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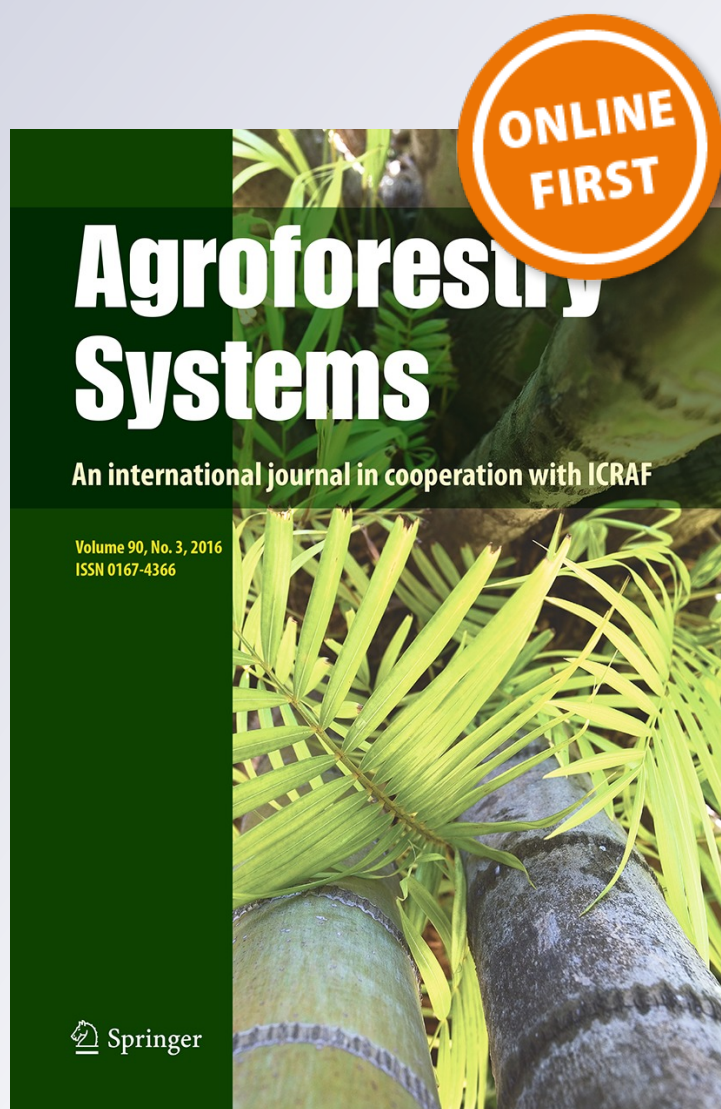
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The chemical composition and in vitro digestibility evaluation of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. Shokoufeh) leaves versus hulls and green versus dry leaves as feed for ruminants

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Abstract The current study aimed to evaluate the chemical composition and in vitro digestibility of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. Shokoufeh) leaves versus hulls, and green versus dry leaves as feed for ruminants. The fresh green almond hulls (GAH) and leaves (GAL) were harvested and spread under a shade to dry. Dry almond leaves (DAL) were collected from under the trees where as dry almond hulls (DAH) were collected 4 weeks after harvesting the fresh samples. The chemical composition of substrates was determined using standard approaches and the metabolisable energy (ME), in vitro dry matter (DMD) and in vitro organic matter (OMD) digestibility were measured using the in vitro gas production (GP) technique. The GAL contained 81 g crude protein (CP) kg^{-1} DM while DAH contained 103 g CP kg^{-1} DM. The CP was higher ($P = 0.0003$) in dry (leaves and hulls) than in green (leaves and hulls) samples. The ash content ranged from 99.2 to 181.5 g kg^{-1} DM in DAH and DAL, respectively, ($P = 0.0041$). The ether extract content ranged from 27 for DAH to 65 g kg^{-1} for DAL ($P = 0.0018$). The acid detergent fibre and neutral detergent fibre content ranged from 185 to 304 and 444 to 620 g kg^{-1} DM ($P = 0.04$), for GAL and DAH, respectively. The DAH had the highest ($P = 0.0001$) GP$_{24}$ and GP$_{96}$. The DAH had the highest ($P = 0.0001$) potential GP (i.e., *b*), while the GP rate was highest for GAL and GAH ($P = 0.034$), ME was highest for DAH ($P = 0.0001$), and in vitro OMD was highest for DAH ($P = 0.0001$). The highest DMD ($P = 0.0001$) values were obtained with DAH followed by GAL, DAL and GAH, respectively. It can be concluded that almond hulls and leaves have a good nutritional potential to cover the maintenance nutrient requirements of small ruminants. Almond hulls and leaves can also be used as supplement to low quality mature pasture and/or crop residues. However, more studies are warranted to better characterize these feeds in in vivo animal feeding trials.

