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Determination of Nutritive Value of *Poa trivialis* Using *in vitro* Methods, Gas Production and Nylon Bag

Research Article

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ABSTRACT

This study was conducted to determine the chemical composition, metabolizable energy, digestibility and ability of gas production of *Poa trivialis*. Samples was collected at three phenological stages including: vegetative growth, flowering and seedling on two different elevations of 1300 to 1500 m and 2000 to 1800 m asl in Neor and Hir region as the first and second sites, respectively. Testing of gas production was performed using Semi-automatic gas producers machine WT-Binder 87532 Model (made in Germany). Fistulated castrated male sheeps were used for preparation of required rumen fluid. Results show that more crude protein and less cell wall were in the vegetative growth stage. Gas production was higher in the first stage in comparison with other growing stages. The amounts of gas production of soluble and insoluble structures at the first and second sites were 75.63 and 81.50 mL, respectively. Metabolizabe energy of *Poa triviali* was 2.38 in the first site and in the second site 2.48 Mcal/kg DM in the vegetative growth stage. Collected samples from the second site had more metabolizabe energy, digestibility, degradability and gas production in comparison with the results of the first site.

KEY WORDS degradability, digestibility, metabolizabe energy, phenological growth stages, *Poa trivialis*.

INTRODUCTION

Identification and evaluation of nutrients and recognition of livestock requirements are two important factors to ensure maximum production with minimum cost, and they are the farm management priorities. The proper nutrition management of ruminants requires that nutritional value of each food and gradients for them are determined correctly according to the standard procedures. Thus, determination of rangeland plants nutritive value, which is one of the main nutritional sources of livestock, has particular importance (Ghourchi, 1996; Mirzaei *et al*. 2014). Rangeland plants are important source of forage for livestock; however there is little information about the nutritional value of those, especially in Ardabil province (Ghanbari, 2008; Eshghi *et al*. 2013). Lack of sufficient information from the nutritional value of rangeland forages has created limitation for the optimum feed use of livestock and increasing production efficiency. On the other hand, factors affecting the nutritional value of rangeland forage are including species, stage of growth, soil type, climate, etc. Plant growth stage at the harvest time can be an affective factor on forage quality more than any other factors (Tabatabaei, 2006). Because of that almost two thirds of the production cost is livestock feed costs, thus nutrition has a major role in economic efficiency and animal performances. However, due to the lack of animal protein and needing to increase the animal production using this forage resources, it is essential to have available information of nutritional value of feed sources (Amirkhani, 2008). *Poa trivialis* (Rough Meadow Grass) is distributed in Europe, north Africa and Asia (Davis, 1985). It is one of those rangeland plants, which is distributed on Golestan, Mazandaran, Gilan, East Azerbaijan, Fars, Hamadan, Kerman, Khorasan, Qazvin, Tehran and Ardabil provinces on 100 m to 3400 m asl (Bor, 1970). It is also distributed on different Ardabil county's rangelands and as Budd (1969) reported it has high quality as the forage for livestock. As it is one of the main rangeland plants of Iran and according to the national plan to study the main rangeland plants from the nutritional value perspective, this species was selected for this study. Thus, determination of nutritional value of rangeland forages is necessary for grazing and livestock production management. In order to generate information for use in livestock and rangeland management, the aim of this study was to determine the nutritional value and metabolizable energy of *Poa trivialis*. at three phenological stages on two elevation classes of Neor region in Ardebil province.

