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RUNNINGHEAD: ORANGE LEAVES IN DAIRY GOATS DIETS

Energy, nitrogen partitioning and methane emissions in dairy goats differ when an isoenergetic and isoproteic diet contained orange leaves and rice straw crop residues

C. Fernández [1,*], T. Romero [1], J. V. Martí [1], V. J. Moya [1], I. Hernando [2], and J. J. Loor [3]

[1]Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, 46022 Valencia, Spain

[2]Facultad de Magisterio y Ciencias de la Educación. Universidad Católica de Valencia, Spain

[3]Department of Animal Sciences, Division of Nutritional Sciences, University of Illinois, Urbana 61801, USA

[*]Corresponding author: cjfernandez@dca.upv.es

ABSTRACT

The aim of this study was to evaluate the effects of incorporating rice straw and orange leaves into the diets for goats. Ten Murciano-Granadina goats at mid-lactation weighting 45 ± 0.3 kg BW were used in a cross-over design. Two isoproteic and isoenergetic diets (180 g/kg DM and 17 MJ/kg DM, respectively) with alfalfa hay as forage source (33% of DM) were fed. A control diet (CON) incorporated barley as energy source and soy hulls as fiber component. The experimental diet (ORG) replaced barley and soy hulls with orange leaves (19% on DM basis), rice straw (12%, on DM basis) and soya oil (2%). Peas and horsebeans were the protein source in both diets. Each goat received the 2 treatments in 2 periods. Goats were fed the experimental diets and after 14 days on their respective treatments moved to individual metabolism cages for another 7 days. Subsequently, feed intake, total fecal and urine output and milk yield were recorded daily over the first 5 days. During the last 2 days, ruminal fluid and blood samples were collected along with individual gas exchange measurements recorded by a mobile open-circuit indirect calorimetry system using a head box. No differences in dry matter intake were detected, and apparent total tract digestibility was greater in CON than ORG. Efficiency of metabolizable energy intake for milk and maintenance also was lower in response to ORG (0.65 vs. 0.63), with energy balance being negative (-12 kJ/kg of $BW^{0.75}$) due to mobilization of fat (-16 g/animal vs. 68 g/animal for ORG and CON, respectively). Although actual milk yield was lower in goats fed ORG (2.32 vs. 2.06 kg/d, respectively), energy-corrected milk did not differ (2.81 kg/d on average). In terms of milk quality, milk fat content and concentrations of monounsaturated (18.54 vs. 11.55 g/100 g milk fat) and polyunsaturated fatty acids (5.75 vs. 3.99 g/100 g milk fat) were greater in goats fed ORG. Based on various indices, the milk produced by ORG would be less atherogenic and thrombogenic than CON milk. Compared with CON, enteric CH_4 emission was lower due

to feeding ORG (reduction of 38 g CH_4/kg milk fat). Data suggest that greater fat mobilization in goats fed ORG might have been due to the apparent lack of synchrony between degradable protein and carbohydrate and the lipogenic nutrients associated with the lower cereal content of the ORG diet. Thus, goats fed ORG seemed to rely more on fat depots to help meet energy requirements and reach optimal performance. As such, the lower content of glucogenic nutrients in ORG did not favor body fat deposition and partitioning of ME into body tissue. Overall, responses in terms of CH_4 emissions and milk quality suggest that inclusion of rice straw and orange leaves in diets for small ruminants could be a valuable alternative to reuse, recycle and revalue agricultural by-products.

Key words: orange leaves, rice straw, dairy goat, methane emission

INTRODUCTION

Total European agricultural area dedicated to crops spans 173 million ha. Spain, with an area for agricultural purposes of 23 million ha, was the second country (EUROSTAT, 2020). In Mediterranean regions, rice and orange production areas are mainly located in Spain, Italy and Greece. According to the latest FAO data, the EU produced approximately 3.7 million tons of rice and 11.1 million tons of citrus fruit in 2018 (FAOSTAT, 2020). Waste management represents a key element in strategies for reducing air and water pollution, greenhouse, gas emissions, and health problems. One of the priority objectives, regarding waste policy and managing, outlined in the 7th Environment Action Programme of the European Union to 2020 was to maximize recycling and re-use (EAP, 2020).

As a result, large amounts of by-product waste were generated. In the case of rice cultivation, straw is generated during harvest, which makes this by-product one of the most-abundant and available in large quantities (10 tons/ha cultivated) around the world (Matias et al., 2019). In the case of citrus, an annual pruning is necessary for physiological control that guarantees optimal production by the plant (Guardiola et al., 2008), and out of 3.92 tons/ha cultivated (dry matter basis) this process generates approximately 50% leaf and 50% wood by-product (EFEAGRO, 2016). Traditionally, the most-common way for rapid and inexpensive disposal of rice straw and citrus waste has been burning in the field (Kumar et al., 2015; Segarra et al., 2019). Clearly, this practice entails an environmental cost as it constitutes a significant source of greenhouse gas emissions into the atmosphere and removes valuable material that can be used in many ways (Segarra et al., 2019). In the case of rice straw, an alternate approach for disposal is through soil incorporation strategies (Ribó et al., 2017). However, the incorporation of stubble and straw to wet soil is associated with an increase in methane emissions (Dominguez-Escribá and Porcar, 2010). In this

scenario, the use of agro-industrial by-products as feed ingredients could represent an important component of the global strategy to reduce this environmental impact.

