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Effect of phenological stage on nutrient composition, *in vitro* fermentation and gas production kinetics of *Plantago lanceolata* herbage

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ABSTRACT: This study was aimed at determining the nutrient composition and *in vitro* ruminal digestion values of *Plantago lanceolata* herbage in different phenological stages. The plant samples were gathered in the vegetative, flowering and early seed stages of the plant. The crude protein, diethyl ether extract, ash, non-fibre carbohydrates, and proanthocyanidins levels of the vegetative and flowering stages were higher than those of the early seed stage ($P < 0.001$). Structural carbohydrate levels ($P < 0.05$) were determined to have a higher value in the early seed stage. Glucose, fructose, Ca, K, Mg, P, Fe and Cu concentrations decreased as the plant matured ($P < 0.001$), but Na, Zn, and Mn concentrations increased ($P < 0.05$). The asymptote gas production, gas production rate ($P < 0.001$), total gas production at 24 h ($P = 0.002$), metabolic energy, net energy lactation and organic matter digestibility values and the number of *Entodinium* ($P < 0.001$) and total bacteria count ($P = 0.026$) of the flowering and vegetative stages were higher than those of the early seed stage. Methane produced by 0.2 g dry matter was similar in the three phenological stages ($P = 0.078$). The bound condensed tannins and saponin contents of plants and ammoniacal-N, number of total protozoa and pH value of rumen fluid were similar in the three different phenological stages ($P > 0.05$). The present study indicates that *P. lanceolata* in the vegetative and flowering stages has, owing to its chemical composition, energy content and digestibility, the potential to be used as a forage source for ruminants in areas affected by drought.

Keywords: *in vitro* gas production; *Plantaginaceae;* ruminal microbiota; vegetative

Forage has significant importance for rumination, digestion physiology and the production of good-quality animals (Van Soest et al. 1991; NRC 2001). The quality of forage changes according to the family of the plant, phenological stage, climatic conditions (annual rainfall, temperature etc.), soil content (irrigation, pH, salty etc.) and preservation form (herbage, hay, silage). In lands which have a high amount of annual rainfall the quality of various plant species in grasslands and pastures is good, and it is easy to cultivate plants. However, in lands which have arid and semi-arid climate conditions, green forage can only be grown at a certain level in the spring and early summer months in grasslands; after early summer most of the plants turn yellow and dry out. In addition, 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 reported that global warming has caused changes in climate types resulting in semi-humid and semi-arid areas changing into semi-arid or arid areas (Altin et al. 2012). For drought lands, research on alternative plant species which have forage properties is required. However, plantago species are suitable for cultivation in arid lands and different kinds of soil types (salty and sandy soil) (Stewart 1996).

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Plantago species, which are widespread in grasslands in warm climates, are perennial or annual, can be cultivated in soil with pH range 4.2–7.8 and with sandy, neutral and basic properties, can grow up to 50 cm (according to climatic conditions) and comprise about 270 species. Besides, this plant family is resistant to cold weather conditions. These species are members of the *Plantaginaceae* family, which is present in many countries (Stewart 1996; Samuelsen 2000). In Turkey, *P. lanceolata* (narrow-leaf species or Ribwort Plantain), *P. major* and *P. asiatica* (broad-leaf species) of the plantago genus are commonly present and grow naturally in pastures, whether they are farmed or not. Plantago species are avidly consumed by grazing animal species due to their peculiar mushroom smell (volatile oct-1-en-3-ol content) and palatability (sorbitol content) (Stewart 1996; Fons et al. 1998).

The negative effects of climate change on quality forage production have become manifest in the lands surrounding the Mediterranean Sea, which include southern Europe and the eastern Mediterranean Levant countries, such as Turkey (IPCC 2014; Cook et al. 2016). In the Mediterranean Levant region, which has arid and semi-arid climatic conditions, the quality and quantity of forage production for harvesting and grazing have decreased. In recent years, researchers in countries where the effects of drought are pronounced have suggested *P. lanceolata* as an alternative forage for grazing during periods of drought over the summer months (Powell et al. 2007; Somasiri et al. 2015). The nutrient quality and dry matter yields of pastures has decreased, especially during periods characterised by low soil humidity, in arid or semi-arid areas. Pure swards of *P. lanceolata* have been shown to yield 8–19 t dry matter/ha/year under drought conditions (with irrigation or non-irrigation) in New Zealand (Minnee et al. 2013; Lee et al. 2015). We hypothesised that Plantago species might represent potential alternative forage for grazing animals in the Mediterranean Levant region and other arid or semi-arid lands (Fraser et al. 1996; Ramirez-Restrepo and Barry 2005). The purpose of this study was to determine the composition of selected nutrients (protein, fibre, minerals etc.) and anti-nutritional factors (saponin, proanthocyanidins, etc.), and *in vitro* ruminal digestibility values of *Plantago lanceolata* herbage in different phenological stages (vegetative, flowering and early seed).

