WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
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Journal of Food, Agriculture & Environment Vol.9 (2): 380-384. 2011 *www.world-food.net*

Phenological stage effects on forage quality of four forbs species

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Received 29 November 2010, accepted 2 April 2011.

Abstract

Information on nutritive values of each plant part in each phenological stage could help range managers choose suitable grazing times to achieve higher animal performance without detriment to vegetation. Thus, nutritive value of different plant parts of four species in three phenological stages (vegetative, flowering and seed production) from two sites was investigated. Species included: *Sanguisorba minor*, *Onobrychis transcaspica, Onobrychis radiata* and *Astragalus brevidens.* Forage quality was evaluated based on crude protein (CP), acid detergent fiber (ADF), dry matter digestibility (DMD) and metabolizable energy (ME) contents. A completely randomized design with a factorial arrangement of species and phenological stage was analyzed with five replicates. The results of chemical analysis indicated that there were significant differences among different phenological stages of various species in terms of their properties. As the plant ages increased, CP, ME and DMD decreased and ADF increased. Significant differences were obtained between forage quality of diverse species ($p<0.01$). There were significant differences between CP contents among forbs species ($P < 0.01$). High ADF percentages were obtained in *Astragalus brevidens* (47.59%). Low ADF contents were in *Sanguisorba minor* (36.9%). *Astragalus brevidens* exhibited the lowest DMD (50.7%). Metabolizable energy ranged from 6.61 for *Astragalus brevidens* to 7.87 MJ/kg for *Sanguisorba minor*.

Key words: Phenological stages, crude protein, acid detergent fiber, dry matter digestibility, metabolizable energy.

Introduction

Understanding of nutrient contents in plants is useful for determining rangeland capacity, the most proper time of utilization of range plants, prediction of malnutrition and evaluation of nutrition requirements of plants. In order to obtain optimum utilization of rangelands, it is necessary to consider temporal variation of forage quality. For determining forage quality, different variables are evaluated.

One of the main objectives of range management is livestock production, which depends on the nutritive value of available forage [32]. Ganskopp and Bohnert [13] proposed that livestock and wildlife managers must be aware of the nutritional dynamics of forages to sustain satisfactory growth and reproduction of their animals and assure fair value of the pasture. Feed quality has been defined as the amount of nutrient material that an animal can obtain from a feed in the shortest possible time [35].Valentine [33] believed that the nutrient balance of animals, whether grazing or pen fed is dependent upon four basic factors including the animal's nutrient requirements and nutrient contents, digestibility and amount of feedstuffs consumed. Knowledge of generalized nutrient trends in the forage plants available to grazing animals will assist in achieving their most timely utilization, help predict nutrient deficiencies and suggest supplementation needs.

Information on the nutritive value of forage by phenological stage could help range managers choose suitable grazing times and stocking rates to achieve higher animal performance without damage to vegetation. Factors that affect forage quality include species, leaf-to-stem ratio, stage of growth, soil agents, climate, harvesting, diseases and pests [7, 17]. McDonald *et al.* [26] reported that in early spring, digestibility of plants might reach 80% or higher and will decrease when plant growth is complete. Digestible energy, metabolizable energy, and digestibility of forage decrease, whereas fiber and lignin increase with maturity of plants. Behnamfar *et al.* [8] and Holchek *et al.* [19] reported reduction of crude protein (CP) and digestibility of forage when plants matured. However, percentages of acid detergent fiber (ADF) and neutral detergent fiber (NDF) increased. Differences among ratios of plant parts in different phenological stages were reported by Jafari [20]. In the primary growth stage, grass stems are short and ratios of leaf to stem are high. When plant growth is complete, stems comprise a major part of the total forage [23].

Assessment of the nutritive value of pastures is mainly concerned with the supply of energy, protein and minerals. Among various common chemical determinations of plant materials, CP, DMD and ME are mainly considered for evaluation of forage quality [4, 5, 7, 10, 27, 30]. Walton [35] stated that digestibility is frequently considered to be the most valuable estimate of forage quality, since it is closely associated with animal productivity. Digestibility may be related to dry matter, energy, or to any component of the nutrient material available in the feed [34]. In this study, the forage quality of 4 forbs in 3 phenological stages (vegetative, flowering and completed growth) was determined and compared.

Materials and Methods

The study was conducted at two study areas. The Sisab region is located in north east of Iran (North Khorasan Province) 35 km east of Bojnourd city. Other area is Asadli region. Asadli is located

on the northern slopes of Aladagh elevations in north east of Iran (North Khorasan Province). This region is situated between 57º02´ to 57º25´ east longitudes and 37º13´ to 37º23´ north latitude. This region is located between the cities of Bojnord and Esfarain. The altitude of the area is 1900 m. The mean annual rainfall is 336.1 mm and the median annual temperature is 8.9ºC. The climate of this region with using of Emberger method is cold semi-arid. Sisab region is situated between 57º27´20" east longitudes and 37º28´15" north latitude. The altitude of the area is 1570 m. The mean annual rainfall is 250 mm and the median annual temperature is 12.2ºC. The climate of this region with using of Demartonne method is semi-arid.