The use of cereal straw as feeds for ruminants is characterized by low protein content, high degree of lignification and low digestibility. Rice straw in unique relative to other cereals straws in being low in lignin and high in silica (Van Soest, 2006). Silica and lignin in that order are the primary limiting factors in rice straw quality.

Besides of the use of a traditional poor-quality by-product as rice straw in ruminant nutrition, the use of citrus leaves provides a source of fiber for ruminal fermentation and is an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids and phenolic compounds (Bampidis and Robinson, 2006; Khettal et al., 2017). These compounds, included at low or moderate levels in the diet, have a positive effect on productive performance and health (Patra et al., 2017; Correddu et al., 2020). Furthermore, essential oils and bioactive compounds present in citrus leaves could be beneficial to reduce CH_4 emissions from fermentation (Patra and Yu, 2012; Knapp et al., 2014; Correddu et al., 2020).

The demand for efficient use of food by-products is increasing due to economic and environmental concerns. Thus, an alternative feedstuff such as the use of these in ruminant diets could be implemented as a way to recycle and reuse these wastes or residues in support of production of human-edible foods such as milk or meat (Cao et al., 2009). In 2017, the global dairy goat population was estimated at 218 million, with production being more common in Mediterranean Countries such as France, Spain, Italy and Greece (Miller and Lu, 2019).

The main objective of the present study was to include orange leaves and rice straw in replacement of barley grain into the concentrate feed for dairy goats, and study the

104 impact on intake, apparent total tract digestibility, energy, carbon (**C**) and nitrogen (**N**)
105 balance, milk performance, and CH_4 emissions.

106 MATERIAL AND METHODS

107 *Ethics Statement*

108 The experimental procedures were approved (2017/VSC/PEA/00182) by the
109 Committee on Animal Use and Care at the Polytechnic University of Valencia (UPV)
110 (Valencia, Spain), and followed the codes of practice for animals used in experimental
111 work proposed by the European Union (EU, 2003). Authors declare that this manuscript
112 does not involve ethical issues or affect any endangered or protected species.

113 *Animals and Diets*

114 The experiment was conducted at the Animal Science Department Experimental
115 Farm (UPV) (Valencia, Spain). Ten multiparous mature Murciano-Granadina dairy goats
116 in mid-lactation were selected and divided into two homogenous groups of five goats based
117 on similar body weight (BW; 45.3 ± 0.3 kg of BW) and milk production in the previous
118 lactation (640.29 ± 60 kg of milk per 210 ± 30 days of lactation).

119 The chemical composition of alfalfa, concentrates and whole mixed diet (forage
120 and pelleted concentrate) is reported in Table 1. Treatments were evaluated in a crossover
121 design (2 treatments crossed with 2 period) using diets fed as total mixed rations. Goats
122 were fed daily 1 kg alfalfa hay and 1.7 kg concentrate (37:63 forage to concentrate ratio).
123 Half the daily ration was offered at 0800 h and half at 1600 h. The concentrate and premix
124 were combined and pelleted. The control (**CON**) group was fed concentrate with 406 g/kg
125 of dry matter (DM) of barley grain, and the test group was fed concentrate in which 300 g
126 of orange leaves/kg DM and 190 g of rice straw/kg DM replaced barley grain (**ORG**). In
127 order to replace barley grain with orange leaves and rice straw, we relied on the nutritional
128 value information for orange leaves determined in our previous work (Fernández et al.

2019a) and literature information for rice straw (Van Soest, 2006). Nutrient requirements followed published recommendations for lactating goats weighing 45 kg of BW and producing 2.5 kg milk per day (Calsamiglia et al., 2019). Although the assessment of the energy content of the diets as gross energy (**GE**) did not guarantee they were isoenergetic, we aimed to achieve isoenergetic and isoproteic diets by adding soybean oil to the ORG diet and the same sources of protein were used in both diets (field peas and horsebeans).

Animals, Experimental Design 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 had two 33 d periods. During the adaptation period, goats were fed the experimental diets in pens for 14 d and then allocated to individual metabolism cages (1.5 m long x 0.53 m wide x 1.65 m high) at thermoneutrality (20-23 ºC determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA) for another 7 d. Subsequently, data on feed offered and refused and total fecal, urine and milk output were recorded daily for each goat during a 5 d period. In addition, BW at the beginning and end of the experimental period were recorded. Over 5 consecutive days for each goat, daily total feces were collected in wire-screen baskets placed under the floor of the metabolism crates and daily total urine was collected through a funnel into plastic buckets containing 100 mL 10% (vol/vol) of H_2SO_4. Acidification of urine was necessary to prevent microbial degradation and loss of volatile ammonium. Feces and urine from each goat were weighed daily and representative samples (10%) of diets, feces and urine stored at -20 ºC until chemical analyses.

