NUTRITIONAL VALUE OF SOME RARE FORAGE PLANTS FED TO SMALL RUMINANTS †

[VALOR NUTRICIONAL DE ALGUNAS PLANTAS FORRAJERAS RARAS ALIMENTADAS A PEQUEÑOS RUMIANTES]

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SUMMARY

Background: There are some plants in the global rangelands whose nutritional value is still unknown to animal nutritionists. **Objective:** This study aimed to evaluate the *in vitro* value of some rare plants including *Marrubium vulgare* L., *Ceratocarpus arenarius* L., *Gypsophila paniculate* L., *Ferula gummosa* Boiss., and *Centaurea virgate* Lam. that are consumed by livestock. **Methodology:** The fresh samples were collected from Iran about mid-June, after the flowering stage. Buffering capacity, chemical and mineral compounds of plants were determined according to the standard methods. An *in vitro* media was also used to measure some fermentation parameters. **Results:** There were significant differences in chemical composition and mineral contents among the plants. The higher CP content was found in *M. vulgare* (173.8 g kg^{-1} DM) and *C. arenarius* (140.1 g kg^{-1} DM), respectively. The lowest ADF (166.6 g kg^{-1} DM), NDF (294.9 g kg^{-1} DM), and ADL (74.9 g kg^{-1} DM) contents were recorded in *M. vulgare*. *C. arenarius* exhibited the lowest DMD (473.3 g kg^{-1} DM), ME (4.12 MJ kg^{-1} DM), RFV (99.46), and gas volume at 24 h (13.57 ml) which were significantly different from all other plant species. The calcium content ranged from 18.15 for *C. virgata* to 37.62 g kg^{-1} DM for *G. paniculata*. The highest Na (0.64 g kg^{-1} DM), K (24.60 g kg^{-1} DM), Co (3.07 mg kg^{-1} DM), and Fe (756.53 mg kg^{-1} DM) were observed in *M. vulgare*. With the addition of *G. paniculata* to the culture medium, a noticeable increase in TVFA (40.65 mM L^{-1}) concentration was observed. The highest acid-base buffering capacity was obtained in *G. paniculata* (191.64 mEq×10^{-3}). Inclusion of *C. virgata* gave significantly higher acetate (64.07%) and butyrate (14.83%) concentration and lower propionate (19.17%) concentration in the culture medium. **Implications:** The available data can take into account in preparing a balanced diet, especially for sheep grazing in the pasture. Due to the secondary metabolites, the forage value of these plants should also be evaluated in the form of *in vivo* experiments with long-term experiment periods. **Conclusion:** According to the *in vitro* results, when these plants are fed together with a diversity of good quality forage, they can provide part of the nutrient requirement for small ruminant in Iran.

Key words: Forage quality; *In vitro*; Livestock; Plant; Pasture

RESUMEN

Antecedentes. Hay algunas plantas en los pastizales del mundo cuyo valor nutricional aún es desconocido para los nutricionistas. **Objetivo**. Este estudio tuvo como objetivo evaluar el valor *in vitro* de algunas plantas raras como *Marrubium vulgare* L., *Ceratocarpus arenarius* L., *Gypsophila paniculate* L., *Ferula gummosa* Boiss. y *Centaurea virgate* Lam. que son consumidos por el ganado. **Metodología**. Las muestras frescas se recolectaron en Irán a mediados de junio, después de la etapa de floración. La capacidad amortiguadora, los compuestos químicos y minerales de las plantas se determinaron según los métodos estándar. También se utilizó un medio *in vitro* para medir algunos parámetros de fermentación. **Resultados.** Hubo diferencias significativas en la composición química y el contenido de minerales entre las plantas. El mayor contenido de PB se encontró en *M. vulgare* (173.8 g kg^{-1} MS) y *C. arenarius* (140.1 g kg^{-1} MS), respectivamente. Los contenidos más bajos de FDA (166.6 g kg^{-1} MS), FDN (294.9 g kg^{-1} MS) y LAD (74.9 g kg^{-1} MS) se registraron en *M. vulgare. C. arenarius* exhibió la DMD más baja (473.3 g kg^{-1} MS), ME (4.12 MJ kg^{-1} MS), RFV (99.46) y volumen de gas a las 24 h (13.57 ml) que fueron significativamente diferentes de todas las demás especies de plantas. El contenido de calcio osciló entre 18.15 para *C. virgata* y 37.62 g kg^{-1} de MS para *G. paniculata*. Los niveles más altos de Na (0.64 g kg^{-1} MS), K (24.60 g kg^{-1} MS), Co (3.07 mg kg^{-1} MS) y Fe (756.53 mg kg^{-1} MS) se observaron en *M. vulgare*. Con la adición de *G. paniculata* al medio de cultivo, se observó un aumento notable en la concentración de TVFA (40.65 mM L-1). La mayor capacidad amortiguadora ácido-base se obtuvo en *G. paniculata* (191.64 mEq×10^{-3}). La inclusión de *C. virgata* dió una concentración de acetato (64.07%) y butirato (14.83%) significativamente más alta y una concentración más baja de propionato (19.17%) en el medio de cultivo. **Implicaciones.** Los datos disponibles se pueden tener en cuenta en la preparación de una dieta equilibrada, especialmente para las ovejas que pastan. Debido a los metabolitos secundarios, el valor forrajero de estas

