Ruminal digestibility, microbial count, volatile fatty acids and gas kinetics of alternative forage sources for arid and semi-arid areas as *in vitro*

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\section*{ABSTRACT}
Atriplex (*Atriplex patula*) and plantago (*Plantago lanceolata*) species are commonly found in grasslands which have arid and semi-arid climatic conditions. The aim of the study was to make comparison of atriplex, plantago and alfalfa (*Medicago sativa*) herbage in terms of nutrient matter content, gas kinetics, methane production, estimated digestion parameters, ammonia-N, volatile fatty acids (VFAs), total bacteria count and numbers of protozoa. *In vitro* gas production was carried out for up to 96 h by using the *in vitro* digestion technique. The molar total VFAs, metabolic energy (ME), net energy lactation (NEL), and organic matter digestibility (OMD) levels, count of total bacteria, number of total ciliate and number of *Dasytricha* sp., *Diplodiniinae* and *Entodiniinae* did not show significant differences between alfalfa and the alternative forages (*p > 0.05*). *In vitro* methane production of atriplex herbage was higher than that of plantago herbage (*p < 0.01*). The ruminal ammonia-N concentration of plantago herbage was lower than those of atriplex and alfalfa herbage (*p < 0.001*). The individual molar proportions of propionic acid of alfalfa herbage were higher than those of the other forages (*p < 0.001*). The results of the present experiment confirmed that *A. patula* and *P. lanceolata*, which are appropriate for the flora of the Mediterranean region, can be used as alternative good quality forage plants to alfalfa hay for grazing animals.

\section*{Introduction}
Global warming has brought about a reduction in annual precipitation levels and for this reason difficulties are now being encountered in the production of high-quality forages especially in arid and semi-arid areas (IPCC 2014). Researchers have showed that global warming has induced variations in climate types resulting in semi-humid and semi-arid areas being turned into semi-arid or arid areas (Altin et al. 2012). In this kind of climate, plants turn yellow at the end of the summer and the vegetation of these areas has started to change to steppe or desert-like steppe. Such areas cannot supply enough high-quality forages for grazing animals. These grazing areas, which are of poor quality, do not meet the required energy and nutrient needs of grazing animals (Budak 2013). In addition, different kinds of soil types, such as salty and sandy soil, are one of the major obstacles to solving the forage production problem (Yulafci & Pul 2005; Tuteja 2007). However, alternative forage sources like atriplex (*Atriplex patula*) and plantago (*Plantago lanceolata*) which are suitable for cultivation in arid areas and different kinds of soil types (salty and sandy soil) in areas with arid and semi-arid climates may be able to provide forage needs.

Plantago species, which are commonly found in warm climates in grasslands and are perennial or annual, can grow up to 30 cm and comprise 265 species. They belong to the *Plantaginaceae* family and have numerous leaves which are large and long. These species are located and grow in many countries around the world. This plant family can grow easily in different types of soils (pH 4.2–7.8) (Stewart 1996). Plantago leaves which can remain green until the end of summer are consumed avidly by grazing animal species (cattle, sheep, deer and horse) because of their peculiar mushroom smell (volatile oct-1-en-3-ol compound) and their palatability (sorbitol accumulation), especially in salty and arid soil conditions (Derrick et al. 1993).

The genus Atriplex is a cosmopolitan group of nearly 200 species. It includes annual hays...
(Atriplex patula, Atriplex rosea and Atriplex prostrata) which are common in arid/semi-arid lands and perennial saltbushes (Atriplex nummularia, Atriplex rhagodioides and Atriplex undulate) which are common in desert lands (Khalil et al. 1986; Bassett & Munro 1987; Silva-Colomer & Passera 1990). The genus occurs on all continents except Antarctica. Atriplex patula (oraches; spreading atriplex; called atriplex in the present study) is an annual, 15–150 cm high, erect or occasionally prostrate and with simple or branched angular stems, which are green and pale-yellowish striped becoming woody towards the base. Atriplex patula is found in disturbed habitats including fields and ornamental plantings. Members of this genus do not contain any toxins and all have leaves that are more or less edible by human.