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Introduction

Almond, (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*) is a species belonging to the Rosaceae family (Sfahlan et al. 2009). The state of California in the USA is the major producer of almond; however, its production is widely distributed (Wijerante et al. 2006) and there is increasing interest to produce almond and its by-products worldwide. Approximately 2,112,815 metric ton of almonds with shell is produced globally with Iran contributing about 110,000 metric ton (FAO 2007). Almond is a mid-size tree with fruit that can grow up to ten meters tall (Chen et al. 2010). The fruit is made of hulls, shell and kernel. Drying almond hulls results in approximately (kg^{-1} DM) 250 g nut, 500 g hulls and 250 g shell (Aguilar et al. 1984; Fadel 1999).

The chemical composition and nutritive value of almond hulls has been investigated by many researchers (Alibes et al. 1983; Pinto et al. 1989). The hulls are by-product with low protein, high N-free extract and reasonable energy content. Studies reported that hulls as a feedstuff contained ($g\ kg^{-1}$): 21–80 of crude protein (CP), 17–30 of ether extract (EE), 280–385 of neutral detergent fibre (NDF), 487–578 of non-fibrous carbohydrate, 596–667 of in vivo dry matter (DM) digestibility (DMD) and 1.9–2.9 Mcal of metabolisable energy (ME) (Alibes et al. 1983; Aguilar et al. 1984; Reed and Brown 1988; Fadel 1999; Alibes et al. 1983; Getachew et al. 2004). In Iran, small ruminants are fed almond tree leaves (green or dry) as a part of diet forage. However, no information is available on the nutritive value of green or dry leaves, as well as dry almond tree hulls. Therefore, the current study aimed to evaluate the chemical composition, in vitro digestibility, and ME of leaves versus hulls, and green versus dry almond tree leaves as feed for ruminants.

Materials and methods

Sampling zone and collection

This experiment was conducted using almond (*Prunus dulcis*, syn. *Prunus amygdalus*; var. Shokoufeh) hulls and leaves from the north-eastern part of Iran (Esferayein, Northern Khorasan). The area is located at an altitude of 1249 m above sea level. The mean annual rainfall is 300 mm with moderate air temperature of about 20–25 °C. Almond hulls and leaves were obtained from 10 almond orchards, where at least 10 different trees were sampled, and the hulls and leaves pooled for each garden before analysis, resulting in 10 samples. The trees were aged 15–20 years with heights ranging from 6 to 10 m. The stem diameters were about 30 cm, and the leave lengths ranged from 9 to 12 cm. The leaves were oval and spear to oval in shape with elongated end.

Chemical composition

The fresh green almond hulls (GAH) and leaves (GAL) were harvested in the autumn. Dry almond leaves (DAL) were collected from beneath the trees i.e., these where dry mature leaves which had fallen off the tree after leaf senescence, dry almond hulls (DAH) were collected 4 weeks after harvesting the green hulls and spread under a shade to dry. The fresh green leaves (GAL) were dried on a bench in the laboratory and ground to pass through 1 mm sieve for subsequent analyses. Samples were analysed for DM (method ID 934.0) by drying the samples at 105 °C overnight and the ash content (method ID 942.05) was determined by igniting the samples in a muffle furnace at 550 °C for 8 h. Nitrogen content (method ID 954.01) was determined using Kjeldahl method (AOAC 1997), where, CP was calculated as N × 6.25. Ether extract (method ID 945.16) was determined according to AOAC (1997). Concentrations of NDF and ADF were determined according to Van Soest et al. (1991) using an ANKOM[200] Fibre Analyser Unit (ANKOM Technology Corp., Macedon, NY, USA) without use of an alpha amylase but with sodium sulphite. Both NDF and ADF are expressed without residual ash. Water soluble carbohydrate (WSC) was measured using the method of MAFF (MAFF 1982). All chemical analyses were carried out in triplicate.