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plantas también debe evaluarse en forma de experimentos *in vivo* con períodos de experimentación a largo plazo. **Conclusión**. Según los resultados in vitro, cuando estas plantas se alimentan junto con una diversidad de forrajes de buena calidad, pueden proporcionar parte del requerimiento de nutrientes para los pequeños rumiantes en Irán.
Palabras clave: Calidad del forraje; *In vitro*; Ganado; Planta; Pastar

INTRODUCTION

Ruminants feed sometimes on various weed forages, some medicinal-range plants or grasses whose nutritional value may be unknown to livestock producers worldwide. Therefore, knowing about the composition and nutritive value of rare plants from different countries of the word is of importance to the proper feeding of ruminants. The nutritional contribution, chemical composition, and the nutritive value of most common forages have been extensively studied. However, some plants produce secondary metabolites, which have different ecophysiological or defensive aspects (Harborne, 1999). When livestock eat plants containing secondary metabolites, these compounds are not considered as nutritional sources, but may affect ruminal fermentation and finally animal performance. Horehound (*Marrubium vulgare* L.) is a perennial herb of the *Lamiaceae* family which is commonly distributed worldwide and some of its nutritional parameters as forage feed have been less studied (Gasmi-Boubaker *et al.,* 2008). The flowers, aerial parts, and the aqueous extract of *M. vulgare* are extensively used in traditional medicine for neurosedative, anti-inflammatory, and treating cough (Mascolo *et al.*, 1987; Wichtl and Anton, 1999; Girre, 2000). It is reported that *C. arenarius* with Persian name of sandy abalak has relatively high CP content when consumed as a fodder in autumn (Jia, 1987). This plant can meet the nutrient requirements of goitred gazelle during winter (Xu *et al.*, 2012). *G. paniculata* (*Caryophyllaceae*) with common name of baby's breath is a small perennial herb that is extensively distributed worldwide and its roots have been used as a traditional Chinese herb to treat fever, consumptive disease, and infantile malnutrition syndrome (Yao *et al.*, 2010). *F. gummosa* (common name: galbanum) belongs to the *Apiaceae* family which is a perennial plant native to central Asia and Iran (Zargari, 1991). The anti-inflammatory, antinociceptive, antipyretic effects (Valencia *et al.*, 1994) and contraceptive action (Singh *et al.*, 1988; Parkash *et al.*, 1991) of *F. gummosa* have been extensively investigated. Squarrose knapweed (*Centaurea virgata*) is commonly consumed in the traditional medicine of Turkey for the treatment of gastric ulcer, diabetes, and allergy (Tüzün *et al.*, 2017). The nutritional value of the plants studied in the current experiment is still unfamiliar for most nutritionists. This study aimed to evaluate the nutritional value of some rare plants including *Marrubium vulgare* L., *Ceratocarpus arenarius* L., *Gypsophila paniculate* L., *Ferula gummosa* Boiss., and *Centaurea virgate* Lam. that are consumed by livestock using different laboratory methods.

MATERIALS AND METHODS

Plants and Study area

Samples of plant species, *Marrubium vulgare* L., *Ceratocarpus arenarius* L., *Gypsophila paniculata* L., *Ferula gummosa* Boiss, and *Centaurea virgata* Lam., were collected at the flowering stage in mid-June 2019 from Torbat-e Jam mountainous area of Razavi Khorasan province, Iran. This area (Revenj village) with an altitude of 1321 m above sea level is located at 35°18′ N and 60° 19′ E. The mean annual rainfall of Torbat-e Jam is 254 mm with a moderate air temperature of 15.7 °C. These herbs are palatable to livestock and important in Iran rangelands (Arzani and Naseri, 2009).

