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The *in vitro* digestion of neutral detergent fibre and other ruminal fermentation parameters of some fibrous feedstuffs in *Damascus* goat (*Capra aegagrus hircus*)

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ABSTRACT. The study aimed to compare *in vitro* gas and methane production and ruminal fermentation patterns of lucerne hay (*Medicago sativa* L.), sugar beet pulp (*Beta vulgaris* L.), maize silage (*Zea mays* L.), plantago hay (*Plantago lanceolata* L.), ajuga hay (*Ajuga bombycina* L.), guelder-rose leaf (*Viburnum opulus* L.), tomato pomace (*Solanum lycopersicum* L.), Jerusalem artichoke hay (*Helianthus tuberosus*) and pomegranate peel (*Punica granatum* L.) in rumen fluid of *Damascus* goats. Ruminal fermentation parameters such as kinetics, gas production, organic matter digestibility-gas (OMd_{gas}), true-dry matter digestibility (T-DMd), true-organic matter digestibility (T-OMd), true-neutral detergent fibre digestibility (T-NDFd), partitioning factor (PF_{24}) and gas yield (GY_{24}) and metabolizable energy (ME) were determined. The highest gas production from insoluble fraction (b_{gas}) and potential gas production $(a+b)_{gas}$ were in tomato pomace ($P < 0.001$). The (b_{gas}) and $(a+b)_{gas}$ values of plantago, ajuga and Jerusalem artichoke hays were higher than those of lucerne hay ($P < 0.001$). The T-DMd, T-OMd, T-NDFd, OMd_{gas}, ME and methane values of tomato pomace and sugar beet pulp were the highest in tested forages ($P < 0.01$). The highest acetic acid concentration in fermentation fluid was in Jerusalem artichoke hay and maize silage; the molarities of volatile fatty acids in fermentation fluids were ranged from 91.84 to 104.21 ($P < 0.001$). It can be concluded that tomato pomace and sugar beet pulp have the digestive potential in the goat rumen, although they promote high methane production. Moreover, hays of plantago, ajuga and Jerusalem artichoke as well as pomegranate peels may be used as alternative forages to common fibrous feedstuffs like lucerne hay in goat nutrition.

Introduction

Nowadays, global warming negatively influences the production of quality forages especially in arid and semi-arid areas. The eastern Mediterranean Levant region has observed the worst drought for about 900 years (IPCC, 2014). Researchers reported that global warming has caused changes in climate that result in turning semi-humid and semi-arid areas into arid areas (Altın et al., 2012). In arid or semi-arid lands, plants turn yellow-brown at the end of the summer and the plant flora of these lands has started to change to steppe or desert-like steppe. Therefore grazing herbivores in arid or semi-arid lands cannot gain required energy and nutrients. In addition, soil types such as sandy and salty constitute also the serious problem to solve (Tuteja, 2007). Therefore, animal nutritionists have been seeking

suitable and quality plants and tree-forages for arid and semi-arid areas (Kara et al., 2015a,b; 2018a,b).

Forages include inevitable diet components for herbivores. Forage-analysing laboratories have started to interpret forages in terms of neutral detergent fibre (NDF) digestibility (Musco et al., 2016; Jang et al., 2017). The high level of NDF digestibility is found in forage/fibrous feedstuffs in the diet of ruminants and can indicate the level of total digestibility of forage/fibrous feedstuffs. There are several important reasons to evaluate NDF digestibility. Generally, it is required for prediction of energy value of forages/fibrous feedstuffs (Traxler et al., 1998). Total tract NDF digestibility in ruminants comprises a fraction digested in rumen (about 93%) and a part digested in hindgut (Lopes et al., 2015a). Generally, the NDF digestibility of legume forages is lower (due to higher lignification – high lignin content) than of grass forages (NRC, 2001). Grasses are characterised by a very wide range of NDF content (46% for pasture, 50–69% for immature-mature hay and 51–66% for silage) and NDF digestibility. Maize silage has moderate NDF (44–54%) and low lignin (acid detergent lignin (ADL); 2.5–3.5%) values and a wide range of NDF digestibility. Total tract NDF digestibility has ranged from 25% to 70% for lucerne hay, from 25% to 80% for maize silage, and from 15% to 80% for grass hay (Combs, 2018). The discrepancies in NDF digestibility of maize silage can occur when maize herbage is harvested at 'the seed solidifies'–'the seed matures' stage (low NDF digestibility), or when it is at 'seed dough formation' stage (high NDF digestibility) (Lopes et al., 2015b). The ruminal digestions of grass and legume forages depend lignin content and lignifications for plant species (Musco et al., 2016).

The ruminal digestibility of the same forage may be different in ruminants such as goat, sheep, cattle or buffalo (Ramos et al., 2011; Musco et al., 2016; Ersahince and Kara, 2017; Jang et al., 2017) due to the microbial community and metabolite profile of the rumen (Lee et al., 2012). The aim of the present study was to compare the *in vitro* digestion parameters of some alternative fibrous feedstuffs, not commonly used in animal diets and with not known nutritional properties, from arid or semi-arid lands: plantago hay (*Plantago lanceolata* L.), ajuga hay (*Ajuga bombycina* L.), guelder-rose leaf (*Viburnum opulus* L.), tomato pomace (*Solanum lycopersicum* L.), Jerusalem artichoke hay (*Helianthus tuberosus*) and pomegranate peel (*Punica granatum* L.), with common forage/fibrous feedstuffs: lucerne hay (*Medicago sativa* L.), sugar beet pulp (*Beta vulgaris* L.), maize silage (*Zea mays* L.) by rumen fluid of *Damascus* goats (*Capra aegagrus hircus*).