MATERIAL AND METHODS

Area from which samples were collected. Plant samples were collected from the Kayseri province in Turkey. Kayseri is located (38°56 N, 34° 24 E) in the centre (Cappadocia district) of Turkey. It is located 1050 m above sea level. Kayseri is vulnerable to desertification and has < 400 mm annual rainfall, which is convectional and frontal type. The vegetation of this place comprises steppe and dry forests. The climate of Kayseri province is arid and semi-arid based on to annual temperature values and rainfall (Altin et al. 2012).

The soil type of the Kayseri province is loamy neutral (pH = 7.11), salt-free (E.C. value is 0.33 mmhos/cm), containing 2.94% organic matter, 21.87% lime, 5.96 P_2O_5 kg/d dry soil and 22.87 K_2O kg/d dry soil in the upper ~ 30 cm layer of soil.

Plant samples. A random sampling method was used from native grassland. The three phenological stages of *P. lanceolata* were investigated. Plant samples were gathered in the vegetative (May 2015) (n = 8), flowering (June 2015) (n = 8) and early seed (July 2015) (n = 8) stages, as shown Table 1. The plant samples were gathered from eight different plants for each phenological stage and included all the parts of the plants. Eight samples of *P. lanceolata* were randomly collected from four different areas in Kayseri. Sampling amount was about 500 g for each replicate, and it was about 4 kg (0.5 kg × 8 = 4 kg) for each phenological stage. The samples included all aerial parts (leaf, stem, or bud-flower) of the plant. Cutting was manually performed at 1 cm above the soil, in the middle of a 1.0-m^2 delimited area. The cut forage was collected in plastic bags

Table 1. Phenological stages of *P. lanceolata* investigated in the present study

Phenological stage	Stage explanation
Vegetative	stem height < 10 cm; stem leafless; no bud or flowers; stalked and green leaves in basal rosette and quite erect; lanceolata and linear leaves
Flowering	stem height > 20 cm; stem leafless; open flowers; stalked and green leaves in basal rosette and quite erect; lanceolata and linear leaves
Early seed	brown flowers; stem leafless; first green pods; stalked and green leaves in basal rosette and quite erect; lanceolata and linear leaves; brown leaves in basal parts

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and weighed. Subsequently, about 500 g representative sample for each phenological stage were dried in a thermostatically controlled cabinet (Lovidond, Switzerland) for 48 hours at 60 °C, and dry matter (DM) content was calculated.

Chemical analysis. The dried samples were milled down to 1 mm diameter (IKA MF10.1, Germany), and the ash, crude protein (CP) and diethyl ether extract (EE) levels were determined according to the AOAC (2000). The neutral detergent fibre, acid detergent fibre, and acid detergent lignin contents were determined according to Van Soest et al. (1991). For determination of neutral detergent fibre, sodium sulphite and α-amylase levels were analysed in plant samples (Kara et al. 2016). These fibre contents did not include the ash residue (abbreviated as; aNDFom, ADFom, and ADL). The phenolic compound (proanthocyanidins (PAC), bound condensed tannin (BCT) and extractable condensed tannin (ECT)) contents of plant samples were determined according to the butanol-HCl method using a spectrophotometer (UviLine 8100, SI Analytics, Germany) (Makkar et al. 1995; Kara 2016). The saponin content was determined according to the methanol-p-anisaldehyde-sulphuric acid method of Ersahince and Kara (2017). The non-fibrous carbohydrate (NFC) composition of samples was calculated using the formula of NRC (2001). All chemical analyses were conducted in duplicate (2) for samples (8) of each phenological stage ($n = 8 \times 2$).

The calcium, magnesium, phosphorus, sodium and potassium macromineral and ferrous, zinc, and manganese micromineral contents in the different phenological stages of *P. lanceolata* were determined using an XRF device (PAN analytical AXIOS Advanced, the Netherlands).

The glucose and fructose concentrations of the different phenological stages of *P. lanceolata* were detected in a high-performance liquid chromatography device (Agilent 1260, USA) according to the method of Guney et al. (2013). The relative feed value of plant samples was calculated using the equations of Jeranyama and Garcia (2004).

***In vitro* Hohenheim gas production technique**. Rumen fluid was taken from two cattle (Simmental, of about 450 kg live weight and 18 months of age), fed with a ration (8.5 kg dry matter/day/cow) containing forage (approximately 60% of total mix feed on a dry matter basis, 2.1 kg of maize silage, 1.8 kg of alfalfa herbage + 0.9 kg of wheat straw) and 3.7 kg of concentrated mix feed with 15% CP and 2700 kcal/kg metabolic energy (approximately 40% of total mix feed on a dry matter basis). Rumen fluid was transferred into a thermos under constant CO_2 gas, and then filtered using muslin with 1–5 µm pore diameter to obtain the inoculum. In the *in vitro* Hohenheim gas production technique (Menke and Steingass 1988), pre-dried *P. lanceolata* herbage samples (0.200 ± 0.010 g; substrate), were incubated with rumen fluid inoculum (10 ml) and buffer mixture (20 ml) in an aerobic glass fermenter (with 100 ml volume, Model Fortuna, Germany) at 39 °C for up to 96 h, in triplicate. Besides, three blank glass fermenters (no samples) were incubated to provide correction values.