Laboratory analysis

Twelve samples from each of these plant species were randomly collected, mixed and immediatelly transferred to the central laboratory. Whole plant samples were oven-dried at 60 °C for 48 h to measure the dry matter (DM) content (AOAC, 1990). Acid detergent lignin (ADL), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were determined using Ankom technology (2005; 2006a; 2006b), Dacron bags. The non-fiber carbohydrate (NFC) of plants was calculated by subtraction of CP, NDF, fat, and ash from total DM (Sniffen *et al.*, 1992). The buffering capacity and pH correlated to each plant were determined using Jasaitis *et al.* (1987) method. The crude fat (Fat), crude ash (Ash), and crude protein contents (CP, Kjeldahl method) were determined using the methods described by AOAC (1999). The mineral content of samples including Ca, K, Na, Mg, Fe, Zn, Mn, and cobalt were determined based on AOAC (1990), using an atomic absorption apparatus (SavantAA, GBC, Australia).

The *in vitro* culture medium was prepared using the method described by Menke and Steingass (1988). The rumen fluid was collected from two fistulated Baluchi male sheep (30±3.5 kg) fed on a diet at the maintenance level. The rumen fluid was strained through four layers of cheesecloth, then transfered to the water bath at 39°C. About 200 mg substrate, ground through 1 mm screen, were weighted into 120 ml serum bottles. The rumen fluid and artificial saliva (1:2 ratio) were added, the serum bottles closed with rubber caps, sealed with aluminium caps and were incubated in a water bath at 39 °C for 3, 6, 9, 12, 24, 48, 72 and 96 h. The gas production was measured according to the method described by Theodorou *et al.* (1994). Four replicates were assigned to each treatment and four

serum bottles without samples were incubated as blanks.

Another four bottles (similar to *in vitro* gas production run) were used to measure ammonia nitrogen (NH_3-N), dry matter digestibility (DMD), pH, and volatile fatty acids (VFA) in the culture medium. After 24 h incubation, the bottles were opened, and their contents were filtered using a Buchner funnel equipped with the polyester filter (45 µm pore size). The filtered residuals were poured into pre-weighed crucibles and oven-dried at 60°C for 48 h. Finally, the DMD of each plant was calculated based on the amount of the initial sample weight (200 mg) and residual weight (Mauricio *et al.*, 2001). The pH of the culture medium was determined by a pH meter (Hana, Model HI 2210-01, USA) after filtering. To measure ammonia nitrogen, 5 ml of the filtered solution was mixed with 5 ml of 0.2 N HCl and stored in a freezer at –18 °C until the next tests (Komolong *et al.*, 2001). Sampling and preparation for VFA assay were performed according to the method of Getachew *et al.* (2004). Total volatile fatty acids (TVFA) and its ingredients were determined by gas chromatography (YL6100 GC; Young Lin Instrument, Anyang, South Korea) equipped with a 50 m silica-fused (0.32 mm ID) column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA, USA). Crotonic acid (trans-2-butenoic acid) and helium were used as internal standard and carrier gas, respectively. The detector and injector temperatures were set at 250 °C. The initial and final oven temperatures were 55 and 195 °C, respectively.

Estimates and data analysis

Metabolizable energy (ME) and net energy for lactation (NEl) were determined based on the equations of Menke and Steingass (1988). The data from the gas test were analyzed using the equation $P = b(1 - e^{-ct})$, where P is the volume of gas produced at time *t*, *b* the potential gas production (ml/200 mg DM), *c* the constant rate of gas production for b (%/h) and t the incubation time (h) (Ørskov and McDonald, 1979). The dry matter intake (DMI, % of live weight) was calculated based on $\text{DMI} = 120/\%\text{NDF}$, where NDF was percentage of neutral detergent fiber (Sanson and Kercher, 1996). The relative feed value (RFV) index was calculated based on the equation RFV = (% DDM×%DMI)/1.29 as described by Sanson and Kercher (1996) in which, DDM was dry matter digestibility [%DDM = 88.9-(0.779×%ADF)] and DMI dry matter intake (persentage of live weight). The data were analyzed in a completely randomized design using SAS software (2002) with the following model: Yij = µ + Ti + eij where; Yij = the value of each observation, µ = total mean, Ti = treatment effect and eij = experimental error. The treatments and fixed effects were subjected to one-way analysis of variance using the GLM of SAS (2000). Statistical differences between treatments were determined at $P < 0.05$ using Duncan's multiple range test.