Material and methods

Fibrous feedstuffs

The fibrous feedstuffs samples were supplied from the Kayseri, Cappadocia district, Turkey (38°56'N, 34°24'E). Semi-arid climate condition is dominant in this area (Altın et al., 2012). In this area, the native meadow for grazing goat and sheep is yellow-brown in the middle-end of summer.

Industrial by-products as sugar beet (*Beta vulgaris* L.) pulp and tomato (*Solanum lycopersicum* L.) pomace were supplied fresh from local factories – sugar factory and tomato paste factory, respectively. Pomegranate (*Punica granatum* L.) peel was receipted as fresh by-product from a fruit-juice production factory in Kayseri province.

Lucerne hay and maize silage were obtained from a local dairy cattle farmer. Other alternative forages were collected from native grassland of Kayseri province. Fresh lucerne (*Medicago sativa* L.) was harvested at vegetative stage, before flowering. Jerusalem artichoke herbage (*Helianthus tuberosus*), grown for tuber production, was cut at flowering stage of plant. Maize (*Zea mays* L.) silage was of moderate quality and it had preserved from maize herbage harvested at seed bulking stage. Plantago herbage (*Plantago lanceolata* L.) and ajuga herbage (*Ajuga bombycina* L.) were collected from native grassland at the same time (in June) at vegetative stage of phenological phase. Leaves of guelder-rose tree (*Viburnum opulus* L.) were collected manually from different trees at maturating stage of fruits. All feedstuffs were prepared in four replicates (500 g as wet each). All fibrous feedstuffs were dried at 55 °C for 48 h in a drying oven (Binder, Tuttlingen, Germany).

Chemical analysis

The dried samples were ground in a grinder mill (IKA A10, IKA-Werke, Staufen im Breisgau, Germany) up to 1 mm. The ash levels were determined after burning at 550 °C for 8 h (AOAC, 1990; method 942.05). Nitrogen (N) content was analysed by the Kjeldahl method; the crude protein content (CP) was calculated as N × 6.25 (AOAC, 1990; method 954.01). The ether extract (EE) and crude fibre (CF) levels were determined according to the method reported by the AOAC (AOAC, 1990; method 920.39; and AOAC, 1980; methods 7.066–7.070). Thermo-

stable α-amylase treated neutral detergent fibre did not include ash (aNDFom) content, which form the cell wall components in the samples, was analysed according to the methods reported by Van-Soest et al. (1991). The total condensed tannin (TCT) content of feedstuffs was determined using the butanol-HCl procedure of Makkar et al. (1995) at 100 °C in the presence of Fe^{3+} (Kara, 2016). To remove the extractable condensed tannins (ECT) from feedstuffs, the samples of feedstuffs were incubated with acetone/water mixture in tubes at room temperature for 24 h. The obtained reddish-yellowish extract, in which ECT were present, was discarded for ECT content reading with the use of spectrophotometer. The samples which had extractable condensed tannins compounds removed were analysed for the determination of bound condensed tannins (BCT) using the aforementioned procedure of Makkar et al. (1995) at 100 °C incubation in the presence of Fe^{3+}. The ECT was calculated with an equation: ECT = TCT − BCT (Kara, 2016). Absorbance values were analysed using a spectrophotometer (UviLine 8100, SI Analytics; Mainz, Germany) at 550 nm wavelength. Non-fibrous carbohydrates (NFC) compositions of fibrous feedstuffs were calculated using the formulation of NRC (2001). Measurements were performed in duplicate.

Rumen fluid inoculum

Rumen fluid required for *in vitro* gas production technique, was obtained from three male *Damascus* goat (*Capra aegagrus hircus*) fatteners (15 months of age, about 70 kg live weight) fed diet containing 0.6 kg/day barley straw, 0.8 kg/day meadow hay, 0.25 kg/day crushed barley grain, 0.3 kg/day wet carrot pulp and 0.25 kg/day wheat bran as feed basis. The diet, including 1.73 kg as DM, 3660 kcal as metabolic energy (ME) and 159 g as CP per day, covered requirements for fattening goat (NRC, 1981). Rumen fluid of approximately 0.25 l (totally 750 ml for three goats) was collected two hours after feeding. The fresh rumen fluid samples were collected using CO_2 gas in a thermos container at 39 °C, and then these rumen fluids were filtered with four layers of cheesecloth in the laboratory.

The *in vitro* digestion procedure

The samples were incubated in rumen fluid and buffer mixture in glass fermenters (Model Fortuna, Haberle Labortechnik, Germany) with 100 ml volumes according to a the *in vitro* gas production procedure (Menke and Steingass, 1988). This procedure was carried out using bi-distilled water, macro-mineral solution, buffer solution, trace-mineral solution, resazurine solution and reducing solution. The dried samples (0.20 ± 0.01 g) were weighed in glass fermenters. The glass fermenters were pre-warmed at 39 °C in a thermostatically controlled cabinet (Incubator TC 256 G, Lovibond, Tintometer GmbH, Amesbury, UK), before 10 ml of rumen fluid and 20 ml of pre-warmed buffer mixture were dispensed anaerobically in each fermenter using an automatic dispenser. The glass fermenters were closed using one position polypropylene clamps and incubated at 39 ± 0.5 °C. Each sample was studied in triplicate for cumulative gas production and also in triplicate for methane analyses and determination of *in vitro* true neutral detergent fibre disappearance (T-NDFd), *in vitro* true organic matter disappearance (T-OMd) and *in vitro* true dry matter disappearance (T-DMd) (in total six measurements for each feedstuff). In addition, three blank fermenters (no template; rumen fluid + buffer mixture) were used to calculate the total gas production. During incubation, the total gas volume was recorded from the calibrated scale on the glass fermenters at 3, 6, 12, 24, 48, 72 and 96 h.

