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Nutritive Value of Rangeland Plants Compared to *Medicago sativa*

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Abstract. Awareness of the forage nutritive value of rangeland species is important to provide a balanced diet in order to meet the nutritional requirements of ruminants and to determine the suitable grazing time and rangeland grazing capacity. Hence, different laboratory methods were used to compare the nutritional value of *Centaurea cyanus*, *Fumaria officinalis*, *Galium tricornutum* and *Vicia sativa* as compared to *Medicago sativa*. Whole plant samples just before flowering were randomly collected from different rangelands of Torbat-e Jam in spring 2018. The means of Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Organic Matter (OM), Acid Detergent Lignin (ADL), Nitrogen-Free Extract (NFE) and Non-Fiber Carbohydrates (NFC) of five studied plants were 19.67, 1.79, 19.16, 35.96, 27.20, 86.98, 9.95, 46.36 and 29.56% of dry matter (DM), respectively. The macro and micro elements were in a reasonable range as compared to *Medicago sativa*. The *in vitro* organic matter degradability (IVOMD), *in vitro* dry matter degradability (IVDMD), 24 h *in vitro* gas production (24 h IVGP), metabolizable energy (ME) and net energy for lactation (NEl) were the highest in *Centaurea cyanus* and *Galium tricornutum*, respectively. Differences in fermentation parameters (NH_3-N, total volatile fatty acids: TVFA and pH) were observed in the media following incubation of plants. The 24 h IVGP was positively correlated ($P<0.0001$) with IVOMD, IVDMD and TVFA and negatively with ammonia nitrogen (NH_3-N; $P<0.0001$), crude protein (CP; $P<0.01$) and ether extract (EE; $P<0.05$). The results indicated that all rangeland plants studied in this experiment had a favorite nutritional value compared to *Medicago sativa*.

Key words: Rangeland plants, *Medicago sativa*, Nutritional value, Livestock, Torbat-e Jam

Introduction

A large amount of feedstuffs will be obtained from rangeland plants, if the annual rainfall of Iran is optimum. There are a lot of rangeland plants whose nutritional value is still unknown to most ranchers. Many studies have evaluated the nutritional value of different rangeland or weed plants in Iran (Kazemi *et al.,* 2009; Towhidi *et al.,* 2011; Kazemi *et al.,* 2012; Naseri *et al.,* 2017; Ezzat *et al.,* 2018). Having knowledge about the nutritional value of different rangeland plants will play an essential role in supplying the dietary requirements of ruminants, especially cattle and sheep. Evaluation of the nutritional value of feed or other forages with common laboratory techniques (*In vitro* gas production: IVGP and ANKOM techniques) is faster and less costly as compared to *in vivo* methods (Getachew *et al.,* 2004). The IVGP technique has been frequently used to evaluate the nutritional value of animal feed (Al-Masri, 2009; Abdalla *et al.,* 2012). *Centaurea cyanus* as a medicinal plant is a member of the *Asteraceae* family, which is also ornamental plant because of its beautiful blue flowers (Chiru *et al.,* 2013; Bruneton, 1995). The extract of *Centaurea cyanus* caused cytotoxicity in *Artemia salina* and human fibrosarcoma cells, and the guaianolides compounds were also found in it (Bruno *et al.,* 2005). The flowers of *Centaurea cyanus* tested as a medicinal plant did not cause any significant effect on some fermentation parameters in the culture medium (Garcia-Gonzalez *et al.,* 2008). The use of *Fumaria officinalis* (Fumariaceae family) and cornflower (*Centaurea cyanus*) is also recommended for ruminant nutrition (Laudato and Capasso, 2013). *Galium tricornutum* with trailing or climbing stems is from *Rubiaceae* family that is found in Middle East, especially Iran. This plant is used as an anti-scorbutic agent, refrigerant, diuretic and aperients agent (Khan *et al.,* 2017). As a forage plant, *Vicia sativa* (Fabaceae family) plays an important role both as forage for livestock (Kaya *et al.,* 2013) and in the human diet (Akpinar *et al.,* 2001), especially when food supply decreased (Enneking, 1994). The common vetch (*Vicia sativa*) is also one of the important annual legumes (Mikic *et al.,* 2009). *Vicia sativa* is now widely cultivated in many areas of the world (Francis *et al.,* 1999) because it suggested a low-cost, rich source of protein and minerals for producers (Akpinar *et al.,* 2001). There was no enough information about the nutritional value of the four rangeland plants (*Centaurea cyanus, Fumaria officinalis, Galium tricornutum, Vicia sativa*) compared to *Medicago sativa.* Hence, the aim of this study was to determine *in vitro* dry matter (IVDMD) and *in vitro* organic matter degradability (IVOMD), the mineral and chemical compounds, and some fermentation parameters by common laboratory methods. The correlation coefficient between some of the measured parameters was also determined.