Determination of *in vitro* cumulative total gas volume and patterns and *in vitro* methane production. The total gas volume and produced substrates were read from the volume lines on the glass fermenter at 3, 6, 12, 24, 48, 72 and 96 h. The amount of methane gas in total gas produced at 24 h was determined in an infrared methane measurement device (Sensor, Europe GmbH, Erkrath, Germany) according to Kara et al. (2015a). The gas production pattern was estimated using the following model of France et al. (2000):

$$y = A\ (1 - e^{-c^*t})$$

where: y = cumulative gas production (ml); t = the time of incubation (h); A = asymptote (total gas produced; ml); c = gas production rate

Estimated metabolic energy, net energy lactation and organic matter digestibility levels. These estimated energy and digestibility values were calculated according to the formulas of Menke and Steingass (1988) and Abas et al. (2005).

The composition of the *in vitro* fermentation fluid after 24 h of incubation. Selected chemical values were determined in the fluid in the *in vitro* glass fermenters which measured methane production after 24 h of incubation. The pH value of the filtered *in vitro* fermentation fluid was determined using a digital pH meter (Mettler Toledo S220, Switzerland). For the determination of ammoniacal-N (NH_3-N, mg/dl) concentration, fermentation fluid was centrifuged at 4000 rpm for 5 min and then distilled in a distillation system. The distillate in 4% (w : w) of H_3BO_3 was titrated with 0.1 N HCl. (Makkar and Becker 1996).

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The 10 ml of fermentation fluid in each fermenter were transferred to tubes. The total numbers and generic compositions of ciliate protozoa in fermentation fluid samples were determined according to the description of Kara et al. (2016). Ruminal total bacteria counts were determined using a spectrophotometer. One hundred microlitres of ruminal fermentation fluid were diluted with 35% formaldehyde (900 µl). The absorbance of the mixture was read at 660 nm wavelength using a spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd, UK) (Minato and Suto 1981).

The VFA concentration of *in vitro* ruminal fermentation fluid (acetic, propionic and butyric acids; mmol/L in fluid and % of total VFA) was detected using a gas chromatograph (Perkin Elmer Auto System XL, USA) device equipped with a flame ionisation detector (GC/FID) (Kara et al. 2016).

Statistical analysis. The obtained data were statistically analysed using SPSS 17.0 software (IBM Corp., Armonk, USA). One-way analysis of variance was conducted for variables tested in different phenological stages of the plant. Data were analysed using the following statistical model:

$$Yij = \mu ij + Si + ei$$

where: Yij = the general mean for each parameter investigated; µ = the mean of plant phenological stage for each tested parameter; S_i = the i^{th} effect of phenological stages of *P. lanceolata* on the observed parameters; e*i* = the standard error

The significance of differences in means were revealed using Tukey's multiple range test at $P < 0.05$. The data were presented as the mean ± standard error of the mean. Correlation coefficient (r) relationships were determined between plant phenological stage and the studied parameters using the aforementioned software.

RESULTS

Chemical composition

The carbohydrate (fibre, NFC, glucose, and fructose), CP, EE, tannin, saponin and macro- and micromineral contents and relative feed value in the different phenological stages of *P. lanceolata* are presented in Tables 2 and 3.

In vitro gas production and fermentation parameters of rumen fluid

The asymptote gas production (A_{gas}), gas production rate (c_{gas}) ($P < 0.001$), Gas24, Gas96 ($P = 0.002$), ME, net energy lactation and organic matter

Table 2. Nutrient matter composition and relative feed value of *P. lanceolata* herbage

Items (g/kg as DM)	Phenological stage			SD	*P*-value
	vegetative	flowering	early seed		
Crude protein	104.80 ± 0.40^{b}	110.80 ± 1.10^{a}	66.40 ± 0.50^{c}	20.80	< 0.001
aNDFom	382.20 ± 14.90^{c}	463.30 ± 2.50^{b}	552.10 ± 9.20^{a}	72.50	< 0.001
ADFom	313.10 ± 17.80	328.10 ± 15.20	377.90 ± 16.10	41.52	0.052
Acid detergent lignin	86.80 ± 6.35^{b}	116.50 ± 9.50^{ab}	148.10 ± 13.70^{a}	32.00	0.025
Diethyl ether extract	21.10 ± 0.10^{a}	17.90 ± 0.10^{b}	16.90 ± 0.10^{c}	1.90	< 0.001
Ash	122.30 ± 0.10^{a}	109.20 ± 0.10^{b}	101.00 ± 0.10^{c}	9.50	< 0.001
Non-fibre carbohydrate	390.80 ± 14.80^{a}	301.30 ± 1.04^{b}	236.30 ± 10.16^{c}	68.93	< 0.001
Proanthocyanidins	10.40 ± 0.40^{a}	6.00 ± 0.20^{b}	4.60 ± 0.20^{c}	2.60	< 0.001
Bound condensed tannin	1.05 ± 0.23	1.70 ± 0.02	0.79 ± 0.31	0.15	0.339
Extractable condensed tannin	6.36 ± 0.06^{a}	2.45 ± 0.14^{b}	2.44 ± 0.14^{b}	0.58	< 0.001
Saponin	3.53 ± 0.23	3.55 ± 0.23	3.87 ± 0.12	0.20	0.399
Glucose	18.90 ± 0.05^{a}	18.20 ± 0.08^{b}	16.60 ± 0.05^{c}	10.50	< 0.001
Fructose	10.60 ± 0.03^{a}	10.40 ± 0.33^{a}	8.30 ± 0.34^{b}	11.70	0.017
Relative feed value	157.54 ± 7.00^{a}	127.20 ± 2.77^{b}	100.17 ± 1.56^{c}	25.76	< 0.001

aNDFom = residual ash-free neutral detergent fibre assayed with α-amylase; ADFom = residual ash-free acid detergent fibre; DM = dry matter