Determination of methane production

After measuring the total gas volume at 24 h (n = 3), the tubing of the plastic fermenter outlet was inserted into the inlet of the methane analyser (Sensor Europe GmbH, Erkrath, Germany) connected with a computer (Samsung, Seoul, South Korea) and the piston was pushed to insert the accumulated gas into the analyser. Methane level was displayed on a computer screen as a percent of total gas produced. This value was used to calculate methane in the total gas (Kara et al., 2015b).

Determination of *in vitro* digestibility

Three of the glass fermenters were stopped after 24 h, and then the fermenting liquor was analysed for volatile fatty acids (VFA), *in vitro* true neutral detergent fibre disappearance (T-NDFd) and *in vitro* true organic matter disappearance (T-OMd) values.

The non-digestible substrate (residue) (n = 3) was determined by filtering the fermentation residues using a vacuum unit (Velp Dietary Fibre Analyzer, Velp Scientific, Usmate, Italy) on pre-weighed glass crucibles (porosity #2, Velp Scientific, Usmate Italy) the fermentation residues. It was dried at 105 °C and weighed after cooling in a desiccator. Then this glass crucible with did residual was burned at 550 °C after cooling in a desiccator and residue DM of feedstuff was determined. The *in vitro* T-DMd was calculated as 1 − [(DM residue − DM blank) / initial DM)] × 100. The *in vitro* T-OMd was calculated as 1 − [(OM residue − OM blank) / initial OM)] × 100.

The NDF content of residue was determined with fibre analysis in residue according to modified method of Van Soest et al. (1991). The residue which was 0.10–0.15 g (can be different for each substrate) in glass crucible was incubated in 50–75 ml of neutral detergent fibre solutions for 30 min in a column fibre analyser (FIWE3, Velp Scientific, Usmate Italy). At the end, the soluble compounds in residue was vacuumed and then filtered with hot distiller water. The *in vitro* T-NDFd was calculated as 1 − [(NDF residue − NDF blank) / initial NDF)] × 100.

Determination of volatile fatty acids (VFA)

The VFA concentrations of *in vitro* digestion fluid in glass fermenters, which were used to determine the total gas and methane volume at 24 h, were analysed as triplicates for each feedstuffs samples.

The VFA analysis was performed according to the method of Ersahince and Kara (2017). The 10 ml of digestion fluid in each fermenter was collected in Falcon tubes and filtered. The 1.50 ml of digestion fluid was filtered and 0.375 ml of 25% meta-phosphoric acid (in distilled water) was mixed in micro centrifuge tube with 2 ml volume added, and stored in a deepfreeze (−20 °C) up to the VFA analyses. The frozen samples in micro-centrifuge tube were thawed at 4 °C and centrifuged at 15000 g for 15 min using a micro-centrifuge (Gyrozen 1524, Yuseong-gu, Daejeon, South Korea). The supernatants in tubes were extracted, filtered and then transferred (Millex Syringe-driven Filter Unit, Merck KGaA, Darmstadt, Germany) into the vials (Chromacol Ltd, Welwyn Garden City, UK). The VFA analysis was carried out by using a gas chromatograph (GC) (TRACE™ 1300, Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector (FID), an autosampler (AI 1310, Thermo Scientific, Waltham, MA, USA) and a polyethylene glycol column (length: 60 m; i.d: 0.25 mm; film thickness: 0.25µm) (TG-WAXMS, Thermo Scientific, Waltham, MA, USA). The helium, carrier gas, flow was 1.5 ml/min. The flows of air and hydrogen were 350 ml/min and 35 ml/min, respectively. The injection were at split mode. The port temperature of injection was at 280 °C. The oven temperature was increased from 160 to 180 °C at a rate 20 °C/min. The FID temperature was 300 °C. The concentrations of VFA as mmol/l were identified using a Xcalibur software program (Thermo Scientific, Waltham, MA, USA). The percentages of VFA [acetic (AA), butyric (BA) and propionic (PA) acids] and A/(B+P) ratio were calculated (Ersahince and Kara, 2017).

Estimation of fermentation parameters

The metabolic energy (ME) contents of the samples and gas organic matter digestibility (OMd_{gas}) was estimated using value of total gas produced at 24 h using the equations of Menke and Steingass (1987) and Blümmel and Ørskov (1993) as follows:

$$OMd_{gas}\ (\%) = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times ash,$$

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136 \times GP + 0.057 \times CP + 0.0029 \times EE^2,$$

where: GP – 24 h net gas production (ml/0.2 g DM), CP – crude protein (g/kg DM), ash – ash content (g/kg DM), and EE – ether extract (g/kg DM).

The gas yields (GY_{24}), partial factor (PF_{24}), and microbial crude protein production levels (MCP) of the samples at 24 h were calculated using the equations:

$$GY_{24} = (Gas24h \times 10^3) / T\text{-}DMd,$$
$$PF_{24} = T\text{-}DMd / Gas24h,$$

where: T-DMd – *in vitro* dry matter disappearance (mg) for g DM at 24 h (mg/g DM), Gas24h – volume (ml) of total gas produced for g DM at 24 h (ml/g DM).