Materials and Methods

Plants collection and laboratory analysis

The plant samples (*Centaurea cyanus, Fumaria officinalis, Galium tricornutum* and *Vicia sativa)* and also *medicago sativa* were randomly collected just before flowering from natural rangelands of Torbat-e Jam (Iran; mean rainfall: 254 mm; mean annual temperature: 26 °C; 928 meter above sea level) in spring 2018. The samples of whole plants after collecting were immediately transferred to the laboratory, dried in an oven (Behdad Co.) at 60 °C for 48 h, ground via a 1-mm mesh screen in a Wiley Mill and were used for chemical analysis, *in vitro* gas production (IVGP), *in vitro* organic matter degradability (IVOMD) and *in vitro* dry matter (IVDMD) degradability (Getachew *et al.,* 2004). The Kjeldahl method (AOAC, 1999, ID 984.13) was applied to crude protein (CP) determination. For dry matter (DM) determination, a sample of

plant was oven dried at 135 °C for 4 h (AOAC, 1999, ID 930.5). The crude fiber (CF; Komarek *et al.*, 1996), acid detergent lignin (ADL), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by the ANKOM technology (ANKOM Technology, 2005; ANKOM Technology, 2006a; ANKOM Technology, 2006b) using solutions described by Van Soest *et al.* (1991). Ether extract (EE) was determined using ether extraction in the Soxhlet device (AOAC, 1999, ID 954.02). The non-fiber carbohydrate (NFC) content of samples was calculated by subtracting CP, NDF, fat, and ash from total DM (Sniffen *et al.*, 1992). The nitrogen-free extract (NFE) was determined by subtracting CP, EE, ash, and CF from 100 (Arshadullah *et al.*, 2009). Some minerals (S, Mg, Mn, Cu, Fe, and Zn) were determined by atomic absorption spectrometry (AOAC, 1990). Phosphorus was determined using the molybdate-vanadate method (spectrophotometry). Calcium, Sodium and potassium were determined by flame photometer (AOAC, 1990). For VFA determination, the rumen fluid was combined with 250 g/l metaphosphoric acid (0.2 ml/ml of ruminal fluid) and reserved in -20 ◦C for further analysis. The VFA was analyzed by gas chromatography. Crotonic acid (trans-2-butenoic acid) and Helium were used as the internal standard and carrier gas, respectively. Initial and final temperatures of oven were 55 and 195 °C, respectively. The temperature of detector and injector was also set at 250 °C (Khorrami *et al.*, 2015).

Laboratory gas test and *in vitro* degradability

About 200 mg of samples were weighed into 120 ml glass bottles. Before filling the bottles, the buffered-mineral solution was kept in a water bath at 39 ◦C under anaerobic conditions with CO_2 infusion. Rumen fluid was collected before the morning feeding from three fistulated Baluchi male sheep (40 ± 4.7 kg live weight) fed by alfalfa hay and corn silage twice (7:30 and 18:30 h) a day at maintenance. The taken rumen fluid via fistula was filtered through four layers of cheesecloth, flushed with CO_2, transferred into pre-warmed thermos flask and then transferred to the laboratory for the next laboratory works. By the use of a dispenser, the glass bottles were then filled with rumen fluid and artificial saliva solution (V: V, 1:2) prepared (30 ml) by Menke and Steingass (1988) method. Afterwards, each glass bottle was plumped with rubber and aluminum caps, was shaken gently and placed in a water bath at 39 ◦C. After 3, 6, 9, 12, 24, 48, 72, 96 and 120 h of incubation, gas production accumulating in the headspace of each bottle with gas volume was measured using a pressure transducer, following the procedure described by Theodorou *et al.* (1994). For the gas test, each plant species was tested in quintuplet. The gas produced during 24 h incubation was used to estimate the metabolizable energy (ME) and net energy for lactation (NEl) according to equations of Menke and Steingass (1988);

$$ME\ (MJ/kg\ DM) = 2.20 + 0.1357GP + 0.0057CP + 0.0002859EE^{\wedge}2$$

; which GP, CP, and EE were 24 h IVGP (ml/200 mg of incubated sample), crude protein (% of DM) and ether extract (% of DM), respectively.

$$NEl = 0.54 + 0.0959GP + 0.0038CP + 0.0001733EE^{\wedge}2$$

; which GP, CP, and EE were also 24 h IVGP (ml/200 mg of incubated sample), crude protein (% of DM) and ether extract (% of DM), respectively. A medium similar to one developed for gas production was used to measure TVFA, pH, and NH_3-N. About 10 ml of bottle content was centrifuged at 1000×g for 20 min. Also, 5 ml of the supernatant was transferred into a 10 ml plastic tube containing 1 ml of 25% metaphosphoric acid centrifuged at 1000×g and then conserved in 18 °C until VFA determination (Getachew *et al.*, 2004). The pH of the media was measured by the

means of a pH meter (Metrohm, 691) after 24 h incubation. After 24 h incubation, the contents of each glass bottle were empty, strained through four layers of cheesecloth and then 10 ml of strained rumen fluid was acidified by 10 ml of 0.2 N HCl for the determination of NH_3-N using the distillation method (Komolong *et al.*, 2001). Finally, all contents remaining in the bottles were filtered through nylon bags (pore size 50 µ) by vacuum pump, oven dried at 60 °C for 48 h and analyzed for IVDMD and IVOMD (Kazemi *et al.*, 2013).

