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Use of orange leaves as a replacement for alfalfa in energy and nitrogen partitioning, methane emissions and milk performance of Murciano-Granadina goats

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Highlights

- Orange leaves were used as a partial replacement of alfalfa in mixed diets for lactating goats.
- Orange leaves reduced the dry mater intake.
- Orange leaves reduced the CH_4 production.
- Orange leaves had no detrimental effect on milk yield and quality.

ABSTRACT

The objective of this experiment was to study the effects of substituting alfalfa with orange leaves on energy, nitrogen and carbon balance, methane emission, and milk performance in dairy goats. Ten Murciano-Granadina dairy goats in mid lactation (43.5 ± 3.59 kg of body weight [BW]) were selected in a crossover design experiment, where each goat received two treatments in 2 periods. One group of five goats was fed a mixed ration with 450 g of pelleted alfalfa and 550 g of pelleted concentrate/kg of dry matter (ALF diet), and the other diet replaced alfalfa with orange leaves (ORG diet). Inclusion of ORG than ALF diet reduced (P = 0.041) dry matter intake. The metabolizable energy intake was identical between treatments (901 kJ/kg of $BW^{0.75}$, on average) and, the efficiency of metabolizable energy for milk production was 0.73. Retention of energy was lower (P = 0.001) in ORG diet than ALF diet. Carbon and nitrogen intake (P = 0.022 and P = 0.004, respectively) was greater for diet ALF than ORG, with no differences in milk carbon and nitrogen. The ORG diet reduced (P = 0.037) milk fat 3 g/kg, and CH_4 (P = 0.001) 6 g/d. One of the milk fatty acids positively correlated with CH_4 production was C16:0; it was greater (P < 0.05) in ALF than ORG diet. When CH_4 was expressed over OM digestibility and milk basis, differences were preserved. Results suggest that orange leaves are effective in reducing CH_4 emission without detrimental effect on nutrients balance and milk yield.

Abbreviations: ALF, mixed diet that incorporate alfalfa; ORG, mixed diet that incorporate orange leaves; C, carbon; N, nitrogen; BW, body weight; DM, dry matter; OM, organic matter; aNDF, neutral detergent fibre assayed with a heat stable amylase; NFC, non-fibrous

carbohydrate; GE, gross energy; E, energy; MEI, metabolizable energy intake; HP, heat production; RE, recovered energy; RQnp, nonprotein respiratory quotient; k_l; efficiency of use of metabolizable energy for milk production.

*Key words***:** lactating goats, orange leaves, methane emissions.

1. Introduction

In Spain, the Valencian Community is one of the oldest citrus production areas in the world and well known for traditional cultivation of mandarins and oranges. According to data released by the Agriculture Department of the Valencian Community and published in EFEagro (2016), the Valencian Community produces 1.85 million tons of oranges. The main residue generated in citrus crops is pruning waste. Around 3.92 tons (dry matter) of pruning waste are generated per cultivated hectare (EFEagro, 2016). Yearly, Spain generates 1.87 million tons of pruning waste in dry matter, of which approximately 500 g/kg is leaf and 500 g/kg wood.

Orange leaves are an important pruning waste and, their use for livestock feeding had not been investigated (Bampidis and Robinson, 2006). Orange leaves could be used as forage for ruminant feeds, so the pruning waste can be transformed into a valuable product for dairy ruminant. Furthermore, orange leaves could be beneficial to reduce methane emissions from ruminants due to its content of essential oils (Knapp et al, 2014).

The aim of this experiment was to investigate in lactating goats the effect of orange leaves as forage supplement on the intake, digestibility, energy, carbon (C) and nitrogen (N) balance, milk performance and their potential to reduce methane emissions.

2. Material and methods

The experimental procedure was approved by the Animal Use and Care Committee of the Universitat Politècnica de València (UPV, Spain) and followed the codes of practice for animals used in experimental works proposed by the European Union (2007).