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

$$Y = a + b \times (1 - \exp^{-ct}),$$

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

Statistical analysis

One-way analysis of variance (ANOVA) was implemented for the data which had homogeneous variances by General Linear Model procedure to test treatment differences. Data was analysed based according to model:

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

where: Yij – general mean common for each variables under research, µij – the mean of different fibrous feedstuffs for each tested parameter, Si – the effect of i – different fibrous feedstuffs on the examined variables, ei – the standard error term. The means were separated by Tukey's multiple range test at $P < 0.05$.

Linear relations among the studied parameters were determined using Pearson's correlation though SPSS 17.0 software (IBM Corp., Armonk, NY, USA).

Results

The CP contents of fibrous feedstuffs used in this study were ranged from about 7 to 23% in DM. The aNDFom contents in tested feedstuffs were changed from approximately 19 to 55%. The ash, NFC, TCT, ECT and BCT contents of fibrous feedstuffs were varied about 6–12%, 26–45%, 0.14–2.6%, 0.06–1.3% and 0.08–1.8%, respectively (Table 1).

The *in vitro* T-NDFd, T-OMd and T-DMd parameters of Jerusalem artichoke hay, ajuga hay, plantago hay, gulder-rose leaf and pomegranate peel were the same as for lucerne hay and maize silage. The highest T-NDFd was in sugar beet pulp ($P < 0.001$). The *in vitro* T-OMd and b_{gas} values of sugar beet pulp and tomato pomace were higher than those of pomegranate peel, Jerusalem artichoke hay and gulder-rose leaf ($P < 0.001$). The T-DMd values of lucerne hay and maize silage were similar to those of plantago hay, pomegranate peel, Jerusalem artichoke hay, ajuga hay and guilder-rose leaf. The *in vitro* T-DMd, T-OMd, T-NDFd, OMd_{gas}, ME, Gas24h, $(a+b)_{gas}$ and methane (ml) values of tomato pomace and sugar beet pulp were the highest in examined forages ($P < 0.001$). The lowest OMd_{gas}, which was calculated from produced total gas, was in pomegranate peel ($P < 0.001$) (Tables 2, 3 and 4).

Table 1. Nutrients content of fibrous feedstuffs, % in dry matter (DM)

Forages	Ash	CP	EE	aNDFom	NFC	TCT	BCT	ECT
Lucerne hay	8.94	22.47	0.51	42.55	25.53	0.61	0.15	0.46
Maize silage	11.56	7.79	2.56	51.15	26.94	0.65	0.17	0.48
Sugar beet pulp	5.67	10.69	0.10	54.32	29.22	0.14	0.06	0.08
Tomato pomace	11.05	13.71	0.92	19.14	44.82	2.30	0.53	1.77
Plantago hay	10.10	6.64	1.69	55.21	26.36	1.01	0.41	0.60
Pomegranate peel	4.32	8.37	0.44	31.50	55.37	0.85	0.55	0.30
Jerusalem artichoke hay	10.16	7.50	1.82	38.22	42.30	0.50	0.29	0.21
Ajuga hay	9.86	8.90	1.97	34.54	44.73	0.54	0.33	0.21
Guelder-rose leaf	10.78	8.20	1.02	37.76	42.24	2.55	1.26	1.29

CP – crude protein; EE – diethyl ether extract; aNDFom – thermostable α-amylase treated neutral detergent fibre content corrected for ash; NFC – non-fibrous carbohydrates; TCT – total condensed tannins; BCT – bound condensed tannins; ECT – extractable condensed tannins

Table 2. *In vitro* neutral detergent fibre, organic matter and dry matter digestions of some fibrous feedstuffs in rumen fluid of *Damascus* goat

Forages	T-NDFd	T-OMd	T-DMd	OMd_{gas}
Lucerne hay	38.43^{c}	63.26^{ab}	55.61^{c}	67.36^{b}
Maize silage	52.85^{bc}	64.59^{ab}	60.19^{c}	66.75^{b}
Sugar beet pulp	73.59^{a}	91.79^{a}	93.32^{a}	89.28^{a}
Tomato pomace	59.88^{b}	90.04^{a}	90.46^{ab}	89.60^{a}
Plantago hay	36.95^{c}	64.61^{ab}	57.06^{c}	72.77^{b}
Pomegranate peel	34.41^{c}	55.38^{b}	56.18^{c}	50.93^{c}
Jerusalem artichoke hay	34.68^{c}	56.49^{b}	57.61^{c}	63.98^{b}
Ajuga hay	35.95^{c}	65.61^{ab}	67.36^{bc}	69.46^{b}
Guelder-rose leaf	36.46^{bc}	59.67^{b}	61.85^{c}	64.21^{b}
SEM	4.74	3.54	3.56	2.09
SD	19.56	14.60	15.11	12.20
P-value	<0.001	<0.001	0.001	<0.001

T-DMd – *in vitro* true dry matter disappearance, as %; T-OMd – *in vitro* true organic matter disappearance, as %; T-NDFd – *in vitro* true neutral detergent fibre disappearance, as %; OMd_{gas} – organic matter digestion estimated using value of total gas production at 24 h, as %; SEM – standard error of means; SD – standard deviation of means