Goats were milked once daily at 0800 h with a portable milking machine (Flaco, model DL-170, J. Delgado S.A., Ciudad Real, Spain). Immediately after milking, individual milk yield was measured and a sub-sample of 250 mL per animal was placed in

154 a bottle and frozen until analysis. In addition, samples were collected into plastic vials (50
155 mL per animal) that contained 20 mg of potassium dichromate as a preservative and taken
156 to the Interprofessional Dairy Laboratory of the Valencia Community Region (LICOVAL,
157 Valencia, Spain) for composition analysis (dry matter, crude protein, fat and lactose). Prior
158 to gas exchange determinations, goats were moved from metabolism cages to pens for 2 d
159 during which ruminal fluid and blood samples were collected. Ruminal fluid samples were
160 collected by stomach tube (50 mL) before the morning feeding following a procedure
161 described previously (Ramos-Morales et al., 2014). Ruminal fluid was strained through 4
162 layers of cheesecloth, and pH determined immediately using a portable pH meter (Model
163 265A, Orion Research Inc., Beverly, MA, USA). A sub-sample of ruminal fluid (4 mL)
164 was acidified with 50% H_2SO_4 and frozen until later determination of ammonium. Further
165 samples (0.9 mL) for analysis of VFA were mixed with H_3PO_4 (0.1 mL) and kept frozen
166 until analysis. Jugular blood was sampled in 10 mL tubes treated with EDTA and
167 immediately centrifuged for plasma separation and storage at – 20ºC.
168 Gas exchange was measured for each goat during 24 h with an indirect calorimetric
169 system based on two ventilated head-box designed for small ruminants (5 d period). The
170 respirometry system was equipped with 2 head hoods, 2 flow-meters (Thermal Mass
171 Flowmeter Sensyflow VT-S, ABB, Alzenau, Germany) and 2 air suctions provided by
172 centrifugal fans (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain).
173 Concentrations of CH_4 and carbon dioxide (CO_2) were measured using the infrared
174 principle, and O_2 measured with the paramagnetic principle (Easyflow Gas Analyzer,
175 model 3020, ABB, Alzenau, Germany). The CH_4 and CO_2 are measured using infrared
176 principle with a range from 0-0.15 and 0-1.5%, respectively. The analysis of O_2 works on
177 the paramagnetic principle with a range from 18-21%. Although the unit was an
178 autocalibrated model, analyzers were calibrated with reference gases before each test. The

179 mobile open-circuit respirometry system used for these measurements was described
180 previously (Fernández et al, 2012; Fernández et al., 2015; Fernández et al., 2019b). The
181 whole system was calibrated by injecting pure nitrogen (N_2) and CO_2 into the head box
182 (McLean and Tobin, 1987), determined gravimetrically using a precision scale (MOBBA
183 mini-SP 0.2–30 kg, Industrial Weighing System, Barcelona, Spain). Calibration factors
184 were calculated as described previously (Brockway et al., 1971). The CH_4 and CO_2
185 production and oxygen (O_2) consumption were calculated as described previously
186 (Aguilera and Prieto, 1986). An initial atmospheric air sample was collected and the gas
187 concentrations were used as reference for calculations.

188 ***Chemical Analysis***

189 Feed, feed refusal and fecal samples were first dried in a forced-air oven at 55 ºC
190 for 48 h then ground to pass a 1 mm screen before analysis. Urine and milk were lyophilized
191 prior to analyses. Chemical analyses of the diet, refusals and feces were conducted
192 according to AOAC (2000) for DM (934.01), ash (942.05) and ether extract (**EE**; 920.39).
193 The DM of diets and feces was determined by oven-drying at 102 ± 2 ºC for 24 h. Ash
194 concentration was measured by incineration in an electric muffle furnace at 550 ºC for 6 h.
195 The EE was extracted with petroleum ether after acid hydrolysis to recover saponified fat
196 (Soxhlet System HT Tecator, Hillerød, Denmark; 1047 Hydrolyzing Unit and 1043
197 Extraction Unit). The NDF and ADF were measured in an ANKOM Fiber Analyzer (A220,
198 ANKOM Technologies, Fairport, NY, USA) according to a published protocol (Mertens,
199 2002) and AOAC (2000), respectively. The NDF was determined using sodium sulfite and
200 alpha amylase. The NFC content of diets was calculated by difference based on chemical
201 analysis of individual feeds according to NRC (2001): NFC = 100 – NDF – ash – CP – EE.
202 The GE content of the dried samples (feed, feces, urine and milk) was analyzed by
203 combustion in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough,

204 UK). Starch content was determined by an enzymatic method (α-amylase obtained from
205 Sigma-Aldrich, Steinheim, Germany) (Batey, 1982). The C and N were analyzed by the
206 Dumas principle (TruSpec CN; LECO Corporation, St. Joseph, MI, USA). Multiplying N
207 by a factor of 6.25 converted the results to CP.

208 Determination of ruminal VFA was based on a method described previously
209 (Jouany, 1982) using a gas chromatograph (Fisons 8000 series; Fisons Instruments SpA,
210 Milan, Italy) equipped with a split/splitless injector and flame ionization detector. Milk
211 composition (fat, protein, lactose, citrate and total milk solids content) was analyzed with
212 an infrared analyzer (MilkoScan FT120 Foss Electric, Hillerød, Denmark). Fatty acid
213 methyl esters of milk were prepared directly as previously described by O'Fallon et al.
214 (2007). The FA methyl esters were analyzed in a Focus Gas Chromatograph (Thermo,
215 Milan, Italy) equipped with a split/splitless injector and a flame ionization detector.
216 Separation of methyl esters was performed in a fused silica capillary column SP™ 2560
217 (Supelco, PA, USA) (100 m × 0.25 mm × 0.2 μm film thickness). The carrier gas was
218 helium at a linear velocity of 20 cm/s. The samples were injected with a split ratio of 1/100.
219 The initial oven temperature was set at 140 ºC held for 5 min and increased to 240 at 4
220 ºC/min and finally maintained at that temperature for 30 min. Both detector and injector
221 temperatures were set at 260 ºC. Two external standards were used for identification of
222 fatty acids and CLA isomers. Supelco 37 Component FAME MIX (CRM47885) for fatty
223 acids and Linoleic acid, conjugated methyl ester isomer mix (Sigma 05632) for CLA
224 isomers. Furthermore, for confirmatory purposes, the chromatographic profile obtained
225 was compared with those described previously by Kramet et al., 1997.