Gas test equation and statistical analysis

Constant rate (cgas) and potential gas production (bgas) were obtained by fitting the gas production data to the nonlinear equation $Y = b(1 - e^{-ct})$ (Ørskov and Mcdonald, 1979); *Y* is the volume of gas produced at time t, *b* is the potential gas production (ml/200 mg DM), and *c* is the constant rate of gas production (ml/h). All data were subjected to one-way analysis of variance using the GLM of SAS (2002). Duncan's multiple-range test was used for separation of means at P= 0.05. Correlation coefficient between different parameters was calculated using SAS program (2002).

Results

Chemical composition

Dry matter and chemical composition (%) of different plants as compared to *Medicago sativa* are shown in Table 1. The results indicated that the range of the chemical compositions of plants was different. The lowest ADF (22.27% of DM), NDF (30.07% of DM) and CF (14.67% of DM) were related to *Fumaria officinalis* and the highest NFC (42.08% of DM) and NFE (58.15% of DM) were both related to *Galium tricornutum*. The highest CP (24.95% of DM) and DM (24.88%) were also related to *Medicago sativa*. The highest OM (91.67 and 91.55% of DM) was observed for *Vicia sativa* and *Medicago sativa*, respectively.

Table 1. Dry matter and chemical composition (%) of different plants compared to *Medicago sativa*

Plant	DM	CP	OM	NDF	ADF	CF	ADL	EE	NFE	NFC
Medicago sativa	24.88^{a}	24.95^{a}	91.55^{a}	36.00^{c}	23.27^{c}	18.86^{bc}	5.12^{e}	1.76^{ab}	45.97^{b}	28.83^{b}
Centaurea cyanus	14.74^{d}	16.24^{c}	81.47^{d}	42.33^{a}	31.53^{a}	20.17^{b}	9.77^{c}	1.14^{b}	43.91^{b}	21.75^{c}
Fumaria officinalis	15.77^{d}	23.14^{b}	83.77^{c}	30.07^{e}	22.27^{c}	14.67^{d}	12.45^{b}	2.33^{a}	43.62^{b}	28.22^{b}
Galium tricornutum	18.18^{c}	10.12^{d}	86.43^{b}	33.00^{d}	28.33^{b}	16.93^{cd}	15.06^{a}	1.23^{b}	58.15^{a}	42.08^{a}
Vicia sativa	20.62^{b}	23.87^{b}	91.67^{a}	38.40^{b}	30.60^{a}	25.15^{a}	7.37^{d}	2.49^{a}	40.14^{c}	26.90^{b}
SEM	0.43	0.32	0.32	0.42	0.63	0.75	0.39	0.32	0.78	0.79
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.004	<0.0001	<0.0001

Means in columns with different superscripts differ significantly (P<0.05).
DM: dry matter; CP: crude protein; OM: organic matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CF: crude fiber; ADL: acid detergent lignin; EE: ether extract; NFE: nitrogen-free extract; NFC: non-fiber carbohydrate.

Mineral composition

Mineral composition of different rangeland plants (g/kg DM) as compared to *Medicago sativa* was presented in table 2. The highest and lowest Ca were observed in *Galium tricornutum* (19.10 g/kg DM) and *Centaurea cyanus* (3.78 g/kg DM), respectively. The amount of P, Mg, Fe and Mn in *Fumaria officinalis* (5.76, 6.30, 0.66 and 0.092 g/kg DM, respectively) was the highest; however, *Medicago sativa* had higher S (2.48 g/kg DM), Cu (0.0077 g/kg DM) and K (28.15 g/kg DM) contents. The amount of Na for *Vicia sativa* (3.94 g/kg DM) was also higher than other plants.

Table 2. Mineral composition of different rangeland plants (g/kg DM) compared to *Medicago sativa*

Plant	P	Ca	K	Mg	Na	S	Mn	Cu	Fe	Zn
Medicago sativa	2.61^{b}	13.29^{b}	28.15^{a}	4.50^{b}	1.30^{b}	2.48^{a}	0.046^{b}	0.0077^{a}	0.26^{d}	0.066^{b}
Centaurea cyanus	1.23^{e}	3.78^{c}	6.25^{e}	0.69^{e}	0.18^{c}	0.32^{e}	0.026^{e}	0.0063^{b}	0.45^{b}	0.019^{d}
Fumaria officinalis	5.76^{a}	12.62^{b}	22.38^{b}	6.30^{a}	0.17^{c}	0.88^{d}	0.092^{a}	0.0015^{e}	0.66^{a}	0.12^{a}
Galium tricornutum	2.20^{c}	19.10^{a}	19.50^{c}	1.39^{d}	0.12^{d}	1.20^{c}	0.043^{c}	0.0033^{d}	0.22^{e}	0.020^{d}
Vicia sativa	1.64^{d}	13.02^{b}	17.92^{d}	2.43^{c}	3.94^{a}	1.63^{b}	0.038^{d}	0.0043^{c}	0.29^{c}	0.038^{c}
SEM	0.044	0.32	0.43	0.04	0.013	0.02	0.0003	0.000118	0.002	0.002
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in columns with different superscripts differ significantly (P<0.05).

