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Ruminal fermentation kinetics of nine halophytic tree species at different growth stages

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Abstract Halophytic plant species have been used for forage from ancient periods due to their nutritional constituents. Additionally, there is little information on the effect of the consumption of halophytic plant species on the emission of fermentation gas by ruminants. This context was aimed to assess the chemical composition, mineral contents, and fermentation gas mitigation properties of nine different halophytic plants species, collected from south-eastern region of Iran. The minerals such as calcium (Ca), sodium (Na), and potassium (K) were estimated using atomic absorption spectroscopy, and phosphorus (P) by spectrophotometer. The mineral contents of halophytes were within the usual ranges. The rate of fermentation gas production (GP) among plants was calculated according to the standard protocol in a time-dependent manner (2–96 h) and revealed the characteristics of these halophytes. The GP was reduced for halophytes from vegetative and flowering stages to seed ripening stage. Parameters such as metabolisable energy, organic matter digestibility, and digestible organic matter in dry matter were estimated and observed to be decreased ($P < 0.05$) among different growth stages of halophytes. The evaluation of chemical constituents, mineral components, and other parameters established the promising role of these halophytic plant species as efficacious forage resources for ruminants. Furthermore, these halophytes may be used as valuable feeding stuffs for ruminants due to the mitigation characteristics of fermentation gas and less use of energy-rich supplements, respectively.

Keywords Chemical composition · Gas production · Halophytes · Mineral components · Ruminants

Introduction

The deficiency of feed resources in arid and semi-arid regions is considered as major concerns and important limitations globally in order to improve the

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productivity and sustainability of animals. Currently, ruminant production had faced problems of low availability of nutritionally rich forages (Rojas Hernández et al. 2015). In livestock systems, the ruminants and their productivity are severely influenced by soil salinisation. As a consequence of this, the rangelands contribution to ruminant feeding reduced drastically (Ben Salem et al. 2010). In fact, rangelands are the pivotal sources for diversified feeds resource-deficient agro-pastoral communities (Larbi et al. 2009). Ruminant livestock produces greenhouse gases such as methane (CH_4), carbon dioxide (CO_2), and other gases through the fermentative process in the presence of available nutrients that increase environmental pollution. According to FAO (FAO 2008), the contribution of CH_4 production and CO_2 emission from livestock accounts for about 18% and 9%, respectively. Thus, there is emerging trend in reducing the emission of greenhouse gas by ruminants without altering the rumen function (Elghandour et al. 2016a). Researchers have already adopted several strategies such as the use of exogenous enzymes (Kholif et al. 2016), organic acid salts (Elghandour et al. 2016b), and yeast (Elghandour et al. 2017) in order to reduce the emission of greenhouse gases. However, new strategies as well as practices need to be employed in order to upgrade sustainability of natural resources, to improve ruminant livestock systems, and to mitigate the emission of detrimental gases in the environment. In connection with this, halophytes could be an appropriate alternative for capturing and sequestering greenhouse gases in order to alleviate its detrimental influence (Glenn et al. 1993).

Halophytes are plants that have the unique capability to tolerate saline and alkaline soils. In several parts of the world, native and introduced halophytes are used as forage, particularly as substitute for feeds which are scanty (Ben Salem et al. 2004). In fact, there has been immense interest in utilising halophytes as forage in the salt-stressed agricultural regions to enhance the productivity of livestock. However, several parameters influence their chemical composition, palatability, and nutritive value (El-Shatnawi and Abdullah 2003).

In the last few years, a significant interest has been grown to determine the nutritional value of halophytes (Moujahed and Kayouli 2005). The feed intake as well as the feeding value of halophytes are affected by high salt and mineral concentrations. These plants are known for osmotic adjustment in response to salt stress (Guo et al. 2005), exhibiting useful role for the growth of plant and livestocks (Norman et al. 2013). In fact, the variable constituents of halophytes play a pivotal role on feeding value. Similarly, the presence of bioactive constituents in halophytes has potentially profound effects on the grazing ruminants.

In the present scenario, the main challenge for researchers in developing halophyte-derived feeding system is to identify plants with appropriate composition that can be easily metabolised by the animals at low metabolic cost and add benefit to the animals as well as environment. This investigation was aimed to evaluate the chemical and mineral composition of nine selected halophyte tree species from south-eastern region of Iran, and further explore the greenhouse gas mitigation ability of halophytes when integrating with the feeding systems.

Materials and methods

Sampling zone and collection

Nine halophytic tree plants species were collected from the south-eastern region of Iran (Zābol, Sistan and Baluchestan Province, Iran). The location is present at an altitude of 483 m above sea level. The mean annual rainfall and temperature are 58 mm, 22 °C, wind *N* at 76 km/h, 24% humidity, respectively. Plant samples were collected from winter of 2015 to summer of 2016 at various growth stages from the rangelands of Sistan region, Iran, using stratified random sampling tool. The first growth in the vegetative (leaf development) stage was collected as cut I from March 30 to April 12. The flowering (floral development) stage was collected after 40 days of the previous cut (cut II, late spring, from May 29 to June 6), then 42 days later (cut III, late summer, July 20 to July 30) seed (seed development and ripening) stage was collected.