226 Analysis of glutamate and free amino groups was according to a published method
227 (Larsen and Fernández, 2017). Creatinine was analyzed according to standard procedures
228 (Siemens Diagnostics® Clinical Methods for ADVIA 1800). Plasma non-esterified fatty

acids (NEFA) were determined using the NEFA C ACS-ACOD assay method, BHB was determined as proposed by Harano et al. (1985). Minor milk constituents such as glucose, glucose-6-phosphate, malate, isocitrate, BHB, and uric acid were determined by enzymatic-fluorometric methods (Larsen and Nielsen, 2005; Larsen and Moyes, 2010; Larsen, 2014; Larsen, 2015). Plasma and milk urea were analyzed by flow injection analyses (FIA) and enzymatic degradation. Application notes given by the manufacturer were followed (Foss Tecator AB, Höganäs, Sweden).

Calculations

Fat corrected milk (**FCM**) at 4% were obtained according to a published equation (Mavrogenis and Papachristoforou, 1988); FCM (4%) = kg of milk × [0.411 + (0.147 × fat (%))].

The ME intake (**MEI**) was calculated as the difference between GE intake (GEI) and energy losses in feces (E_{feces}), urine (E_{urine}) and CH_4 (E_{CH4}; with an energy equivalent value of 39.5 kJ/L CH_4; Brouwer, 1965).

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

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

where gases were expressed in liters per day and N_{urine} in grams per day. Recovered energy was the difference between ME and HP. Energy retention in the body (RE_{body}) was calculated as the difference between recovered energy and milk energy (E_{milk}).

Energy associated with the oxidation of protein (**OXP**), carbohydrate (**OXCHO**) and fat (**OXF**) was calculated by published methods for ruminants (Brouwer, 1958; Chwalibog et al., 1997). The CO_2 production from oxidation (CO_{2x}) was calculated as $CO_2 - (2 \times CH_4)$, according to Fahey and Berger (1988). Calculations were as follows:

$$OXP = 6.25 \times N_{urine} \times 18.42\ (kJ/g),$$

254 $OXCHO = (-2.968 \times O_2 + 4.174 \times CO_{2x} - 2.446 \times N_{urine}) \times 17.58$ (kJ/g),

255 $OXF = (1.719 \times O_2 - 1.719 \times CO_{2x} - 1.963 \times N_{urine}) \times 39.76$ (kJ/g).

256 Then, HP from oxidation (HPx) was:

257 $HPx\ (kJ) = 16.18 \times O_2 + 5.02 \times CO_{2x} - 5.99 \times N_{urine}$.

258 Gases were expressed in liters per day and N_{urine} in grams per day. Heat of
259 fermentation (HPf) was estimated subtracting HP from HPx. Non-protein respiratory
260 quotient from oxidation of nutrients (RQnpx) was determined as: $RQnpx = (CO_{2x} – (N_{urine}$
261 $\times 6.25 \times 0.774)) / (O_2 – (N_{urine} \times 6.25 \times 0.957))$. For C and N balance, we followed the
262 equations and values proposed previously (McLean and Tobin, 1987), and the grams
263 retained in protein ($R_{protein}$) and fat (R_{fat}) were calculated.

264 Efficiency of ME to milk and maintenance (kls) was calculated according to INRA
265 (2018); kls = 0.65 + 0.247 x (q – 0.63), being q the metabolisability (ME/GE).

266 ***Statistical Analysis***

267 The experiment was conducted as a crossover design: each goat received both
268 treatments in 2 periods. Effects of diet on intake, digestibility, ruminal fermentation, milk
269 performance, energy and C and N balances, and oxidation of nutrients were analyzed using
270 a mixed model (lme function from the nlme library) in R (2016). The following statistical
271 model was used:

272 $$Y = \mu + D + T + D \times T + goat + \varepsilon$$

273 Where: Y is the dependent variable, μ is the overall mean, and D and T are the fixed
274 effects of diet and period of time, respectively, and their interaction; goat is the random
275 effect of goat; and ε is the random error. Least squares mean were reported throughout and
276 differences were considered significant at $P < 0.05$.

277 **RESULTS AND DISCUSSION**

No significant effect was observed for period and their interaction in the crossover design; thus, tables report only the effect of diet. The average value for the calibration factor of O_2, CO_2 and CH_4 was 1.0021 ± 0.00110 (n = 4), 1.0015 ± 0.00922 (n = 4) and 0.9798 ± 0.00762 (n = 4), respectively. The consistent values confirmed the absence of leaks from the entire open circuit indirect calorimetry system.