Samples were collected in three phenological stages: primary growth (rapid growth, vegetative), flowering and seed production (plant growth completed). At each site, 5 points were randomly selected for sample collections of each species. Plant parts were collected, oven dried at 70ºC for 24 hours and weighed. Finally, samples were milled and passed through a 0.5 mm sieve for chemical analysis.

Sanguisorba minor, Onobrychis transcaspica, Onobrychis radiata and *Astragalus brevidens* species were selected for study. These species grow in most dry rangelands of Iran and are relatively palatable to domestic grazing animals and able to regrow after grazing or harvesting. They belong to the families of Rosaceae and Fabaceae. CP was calculated on the basis of nitrogen percentage (N% 6.25) measured by the Kjeldahl technique [1]. It was multiplied by a factor of 10 to convert to g per kg. ADF was measured with the Fiber Tech System [1]. Dry matter digestibility was estimated by the formula [28]:

$$DMD\% = 83.58 - 0.824ADF\% + 2.626N\%$$

Metabolically energy was predicted with the equation [1]:

$$ME = 0.17DMD\% - 2$$

Effects of species and phenological stage were analyzed in a factorial arrangement of a completely randomized design with five replications of each species collected for each stage at each site. Each plant yielded information on two (leaf and stem in phenological stages 1, 2 and 3) or three (leaf, stem, and flower in stages 2 and 3) plant parts. Thus, plant part was included in the analysis as a subplot factor in a split plot arrangement. Locations were combined into analysis following Cochran and Cox[9]. When factors interacted, simple effects were analyzed with a least significant difference test.

Analysis of variance (ANOVA) was used to determine the existence of differences in chemical composition of species and life forms. Comparison of the species effect and phenological stages were carried out using Minitab statistical package. Duncan's method was also used for comparison and grouping of nutrients.

Results

Significant differences were obtained between forage quality of *Sanguisorba minor, Onobrychis transcaspica, Onobrychis radiata* and *Astragalus brevidens* species ($p<0.01$) (Table 1). Crude protein content of forage species ranged from 11.62 to 15.04% (Table 2). The minimum CP content was recorded for *Sanguisorba minor* and the maximum CP for *Astragalus brevidens*. Crude protein contents of legume species were generally greater than for *Sanguisorba minor* species. There was a significant difference in protein content among growth stages of *Sanguisorba minor* ($p<0.01$). The highest protein content was 17.04% in vegetative growth stage and the lowest value (5.21%) was in seed ripening stage. The crude protein content of flowering stage was 12.61%. Crude protein contents of *Onobrychis transcaspica* at vegetative growth stage and seed ripening stage were significantly different ($p<0.05$), but flowering stage was not significantly different from vegetative growth and seed ripening stages. The results revealed that there was a significant difference in protein contents among growth stages of *Onobrychis radiata* and *Astragalus brevidens* ($p<0.01$). Significant differences were not observed among effects of the species and phenological stages for CP contents.

There were high ADF percentage for *Astragalus brevidens* and low content for *Sanguisorba minor*. There was significant difference in ADF percentage of *Sanguisorba minor* at different stages of plant growth ($p<0.01$). The highest ADF percentage was 50.28% at seed ripening stage and the lowest value (24.77%) was at vegetative growth stage (Table 2). The ADF percentage at flowering stage was 35.65%. The results indicated also that the ADF percentage of *Onobrychis transcaspica, Onobrychis radiata* and *Astragalus brevidens* were not significantly different at three phenological stages. There were not significant differences between effect of the species and phenological stages for ADF contents.

Table 1. The results of variance analysis (crude protein (CP), acid detergent fiber (ADF), dry matter digestibility (DMD) and metabolizable energy (ME) contents) of forage species in different phenological stages.