RESULTS AND DISCUSSION

The chemical composition differed among the plant species (Table 1). The CP content of plants ranged from 66.9 for *C. virgata* to 173.8 g kg^{-1} DM for *M. vulgare*. The minimum ADF (166.6 g kg^{-1} DM), NDF (294.9 g kg^{-1} DM), and ADL (74.9 g kg^{-1} DM) contents were recorded for *M. vulgare* ($P < 0.05$). The highest fat (73 g kg^{-1} DM), ash (147.1 g kg^{-1} DM), and DM (447.2 g kg^{-1} fresh weight) contents were obtained in *F. gummosa*, *M. vulgare*, and *C. virgata*, respectively ($P < 0.05$). Determining the chemical composition of different plant species can help animal nutritionists to easily evaluate their nutritional value (Kazemi and Valizadeh, 2019). With this purpose, ME, NDF, ADF, DMD, and CP contents are often considered as indicators of nutritional value (Aydin *et al.*, 2007; Karabulut *et al.*, 2007; Arzani *et al.*, 2010). The plants available in the rangelands of Torbat-e Jam are usually considered as forage sources with high nutritional quality during spring and summer. However, forage quality decreases as plants mature (Arzani *et al.*, 2006). The current results showed that the evaluated plant species had different nutritive values. The chemical content of plant species may differ because of an inherent ability to extract certain nutrients from the soil and to accumulate them in their tissues (Cook and Stubbendieck, 1986). The nutritional value of range plants may also vary due to their different yields of leaves, stems, and flower stalks during different phenolic stages (Arzani, 1994; Ghodsi Rasi and Arzani, 1997; Arzani *et al.*, 2004). Crude protein is one of the most important nutrients in livestock feeding. Ewes require a diet of 70–80 g CP per day to maintain body weight (Uzun, 2010). In the current study, the CP content of all the plant species except *C. virgata* was above 7%. Therefore, feeding the ewes with presented plants (except *C. virgata*) will be able to meet the CP requirements at the maintenance level. In a study, the live weight of grazing livestock in the Zagros rangelands of Iran reported 50 kg per head (Esmaelli and Ebrahimi, 2003). Therefore, according to NRC (2007) recommendations, the plant species in the current study can easily meet the CP requirements of sheep at the maintenance level. Hence, the protein will not be a limiting nutritional factor for the sheep that use these plants. Corn and alfalfa forages are two major nutrient sources in livestock feeding. Therefore, a comparison of the chemical composition of the studied five plant species with alfalfa or maize might be helpful to determine

Table 1. Dry matter (g kg^{-1} of fresh weight) and chemical composition (g kg^{-1} of dry matter) of some rare forage plants.

	DM	Ash	ADF	NDF	ADL	NFC	CP	Fat
Marrubium vulgare L.	254.1^{d}	147.1^{a}	166.6^{d}	294.9^{c}	74.9^{d}	317.4^{b}	173.8^{a}	66.7^{b}
Ceratocarpus arenarius L.	333.8^{b}	87.5^{b}	271.7^{a}	504.3^{a}	127.4^{b}	249.1^{c}	140.1^{b}	19.0^{d}
Gypsophila paniculata L.	340.8^{b}	80.6^{bc}	188.1^{c}	372.7^{b}	100.5^{c}	388.1^{a}	119.5^{c}	39.0^{c}
Ferula gummosa Boiss.	298.8^{c}	73.5^{c}	214.0^{b}	372.0^{b}	85.6^{cd}	376.4^{a}	105.1^{c}	73.0^{a}
Centaurea virgata Lam.	447.2^{a}	39.2^{d}	266.2^{a}	505.7^{a}	148.8^{a}	364.5^{a}	66.9^{d}	23.7^{d}
SEM	4.8	2.9	4.8	5.8	5.8	9.4	5.6	2.0

$^{a-d}$ Means within each column with different superscript letters are significantly different at $P < 0.05$.
DM: dry matter; ADF: acid detergent fiber; NDF: neutral detergent fiber: ADL: acid detergent lignin; NFC: non-fiber carbohydrates; CP: crude protein; SEM: standard error of the mean.

