, ME and OMD for plants, especially in grasses (*Festuca ovina* and *Alopecurus textilis*) throughout the year, second and third stages of sampling (mid and late summer) and comparing the nutrient requirements of Moghanian sheep (native breed in Ardabil province) with these data show that only at early stage of sampling (late spring and early summer), one can supply sufficient nutrient for domestics whereas the ewes are pregnant at mid and end of summer and these nutrients are insufficient for them therefore, we have recommended the use of supplementary feeding in diets of grazing ewes for ideal productivity performance.

Key words: Quality traits, Gas production, *Trifolium montanum*, *Festuca ovina*, *Alopecurus textilis*.

Introduction

In many traditional stock framings of ruminants such as Sabalan rangelands in north west of Iran, fresh forage represents the main component of diets for ruminants during the spring and summer seasons. Knowledge of nutritive value of forages is more important for planning the forage utilization in grazing seasons and developing an optimal feeding regime for ruminants. Furthermore, the optimization of feeding strategy is essential to control the forage wastage and minimize the environmental effects. The chemical composition and digestibility of fresh forage can be estimated on the basis of herbage species, mix samples of forages, maturity stage, climatic conditions and fertilizer application (Adesogan, 2002). In order to facilitate the production efficiency, small and large herds are assembled during the grazing seasons in Sabalan rangelands. Sabalan rangelands are located in North West of Iran in Ardabil province with temperate weather in spring and summer and cold weather in fall and winter. The Sabalan Mountain contains approximately 471 plant species in 200,000 Km^2. The quality of forages affected by the maturity stage has been intensively studied throughout the history of animal science because of its importance to the biological and economical performance of ruminant based production systems. There are some In vitro techniques available to evaluate the nutritive value of feeds at relatively low cost. The use of In vitro gas method to estimate the digestion of feed is based on the measured relationships between the In vivo digestibility of feeds and In vitro gas production in combination with the feed's chemical composition (Menke and Steingass, 1988). The In vitro gas production technique developed by Menke *et al.*, (1979) is a very useful tool for the rapid screening of feeds to assess their potential as energy sources for ruminant animals (Blummel and Becker, 1997) assuming that the volume of produced gas reflects the end result of the substrate fermentation to Short Chain Fatty Acids (SCFA), microbial biomass and the neutralization of SCFA. This technique has been used by Blummel and Qrskov (1993) to determine gas production at several incubation times. The obtained values could describe the pattern of feed fermentation using the model proposed by Larbi *et al.*, (1996). The gas produced from incubating 200 mg of food dry matter during 24 hours can be used with the concentration of Crude Protein (CP) and ash to estimate the Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) (Menke *et al.*, 1979). In vitro gas production technique has been found to predict the nutritive value of forages reliably (Makkar *et al.*, 1997). Blummel and Ørskov (1993) reported high positive correlation between In vitro gas production and degradability values of dry matter at various incubation periods (r=0.95 to 0.97). The objective of this study was to estimate the In vitro gas production of three indigenous and dominant plants namely *Festuca ovina*, *Alopecorus textilis* (as grasses) and *Trifolium montanum* (as legume) with ascertaining their nutritive value, determining the effect of grazing stages and growing period on chemical composition, gas production, OMD and ME and calculating the correlation coefficients among chemical composition, sampling stage and gas production rate (at 24 hour incubation) of these plants.

Materials and Methods

Study area

This study was conducted in west of Ardabil province around Sabalan Mountain at an elevation of 1800 to 3800 m above sea level. The extent of study area was 200000 Km^2 with 471 plant species. Mean annual rainfall is 500 mm. The biomass production of study area is ranged from 250 in Shrublands to 750 kg/ha in Forbs and Grasslands. This area is consisted of 60-70 percents of grasses, 15-25 percents of forbs and the rest consisted of other plants such as shrubs. The predominant vegetative cover was *Festuca ovina*. Studied plants in this assay are dominant plant types in Sabalan rangelands.