Table 3. *In vitro* gas kinetics of some fibrous feedstuffs in rumen fluid of *Damascus* goat

Forages	c_{gas}	a_{gas}	b_{gas}	$(a+b)_{gas}$
Lucerne hay	0.10^{a}	-0.93^{f}	57.55^{h}	56.62^{g}
Maize silage	0.06^{e}	-0.07^{d}	67.90^{d}	67.82^{d}
Sugar beet pulp	0.09^{b}	-0.62^{e}	87.53^{a}	86.90^{b}
Tomato pomace	0.07^{d}	3.91^{b}	87.74^{a}	91.66^{a}
Plantago hay	0.08^{c}	-0.05^{d}	72.67^{b}	72.62^{c}
Pomegranate peel	0.05^{f}	4.10^{a}	61.97^{e}	66.08^{e}
Jerusalem artichoke hay	0.08^{c}	3.22^{c}	59.16^{g}	62.39^{f}
Ajuga hay	0.06^{e}	-0.73^{e}	68.97^{cd}	68.24^{d}
Guelder-rose leaf	0.07^{d}	-1.16^{g}	60.49^{f}	59.32^{h}
SEM	0.003	0.506	2.62	2.72
SD	0.016	2.142	11.15	11.55
P-value	<0.001	<0.001	<0.001	<0.001

c_{gas} – gas production rate (0.2 g DM); a_{gas} – gas production (ml/0.2 g DM) from quickly soluble fraction; b_{gas} – gas production (ml/0.2 g DM) from insoluble fraction; $(a+b)_{gas}$ – potential gas production (ml/0.2 g DM); SEM – standard error of means; SD – standard deviation of means; $^{a-h}$ – means with different superscripts within each column are significantly different

Table 4. *In vitro* gas production and estimated digestion values of some fibrous feedstuffs in rumen fluid of *Damascus* goat

Forages	Gas24h	GY_{24}	PF_{24}	ME	CH_4
Lucerne hay	49.45^{b}	457.75	2.18	8.91^{b}	7.58^{b}
Maize silage	50.19^{b}	427.04	2.34	9.01^{b}	8.50^{b}
Sugar beet pulp	80.44^{a}	417.79	2.40	13.11^{a}	12.12^{a}
Tomato pomace	75.86^{a}	415.04	2.54	12.49^{a}	11.51^{a}
Plantago hay	57.51^{b}	507.34	2.27	10.00^{b}	8.51^{b}
Pomegranate peel	36.70^{c}	353.58	3.05	7.18^{c}	6.13^{b}
Jerusalem artichoke hay	49.18^{bc}	426.82	2.34	8.87^{bc}	7.77^{b}
Ajuga hay	52.42^{b}	366.26	2.78	9.31^{b}	8.53^{b}
Guelder-rose leaf	47.33^{bc}	385.54	2.59	8.62^{bc}	6.26^{b}
SEM	2.36	20.60	0.11	0.32	0.40
SD	13.76	87.40	0.50	1.86	2.11
P-value	<0.001	0.883	0.875	<0.001	<0.001

Gas24h – the *in vitro* total gas volume (ml) produced at 24 h; GY_{24} – gas yield is total gas volume (ml) produced for g T-DMd at 24 h; PF_{24} – partial factor is ratio T-DMd to Gas24h; ME – metabolizable energy as MJ/kg DM; CH_4 – *in vitro* methane production as ml/0.2 g DM at 24 h; SEM – standard error of means; SD – standard deviation of means; ^{abc} – means with different superscripts within each column are significantly different

Table 5. The composition of volatile fatty acids of fibrous feedstuffs digested *in vitro* in rumen fluid of *Damascus* goat

Forages	mmol/l, in digestion fluid				%, in TVFA			A/(P+B)
	AA	PA	BA	TVFA	AA	PA	BA	
Lucerne hay	57.29^{cd}	28.15	12.79	98.23^{ab}	57.60	29.99^{ab}	12.40	1.35
Maize silage	62.89^{ab}	29.30	12.01	104.21^{a}	60.32	28.15^{bc}	11.51	1.52
Sugar beet pulp	59.90^{bc}	29.11	13.00	102.02^{ab}	58.71	28.54^{abc}	12.74	1.42
Tomato pomace	56.86^{cd}	28.31	12.61	97.78^{ab}	58.09	29.19^{abc}	12.71	1.38
Plantago hay	56.52^{cd}	28.95	12.11	97.59^{ab}	57.91	29.67^{abc}	12.41	1.37
Pomegranate peel	55.43^{d}	26.96	12.21	94.61^{ab}	58.59	28.50^{bc}	12.90	1.41
Jerusalem artichoke hay	64.01^{a}	28.94	13.09	106.05^{a}	60.35	27.29^{c}	12.34	1.52
Ajuga hay	56.55^{cd}	27.76	12.30	96.62^{ab}	57.37	31.03^{a}	11.59	1.34
Guelder-rose leaf	55.39^{d}	26.62	9.82	91.84^{b}	60.39	28.96^{abc}	10.63	1.53
SEM	1.22	0.32	0.34	1.09	0.32	0.27	0.21	0.08
SD	5.19	1.39	1.44	4.63	1.37	1.16	0.90	0.01
P-value	<0.001	0.191	0.186	0.010	0.051	0.007	0.169	0.054

AA – acetic acid; PA – propionic acid; BA – butyric acid; TVFA – acetic + propionic + butyric acids, as mmol/l in digestion fluid; A/(P+B) – (acetic acid)/(propionic acid + butyric acid); SEM – standard error of means; SD – standard deviation of means; $^{a-d}$ – means with different superscripts within each column are significantly different

The c_{gas} value of lucerne hay was the highest and the lowest for pomegranate pomace ($P < 0.001$). Besides the a_{gas} values of pomegranate peel, tomato pomace and Jerusalem artichoke were higher than those of other fibrous feedstuffs in the *in vitro* fermentation with goat rumen fluid ($P < 0.001$). The *in vitro* GY_{24}, PF_{24} and molarities of PA and BA for different fibrous feedstuffs were similar after forages fermentation with goat rumen fluid ($P > 0.05$) (Tables 3, 4 and 5).