Feed Intake and Digestibility

No difference in total DMI ($P > 0.05$) was observed between diets (2.23 kg/d, on average) indicating animals in each group consumed the same amount of concentrate (Table 2). Although both diets were formulated to be isoenergetic and soy hulls were added to the CON diet to match its fiber content with ORG, the barley diet was more digestible (Table 1). Thus, inclusion of rice straw into the ORG diet had a negative impact on digestion as illustrated by the lower NDF digestibility with ORG than CON (37 vs. 55%, respectively). This response was probably associated with the high lignin content in rice straw (ADF digestibility was 34 and 48% for ORG and CON, respectively). The CP and EE apparent total tract digestibility was lower ($P < 0.001$) in CON than ORG diet. Similar results were obtained in a previous study (Romero et al., 2020) in dairy goats fed lemon leaves and rice straw. Atikah et al. (2018) supplemented the diet with olive, palm or sunflower oil and also reported greater apparent digestibility of CP and EE. Classical studies (Palmquist and Jenkins, 1980) indicated that high dietary lipid was more likely to inhibit fiber degradability with a concomitant reduction in fermentation, possibly due to coating of feed particles and preventing bacterial attachment. Orange leaves (like other citrus plants) also contain essential oils (Fernández et al., 2019a) that could contribute to decreasing fiber and DM apparent digestibility through a direct effect on microorganisms.

Ruminal Fermentation

302 Average ruminal pH never fell below 6.5 (Table 3) and was within a range 6.0–7.0,
303 suggesting that values obtained were sufficiently high to maintain normal ruminal
304 fermentation (Ørskov and Fraser, 1975). No differences between diets were observed for
305 NH_3-N, urea and VFA. Acetic and butyric acids are considered lipogenic substrates and
306 propionic acid is considered a glucogenic substrate (Knegsel et al., 2007). Differences (P
307 < 0.05) were detected when the ratio of acetic to propionic acid was determined, being
308 greater for ORG than CON. Thus, based on Knegsel et al. (2007), we speculate that the
309 CON diet had a tendency to behave like a glycogenic diet while the ORG diet seemed to
310 have a lipogenic behavior.
311 Free amino groups and glutamate were lower in ruminal fluid from goats fed ORG.
312 Dietary lipid has a strong inhibitory effect on protozoa and cellulolytic bacteria that can
313 cause reductions in carbohydrate fermentation and asynchrony between protein and
314 carbohydrate digestion for microbial protein synthesis (Casper et al., 1999), and ORG diet
315 had soya oil added and the lipids content from orange leaves. In contrast, isobutyric and
316 isovaleric acids were greater (P < 0.05) in response to ORG. Atikah et al. (2018) observed
317 greater isobutyric acid concentration in goats fed diets supplemented with olive oil and
318 Romero et al. (2020) a greater concentration of isovaleric acid in goats fed a ration that
319 included lemon leaves. Isobutyric and isovaleric acids are mainly generated during
320 degradation of branched-chain amino acids, thus, greater concentrations observed in goats
321 fed ORG suggested greater ruminal protein degradation and potentially a decrease in the
322 use of amino groups for microbial protein synthesis.
323 Plasma metabolites are reported in Table 3. Urea in plasma was greater (P < 0.05)
324 with ORG than CON perhaps because ORG had a little more protein, and it was slightly
325 more digestible. Greater (P < 0.05) plasma NEFA were observed with ORG than CON,
326 which agrees with the idea that ORG was more lipogenic than glucogenic. We speculate

that the greater plasma NEFA concentration with the ORG diet likely originated from mobilized body fat reserves as indicated by the negative energy balance and fat retention observed with ORG diet (discussed below).

Energy Balance

Due to similar DMI, no significant differences ($P > 0.05$) in GEI (2,203 kJ/kg of $BW^{0.75}$, on average) were observed (Table 4). The lower digestibility in response to feeding ORG led to greater energy losses in feces ($P < 0.001$). Urine energy losses also were greater ($P < 0.001$) with ORG vs. CON, but a reduction ($P = 0.001$) in energy losses in CH_4 with the ORG diet was detected (96 vs. 88 kJ/kg of $BW^{0.75}$ for CON and ORG, respectively). Therefore, this response supported that higher urine losses could be because ORG animals ate more digestible protein and, the significantly large decrease in NDF digestibility observed in ORG diet (probably because of rice straw) possibly cause less CH_4. Besides, ORG diet had soybean oil added, and it was known to disrupt rumen bacteria populations leading to shift in rumen biohydrogenation pathways and also rumen fermentation (such as VFA and CH_4). Patra et al. (2017) and Correddu et al. (2020) reported that some plant secondary metabolites, such as essential oils and polyphenols, may exert inhibitory effects on the ruminal methanogenic activity, and orange leaves contain essential oils and tannins. Larsen et al. (2016) reported that increasing the level of dietary starch and lipid and decreasing NDF and ADF reduced CH_4 production.