Halophyte plants included in the present study were four grasses of the family *Poaceae*, namely *Aeluropus lagopoides* (L.) Thwaites, *Aeluropus littoralis* (Gouan) Parl., *Lolium perenne* L. and *Avena fatua* L., the two *Compositae Carthamus lanatus* L. and *Artemisia maritima* L. and three species of other families *Atriplex leucoclada* Boiss. (*Amaranthaceae*), *Prosopis farcta* (Banks & Sol.) J.F.Macbr.

(*Leguminosae*), and *Zygophyllum eurypterum* Boiss. & Buhse (*Zygophyllaceae*). Each species corresponds to 25 individual plants. In view of homogeneity, samples from each species were collected in the vegetation community. The fresh-cut samples were dried inside the laboratory at room temperature (28–30 °C) and ground for experimental analyses.

Chemical and mineral composition

Dry matter (DM) was estimated by drying the samples overnight at 105 °C (AOAC 1990). Dried samples were also analysed for the presence of ether extract (EE) according to AOAC (2000). The ash was obtained by ignition of the samples for 8 h in a muffle furnace at 550 °C (AOAC 1990). Nitrogen level was estimated by the Kjeldahl method (AOAC 1990). Crude protein (CP) was estimated as nitrogen (N) × 6.25 (AOAC 1990). Concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) of samples were determined according to the methodology of Van Soest and Robertson (1990). The minerals such as calcium (Ca), sodium (Na), and potassium (K) were estimated using atomic absorption, and phosphorus (P) by spectrophotometer (AOAC 1990). All chemical and mineral analyses were carried out in triplicate.

Gas production

Three fistulated native cattle, fed twice daily on a diet of lucerne hay (60%) and concentrate (40%), were used for obtaining rumen fluid. The rate of GP was estimated according to the methodology of Menke and Steingass (1988) with slight modifications. Approximately 200 mg of samples was added to 30 ml of rumen fluid/buffer (1:2) mixture in calibrated glass syringes. Further, the mixture was incubated at 39 °C in a water bath. Blank included buffered rumen fluid only. Prior to dispensing 30 ml of rumen fluid–buffer mixture into each syringe, the syringes were pre-warmed at 39 °C. The rate of GP was recorded before incubation (t_0) and up to 96 h of incubation. Total gas values were corrected for blanks at each incubation time. Volumes of gas at standard temperature (273.15 K) and pressure (10^5 Pa) were calculated from the actual recorded gas volumes and the temperature and atmospheric pressures at the time of measurement (López et al. 2007) The fermentation gas standard volumes were fit to the equation given below, as mentioned by Orskov and McDonald (1979):

$$P = b(1 - \mathrm{e}^{-ct}), \tag{1}$$

where P is the gas production at time t, b is the potential gas production (ml), c is the gas production rate constant (ml/h), and t is the incubation time (h).

The metabolisable energy (ME; MJ/kg DM) of samples was estimated according to Menke et al. (1979) as given below:

$$\mathrm{ME}\ (\mathrm{MJ/kg\ DM}) = 2.20 + 0.136\mathrm{GP} + 0.057\mathrm{CP}\ \left(R^2 = 0.94\right), \tag{2}$$

where GP is 24-h biogas production (ml/200 mg) and CP is crude protein (%).

The in vitro organic matter digestibility (OMD) of foliages was estimated using the equation of Menke et al. (1979) as follows:

$$\mathrm{OMD}\ (\%) = 14.88 + 0.889\mathrm{GP} + 0.45\mathrm{CP} + 0.0651\mathrm{XA}, \tag{3}$$

where GP is 24-h gas production (ml/200 mg), CP is crude protein (%), and XA is ash content (%).

Digestible organic matter in dry matter (DOMD) is calculated as:

$$\mathrm{DOMD}\ (\%) = \mathrm{OMD} \times \mathrm{OM}, \tag{4}$$

where OMD is organic matter digestibility and OM is organic matter

Statistical analyses

General linear model (GLM) of SAS (2002) was used for estimating chemical composition, in vitro GP kinetics, OMD, and ME contents of samples. The model includes:

$$Y_{ijk} = \mu + S_i + \varepsilon_{ijk}, \tag{5}$$

where Y_{ijk} indicates observation on chemical composition, in vitro GP kinetics, OMD, and ME contents; S_i represents the influence of species on the parameters, and ε_{ijk} is the standard error term.

Turkey's multiple range test was used for calculating significant differences between means, and values representing $P < 0.05$ were considered significant. Residual mean square in the analysis of variance was used for calculating standard errors of means.

Data were also reanalysed according to the equation given below:

$$Y_{ij} = \mu + \text{block}_i + T_j + e_{ij} + \varepsilon_{ijk}, \qquad (6)$$

where i is 1…3 stage, j is 1…9 treatment, and k is 1…3 sample.

Results

Chemical composition

Among various halophytes, DM increased from 93.9% (*C. lanatus*), 94.6% (*A. littoralis*), 94.8% (*P. farcta*), 94.9% (*A. leucoclada*), 95.4% (*Z. eurypterum*), 95.6% (*A. maritima*), 96.0% (*A. fatua*), 96.1% (*L. perenne*) to 96.5% (*A. lagopoides*). The percentage DM was found to be increased at different growth stages, viz. vegetative (94.7%), flowering (95.3%), and seed (95.9%) - (Table 1).