In vitro gas test and other fermentation parameters

The gas production after 12, 24, 48 and 72 h of incubation and estimated (bgas and cgas) parameters are presented in Table 3. The IVGP after 24, 48 and 72 h of incubation as well as bgas and cgas changed when four different rangeland plants were incubated in the medium. Despite having higher 24, 48 and 72 h IVGP (*Centaurea cyanus*) as compared to other plants, there was no significant difference for these parameters between *Centaurea cyanus* and *Galium tricornutum*. The cgas and bgas were the highest for *Vicia sativa* and *Centaurea cyanus*, respectively.

Table 3. The gas production after 12, 24, 48 and 72 h incubation and estimated (bgas and cgas) parameters

Plant	bgas (ml/200mgDM)	cgas (ml/h)	gas 12 h (ml/200mgDM)	gas 24 h (ml/200mgDM)	gas 48 h (ml/200mgDM)	gas 72 h (ml/200mgDM)
Medicago sativa	59.58^{c}	0.091^{c}	38.18^{c}	49.25^{c}	56.00^{b}	59.22^{b}
Centaurea cyanus	71.31^{a}	0.076^{d}	42.75^{ab}	57.56^{a}	65.81^{a}	70.43^{a}
Fumaria officinalis	60.33^{bc}	0.099^{b}	40.96^{b}	52.72^{b}	56.92^{b}	59.30^{b}
Galium tricornutum	63.70^{b}	0.101^{b}	43.69^{a}	55.94^{ab}	63.06^{a}	68.02^{a}
Vicia sativa	51.58^{d}	0.12^{a}	37.30^{c}	44.37^{d}	47.91^{c}	51.72^{c}
SEM	1.2	0.002	0.74	1.03	1.28	1.39
P value	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001

Means in columns with different superscripts differ significantly (P<0.05).
bgas: potential gas production; cgas: constant rate of gas production; gas 12, 24, 48 and 72 h: *in vitro* gas production after 12, 24, 48 and 72 h incubation.

The NH_3-N, pH, IVDMD, ME, NEl and IVOMD of different plants incubated in the media have been shown in Table 4. The IVOMD (74.91% of DM), ME (10.10 MJ/kg DM), NEl (6.12 MJ/kg DM), and IVDMD (71.55% of DM) values were the highest in *Centaurea cyanus* and lowest in *Vicia sativa* (62.98% of DM, 8.36 MJ/kg DM, 4.89 MJ/kg DM and 59.61% of DM), respectively. The lowest NH_3-N (15.28 mg/dL) and pH (6.39) were observed for *Centaurea cyanus* and *Galium tricornutum* when incubated in the media, respectively.

Table 4. The NH_3-N, pH, *in vitro* organic matter degradability (IVDMD), metabolizable energy (ME), net energy for lactation (NEl) and *in vitro* dry matter degradability (IVOMD) of different plants incubated in the media

Plant	NH_3-N (mg/dL)	pH	IVOMD (% of DM)	ME (MJ/kg DM)	NEl (MJ/kg DM)	IVDMD (% of DM)
Medicago sativa	16.08^{b}	6.45^{a}	67.77^{c}	9.03^{c}	5.36^{c}	64.08^{c}
Centaurea cyanus	15.28^{d}	6.40^{bc}	74.91^{a}	10.10^{a}	6.12^{a}	71.55^{a}
Fumaria officinalis	15.74^{c}	6.43^{ab}	70.75^{b}	9.49^{b}	5.68^{b}	67.43^{b}
Galium tricornutum	15.43^{cd}	6.39^{c}	72.64^{ab}	9.85^{ab}	5.94^{ab}	69.59^{ab}
Vicia sativa	16.55^{a}	6.41^{abc}	62.98^{d}	8.36^{d}	4.89^{d}	59.61^{d}
SEM	0.1	0.012	0.86	0.14	0.1	0.93
P value	<0.0001	0.03	<0.0001	<0.0001	<0.0001	<0.0001

Means in columns with different superscripts differ significantly (P<0.05).
NH_3-N: ammonia nitrogen; IVOMD: *in vitro* organic matter degradability; ME: metabolizable energy; NEl: net energy for lactation; IVDMD: *in vitro* dry matter degradability.

Volatile fatty acids produced from the incubation of different plants in the media have been shown in Table 5. Referring to *Medicago sativa* as control plant, the TVFA as well as molar proportions of acetate, propionate and butyrate were affected by the treatments. The minimum and maximum TVFA concentration was observed in *Vicia sativa* and *Centaurea cyanus*, respectively. The highest and lowest acetate concentration was related to *Vicia sativa* and *Galium tricornutum*, respectively. Between different range plants, *Galium tricornutum* had also the highest propionate concentration.