Gas production

The amount of gases produced can be a good indicator of feed fermentability and microbial activity (Elahi et al. 2014; Elghandour et al. 2015a). As described in Cedillo et al. (2014), rumen fluid was obtained from

three fistulated sheep fed twice daily a diet containing lucerne hay (600 g kg^{-1}) and concentrates mixture (400 g kg^{-1}) formulated to cover their nutrient requirements (NRC 1985). Sheep had full access to fresh water at all times during the rumen inoculum collection phase. About 200 mg of samples were incubated with 30 mL of rumen fluid-buffer mixture (ratio of 1:2) in calibrated glass syringes as described by Menke and Steingass (1988). In three runs in 3 different weeks, one hundred and twenty 100-mL calibrated glass syringes (Model Fortuna, Haberle Labortechnik, Germany) with or without substrate (2 foliage types (hulls and leaves) and 2 harvest types (fresh green and dry) × 10 samples (from 10 gardens) × 3 syringes of each triplicate sample plus three syringes as blanks (i.e., rumen fluid only) were used. Syringes were pre-warmed at 39 °C before the injection of 30 mL of rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39 °C. Reading of gas production (GP) was recorded after 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h of incubation. Total GP values were corrected for syringes blank incubated at the same time with other syringes contained samples. The cumulative GP data were fitted to the exponential equation: $P = b\,(1 - e^{-ct})$ of Ørskov and McDonald (1979) where P is the GP at time t; b is the potential GP (mL 200 mg^{-1} DM), c is the GP rate constant (h^{-1}), t incubation time (h).

The ME (MJ kg^{-1} DM) was calculated using equation of Menke et al. (1979) as follows: ME (MJ kg^{-1} DM) = 2.20 + 0.136 GP + 0.057 CP ($R^2 = 0.94$) where, GP is 24 h net GP (ml 200 mg^{-1} DM), CP is crude protein (%). The in vitro organic matter digestibility (OMD) of foliage was calculated using equation of Menke et al. (1979) as follows: in vitro OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA where, GP is 24 h net gas production (ml 200 mg^{-1} DM), CP is crude protein (%) and XA is ash content (%).

The DMD was determined at the end of the incubation period as previously described in Vallejo et al. (2016). Briefly, after 96 h of incubation, the contents of each syringe were filtered through sintered glass crucibles under vacuum and the fermentation residues were dried at 105 °C overnight. Weight loss after drying was taken as undegradable DM. The DMD (mg g^{-1} DM) at 96 h of incubation was calculated as the difference between substrate DM content and its undegradable DM.

Statistical analysis

The chemical composition, in vitro GP kinetics, in vitro OMD, DMD and ME contents data was analysed as completely randomized design with a 2 × 2 factorial treatment arrangement (i.e., 2 foliage types (hulls and leaves) with 2 harvest types (fresh green and dry), using the GLM of SAS (2002). Before analysis, the data of three runs within the same sample were averaged. The statistical model was:

$$Y_{ijk} = \mu + S_i + R_j + S_i \times R_j + \varepsilon_{ijk}$$

where Y_{ijk} represents the general observation of chemical composition, in vitro GP kinetics, in vitro OMD, DMD and ME contents, S_i the ith effect of foliage type on the observed parameters; R_j is the jth effect of harvest types on the observed parameters. The $S_i \times R_j$ term presents ith and jth interaction effects of foliage and harvest types on chemical composition, in vitro GP kinetics, in vitro OMD, DMD and ME contents, and ε_{ijk} the standard error term common for all observations. Mean differences were declared significant at $P < 0.05$. The means were separated using the Tukey's Multiple Range Test (Pearse and Hartley 1966).