their forage value. The CP (173.8 g kg^{-1} DM) content of *M. vulgare* was in the range of different alfalfa varieties (150.5–213.9 g kg^{-1} DM) reported by Kamalak *et al.* (2005). Gasmi-Boubaker *et al.* (2008) reported that *M. vulgare* composition in terms of DM, CP, ADF, NDF and ash was 269.2, 188.8, 241.7, 399.3, and 164.2 g kg^{-1} DM, respectively. These values of DM, CP and ash did not differ significantly with the contents in the current study (254.1, 173.8 and 147.1 g kg^{-1} DM, respectively). However, the values of ADF and NDF were higher than those recorded in the current study (166.6 and 294.9 g kg^{-1} DM, respectively). The CP was lower and ADF higher than those reported by Arzani *et al.* (2006) for *C. virgata* (83 and 474 g kg^{-1} DM, respectively). The NDF (442.5-467.3 g kg^{-1} DM) and ADF (241.5-272.4 g kg^{-1} DM) contents reported for two maize varieties by Bernard *et al.* (2004) were higher than those reported in the current study for *M. vulgare* (NDF and ADF at 294.9 and 166.6 g kg^{-1} DM, respectively). The NDF and ADF in *F. gummosa* and *G. paniculata* (372 and 372.7; 214 and 188.1 g kg^{-1} DM, respectively) were higher than those of *M. vulgare* in the current study.

Significant differences for some estimated parameters were found among the five plant species ($P < 0.05$; Table 2). The ME value of the plant species ranged from 4.12 to 7.12 MJ kg^{-1} DM for *C. arenarius* and *G. paniculata*, respectively. The RFV (230.90) and DMD (620 g kg^{-1} DM) of *G. paniculata* were significantly higher than those of the other plant species ($P < 0.05$). The ME for *G. paniculata* was within the range of values (6.86 to 9.87 MJ kg^{-1} DM) reported for some rangeland plants by Arzani *et al.* (2006). Among the plant species of the present study, *M. vulgare* showed the highest DMI percentage ($P < 0.05$). The range of ME in the present study was lower than those of reported for different maize varieties (8.65–9.76 MJ kg^{-1} DM) (Kamalak *et al.*, 2005). The DMD for *M. vulgare* (533.3 g kg^{-1} DM) was lower than that (612 g kg^{-1} DM) reported by Gasmi-Boubaker *et al.* (2008). However, DMD of *G. paniculata* at 620 g kg^{-1} DM was similar to the value recorded by Gasmi-Boubaker *et al.* (2008). The nutritional value of plants can be influenced by plant species and phenological stages (Asaadi and Daadkhah, 2010). The CP, ME, and DMD values decrease as the plant matures, and the ADF and NDF values increase (Asaadi and Daadkhah, 2010). In recent years, the relative feed value (RFV) index has been used to assess the quality of legume and grass forages and to compare plant varieties and costs associated with feeding (Redfearn *et al.*, 2008). The forages with an RFV above 151 were classified as high quality (Redfearn *et al.*, 2008). Except for *C. arenarius* (99.46), the other plants had RFV above 151 hence were considered as high-quality forages.

Table 2. Some nutritional parameters for some rare forage plants.

	ME	NEl	DMI	DMD	RFV
Marrubium vulgare L.	6.03^{c}	3.24^{c}	4.07^{a}	533.3^{d}	190.77^{b}
Ceratocarpus arenarius L.	4.12^{d}	1.89^{d}	2.38^{c}	473.3^{e}	99.46^{c}
Gypsophila paniculata L.	7.12^{a}	4.01^{a}	3.22^{b}	620.0^{a}	230.90^{a}
Ferula gummosa Boiss.	6.26^{c}	3.40^{c}	3.22^{b}	555.0^{c}	190.69^{b}
Centaurea virgata Lam.	6.57^{b}	3.63^{b}	2.37^{c}	581.7^{b}	191.82^{b}
SEM	0.08	0.06	0.05	5.8	3.36

$^{a-e}$ Means within each column with different superscript letters are significantly different at $P < 0.05$.
ME (MJ Kg^{-1} DM), metabolizable energy; NEl (MJ kg^{-1} DM), net energy for lactation; DMI (% of live weight), dry matter intake; DMD (g kg^{-1} DM), dry matter digestibility; RFV, relative feed value; SEM: standard error of the mean.

Table 3. Mineral composition of some unknown nutritional plants.