Table 5. Volatile fatty acids produced from incubation of different plants in the media

Plant	TVFA (mmol/L)	Molar proportions (%)			
		Acetate	Propionate	Butyrate	Other
Medicago sativa	30.03^{c}	65.45^{cd}	20.93^{b}	8.40^{a}	5.22^{ab}
Centaurea cyanus	34.64^{a}	68.67^{ab}	20.80^{b}	5.90^{bc}	4.30^{c}
Fumaria officinalis	31.95^{b}	67.30^{cb}	20.97^{b}	6.25^{b}	5.48^{a}
Galium tricornutum	33.74^{ab}	65.08^{d}	25.18^{a}	5.31^{bc}	4.43^{bc}
Vicia sativa	27.32^{d}	69.33^{a}	20.75^{b}	4.97^{c}	4.95^{abc}
SEM	0.57	0.59	0.45	0.30	0.26
P value	<0.0001	0.001	<0.0001	0.0001	0.04

Means in columns with different superscripts differ significantly (P<0.05).
TVFA: total volatile fatty acids.

Correlation coefficient between different parameters

Correlation between some studied parameters was shown in Table 6. The IVGP after 24 h as well as bgas (P<0.0001) was positively correlated with IVOMD, IVDMD and TVFA, and was negatively correlated with NH_3-N, CP, and EE. No correlation was observed between 24 h gas production and NDF or ADF.

Table 6. Correlation between some studied parameters

	gas 24 h	bgas	IVOMD	IVDMD	TVFA	NH_3-N	CP	EE	NFE	NFC	CF	NDF	ADF
gas 24 h	1.0												
bgas	0.84****	1.0											
IVOMD	0.98****	0.86****	1.0										
IVDMD	0.99****	0.85****	0.98****	1.0									
TVFA	0.98****	0.85****	0.98****	0.99****	1.0								
NH_3-N	-0.98****	-0.084****	-0.98****	-0.99****	-0.99****	1.0							
CP	-0.71**	-0.58*	-0.65**	0.68**	-0.71**	0.71**	1.0						
EE	-0.58*	-0.52*	-0.58*	-0.56*	-0.58*	0.58*	0.58*	1.0					
NFE	0.50	0.32	0.44	0.46	0.50*	-0.50*	-0.79***	-0.51*	1.0				
NFC	0.15	-0.10	0.09	0.11	0.15	-0.15	-0.59*	-0.22	0.88****	1.0			
CF	-0.56*	-0.43	-0.57*	-0.57*	-0.56*	0.45	0.24	0.15	-0.50*	-0.34	1.0		
NDF	0.01	0.27	0.02	0.009	0.01	-0.01	-0.02	-0.27	-0.36	-0.59*	0.67**	1.0	
ADF	0.11	0.21	0.07	0.09	0.11	-0.11	-0.45	-0.18	-0.03	-0.14	0.63**	0.73**	1.0

Gas 24 h: *in vitro* gas production after 24 h incubation (ml/200 mg DM); bgas: potential gas production (ml/200 mg DM); IVOMD: *in vitro* organic matter degradability (g/kg DM); IVDMD: *in vitro* dry matter degradability (g/kg DM); TVFA: total volatile fatty acids (mmol/L); NH_3-N: ammonia nitrogen (mg/dL); CP: crude protein (% of DM); EE: ether extract (% of DM); NFE: nitrogen-free extract (% of DM); NFC: non-fiber carbohydrate (% of DM); CF: crude fiber (% of DM); NDF: neutral detergent fiber (% of DM); ADF: acid detergent fiber (% of DM). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Discussion