2.1. Orange leaves, animals and diets

The experiment was conducted at the Animal Science Department Experimental Farm, Valencia (Spain). Ten multiparous mature Murciano-Granadina dairy goats in mid-lactation were selected and allocated to two homogenous groups of five goats based on similar body weight (BW; 43.5 ± 3.59 kg), milk production in previous lactations (655.3 ± 66 kg of milk during 210 ± 30 days of lactation) and milk yield at the beginning of the experiment (2.0 ± 0.3 kg of milk/d), in a crossover design (2 treatments crossed with 2 period). Treatments consisted of two different mixed rations (Table 1). The concentrate (pelleted) was the same for the two groups whereas the forage (pelleted) was different. The experimental diets consisted in concentrate and alfalfa (ALF diet) and concentrate and orange leaves (ORG diet), both following the nutrient recommendation by Calsamiglia et al. (2009) for goats in lactation. The daily amount of feed offered was 2.2 kg in a forage to concentrate ratio of 45:55. Half of daily ration was offered at 0900 h and half at 1600 h, respectively. Goats had access to water.

2.2. Experimental schedule and measurements

Apparent total tract digestibility, gas exchange, energy partitioning, C and N balance, oxidation of nutrients, and milk composition and yield were determined. The experiment was conducted in a crossover design in two 38 days period. During adaptation, goats were in pens and fed the experimental diets in pens for 14 days and then assigned to individual metabolism cages at thermoneutrality (20-23 ºC; determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA) for 7 days. Feed offered and refused and the total fecal, urine and milk output were recorded daily for each goat for 5 consecutive days, as well as BW at the beginning and end of the period. Feces were collected in wire-screen baskets placed under the floor of the metabolism cages and urine was collected through a funnel into plastic buckets containing 100 mL 10% (vol/vol) of H_2SO_4 to prevent microbial degradation and the loss of volatile ammonia-N (NH_3-N). Representative samples (10%) of diet, feces and urine were collected daily, stored at -20 ºC and pooled for chemical analysis. The goats were milked once daily at 0800 h with a portable milking machine (Flaco, model DL-170, J. Delgado S.A., Ciudad Real, Spain). The individual milk yield was measured and a sample of 10% was placed in a bottle and frozen until analysis. In addition, samples were collected into plastic vial and immediately taken to the Interprofessional Dairy Laboratory of the Valencia Community Region (LICOVAL, Valencia, Spain) for compositional analysis (dry matter, crude protein, fat and lactose). Ruminal fluid samples were collected using an esophageal tube before the morning feeding the last day of the digestibility trial. Ruminal sample was strained through four layers of cheesecloth. Ruminal fluid pH was immediately determined using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA, USA). A ruminal fluid sample was acidified with 50% H_2SO_4 and frozen until determination of NH_3-N. Samples for analysis of VFA were mixed with H_3PO_4 and kept frozen until analysis. Then, goats were moved from metabolism cages to pens for 2 days, previous to gas exchange determinations.

Gas exchange was measured for each goat during 24 h using an indirect calorimetric system based on a ventilated head-box designed for small ruminants (Fernández et al., 2012; 2015). The whole system was calibrated according to McLean and Tobin (1987), and calibration factors were calculated according to Brockway et al. (1971). The digestibility-balance period included 10 days of gas exchange determinations in the crossover design.

2.3. Chemical analysis

Feed, feed refusal and feces samples were first dried in a forced air oven at 55ºC for 48 h. Urine was dried by lyophilization. DM of diets, refusal and feces was determined by oven-drying at 102 ± 2ºC for 24 h (no. 934.01, AOAC, 2008). Ash concentration (no. 942.05, AOAC, 2008) was measured by incineration in an electric muffle furnace at 550 ºC for 6 h to determine organic matter (OM). Feed offered, orts and feces were analysed for neutral detergent fibre (aNDF) and acid detergent fibre (ADF) using the ANKOM Fibre Analyzer (A220, ANKOM Technologies, Fairport, NY, USA), and lignin (sa) was determined by solubilization of cellulose with sulphuric acid, following procedures of Van Soest and Wine (1968). The aNDF was NDF assayed with a heat stable amylase and expressed inclusive of residual ash. Ether extract (EE) was extracted with petroleum ether after acid hydrolysis to recover saponified fat (Soxhlet System HT Tecator, Hillerød, Denmark; 1047 Hydrolyzing Unit and 1043 Extraction Unit) using no. 920.39 of AOAC (2008). Starch content was determined by enzymatic method (α-amylase; from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). Amounts of C and N were analysed by Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA) in a total combustion method (no. 968.06, AOAC, 2008). The NFC content of diets was calculated by difference method, based on chemical analysis of individual feeds as

recommended by NRC (2001); NFC = 100 – (NDF + ash + CP + EE). Gross energy content of the dried samples (feed, feces and urine) was analyzed by combustion in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK).