Properties	Treatment (Source)	DF	Seq SS	Adj MS	F	P
Crude protein	Species	3	109.637	36.546	6.19	0.001
	Stages	2	844.714	422.357	71.55	0.000
	Species*Stages	6	48.403	8.067	1.37	0.249
	Error	44	259.724	5.903		
	Total	59	1277.159			
ADF%	Species	3	1193.99	398.00	7.26	0.000
	Stages	2	1600.23	800.12	14.60	0.000
	Species*Stages	6	709.90	118.32	2.16	0.065
	Error	44	2411.46	54.81		
	Total	59	6189.76			
DMD%	Species	3	679.67	226.56	6.27	0.001
	Stages	2	2041.46	1020.73	28.23	0.000
	Species*Stages	6	554.40	92.40	2.56	0.033
	Error	44	1591.05	36.16		
	Total	59	5069.46			
ME(Mj/kg)	Species	3	19.643	6.548	6.27	0.001
	Stages	2	58.998	29.499	28.23	0.000
	Species*Stages	6	16.022	2.670	2.56	0.033
	Error	44	45.981	1.045		
	Total	59	146.507			

Table 2. Variation in crude protein (CP %) and acid detergent fiber (ADF %) between species and within plant and phenological stages of *Sanguisorba minor, Onobrychis transcaspica, Onobrychis radiata* and *Astragalus brevidens.*

Plant species	Crude protein (CP %)			Acid detergent fiber (ADF %)		
	Vegetative growth	Flowering	Maturity	Vegetative growth	Flowering	Maturity
Sanguisorba minor	aCD17.04	bB12.61	cA5.21	aA24.77	bA35.65	cA50.28
Astragalus brevidens	aD20.49	bB14.45	cC10.16	aC45.28	aBC47.85	aA49.64
Onobrychis transcaspica	aCD18.52	abB14.82	bC11.1	aBA31.77	aBA41.48	aA42.91
Onobrychis radiata	aCD18.68	bB13.65	cC10.19	aCB43.5	aCBA43.6	aBA51.14

* Means in columns with different superscripts (A, B …) between species and in rows (a, b...) between phenological stages are different (P<0.05).

There were significant differences (p<0.01) in digestible dry matter (DMD)contents of *Sanguisorba minor* at different vegetative stages (Table 1). The highest value of DMD at vegetative growth stage was 70.33% and the lowest one at seed ripening stage 44.34% (Table 3). DMD content of flowering was also 59.50%. The DMD contents at different vegetative stages of *Onobrychis transcaspica* and *Astragalus brevidens* showed significant differences (p<0.05). No significant difference was observed in DMD content of *Onobrychis radiata* (Table 3). According to the results, significant differences were observed among effect of the species and phenological stages for DMD contents (p<0.05).

Metabolizable energy (ME) ranged from 6.61 to 7.87 MJ/kg dry matter. *Astragalus brevidens* had the lowest ME, while *Sanguisorba minor* had the highest value. The ME content at different vegetative stages of *Sanguisorba minor* showed significant differences (p<0.01). The highest value of ME at vegetative stage was 9.96 MJ/kg while the lowest value in seed ripening period was 5.54 MJ/kg. Furthermore, the ME of flowering was 8.11 MJ/kg (Table 3). The ME content at different vegetative stages of *Onobrychis transcaspica* and *Astragalus brevidens* showed significant differences (p<0.05). No significant difference was observed in ME content of *Onobrychis radiata* (Table 3). According to the results, significant differences were observed among effects of species and phenological stages for ME contents (p<0.05).

Discussion

Arid and semi-arid regions of Iran have diverse and rich collection of plants. Vegetation cover of such regions has high resistance to difficult environmental conditions. Also, the forage, nutritional, industrial, medicinal and conservative values of these plants are of high importance. The nutrient value of range forage depends on plant composition and stage of growth. The close matching of nutrients requirements and feed quality is necessary for efficient animal production. This study suggests that adequate nutrients are available in vegetation communities including the evaluated species.

Range forage quality has spatial and temporary variations. The chemical analysis of range forage plants serves as a comparative measure of differences between species and changes with season or phenology. Rangelands of Asadli and Sisab in poor condition usually supply livestock during spring and early summer. However, forage quality declines as plants mature. The results showed the evaluated forage species had different nutritive values. As Cook and Stubbendieck [10] reported the chemical content of plant species may differ because of an inherent ability to withdraw certain nutrients from the soil and to concentrate them in tissues. Plants may also vary in susceptibility to leaching, or may produce different proportions of leaves, stems, and flower stalks at various stages of maturity or because of previous grazing treatments [3, 5, 14]. Legumes do not lose quality with maturity. Feed quality of legumes is generally high. Seasonal changes of CP during different phenological stages were reported by White [36], Akbarinia and Koocheki [2] and Arzani *et al.* [7]. They found that when plants became older, CP decreased. In this study, CP of *Sanguisorba minor, Onobrychis transcaspica, Onobrychis radiata* and *Astragalus brevidens* were different between phenological stages. Results also showed differences (P<0.01) between CP content in different species. Among species in the present study *Sanguisorba minor* showed the lowest CP percentage. Stoddart *et al.* [32] stated that declines in nutrient contents and leaching are especially serious in the case of herbaceous plants and forbs. In the same region, as the protein of bur clover (*Medicago hisida*) was leached, an increasing wide nutritive ratio resulted [16].