The OM in the halophytes ranged from 64.6% (*A. fatua*) to 94.9% (*C. lanatus*). However, the OM was found to be decreased at vegetative (82.6%), flowering (80.6%), and seed stages (75.6%).

The ash content varied significantly ($P < 0.05$) from 5.1% in *C. lanatus* to 35.4% in *A. fatua.* The highest ash content (24.5%) was recorded in the seed stage, followed by flowering (19.5%) and vegetative stages (17.4%). Variations between ash contents of halophytes were found to be non-significant at all growth stages.

The CP content among halophytic plants ranged from 7.9% (*A. maritima*), to 14.3% (*A. lagopoides*) and varied significantly ($P < 0.05$). The CP content was decreased significantly ($P < 0.05$) from vegetative (13.7%) and flowering (10.6%) to seed stages (8.2%).

The differences between ether extract (% EE) content of halophytes were non-significant and estimated in the increasing order of *C. lanatus* $<$ *A.*

Table 1 Chemical composition and mineral contents of halophytic plant species

	Chemical composition (%)							Mineral contents (%)			
	DM	OM	Ash	CP	EE	ADF	NDF	Ca	P	Na	K
Plant species											
Aeluropus littoralis	94.6	92.1^{a}	7.9^{c}	9.4	4.1	48.4^{a}	54.1^{ab}	0.80	0.16	0.55^{d}	0.66
Aeluropus lagopoides	96.5	67.8^{bc}	32.2^{ab}	14.3	4.2	28.2^{c}	37.4^{c}	0.21	0.27	19.01^{b}	0.18
Carthamus lanatus	93.9	94.93^{a}	5.07^{c}	14.0	2.6	40.4^{abc}	46.1^{bc}	0.93	0.16	0.67^{d}	0.53
Avena fatua	96.0	64.6^{c}	35.4^{a}	10.1	3.4	34.7^{bc}	43.2^{bc}	0.35	0.30	24.89^{b}	0.18
Artemisia maritima	95.6	88.0^{ab}	12.0^{bc}	7.9	2.7	41.6^{ab}	48.3^{abc}	1.27	0.30	1.38^{d}	0.45
Atriplex leucoclada	94.9	68.3^{bc}	7.9^{c}	13.4	3.3	29.3^{bc}	39.6^{bc}	0.23	0.23	38.79^{a}	0.18
Lolium perenne	96.1	67.3^{bc}	32.7^{ab}	9.5	3.9	29.4^{bc}	41.2^{bc}	0.36	0.39	16.02^{bc}	0.147
Zygophyllum eurypterum	95.4	86.0^{abc}	14.0^{abc}	8.5	3.1	38.9^{abc}	61.4^{a}	0.40	0.10	3.73^{cd}	1.04
Prosopis farcta	94.8	86.9^{abc}	13.1^{abc}	10.6	3.1	35.7^{bc}	42.7^{bc}	1.07	0.13	0.53^{d}	3.53
SEM	0.459	0.962	0.962	0.465	0.451	0.589	0.458	0.083	0.119	0.997	0.071
P value	0.511	0.0005	0.0005	0.0361	0.193	0.0004	0.0006	0.012	0.193	< 0.0001	0.1117
Growth stage											
Vegetative	94.7	82.6	17.4	13.7^{a}	4.0^{a}	30.6^{b}	40.6^{b}	0.60	0.23	13.9	1.37
Flowering	95.3	80.5	19.4	10.6^{b}	3.3^{ab}	35.5^{b}	45.7^{ab}	0.58	0.28	11.9	0.66
Seed	95.9	75.5	24.4	8.2^{b}	2.7^{b}	42.7^{a}	51.6^{a}	0.66	0.17	9.4	0.26
P value	0.275	0.188	0.188	0.001	0.014	< 0.0001	0.0011	0.898	0.218	0.151	0.222

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *ADF* acid detergent fibre, *NDF* neutral detergent fibre, *Ca* calcium, *P* phosphorus, *Na* sodium, *K* potassium, *SEM* standard error of the mean, $P < 0.05$—significant, $P > 0.05$—non-significant

^{a,b,c,d}Mean values within each factor with different letters differ ($P < 0.05$)

maritima < *Z. eurypterum* < *P. farcta* < *A. leucoclada* < *A. fatua* < *L. perenne* < *A. littoralis* < *A. lagopoides*. The highest EE contents of halophytes were achieved at vegetative stage (4.0%), which was further narrowed down to flowering (3.3%) and seed stage (2.7%) at significant level.

The structural constituents (ADF and NDF) of halophytes were observed significantly different ($P < 0.05$). The ADF content varied from 28.15% (*A. lagopoides*) to 48.4% (*A. littoralis*). The percentage of ADF content in different growth stages was found to be increased from vegetative (30.6%) and flowering (35.5%) to seed stages (42.7%) and varied significantly. Similarly, the NDF contents of halophytes were estimated in the order of *A. lagopoides*, *A. leucoclada*, *L. perenne*, *P. farcta*, *A. fatua*, *C. lanatus*, *A. maritima*, *A. littoralis* and *Z. eurypterum*. Likewise, the NDF contents at different growth stages varied significantly and were estimated in the order of 40.6% (vegetative) < 45.7% (flowering) < 51.6% (seed).