Milk composition (dry matter, fat, protein and lactose) was analyzed with an infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark). Fatty acid methyl esters of total milk lipids were prepared directly as described O´Fallon et al. (2007). The FA methyl esters were analyzed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector. Separation of methyl esters was performed in a fused silica capillary column SP^{TM} 2560 (100 m x 0.25 mm x 0.2 µm film thickness; Supelco, PA, USA). The carrier gas was Helium at a linear velocity of 20 cm/s. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140 ºC held for 5 min and increased to 240 at 4 ºC/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260 ºC. Milk urea content was analyzed using a flow injection analyzer (Foss Tecator AB, Höganäs, Sweden).

The NH_3-N content of ruminal fluid samples was analyzed by direct distillation using the Kjeldahl method (2300 Kjeltec Analyzer Unit Foss Tecator, Hillerød, Denmark). Determination of ruminal VFA was based on the method described by Jouany (1982) using a gas chromatograph (Fisons 8000 series; Fisons Instruments SpA, Milan, Italy) equipped with a split/splitless injector and flame ionization detector.

2.4. Calculations

The ME intake (MEI) was calculated as the difference between GE intake (GEI) and energy losses in feces, urine and CH_4 (with an energy equivalent value of 39.5 kJ/L CH_4; Brouwer, 1965).

Daily heat production (HP) was determined from measurements of O_2 consumption, CO_2 and CH_4 production (L), and urine N (N_{urine}, g), using the equation of Brouwer (1965):

$$HP\ (kJ) = 16.18 \times O_2 + 5.02 \times CO_2 - 2.17 \times CH_4 - 5.99 \times N_{urine}$$

The body tissue energy (RE_{body}) was calculated as MEI - HP - milk energy (E_{milk}).

The non-protein respiratory quotient (RQnp) was determined as: $RQnp = (CO_2 – (N_{urine} \times 6.25 \times 0.774)) / (O_2 – (N_{urine} \times 6.25 \times 0.957))$. For C and N balance, we followed the equations and values proposed by McLean and Tobin (1987), and the retained protein ($R_{protein}$) and fat (R_{fat}) were calculated (g).

The efficiency of use of ME for lactation (k_l)was calculated according to AFRC (1993). The corrected milk energy was estimated as E_{milk} + (0.84 x negative energy retention) + (1.05 x positive energy retention). The efficiency of use of ME for milk production (k_l) was calculated according to AFRC (1993) as corrected milk energy/(ME - ME_m). ME_m was obtained from the estimation of Aguilera et al. (1990) for Granadina goats from both positive and negative energy retentions (401 kJ/kg of $BW^{0.75}$). Net energy for lactation (NE_L) was computed as MEI x k_l.

2.5. Statistical analysis

The effects of alfalfa substitution by orange leaves on intake, digestibility, ruminal fermentation, milk performance, energy and C-N balances, and oxidation of nutrients were analyzed using the PROC MIXED of SAS (2001). The model for the dependent variables included the fixed effect of diet and period with goat as random effect. The following

statistical model was used: $Y = \mu + D + T + goat + \varepsilon$ where Y is the dependent variable, μ is the overall mean, and D and T are the fixed effects of diet and period of time, respectively; goat is the random effect of goat; and ε is the random error. Least squares mean are reported throughout and differences were considered significant at $P < 0.05$.

3. Results

There was no significant effect for the fixed effect period of time, so the tables report only the effect of diet. The average calibration factor value from the indirect calorimetry unit was 1.0043 ± 0.00126 (n = 4), 0.9951 ± 0.00982 (n = 4) and 0.9655 ± 0.00623 (n = 4) for O_2, CO_2 and CH_4, respectively.

The DM intake was lower ($P = 0.041$) in ORG than ALF diet (Table 2). Apparent digestibility coefficients (DM, OM, EE and GE) were significantly greater ($P < 0.05$) in ORG than ALF diet and no effect in fibre digestibility was observed. No difference was found in milk yield and greater ($P = 0.037$) values of milk fat was found in ALF than ORG diet.

Differences ($P < 0.05$) were found in the content of ruminal acetic, propionic and butyric acids between diets (Table 4). Acetic acid increased significantly in ALF than ORG diet, whereas propionic and butyric acid decrease.