Chemical composition

This project has provided valuable data about the nutritive value of four rangeland plants (compared to *Medicago sativa*) supplied for use as animal feed in ruminants. The present data can supply some potential nutritional issues for ruminants when adjusting the diet. Some rangeland plants in good growth conditions can usually supply many nutrients for livestock (Kazemi *et al.*, 2009; Kazemi *et al.*, 2012). The results (Table 1) indicated that four evaluated rangeland plants have different chemical compositions as compared to *Medicago sativa*. In agreement with the present study, the OM content of *Centaurea cyanus* was reported as 18.53% of DM (Knight *et al.*, 1972). The results of Rop *et al.* (2012) indicated that edible flowers of *Centaurea cyanus* have low DM (9.74%) and CP (6.73% of DM). The CP content of the rangeland plants studied herein is much more than the minimum level of 7-8% of DM required for balanced rumen function and dry matter intake in ruminant (Van Soest, 1994). Inclusion of the feedstuffs with a lower CP content (7% of DM) in the diet of ruminant will require a supplementation of nitrogen to equilibrate their ingestion and digestion (Paterson *et al.*, 1996). The low CP contents in *Galium tricornutum* can be probably due to higher stem to leaf ratio. Susceptibility of different plants may be different to leaching or may be consisted of different proportions of stems, leaves, and flower stalks at various stages of maturity which will be effective in the nutritional value of the plant when harvesting (Arzani *et al.*, 2004). Similar results have been reported in Iran, where the CP of weed plants was observed to range between 13.28 to 25.50% of DM (Kazemi *et al.*, 2009). The chemical composition of four rangeland plants reported in this study was comparable with *Medicago sativa*. These plants are also good sources of OM with the highest concentration of NFE. The DM content of the four plants compared to *Medicago sativa* is very low (less than 24.82%). The low DM content reveals that these plants need appropriate preservation as they will be prone to deterioration. So if it is required to keep these plants longer, they should be dried in the sun. The EE content was the highest in both *Fumaria officinalis* and *Vicia sativa* as compared to *Medicago sativa* (Table 1). The amount of crude fiber for *Vicia sativa* (25.15% of DM) was lower than those reported by Al-Masri (2009) at different stages of maturity (13.87 to 21.88% of DM), but the NDF was in consistent with that study (30.61 to 42.53% of DM). The means of NDF (30.49 to 43.46% of DM), ADF (25.01 to 27.79% of DM) and ADL (3.51 to 5.75% of DM) reported by Al-Masri (1998) under different harvesting regimens were at the same range as compared to those reported for *Vicia sativa* in this experiment. It is reported that *Vicia sativa* has high crude protein (Ebelhar *et al*., 1984) compared with barley which is in consistent with the present study (23.87% of DM). The reported results of chemical composition about *Vicia sativa* were also comparable to those reported by other works (Lanyasunya *et al.*, 2006). The CP of plant is an important and limiting parameter for growth of microorganisms (Cone and Van Gelder, 1999; Blummel *et al.*, 2003). However, the CP of rangeland plants studied in this experiment was higher than that required by microorganisms in the rumen to supplying optimum activity (Gutteridge and Shelton, 1994). Both ADF and NDF values of *Medicago sativa* in this experiment were similar to those reported by National Research Council (NRC, 1989).

Mineral composition

Minerals play a critical role in many biochemical reactions of animal body (Underwood and Suttle, 1999). The mineral composition was different among four rangeland plants as compared to *Medicago sativa*. The calcium which is

essential for skeletal system and muscle development was the maximum in *Galium tricornutum* (19.10 g/kg DM). Some of essential minerals are found in insufficient amounts in diets especially consumed by ruminants. The mineral concentrations within feedstuffs are different. In addition to differences among feed type and plant species, mineral contents can be affected by soil type and pH (NRC, 2007). Different climatic conditions and plant maturity can change mineral content (NRC, 2007). Potassium and calcium were the predominant minerals in the five studied plants. The richest source of potassium in this study was related to *Medicago sativa* and other plants showed also high K contents. The daily requirements of a goat mature to potassium are about 1800-2500 mg/kg DM (NRC, 2007). It seems that the rangeland plants studied in this experiment contain more potassium (more than 8.5 times) to meet the requirements of the adult goats. Maintenance calcium requirement of goats was recommended as 20 mg/kg body weight (Haenlein, 1992). The amount of calcium in studied plants seems to be able to meet the calcium requirements of animals with an acceptable level. The lowest magnesium content was observed in *Fumaria officinalis* (6.30 g/kg DM). Magnesium plays a critical role in activating many of the enzymes and chemical reactions of the animal body (Li *et al.*, 2007). The richest source of Zinc was observed for *Fumaria officinalis* (0.12 g/kg DM). Zinc is an important mineral for all organisms, which is effective in immune system, insulin secretion (Chausmer, 1998), and release of vitamin A from liver reservoirs (Hwang *et al.*, 2002) and contributes to the structure of some key enzymes such as superoxide dismutase (Boron *et al.*, 1988; Hwang *et al.*, 2002). Underwood and Suttle (1999) have reviewed the problems that may arise due to the deficiency of some minerals for animals. The results of this study confirmed that four rangeland plants are species that will provide reasonable levels of some minerals compared to *Medicago sativa*. Iron is needed for hemoglobin synthesis and many cellular processes, but its deficiency can cause anemia and other diseases (Kaya and Incekara, 2000). At the present study, *Fumaria officinalis* with the appropriate level of iron (0.66 g/kg DM) can prevent anemia after feeding. The Mg (3.8 g/kg DM), Na (2.1 g/kg DM), P (1.3 g/kg DM), Ca (23.0 g/kg DM) and Fe (0.4 g/kg DM) contents of *Vicia sativa* reported by Gasmi-Boubaker *et al.* (2012) were relatively in range of this study.