MATERIALS AND METHODS

Characteristics of the selected sites and specimen sample collection

Specimens sample were collected in the Nour region at the elevation of 1300 to 1500 m asl as the first site (48˚; 27 'E and 38˚; 03 'N). According to the meteorological stations of Ardabil, Kourayim and Fouladlou, the average annual rainfall and average temperature were 370 mm and 9 ˚C, respectively. The second site was selected at the elevation of 1800 to 2000 m asl in the following coordinates (48˚; 30 'Eand 38˚; 02 'N). According to the meteorological stations, average annual rainfall is about 480 mm and the average temperature is 8 ˚C. In general, Neor region climate is semi-humid and cold. Soil texture is loamy-clay and depth is moderate to high (Fertile soil). Dominant vegetation type is shrub, forbs and grasses including: *Achillea talagonica*, *Bromus tomentellus* Boiss., *Artemisia fragrans*, *Dactylis glomerata*, *Kochia prostrate*, *Astragalus pinetorum*, *Cousinia pinarocephala*, *Poa bulbosa*, *Vicia canescens*, *Dianthus orientalis* and *Gundelia tournefortii*, which are distributed on site one, and on site two species such as *Achillea talagonica*, *Agropyron tauri*, *Alopecurus textails*, *Anchusa italic*, *Artemisia aucheri*, *Astragalus aegobromus*, *Astragalus angustifolius*, *Astragalus lisaricus*, *Astragalus persicus*, *Astragalus pinetorum*, *Bromus tomentellus*, *Lathyrus pratensis*, *Onobrychis cornuta*, *Festuca ovina*, *Papaver fugax*, *Poa pratensis*, *Potentilla argentea*, *Trifolium repens*, *Thymus kotschyanus* and *Vicia ervilia* are distributed. Samples of *Poa trivialis* were collected in three phenological stages of vegetative growth, flowering and seedling stages from two elevation classes. According to the size of *Poa trivialis*, in each growing stage minimum of at least 1000 of that were collected.

Experiments and analysis

Processes of experiment were conducted in the Physiology and Animal Nutrition Laboratories at the University of Mohaghegh Ardabili and Animal Science and Research Institute of Iran-Karaj. Chemical analysis of *Poa trivialis* samples were performed using proposed AOAC (2000) methods. All measurements in this section are based on 100% dry matter with three replications. Testing of gas production was performed using semi-automatic gas producers machine WT-Binder 87532 Model (made in Germany). In this device, there are syringes with glass piston syringe. Feed mill samples are placed in the syringes and gas production is measured at different times (Menke, 1988). The samples were milled with 1 mm sieve and then 200 mg of each sample was poured into each syringe. Used syringes were heated in a water bath at 39 ˚C. Then, rumen fluid and artificial saliva were added to the syringes. Gas production was read at times of 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. Fistulated castrated male sheeps were used for preparation of required rumen fluid. These animals were fed with 10 percent more than maintenance and with forage (hay and straw) and concentrates (barley, soybean meal and wheat bran). Animal diets were 2.2 kg of dry matter per day, containing 65% forage and 35% concentrate. Basal diet was included 14.2 Mcal/kg of dry matter metabolizable energy and content of 12% crude protein. Dry matter degradability was performed using the nylon bag method (Orskov and McDonald, 1979) and four fistulated castrated male sheeps. Milled feed samples (4 g) were placed inside the bag and degradability was measured at 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours with three replications. Animal nutrition was performed twice a day (morning and evening), in the maintenance level. Their diet consisted of 65% forage (hay and straw) and 35% concentrate (barley, soybean meal and wheat bran).

Statistical analysis

Data were analyzed in a completely randomized design. Degradation parameters were calculated using Fitcurve and Neway software for gas and nylon bags. For data analysis SAS 9.1 software was used SAS (2003). Mixed model was used to compare the different stages. Mixed models are particularly useful in settings where repeated measurements are made on the same statistical units, or where measurements are made on clusters of related statistical units. Because of their advantage to deal with missing values, mixed effects models are often preferred over more traditional approaches such as repeated measures ANOVA (rANOVA). The time before the correspondence time in gas production rate is the fixed factor for its next time gas production. The statistical model was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Where:
μ: total average.
α: elevation of the site factor (i=1-2).
β_j: sampling time factor (j=1-3).
$\alpha\beta_{ij}$: interaction between site and stage.
ε_{ijk}: variance between experimental units measurements.

RESULTS AND DISCUSSION

Chemical composition of *Poa trivialis*

The results of chemical composition of *Poa trivialis* (Table 1) showed that the crude protein content was high in both sites and it was reduced with increasing the age of plant (P<0.05). Crude protein is higher in the early growing stages of plant because young plants have higher proportion of leaf to stem.