Energy loss in feces and methane was bigger ($P < 0.05$) for ALF in comparison with the ORG diet (Table 5). Highest ($P = 0.001$) energy retention was found in ALF diet. The RQnp was superior ($P = 0.001$) in ALF than ORG diet. Differences in C and N intake and feces (Table 6) were greater ($P < 0.05$) in ALF than ORG diet, as well as for the C from the gas exchange. N intake, urine and feces was greater ($P < 0.05$) in ALF than ORG diet. The C and N were expressed as C:N ratio and significant ($P < 0.05$) greater values of ORG than ALF was observed for intake, feces and urine.

Differences ($P < 0.05$) were found in C16:0 and C22:0, being higher in ALF than ORG diet (Table 7). The polyunsaturated fatty acid C17:1, C18:1n9c, C18:2n6c, C18:3n6, CLA and C22:0 were higher ($P < 0.05$) in ORG than ALF diet. Goats fed ORG diet produced less ($P = 0.001$) CH_4 than ALF diet (Table 8). These differences were consistent when CH_4 was expressed over OM digested and milk production.

4. Discussion

4.1. Feed intake, digestibility and rumen fermentation

The lower DM intake associated with ORG diet could be due to the fat quality, as it was reported in dairy cows supplemented with rice bran oil (Lunsin et al., 2012). Consumption of concentrate and forage were recorded separately. The intake of concentrate was greater in ORG than ALF (0.70 in ORG compared to 0.65 in ALF diet). Maybe the higher apparent digestibility coefficient of DM, OM, NFC and GE in ORG than ALF diet was related to this fact. Therefore, the ingested ORG diet had a lower forage to concentrate ratio and these intake variations could explain higher digestibility with ORG diet mentioned above. Others studies observed increases in OM digestibility when supplementation with citrus by-products. Villarreal et al. (2006) in beef cattle reported that digestion of total diet DM and OM tended to increase linearly with increasing citrus by-products supplementation (pelleted citrus pulp), and similar values for OM and energy digestibility were observed with dried orange by Madrid et al. (1996), although these citrus by-products was not equivalent to the orange leaves used in our trial.

In previous studies in our lab with the same breed and stage of lactation, average milk yield was greater (2.1 kg/d; Criscioni and Fernández, 2016) than in the present study (1.29 kg/d; Table 2). Content of milk fat was 3 g/kg greater in ALF than ORG diet associated with the fibre content, and the greater intake of forage for this first. Fegeros et

al. (1995) in lactating ewes fed dried citrus pulp found similar trend in milk composition as in our study.

Goats fed ALF presented higher acetic acid content associated with less concentrate intake compared with ORG diet (63.3 vs. 53.5 mol/100mol, respectively). The ORG was richer in starch, NFC and lipid than ALF diet. Also, greater amount of propionic acid was found (26.7 vs. 19.7 mol/100mol).

4.2. Energy balance

The GE intake was 286 kJ/kg of $BW^{0.75}$ greater in ALF compared to ORG diet (Table 5). Energy losses in feces were higher for ALF than ORG diet, associated with greater fibre content and lower digestibility. The ORG diet reduced energy loss (18 kJ/kg of $BW^{0.75}$) as CH_4, indicating a diminution effect when the level of starch and NFC in diet increased (Knapp, 2014). Daily average MEI (901 kJ MEI/kg of $BW^{0.75}$) obtained was less than reported by other authors for the same breed under similar stage of lactation (1,254 kJ MEI/kg of $BW^{0.75}$) for Murciano-Granadina goats (Criscioni and Fernández, 2016) or for Saanen goats (1,608 kJ MEI/kg of $BW^{0.75}$; Bava et al., 2001). The DM intake was lower than that observed in other studies, subsequently MEI and milk yield was lower than the predictable values. No differences were found in HP and E_{milk}, with average values of 536 291 kJ/kg of $BW^{0.75}$, respectively. However, the energy recovered in the body was greater in ALF than ORG diet (110 vs. 36 kJ RE_{body}/kg of $BW^{0.75}$) because goats fed ALF had higher GEI and the RQnp was around 1. Conversely, goats fed ORG had lower RQnp than 1 indicating lipid reserves mobilization as suggested Chwalibog et al. (1997), and therefore less fat retention.

