



## Comparison of nutrient composition and anti-methanogenic properties of different *Rosaceae* species

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**ABSTRACT.** The aim of the study was to compare different parts (leaves, fruits, seeds) of three *Rosaceae* species (*Rosa canina*, *Crataegus orientalis* and *Crataegus monogyna*) in terms of nutrient composition and *in vitro* ruminal fermentation parameters. The *in vitro* total gas production levels of the leaves of all *Rosaceae* species were similar ( $P > 0.05$ ). The *in vitro* total gas production of *R. canina* fruits and seeds was lower compared with those of *C. orientalis* and *C. monogyna* fruits ( $P = 0.001$ ) and seeds ( $P = 0.008$ ). The *in vitro* methane production of *R. canina* seeds was lower (13.66%) than that of the *Crataegus* seeds (16.50%–16.80%;  $P < 0.01$ ). The methane production of the leaves and fruits of all three *Rosaceae* species was similar ( $P > 0.05$ ). There were no differences among the *in vitro* short-chain fatty acid (SCFA), organic matter digestibility (OMD), metabolizable energy (ME) and net energy lactation (NE<sub>L</sub>) levels of leaves of the three *Rosaceae* species. The ME, NE<sub>L</sub>, SCFA and OMD levels of *Rosaceae* fruits and seeds differed significantly ( $P < 0.01$ ). The *Rosaceae* fruits and seeds could be ordered as *C. orientalis* > *C. monogyna* > *R. canina* in terms of ME, NE<sub>L</sub>, SCFA and OMD. The leaves of the *Crataegus* species, when compared in terms of roughage source for grazing animals in scrublands, contain a moderate level of crude protein and fibre, but the crude protein level in the leaves of *R. canina* was rather low. The leaves and fruits of all tested *Rosaceae* species and the seeds of *R. canina* can be used as rumen modulators with an anti-methanogenic effect. These results suggest that *Crataegus* leaves have the advantage of maintaining their nutritive value for ruminants throughout the dry season (especially in late summer) when grasses dry up.

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### Introduction

Browse species (shrub and tree forages) are commonly used in animal nutrition in many regions of the world (Vandermeulen et al., 2013). Tree forages usually provide supplemental protein and energy when grasses are mature and of low value and are a reserve of feed that can be utilized in time of drought in arid and semiarid regions of the world (Kibont and Ørskov, 1993; Ramirez-Orduña et al.,

2003). Tree forage species are also rich in secondary metabolites such as tannins, and are called tanniferous plants. Recent studies have revealed the inhibitory effect of tannins and tanniferous plants on methane emission by ruminants (Dong et al., 2010; Szumacher-Strabel et al., 2011). The phytochemicals, such as tannins and flavonoids, occurring in the structures of *Rosa canina* (in fruits, seeds, leaves) may reduce ruminal methane production (Woodward et al., 2002; Szumacher-Strabel

et al., 2011). Dong et al. (2010) showed that addition of herbal medicine mixture consisting of *Crataegus pinnatifida* to a goat diet reduced methane production, increased propionate concentrations and decreased protozoa numbers. *Rosaceae* species comprise spiny shrubs and trees native to the temperate regions of the world. The studied *Crataegus* species and *Rosa canina* are members of the *Rosaceae* family (Ercisli, 2004; Hummer and Janick, 2009).

In Mediterranean countries like Turkey, members of the *Rosaceae* family, including the *Crataegus* species, are aggressive pioneers of distributed sites, and often cover overgrazed pastures and abandoned farm fields, especially in late summer. These plant species may be used as feeds for grazing cattle, and may also have anti-methanogenic properties. This study was conducted to determine the effects of different parts of some *Rosaceae* species on methane emission and some ruminal fermentation parameters.

## Material and methods

### Plant samples

In the study, the leaves, seeds and fruits of *Rosa canina*, *Crataegus orientalis* and *Crataegus monogyna* were investigated. Plant samples were gathered at the time of the fruits' maturity (October) in the provinces of Kayseri and Nevşehir (Cappadocia) in Turkey's Central Anatolia region. The samples were clipped with scissors from three different sections and included the fruits and leaves of eight different plants of each species. The seeds were removed from the fruit samples from each plant. In the laboratory, the leaves and fruits were manually separated from the original cuttings. These plant parts (seeds, fruits and leaves) were prepared separately for each plant species. Chemical analysis and *in vitro* gas production were carried out separately with these plant parts.