^{a,b,c}Values within a row with different superscripts differ significantly at $P < 0.05$

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Table 3. Macromineral (g/kg dry matter) and micromineral (mg/kg dry matter) contents of *P. lanceolata* herbage in different phenological stages

Items		Phenological stage			SD	*P*-value
		vegetative	flowering	early seed		
Macrominerals	Ca	10.93 ± 0.09[a]	9.83 ± 0.01[b]	9.11 ± 0.03[c]	0.82	0.002
	K	8.37 ± 0.01[a]	7.30 ± 0.01[b]	6.69 ± 0.03[b]	0.78	0.002
	P	0.43 ± 0.005[a]	0.41 ± 0.007[a]	0.37 ± 0.006[b]	0.03	0.007
	Mg	0.21 ± 0.002[a]	0.17 ± 0.009[b]	0.14 ± 0.003[b]	0.03	0.010
	Na	0.01 ± 0.001[b]	0.02 ± 0.001[a]	0.02 ± 0.001[a]	0.004	0.006
Microminerals	Fe	205.00 ± 4.70[a]	192.35 ± 0.85[a]	125.85 ± 1.25[b]	38.15	< 0.001
	Zn	35.50 ± 0.50[c]	37.85 ± 0.45[b]	42.61 ± 0.61[a]	3.29	0.003
	Cu	32.05 ± 0.75[a]	28.56 ± 0.46[b]	14.16 ± 0.04[c]	8.50	< 0.001
	Mn	27.85 ± 0.65[c]	35.01 ± 0.29[b]	39.48 ± 1.08[a]	5.31	< 0.001

[a,b,c]Values within a row with different superscripts differ significantly at $P < 0.05$

digestibility levels and number of *Entodinium* ($P < 0.001$) and total bacteria count ($P = 0.026$) of the flowering and vegetative stages were higher than those of the early seed stage (Tables 4 and 5).

The estimated metabolic energy, net energy lactation and organic matter digestibility values were negatively correlated with maturation of the plant ($r = -0.78$), aNDFom ($r = -0.81$) and ADL ($r = -0.70$); however, they were positively correlated with the NFC ($r = 0.80$) and PAC ($r = 0.72$) (for 4.60–10.40 g/kg PAC) ($P < 0.01$) (Table 6).

DISCUSSION

The results of the present study demonstrated that the highest CP content of *P. lanceolata* herbage was in the flowering stage. Plant CP content was reduced with maturation. In addition, previous studies stated that the leaf parts alone of *P. lanceolata* harboured from about 110 to 160 g/kg CP in different phenological stages (Guil-Guerrero 2001; Kara et al. 2015b). Guil-Guerrero (2001) reported a CP value of 186.10 g/kg for *P. major* leaves and 229.60 g/kg for *P. media* leaves. In the present study, the fibre composition of the plant increased with plant maturation. The composition of the cell wall carbohydrate in the present study was different from the results of previous studies (Jackson et al. 1996; Kara et al. 2015b); this may be related with the plant parts (leaves or all parts), phenological stage of the plant and season. In addition, Kamalak et al. (2005) stated that the CP content of fourteen different alfalfa varieties harvested at the beginning of flowering, which is a common practice in Turkey for quality forage production, varied between 150.5 and 213.9 g/kg. The EE content of *P. lanceolata* was

Table 4. Gas kinetics and fermentation parameters of *P. lanceolata* herbage in different phenological stages

Items	Phenological stage			SD	*P*-value
	vegetative	flowering	early seed		
A_{gas} (ml/0.2 g DM)	55.57 ± 0.11[a]	55.43 ± 0.06[a]	49.47 ± 0.17[b]	3.02	< 0.001
c_{gas} (h)	0.090 ± 0.001[a]	0.084 ± 0.001[b]	0.062 ± 0.001[c]	0.01	< 0.001
Gas24 (ml/0.2 g DM)	48.33 ± 1.42[a]	45.16 ± 0.40[a]	43.33 ± 0.21[b]	2.91	0.002
Gas96 (ml/0.2 g DM)	57.00 ± 0.63[a]	54.88 ± 0.61[a]	52.33 ± 0.91[b]	2.62	0.002
Metabolisable energy (MJ/kg DM)	9.24 ± 0.21[a]	8.90 ± 0.07[a]	8.31 ± 0.03[b]	0.50	< 0.001
Net energy lactation (MJ/kg DM)	5.58 ± 0.18[a]	5.29 ± 0.06[a]	4.78 ± 0.03[b]	0.43	< 0.001
Organic matter digestibility (%)	59.94 ± 1.35[a]	57.69 ± 0.44[a]	53.99 ± 0.19[b]	3.17	< 0.001
Methane (ml/0.2 g DM)	7.97 ± 0.23	7.61 ± 0.06	8.09 ± 0.04	0.80	0.078
Ammoniacal-N (mg/l)	37.40 ± 0.43	36.75 ± 1.41	35.77 ± 1.98	2.25	0.854
pH	6.85 ± 0.01	6.86 ± 0.02	6.85 ± 0.02	0.02	0.730