	Ca	Mg	Na	K	Zn	Mn	Co	Fe
Marrubium vulgare L.	26.60^{c}	5.18^{b}	0.64^{a}	24.60^{a}	34.53^{b}	61.10^{b}	3.07^{a}	756.53^{a}
Ceratocarpus L. *arenarius*	25.57^{c}	3.20^{c}	0.47^{b}	16.25^{b}	20.40^{c}	54.33^{c}	2.87^{ab}	572.10^{b}
Gypsophila paniculata L.	37.62^{a}	6.23^{a}	0.38^{b}	6.13^{d}	19.50^{c}	38.80^{d}	2.47^{bc}	447.90^{c}
Ferula gummosa Boiss.	33.07^{b}	3.01^{c}	0.40^{b}	12.76^{c}	56.50^{a}	96.43^{a}	2.60^{bc}	230.80^{d}
Centaurea virgata Lam.	18.15^{d}	2.86^{c}	0.39^{b}	5.93^{d}	22.47^{b}	37.87^{d}	2.37^{c}	263.03^{d}
SEM	1.26	0.14	0.05	0.38	1.69	1.64	0.14	25.25

$^{a-d}$ Means within each column with different superscript letters are significantly different at $P < 0.05$.
Ca (g kg^{-1} DM): calcium; Mg (g kg^{-1} DM): magnesium; Na (g kg^{-1} DM): sodium; K (g kg^{-1} DM): potassium; Zn (mg kg^{-1} DM): zinc; Mn (mg kg^{-1} DM): manganese; Co (mg kg^{-1} DM): cobalt; Fe (mg kg^{-1} DM): iron; SEM: standard error of the mean.

The highest Ca (37.62 g kg^{-1} DM) and Mg (6.23 g kg^{-1} DM) concentrations were recorded in *G. paniculata* (Table 3). The highest Na and K (0.64 and 24.60 g kg^{-1} DM); Co and Fe (3.07 and 756.53 mg kg^{-1} DM) concentrations were recorded in *M. vulgare*. *F. gummosa* had the highest Mn and Zn (96.43 and 56.50 mg kg^{-1} DM). Cobalt content ranged from 2.37 mg kg^{-1} DM for *C. virgata* to 3.07 mg kg^{-1} DM for *M. vulgare*. The mineral contents in *M. vulgare* reported by Gasmi-Boubaker *et al.* (2008), except Zn, were lower than the concentrations recorded in the current study. However, the Zn concentration in the current study was similar to the value reported by Gasmi-Boubaker *et al.* (2008). The concentration of Zn in alfafa was higher than that in *M. vulgare* but the content of all the other minerals were higher in *M. vulgare*. The content and composition of minerals in plants grazed by livestock play a major role in meeting the mineral requirements of the livestock. Underwood and Suttle (1999) have presented numerous reports about mineral requirements and problems associated with mineral deficiency in animals. Hence, the determination of minerals concentration in plants grown in the rangelands is useful to prevent occurrence of mineral deficiencies in livestock that graze on these plants.

There was a significant difference for gas production parameters among the different plant species (Table 4) ($P < 0.05$). The gas production at 12, 24, 48, and 72 h incubation (28.93, 35.70, 40.82, and 45.90 ml, respectively) and potential gas production (bgas, 44.82 ml) were highest for *G. paniculata* ($P < 0.05$). *F. gummosa* showed the highest constant rate of gas production (cgas, 0.11 %/h, $P < 0.05$). The gas produced at 24 h ranged from 13.57 in *C. arenarius* to 35.70 ml in *G. paniculata*. The low cost and high availability of the gas test method have made it popular in use for *in vitro* studies. The method has been used for many years to determine the nutritive value of some plants under artificial ruminal fermentation conditions (Kazemi and Valizadeh, 2019; Kazemi, 2019). One of the most important reasons for using the gas production technique is to determine the digestibility and energy value of ruminant feeds (Krishnamoorthy *et al.*, 2005). Kazemi (2019) found a strong positive correlation between 24 h gas production and DMD. It is suggested that part of the increase in the gas production of *G. paniculata* might be due to an increase in the DMD. Having a strong positive correlation between bgas and TVFA (Kazemi and Valizadeh, 2019; Kazemi, 2019), it is suggested that the increased gas production could be due to excess TVFA produced by *G. paniculata*.