Due to greater losses in feces and urine with the ORG diet, MEI was lower in response to feeding ORG (a reduction of 158 kJ/kg of $BW^{0.75}$). In agreement with digestibility results, the ORG diet was characterized by the lowest content and digestible ME. Thus, results suggest that the CON compared with ORG diet, which was rich in starch and had a fairly high NDF digestibility, supplied more ME. No differences were observed in HP (730 kJ/kg of $BW^{0.75}$, on average). These values were in the range of previous work

with goats. In mid-lactating animals fed diets with 60% concentrate, Bava et al. (2001) reported average values of 855 kJ/kg of $BW^{0.75}$ for Saanen goats and Tovar-Luna et al. (2010) an average of 737 kJ/kg of $BW^{0.75}$ with Alpine goats. The E_{milk} was greater in ORG than CON (476 vs. 446 kJ/kg of $BW^{0.75}$, respectively), the RE_{body} was lower ($P < 0.001$) averaging 163 vs. -12 kJ/kg of $BW^{0.75}$ for CON and ORG, respectively.

These data indicated that goats fed ORG had greater energy in milk as a consequence of the mobilization of lipid reserves (as observed with the negative RE_{body} in ORG). In lactating ruminants, lipogenic nutrients can increase the partitioning of ME into milk (increasing milk fat yield), and consequently decrease partitioning of ME into body reserves (Van Knegsel et al., 2007). Thus, the present observations suggested that the lower content of glucogenic nutrients in ORG (i.e., diet CON was greater in barley grain) did not favor body fat deposition.

Efficiency of ME for milk and maintenance (kls), as defined by INRA (2018), was lower ($P < 0.05$) in response to feeding ORG (0.65 vs. 0.63, respectively). Similar values (0.67 and 0.63, respectively) were reported for Granadina (Aguilera et al., 1990) and Alpine goats (Tovar-Luna et al., 2010), respectively.

Differences between diets ($P = 0.03$) were detected for ME when expressed per kg of DM; 11 and 9 MJ/kg of DM for CON and ORG, respectively, indicating that diets were not isoenergetic.

Oxidation of Nutrients

Production of CO_2 is derived from nutrient oxidation and ruminal fermentation. Thus, separation between these two components is necessary to calculate substrate oxidation and the proportion that supports total HP associated with oxidative processes. Diet had no effect on HPx and differences ($P < 0.05$) were observed in HPf, with lower values in response to feeding ORG (Table 5). Oxidation of nutrients as OXP was greater

($P < 0.001$) in ORG than CON (19% vs. 14%, respectively), which agreed with the greater energy losses (Table 4) and N excreted in urine (Table 6). The OXCHO was lower ($P < 0.001$) (41% vs. 56%, respectively) and OXF greater ($P < 0.05$) with ORG than CON (40% and 30%, respectively). Namely, the OXCHO was predominant in the diet with more cereal and greater digestibility, while in the diet with more fibrous by-products, OXF was major. It is possible that protein metabolism and lipid mobilization supported the greater milk energy (Table 4) when the ORG diet was fed. We must bear in mind that the gas exchange method does not discriminate between oxidation of exogenous and endogenous glucose, thus, the data more closely represented net catabolism of glucose. Goats fed ORG had a reduction of OXCHO as a result of lower starch intake.

Because dietary fat content in ruminant diets is typically low, the greater contribution of OXF with the ORG diet probably originated from mobilization of reserves (Derno et al., 2013). Few studies in ruminants related to oxidation of nutrients are available. Chwalibog et al. (1997) using calves with positive retained energy as fat suggested that a part of OXF originates from ingested carbohydrate, mainly fiber. A significant difference ($P < 0.001$) was observed for RQnpx, with ORG resulting in lower values than CON (0.83 vs. 0.90, respectively). These responses might have been the result of differences in starch content in the diet and the consequent increase in HP associated with oxidation of carbohydrates. An RQnpx value lower than 1 indicates predominance of OXF compared with OXCHO (Chwalibog et al., 1997), as was observed in this study.

Carbon and Nitrogen Balance

The C and N balance are shown in Table 6. The C balance followed the same tendency than for energy balance. Although greater N intake ($P < 0.05$) and N in urine were detected due to feeding ORG (0.3 g/kg of $BW^{0.75}$), no differences were observed for N in feces. Urinary N is largely represented by urea, and is therefore more rapidly nitrified with

consequent emission of ammonia. Thus, urinary N is less desirable and shifting N excretion from urine to feces may be useful as Brito and Broderick (2007) reported. Total N excretion was greater ($P < 0.05$) (3.03 vs. 2.79 g/kg of $BW^{0.75}$), N in milk lower (0.70 vs. 0.73 g/kg of $BW^{0.75}$), and N retained in the body greater when ORG was fed (0.22 vs. 0.16 g/kg of $BW^{0.75}$). From the C and N balance data obtained we estimated retention of protein and fat (according to McLean and Tobin, 1987). These theorical estimates indicated that feeding CON led to more fat retention, while feeding ORG led to more fat mobilization. Thus, the R_{fat} was approximately 68 g/animal in CON and -16 g/animal in ORG (Table 6).