Mineral composition

Total content of Ca in all halophytes varied significantly, showing highest amount of Ca in *A. maritima*, followed by *P. farcta*, *C. lanatus*, *A. littoralis*, *Z. eurypterum*, *L. perenne*, *A. fatua*, *A. leucoclada*, and *A. lagopoides*. However, the seed stage showed maximum content of Ca in comparison with vegetative and flowering stages (Table 1).

The highest and lowest contents of P were estimated in *L. perenne* and *Z. eurypterum*, respectively. The *P* content in the growth stages of halophytes was observed in the order of 0.28% (flowering) > 0.22% (vegetative) > 0.175% (seed). The maximum amount of Na and K was estimated in *A. leucoclada* and *P. farcta*, respectively, whereas *P. farcta* and *L. perenne* depicted the lowest amount of Na and K, respectively. The Na content in the growth stages of halophytes was observed to be decreased from vegetative and flowering to seed stage. Similarly, K content in the growth stages of halophytes was observed in the order of seed (0.26%) < flowering (0.66%) < vegetative (1.37%). The differences between Na and K contents of halophytes were observed non-significant ($P > 0.05$) at all growth stages.

Gas production volume

P. farcta was found to be a potent halophyte as a fodder, producing minimum amount of gas after 24 h and 96 h. However, *A. littoralis* showed maximum amount of gas production in a time-dependent manner, especially after 24 h and 96 h. The other plant species showed moderate level of gas production after 96 h. The variations among the GP ability were found to be significantly ($P < 0.05$) among halophyte species. Likewise, the gas emitted in the presence of halophytes was found to be mitigated at seed stage, followed by flowering and vegetative stages in a time-dependent manner. After 24 h, 24.6, 28.7, and 31.6 ml of gases per 200 mg DM were produced at seed, flowering, and vegetative stages, respectively, whereas 32.4, 37.9, and 40.0 ml of gases per 200 mg DM were produced at seed, flowering and vegetative stages, respectively after 96 h (Table 2).

Estimation of cumulative gas production and other parameters

Among halophytes incorporated as fodder, *P. farcta* depicted promising gas mitigation property in terms of the lowest amount of gas produced. *A. littoralis* caused the highest amount of gas production from the ruminants. Apart from this, other halophytes showed moderate level of gas production in the order of *C. lanatus* (41.9 ml) > *A. lagopoides* (38.6 ml) > *A. leucoclada* (37.3 ml) > *A. maritima* (35.8 ml) > *L. perenne* (34.4 ml) > *A. fatua* (33.3 ml) > *Z. eurypterum* (26.0 ml). The seed stage of halophytes released lower ($P < 0.05$) amount of gas in comparison with vegetative and flowering stages (Table 3).

In Table 3, The ME was the highest in *C. lanatus*, followed by *A. littoralis*, *A. maritima*, *A. leucoclada*, *A. fatua*, *A. lagopoides*, *Z. eurypterum*, *L. perenne*, and then *P. farcta*. Vegetative stage showed the highest ($P < 0.05$) ME, followed by flowering and seed stages.

The OMD for halophytes varied from 38.6% (*P. farcta*) to 59.4% (*C. lanatus*). The OMD values for growth stage varied from 44.5% (seed) and 49.3% (flowering) to 53.5% (vegetative).

The DOMD values among halophytic plants species fluctuated significantly from 30.27% in *L. perenne* to 56.4% in *C. lanatus*. The DOMD content in

Table 2 Gas production volume (ml/200 mg DM) of halophytic plant species at different incubation periods

	2 h	4 h	6 h	8 h	12 h	24 h	48 h	72 h	96 h
Plant species									
Aeluropus littoralis	4.7^{bc}	10.7^{bc}	13.2^{bc}	16.5^{bc}	23.7^{ab}	35.9^{a}	43.5^{a}	46.6^{a}	48.6^{a}
Aeluropus lagopoides	3.7^{cd}	6.3^{d}	9.2^{c}	10.9^{c}	15.7^{bc}	27.2^{bc}	33.7^{bc}	37.0^{bc}	38.0^{bc}
Carthamus lanatus	7.6^{a}	17.0^{a}	21.8^{a}	26.3^{a}	30.4^{a}	35.7^{ab}	40.0^{ab}	42.9^{ab}	44.9^{ab}
Avena fatua	3.8^{cd}	8.6^{cd}	12.2^{bc}	15.4^{bc}	23.4^{ab}	28.0^{abc}	30.1^{cd}	33.4^{cd}	34.7^{cd}
Artemisia maritima	6.2^{abc}	14.3^{ab}	17.8^{ab}	21.8^{ab}	26.6^{a}	30.8^{abc}	33.5^{bc}	37.0^{bc}	38.3^{cd}
Atriplex leucoclada	4.1^{cd}	8.8^{cd}	14.0^{bc}	17.1^{bc}	22.9^{ab}	30.7^{abc}	35.3^{bc}	37.1^{bc}	38.1^{bc}
Lolium perenne	3.9^{cd}	7.1^{cd}	9.4^{c}	11.2^{c}	14.6^{bc}	25.4^{cd}	31.4^{cd}	33.8^{cd}	33.8^{cd}
Zygophyllum eurypterum	7.2^{ab}	14.4^{ab}	16.6^{ab}	19.2^{ab}	21.9^{abc}	24.1^{cd}	24.7^{de}	26.9^{de}	28.1^{d}
Prosopis farcta	1.7^{d}	6.7^{d}	8.3^{c}	10.6^{c}	13.4^{c}	17.0^{d}	20.4^{e}	24.4^{e}	26.6^{d}
SEM	0.49	0.68	1.06	1.46	1.72	1.57	1.48	1.47	1.55
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Growth stage									
Vegetative	4.9	10.9	14.7^{a}	18.4^{a}	24.4^{a}	31.6^{a}	35.7^{a}	38.8^{a}	40.0^{a}
Flowering	4.9	10.6	13.8^{ab}	16.5^{ab}	21.3^{ab}	28.7^{a}	33.8^{a}	36.5^{a}	37.9^{a}
Seed	4.5	9.7	12.3^{b}	14.7^{b}	18.5^{b}	24.6^{b}	28.0^{b}	31.0^{b}	32.4^{b}
P value	0.587	0.116	0.046	0.022	0.003	0.0002	< 0.0001	< 0.0001	< 0.0001