Table 4. *In vitro* gas production parameters for some rare forage plants.

	bgas	cgas	gas12 h	gas 24 h	gas 48 h	gas 72 h
Marrubium vulgare L.	37.50^{c}	0.066^{c}	19.50^{c}	27.41^{d}	32.77^{d}	34.68^{cd}
Ceratocarpus arenarius L.	35.77^{d}	0.072^{c}	8.10^{d}	13.57^{e}	20.92^{e}	32.37^{d}
Gypsophila paniculata L.	44.82^{a}	0.089^{b}	28.93^{a}	35.70^{a}	40.82^{a}	45.90^{a}
Ferula gummosa Boiss.	35.84^{d}	0.11^{a}	25.03^{b}	29.37^{c}	35.72^{c}	36.75^{c}
Centaurea virgata Lam.	40.83^{b}	0.085^{b}	25.0^{b}	31.95^{b}	38.37^{b}	41.95^{b}
SEM	0.48	0.002	0.59	0.62	0.78	0.77

$^{a-e}$ Means within each column with different superscript letters are significantly different at $P < 0.05$.
bgas: potential gas production (ml); cgas (%/h): constant rate of gas production; gas 12, 24, 48 and 72 h (ml/200 mg DM of sample): cumulative gas production after 12, 24, 48, and 72 h incubation; SEM: standard error of the mean.

Table 5. Total volatile fatty acid (mM L^{-1}), individual volatile fatty acid (% of TVFA), NH_3-N (mg dL^{-1}) and pH of media after rare plants incubation.

	TVFA	Acetate	Propionate	Butyrate	Valerate	Isovalerate	pH	NH_3-N
Marrubium vulgare L.	37.08^{d}	62.70^{ab}	21.97^{b}	12.77^{b}	1.37	0.50	6.95^{ab}	32.08^{a}
Ceratocarpus arenarius L.	31.13^{e}	59.90^{c}	24.20^{a}	13.40^{b}	1.42	0.52	6.98^{a}	30.67^{ab}
Gypsophila paniculata L.	40.65^{a}	61.50^{b}	22.63^{b}	13.33^{b}	1.37	0.43	6.82^{c}	28.97^{bc}
Ferula gummosa Boiss.	37.93^{c}	62.67^{ab}	22.10^{b}	13.40^{b}	1.25	0.43	6.89^{bc}	28.50^{bc}
Centaurea virgata Lam.	39.04^{b}	64.07^{a}	19.17^{c}	14.83^{a}	1.33	0.42	6.87^{bc}	27.77^{c}
SEM	0.27	0.50	0.33	0.39	0.08	0.05	0.02	0.84

$^{a-e}$ Means within each column with different superscript letters are significantly different at $P < 0.05$.
TVFA: Total volatile fatty acids; SEM: standard error of the mean.

The highest TVFA (40.65 mM L^{-1}), propionate (24.20%) and butyrate (14.83%) were recorded in *G. paniculata*, *C. arenarius*, and *C. virgata*, respectively. Acetate concentration ranged from 59.9% for *C. arenarius* to 64.07% for *C. virgate* (Table 5). Valerate and isovalerate concentrations were not affected by the experimental treatments. The range of pH and NH_3-N was 6.82–6.98 and 27.77–32.08 mg dL^{-1}, respectively. The fermentation in the rumen can be affected by the type of feed consumed by the animals. On the other hand, the rumen also produces various other products such as VFA, ammonia nitrogen and microbial protein (Blümmel *et al.*, 1997; Megías *et al.*, 2014). The VFA are the most important by-products of the fermentation of carbohydrates in the rumen and the main energy source for ruminants. The VFA contribute 50–75% of the total energy supply to the ruminant (Faverdin, 1999). Acetate is the major precursor of milk fat synthesis in the ruminants and propionate is a precursor of milk sugar synthesis (Tagang *et al.*, 2010). A positive correlation between CP and NH_3-N concentration in the culture medium supported the high CP degradability in all the five plant species as reported by Getachew *et al.* (2004). In the current study, it would seem that *M. vulgare* produced more NH_3-N in the medium due to its higher protein content.