A_{gas} = asymptote (total gas produced; ml/0.2 g DM); c_{gas} = gas production rate (h); DM = dry matter; Gas24 = volume of total gas produced after 24 h of incubation; Gas96 = volume of total gas produced after 96 h of incubation; Methane = volume of *in vitro* methane produced as ml/0.2 g DM at 24 h [methane production, ml = (Gas24, ml × methane as percent in Gas24)/100]

[a,b,c]Values within a row with different superscripts differ significantly at $P < 0.05$

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Table 5. Effects of *P. lanceolata* herbage in different phenological stages on ruminal bacteria count (× 10^9/ml rumen fluid) and number of protozoa (× 10^4/ml rumen fluid) and VFA concentration

Items	Phenological stage			SD	*P*-value
	vegetative	flowering	early seed		
Total bacteria	3.01 ± 0.15^a	2.65 ± 0.19^{ab}	2.17 ± 0.12^b	0.43	0.026
Total protozoa	47.03 ± 0.23	47.80 ± 0.81	43.72 ± 0.37	2.27	0.850
Protozoa numbers					
Isotricha	0.16 ± 0.02^b	0.16 ± 0.01^b	0.33 ± 0.01^a	0.08	< 0.001
Dasytricha	0.51 ± 0.02^b	0.47 ± 0.01^b	0.80 ± 0.09^a	0.17	0.011
Entodinium	43.13 ± 1.67^a	43.90 ± 1.53^a	27.93 ± 0.07^c	6.91	< 0.001
Total VFA (mmol/l)	94.68 ± 3.57	99.15 ± 6.37	85.38 ± 5.05	9.81	0.233
Molar proportions of VFA (mmol/l)					
Acetic acid	56.01 ± 0.50	55.92 ± 5.18	48.69 ± 3.29	6.46	0.318
Propionic acid	28.30 ± 0.65^a	22.60 ± 1.19^b	19.36 ± 1.70^b	4.35	0.007
Butyric acid	11.83 ± 0.17	12.40 ± 0.29	11.71 ± 0.38	0.55	0.286
Individual percent proportions in VFA (%)					
Acetic acid	59.34 ± 2.52	56.17 ± 1.72	56.99 ± 1.26	3.19	0.516
Propionic acid	29.99 ± 1.52^a	22.82 ± 0.28^b	22.61 ± 1.06^b	3.98	0.005
Butyric acid	12.52 ± 0.29	12.66 ± 1.17	13.77 ± 0.53	1.29	0.491
(A + B)/P	2.39 ± 0.03^b	3.01 ± 0.06^a	3.14 ± 0.18^a	0.38	0.008

Total VFA = acetic acid + propionic acid + butyric acid as mmol/l in rumen fluid; (A + B)/P = (acetic acid + butyric acid)/propionic acid

^{a,b,c}Values within a row with different superscripts differ significantly at $P < 0.05$

similar to that reported by Kamalak et al. (2005) for alfalfa herbages.

The ash content of *P. lanceolata* herbage was at the same level as that of plants which are grown in arid or semi-arid lands (Guil-Guerrero 2001; Harrington et al. 2006; Kara et al. 2015b). The ash contents of *P. lanceolata* herbages were at the same level as those found in quality forages (alfalfa,

Table 6. Correlation coefficients (*r*) for the relationship of phenological stage and chemical composition with gas production pattern and estimated *in vitro* digestion parameters

	Maturity of plant	NFC	aNDFom	PAC	ADL
Metabolisable energy	–0.784**	0.799**	–0.816**	0.716**	–0.708**
Net energy lactation	–0.783**	0.797**	–0.814**	0.717**	–0.707**
Organic matter digestibility	–0.789**	0.804**	–0.819**	0.723**	–0.702**
c_{gas}	–0.933**	0.883**	–0.936**	0.523	–0.918**
A_{gas}	–0.875**	0.808**	–0.866**	0.394	–0.872**
Ammonia-N	–0.125	0.132	–0.120	0.305	–0.023
Methane	0.277	–0.335	0.307	–0.218	0.180
Acetic acid	–0.491	0.409	–0.438	0.200	–0.656
Propionic acid	–0.890**	0.876**	–0.849**	0.738*	–0.813**
Butyric acid	–0.098	–0.006	–0.069	–0.261	–0.095
VFA	–0.410	0.281	–0.339	0.069	–0.629
(A + B)/P	0.836**	–0.859**	0.811**	–0.782*	0.622