Results

There were considerable variations ($P < 0.05$) between almond hulls and leaves in terms of chemical compositions. The GAL contained 81 g CP kg^{-1} DM while DAH contained 103 g CP kg^{-1} DM. The CP was higher ($P = 0.0003$) in dry (leaves and hulls) than in green (leaves and hulls) samples. The ash content ranged from 99.2 to 181.5 g kg^{-1} DM in DAH and DAL, respectively, ($P = 0.0041$). Leaves had higher ($P = 0.0001$) ash content than hulls. The EE content of the leaves was higher ($P = 0.0001$) than hulls. Moreover, the dry almond had higher ($P = 0.0036$) EE content than the fresh green. The EE content ranged from 27 for DAH to 65 g kg^{-1} for DAL ($P = 0.0018$). The ADF and NDF content ranged from 185 to 304 and 444 to 620 g kg^{-1} DM ($P = 0.04$), for GAL and DAH, respectively. The hulls showed higher ($P = 0.0001$) content of both ADF and NDF than the leaves. The WSC content was highest ($P = 0.0001$) in almond leaves (Table 1).

Table 1 Chemical composition (g kg^{-1} DM) of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. Shokoufeh) leaves versus hulls, and green versus dry leaves as feed for ruminants (n = 10 samples)

	GAL	DAL	GAH	DAH	SEM	*P* value	Hulls	Leaves	*P* value	Dry	Fresh green	*P* value
DM	970.5[b]	980.3[a]	967.5[b]	971.2[b]	12.81	0.0339	969.4[b]	975.4[a]	0.0062	975.8[a]	969.0[b]	0.0032
OM	847.9[b]	818.5[c]	885.2[a]	900.8[a]	9.82	0.0041	893.0[a]	833.2[b]	0.0001	859.7	866.6	0.2586
Ash	152.1[b]	181.5[a]	114.8[c]	99.2[c]	2.98	0.0041	106.9[b]	166.7[a]	0.0001	140.2	133.3	0.2586
CP	81.3[b]	103.1[a]	81.1[b]	103.4[a]	3.62	0.4480	92.3	92.2	0.9863	103.3[a]	81.2[b]	0.0003
EE	52.1[b]	64.7[a]	29.6[c]	26.7[c]	1.27	0.0018	26.3[b]	58.1[a]	0.0001	45.3[a]	39.0[b]	0.0036
ADF	185.0[b]	200.1[a]	296.6[a]	303.5[a]	1.2	0.0566	300.0[a]	192.5[b]	0.0001	251.8	240.8	0.1512
NDF	444.0[d]	473.3[c]	598.3[b]	619.8[a]	10.75	0.0400	609.0[a]	458.6[b]	0.0001	546.5[a]	521.2[b]	0.0004
WSC	186.9[a]	188.4[a]	126.3[c]	141.3[b]	4.35	0.0100	132.8[b]	187.6[a]	0.0001	164.8[a]	156.6[b]	0.0036

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *ADF* acid detergent fibre, *NDF* neutral detergent fibre, *WSC* water soluble carbohydrates, *GAL* green almond leaf, *DAL* dry almond leaf, *GAH* green almond hulls, *DAH* dry almond hulls

Means within same row with different superscript letters are significantly different ($P < 0.05$)

The highest GP was observed for DAH compared to the other treatments (Fig. 1). The DAH had the highest ($P = 0.0001$) GP$_{24}$ and GP$_{96}$. The DAH had the highest ($P = 0.0001$) potential GP (i.e., *b*), while the values GP rate (i.e., *c*) was highest for GAL and GAH ($P = 0.034$), ME was highest for DAH ($P = 0.0001$), and in vitro OMD was highest for DAH ($P = 0.0001$). The highest DMD ($P = 0.0001$) values were obtained with DAH followed by GAL, DAL and GAH, respectively (Table 2).