### Chemical analysis

The dry matter (DM) levels of the fruit parts (without seeds) ( $n = 8$ ) were determined after drying for 48 h at 60°C in a thermostatically controlled cabinet (Lovidond, Switzerland). The DM levels of the leaves ( $n = 8$ ) and seeds ( $n = 8$ ) were determined after 24 h at 105°C. After drying, the samples were milled through a 1 mm sieve (IKA MF10.1; 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 according to AOAC 1990 (method 942.05). Nitrogen (N) content was measured by the Kjeldahl

method (AOAC, 1990; method 954.01) and crude protein (CP) was calculated as  $N \times 6.25$ . The ether extract (EE) (AOAC, 1980; method 920.39) and crude cellulose (CC) (AOAC, 1990; methods 7.066–7.070) levels were determined. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analysed using Fibre Analyser (Velp FIWE3; Italy) according to Van Soest et al. (1991). NDF was determined using sodium sulphite and heat-stable amylase. Neither NDF nor ADF were inclusive of residual ash. The total condensed tannin (CT) contents of the samples were determined by the butanol-HCl method reported by Makkar et al. (1995) using a spectrophotometer (Shimadzu 1208 UV/VIS; Japan). Chemical analyses were carried out in duplicate.

### *In vitro* Hohenheim gas production technique

*In vitro* gas production was carried out using the Hohenheim gas test. The samples of leaves, fruits and seeds, as well as a mixture of each part of *R. canina*, *C. orientalis* and *C. monogyna* were collected. The rumen fluid used in this technique was obtained from two beef cows fed a diet containing lucerne hay-maize silage (about 60%) and concentrate feed mixture (about 40%). Rumen fluid was collected into a thermos containing water at 39°C under CO<sub>2</sub> gas, and filtered through four layers of cheesecloth in the laboratory. The technique was carried out according to the procedures of Menke and Steingass (1988). The plant samples were incubated in rumen fluid and buffer mixture in glass syringes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettleschie; Germany). The dry samples ( $200 \pm 10$  mg) were weighed in triplicate into glass syringes. Thirty millilitres of the rumen fluid + buffer mixture in a 1:2 (v/v) ratio were injected into each syringe. After closing the clips on the silicon tubes 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. Then, the clips were closed, the initial volume recorded and the syringes incubated in a water bath at 39°C for 24 h. The *in vitro* gas production analyses were carried out in triplicate. Three blank syringes (no sample; rumen fluid + buffer mixture) were used to calculate total gas production.

### Determination of *in vitro* total gas and methane production

After 24 h of incubation, the total gas volume was recorded from the calibrated scale on the syringe. After measuring the total gas volume, the tubing of the plastic syringe outlet was inserted into the inlet

of a methane analyser (Sensor, Europe GmbH; Erkrath, Germany) connected with a computer (Samsung, South Korea) and the piston was pushed to insert the accumulated gas into the analyser. Methane as production was displayed on a computer screen as a percent of total gas produced. This value was used to calculate methane in the total gas volume (Goel et al., 2008).

### Calculation and statistical analysis

Methane production was calculated using the following formula:

$$\text{Methane production (ml)} = [\text{in vitro total gas produced (ml)} \times \text{methane produced (\%)}] / 100$$

The ME and OMD contents of different parts of the plants were calculated using the equations of Menke et al. (1979) and Menke and Steingass (1988) and were as follows:

$$\text{ME (MJ} \cdot \text{kg}^{-1} \text{ DM)} = 2.20 + 0.136 \times \text{GP} + 0.057 \times \text{CP}$$

$$\text{OMD (mg} \cdot \text{kg}^{-1} \text{ DM)} = 14.88 + 0.889 \times \text{GP} + 0.45 \times \text{CP} + 0.0651 \times \text{A}$$

The  $\text{NE}_L$  was calculated according to Blümmel et al. (1997):

$$\text{NE}_L \text{ (MJ} \cdot \text{kg}^{-1} \text{ DM)} = 0.115 \times \text{GP} + 0.0054 \times \text{CP} + 0.014 \times \text{EE} - 0.0054 \times \text{A} - 0.36$$

SCFA were calculated using the equation of Makkar (2005):

$$\text{SCFA (mmol} \cdot \text{g}^{-1} \text{ DM)} = 0.0222 \times \text{GP} - 0.00425 \times 5$$

where: GP – 24 h net gas production ( $\text{ml} \cdot 200 \text{ mg}^{-1}$ ), CP – crude protein ( $\text{g} \cdot \text{kg}^{-1} \text{ DM}$ ), A – ash content ( $\text{g} \cdot \text{kg}^{-1} \text{ DM}$ ), EE – ether extract ( $\text{g} \cdot \text{kg}^{-1} \text{ DM}$ ).