A_{gas} = asymptote (total gas produced; ml/0.2 g DM); (A + B)/P = (acetic acid + butyric acid)/propionic acid; ADL = acid detergent lignin; aNDFom = residual ash-free neutral detergent fibre assayed with α-amylase; c_{gas} = gas production rate (h); DM = dry matter; Methane = *in vitro* methane production as ml/0.2 g DM at 24 h; NFC = non-fibre carbohydrate; PAC = proanthocyanidins; Total VFA = acetic + propionic + butyric acids as mmol/l in rumen fluid

*Correlation is significant at 0.01 level; **correlation is significant at 0.05 level

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legume pastures and grass-legume mixtures) according to the NRC (2001). The macro- and micromineral compositions of *Plantago* herbages were consistent with the results of previous studies (Yildirim et al. 2001; Harrington et al. 2006; Stef et al. 2010). The Ca, Cu, Fe, Mn and Zn compositions of *P. lanceolata* herbages in the current study were at the same level as those of alfalfa, legume pasture, grass pasture and grass-legume mixture (NRC 2001). The results of the present study showed that the Ca, P, K, Mg, Fe and Cu contents were reduced in *P. lanceolata* with the progression of phenological stage; however, Na, Zn and Mn contents increased. Accordingly, the satisfactory mineral composition of *P. lanceolata* herbage has showed that it is suitable for quality forage for grazing animals in countries which have a climate prone to drought.

The effects of PAC on digestion activity are determined by its concentration in animal diet. Low levels of PAC (10–30 g/kg) in diet have been reported to minimise ruminal methane production and to provide by-pass properties to protein (Min et al. 2006). In contrast, high PAC content (> 50 g/kg) in diet may lead to negative effects on the digestion and the ruminal digestion of protein and other nutrients and may block the absorption of nutrient matter by the gut tract (Barry 1987). In the present study, the PAC contents of the different phenological stages of *P. lanceolata* may be characterised as low according to Jackson et al. (1996). The decrease in ECT content during plant maturation was similar to the results of Ersahince and Kara (2017). In a previous study, it was determined that herbage of *P. lanceolata* contained 13.80 g/kg PAC at the start of the flowering stage (Kara et al. 2016).

The relative feed value of *P. lanceolata* for the studied phenological stages was similar to the relative feed value of alfalfa hay for the pre-bud and full flowering stages (Jeranyama and Garcia (2004). Kara et al. (2015b) reported that the relative feed value of *P. lanceolata* leaves was 196% at the seed bulking stages. The high CP and moderate aNDFom and PAC contents of *P. lanceolata*, especially in the flowering stage, were sufficiently high to warrant consideration for use as a source of alternative forage.

In the conducted studies, the differences in the nutrient content of *Plantago* species may be related with phenological stage, plant variety, nutrient content of soil and stress factors (such as irrigation, salinity). There are not many studies on the nutrient composition of *Plantago* species in terms of livestock nutrition. Our study will contribute to the literature concerning alternative forage which can be cultivated in arid and semi-arid lands.

Although high PAC content in forage which is consumed by ruminants has negative effects on digestion and assimilation activity, high PAC can reduce the number of protozoa as well as methane production in the rumen environment (Min et al. 2006; Kara et al. 2016). In the present study, the low PAC content of *P. lanceolata* herbage did not change methane production or the number of total protozoa. Rumen protozoa can be classified into two major types: *Entodinium* (*Oligoisotricha*) and *Holotricha* (*Isotricha, Dasytricha*) ciliates (Gocmen and Ozbel 2001). The increased number of *Isotricha* and *Dasytricha* in the early seed stage of *P. lanceolata* found in the present study did not affect methane production. In addition, the reduction in both the number of *Entodinium* and the total bacteria count in the early seed stage of *P. lanceolata* may be due to the reduction in CP and NFC content and the increase in fibre content (especially decreased ADL content) (Takenaka et al. 2004; Martinele et al. 2010).

The *in vitro* gas production level is affected by the nutrient composition of tested feedstuff, the presence of compounds inhibiting (such as tannins) gas production, the microflora and microfauna content of the rumen fluid (donor animal's diet) and the quality of fermentation provided (Blummel and Orskov 1993; Kara et al. 2015a; Kara et al. 2016). In the present study, the asymptote gas production, gas production rate, gas24 and gas96 values of *P. lanceolata* in the vegetative and flowering stages were higher than those of the early seed stage. *P. lanceolata* herbage had high *in vitro* fermentation values in all phenological stages. These results demonstrate that even if *P. lanceolata* can be easily digested in all phenological stages up until seed bulking. The decreased *in vitro* total gas production with plant maturation may be related with the increase in structural components of the plant cell wall, decrease in ruminal total bacteria count and NFC content. There was a negative correlation between cell wall structural carbohydrates (aNDFom and ADL) and *in vitro* gas production; also, there was a positive correlation between the level of easily soluble carbohydrates (NFC) and *in vitro* gas production values. In the current study, it was

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found that, owing to its digestibility and energy levels, *P. lanceolata* herbage could represent an alternative to traditional forages (legume and grass pastures and also alfalfa herbage), which require much irrigation, for the Mediterranean Levant region (Kamalak et al. 2005; Kara et al. 2016). Thus, *P. lanceolata* can be cultivated as quality forage with high energy and organic matter digestibility values for arid and semi-arid or salty lands.