Buffers are substances that neutralize the excess acid produced by the digestion of nutrients in the rumen fluid and prevent sudden changes in pH that would occur as a result of excessive consumption of cereals (Bujňák *et al.*, 2011). Among the plant species, *G. paniculata* had the highest plant pH (6.27), and titratable acidity (273.75 $mEq\times10^{-3}$) ($P < 0.05$). The higher titratable alkalinity (243.0 $mEq\times10^{-3}$) and base-buffering capacity (71.53 $mEq\times10^{-3}$) were obtained in *M. Vulgare* ($P < 0.05$). *C. virgata* had the lowest acid-base buffering capacity (129.89 $mEq\times10^{-3}$) ($P < 0.05$). The buffering system in the rumen is controlled by three major mechanisms, including the salivary buffer system, the buffering capacity of feed and the buffering additives (Moharrery, 2007). It is reported that the initial pH and the titratable acidity are the two major indices that affect the rumen pH. The titratable acidity shows the number of acid equivalents required to reduce pH to 4 (Jasaitis *et al.*, 1987). In the present study, the lowest titratable acidity was observed in *C. virgata* (116.25 $mEq\times10^{-3}$), indicating a lower resistance to acidification. Due to the different ash contents for the studied plants, different buffering capacities were recorded among them. The buffering capacity of some protein sources and leguminous fodder have been reported to be greater than 85 $mEq\times10^{-3}$ (Montanez-Valdez *et al.*, 2013), which is consistent with the current study. The titratable alkalinity indicates the number of base equivalents required to increase the pH to 9 (Jasaitis *et al.*, 1987). The lowest titratable alkalinity in the current study was recorded in *C. arenarius* at 190.50 $mEq\times10^{-3}$. Indeed, the acid-buffering capacity is the amount of acid needed to change a pH unit in a feed sample (water soluble). The highest acid-buffering capacity in *G. paniculata* (120.33 $mEq\times10^{-3}$), indicated that more acid is needed to achieve a unit change in the pH of its water-soluble sample.

The base-buffering capacity is the number of base equivalents required (such as 0.1 N NaOH) for a unit pH change in a feed sample (water-soluble). The highest value of base-buffering capacity was observed in *M. vulgare* and *G. paniculata*, respectively. The highest value of acid-base buffering capacity in *M. vulgare* and *G. paniculata* ($P < 0.05$) indicated that these plants were more efficient in ruminal pH control.

Table 6. The pH and buffering capacity of some rare forage plants.

	Plant pH	Titratable acidity ($mEq\times10^{-3}$)	Acid-buffering capacity ($mEq\times10^{-3}$)	Titratable alkalinity ($mEq\times10^{-3}$)	Base-buffering capacity ($mEq\times10^{-3}$)	Acid-base buffering capacity ($mEq\times10^{-3}$)
Marrubium vulgare L.	5.90^{b}	234.50^{b}	123.63^{a}	243.0^{a}	71.53^{a}	195.16^{a}
Ceratocarpus arenarius L.	5.86^{b}	152.0^{d}	81.70^{c}	190.50^{c}	57.60^{d}	139.30^{c}
Gypsophila paniculata L.	6.27^{a}	273.75^{a}	120.33^{a}	211.25^{b}	71.31^{a}	191.64^{a}
Ferula gummosa Boiss.	5.86^{b}	173.75^{c}	93.54^{b}	213.25^{b}	64.48^{b}	158.02^{b}
Centaurea virgata Lam.	5.65^{c}	116.25^{e}	70.56^{d}	210.75^{b}	59.33^{c}	129.89^{d}
SEM	0.02	2.54	1.70	1.43	0.53	1.80

[a-d] Means within each column with different superscript letters are significantly different at $P < 0.05$.
SEM: standard error of the mean.

CONCLUSIONS

This study showed that some rare forage plants including *Marrubium vulgare* L., *Ceratocarpus arenarius* L., *Gypsophila paniculata* L., *Ferula gummosa* Boiss., and *Centaurea virgata* Lam. have potential nutritional value for livestock in terms of their sources of dry matter and its nutrient composition, macro and micro nutrients. Concerning some chemical composition, *in vitro* gas production, TVFA, ME, RFV, and DMD, it seemed that *G. paniculata* had the highest potential to be used as a good forage source in small ruminants feeding compared with all the studied plant species. The *M. vulgare* can also be considered as a valuable plant for feeding small ruminans because of its relatively high CP and low NDF and ADF contents. Overall, the physiological and functional responses of ruminants to the consumption of these plants should also be investigated.

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Compliance with ethical standard. All experimental procedures in this project were approved by the research Committee at the University of Torbat-e Jam.

Data availability. Data are available with Mohsen Kazemi (email: phd1388@gmail.com), upon reasonable request.

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