Arzani *et al.* (2008) in the study of *Poa bulbosa* was reported the same results with decreasing crude protein from 14.85, 4.84 and 2.48 for vegetative growth, flowering and seeding stages, respectively. While crude protein of *Poa trivialis* was 6.73, 5.25 and 4.19 during the mentioned growing stages in the first sites, respectively. Kamalak (2005) made an experiment about the nutritional value of wheat and barley straw and he concluded that the chemical composition of the wheat and barley straw crude protein content were 3.14 and 4.22, respectively. This result was similar to *Poa trivialis* CP in the third stage of this study. There is a direct proportion between the nutritional value and crude protein of plants and inverse ratio in relation to the quality and nutritional value of plants and crude fiber. Thus, it may been concluded that the nutritive value of *Poa trivialis* has high quality in the early stages of growing in comparison with later stages. Maintenance and strength of tissues were increased with plant growth stages, which are caused by the increase of carbohydrates such as cellulose, hemi cellulose and lignin. Thus, plant growth stages may be considered to increase the carbohydrate structure. These results are supported by the reports of Stodart *et al.* (1975). Shirmardi *et al.* (2004) also reported that rangeland forage quality changes by increasing the age, and the chemical composition of rangeland forage is affected by climate changes. Givens *et al.* (1990) found that the chemical composition of rangeland forage is affected by grazing period, climate and grazing intensity.

Daalkhaijav *et al.* (2000) measured the cell wall of desert steppe plants and they found a range of values going from 55.05% to 71.96%. In our study, the *Poa trivialis* cell wall values obtained ranging between 56.80 to 78.80%. As can be seen in Table 1, *Poa trivialis* cell wall was similar to cell wall composition of the desert steppe rangeland plants.

Gas production rate

Poa trivialis gas production parameters are shown in Table 2. Study of gas production from *Poa trivialis* fast degradable parts (a) showed a negative effect due to delayed fermentation substrate and delay in gas production (Khazaal *et al.* 1993). Soluble parts were very quickly attacked by microorganisms and gas production was increased. Lowest gas production was observed from (a) portion of third stage of first site (P<0.05), that showed delayed in accumulation of bacteria and delayed in the substrate fermentation. Songsak *et al.* (2006) conducted a study in sugarcane and they estimated that the portion of gas production was 0.49 mL and it is similar to the gas production of *Poa trivialis* in the first stage of the first site. Rate of gas production (c) of *Poa trivialis* was significantly different in the first stage from the second and third stages in the second site (0.07 mL per hour) (P<0.05). It showed that degradability of soluble fraction (a) was higher according to the type of its carbohydrate (fructose and other carbohydrates in solution) and so gas production rate was increased. Rate of gas production was calculated 0.03 mL per hour in the first site, which may been related to late degradation of carbohydrates. For example, cellulose and hemi cellulose were higher in the third stage of growth. The total gas production was higher in the first stage of the second site and was lower in the second stage of the first site (P<0.05). Thus, the rate of gas production is associated with the total gas. In a study conducted on *Alopecurus textail*, *Festuca ovina* L. and *Trifolium montanum* gas production rate was 0.03 mL per hour in the third stage (Ghanbari, 2008). This was corresponded to gas production rate in the third stage of first site of this study. Effects of sampling procedures showed significant difference on *Poa trivialis* and total gas production in the first site (P<0.05). This difference is due to higher gas production from the part b of the first step, which corresponded to gas production in the first stage of the second site in the study of Ghanbari (2008) in three stages.

Metabolizable energy, digestible organic matter and digestible organic matter in dry matter of *Poa trivialis* using gas

Metabolizable energy, digestible organic matter and digestible organic matter in dry matter using gas test is shown in Table 3. Metabolizable energy of *Poa trivialis* was higher in the first stage at both sites (P<0.05).