In the present study, our aim was to compare atriplex (Atriplex patula) and plantago (Plantago lanceolata) with alfalfa (Medicago sativa), which is commonly used as a forage with high quality, at the start of the flowering stage in terms of in vitro gas kinetics, methane production, estimated digestion parameters, ammonia-N, volatile fatty acids (VFAs), total bacteria count and protozoa number.

Materials and methods

The sampling area

Plant samples were collected from the Kayseri, Turkey. The Kayseri is located (38° 56’ N, 34° 24’ E) in central (Cappadocia district) of Turkey. It is located 1050 m above sea level. The Cappadocia district, which is located in the southern part of the study area, is vulnerable to desertification. Steppe and dry forests are the dominant vegetation in this location. The mean January temperature is 0°C and the mean July temperature is above 20°C. The mean annual rainfall amount is below 400 mm. Rain type is convectional and frontal. Semi-arid climate condition is dominant in Kayseri depending on temperature and rainfall amounts (Altin et al. 2012).

In the study, samples of atriplex (Atriplex patula), plantago (Plantago lanceolata) and alfalfa (Medicago sativa) were gathered at the start of the flowering stage (about flowering 10%). Plantago lanceolata and Atriplex patula samples were randomly collected from six samples (about 500 g for each samples) from six different areas. Samples of alfalfa herbage also were randomly collected from six samples (about 500 g) in different fields of private forage farmer in Kayseri. Samples included aerial all parts (leaf, stem, and bud) of the plants.

Chemical analysis

The samples of plant were dried in a thermostatically controlled cabinet (Lovidond, Dortmund, Germany) for 48 h at 55°C. After drying, the samples were milled through a 1 mm sieve (IKA-Werke, Staufen im Breisgau, Germany) for use in wet chemical analysis and in vitro gas production. The ash contents were detected by igniting the samples in a muffle furnace at 525°C for 8 h (AOAC 1990; method 942.05). Nitrogen (N) content was measured by the kjeldahl method. Crude protein (CP) was calculated as N × 6.25 (AOAC 1990; method 942.01). The diethyl ether extract (EE) and crude fibre (CF) levels were determined according to the methods (method 920.39 and 7.066–7.070) reported by the AOAC (1990). The neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) contents forming the cell wall components in these samples were analysed by using a fibre analyser (Velp FIWE3, Italy) according to the methods reported by Van Soest et al. (1991). The NDF was determined using sodium sulphite and thermo-stable α-amylase (aNDF). Neither NDF nor ADF was inclusive of residual ash (aNDFom and ADFom). Total condensed tannin (TCT) contents of the samples were determined by the butanol-HCl method reported by Makkar et al. (1995) using a spectrophotometer (UviLine 8100, SI Analytics, Germany).

The relative feed values (RFV) of the plants were calculated using the equations of Jeranyama & Garcia (2004) as follows:

\[
DDM = \text{Digestible Dry Matter} = \frac{88.9 - (0.779 \times \% \ ADFom)}{\% \ aNDFom}
\]

\[
DMI = \text{Dry Matter Intake (\% of BW)} = \frac{120(\% \ aNDFom)}{DMM}
\]

\[
RFV = \frac{(DDM \times DMI)}{1.29}
\]