The value obtained in the present work for k_l was the same as that the reported by Bava et al. (2001) in Saanen goats (0.73), although Aguilera et al. (1990) reported lower

values with lactating Granadina goats (0.67). Tovar-Luna et al. (2010) in Alpine lactating goats reported values ranging from 0.66 to 0.78. Discrepancies in k_l values could be due to differences in experimental conditions. The NE_L (MJ/kg DM) obtained was 10 for both ALF and ORG diet.

4.3. Carbon and nitrogen balance

The greater C intake in ALF respect to ORG diet was due to the higher intake. Higher values in C_{feces} were related with lower DM and OM digestibility in ALF vs. ORG diet. The greater C in CO_2 and CH_4 for ALF diet was due to the higher DM intake. The C secreted into the milk was not affected by treatment with a positive retention. The efficiency of milk C output regarding to C ingested was 0.16 on average for both diets, a lower value compared to others studies testing mixed diets in the same breed of goat (0.25; Criscioni and Fernández, 2016).

The same trend was observed for N balance. N intake, feces and urine were greater in ALF than ORG diet (Table 6). The N efficiency (N_{milk}/N_{intake}) was lower in ALF than ORG diet; 0.18 and 0.24, respectively, due that lower N_{intake} in ORG diet. But both values were lower than the obtained previously (0.31) by Criscioni and Fernández (2016).

If C and N were expressed as C:N ratio, differences in C:N intake were observed. A lower C:N ratio means the diet is higher in N (ALF diet). This supports the view that animals seek out the richer nitrogen diets, coextensive with the greater DM intake in ALF diet. The reason animals will select the more palatable C3 plants is in their quest to maximize protein intake (Lauder, 2000). The lower C:N in feces and urine for ORG diet showed us the higher excretion of N, which would indicate an inefficient ruminal proteosynthesis due plant secondary metabolites, from orange leaves, interference in microbial activity (Palmquist and Jenkins, 1980; Patra et al., 2017).

Although no differences in retention of fat was observed (more fat retention in ALF than ORG), the RE_{body} was greater (P = 0.001) in ALF than ORG.

4.4. Milk fatty acids

Higher ruminal propionate increases the proportion of odd-chain fatty acids in milk fat, and the desaturation of C17:0 in the mammary gland by delta-9 desaturase resulting in the formation of C17:1 (Vlaeminck et al., 2015). The increase of C17:1 in ORG than ALF diet was accompanying with greatest rumen propionate in ORG diet. ORG diet was richer in starch and milk C18:2n6c (0.55 g/100 g) was greater than ALF diet. Vlaeminck et al. (2006; 2015) suggested that the amount of starch increased milk C18:2n6c content. Greater content of C18:1n9c (3.36 g/100 g fat) in ORG than ALF diet possibly reflects a larger contribution of mobilized fat (Fievez et al., 2012). We observed lower RE_{body} and retention of fat in ORG than ALF diet (Tables 5 and 6), with RQnp < 1 that is an indicator of fat mobilization. Goats fed ORG diet shown greater content of CLA 9c11t + 9t11c than goats fed ALF. A greater amount of C18:2n6c (0.18 mg/100 mg) and C18:3n6 (0.14 mg/100 mg) in orange leaves than alfalfa hay was the reliable of these higher values (Table 3).

The most positive relationships between CH_4 production and milk FA concentrations were obtained for saturated fatty acids (C6:0 to C16:0). In our study, the C16:0 was 3.34 g/100g greater in ALF than ORG diet. Milk C16:0 result mainly from mammary *de novo* fatty acids synthesis, based primarily on the use of acetate produced in the rumen during fibre digestion (Fievez et al., 2012). In our study content of ruminal acetate was 9.8 mol/100mol greater for ALF than ORG diet.

4.5. Methane emissions

The goats fed ORG produced less CH_4 because the DM intake was lower, also the DM intake of forage was 180 g lower than ALF diet. These differences were preserved when CH_4 was expressed as OM digested and milk yield basis (Table 8). So, a reduction of 3.8 g CH_4 / kg of OM digested and milk in ORG diet was found respect to ALF. Factors responsible for differences among finding are unclear but may include variation in chemical composition. Patra et al. (2017) reported that some plant secondary metabolites may exert inhibitory effects on the methanogenic activity, and orange leaves contain essential oil and tannins. According to Johnson and Johnson (1995), fermentation of fibrous carbohydrates produces more CH_4 than fermentation of soluble sugars, which in turn produce more CH_4 than fermentation of starch. Grainger and Beauchemin (2011) reported that increasing the level of starch and lipids, and decreasing aNDF and ADF in diet, reduced the CH_4 production.