Discussion

Chemical composition

In the current study, dry almond sample (leaves and hulls) had higher DM, CP, EE and NDF contents than the fresh green sample (leaves and hulls). Additionally, hulls had higher OM, ADF and NDF contents than leaves. The chemical composition and nutritive value of plants and plant parts is affected
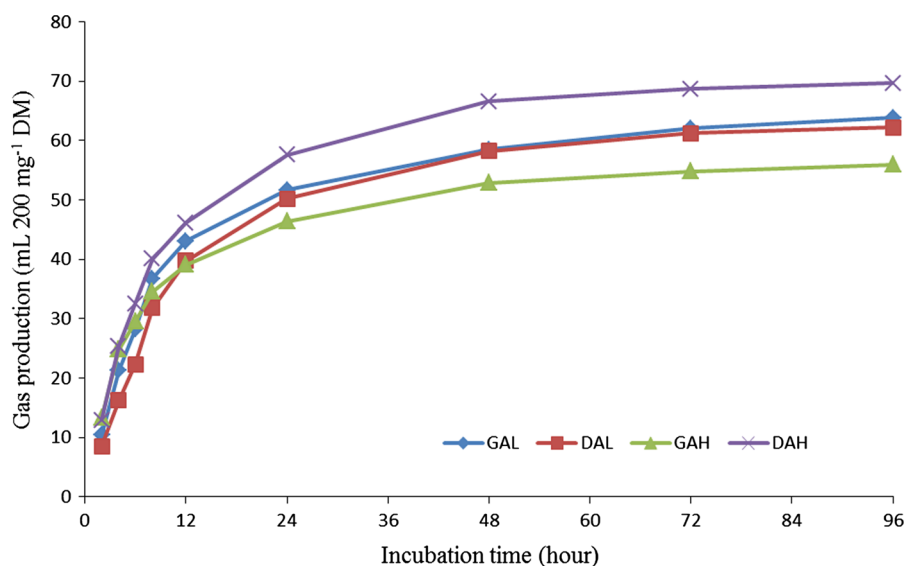

Fig. 1 In vitro gas production profile of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. Shokoufeh) leaves versus hulls, and green versus dry leaves as feed for ruminants (mL gas 200 mg^{-1} DM). *GAL* green almond leaf, *DAL* dry almond leaf, *GAH* green almond hulls, *DAH* dry almond hulls

Table 2 Gas production after 24 and 96 h, kinetics and digestibility of almond tree (*Prunus dulcis* D. A. Webb syn. *Prunus amygdalus*; var. Shokoufeh) leaves versus hulls, and green versus dry leaves as feed for ruminants (n = 10 samples)

	GAL	DAL	GAH	DAH	SEM	*P* value	Leaves	Hulls	*P* value	Dry	Fresh green	*P* value
Gas production and kinetics												
GP$_{24}$	51.7[b]	50.2[b]	46.6[c]	57.6[a]	0.80	0.0001	50.9[a]	52.0[a]	0.0523	49.04[b]	53.90[a]	0.0001
GP$_{96}$	63.8[b]	62.2[b]	56.0[c]	69.67[a]	1.08	0.0001	63.0[a]	62.8[a]	0.7427	59.90[b]	65.95[a]	0.0001
b	60.06[b]	60.72[b]	46.5[c]	63.4[a]	1.10	0.0001	60.4[a]	54.9[b]	0.0001	53.28[b]	62.04[a]	0.0001
c	0.100[a]	0.084[c]	0.099[a]	0.091[b]	0.0021	0.0340	0.092[a]	0.095[a]	0.1323	0.099[a]	0.088[b]	0.0001
In vitro OMD	621.9[b]	611.7[c]	573.1[d]	672.1[a]	1.40	0.0001	616.8[b]	622.6[a]	0.0001	597.5[b]	641.9[a]	0.0001
In vitro DMD	527.3[b]	500.5[d]	507.3[c]	605.0[a]	1.30	0.0001	513.9[b]	556.1[a]	0.0001	517.3[b]	552.7[a]	0.0001
ME	9.26[b]	9.07[b]	8.54[c]	10.07[a]	0.112	0.0001	9.16[a]	9.30[a]	0.0691	8.90[b]	9.57[a]	0.0001