In vitro Hohenheim gas production technique

Rumen fluid was obtained from two beef cattle (Simmental breed, at 18 months of age and about 650 kg body weight) fed with a diet containing rough
feed (approximately 60% of total mix feed on a dry matter basis, maize silage + alfalfa hay + wheat straw) and concentrate feed (approximately 40% of total mix feed on a dry matter basis). Rumen fluid was collected after two hours than morning feeding. Rumen fluid was collected into a thermos including water at 39 °C under CO₂ gas, and filtered with four layers of cheesecloth in the laboratory. The technique was carried out according to the procedures of Menke & Steingass (1988). The plant samples were incubated in rumen fluid and buffer mixture in 100 ml glass syringes (Model Fortuna, Germany). One litre of buffer mixture included 474 ml of bi-distilled water, 237.33 ml of macro-mineral solution (5.7 g of Na₂HPO₄, 6.2 g of KH₂PO₄ and 0.6 g of MgSO₄ in 1 L of bi-distilled water), 237.33 ml of buffer solution (35 g of NaHCO₃ and 4 g of NH₄HCO₃ in 1 L of bi-distilled water), 0.12 ml of trace-mineral solution (13.2 g of CaCl₂·2H₂O, 10 g of MnCl₂·4H₂O, 1 g of CoCl₂·6H₂O and 0.8 g of FeCl₃·6H₂O in 100 ml of bi-distilled water), 1.22 ml of resazurin solution (0.1 g of resazurin in 100 ml of bi-distilled water) and 50 ml of reducing solution (285 mg of Na₂S·7H₂O and 4 ml of 1 N NaOH in 96 ml of bi-distilled water). Dried plant samples (200 ± 10 mg) were weighed in triplicate into glass syringes. Thirty millilitres of the rumen fluid + buffer mixture at a 1:2 (v/v) ratio was added to each syringe. In addition, three blank syringes (no template; rumen fluid + buffer mixture) were used to calculate the total gas production. After closing the clips on the silicon tube of the syringe, the syringes were gently shaken and the clips were opened to remove gas by pushing the piston upwards to achieve complete gas removal. The clip was closed, the initial volume recorded and the syringes were incubated in a water bath at 39 °C for up to 96 h.

**Determination of total gas and methane production**

In incubation, the total gas volume was recorded from the calibrated scale on the syringe for 3, 6, 12, 24, 48, 72, and 96 h. After measuring the total gas volume at 24 h, the tubing of the plastic syringe outlet was inserted into the inlet of the methane analyser (Sensor, Europe GmbH, Erkrath, Germany) and the piston was pushed to insert the accumulated gas into the analyser. The methane as a percent (%) of the total gas was displayed on a PC. This value was used for the calculation of methane in the total gas volume (Kara et al. 2015a). The production of methane was calculated using the following formula: Methane production (ml) = [in vitro total gas produced (ml) × methane (%)]/100

Cumulative gas production data were fitted to the exponential equation of Orskov & McDonald (1979):

\[ Y = a + b \left(1 - e^{-ct}\right) \]

where: \( a \) = the gas production from the immediately soluble fraction (ml), \( b \) = the gas production from the insoluble fraction (ml), \( c \) = the gas production rate constant \( a + b \) = the potential gas production (ml), \( t \) = the incubation time (h), \( Y \) = the gas produced at the time \( t \).

The fermentation kinetics were estimated using a computer package programme called Fig P (Biosoft, Cambridge, UK).

**Estimated levels of metabolic energy (ME), net energy lactation (NEₗ), and organic matter digestibility (OMD)**

The ME and OMD contents of the plants were calculated using the equations of Menke & Steingass (1988) as follows:

\[
\begin{align*}
\text{ME (MJ/kg DM)} &= 2.20 + 0.136 \times \text{GP} + 0.057 \times \text{CP} \\
\text{OMD (% DM)} &= 14.88 + 0.889 \times \text{GP} + 0.45 \times \text{CP} + 0.0651 \times \text{Ash}
\end{align*}
\]

The NEₗ was calculated according to the method of Blümmel & Orskov (1993):

\[
\begin{align*}
\text{NEₗ (MJ/kg DM)} &= 0.115 \times \text{GP} - 0.0054 \times \text{CP} + 0.014 \times \text{EE} - 0.0054 \times \text{Ash} - 0.36 \\
\text{GP} &= 24 \text{ h net gas production (ml/200 mg),} \\
\text{CP} &= \text{Crude protein (g/kg DM),} \\
\text{Ash} &= \text{Ash content (g/kg DM),} \\
\text{EE} &= \text{Ether extract (g/kg DM).}
\end{align*}
\]