The statistical analysis of data was performed using SPSS 17.0 software (2008). The statistical significance among groups was determined by one-way ANOVA. Levene's test was performed to check the homogeneity of variances. Tukey's Multiple Range Test, one of the multiple comparison tests, was used when the difference among groups was found to be statistically significant. The data were presented on the basis of mean and  $\pm$  standard error of mean.

The correlations ( $r$ ) among the nutrient components and gas production were determined using the SPSS procedure (SPSS 17.0 software; 2008).

## Results

The nutrient contents of different parts of the tested *Rosaceae* species are presented in Table 1. The CP content ranged from 101 to 111  $\text{g} \cdot \text{kg}^{-1}$  of *Crataegus* leaves and was about 87  $\text{g} \cdot \text{kg}^{-1}$  of *R. canina* leaves.

The CP content of the studied *Rosaceae* fruits and seeds ranged from 41 to 78  $\text{g} \cdot \text{kg}^{-1}$ . The NDF ranged from 286 to 304  $\text{g} \cdot \text{kg}^{-1}$  in *Crataegus* leaves and 224  $\text{g} \cdot \text{kg}^{-1}$  in *R. canina* leaves. The *Rosaceae* fruits contained from 180 to 201  $\text{g} \cdot \text{kg}^{-1}$  NDF and their seeds had from 553 to 598  $\text{g} \cdot \text{kg}^{-1}$  NDF. The levels of total CT were in a narrow range from 21 to 44  $\text{g} \cdot \text{kg}^{-1}$  for different parts of *R. canina* and in a wide range from 17 to 126  $\text{g} \cdot \text{kg}^{-1}$  from 17 to 18  $\text{g} \cdot \text{kg}^{-1}$  in fruits and seeds, from 106 to 126  $\text{g} \cdot \text{kg}^{-1}$  in leaf in different parts of *Crataegus* species plants.

The total gas production and methane production at 24 h are shown in Table 2 and Figures 1 and 2. The total gas production of the different leaves ranged from 38.38 to 41.87  $\text{ml} \cdot 200 \text{ mg}^{-1} \text{ DM}$ . The total gas production of *R. canina* fruits and seeds was lower than of *C. orientalis* and *C. monogyna* fruits ( $P \leq 0.001$ ) and seeds ( $P < 0.01$ ). The *in vitro* gas production of *Rosaceae* family species (a mixture of three plants) was determined as 55.34  $\text{ml} \cdot 200 \text{ mg}^{-1} \text{ DM}$  in fruits, 39.69  $\text{ml} \cdot 200 \text{ mg}^{-1} \text{ DM}$  in leaves, and 23.55  $\text{ml} \cdot 200 \text{ mg}^{-1} \text{ DM}$  in the seeds ( $P < 0.001$ ; Figure 1). Total gas production when a mixture of *Rosaceae* fruits was used was higher than for the seed and leaf mixes ( $P < 0.001$ ; Figure 1). The *in vitro* methane production of *R. canina* seeds was lower than of the *Crataegus* species seeds ( $P < 0.01$ ). However, the methane production of the different leaf and fruit samples was similar ( $P > 0.05$ ; Table 2). The *in vitro* methane production of the *Rosaceae* leaf mixture (12.37%) was lower (15.65%) than of the seed mixture and similar (13.66 %) as that of the fruit mixture ( $P < 0.001$ ; Figure 2).

There were no differences among the *in vitro* ME,  $\text{NE}_L$ , SCFA and OMD levels of *R. canina*, *C. orientalis* and *C. monogyna* leaves (Table 3). ME,  $\text{NE}_L$ , SCFA and OMD levels in the fruits and seeds of *Rosaceae* species differed ( $P < 0.01$ ). The fruits and seeds could be ordered as *C. orientalis* > *C. monogyna* > *R. canina* in terms of ME,  $\text{NE}_L$ , SCFA and OMD.