Although the estimated gas production parameters were low at plant maturation, ruminal ammoniacal-N concentrations did not change. Normally, high gas production would indicate high ruminal degradability, but feedstuffs which are rich in terms of CP can produce low total gas during fermentation. This is because protein and non-protein nitrogen fermentation in rumen produces ammoniacal-N, which affects the carbonate buffer equilibrium by neutralising H^+ ions from volatile fatty acids without the release of carbon dioxide.

In the present study, the ruminal total VFA level (85.38–94.68 mmol/l) in the fermentation process of *P. lanceolata* herbage was at an ideal level for the normal ruminal ecosystem. Molar propionic acid and (A + B)/P in rumen fluid were highest in the vegetative stages compared to the flowering and early seed stages; this may be related with soluble carbohydrates levels, aNDFom, ADL and bacteria – protozoa counts. In addition, the fact that molar proportions and individual percent proportions in VFA of acetic and butyric acids did not different among phenological stages may be associated with the high digestibility of *P. lanceolata* herbage at all stages. Rumen microorganisms alter the basic parameters of the ruminal process, such as digestibility, methane production, ammoniacal-N concentration or VFA profile.

The results of the present work have demonstrated that *P. lanceolata,* which is appropriate for warm climates and the flora of Central Turkey, can be used in ruminant feeding as an alternative quality forage source, especially in the vegetative or flowering stages (containing approximately 110 g/kg CP, 380–460 g/kg aNDFom, 9 MJ/kg metabolic energy, 60% organic matter digestibility and high macro- and micromineral contents). Therefore, antinutritional factors (saponin, proanthocyanidins and bound and extractable condensed tannins) in *P. lanceolata* herbage were not present at toxic levels for grazing animals. In addition, it is suggested that *P. lanceolata* should be planted to improve the quality of pasture. There is need for further studies on *Plantago* species cultivation in southern Europe and the eastern Mediterranean Levant region and on *in vivo* feeding studies in grazing animals.

REFERENCES

Abas I, Ozpinar H, Can KH, Kahraman R (2005): Determination of the metabolisable (ME) and net energy lactation (NEL) contents of some feeds in the Marmara Region by in vitro gas technique. Turkish Journal of Veterinary and Animal Sciences 29, 751–757.

Altin TB, Barak AE, Altin BN (2012): Change in precipitation and temperature amounts over three decades in Central Anatolia, Turkey. Atmospheric and Climate Science 2, 107–125.

AOAC – Association of Official Analytical Chemists (2000): Official Methods of Analysis. 17th edn. AOAC International, Gaithersburg, USA.

Barry TN (1987): Secondary compounds of forages. In: Hacker JB, Ternouth JH (eds): Nutrition of Herbivores. Academic Press, Sydney. 91–120.

Blummel M, Orskov ER (1993): Comparison of in vitro gas production and nylon bag degradability of roughages in predicting of food intake in cattle. Animal and Feed Science Technology 40, 109–119.

Cook BI, Anchukaitis KJ, Touchan R, Meko DM, Cook ER (2016): Spatiotemporal drought variability in the Mediterranean over the last 900 years. Journal of Geophysical Research: Atmospheres 121, 2060–2074.

Ersahince AC, Kara K (2017): Nutrient composition and in vitro digestion parameters of Jerusalem artichoke (Helianthus tuberosus L.) herbage at different maturity stages in horse and ruminant. Journal of Animal and Feed Sciences 26, 213–225.

Fons F, Rapior S, Gargadennec A, Andary C, Bessiere JM (1998): Volatile components of Plantago lanceolata (Piantaginaceae). Acta Botanica Gallica 145, 265–269.

France J, Dijkstra J, Dhanoa MS, Lopez S, Bannink A (2000): Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: Derivation of models and other mathematical considerations. British Journal of Nutrition 83, 131–142.

Fraser TJ, Scott SM, Rowarth JS (1996): Pasture species effects on carcass and meat quality. Proceedings New Zealand Grassland Association 58, 63–66.

Gocmen B, Ozbel Y (2001): Flagellated (Mastigophora) and Holotrichous ciliated (Ciliophora) protozoa inhabiting the rumen. Turk Parazitoloji Dergisi 25, 405–425.

https://doi.org/10.17221/2/2017-VETMED

Guil-Guerrero JL (2001): Nutritional composition of Plantago species (P. major L., P. lanceolata L., and P. media L.). Ecology of Food and Nutrition 40, 481–495.

Guney D, Bak ZD, Aydinoglu F, Turna I, Ayaz FA (2013): Effect of geographical variation on the sugar composition of the oriental beech (Fagus orientalis Lipsky). Turkish Journal of Agriculture and Forestry 37, 221–230.

Harrington KC, Thatcher A, Kemp PD (2006): Mineral composition and nutritive value of some common pasture weeds. New Zealand Plant Protection 59, 261–265.