**In vitro rumen fluid characteristics**

The pH of the rumen medium was determined using a digital pH metre (Mettler Toledo S220; Mettler Toledo, Greifensee, Switzerland). The ammonia-N (NH₃-N, mg/dL) concentration of the digestion fluid was estimated by a distillation system (Makkar & Becker 1996), without acid digestion and after distillation with potassium hydroxide (2 N) in boric acid and titration with diluted hydrochloric acid (0.1 N), after previous centrifugation of the sample to 1000 × g for 15 min (Souza et al. 2010).

After the total gas volume at 24 h of in vitro incubation was measured, approximately 10 ml rumen fluid from each syringe was received into falcon tubes. Total numbers and generic composition of ciliate protozoa were determine according to the...
procedures described by Dehory (1984). Rumen fluid (0.1 mL) samples were collected and fixed by 0.9 mL methyl green-formal-saline solution (100 mL formaldehyde (35%), 900 mL distilled water, 0.6 g methyl green, and 8.0 g NaCl). The diluted sample was pipetted into a Sedgewick Rafter counting chamber by a wide-orifice pipette. Total numbers and generic composition of ciliate protozoa were determined using a microscope (Nikon Eclipse E-100, The Netherlands). Determination of total bacteria count was carried out using a spectrophotometer (T80 + UV/VIS Spectrophotometer, PG Instruments Ltd, UK).

Ruminal VFAs [acetic (A), propionic (P) and butyric (B) acids, mmol/L of rumen fluid] concentration assessed by gas chromatograph (Perkin Elmer Auto System XL, USA) equipped with Flame Ionisation Detector (GC/FID) as described by Erwin et al. (1961). The ruminal fluid was squeezed through four layers of cheesecloth, mixed with 25% (w/v) meta-phosphoric acid and kept frozen (−20 °C) for the analysis of VFA. The frozen samples were thawed at 4 °C and centrifuged. The supernatant (0.5 mL) was mixed with the same volume of 20 mmol 4-methyl n-valeric acid as an internal standard. The analysis was performed under the following conditions: capillary column, Varian CP-Sil 88 (50 m × 0.25 mm, film thickness 1.20 μm); injector and detector temperature, 240 °C; stove heat programme, from 80 °C (1 min. hold) to 240 °C rising at 10 °C/min., and 20 min. hold at 240 °C; flow speed, 15 psi; detector: 70 eV; ionisation type, Ei; carrier gas, helium (20 mL/min.); sample injected 1 μL. Identification of constituents was carried out with the help of retention times of standard substances (Analytic Fluka).

Statistical analysis
The data of the experiment were first subjected to Levene’s test to detect the variance homogeneity. One-way variance analyses (ANOVA) were implemented for homogeneous variances by General Linear Model procedures to test treatment differences. Data were analysed based on the statistical model: Yij = μ + Si + eij. Where, Yij = the value for each parameter under investigation in the i-th forage source at the j-th observation, μ = the general mean common of forage sources for each parameter under investigation, Si = the effect of alternative forage sources on the observed parameters, eij = the random error term at the ij-th observation. The means were separated by Tukey’s multiple range test at p < .05.

Analyses were performed using SPSS 17.0 software (IBM Corp., Armonk, NY).

Results

Nutrient matter composition
The nutrient matter contents and RFV of the plants are presented in Table 1. The CP content in alfalfa was higher than in atriplex and plantago (p < .001). The EE and TCT content of plantago were higher than in the other forages (p ≤ .001). Ash of atriplex was found to have the highest value compared to the others (p < .001). The aNDFom (p ≤ .001) and HC (p < .001) contents of atriplex herbage had the highest values in of the forages studied. The ADL (p < .001) content of plantago had higher values than those of atriplex and alfalfa herbage (p < .001). The ADFom content of atriplex was lower than those of alfalfa and plantago herbage. The CF content of alfalfa herbage was higher than those of the other forages (p < .001). The RFV values of forage sources were similar (p > .05) (Table 1).