5. Conclusions

Inclusion of orange leaves as forage in a regular diet for lactating goats reduced the DM intake with no detrimental effect on milk production and k_l. In addition, methane emission was reduced.

Conflict of interest

None

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Table

Table 1

Ingredients and chemical composition of the diets

	FORAGES		CONCENTRATE	Diet[a]	
Item	Alfalfa	Orange leaves	Compound feed	ALF	ORG
Ingredients, g/kg DM[b]					
Alfalfa pellet	1000			450	
Orange leaves pellet		1000			450
Barley			350	193	193
Corn			309	170	170
Wheat bran			150	83	83
Soy meal (44% CP)			148	81	81
Calcium carbonate			22	12	12
Sodium chloride			11	6	6
Bypass fat[c]			5	3	3
Premix[d]			5	3	3
Chemical composition, g/kg DM					
Dry matter	918	910	909	913	909
Organic matter	834	879	920	881	902
Ash	166	121	80	119	98
Crude protein	169	111	177	173	147
Ether extract	12	20	26	20	23
Neutral detergent fibre	494	376	168	315	262
Acid detergent fibre	321	263	53	173	147

Lignin	71	67	5	34	33
NFC[e]	160	373	548	374	470
Starch	11	62	495	277	300
Carbon				405	397
Nitrogen				28	25
Carbon : Nitrogen				15	16
Gross energy, MJ/kg DM	16	17	17	16	17
[a] ALF = Alfalfa; ORG = Orange leaves.					
[b] DM = dry matter.					
[c] Bypass fat of palm fatty acid distillate (Norel Animal Nutrition, Norel S.A., Spain).					
[d] Premix composition (ppm or IU/kg): Se, 40 mg/kg; I, 250 mg/kg; Co, 80 mg/kg; Cu, 3,000 mg/kg; Fe, 6,000 mg/kg; Zn, 23,400 mg/kg; Mn, 29,000 mg/kg; S, 60,000 mg/kg; Mg, 60,000 mg/kg; vitamin A, 2,000,000 IU/kg; vitamin D3, 400,000 IU/kg; vitamin E, 2,000 ppm; nicotinic acid, 10,000 ppm; choline, 20,300 ppm (NACOOP S.A. España).					
[e] NFC = non fibrous carbohydrate content: 100 - (NDF+ash+CP+EE); NDF=neutral detergent fibre, CP=crude protein, EE=ether extract.					

Table 2

Body weight, intake, apparent digestibility coefficients and milk performance of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet.

	Diet[b]			
Item[a]	ALF	ORG	SEM[c]	P-value
BW, kg	44.1	42.5	0.64	0.244
DMI, kg/d	1.69	1.36	0.078	0.041
Concentrate	1.10	0.95	0.043	0.131
Forage	0.59	0.41	0.049	0.058
Digestibility, g/kg DM				
DM	635	711	20.1	0.033
OM	666	730	18.8	0.049
CP	666	723	20.6	0.190
EE	565	701	30.9	0.031
aNDF	373	335	36.3	0.615
ADF	383	381	36.2	0.980
Lignin	218	307	46.8	0.366
NFC	895	931	7.9	0.031
GE	659	718	19.5	0.046
Milk yield, kg/day	1.33	1.25	0.070	0.569
Chemical composition, g/kg				
Dry matter	153	148	2.8	0.465
Fat	57.2	54,0	0.20	0.037
Protein	40.1	42.3	0.85	0.297
Lactose	46.0	47.2	0.41	0.140
Urea, mmol/L	7.10	6.67	0.83	0.823

[a] BW = body weight; DMI = dry matter intake; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; aNDF = neutral detergent fibre; ADF = acid detergent fibre; NFC = non fibrous carbohydrate; GE = gross energy.		
[b] ALF = Alfalfa; ORG = Orange leaves.		
[c] SEM = standard error of the mean.		

Table 3

Fatty acid profile from Alfalfa and Orange leaves (mg/100 mg).