SEM standard error of mean, $P < 0.05$—significant, $P > 0.05$—non-significant

^{a,b,c,d,e}Mean values within each factor with different letters differ ($P < 0.05$)

the growth stages of halophytes was observed ($P < 0.05$) in the order of seed < flowering < vegetative.

Discussion

The livestock productivity in tropical regions is related to the nutritional content of feeds as basal diet (Olivares-Pérez et al. 2017). The quality of halophytic plants as forage is the indication for achieving the demand for livestock industries. In fact, the parameters that influence the quality of forage basically represent not only the chemical composition and mineral constituents, but also the ability to mitigate the production of detrimental gases from the ruminants, making a cleaner and sustainable environment for the societies.

The chemical composition, mineral constituents, and other efficacious parameters have been known to differ for halophytes. The nutritional parameter of animal's feed is mainly evaluated by its chemical constituents. In general, the chemical constituents of plants vary due to variations in the varieties of plants, fertility of soil, abiotic factors, maturity stage, etc. (Elahi et al. 2017; Vazquez-Mendoza et al. 2017). Thus, it is mandatory to estimate the composition of halophytes for their promising application as livestock fodder.

The DM and OM are considered decisive components, reflecting upon the nutritional values of halophytes as feeds for ruminants. In the present investigation, the DM values were found to be varied for nine different types of halophytic plants species studied. There was not much variation reported between the DM values at different growth stages of halophytes. It was reported that DM of halophytes increased with maturity. The results were consistent with the reports of Kramberger and Klemencic (2003) and Hussain and Durrani (2009) who also reported increased DM with the maturity of fodder plants. Likewise, in the present study, the OM varied significantly ($P < 0.05$) among the selected halophytes. The findings agree with the reports of Sun and Zhou (2010) who demonstrated that OM intakes significantly increased when the lambs were fed with varied concentrations of *Suaeda glauca* seeds in diet.

Table 3 Gas production parameters of halophyte plant species

	b	*c*	ME	OMD	DOMD
Plant species					
Aeluropus littoralis	47.9^{a}	0.056^{cd}	8.6^{ab}	57.8^{ab}	53.2^{a}
Aeluropus lagopoides	38.6^{bc}	0.047^{d}	6.7^{dce}	47.1^{dc}	32.1^{c}
Carthamus lanatus	41.9^{ab}	0.115^{b}	8.8^{a}	59.4^{a}	56.4^{a}
Avena fatua	33.3^{cd}	0.080^{c}	6.8^{dc}	47.2^{dc}	30.9^{c}
Artemisia maritima	35.8^{bc}	0.113^{b}	7.65^{bc}	51.9^{bc}	45.6^{ab}
Atriplex leucoclada	37.3^{bc}	0.074^{cd}	7.3^{dc}	50.2^{dc}	34.7^{bc}
Lolium perenne	34.4^{bcd}	0.052^{cd}	6.4^{ed}	44.4^{ed}	30.3^{c}
Zygophyllum eurypterum	26.0^{de}	0.172^{a}	6.6^{cde}	45.3^{cde}	38.9^{bc}
Prosopis farcta	24.3^{e}	0.062^{cd}	5.6^{e}	38.6^{e}	33.3^{c}
SEM	1.54	0.0056	1.162	1/139	0.928
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Growth stage					
Vegetative	38.9^{a}	0.088	7.82^{a}	53.4^{a}	44.53^{a}
Flowering	36.7^{a}	0.082	7.18^{b}	49.3^{b}	39.84^{a}
Seed	30.8^{b}	0.087	6.43^{c}	44.5^{c}	34.08^{b}
P value	< 0.0001	0.380	< 0.0001	< 0.0001	0.0001

b potential gas production (ml), *c* gas production rate constant (ml/h), *ME* metabolisable energy (MJ/kg), *OMD* organic matter digestibility (%), *DOMD* digestible organic matter in dry matter (%), *SEM* standard error of mean, $P < 0.05$—significant, $P > 0.05$—non-significant

^{a,b,c,d,e}Mean values within each factor with different letters differ ($P < 0.05$)

On the other hand, the current study showed that the OM values were found to be reduced from vegetative to the seed stage of halophytes, establishing the finding against the data of Sultan et al. (2008) who reported increased OM values from early broom stage to maturity stage of grasses.