In vitro gas production and fermentation parameters of rumen fluid
The gas production from insoluble fraction (bgas), gas production rate (cgas) and potential gas production ((a + b)gas) values of the other forage sources were similar to those of alfalfa herbage (p > .05). There is no difference between the alternative forages and alfalfa herbage in terms of cumulative gas production values up to 96 h (p > .05) (Table 2).

The ME, NEL and OMD values were similar for all forages (p > .05). The methane production of atriplex herbage was higher than that of plantago herbage, but similar to that of alfalfa herbage (p < .01). The ammonia-N concentration of plantago herbage

| Table 1. Nutrient compositions and RFV of different alternative forages (n = 6). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Alfalfa         | P. patula       | P. lanceolata   | SD SEM          | p value         |
| TCT             | 0.37b           | 0.46b           | 1.38a           | 0.48            | 0.16            | <.001           |
| CP              | 19.18a          | 14.64b          | 10.74c          | 3.70            | 1.23            | <.001           |
| EE              | 1.54b           | 1.40b           | 1.95a           | 0.26            | 0.08            | <.001           |
| Ash             | 8.45a           | 14.56a          | 11.59b          | 2.65            | 0.88            | <.001           |
| CF              | 30.66a          | 23.99a          | 24.71b          | 3.23            | 1.07            | <.001           |
| aNDFom          | 38.01b          | 41.04b          | 37.29a          | 1.81            | 0.60            | ≤ .001          |
| ADFom           | 32.78a          | 27.92a          | 31.88b          | 3.64            | 1.21            | ≤ .001          |
| HC              | 5.22b           | 13.12a          | 5.41a           | 5.31            | 1.77            | <.001           |
| ADL             | 6.48b           | 4.86a           | 10.20a          | 4.79            | 1.69            | <.001           |
| RFV             | 155.06          | 152.22          | 159.01          | 3.21            | 1.07            | <.492           |

SD: Standard deviation of means; SEM: Standard error of means; TCT: total condensed tannin; CP: crude protein; EE: diethyl ether extract; CF: crude fibre; aNDFom: neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom: acid detergent fibre expressed exclusive of residual ash; HC: hemicellulose (aNDFom-ADFom); ADL: acid detergent lignin determined by solubilisation of cellulose with sulphuric acid; RFV: relative feed value.

Means within the same line with differing superscripts are significantly different.
was lower than those of atriplex and alfalfa herbage (\(p < .001\)) (Table 3).

The molarities of total VFAs, proportions of individual acetic acid, butyric acid and other volatile acid in rumen fluid were similar in the forages (\(p > .05\)). The proportion of individual propionic in rumen fluid for alfalfa herbage was higher than those of the other forages (\(p < .001\)). Therefore the \((A + B)/P\) ratio in rumen fluid for alfalfa herbage was lower than those of the alternative forages (\(p < .05\)) (Table 4).

The total bacteria count, number of total ciliate protozoa and numbers of *Dasytricha sp.*, *Diplodiniinae*, and *Entodiniinae* had similar values for all forages (\(p > .05\)). In addition, the number of *Isotricha spp.* for the fermentation of plantago herbage was higher than those of alfalfa and atriplex herbage (\(p < .05\)) (Table 4).

### Discussion

Nowadays, the negative effects of global warming on water resources are a significant source of concern in the regions surrounding the Mediterranean Sea which includes southern Europe, northern Africa, and the eastern Mediterranean Levant region (Cyprus, Israel, Jordan, Lebanon, Palestine, Syria, and Turkey). These lands have observed the worst drought of the past nine centuries (IPCC 2014; Cook et al. 2016). Plants which grow in arid and semi-arid lands such as the Mediterranean region have been seriously affected by climatic changes and decreased forage quality (Moore & Jung 2001). Therefore, researchers have studied suitable plants for the grasslands of arid and semi-arid areas (Kamalak et al. 2010; Kara et al. 2015b; Ozkan 2016).