For leaves of *Rosaceae* family species, the *in vitro* methane production was negatively correlated ( $P < 0.01$ ) with the contents of NDF ( $r = -0.88$ ), CT ( $r = -0.94$ ), CP ( $r = -0.83$ ). The NDF contents in leaves of the *Rosaceae* family species were positively correlated with the CT ( $r = 0.98$ ) and CP ( $r = 0.88$ ) contents ( $P < 0.01$ ). For *Rosaceae* fruits, the *in vitro* total gas production was negatively correlated with the NDF ( $r = -0.88$ ) and CT ( $r = -0.89$ ), but positively correlated with the CP ( $r = 0.84$ ) contents ( $P < 0.01$ ). The NDF contents in the fruits of *Rosaceae* family species were positively cor-

**Table 1.** Chemical composition of different parts of *Rosaceae* species

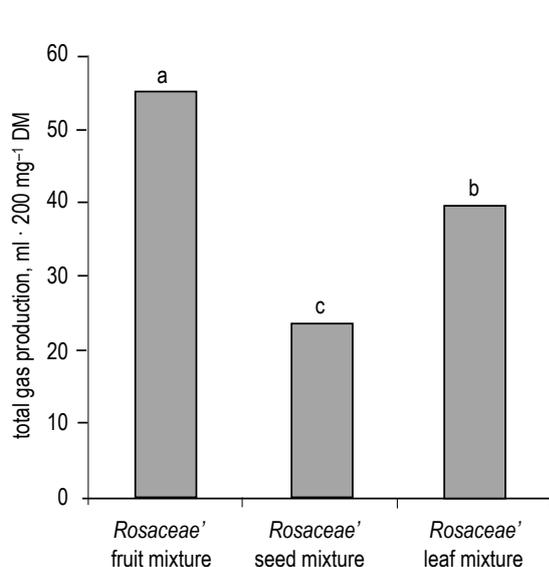
Parts of plant	<i>Rosaceae</i> species	DM g · kg <sup>-1</sup>	Ash	CP	EE	CC	ADF	NDF	CT
Leaf	<i>R. canina</i>	587.40	107.60	87.57	72.96	127.35	152.38	224.43	44.03
	<i>C. orientalis</i>	573.33	91.25	111.88	87.75	146.74	217.68	304.62	106.93
	<i>C. monogyna</i>	569.36	85.78	101.57	87.61	126.45	187.29	286.34	126.90
Fruit	<i>R. canina</i>	541.73	52.71	41.62	9.48	161.20	175.43	201.38	21.59
	<i>C. orientalis</i>	329.81	59.86	47.96	13.69	139.58	171.76	180.90	18.74
	<i>C. monogyna</i>	247.87	58.48	42.75	21.24	136.80	181.19	189.26	18.85
Seed	<i>R. canina</i>	785.65	22.73	78.89	74.89	150.30	538.39	553.23	26.64
	<i>C. orientalis</i>	733.52	28.35	45.43	44.07	142.14	534.29	598.67	17.33
	<i>C. monogyna</i>	647.21	22.97	55.62	41.84	141.50	532.51	567.74	17.61

DM – dry matter, CP – crude protein, EE – ether extract, CC – crude cellulose, NDF – neutral detergent fibre, ADF – acid detergent fibre, CT – total condensed tannins

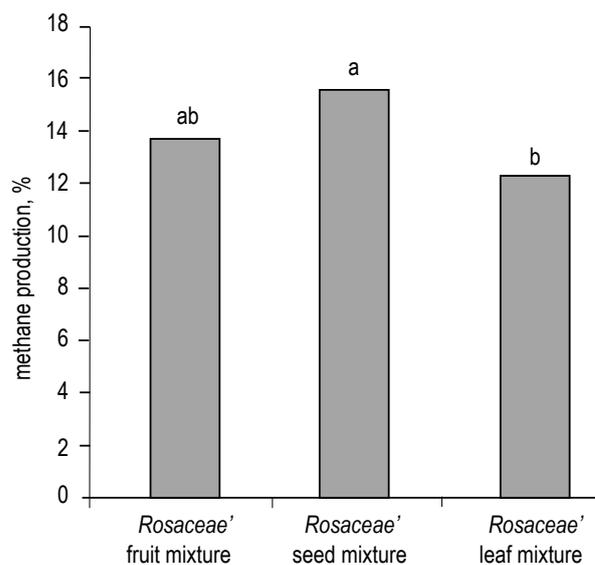
**Table 2.** Effect of different parts of *Rosaceae* species on *in vitro* total gas and methane production at 24 h of *in vitro* rumen incubation