Sampling and Experimental Analyses

Samples were collected from forb (*Trifolium montanum*) and grasses (*Festuca ovina* and *Alopecurus textilis*) of Sabalan rangeland. They were clipped manually with special scissors at three grazing stages in 25th May (flowering stage), 13th July (seeding stage) and 27th August (ripening stage) in 2006 from study area and finally, 45 main samples were collected. All samples were ground through a 1 mm screen for In vitro gas production technique, incubation and chemical analysis. Dry Matter (DM), Crude Protein (CP), Ether Extract (EE) and ash content of samples were determined (AOAC, 1990). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were examined using the method proposed by Van Soest *et al.,* (1994). Rumen fluid inoculum was collected from the animal before the morning feeding under vacuum pressure via the rumen fistula into a 2 liter glass flask and into two pre-warmed 1 litter thermos flasks which were then transported to the laboratory. The medium preparation was described by Makkar *et al.,* (1997). Mixed rumen fluid inoculums were obtained from two fistulated taleshian native steers (weighed 240±10 kg). The animals were fed twice a day. Water and a mineral lick were available *in libitum*. The feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 50 ml serum bottle (Sommart *et al.,* 2000). The bottles were pre-warmed in a hot air oven at 39°C for about 1 h prior to the injection of 40 ml of rumen fluid medium (using a 60 ml syringe) to each bottle. The bottles were closed with rubber stoppers, sealed and incubated in a hot air oven at 39°C. The rate of gas production was measured by reading and recording the gas volume after incubation using a 20 ml glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 2 to 96 h after incubation periods. Amounts of cumulative gas volume at 2, 4, 6, 12, 24, 48, 72 and 96 h after incubations were fitted using the equation $Y = a+b [(1-Exp(-ct)]$ (Ørskov and McDonald, 1979) where a is the intercept which ideally reflects the fermentation of soluble fraction, b is the fermentation of insoluble fraction, c is the rate of gas production, (a+b) is potential extent of gas production and Y is gas production at time ‘t’. In vitro digestibility of organic matter was measured at 24 h after incubation as OMD =14.88 + 0.889 GP + 0.45 CP + 0.0651 XA. The ME will be calculated as ME, MJ DM =2.20 + 0.136 GP + 0.057 CP + 0.0029 CP^2 where GP is gas volume at 24 h and CP is the percentage of crude protein in feedstuffs.

Statistical Analyses

All data obtained from the trials were subjected to the analysis of variance procedure of SAS, 2001. Means were separated by Duncan Multiple Range Test (DMRT). The level of significance was determined at $P<0.05$. In vitro gas production data were fitted to the asymptote exponential model using Naway-Excel computer program (Macaulay Institute, Aberdeen, UK). Analysis of variance was carried out on chemical composition, In vitro gas production, organic matter digestibility and ME contents using the GLM procedure.

Results

Chemical Composition of Selected Plants

The chemical compositions of selected plants are presented in (Table 1). The crude protein content of the plants is ranged from 8.01 in *Alopecurus textilis* to 18.65% in *Trifolium montanum*.

The CP content of *Trifolium montanum* was considerably higher than two other species. There is not enough information available in terms of chemical composition for *Trifolium montanum* and *Alopecurus textilis* but there are some accessible data for *Festuca ovina*. There was considerable variation between forage samples for *Trifolium montanum* as compared to forage samples for two other species in terms of some chemical composition. Cell wall contents (NDF and ADF) which represent the most important fraction of dry matter for all forages are ranged from 41.83 to 73.03 and 26.97 to 42.18, respectively. Ash content of forages is ranged from 5.97 to 8.95%. *Trifolium montanum* had the highest ash content while *Alopecurus textilis* had the lowest. Significant differences in some chemical compositions were observed in the forages among three stages of maturity (grazing stage) in this experiment (Tables 2, 3 & 4).