The results of ADF showed significant differences in phenological stages. Also, ADF showed an increasing trend during the development stages which is in accordance with Heshmati *et al.* [18]. Among species in the present study *Astragalus brevidens* had the highest ADF percentage. Young plant cells

Table 3. Variation in dry matter digestibility (DMD %), metabolizable energy (ME, MJ/kg) between species and within plant and phenological stages of *Sanguisorba minor, Onobrychis transcaspica, Onobrychis radiata* and *Astragalus brevidens.*

Plant species	Dry matter digestibility %(DMD)			Metabolizable energy (ME, MJ/kg)		
	Vegetative growth	Flowering	Maturity	Vegetative growth	Flowering	Maturity
Sanguisorba minor	aC70.33	bC59.5	cB44.34	aC9.96	bC8.11	cB5.54
Astragalus brevidens	aBA54.87	abCBA50.22	bCB46.94	aBA7.33	abCBA6.54	bCB5.98
Onobrychis transcaspica	aCB65.19	abC55.63	bC52.88	aCB9.08	abC7.46	bC6.99
Onobrychis radiata	aBA55.58	aCB53.41	aB45.72	aBA7.45	aCB7.08	aB5.77

*Means in columns with different superscripts (A, B …) between species and in rows (a, b...) between phenological stages are different (P<0.05).

have one external layer called a primary cell wall, but when they become mature, a secondary cell wall is also formed. Because of storage tissues in seeds, ADF and NDF contents varied with seed maturity between phenological stages and species. Arzani *et al.* [4] also reported that with progress of plant growth, ratios of protector and firmness tissues, which mostly consist of structural carbohydrates such as celluloses, hemicelluloses and lignin, are increased. Therefore, maturity of plants and an increase in structural carbohydrates cause higher fiber amounts in forage late in the growing season.

Dry matter digestibility of plant parts mainly decreased with growth progress, and DMD for all species in all phenological stages were different. This agreed with results obtained by Akbarinia and Koocheki [2]. They reported that a reduction of DMD with maturity of plants is due to increasing structural tissues in stems. Pinkerton [29] also reported a close relationship between digestibility and cell wall characteristics. In contrast, the chemical structure of cell walls changes with plant growth. As plant growth continues, fiber content increases and digestibility decreases. Reduction of digestibility in matured plants also was reported by Kashki [22] and Linn and Kuehn [24].

In our experiment for different species DMD values of forages were above 50%. Generally, about 50% digestibility is sufficient for animal maintenance [15, 31, 36]. The low digestibility of *Astragalus brevidens* might be due to their anatomic characteristics and in addition the phenology that caused the forage to be stems with higher fiber content than other species at the time of sample collection. As El-Shatnawi and Mohawesh [11] and Ganskopp and Bohnert [12] also reported, stems have relatively high fiber content. The results of measured ME showed significant differences in phenological stages. Information on ME content could guide range managers to estimate forage requirements of grazing animals based on energy required for particular physiological status. The basic energy requirement for maintenance of sheep is derived from the metabolic weight relationship: kcal required = $70W^{0.75}$, where W is the weight of animal in kg [32].

The results suggest that palatability, digestibility and nutritional values are decreased during the growth period due to accumulation of fiber in plant tissues. Arzani *et al.* [6] proposed that metabolic energy is decreased during vegetative growth period. Current research showed that different species in this study are rich in DMD and ME and are considered as a suitable source for livestock nutrition.

Generally, comparison of forage quality of the plants shows that crude protein, metabolic energy and percentage of digestible dry matter decreased during vegetative growth stage while ADF increased. During the plant development, digestibility and nutritional content decreased and the content of non-digestible nutrients also decreased due to fiber increase of plant tissues. Consequently, forage quality will be decreased.

Conclusions

Phenological stage of growth had a significant influence on forage quality. The close matching of nutrient requirements and feed quality is necessary for efficient animal production. With increased plant development, CP, DMD and ME are reduced. Higher forage quality was recorded for the 1st stage of growth. This study suggests that adequate nutrients are available in vegetation communities including the evaluated species. However, most vegetation communities of Asadli and Sisab are classified as poor condition because of severe overgrazing [25]. Therefore, in their improvement plans, grazing strategies should be developed to reduce pressure on plant species and to improve botanical composition of grazing area. We suggest that rangelands with a diversity of good forage quality species, that exploit all levels of the soil profile, will provide adequate forage quality for longer time periods than rangeland with poor diversity. Due to diversity of native plant communities and limitation in time, cost and variety of factors indicating forage quality, N and ADF are the best factors that should be measured for nutritive evaluation of forage. This recommendation is based on relationship between factors investigated in this experiment and also results of Kabuli [21],

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