The *in vitro* cumulative gas production values of tomato pomace and sugar beet pulp were >100 ml/0.2 g DM at 96 h of incubation and these values were higher than those of other forages ($P < 0.05$) (Figure 1). Besides, plantago hay had at high level digestibility characteristics for *in vitro* goat ruminal digestion model. The $(a+b)_{gas}$ and cumulative gas production values at 96 h of tomato pomace and sugar beet pulp were higher than those of other alternative forages ($P < 0.05$) (Figure 1 and Table 3). The $(a+b)_{gas}$ and cumulative gas production values up to 96 h of plantago hay were higher than those of lucerne hay and maize silage ($P < 0.05$). The b_{gas}, $(a+b)_{gas,}$ Gas24h and gas production up to 96 h of ajuga hay were similar to those of maize silage ($P > 0.05$) (Tables 3 and 4, Figure 1). The cumulative gas production values up to 96 h of Jerusalem artichoke hay and guilder-rose leaf were very close to those of maize silage (Figure 1). But the cumulative gas production value up to 24 h of pomegranate peel was lower than that of maize silage; and was similar with that of maize silage at 48–96 h (Figure 1, Table 4).

The highest CH_4 production and ME values were in tomato pomace and sugar beet pulp ($P < 0.05$), these values were higher than those of common

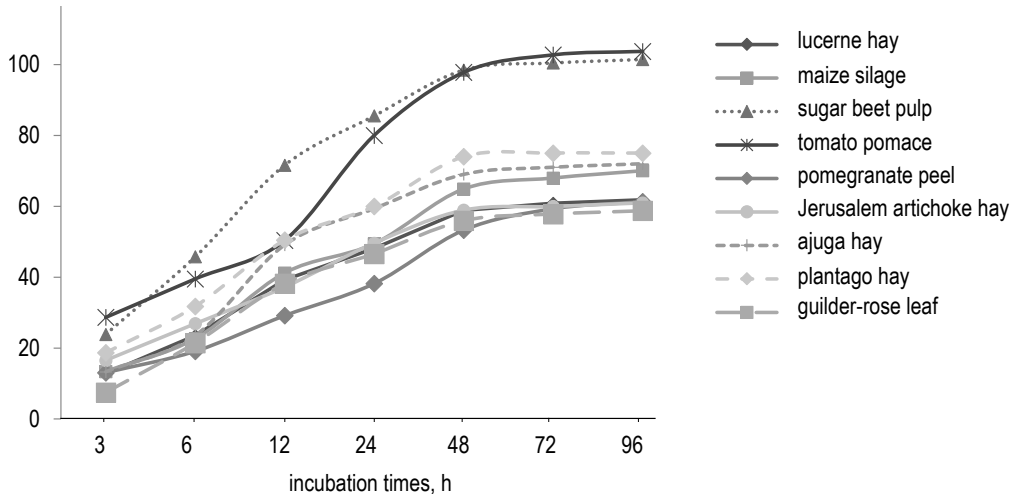

Figure 1. The *in vitro* cumulative total gas production values up to 96 h of examined fibrous feedstuffs

Table 6. Correlations between tested *in vitro* digestion and fermentation parameters

	PF_{24}	T-DMd	ME	OMd_{gas}	CH_4	T-NDFd	T-OMd	c_{gas}	a_{gas}	b_{gas}	$(a+b)_{gas}$	AA	PA	BA	TVFA
GY_{24}	-0.925**	-0.311	0.423	0.438	0.419	-0.044	-0.325	0.345	-0.131	0.054	0.028	0.156	0.258	0.198	0.217
PF_{24}	1	0.255	-0.451	-0.473*	-0.438	0.004	0.272	-0.421	0.213	-0.022	0.018	-0.313	-0.266	-0.222	-0.325
T-DMd		1	0.722**	0.698**	0.689**	0.867**	0.998**	0.156	0.076	0.867**	0.851**	0.007	0.216	0.171	0.104
ME			1	0.988**	0.962**	0.797**	0.710**	0.411	-0.021	0.842**	0.808**	0.166	0.392	0.324	0.290
OMd_{gas}				1	0.951**	0.734**	0.684**	0.431	-0.086	0.818**	0.773**	0.134	0.390	0.293	0.261
CH_4					1	0.745**	0.678**	0.317	0.050	0.861**	0.841**	0.587*	0.504*	0.432*	0.424*
T-NDFd						1	0.855**	0.382	-0.093	0.805**	0.759**	0.159	0.327	0.228	0.244
T-OMd							1	0.136	0.115	0.863**	0.854**	0.006	0.204	0.183	0.103
c_{gas}								1	-0.473*	0.102	0.010	0.265	0.343	0.228	0.316
a_{gas}									1	0.094	0.277	0.123	-0.046	0.277	0.138
b_{gas}										1	0.983**	-0.022	0.326	0.245	0.133
$(a+b)_{gas}$											1	0.002	0.306	0.288	0.154
AA												1	0.638**	0.475*	0.924**
PA													1	0.729**	0.851**
BA														1	0.748**

abbreviations – see Tables 2–5; * – correlation is significant at the 0.05 level; ** – correlation is significant at the 0.01 level

forages (lucerne hay and maize silage) and other examined uncommon forages ($P < 0.05$) (Table 4). The CH_4 production and ME values of lucerne hay and maize silage were similar to those of alternative forages (Table 4).