Gas production and some fermentation factors

The IVGP parameter was different ($P<0.05$) among four rangeland plants compared to *Medicago sativa*. The mean of IVOMD (78.43 to 84.02% of DM) reported by Al-Masri (1998) at different harvesting times was higher than that reported for *Vicia sativa* (62.98% of DM) in this experiment. The bgas was reported (71.5 ml/0.2 g DM; Berhane *et al.*, 2006) in fresh cut vetch (*Vicia sativa*) which is higher than present study (51.6 ml/0.2 g DM). The *in sacco* degradation of organic matter and dry matter was 69.78 and 69.58% of DM (Lanyasunya *et al.*, 2006), respectively which is more than what we reported for *Vicia sativa* by *in vitro* technique. In another study, dry matter digestibility was reported as 64.8-71.8% of DM for different species of *Vicia* (Badrzadeh *et al.*, 2008). The 12, 24, 48, and 72 h IVGP and bgas reported for *Vicia sativa* were 41.67, 58.33, 69, and 64.83 ml/200 mg DM, respectively (Karabulut *et al.*, 2007). The IVGP has been used to assess the nutritional value of forage plants as an indicator of degradability (Kazemi *et al.*, 2012; Kazemi *et al.*, 2009). Several researchers have used IVGP characteristics to assess forage or feed quality and have found results which are comparable to degradability measures obtained by *in vitro* and *in situ* techniques (Kazemi *et al.*, 2012; Schofield and Pell, 1995; Mir *et al.*,

1998). The VFA produced from ruminal fermentation are used as the major energy source; the molar proportion of acetate, propionate and butyrate is about 95% of TVFA (Sun and Zhao, 2009). In addition to providing energy sources, rumen VFA has significant effect on nitrogen metabolism in the ruminants. In the present study, TVFA concentration and molar proportions of acetic, propionic and butyric acids in media were affected by the incubation of different plants. Molar proportions of acetic and propionic acids for *Medicago sativa* after 24 h incubation were generally in agreement with the previous reports (Schofield and Pell, 1995; Mir *et al.,* 1998). The concentration of 30.03 mmol/L TVFA for alfalfa after 48 h incubation in this study is almost two half times the value of 81.5 mmol/L reported by Mir *et al.* (1998). It is reported that TVFA production during *in vivo* and *in vitro* fermentation decreased when the substrate contained different amounts of tannin. Although the amounts of tannin present in plants of this study were not measured, the differences in TVFA production may be due to variability of tannin. Decreasing pH with the inclusion of *Galium tricornutum* in the media can be attributed to greater TVFA production in the rumen and higher NFC content. Ammonia plays a key role in the metabolism of ruminal nitrogen. The lowest concentration of NH_3-N required to achieve maximum microbial growth under *in vitro* condition is 2-5 mg/dL (Satter and Slyter, 1974), which all incubated plants in this experiment produced more ammonia nitrogen content. The ME is commonly used as a parameter for forage quality determination, but its measurement is difficult and is not always a good predictor of feeding value (Waghorn, 2007). The NEl is the net energy value of feeds or forages for maintenance, gain, pregnancy, and milk production of lactating cows (Rust *et al.,* 1995). The highest amount of ME and NEl among rangeland plants was related to *Centaurea cyanus* (10.10 MJ/kg DM and 6.12 MJ/kg DM) and *Galium tricornutum* (9.85 MJ/kg DM and 5.94 MJ/kg DM). The IVGP of alfalfa hay was similar to those reported by Ozturk *et al.* (2006) and Karabulut *et al.* (2007). The use of forage plants in animal feed is commonly limited by its low digestibility and voluntary intake; however, the IVDMD and IVOMD of four plants studied in this experiment was within acceptable range (IVDMD: 59.61 to 71.55% of DM; IVOMD: 62.98 to 74.91% of DM), respectively.

Correlation relations

The strong correlation between 24 h gas production with TVFA is in consistent with that given by Kazemi *et al.* (2012) and Getachew *et al.* (2004). The strong negative correlation between CP and 24 h gas production is not agreed with the previous reports (Ndlovu and Nherera, 1997; Larbi *et al.,* 1998) but is in consistent with Getachew *et al.* (2004). The positive correlation between 24 h gas production and VFA production ($p<0.0001$) was significantly higher than that reported by Getachew *et al.* (2004) on feed species. It seems that VFA production can be effective in gas production. Although there was a positive correlation between gas production and amount of substrate used for VFA production, it has also been reported that gas production is positively related to microbial protein synthesis (Krishnamoorthy *et al.,* 1991) and feed intake (Blummel and Ørskov, 1993). The reason of correlation between 24 h gas production and the VFA is probably related to the production of more propionate as a result of the incubation of NFC-rich feeds which is agreed with Getachew *et al.* (2004). Blummel and Ørskov (1993) reported a strong positive relationship between IVGP and short chain fatty acids (SCFA). Cone and Van Gelder (1999) found that high NH_3-N concentration due to its highly basic nature might prevent from the release of gas in the media. The existence of a negative