Tables 2, 3 and 4 show that differences in chemical composition among three grazing stages in *Trifolium montanum* (as a Forb, Legume) were lower than two other species (as Gramineae). The CP, ADF and NDF contents in *Trifolium montanum* did not show substantial variation but in grasses, there was significance variation among the grazing stages ($P<0.05$).

Gas Production Characteristics and Prediction of ME and OMD

Chemical analysis is essential for understanding the nutritional potential of forages but it is not sufficient. Data of gas production during the fermentation period, ME and OMD are given in (Table 5). Gas production from the fermentation of forages was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. A comparison of gas production characteristics and estimated parameters of treatments indicated some significant differences among them except a and b intercepts ($P<0.05$). The values of *a* intercept for all samples were negative at the incubation times in this study.

The results indicate that cumulative gas volumes at all incubations were significantly different ($P<0.05$) between forages. Curves of cumulative gas production for each treatment are presented in (Fig. 1). Study of correlation between chemical composition and gas test parameters at 24 hours showed some significance differences almost in all cases.

Discussion

Chemical Composition of Selected Plants

There are many factors that affect CP content such as stage of growth (Promkot and Wanapat, 2004), maturity, species or variety (Von Keyserlingk *et al.,* 1996), soil types (Baloyi *et al.,* 1997) and high leaf-stem ratio of *Trifolium montanum*. These results are in agreement with the suggestion of Jerry *et al.*, (1989). And also, there are many factors that may affect fibrous (NDF and ADF) content such as growth stage (Promkot and Wanapat, 2004), maturity and species or variety (Agbagla-Dohnani *et al*., 2001; Von Keyserlingk *et al.*,1996), drying methods, growth environment (Mupangwa *et al*., 1997) and soil types (Thu and Preston, 1999). The difference of ash content was probably due to the species. Turhan *et al.* (1997) pointed out that the ash content average of *Festuca ovina* was 16.22%. The variation of present study is in agreement with the studies carried out by Givens *et al.*, (1990) indicating that chemical composition, DM digestibility and energy value of forages varied significantly according to the herbage variety, growth stage, year of harvest and vertical belts. Daalkhaijav (2000) showed that they tend to become more mature and their nutritive values generally decrease as the plant age is increased. Differences among grasses and legume in terms of chemical composition at three stages of maturity are due to high leaf-stem ratio of *Trifolium montanum* and a low decrease in leaf chemical compositions. Regardless of maturity stage, leaves were always more digestible than stems confirming Lambert *et al.*, (1989). It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignification as lignin is an indigestible fraction and acts as a barrier that limits the access of microbial enzymes to the structural polysaccharides of the cell wall. Ammar (2002) reported that NDF, ADF and lignin were significant and negatively correlated with In vitro digestibility.

Gas Production Characteristics and Prediction of ME and OMD

These data suggested that a lag phase may occur in the early state of incubation due to the delay in microbial colonization of substrate. Several authors (Blummel and Ørskov, 1993; Khazaal *et al.*,1995) have also reported negative values with various substrates while using mathematical models to fit gas production kinetics. This is because of a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to the microbial colonization. It is well known that the value for absolute a (|a|) that is described ideally reflects the fermentation of the soluble fraction. The soluble fraction could be easily attached by microorganisms in the rumen and increase the gas production (Table 5). In this study, the absolute a was the highest for *Trifolium montanum*. The gas volume at asymptote (b) described the fermentation of the insoluble fraction that in *Trifolium montanum*, is lower than two others. This result may reflect the proportion of their fiber components and protein contents. Menke *et al.*, (1979) suggested that gas volume at 24 hours after incubation has a relationship with metabolizable energy in feedstuffs. Sommart *et al.*, (2000) suggested that gas volume is a good parameter to predict the digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the In vitro system. Additionally, In vitro OMD was shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003). These data suggested that a lag phase may occur in the early state of incubation due to the delay in microbial colonization of the substrate. Several authors (Blummel and Ørskov, 1993; Khazaal *et al.*, 1995) have also reported negative values with various substrates while using the mathematical models to fit gas production kinetics. This is due to a deviation from the exponential cause of fermentation or delays in the onset of fermentation because of microbial colonization. It is well known that the value of absolute a (|a|) described ideally reflects the fermentation of soluble fraction. The soluble fraction could be easily attached by microorganisms in the rumen and increase gas production (Table 5). In this study, the absolute a was the highest for *Trifolium montanum*. The gas volume at asymptote (b) described the fermentation of the insoluble fraction that is lower than two others in *Trifolium montanum*. This result might reflect the proportion of their fiber components and protein contents. Menke *et al.*, (1979) suggested that gas volume at 24 h after incubation has a relationship with metabolizable energy in feedstuffs. Sommart *et al.*, (2000) suggested that gas volume is a good parameter to predict the digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the In vitro system. Additionally, In vitro organic matter digestibility was shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003).