Ash contents in plants play crucial role in promoting balanced growth of ruminants. The increased ash content is a significant property of halophytic plants that may counteract for mineral deficiency in animals. In the present investigation, the highest content of ash was estimated for *A. fatua.* The ash contents of all the nine halophytes varied significantly. In general, the ash contents were increased at pre-reproductive stage, which further declined at maturity. However, in our study, the ash contents were found to be increased from vegetative to seed stage, agreeing completely with the results of Wahid (1990) who reported improved ash contents with increased maturity of plant. Hussain and Durrani (2009) also estimated increased ash contents in some grasses at advanced growth stage. In contrary to our reports, Kilcher (1981) observed decline in the ash contents of forage with maturity. It might be due to the variation in soil, physiological distribution, and other abiotic factors. Additionally, the metabolic processes occurring in various plants differ based on the plant species, thus altering the ash contents due to the fructification process.

Crude protein refers to the overall nitrogenous compounds present in the forage feed, and they correspond to digestibility and mineral contents. Proteins play significant role in salt acclimation (Parker et al. 2006; Kosová et al. 2013). The proteins are essential for ruminants in order to maintain their metabolic processes. The protein deficiency causes reduced appetite, low feed intake, and poor food efficiency, resulting poor growth and development of animals. The present study showed that halophytes generally contained high CP, *A. lagopoides* estimating the maximum. Our findings are consistent with the reports of Holechek et al. (1998) and Robles and Boza (1993), who also estimated increased CP contents in various shrubs. In general, the concentration of CP was improved at pre-reproductive stages (Ganskopp and Bohnert 2001). In line of the above study, our results also revealed significant reduction ($P < 0.05$) in the CP content from vegetative to seed stages. In fact, a continuous implementation of CP in forage is required for the healthy and enhanced productivity of animals (Holechek et al. 1998). The health and productivity of livestock might decline with low availability of protein in the fodder.

The present study showed moderate level of EE in the diverse halophytes exploited. The differences were significant at different growth stages. The EE percentage decreased from vegetative to seed stage, showing partial agreement with the findings of Hussain and Durrani (2009) who demonstrated mixed rate of EE in plants at pre-reproductive, reproductive, and post-reproductive stages.

The ADF represents extract of acidic detergent hydrolysis that constitutes cellulose, lignin, acid detergent insoluble nitrogen, and acid-insoluble ash. The content of ADF improves with the maturity of plants. Therefore, the energy availability to the livestock depends on the reduced ADF. On the other hand, the NDF represents the bulky characteristics of feed and constitutes the fibres in ADF and hemicellulose. In fact, low NDF rations are often considered as fodder. In view of the above-mentioned facts, the present study estimated lower content of ADF and NDF in *A. lagopoides,* suggesting it a promising halophyte for ruminant feed. In like manner, the study depicted the increased content of ADF and NDF from vegetative to seed stages of halophytes. Our findings strongly agree with the report of Sultan et al. (2008), who estimated improvement in ADF and NDF levels with maturity of plants. In like manner, Ashraf et al. (1995) reported increased ADF and NDF contents at various growth stages of fodders and our findings completely favour. The NDF of plants corresponds to the phenology, as both the NDF and ADF are influenced by plants maturation (Wahid 1990). In fact, the halophytic fodders digestibility by ruminants could be limited by the high levels of fibre.

Apart from the chemical constituents of halophytes, a pivotal parameter, i.e. mineral composition, needs to be addressed for the quality evaluation of halophytes as feed. Halophytes may represent promising sources of minerals to ruminants and may affect their production to a greater extent. The present investigation showed the presence of high level of Na and moderate level of K in the halophytic plants. Few of the halophytes showed K concentration within the recommended range. On the other hand, our halophytes were generally low in Ca and P content. The results showed complete agreement with Swingle et al. (1996) and Riasi et al. (2008) who recorded high K and Na content but low Ca and P in the halophytic plants. However, it is important to use halophytes as forage, containing normal ranges of minerals because excessive or deficient concentrations of minerals may cause toxicity and other severe side effects to the livestock.

The microbial fermentation of feed in the rumen causes the emission of greenhouse gases in the environment which definitely needs to be overcome for maintaining the safer environment. In the present context, various halophytic plants showed decreased production of gases in a time-dependent manner up to 96 h, demonstrating the ability of these halophytes to mitigate the global warming effects. The mitigation of gas emission might be because of the high cell wall constituents of plants, which later showed reduction in microbial activity, causing decreased gas emission. Similar reports were recorded by Broudiscou et al. (2002) who estimated the reduced emission of gases due to the inclusion of plant extracts. Additionally, the halophytes demonstrated the decreased production of gases from 2 to 96 h in the order of vegetative > flowering > seed stages, suggesting the crucial role of halophytes in mitigating GP with maturity. Similarly, the GP from the fermentable fraction was found to be mitigated after the supplementation of *P. farcta* as forage. Gas production values varied significantly ($P < 0.05$) between the growth stages of halophytes, revealing the potentiality of these tested plants to modify and improve ruminal fermentation.