Table 1 Chemical composition of *Poa trivialis* in different phenological stages in site 1 and site 2 (%)

	Growing stages in Neor			Growing stages in Hir		
	Vegetative growth	Flowering	Seeding	Vegetative growth	Flowering	Seeding
CP	6.73	5.25	4.19	7.44	5.11	3.26
CF	31.20	34.20	36	28.20	32	34.20
NDF	65.80	75.60	78.80	56.80	74.80	74
ASH	4.90	5.30	5.10	4.20	4.30	4.05
OM	95.10	94.70	94.90	95.80	95.70	95.95

CP: crude protein; CF: crude fiber; NDF: cell wall and OM: organic matter.

Table 2 Average parameters of fermentation or gas production in *Poa trivialis*

Parameters	a	b	c	RSD	a + b
Vegetative growth in site 1	0.40^{e}	75.23^{c}	0.06^{b}	1.13	75.63^{b}
Vegetative growth in site 2	-0.43^{d}	81.93^{a}	0.07^{a}	1.46	81.50^{a}
Flowering in site 1	-2.37^{b}	78.67^{ab}	0.04^{c}	2.15	76.30^{b}
Flowering in site 2	-1.83^{c}	78.33^{ab}	0.04^{c}	1.75	76.50^{b}
Seeding in site 1	-3.27a	77.37^{c}	0.03^{d}	2.80	74.10^{b}
Seeding in site 2	-2.40b	77.63^{c}	0.04^{c}	2.33	75.23^{b}
SEM	0.19	1.98	-	-	1.95

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
a: gas production from degradable portion (mL).
b: gas production from the insoluble part (mL).
c: gas production rate (mL in hour of 200 mg of dry matter).
RSD: standard deviation of error.
a + b: gas production of soluble and insoluble (mL).
SEM: standard error of the means.

Table 3 Calculated parameters using the rate of gas production in *Poa trivialis*

Parameters	ME (MJ/Kg)	OMD (%)	DOMD (%)
Vegetative growth in site 1	10.16^{b}	67.16^{b}	63.87^{b}
Vegetative growth in site 2	11.42^{a}	75.50^{a}	72.33^{a}
Flowering in site 1	8.63^{c}	57.03^{c}	54.01^{d}
Flowering in site 2	8.89^{c}	58.68^{c}	56.16^{c}
Seeding in site 1	8.06^{d}	53.21^{d}	50.50^{e}
Seeding in site 2	8.26^{d}	54.35^{d}	52.15^{de}
SEM	0.17	1.12	1.06

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
ME: metabolizable energy (MJ/kg).
OMD: organic matter digestibility (%).
DOMD: digestibility of organic matter in dry matter (%).
SEM: standard error of the means.

Crude protein and soluble carbohydrates of *Poa trivialis* was higher in the first stage, thus metabolizable energy was also greater. Arzani *et al.* (2008) reported that metabolizable energy of *Poa bulbosa* was 8.55, 6.84 and 5.30 MJ/kg at different stages of vegetative growth, flowering and seeding, respectively. While the metabolizable energy of *Poa trivialis* was estimated as 11.42, 8.89 and 8.26 MJ/kg in the second site at the vegetative growth, flowering and seeding stages, respectively.

This difference is probably due to species differences and it also depends of climate conditions. *Poa trivialis* metabolizable energy was estimated as 1.93 (Mcal/kg) in the third stage of the first site, which is equal to estimated metabolizable energy of *Festuca ovina* (1.93) (Ghanbari, 2008). Digestion of rangeland forage, especially in the off-season rangeland plant, is often limited due to the lack of degradable nitrogen and minerals such as sulfur deficiency (Mansouri, 2002).

Due to the high crude protein and crude ash in the first stage of the first and second site, digestibility of their organic matter are also higher than in other stages (P<0.05). Datt and Singh (1995) reported that wheat straw treated with urea, molasses and minerals increased dry matter digestibility and gas production and there was a positive correlation between the levels of protein, gas production, dry matter digestibility and organic matter digestibility. In the study of Sallam *et al.* (2007), digestibility of *Trifolium alexanderium* was estimated as 54.20%, which was similar to that of *Poa trivialis* in the third stage of the second site (54.35%). Pariss *et al.* (2000) found low digestibility of organic matter in plants (such as clover), which is due to the secondary metabolite that acts as an inhibitory factor of digestibility. Digestibility of organic matter in dry matter content of *Poa trivialis* was estimated as 72.33% in the first stage of the second site that is more than the other stages (P<0.05).