Fatty acids[a]	Alfalfa	Orange leaves
C4:0	0.00	0.00
C6:0	0.00	0.00
C8:0	0.00	0.00
C10:0	0.00	0.00
C11:0	0.00	0.00
C12:0	0.01	0.02
C13:0	0.12	0.12
C14:0	0.01	0.03
C14:1	0.00	0.00
C15:0	0.01	0.00
C16:0	0.25	0.27
C16:1	0.01	0.00
C17:0	0.01	0.01
C17:1	0.00	0.00
C18:0	0.04	0.04
C18:1n9t	0.00	0.00
C18:1n9c	0.06	0.06
C18:1n7	0.01	0.01
C18:2n6t	0.00	0.00
C18:2n6c	0.14	0.33
C20:0	0.01	0.01
C18:3n6	0.01	0.01
C20:1	0.00	0.00
C18:3n3	0.16	0.29

CLA 9c11t + 9t11c	0.00	0.00
C20:2	0.00	0.00
C22:0	0.02	0.02
C20:3n6	0.01	0.01
C22:1n9	0.00	0.00
C20:3n3	0.01	0.01
C24:0	0.02	0.02
[a] CLA = conjugated linoleic acid.		

Table 4

pH, content of ammonia-N (NH_3-N), and VFA in ruminal fluid of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet.

	Diet[b]			
Item[a]	ALF	ORG	SEM[c]	P-value
pH	7.19	7.25	0.050	0.978
NH_3-N, mg/dL	19.67	16.61	4.620	0.779
Total VFA, mM	26.73	33.04	3.537	0.428
Individual VFA, mol/100 mol				
Acetic acid	63.3	53.5	2.11	0.039
Propionic acid	19.7	26.7	2.27	0.048
Butyric acid	8.18	13.1	1.22	0.047
Iso-Butyric acid	2.38	1.95	0.236	0.410
Iso-Valeric acid	2.55	2.05	0.310	0.471
N-Valeric acid	1.96	1.88	0.186	0.851
N-Caproic acid	0.78	0.42	0.184	0.381
Heptanoic acid	1.07	0.40	0.334	0.367
[a] NH_3-N = ammonia nitrogen.				
[b] ALF = Alfalfa; ORG = Orange leaves.				
[c] SEM = standard error of the mean.				

Table 5 Daily energy partitioning (kJ/kg of $BW^{0.75}$) of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet.

	Diet[b]			
Item[a]	ALF	ORG	SEM[c]	P-value
GEI	1620	1334	72.1	0.033
E_{feces}	570	391	41.5	0.034
E_{urine}	45	48	1.9	0.472
$E_{methane}$	59	41	2.3	0.001
MEI	947	854	46.9	0.428
E_{milk}	301	281	14.5	0.575
HP	536	537	13.3	0.962
RE_{body}	110	36	8.5	0.001
RQnp	1.04	0.85	0.018	0.001

[a] GEI = gross energy intake; E_{feces} = energy losses in feces; E_{urine} = energy losses in urine; $E_{methane}$ = energy losses in methane; MEI = metabolizable energy intake; HP = heat production; E_{milk} = recovered energy in milk; RE_{body} = recovered energy in tissue (REbody = MEI − HP − Emilk); RQnp = nonprotein respiratory quotient {[CO_2 − (Nurine× 6.25 × 0.774)]/[O_2 − (Nurine × 6.25 × 0.957)]}.

[b] ALF = Alfalfa; ORG = Orange leaves.

[c] SEM = standard error of the mean.

Table 6

Carbon and nitrogen balance (g/kg of $BW^{0.75}$) of Murciano-Granadina goats (n = 10) during midlactation according to the type of diet.

	Diet[b]			
Item[a]	ALF	ORG	SEM[c]	P-value
C_{intake}	40.1	32.5	1.77	0.022
C_{feces}	15.5	10.8	1.12	0.037
C_{urine}	1.17	1.12	0.047	0.587
C_{CO2}	14.1	12.2	0.04	0.018
C_{CH4}	0.80	0.73	0.012	0.006
C_{milk}	5.98	5.59	0.327	0.576
$C_{retained\ body}$	2.47	2.11	1.009	0.578
N_{intake}	2.75	2.05	0.123	0.004
N_{feces}	0.94	0.54	0.067	0.002
N_{urine}	0.81	0.51	0.045	0.001
N_{milk}	0.47	0.46	0.025	0.800
$N_{retained\ body}$	0.52	0.53	0.099	0.954
C:N intake	14.6	15.8	0.22	0.003
C:N feces	16.6	19.9	0.47	0.001
C:N urine	1.49	2.20	0.099	0.001
C:N milk	12.8	12.2	0.22	0.059
C:N retained body	2.62	3.98	0.750	0.308
Protein and fat retention				
$R_{protein}$, kJ/kg of $BW^{0.75}$	77.7	79.0	14.89	0.954
R_{fat}, kJ/kg of $BW^{0.75}$	40.1	20.0	10.15	0.253