Parts of plant	<i>Rosaceae</i> species	Gas production		Methane	
		ml · 200 mg <sup>-1</sup> DM		%	
Leaf	<i>R. canina</i>	38.81 ± 2.93	4.80 ± 0.16	12.36 ± 0.24	
	<i>C. orientalis</i>	41.87 ± 1.89	4.92 ± 0.35	11.76 ± 0.12	
	<i>C. monogyna</i>	38.38 ± 0.69	4.55 ± 0.14	11.86 ± 0.21	
	<i>P</i>	0.469	0.204	0.155	
Fruit	<i>R. canina</i>	43.57 ± 1.69 <sup>c</sup>	5.93 ± 0.16	13.60 ± 0.38	
	<i>C. orientalis</i>	65.89 ± 2.53 <sup>a</sup>	9.03 ± 1.18	13.70 ± 0.46	
	<i>C. monogyna</i>	56.56 ± 2.45 <sup>b</sup>	7.71 ± 0.92	13.63 ± 1.05	
	<i>P</i>	0.001	0.333	0.149	
Seed	<i>R. canina</i>	17.76 ± 1.28 <sup>b</sup>	2.43 ± 0.35 <sup>b</sup>	13.66 ± 1.43 <sup>b</sup>	
	<i>C. orientalis</i>	29.12 ± 2.11 <sup>a</sup>	4.89 ± 0.44 <sup>a</sup>	16.80 ± 1.01 <sup>a</sup>	
	<i>C. monogyna</i>	23.77 ± 1.34 <sup>a</sup>	3.92 ± 0.24 <sup>a</sup>	16.50 ± 0.20 <sup>a</sup>	
	<i>P</i>	0.008	0.009	0.034	

<sup>a,b,c</sup> different letters in the same column show significant difference for each part of plant



**Figure 1.** Effect of different parts of *Rosaceae* species (*R. canina* + *C. orientalis* + *C. monogyna* mixture) on *in vitro* total gas production; <sup>a,b,c</sup> different letters show significant difference ( $P < 0.001$ )



**Figure 2.** Effect of different parts of *Rosaceae* species (*R. canina* + *C. orientalis* + *C. monogyna* mixture) on *in vitro* methane production; <sup>a,b</sup> different letters show significant difference ( $P < 0.001$ )

**Table 3.** Effect of different parts of *Rosaceae* species on ME, NE<sub>L</sub>, SFCA and OMD levels at 24 hours of *in vitro* rumen incubation

Parts of plant	<i>Rosaceae</i> species	ME	NE <sub>L</sub>	SCFA	OMD
		MJ · kg <sup>-1</sup> DM		mmol · g <sup>-1</sup> DM	%
Leaf	<i>R. canina</i>	7.98 ± 0.40	4.19 ± 0.08	4.30 ± 0.33	54.02 ± 2.61
	<i>C. orientalis</i>	8.53 ± 0.25	4.59 ± 0.22	4.65 ± 0.21	57.74 ± 1.68
	<i>C. monogyna</i>	8.01 ± 0.39	4.20 ± 0.34	4.26 ± 0.34	54.15 ± 0.62
	<i>P</i>	0.347	0.437	0.695	0.330
Fruit	<i>R. canina</i>	8.36 ± 0.23 <sup>c</sup>	4.65 ± 0.19 <sup>c</sup>	4.83 ± 0.19 <sup>c</sup>	55.83 ± 1.50 <sup>c</sup>
	<i>C. orientalis</i>	11.41 ± 0.34 <sup>a</sup>	7.23 ± 0.29 <sup>a</sup>	7.31 ± 0.28 <sup>a</sup>	76.01 ± 2.24 <sup>a</sup>
	<i>C. monogyna</i>	10.13 ± 0.33 <sup>b</sup>	6.16 ± 0.28 <sup>b</sup>	6.27 ± 0.27 <sup>b</sup>	67.47 ± 2.18 <sup>b</sup>
	<i>P</i>	0.001	0.001	0.001	0.001
Seed	<i>R. canina</i>	5.07 ± 0.17 <sup>b</sup>	1.82 ± 0.15 <sup>b</sup>	1.97 ± 0.14 <sup>b</sup>	34.37 ± 1.14 <sup>b</sup>
	<i>C. orientalis</i>	6.42 ± 0.29 <sup>a</sup>	3.06 ± 0.24 <sup>a</sup>	3.23 ± 0.23 <sup>a</sup>	43.00 ± 1.87 <sup>a</sup>
	<i>C. monogyna</i>	5.75 ± 0.18 <sup>ab</sup>	2.45 ± 0.15 <sup>ab</sup>	2.63 ± 0.14 <sup>a</sup>	38.66 ± 1.19 <sup>ab</sup>
	<i>P</i>	0.014	0.010	0.008	0.016