GP$_{24}$–GP$_{96}$: gas production (mL 200 mg^{-1} DM) after incubation time of 24 and 96 h; b: potential gas production (mL 200 mg^{-1} DM); c: gas production rate constant (h^{-1}); ME: metabolisable energy (MJ kg^{-1} DM); in vitro DMD: in vitro dry matter digestibility (mg g^{-1} DM); in vitro OMD: In vitro organic matter digestibility (mg g^{-1} DM)

Means within same row with different superscript letters are significantly different ($P < 0.05$)

GAL green almond leaf, *DAL* dry almond leaf, *GAH* green almond hulls, *DAH* dry almond hulls

by many factors including genotype, growing conditions, stage of growth, environment, and the interaction between genotype and environment (Arhab et al. 2009; Elghandour et al. 2014). Environmental conditions include the soil, agronomic practice, climatic conditions, harvesting and post harvesting treatments (Arhab et al. 2009; Elghandour et al. 2014).

Bagheripour et al. (2008) reported that sun drying of pistachio by-product samples increased its CP, NDF, ADF and lignin contents. These changes are due to losses of water and hexoses as plant cells respire resulted in concentration of other constituents (Salem et al. 2012). Besides, Van Soest (1994) observed increased NDF, lignin and N contents with sun drying through the disproportionate loss of carbon dioxide, which is in agreement with the results of the current study. However, sun drying of foliages *per se* does not usually result in changes in its nutrient contents. Salem et al. (2012) reported that sun drying of *Atriplex halimus* foliage did not affect its OM, CP, EE and dietary fibre contents. It should be noted that the dry almond leaves (DAL) were collected from under the trees i.e., these where dry mature leaves which had fallen off after leaf senescence.

In ruminant animals, the extent to which foliages are utilized depends on its chemical composition (Ahmed et al. 2015a, b; Kholif et al. 2015, 2016). The CP content of almond tree hulls reported in the current

study (i.e., 92.3 g kg^{-1} DM) was higher than previously reported (Getachew et al. 2004; Yalchi and Kargar 2010). The CP content of almond tree hulls has been reported to range from 48.7 (Reed and Brown 1988) to 80.0 g kg^{-1} DM (Getachew et al. 2004). Yalchi (2010) reported the CP content of almond tree hulls was 28.6 g kg^{-1} DM where as Jafari et al. (2011) observed that the CP content of three Iranian almond varieties ranged from 23.2 to 32.7 g kg^{-1} DM. Elsewhere, Homedes (1985) reported that the American almond tree hulls contain 54.0 to 64.0 g CP kg^{-1} DM. These results are lower than those of the current study. Since the foliage (hulls and leaves) contains over 80.0 g CP kg^{-1}, it can be consider as intermediate quality roughages. This range of CP is sufficient to produce ammonia required for the growth and activity of ruminal microorganisms (Kamalak et al. 2005).

In the current study, the ash content of the almond hulls was 107.0 g kg^{-1} DM which is higher than the level reported by Homedes (1985) for the American almond hulls. The NDF and ADF levels ranged from 444.0 to 620.0 g kg^{-1} DM and from 185.0 to 304.0 g kg^{-1} DM, respectively. The range of NDF and ADF reported by Homedes (1985) were 210.0 to 290.0 g kg^{-1} DM for NDF and 244.0 to 296.0 g kg^{-1} DM for ADF. Norollahi et al. (2005) reported NDF and ADF contents of 211 and 117 g kg^{-1} DM, respectively, for almond hulls from the central region

of Iran. Jafari et al. (2015) observed that almond hulls contain NDF and ADF contents of 300 and 220 g kg^{-1} DM, respectively. These results compared to those of the current study, indicates a wide difference in NDF and ADF contents even from among Iranian almond hulls only. The ADF content is a good index of feed nutritive value and has a high correlation with digestible energy in almond hulls (Aguilar et al. 1984).