Milk Production, Metabolites and Fatty Acids

Diet had an effect on milk yield with goats fed ORG producing on average 0.26 kg/d less (Table 7). However, when milk yield was expressed as FCM, no differences were detected (2.81 kg/d on average), but we have to keep in mind that goats fed ORG diet were in negative energy balance. No differences were detected when milk production was expressed relative to DMI; 0.98 for milk yield/DMI and 1.27 for FCM/DMI. Milk composition was similar to data reported for the Murciano-Granadina breed (Beltrán et al., 2013). However, milk from goats fed ORG had greater content of DM, fat and cheese extract. The ORG diet was richer in lipogenic nutrients and greater milk fat percentage, in contrast, the glucogenic nutrients in the CON diet decreased milk fat components, as hypothesized Knegsel et al. (2007). In recent years, goat's milk production has risen markedly in the countries traditionally producers such as Spain, which produces 22.6 % of the goat's milk in the European Union (FAOSTAT, 2020) ranking second after France (31.9 %). In Spain, farmers are paid based on two components in the milk; protein and fat (protein plus fat is the cheese extract). The cheese extract was greater ($P < 0.001$) in ORG than CON (10.7 vs. 9.6%, respectively). Thus, the present data suggest that inclusion of orange leaves and rice straw in the diet of dairy goats positively affected the commercial

427 value of milk in a payment system based on cheese extract such as in Spain (Pirisi et al.,
428 2007), avoiding the economic loss due to the reduction of milk yield.

429 No significant differences were observed for most milk metabolites studied. As
430 expected, milk urea nitrogen followed same pattern as plasma urea nitrogen. Plasma
431 glucose is the obligatory precursor needed for milk lactose synthesis, where glucose 6
432 phosphate is an intermediate component. Glucose content of milk was lower ($P < 0.05$) in
433 response to feeding ORG, a trend reported previously (Larsen et al., 2016) in cows fed a
434 high- vs. low-digestible diets. Because goats fed ORG were in negative energy balance,
435 metabolites related to fat mobilization such as BHB were greater ($P < 0.05$) in milk (77 vs.
436 74 μ*M* in ORG and CON diet, respectively).

437 Under negative energy balance, body fat mobilization coincided with high plasma
438 concentration of BHB (Xu et al., 2020) as expected. The isocitrate concentration with the
439 ORG compared with CON diet (140 vs. 103 m*M*, respectively) was greater ($P < 0.05$),
440 suggesting that isocitrate increased with negative energy balance and lipid mobilization.
441 Ours results were in line with others studies in dairy cows reporting that milk citrate was
442 an indicator of negative energy balance and reflected its role in fat synthesis in the
443 mammary gland (Xu et al., 2020).

444 Diet (e.g., forage-to-concentrate ratio, forage type) is the main environmental
445 parameter regulating milk fat synthesis and fatty acid composition in ruminants (Bernard
446 et al., 2009; Chilliard et al., 2013; Nudda et al., 2013). In the present study, replacement of
447 barley grain with byproducts (fibrous and with higher polyunsaturated FA (PUFA) content)
448 and the addition of soybean oil to equilibrate dietary energy, led to greater milk fat content
449 (6.5% vs. 5.5%, ORG and CON). Fatty acid composition of milk fat (Table 8) was similar
450 to values reported for the Murciano-Granadina breed (Sanz Ceballos et al., 2009).
451 According to a previous study (Chilliard et al., 2003), the content of FA with 16 or fewer

carbon atoms derives from *de novo* synthesis, whereas those with 18 or more carbons arise from the diet or lipid mobilization. In our study no differences were found between diets in FA with 16 or fewer carbon atoms (29.62 g/100 g of milk fat, on average), whereas greater values ($P < 0.05$) with 18 or more carbon atoms were found for ORG than CON (29.68 vs. 17.66 g/100 g of milk fat, respectively). Concentration of FA with C12:0, C14:1, C16:0 and C17:0 was lower ($P < 0.05$) in goats fed ORG. Milk C16:0 was 8% lower in goats fed ORG. Milk C16:0 results mainly from *de novo* FA synthesis in mammary tissue using acetate produced in the rumen during fiber digestion, with CH_4 output positively correlated with milk C16:0 (Fievez et al., 2012).

Greater values ($P < 0.05$) of C4:0, C6:0, C8:0, C18:0, C18:1, C18:2, C20:0, C18:3n3 and CLA were observed when we feeding ORG (Table 8). The increase in C18:0 and CLA with ORG probably was associated with greater intake of PUFA (soybean oil added to ORG diet) and, thus, greater rate of biohydrogenation (Loor et al., 2002). High rates of fat mobilization led to marked increases in plasma concentrations of NEFA, BHB and accumulation of triacylglycerol in the liver, and also could increase milk fat content (Bjerre-HarpØth et al., 2012). As NEFA are particularly rich in long-chain FA such as C18:1n9c and C18:0 (Hosten et al., 2012; Vlaeminck et al., 2015), concentrations in milk fat of those FA might be linked to negative energy balance. Our results were similar to those reported recently (Fernández et al., 2018; Romero et al., 2020) when animals were fed lemon leaves. Atherogenicity and thrombogenic indices in milk were calculated as indicated previously (Ulbricht and Southgate, 1991), and from a human health standpoint the lower indices observed when ORG was fed suggested not only that orange leaf and rice straw are effective at maintaining milk quality, but also could enhance its beneficial properties.

Methane Emissions

Decreasing overall carbohydrate digestion was accompanied by a moderate change in CH_4 emissions (Table 9), i.e., goats fed ORG produced less ($P < 0.001$) CH_4 (27.2 vs. 30.2 g/d). Because ruminants lose between 2-12% of their dietary GEI as CH_4, a decrease in production of CH_4 represents an improvement in feed efficiency (Johnson and Johnson, 1995). In our study, a reduction ($P < 0.05$) from 4.4 to 4.0% in Ym was observed when orange leaves were fed.