The ME is the commonly used parameter to express the amount of energy in feed for the maintenance and productivity, and to combat nutritional deficiency of ruminants. The present context revealed that the tested halophytes may be potential energy source for livestock. In general, the ME depends widely on the maturity of plants. The present study indicated that there were significant reductions observed between ME at all phenological stages of halophytes. Similar reports were observed by Sultan et al. (2008) and Hussain and Durrani (2009) who demonstrated reduction in ME with increase in grass maturity and advanced growth stages of plants, respectively. In contrast with our findings, Robles and Boza (1993) observed non-significant variations in ME levels of shrubs, perennial herbaceous species, and annual rangeland species. In fact, the variations in the ME depend on the environmental factors and physiological activities of ruminants.

The findings of the present context showed decreased OMD and DOMD from vegetative and flowering stages to seed stage. Similar findings were reported by Sultan et al. (2008) and Skerman and

Riveros (1990) who revealed decreased DOMD values with increased maturity of plants. The results of our investigation were also in line of Revell et al. (1994) and Sultan et al. (2008) who observed a significant correlation between CP and digestibility. Further, a negative correlation reported between ADF, NDF, and DOMD of halophytes in the present study favoured the findings of Perveen (1998).

Conclusion

Halophytes showed acceptable ranges of DM, OM, ash content, CP, EE, ADF, NDF, and minerals which makes these plant species suitable resources as forage for ruminants. Most importantly, halophytes studied in the context of this research appear to have a promising impact on rumen fermentation by emitting low amount of greenhouse gases after a constant period, thereby indicating a valuable role of these halophytes as forage in order to reduce the emission of ruminal fermentation gas to the atmosphere. The inclusion of some of the tested potent halophytes in diet would undoubtedly be a promising approach with sustainable mitigation of gas production. Additionally, the acceptable energy content of these halophyte plant species make them promising candidates as roughage feedstuffs for ruminants.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ashraf Y, Gillani AH, Nagra SA (1995) Effect of harvesting interval and varieties on the chemical composition of indigenous fodder. I: proximate composition. J Agric Res 33:31–43

Association of Official Analytical Chemists (AOAC) (1990) Official methods of analysis, 13th edn. Association of Official Analytical Chemists (AOAC), Washington, DC

Association of Official Analytical Chemists (AOAC) (2000) Official methods of analysis, 17th edn. Official Methods of Analysis of AOAC International, Gaithersburg

Ben Salem H, Nefzaoui A, Ben Salem L (2004) Spineless cactus (*Opuntia fi cus-indica f.* inermis) and Oldman saltbush (*Atriplex nummularia* L.) as alternative supplements for growing Barbarine lambs given straw based diets. Small Rumin Res 51:65–73

Ben Salem H, Noman HC, Nefzaoui A, Mayberry DE, Pearce KL, Revell DK (2010) Potential use of Oldman saltbush (*Atriplex nummularia* Lindl.) in sheep and goat feeding. Small Rumin Res 91:13–28

Broudiscou LP, Papon Y, Broudiscou AF (2002) Effects of dry plant extracts on feed degradation and the production of rumen microbial biomass in a dual outflow fermenter. Anim Feed Sci Technol 101:183–189

Elahi MY, Kargar H, Dindarlou MS, Kholif AE, Elghandour MMY, Rojas-Hernández S, Odongo NE, Salem AZM (2017) The chemical composition and in vitro digestibility evaluation of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. Shokoufeh) leaves versus hulls and green versus dry leaves as feed for ruminants. Agrofor Syst 91:773. https://doi.org/10.1007/s10457-016-9964-5

Elghandour MMY, Kholif AE, Salem AZM, deOca RM, Barbabosa A, Mariezcurrena M, Olafadehan OA (2016a) Addressing sustainable ruminal methane and carbon dioxide emissions of soybean hulls by organic acid salts. J Clean Prod 135:194–200

Elghandour MMY, Kholif AE, Salem AZM, Olafadehan OA, Kholif AM (2016b) Sustainable anaerobic rumen methane and carbon dioxide productions from prickly pear cactus flour by organic acid salts addition. J Clean Prod 139:1362–1369

Elghandour MMY, Vázquez JC, Salem AZM, Kholif AE Cipriano MM, Camacho LM, Márquez O (2017) *In vitro* gas and methane production of two mixed rations influenced by three different cultures of *Saccharomyces cerevisiae*. J Appl Anim Res 45:389–395

El-Shatnawi MKJ, Abdullah AY (2003) Composition changes of *Atriplex nummularia L.* under mediterranean arid environment. Afr J Range Forage Sci 20:253–257

FAO (Food and Agriculture Organization of the United Nations) FAOSTAT (2008) http://faostat.fao.org/. Accessed 24 March 2008

Ganskopp D, Bohnert D (2001) Nutritional dynamics of 7 northern Great basin grasses. J Range Manag 54:640–647

Glenn E, Squires V, Olsen M, Frye R (1993) Potential for carbon sequestration in the drylands. Water Air Soil Pollut 70:341–345

Guo CY, Wang XZ, Chen L, Ma LN, Wang RZ (2005) Physiological and biochemical responses to saline-alkaline stress in two halophytic grass species with different photosynthetic pathways. Photosynthetica 53:128–135

Holechek JL, Pieper RD, Herba CH (1998) Range management, principles and practices, 3rd edn. Prentice Hall, Upper Saddle River, p 07458