Table 1. Chemical Composition of Plants (DM basis)

parameters	*A. textilis*		*T. montanum*		*F. ovina*		Sig.
	Mean	SD	Mean	SD	Mean	SD	
DM	95.86	0.24	95.48	0.49	95.92	0.22	ns
CP	b 8.01	3.47	a 18.65	2.8	b 9.37	2.59	*
NDF	a 73.03	7.43	b 41.83	7.9	a 68.63	5.59	*
ADF	a 42.18	8.03	b 26.97	5.1	a 36.87	5.2	*
ASH	b 5.97	1.55	a 8.95	1.4	b 6.77	1.7	*
EE	0.77	0.4	1.04	0.26	1.09	0.48	ns

Means in rows with no common letters (a–d) differ significantly (p < 0.05).

Table 2. Effect of sampling stage on chemical composition of *Festuca ovina*

Parameters	Stage 1		Stage 2		Stage 3		Sig.
	X	SD	X	SD	X	SD	
DM	96.2a	0.22	95.82b	0.25	95.76b	0.14	*
CP	11.6a	2.27	8.75b	1.77	7.66b	1.4	*
NDF	61.88b	2.5	71.98a	5.32	72.02a	3.2	*
ADF	31.14b	2	37.31a	4.12	42.14a	3.08	*
ASH	8.27a	1.17	6.27b	3.22	5.75b	1.55	*
EE	1.47a	0.26	0.98 ab	0.53	0.79b	0.29	*

Means in rows with no common letters (a–d) differ significantly (p < 0.05).

Table 3. Effect of sampling stage on chemical composition of *Alopecurus textilis*

Parameters	Stage 1		Stage 2		Stage 3		Sig.
	X	SD	X	SD	X	SD	
DM	96.02a	0.8	95.97a	0.57	95.52b	0.57	*
CP	10.11a	1.7	8.41ab	5.2	4.64b	0.32	*
NDF	65.37b	5.1	75.40a	5.8	78.65a	1.33	*
ADF	35.5b	7.18	46.9ab	5.65	47.5a	3.53	*
ASH	6.7	1.3	5.45	2.16	5.4	1.55	ns
EE	0.89	0.43	0.79	0.66	0.57	0.31	ns

Means in rows with no common letters (a–d) differ significantly (p < 0.05).

Table 4. Effect of sampling stage on chemical composition of *Trifolium montanum*

Parameters	Stage 1		Stage 2		Stage3		Sig.
	X	SD	X	SD	X	SD	
DM	95.77	0.33	95.1	0.45	95.23	0.77	ns
CP	20.03	2.77	17.09	2.61	16.52	3.81	ns
NDF	35.8b	5.77	48.43a	6.1	48.67a	7.07	*
ADF	23.3	3.74	31.61	3.95	30.33	5.37	ns
ASH	9.87	0.8	7.7	1.41	8.13	2.11	ns
EE	1.1	0.21	1	0.19	0.97	0.5	ns

Means in rows with no common letters (a–d) differ significantly (p < 0.05).