[a] C_{intake} = C intake; C_{feces} = C losses in feces; C_{urine} = C losses in urine; C_{CO2} = C losses in CO_2; C_{CH4} = C losses in methane; C_{milk} = C in milk; $C_{retained\ body}$ = recovered C in tissue; N_{intake} = N intake; N_{feces} = N losses in feces; N_{urine} = N losses in urine; N_{milk} = N in milk; $N_{retained\ body}$ = recovered N in tissue; R = retention.				
[b] ALF = Alfalfa; ORG = Orange leaves.				
[c] SEM = standard error of the mean.				

Table 7

Fatty acid composition (g of identified fatty acids / 100 g of milk fat) for goats fed the experimental diets (n=10).

	Diet[b]			
Item[a]	ALF	ORG	SEM[c]	P-value
C4:0	0.68	0.68	0.025	0.968
C6:0	1.64	1.59	0.048	0.672
C8:0	2.12	2.01	0.112	0.844
C10:0	8.15	7.67	0.569	0.701
C11:0	0.29	0.31	0.027	0.717
C12:0	4.83	4.99	0.457	0.870
C13:0	6.39	6.74	0.374	0.669
C14:0	7.97	7.41	0.567	0.653
C14:1	0.19	0.19	0.025	0.999
C15:0	0.59	0.58	0.070	0.970
C16:0	23.83	20.49	1.189	0.017
C16:1	0.71	0.74	0.060	0.818
C17:0	0.37	0.40	0.074	0.863
C17:1	0.17	0.27	0.029	0.035
C18:0	3.97	4.03	0.660	0.970
C18:1n9t	0.59	0.74	0.106	0.276
C18:1n9c	10.95	14.31	1.102	0.042
C18:1n7	0.29	0.30	0.047	0.594
C18:2n6t	0.14	0.15	0.012	0.607
C18:2n6c	2.49	3.04	0.127	0.030
C20:0	0.10	0.08	0.005	0.059
C18:3n6	0.01	0.22	0.008	0.012

C20:1	0.04	0.05	0.004	0.427
C18:3n3	0.24	0.26	0.023	0.713
CLA 9c11t + 9t11c	0.36	0.45	0.028	0.042
C20:4n6	0.20	0.20	0.013	0.238
C22:0	0.04	0.02	0.016	0.034
Total fatty acids	77.4	77.9	1.15	0.977
Short-chain fatty acids	2.32	2.27	0.059	0.718
Medium-chain fatty acids	21.8	21.8	1.28	0.998
Long-chain fatty acids	53.3	53.7	1.91	0.912
Saturated fatty acids	61.0	57.1	1.79	0.302
Monounsaturated fatty acids	12.9	16.6	2.19	0.432
Polyunsaturated fatty acids	3.44	4.07	0.303	0.324
AI	3.98	3.19	0.426	0.381

[a] CLA = conjugated linoleic acid; AI = Atherogenicity index calculated as C12:0 + 4 × C14:0 + C16:0/unsaturated fatty acids (Ulbricht and Southgate, 1991).

[b] ALF = Alfalfa; ORG = Orange leaves.

[c] SEM = standard error of the mean.

Table 8

Methane emission of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet.

	Diet[b]			
Item[a]	ALF	ORG	SEM[c]	P-value
CH_4, g/d	18.1	12.3	0.77	0.001
Ym, %	3.6	3.1	0.25	0.197
CH_4/DMI, g/kg	10.7	9.0	1.02	0.289
CH_4/OMd, g/kg	16.1	12.4	0.86	0.024
CH_4/milk, g/kg	13.6	9.8	0.81	0.032

[a] Ym = methane energy/gross energy intake; DMI = dry matter intake; OMd = organic matter digested.

[b] ALF = Alfalfa; ORG = Orange leaves.

[c] SEM = standard error of the mean.