ME – metabolizable energy, NE<sub>L</sub> – net energy lactation, OMD – organic matter digestibility, SCFA – short chain fatty acids; <sup>a,b,c</sup> different letters in the same column show significant difference for each part of plant

**Table 4.** Correlation coefficient (*r*) between total gas production and some nutrient values in different parts of *Rosaceae* species

Parts of plant	MP	TGP	NDF	CT	CP	
Leaf	MP	1	0.124	-0.882**	-0.936**	-0.834**
	TGP		1	0.285	0.183	0.172
	NDF			1	0.977**	0.888**
	CT				1	0.894**
	CP					1
Fruit	MP	1	-0.353	0.268	0.068	-0.535
	TGP		1	-0.883**	-0.895**	0.837**
	NDF			1	0.964**	-0.743**
	CT				1	0.673
	CP					1
Seed	MP	1	0.509	0.261	-0.807**	-0.640
	TGP		1	-0.766**	-0.753*	0.521
	NDF			1	0.064	0.376
	CT				1	0.895**
	CP					1

MP – methane production, TGP – total gas production, CP – crude protein, NDF – neutral detergent fibre, CT – total condensed tannin; \* correlation is significant at 0.01 level, \*\* correlation is significant at 0.05 level

related ( $P < 0.01$ ) with the CT content ( $r = 0.96$ ), but negatively correlated with that of CP content ( $r = -0.74$ ). For the seeds of *Rosaceae* species, methane production was negatively correlated with the CT content ( $r = -0.81$ ;  $P < 0.01$ ). The *in vitro* total gas production for the seeds of *Rosaceae* family species was negatively correlated ( $P < 0.01$ ) with the contents of NDF ( $r = -0.77$ ;  $P < 0.01$ ) and CT ( $r = -0.75$ ;  $P < 0.05$ ). The CT contents in leaves and seeds of *Rosaceae* family species were positively correlated ( $r = 0.89$ ;  $P < 0.01$ ) with the CP contents (Table 4).

## Discussion

**Nutrient composition.** Browse species play a significant role in providing forage for ruminants in arid and semiarid regions of the world. Most browse species have the advantage of maintaining their greenness and nutritive value throughout the dry season when grasses dry up and deteriorate both in quality and quantity. The leaves of browse species generally contain high or moderate levels of protein and are used as a dry-season supplement to poor quality natural pasture and/or fibrous crop residues (Tolera et al., 1997). In the present study, the foliage of the *Crataegus* species contained a high level of CP, even at the mature stage. The CP contents in the leaves of these species were sufficiently high to warrant consideration of their use as a protein supplementation to poor-quality pasture and a source of alternative roughage, similarly to *R. canina* leaves. The CP contents in samples suggest that *Crataegus* leaves have the advantage of maintaining their nutritive value throughout the dry season. The CP, NDF and ADF levels in the leaves, seeds and fruits to *R. canina* were similar to the findings of Ammar et al. (2004). Previous studies reported low levels of CP ( $< 100$  g · kg<sup>-1</sup> DM) in the leaves of some browse plants, especially at mature stages (Tolera et al., 1997; Paton et al., 1999; González-Hernández and Silva-Pando, 2009).

The effects of CTs on digestion vary depending on their amount in the animal diet. In particular, low levels (from 20 to 30 g · kg<sup>-1</sup>) of CTs in the diet have been reported to prevent the breakdown of some proteins in the rumen and allow absorption (as by-pass) in the duodenum (Barry and McNabb, 1999). This activity is a positive effect of condensed

tannins. In contrast, a high condensed tannin content in feed may lead to negative effects on the digestion and absorption of protein and other nutrients (Kumar and Singh, 1984; Frutos et al., 2004).