The highest acetic acid concentration in digestion fluid was found in Jerusalem artichoke hay ($P < 0.001$) but it did not differ from maize silage; and the acetic acid concentrations in digestion fluid for ajuga hay, guilder-rose leaf, pomegranate peel, tomato pomace, plantago hay and lucerne hay were similar. The TVFA molarities in digestion fluid for Jerusalem artichoke and maize silage were similar to those of lucerne hay, sugar beet pulp, tomato pomace, plantago hay, pomegranate peel and ajuga hay; but higher than that of guilder-roe leaf ($P = 0.01$; Table 5). The percentages of AA and BA in VFA and A/(B+P) for *in vitro* digestion fluid of forages did not differ ($P > 0.05$; Table 5).

The T-NDFd was positively correlated with T-DMd ($r = 0.867$), T-OMd ($r = 0.855$), CH4 ($r = 0.745$), b_{gas} ($r = 0.805$), $(a+b)_{gas}$ ($r = 0.759$), ME ($r = 0.797$) ($P < 0.01$). The T-OMd was positively correlated with T-DMd ($r = 0.998$), T-NDFd ($r = 0.855$) methane ($r = 0.678$), $(a+b)_{gas}$ ($r = 0.854$), and ME ($r = 0.710$) ($P < 0.01$). The T-DMd was positively correlated with ME ($r = 0.722$), OMd_{gas} (r=0.698), CH_4 (r=0.689), T-NDFd (r=0.867), T-OMd ($r = 0.998$) and $(a+b)_{gas}$ ($r = 0.851$) ($P < 0.01$). The CH_4 production was positively correlated with T-NDFd ($r = 0.745$), T-OMd (r =0.678), TVFA ($r = 0.424$), b_{gas} ($r = 0.861$) and $(a+b)_{gas}$ ($r = 0.808$) ($P < 0.01$) (Table 6).

Discussion

The condensed tannins compounds are flavonoid polymers found in many plant species consumed by goats. The effects of TCT on digestion activity are determined by its concentration in animal diet. Low levels of TCT (up to 3% in DM basis) in the diet provide by-pass properties to protein (Min et al., 2006). In contrast, high TCT content (>5% in DM basis) in the diet may lead to negative effects on digestion in overall and ruminal digestion of protein and other nutrients, may block the absorption of nutrient matter by the gastrointestinal tract and reduce palatability (Barry and Blaney, 1987). Jakhesara et al. (2010) emphasised that ruminal microflora profile of goat fed total diet with 3.5% TCT (*Acacia nilotica* leaf), changed in favour of Actinobacteria (+35%), Clostridia (+38%) and Proteobacteria (+43%) microorganisms. The goat ruminal microbiota can adapt against plant secondary compounds (Jakhesara et al., 2010). In the present study, up to 2.55% of TCT did not negatively affect *in vitro* gas production and digestion parameters in goats.

Rate of *in vitro* gas production is related to feed digestibility (Menke and Steingass, 1988). The end-products of carbohydrate compounds fermentation, which are structural (also known as plant cell wall substances) or easy-digestible carbohydrates, comprise the *in vitro* total gases (carbon dioxide, methane, and other trace gases). Plant cell wall substances: cellulose, hemicellulose and lignin, are the major source of carbohydrates in forage/

fibrous feedstuffs. The amounts of cellulose, hemicellulose and lignin are different in various types of forage and so differently affect digestibility. In the present study, high T-NDFd, T-DMd and T-OMd values of sugar beet pulp and tomato pomace paralleled with high *in vitro* gas production and ME values. It is known that ruminal methane production is high in feeds that are easily digestible (Hook et al., 2010). The digestion of structural- and easy digestible-carbohydrates in the rumen exposes hydrogen as a by-product, which is essential to the growth of archaea methanogens (Wang et al., 2016). In the current study, methane production was positively correlated with T-DMd, T-NDFd, T-OMd and $(a+b)_{gas}$. Besides, the highest methane production (ml/g DM) and the highest digestibility were found in sugar beet pulp. The high NDF digestibility of forage can increase the digestibility of DM and OM in forage. Generally, the T-NDFd values of forages used in the current study were positively correlated with T-OMd and T-DMd. The T-NDFd of different forages ranging from 34 to 74% may be related with structural carbohydrates, soluble or digestible carbohydrates and industrial or fermentative processes.