relationship between NH_3-N and 24 h gas production in the current experiment may be in consistent with the above hypothesis. It also reported a positive correlation ($P<0.01$) between gas 12, 24 and 48 h with IVOMD (Parissi *et al.*, 2005). In consistent with Kazemi *et al.* (2012) reports, the CP was negatively correlated (r=-0.71) with 24 h gas production. Wolin (1960) reported that the gas produced from protein fermentation is relatively smaller than carbohydrate fermentation (Wolin, 1960). The contribution of fat to gas production is very low when 200 mg coconut oil, palm kernel and/or soy bean oil were supplemented in the media, only 2.0 to 2.8 ml of gas was produced; however, when carbohydrates such as casein and cellulose were incubated, 23.4 and 80 ml gas were produced, respectively (Menke and Steingass, 1988; Getachew *et al.*, 1998). At the present study, the negative correlation ($P<0.01$) was observed between EE and 24 h IVGP (r=-0.58). In consistent with this report, a positive correlation was reported between NH_3-N and NFC by Tylutki *et al.* (2008). A poor correlation between 24 h IVGP and *in vitro* true digestibility of DM was reported by Getachew *et al.* (2004) that could be due to feed compositions such as protein and fat, which play a little role in gas production but are degraded in the culture medium.

Conclusion

Data reported about forage nutritive value of rangeland species in this experiment will be important for livestock husbandries in providing a balanced diet. Four rangeland plants (*Centaurea cyanus*, *Fumaria officinalis*, *Galium tricornutum* and *Vicia sativa*) can be recommended in ruminant (especially sheep and goat) feeding due to relatively high levels of ME, NE, IVOMD, IVDMD and optimum minerals and chemical composition as compared to *Medicago sativa*. However, further *in vivo* experiments are needed to confirm the nutritive value of these rangeland plants. The results indicate that *Centaurea cyanus* and *Galium tricornutum* grown in rangelands of Iran are two rangeland plants with high IVOMD, IVDMD, TVFA, and IVGP (12, 24, 48 and 72 h incubation) and have the potential to be a good-quality forage. Other feeding trials are also needed to determine the dry matter intake, palatability and nutritional value of these rangeland plants for small and large ruminants.

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ارزش تغذیه‌ای برخی از گیاهان مرتعی در مقایسه با یونجه

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چکیده. آگاهی از ارزش غذایی-علوفه‌ای گونه‌های مرتعی در راستای تهیه یک جیره‌ی متعادل به منظور برآورده ساختن احتیاجات تغذیه‌ای دام‌های نشخوارکننده، تعیین ظرفیت چرا و زمان مناسب چرای دام از اهمیت ویژه‌ای برخوردار می‌باشد. از این‌رو با استفاده از روش‌های مختلف آزمایشگاهی، ارزش غذایی چهار گونه مرتعی*Centaurea cyanus*، *Fumaria officinalis*، *Galium tricornutum* و *Vicia sativa* در مقایسه با یونجه (*Medicago sativa*) مورد بررسی قرار گرفتند. نمونه‌های کاملی از گیاهان فوق دقیقاً قبل از گلدهی به‌صورت تصادفی از مراتع تربت‌جام در بهار سال ۱۳۹۷ جمع‌آوری شدند. میانگین پروتئین‌خام، چربی‌خام، فیبرخام، الیاف نامحلول در شوینده‌ی خنثی، الیاف نامحلول در شوینده‌ی اسیدی، ماده آلی، لیگنین نامحلول در شوینده‌ی اسیدی، عصاره‌ی عاری از ازت و کربوهیدرات‌های غیرفیبری برای پنج گیاه مورد مطالعه به‌ترتیب برابر با ۱۹/۶۷، ۱/۷۹، ۱۹/۱۶، ۳۵/۹۶، ۲۷/۲۰، ۸۶/۹۸، ۹/۹۵، ۴۶/۳۶ و ۲۹/۵۶٪ ماده‌خشک بود. عناصر میکرو و ماکروی اندازه‌گیری شده در یک دامنه قابل قبول در مقایسه با یونجه قرار داشتند. میزان تجزیه‌پذیری ماده آلی و ماده‌خشک به‌روش برون‌تنی، تولید گاز در زمان ۲۴ ساعت انکوباسیون، انرژی قابل متابولیسم و انرژی خالص برای شیردهی به‌ترتیب برای *Centaurea cyanus* و *Galium tricornutum* بیشترین بود. پارامترهای تخمیری متفاوتی (نیتروژن آمونیاکی، اسیدهای چرب فرار و pH) در اثر انکوباسیون این گیاهان در محیط کشت مشاهده شد. تولید گاز در زمان ۲۴ ساعت انکوباسیون با تجزیه‌پذیری ماده آلی به‌روش برون‌تنی، تجزیه‌پذیری ماده‌خشک به‌روش برون‌تنی و کل اسیدهای چرب فرار همبستگی مثبتی داشته ($P<0/0001$) ولی با نیتروژن آمونیاکی محیط کشت ($P<0/0001$)، پروتئین‌خام ($P<0/01$) و چربی‌خام ($P<0/01$) گیاهان همبستگی منفی داشت. نتایج کلی نشان داد که تمام گیاهان مرتعی مورد مطالعه در این پژوهش از ارزش تغذیه‌ای مطلوبی در مقایسه با یونجه برخوردار بودند.

کلمات کلیدی: گیاهان مرتعی، یونجه، ارزش تغذیه‌ای، دام، تربت‌جام