***In vitro* gas production and estimated parameters.** *In vitro* gas production is influenced by the nutrient composition (cell wall components, starch, sugar) of the tested feed, the presence of compounds inhibiting gas production (such as condensed tannins, polyethylene glycol), the microflora and protozoa content of the rumen fluid (donor animal's diet feeding direction) and fermentation quality provided (Johnson and Johnson et al., 1995; Goel et al., 2008; Hook et al., 2010). In our study, the different parts of *Rosa canina* could be ordered as fruit > leaf > seed in terms of *in vitro* total gas production. The differences in *in vitro* total gas production in both the plant species and plant parts are thought to result from differences in the level of NDF and ADF constituting the cell walls. The highest NDF and ADF contents and the lowest gas production were determined for the seeds. The higher total gas production when the *Rosaceae* fruit mixture was used than with *Rosaceae* seed and leaf mixtures may be connected to the low fibre content in the fruit. Blümmel and Ørskov (1993) stated that there was a negative correlation between plant fibre content and *in vitro* gas production. Tolera et al. (1997) showed that *in vitro* gas production parameters (*in vitro* potential degradability) of browse species were negatively correlated with the total concentration of tannins and condensed tannins (CT). In the present study, the *in vitro* total gas production with the seed and fruit mixtures of *Rosaceae* species was negatively correlated with NDF and CT contents. The negative effect of CT on gas production may be due to its negative effect on fibre digestion and protozoa count (Bodas et al., 2009; Szumacher-Strabel and Cieślak, 2010). In the current experiment, *in vitro* gas production of the *Rosaceae* fruit mixture was positively correlated with the CP content, similarly as found by Menke and Steingass (1988). Ammar et al. (2004) stated that *in vitro* gas production after 24 h of incubation was determined at about 35 ml · 200 mg<sup>-1</sup> DM for *R. canina* leaves and about 33 ml · 200 mg<sup>-1</sup> DM for *R. canina* fruits. The results of Ammar et al. (2004) are similar with the *in vitro* gas production results of the present study.

There is a positive correlation between *in vitro* gas production with the estimated values of ME, NE<sub>L</sub>, OMD and SCFA (Menke and Steingass, 1988); also, gas production is an important predictor of these values, which are positively related to

microbial mass production (Liu et al., 2002). The estimated ME, NE<sub>L</sub>, OMD and SCFA values of *R. canina* fruits and seeds were lower than those of *Crataegus* fruits and seeds, and may be connected with the *in vitro* gas production values.

***In vitro* methane production.** According to the results of Woodward et al. (2001), phenolic compounds (tannins) may reduce ruminal methane production in sheep and cattle. The mechanism of action is based on both direct effects on methanogen activity and indirect effects on fibre digestion in rumen (Bodas et al., 2009; Szumacher-Strabel and Cieślak, 2010). The *in vitro* methane production of the leaf mixture and seed mixture of *Rosaceae* family species was negatively correlated with the CT content. The *in vitro* methane production in the *Rosaceae* leaf mixture was lower than with seeds mixture and fruits mixture and may be related to its high CT contents. López et al. (2010) reported that a feed (or supplement) can be classified as having low (>11% and ≤14%), moderate (>6% and <11%) and high (>0% and <6%) anti-methanogenic potential according to the methane percentage in the produced total gas in the fermentation results. According to the findings, the seeds of *Crataegus* species do not have any anti-methanogenic activity; all parts of *R. canina*, and the leaf and fruit of *Crataegus* species can be said to have anti-methanogenic properties, even if at low levels. The tannins in the plants have been shown to reduce methane production (Frutos et al., 2004; Dong et al., 2010). In the present experiment, anti-methanogenic activity was found in the plant parts containing from 1.8% to 13.4% of CT, but this effect was not observed in the plant parts containing less than 1.8% of CT. In line with our study, Szumacher-Strabel et al. (2011) determined in a ruminal batch culture system study that the seed oil from *R. canina* reduced methane production.

## Conclusions

The *in vitro* ruminal digestion values in the fruits and seeds of the studied *Crataegus* species were higher than those of *Rosa canina*. The leaves of *R. canina* are not a high quality forage for grazing animals in scrublands, but its seeds and fruit can be used as rumen modulators. The leaves of *Crataegus* have both a moderate level of crude protein and an anti-methanogenic effect. These results suggest that *Crataegus* leaves have the advantage of maintaining their nutritive value throughout the dry season when grasses dry up and deteriorate both in quality and quantity for grazing animals.

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