### Nutrient matter composition

In the present study, the CP contents of atriplex and plantago herbage were slightly lower than those of alfalfa herbage at the start of the flowering stage (19.18% versus 10.74–14.64% in DM), but these herbage may qualify as forage sources with moderate

### Table 2. In vitro gas kinetics and cumulative gas productions of different alternative forage (\(n = 6\)).

|                      | Alfalfa | A. patula | P. lanceolata | SD   | SEM | \(p\) value |
|----------------------|---------|-----------|---------------|------|-----|-------------|
| **Gas kinetics**     |         |           |               |      |     |             |
| \(c_{gas}\)          | 0.05    | 0.05      | 0.05          | 0.02 | 0.01| .788        |
| \(b_{gas}\)          | 52.79   | 55.75     | 52.22         | 2.44 | 0.99| .372        |
| \((a + b)_{gas}\)    | 53.26   | 56.95     | 52.17         | 2.96 | 1.20| .280        |
| **Cumulative gas production** |         |           |               |      |     |             |
| 3 h                  | 9.83    | 9.66      | 10.58         | 1.60 | 0.32| .212        |
| 6 h                  | 20.00   | 20.16     | 20.75         | 2.01 | 0.41| .732        |
| 12 h                 | 33.66   | 33.00     | 33.23         | 2.94 | 0.60| .930        |
| 24 h                 | 41.33   | 41.16     | 42.00         | 3.49 | 0.71| .878        |
| 48 h                 | 56.66   | 54.33     | 53.16         | 2.83 | 0.81| .372        |
| 72 h                 | 57.33   | 55.33     | 55.16         | 3.30 | 0.95| .674        |
| 96 h                 | 57.33   | 56.83     | 55.83         | 3.72 | 1.07| .874        |

SD: Standard deviation of Means; SEM: Standard Error of Means; \(c_{gas}\): gas production rate (\%, 0.2 g DM); \(b_{gas}\): gas production from insoluble fraction (ml/0.2 g DM); \((a + b)_{gas}\): potential gas production (ml/0.2 g DM).

### Table 3. Estimated gas production parameters, ruminal ammonia-N concentration and in vitro ruminal methane production of different alternative forages (\(n = 6\)).

|                      | Alfalfa | A. patula | P. lanceolata | SD   | SEM | \(p\) value |
|----------------------|---------|-----------|---------------|------|-----|-------------|
| **ME**               | 7.91    | 7.85      | 7.96          | 0.47 | 0.09| .915        |
| **NE**               | 4.50    | 4.48      | 4.49          | 0.39 | 0.08| .990        |
| **OMD**              | 51.33   | 51.99     | 51.87         | 2.96 | 0.60| .886        |
| **Ammonia-N**        | 47.85   | 48.02     | 36.50         | 6.23 | 1.80| < .001      |
| **Methane**          | 19.23   | 22.06     | 16.69         | 2.84 | 0.81| .007        |

SD: Standard deviation of Means; SEM: Standard Error of Means; ME: estimated metabolisable energy (MJ/kg DM); NE: estimated net energy lactation (MJ/kg DM); OMD: estimated organic matter digestibility (%); Ammonia-N: ammonia-N concentration of rumen fluid at 24 h (mg N/L); Methane: it expresses methane production as percent in total gas produced at 24 h.

### Table 4. Effects of different forage sources on total VFAs (mmol/L), molar proportions of individual VFAs (% of total VFAs), bacteria (\(\times 10^9\)/ml) and protozoa numbers (\(\times 10^9\)/ml) in rumen fluid (\(n = 6\)).