IPCC – Intergovernmental Panel on Climate Change (2014): Summary for policymakers. In: Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, Adler A, Baum I, Brunner S, Eickemeier P, Kriemann B, Savolainen J, Schlomer S, von Stechow C, Zwickel T, Minx JC (eds): Climate Change 2014: Mitigation of climate change. Contribution of working group iii to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, USA.

Jackson FS, McNabb WC, Barry TN, Fooc YL, Jason P (1996): The condensed tannin content of a range of subtropical and temperate forages and the reactivity of condensed tannin with ribulose- 1.5-bis-phosphate carboxylase (rubisco) protein. Journal of the Science of Food and Agriculture 12, 483-492.

Jeranyama P, Garcia AD (2004): Understanding relative feed value (RFV) and relative forage quality (RFQ). College of Agriculture and Biological Sciences. South Dakota State University, South Dakota-USA. Available at: http://agbiopubs.sdstate.edu/articles/Ex8149.pdf.

Kamalak A, Canbolat O, Erol A, Kilinc C, Kizilsimsek M, Ozkan CO, Ozkose E (2005): Effect of variety on chemical composition, in vitro gas production, metobolizable energy and organic matter digestibility of alfalfa hays. Livestock Research for Rural Development 17. Available at www.lrrd.org/lrrd17/7/kama17077.htm.

Kara K, Guclu BK, Baytok E (2015a): Comparison of nutrient composition and anti-methanogenic properties of different Rosaceae species. Journal of Animal and Feed Sciences 24, 308–314.

Kara K, Guclu BK, Aktug E, Baytok E (2015b): The determination of nutrient matter composition and in vitro digestion parameters of narrow-leaf plantain (Plantago lanceolata) in ruminant. Saglik Bilimleri Dergisi 24, 149–155.

Kara K, Aktug E, Ozkaya S (2016): Ruminal digestibility, microbial count, volatile fatty acids and gas kinetics of alternative forage sources for arid and semi-arid areas as in vitro. Italian Journal of Animal Science 15, 673–680.

Lee JM, Hemmingson NR, Minnee EMK, Clark CEF (2015): Management strategies for chicory (Cichorium intybus L.) and plantain (Plantago lanceolata L.): Impact on dry matter yield, nutritive characteristics and plant density. Crop and Pasture Science 66, 168–183.

Makkar HPS, Becker K (1996): Nutritional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. Animal and Feed Science Technology 63, 211–228.

Makkar HPS, Blummel M, Becker K (1995): Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and their implication in gas production and true digestibility in vitro techniques. British Journal of Nutrition 73, 897–913.

Martinele I, Santos GRA, Matos DS, Batista AMV, Dagosto M (2010): Diet botanical composition and rumen protozoa of sheep in Brazilian semi-arid area. Archivos de Zootechia 59, 169–175.

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

Min BR, Pinchak WE, Anderson RC, Fulford JD, Puchala R (2006): Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat. Journal of Animal Science 84, 2546–2554.

Minato H, Suto T (1981): Technique for fractionation of bacteria in rumen microbial ecosystem. 4. Attachment of rumen bacteria to cellulose powder and elution of bacteria attached to it. Journal of General and Applied Microbiology 27, 21–31.

Minnee EMK, Clark CEF, Clark DA (2013): Herbage production from five grazable forages. Proceedings of the New Zealand Grassland Association 75, 245–250.

NRC – National Research Council (2001): Nutrient requirements of dairy cattle. 7th edn. Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture and Natural Resources, National Research Council. National Academy Press, Washington, D.C.

Powell AM, Kemp PD, Jaya IKD, Osborne MA (2007): Establishment, growth and development of plantain and chicory under grazing. Proceedings of the New Zealand Grassland Association 69, 41–45.

Ramirez-Restrepo CA, Barry TN (2005): Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. Animal and Feed Science Technology 120, 179–201.

Samuelsen AB (2000): The traditional uses, chemical constituents and biological activities of Plantago major L. A review. Journal of Ethnopharmacology 71, 1–21.

Somasiri SC, Kenyon PR, Kemp PD, Morel PCH, Morris ST (2015): Growth performance and carcass characteristics

https://doi.org/10.17221/2/2017-VETMED

of lamb grazing forage mixes inclusive of plantain (Plantago lanceolata L.) and chicory (Cichorium intybus L.). Small Ruminant Research 127, 20-27.

Stef DS, Gergen I, Trasca TI, Harmanescu M, Stef L, Druga M, Biron R, Heghedus-Mindru G (2010): Screening of 33 medicinal plants for the microelements content. Journal of Animal Science and Biotechnology 43, 127–132.

Stewart AV (1996): Plantain (Plantago lanceolata) – a potential pasture species. Proceedings of the New Zealand Grassland Association 58, 77–86.

Takenaka A, Tajima K, Mitsumori M, Kajikawa H (2004): Fiber digestion by rumen ciliate protozoa. Microbes and Environments 19, 203–210.

Van Soest PJ, Robertson JB, Lewis BA (1991): Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.

Yildirim E, Dursun A, Turan M (2001): Determination of the nutrition contents of the wild plants used as vegetables in upper Coruh Valley. Turkish Journal of Botany 25, 367–371.

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