Table 5. In vitro gas production at incubation time (ml/g DM), predicted Metabolizable Energy (ME) (MJ/kg DM) and organic mater of digestibility content of forages

	A. textilis		*T. montanum*		*F.ovina*		
Incubation time	Mean	SD	Mean	SD	Mean	SD	Sig.
2	3.84 b	0.69	5.99 a	0.43	4.62 b	0.53	*
4	5.74 b	2.2	10.31 a	0.84	6.42 b	1.75	*
6	7.85 b	3.66	17.56 a	3.14	8.44 b	2.75	*
8	10.09 b	5.3	25.87 a	6.0	11.22 b	3.84	*
12	16.29 b	8.0	34.90 a	6.7	17.25 b	5.39	*
24	35.46 b	9.57	50.96 a	7.05	40.04 b	5.63	*
48	49.24 b	7.73	58.15 a	4.21	56.76 a	2.45	*
72	57.53 b	7.32	61.43 ab	7.35	64.48 a	3.98	*
96	67.91 b	10.92	66.05 ab	11.38	74.99 a	10.09	*
Parameters							
C	0.042 b	0.014	0.069 a	0.014	0.03 b	0.01	*
$\lvert a \rvert$	2.26	0.78	4.09	1.48	2.7	0.62	ns
A	2.26	-	4.09	-	2.7-	-	ns
B	78.2 ab	9.47	67.3 b	1.7	84.62 a	7.27	*
$\lvert a+ b \rvert$	80.5 ab	9.8	71.4 b	3.15	86.77 a	7.25	*
a+ b	75.97 ab	-	63.23 b	-	81.37 a	-	*
ME	1.75 b	0.31	2.31 a	0.13	1.93 b	0.2	*

Means in rows with no common letters (a–d) differ significantly ($p < 0.05$).

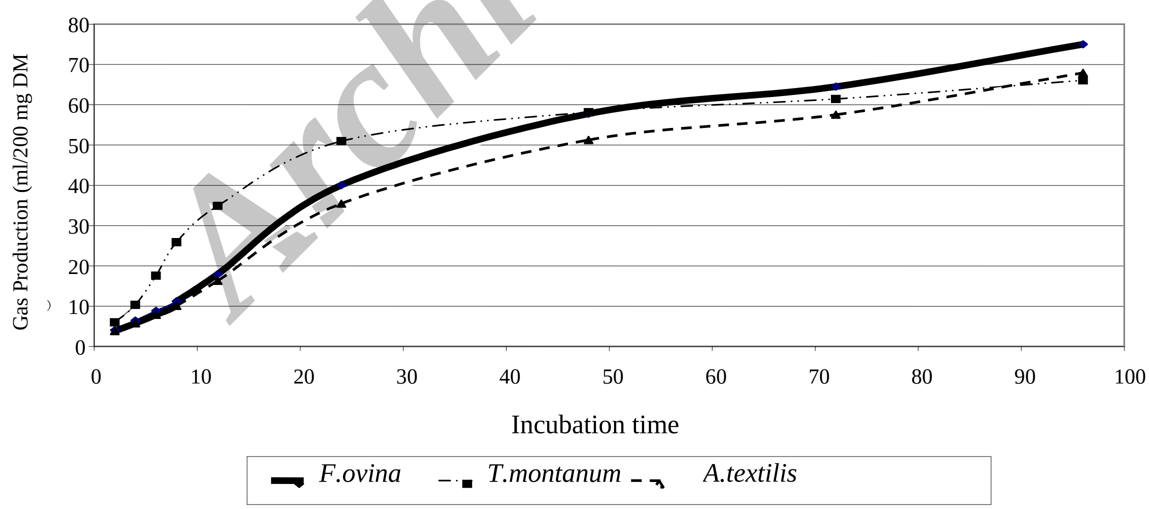

Fig. 1. Cumulative gas production of plants at different times of incubation time

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