In the *in vitro* rumen model for goat, the *in vitro* T-NDFd, T-OMd and T-DMd parameters, and gas amount produced by Jerusalem artichoke hay, ajuga hay, plantago hay, guilder-rose leaf and pomegranate peel were the same with those of lucerne hay and maize silage. These findings demonstrated that these forages could be considered as alternative feed for goat. However, the positive results may differ due to the plant phenological stage, soil-climate-conditions (hay) and industrial processes and preservation methods (pomace and silage) (Tuteja, 2007; Kara et al., 2015a,b). In goat the *in vitro* ruminal digestibility of sugar beet pulp (although 54.32% aNDFom) and tomato pomace (although 1.77% ECT) in the present study was high. Hays of plantago, ajuga and Jerusalem artichoke and the pomegranate peels could be used as alternative forages to lucerne in goat diets in arid and semi-arid areas. Plantago species and ajuga, which are plants growing naturally in meadows, can be applied for animals breed on pastures lacking in this type of plants. In our previous *in vitro* study we stated that plantago hay can be used in beef cattle feeding as an alternative quality forage source, containing approximately 110 g/kg CP, 380–460 g/kg aNDFom, 9 MJ/kg ME, 60% organic matter digestibility and high macro- and micromineral contents (Kara et al., 2018b). Therefore, anti-nutritional factors (saponin, proanthocyanidins and bound and extractable condensed tannins) in *P. lanceolata* herbage were not present at toxic levels for grazing animals (Kara et al., 2018b). In the present study, the *in vitro* estimated ME values and gas production of plantago hay for *Damascus* goat were higher than those found in the study of Kara et al. (2018b) conducted on the beef cattle rumen fluid. The high values of gas production of plantago hay in the present study can be associated with effective roughage digestion by goat rumen flora (Muir, 2011).

The gas production from quickly soluble fraction (a_{gas}) was high (positive values) for pomegranate peel, tomato pomace, ajuga hay and Jerusalem artichoke and can resulted from high NFC content. Whereas T-NDFd values of pomegranate peel, ajuga hay and Jerusalem artichoke were not high and ranged from about 34 to 36%. It can be stated that the digestion of plant cell wall substances from previously mentioned fibrous feedstuffs is not effective in terms of total gas production. Otherwise, tomato pomace in the present study was characterized by high level of T-NDFd; at low level plant cell wall substances may be not very effective.

The *in vitro* total gas and digestion values of pomegranate peel in the present study on rumen goat were higher than those of pomegranate pomace in beef cattle reported by Ebrahimi (2012) and Kara et al. (2018a). Mirzaei-Aghsaghali et al. (2011) found that *in vitro* ME and OMd_{gas} values of pomegranate peel for rumen fluid of steer beef were 8.85 MJ/kg DM and 59.0%, respectively. The results of Mirzaei-Aghsaghali et al. (2011) were higher than those obtained in the present study. The *in vitro* gas production of Jerusalem artichoke hay for cattle has reached 31–44 ml/0.2 g DM at 24 h and 37–48 ml/0.2 g DM in the study of Ersahince and Kara (2017). Whereas T-DMd and total gas production values of Jerusalem artichoke noted by Ersahince and Kara (2017) study were lower than those found in our study. Such difference may be related to the bacterial community and metabolite profiles of the goat rumen (Lee et al., 2012). Wang et al. (2016) demonstrated that Bacteroidetes, Proteobacteria, and Firmicutes were identified as the dominant phyla across all age groups in the goat rumen. In a previous study, in which the level of digestibility was examined in various herbivores managed together in a paddock (22.3 ha) with 76% of heathlands and 24% of improved pasture area, goats were found to have the highest dry matter digestion (791 g/kg DM) in comparison to sheep (749 g/kg DM), cattle (733 g/kg DM) and horses (569 g/kg DM) (Osoro et al., 2017). Although the carbohydrates content of sugar beet pulp had higher aNDFom and lower NFC than those of tomato pomace. Sugar beet pulp had the same *in vitro* cumulative gas production volume as tomato

pomace (due to good level of fibre digestibility). The lucerne hay, plantago hay and maize silage have included lower NFC than Jerusalem artichoke hay, pomegranate peel, ajuga hay and guelder-rose leaf. However, lucerne hay, plantago hay and maize silage had reached the same *in vitro* cumulative gas production volume as Jerusalem artichoke hay, pomegranate peel, ajuga hay and guelder-rose leaf. It can be the effect of good digestion of structural carbohydrates substances. The greatest difficulty in the degradation of the plant cell wall in the rumen is probably the occurrence of a cross-link between cellulose, hemicellulose, lignin and other compounds limiting the access of enzymes to the forage substrate. Free phenolic acids and soluble phenolic-carbohydrate complexes have both been shown to inhibit rumen microbial activity and may prevent microbial attachment to the surface of feed particles (Wang and McAllister, 2002).

The highest concentrations of short chain VFA in the rumen fluid had AA, PA and BA. The molarity of ruminal VFA in the present study was positively correlated with ruminal digestibility and was in agreement with the results of Ersahince and Kara (2017). The increased molarity of AA in comparison to other acids in the rumen fluid may be due to increased fibre digestion. The molarities of PA and BA change in line with the digestibility of starch, organic acid and other substances (Allen, 1997). Molarities of VFA in the current study were in range 94.61–106.05 mmol/l according to gas chromatograph analysis. The high AA molarities in Jerusalem artichoke hay and maize silage may be connected with fibre digestion. The VFA molarities in all forages samples used in the present study were at optimum level for rumen physiology and acidity (Allen, 1997).

Conclusions

It can be concluded that tomato pomace and sugar beet pulp have the potential for digestion in the rumen of goats, although they promote high methane production. Moreover, in goat nutrition hays of plantago, ajuga and Jerusalem artichoke as well as pomegranate peels may be used as alternative forages to common fibrous feedstuffs like lucerne hay.

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