Hussain F, Durrani MJ (2009) Nutritional evaluation of some forage plants from Harboi rangeland, Kalat, Pakistan. Pak J Bot 41:1137–1154

Kholif AE, Elghandour MMY, Rodríguez GB, Olafadehan OA, Salem AZM (2016) Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product. J Clean Prod 142:2649–2655

Kilcher MR (1981) Plant development, stage of maturity and nutrient composition. J Range Manag 34:363–364

Kosová K, Prášil IT, Vítámvás P (2013) Protein contribution to plant salinity response and tolerance acquisition. Int J Mol Sci 14:6757–6789

Kramberger B, Klemencic S (2003) Effect of harvest date on the chemical composition and nutrient value of *Ceratium holosteoides*. Grass Forage Sci 58:12–16

Larbi A, Khatib-Salkini A, Jamal PB, Iniguez L (2009) Shrub yield and fodder quality variations in a non-tropical dryland environment in West Asia. Agrofor Syst 75:147–155

López S, Dhanoa MS, Dijkstra J, Bannink A, Kebreab E, France J (2007) Some methodological and analytical considerations regarding application of the gas production technique. Anim Feed Sci Technol 135:139–156

Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Dev 28:7–55

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979) The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J Agric Sci 93:217–222

Moujahed N, Kayouli C (2005) Supplementation of *Acacia cyanophylla* Lindl. foliage-based diets with feed blocks and PEG 4000 and its effects on in vitro fermentation and performance in sheep. In: Molina Alcaide E, Ben Salem H, Biala K, Morand-Fehr P (eds) Sustainable grazing, nutritional utilization and quality of sheep and goat products. Zaragoza, CIHEAM, pp 333–338 **(Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 67)**

Norman HC, Masters DG, Barrett-Lennard EG (2013) Halophytes as forages in saline landscapes: interactions between plant genotype and environment change their feeding value to ruminants. Environ Exp Bot 92:96–109

Olivares-Pérez J, Rojas-Hernandez S, Camacho-Diaz LM, Cipriano-Salazar M, Salem AZM (2017) Fruits chemical composition and potential ruminal digestion of nine tree species in dry tropic region of Mexico. Agrofor Syst. https://doi.org/10.1007/s10457-017-0161-y

Orskov ER, McDonald L (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J Agric Sci 92:499–503

Parker R, Flowers TJ, Moore AL, Harpham NV (2006) An accurate and reproducible method for proteome profiling of the effects of salt stress in the rice leaf lamina. J Exp Bot 57:1109–1118

Perveen S (1998) Nutritive evaluation of some fodder tree leaves through in vitro digestibility techniques. Technical paper, NWFP Agricultural University, Peshawar

Revell DK, Baker SK, Purser BB (1994) Estimates of the intake and digestion of nitrogen by sheep grazing a Mediterranean pasture as it matures and senesces. Proc Aust Soc Anim Prod 20:217–220

Riasi A, Mesgaran MD, Stern MD, Ruiz Moreno MJ (2008) Chemical composition, in situ ruminal degradability and post-ruminal disappearance of dry matter and crude protein from the halophytic plants *Kochia scoparia*, *Atriplex dimorphostegia*, *Suaeda arcuata* and *Gamanthus gamacarpus*. Anim Feed Sci Technol 141:209–219

Robles CAB, Boza LJ (1993) Native forage flora of southeastern Spain. II. Nutritive value. Pastos 29:47–60

Rojas Hernández S, Olivares Pérez J, Elghandour MMY, Cipriano-Salazar M, Ávila-Morales B, Camacho-Díaz LM, Salem AZM, Cerrillo Soto MA (2015) Effect of polyethylene glycol on *in vitro* gas production of some nonleguminous forage trees in tropical region of the south of Mexico. Agrofor Syst 89:735. https://doi.org/10.1007/s10457-015-9796-8

SAS (2002) User's guide statistics, version 9.0. SAS Institute, Cary

Skerman PJ, Riveros F (1990) FAO plant production and protection series, No. 23. FAO, Rome

Sultan JI, Inam-ur-Rahim Yaqoob M, Nawaz H, Hameed M (2008) Nutritive value of free rangeland grasses of Northern grasslands of Pakistan. Pak J Bot 40:249–258

Sun HX, Zhou DW (2010) Effect of dietary supplement of seed of a halophyte (*Suaeda glauca*) on feed and water intake, diet digestibility, animal performance and serum biochemistry in lambs. Livest Sci 128:133–139

Swingle RS, Glenn EP, Squires VR (1996) Growth performance of lambs fed mixed diets containing halophyte ingredients. Anim Feed Sci Technol 63:137–148

Van Soest PJ, Robertson JB (1990) Methods of dietary fiber, NDF and non starch polysaccharides. In: Symposium paper ADSA June 1990. Depaetment of Animal Science, Cornell University, Ithaca

Vazquez-Mendoza P, Miranda-Romero LA, Aranda-Osorio G, Burgueño-Ferreira JA, Salem AZM (2017) Evaluation of eleven Mexican cultivars of prickly pear cactus trees for possibly utilization as animal fed: *in vitro* gas production. Agrofor Syst 91:749. https://doi.org/10.1007/s10457-016-9947-6

Wahid A (1990) Dietary composition and nutritional status of sheep and goats grazing two rangeland types in Balochistan, Pakistan. Ph.D. Thesis, Oregon State University