| Items                        | Alfalfa | A. patula | P. lanceolata | SD   | SEM | \(p\) value |
|------------------------------|---------|-----------|---------------|------|-----|-------------|
| **Total VFAs**               | 110.36  | 111.78    | 113.09        | 40.20| 11.60| .602        |
| Acetic acid (A)              | 47.40   | 53.30     | 60.57         | 8.49 | 2.45| .062        |
| Propionic acid (P)           | 28.38\(a\) | 19.51\(b\) | 20.75\(b\) | 3.80 | 1.09| < .001      |
| Butyric acid (B)             | 15.46   | 18.97     | 12.66         | 4.97 | 1.43| .205        |
| Other volatile acids         | 8.75    | 8.20      | 6.01          | 2.01 | 0.58| .088        |
| \((A + B)/P\)                | 2.22\(a\) | 3.71\(a\) | 3.54\(a\) | 0.66 | 0.19| < .001      |
| **Total bacteria**           | 2.52    | 2.96      | 2.45          | 0.40 | 0.11| .397        |
| **Total ciliate protozoa**   | 42.92   | 43.08     | 42.59         | 8.86 | 2.55| .419        |
| **Isotricha spp.**           | 0.26\(a\) | 0.20\(a\) | 0.16\(b\) | 0.06 | 0.02| .049        |
| **Dasytricha sp.**           | 0.62    | 0.58      | 0.57          | 0.14 | 0.04| .913        |
| **Diplodiniinae**            | 10.63   | 12.20     | 16.00         | 4.19 | 1.21| .149        |
| **Entodiniinae**             | 31.41   | 30.10     | 25.86         | 9.13 | 2.63| .213        |

SD: Standard deviation of means; SEM: Standard error of means; Total VFAs: Acetic + propionic + butyric + other volatile acids. Other volatile acids: iso-butyric acid + valeric acid + iso-valeric acid.

\(a,b\) Means within the same line with differing superscripts are significantly different.
CP contents for grazing animals. In addition, Kamalak et al. (2005) stated that the CP content for fourteen different alfalfa varieties which are commonly grown in Turkey for hay production varied between 15.05 to 21.39% at the beginning of the flowering stage. Similar to the results of the current study, Kara et al. (2015b) demonstrated that Plantago lanceolata leaf consisted of 12.82% CP at the seed bulking stage of the plant phenological period. In contrast, Guil-Guerrero (2001) stated that CP contents of different Plantago species' leaves ranged widely from 15.82 to 22.96%. Atriplex patula which is used in the present experiment is different from other Atriplex species which mostly accumulate salt and grow naturally in desert lands (Khalil et al. 1986; Bassett & Munro 1987; Silva-Colomer & Passera 1990). In previous studies, the CP content of different Atriplex species (A. nummularia, A. rhagodioides and A. undulate), which grow naturally in desert lands, contained more than about 16% in DM (Khalil et al. 1986; Silva-Colomer & Passera 1990). In another study, the CP content of Atriplex acanthocarpa leaves ranged from 19 to 32% and that of Atriplex canescens leaves ranged from 12 to 24% (Garza & Fullbright 1988). The TCT contents of the alternative forages were low; also, the TCT content of the Plantago herbage reduced ruminal methane production and provided by-pass properties to protein (Min et al. 2006). In a previous study, it was determined that the leaves of P. lanceolata contained 0.64% TCT in the seed bulking stages (Kara et al. 2015b). The plant cell wall substances of the alternative forages used in the present study had normal levels and were as expected from forage. Fibre contents and similar RFV values in forage, which are important criteria that reveal forage, are necessary for a healthy ruminal ecosystem and rumination of ruminants (Jeranyama & Garcia 2004; Kara et al. 2015b). The high ash content of plantago and atriplex herbage was at level for plants that are grown in arid or semi-arid regions (Guil-Guerrero 2001; Harrington et al. 2006; Kara et al. 2015b). The differences in the present study and previous studies on plantago or atriplex forages in terms of some nutrient compositions may have been caused by different climatic types (desert, arid, semi-arid), phenological stage, plant variety, soil nutrient content and stress factors (irrigation, salinity, etc.). There are not many studies on the nutrient composition of Plantago lanceolata and Atriplex patula herbage in terms of livestock nutrition. Our study will contribute to the literature on this subject.