As well as digestibility of organic matter in dry matter of *Poa trivialis* was estimated as 50.50% in the second stage of the first site, which is lower than that of other stages (P<0.05), because its organic matter digestibility was lower than that of the other stages. Solario *et al.* (2004) reported that the chemical composition and digestibility of forages may affect the nutritional value of the forage and it is influenced by species, season, maturity and soil type.

Degradability using the nylon bag technique

Poa trivialis dry matter degradability is shown in Table 4. According to this table, most of the fast degradable dry matter (a) of *Poa trivialis* was found in the first stage (27.20%) and the lowest was found in the third stage (10.47%) of the second site (P<0.05). Related to harvest time, *Poa trivialis* was young and has more degradable organic matter.

Table 4 DM degradability parameters using nylon bag in *Poa trivialis*

Parameters	a	b	c	RSD	a + b	P(k=0.02)
Growing in site 1	24.40[b]	54.70[c]	0.04	3.00	79.10[b]	60.87[b]
Growing in site 2	27.20[a]	56.43[bc]	0.04	1.83	83.63[a]	63.93[a]
Flowering in site 1	18.77[c]	55.50[bc]	0.05	1.92	74.27[d]	57.40[d]
Flowering in site 2	19.53[c]	57.83[b]	0.04	2.28	77.37[bc]	59.17[c]
Seeding in site 1	10.80[d]	65.63[a]	0.04	2.51	76.43[c]	54.03[e]
Seeding in site 2	10.47[d]	65.5[a]	0.04	2.26	75.97[cd]	53.40[e]
SEM	0.76	1.41	-	-	1.2	0.86

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
a: fast degradable dry matter.
b: slow degradable dry matter.
c: degradation rate (percent in hour).
RSD: standard deviation of error.
a + b: total degradation.
SEM: standard error of the means.
P(k=0.02): degradation percent in 2% pass rate.

Soluble carbohydrates decreased with increasing age of the *Poa trivialis* and are added to the structural carbohydrates. It is required additional time for their degradation, because microbes influence its capacity to break. Daalkhaijav *et al.* (2000) showed that the maximum amount of the fast degradable part was found in summer and the lowest in winter in the desert steppe rangeland plants (grass and shrub) and it was reduced in winter to 6 times. The amount of slow degradable dry matter (b) of *Poa trivialis* in the second stage was higher than in the others (P<0.05) and for the first and second sites were 55.50 and 57.83%, respectively. Most of the part b is concerned to the type of carbohydrates and if microorganisms have enough time to break them. In contrast, the lowest part of b was in the third stage. Especially, it was lower in grasses durin summer (Daalkhaijav *et al.* 2000). In the study conducted by Cone *et al.* (2002), part b changes were lower during the year in grasses such as *Poa trivialis*. Total degradation have significant difference between the first stage of these condsite and the second and third stages in site 2 (P<0.05), because degradation of dry matter depends on degradability of crude protein and carbohydrates. Since the degradation of the components were higher in the first stage, thus dry matter degradation was greater. Degradation percentage differed significantly at different stages of development and decreased with increasing age (P<0.05). Generally, degradation of the desert steppe rangeland grasses was lower in mountain rangelands (Daalkhaijav *et al.* 1997). Rangeland grasses become lignified because of the desert climate.

CONCLUSION

Chemical composition, gas production and nylon bag technique, degradability of crude protein, metabolizable energy and digestible organic matter of *Poa trivialis* in the vegetative growth stage and flowering stage were at the maximum for animal. *Poa trivialis* cell wall was higher than the other composition, and then metabolizable energy of that was lower. In addition, metabolizable energy and organic matter digestibility was higher in the first stage. Gas production from degradable part of *Poa trivialis* was also higher.

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