Getachew et al. (2004) observed that WSC content of almond hulls was higher than that of alfalfa and was comparable to that of sugar beet pulp. The WSC content of the dry almond was higher than that for the fresh green almond. These changes are due to losses of water as plant cells respire resulted in concentration of other constituents (Salem et al. 2012) resulting in lower in vitro OMD and DMD for the dry foliage. This is in contrast to Salem et al. (2012) who found that sun drying *A. halimus* reduced its WSC content compared to fresh foliage. From the current chemical composition results and others in vivo experiments in sheep (Norollahi et al. 2005), dairy cow (Aguilar et al. 1984), and goat (Reed and Brown 1988), almond shows a good nutritive value equivalent to 65–90 % energy value of barley.

Gas production

Ruminal fermentation of structural and non-structural carbohydrates produces gases, acetate, propionate and butyrate (Makkar et al. 1995; Salem et al. 2014; Rodriguez et al. 2015). The rate and extent GP can be considered a good indicator of the digestibility and fermentability of feeds and microbial protein synthesis (Elahi et al. 2014; Elghandour et al. 2015a, b). The different kinetics of GP depends on the chemical composition of the fermented substrates that illuminates their nutritional value as feeds (Elghandour et al. 2015a, b). In the current study, potential GP (i.e., *b* fraction) was correlated to CP contents of incubated substrates; however, fermentability of protein produces relatively little gas compared to carbohydrate fermentation (Makkar et al. 1995). The DAH substrate produced the highest *b* value compared to other substrates, which reveals the effect of CP content on GP compared to the WSC contents, which was low in the DAH substrate. The foliage type and harvesting time are two other factors that significantly affect in vitro GP and fermentation kinetics. The higher GP during the first 24 h of incubation with DAH suggests

that greater fermentation occurred during the first 24 h of incubation compared with the other treatments. The tendency ($P = 0.0523$) for differences in GP (at 24 h of fermentation) among hulls and leaves could be due to difference in NDF and ADF fractions (Rubanza et al. 2005). The results of the current study were higher than those of Jafari et al. (2011) who reported that GP of four varieties of almond hulls varied between 64.0 and 81.0 mL g^{-1} DM.

Foliage of DAH had the highest GP during the incubation period. However, potential GP (*b* fraction) of almond hulls was lower than almond leaves. The DAH had the highest ME, in vitro OMD, and DMD. These differences could be due to variations in CP and NDF contents of the foliage. The higher in vitro OMD, DMD, ME and *b* fraction for DAH indicate its higher fermentability and digestion. It is speculated that when hulls and leaves were sun-dried, this could have lowered the concentration of phenolic compounds in them thus improving their nutritive value. However antinutritional factors were not measured in the current study. Salem et al. (2012) reported that sun-drying reduced the concentration of condensed tannins, total phenolics, saponins, alkaloids, and aqueous fraction by 52.0, 63.0, 30.0 and 73.0 %, respectively relative to the fresh *A. halimus* foliage resulting in a lower inhibitory effect on ruminal bacteria when animals were fed such feeds.

Fresh almond green and hulls have fairly high energy value for ruminant animals and have comparable energy value to barley (Aguilar et al. 1984). The National Research Council (NRC 1981) nutrient requirement tables for goats show that a goat with an average body weight of 30 kg and producing 1 kg of milk with 35 g fat kg^{-1} requires 5.43 MJ of ME as a maintenance requirements plus 5.06 MJ of ME for the production of 1 kg milk (35 g fat kg^{-1}) totalling 10.5 MJ as a total ME requirements. In the present study, the ME energy concentrations ranged between 8.54 and 10.07 for GAH and DAH, respectively suggesting that almond can be fed alone to cover the maintenance requirements of goats. However, for milk or meat production, feed supplementation with concentrates would be required.

Conclusions

The results of the current study suggest that almond hulls and leaves have a good nutritional potential to

cover the maintenance nutrient requirements of small ruminants especially during periods of feed scarcity or during dry-seasons. Moreover, almond hulls and leaves can be used as supplement to low quality mature pasture and crop residues. However, more studies are warranted to better characterize these feeds in in vivo animal feeding trials.

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