**In vitro total gas – methane production and fermentation parameters of rumen fluid**

The effects of TCT on digestion activity change depending on the concentration in animal diet. Low levels of TCT (1–3%) in diet have been reported to reduce ruminal methane production and to provide by-pass properties to protein (Kumar & Singh 1984; Min et al. 2006). In the present study, the TCT contents of atriplex and plantago herbage may be characterised as a plant containing low CT, according to Jackson et al. (1996). In the present experiment, the in vitro methane production of plantago herbage with 1.38% TCT content was lower than that of atriplex herbage with 0.46% TCT content. Similar to the findings of the present study, Kara et al. (2015b) stated that the TCT in the leaf of P. lanceolata was negatively correlated with in vitro ruminal methane production.

Tannin compounds have a decreasing effect on ruminal protozoa number (Min et al. 2006; Abarghuei et al. 2014). In agreement with this knowledge, the number of Isotricha spp decreased with 1.38% TCT content in previous studies. The mechanism of protozoal decrease is thought to occur due to the change in the protozoal cell membrane permeability of plant secondary metabolites and the formation of complexes with cholesterol in the protozoal cell membrane which results in cell lysis (Francis et al. 2002). Ruminal methanogenesis was decreased exponentially with the decrease in the number of protozoa by fermentation of plantago herbage (Schönhusen et al. 2003; Hook et al. 2010; Kara et al. 2014). In previous studies it was observed that holotrich protozoa seem to be key players in rumen methanogenesis (Belanche et al. 2012, 2015). In the present study plantago, which has a lower number of Isotricha spp than those of the other forages, produced less methane than that others. Similar with the findings of present study, the in vitro ruminal methane production of P. lanceolata leaf was determined as 13.43% in produced total gas at 24 hours incubation by Kara et al. (2015b). In addition, Kamra (2005) stated that Entodiniinae species, which are in close symbiosis with methanogens and methane production. However, in this study it was evaluated that methane production did not have a relation with Entodiniinae.

The total gas production level of in vitro fermentation depends on the composition of nutrient content (plant cell walls, starch, sugar, etc.), presence of inhibitor for gas production (condensed tannins, polyethylene glycol), diet of donor animal and the quality of fermentation which provided by the microflora in the rumen fluid (Johnson & Johnson 1995;
In the present study, the ME, NEL and OMD values of alfalfa herbage were 7.91 MJ/kg, 4.50 MJ/kg and 51.33% at 24 h incubation. Kamalak et al. (2005) also stated that the ME and OMD values of alfalfa hays ranged from 8.65 to 9.76 MJ/kg and 59.5 to 66.32%, respectively. These differences may be due to the variety of alfalfa, climatic properties, and time of harvest (phenological stage). In the current experiment, the estimated ME, NE\(_L\) and OMD values of \(P.\) lanceolata and \(A.\) patula herbages were 7.85–7.96 MJ/kg, 4.48–4.49 MJ/kg and 51.19–51.87%, respectively. In addition, an in vitro study determined that the leaf of \(P.\) lanceolata at the seed bulking stage has 7.24 MJ/kg ME, 3.88 MJ/kg NE\(_L\) and 47.47% OMD values for ruminant (Kara et al. 2015b). In that study, the low value of ruminal ammonia-N concentration in plantago herbage according to atriplex and alfalfa herbages may be related with low HC and high ADL contents or TCT value. These digestibility results indicated that alternative forage sources can be cultivated in salty or non-irrigated fields and can be used in grazing animal nutrition and for countries which have arid or semi-arid areas.

Conclusions

The results of the present experiment confirmed that \(A.\) patula and \(P.\) lanceolata, which are appropriate for warm climates and the flora of Anatolia (Mediterranean) can be used for grazing animals as alternative good quality forage plants to alfalfa hay. In addition, it is suggested that \(A.\) patula and \(P.\) lanceolata should be cultivated to improvement the quality of pasture. There is need for further studies about these arid and semi-arid plants, especially in vivo studies.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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