Although CH_4 emission is most-commonly expressed in the literature relative to GE intake, it is necessary to correct the production of CH_4 to the amount of DMI. When CH_4 was expressed relative to DMI statistical differences ($P < 0.05$) remained. But, the most meaningful expression is relative to unit of product, feeding ORG reduced ($P < 0.05$) the amount of CH_4 by 38 g/kg of milk fat and 13 g/kg of cheese extract. Due to inherently low digestibility, the large number of fibrous residues potentially available to feed ruminants have only limited application as basal components in ruminant feed. However, when combined with other ingredients with greater nutrient content the whole nutritive value increases. The nutritional strategy used in the present study, designing a diet that replaced cereal with by-product in the concentrate, transformed the glucogenic diet (CON) into a lipogenic diet (ORG) and was successful in reducing CH_4 production while enhancing cheese yield and milk quality. Although it is necessary to pay attention to the negative energy balance obtained in the diet with these by-products. Diets with different formulations or inclusion rates of rice straw and orange leaves likely will be needed to eliminate the negative energy balance observed in the present study.

CONCLUSIONS

Inclusion of orange leaves and rice straw into the concentrate fed to dairy goats increased the cheese extract while reducing milk thrombogenic and atherogenic indices and CH_4 emissions without affecting feed intake. Thus, formulation of diets based on

agricultural by-products could serve as a viable alternative to reduce controlled burning while recycling these wastes towards milk production, and reduction of greenhouse gas emissions.

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734

Table 1. Ingredients and chemical composition of the diets

	Test Feeds		Forage	Compound Feed		Diet[1]	
Item	Rice Straw	Orange Leaves	Alfalfa	Control	Orange & Rice	CON	ORG
Ingredients, g/kg DM							
Alfalfa hay			1000			370	370
Barley				406	50	256	32
Orange leaves		1000			300	0	189
Rice straw	1000				190	0	120
Soy hulls				350		221	0
Field pea, spring				100	200	63	126
Horsebeans				100	190	63	120
Beet molasses				20	20	13	13
Soybean oil					30	0	19
Calcium carbonate				13	10	8	6
Sodium chloride				4	3	3	2
Dicalcium phosphate				3	3	2	2
Premix[2]				4	4	3	3
Chemical composition, % of DM							
DM	88	91	93	93	94	93	94
OM	75	81	83	87	83	86	83
Ash	13	9	10	6	11	7	11
CP	4	12	22	15	16	18	18
Ether extract	0.8	2.1	2.0	1.7	3.3	1.8	2.8
NDF	57	30	42	43	30	42	35
ADF	40	23	28	23	18	25	22
ADL	5.2	5.6	7.1	0.9	2.2	3.2	4.0
NFC[3]	26	47	24	35	39	31	33
Starch	1.4	0.5	1.4	27	19	18	12
Carbon	41	42	42	41	41	42	42
Nitrogen	0.6	1.9	3.6	2.4	2.6	2.8	2.9
Carbon : Nitrogen	70	22	12	17	16	15	15
Gross energy, MJ/kg DM	16	17	16	17	17	17	16

[1] CON = Control; ORG = orange leaves and rice straw.

[2] Premix = Provided by NACOOP S.A. España composition (ppm or IU per kilogram of premix): 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.

[3]NFC = non fibrous carbohydrate content: 100 - (NDF + ash + CP + EE).

Table 2. Body weight, intake, and apparent digestibility coefficients (% of DM) of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet

	Diet[1]			
Item	CON	ORG	SEM[3]	*P*-value
BW, kg	45.7	45.0	0.52	0.491
DMI, kg/d	2.20	2.26	0.023	0.2397
Concentrate DMI, kg/d	1.47	1.51	0.011	0.3144
Forage DMI, kg/d	0.73	0.75	0.020	0.5734
Apparent total-tract digestibility, %				
DM	68	60	0.61	0.001
OM	70	63	0.54	0.001
CP	70	72	0.42	0.001
Ether extract	67	71	1.00	0.001
NDF	55	37	1.15	0.001
ADF	48	34	1.03	0.001
NFC[2]	91	86	0.43	0.001
Energy	69	62	0.54	0.001

[1] CON = Control; ORG = orange leaves and rice straw.

[2] NFC = non fibrous carbohydrate content: 100 - (NDF + ash + CP + ether extract).

Table 3. pH, ammonia-N (NH_3-N), and VFA from rumen and plasma metabolites of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	ORG	SEM	*P*-value
pH	6.5	6.8	0.10	0.0553
NH_3-N, mg/dL	14.4	14.3	0.44	0.913
Urea, m*M*	9.8	7.3	0.82	0.130
Free amino groups, mEq/L	2.62	1.34	0.292	0.025
Glutamate, m*M*	398	210	42.7	0.023
Total VFA, m*M*	38.8	37.2	3.54	0.830
Individual VFA, m*M*				
Acetic acid	20.50	21.38	1.913	0.825
Propionic acid	7.95	7.00	0.850	0.591
Isobutyric acid	0.40	0.55	0.026	0.002
Butyric acid	8.51	6.67	0.764	0.239
Isovaleric acid	0.47	0.67	0.039	0.006
n-Valeric acid	0.82	0.72	0.083	0.528
n-Caproic acid	0.13	0.11	0.021	0.708
Heptanoic acid	0.04	0.14	0.026	0.053
Acetic/Propionic ratio	2.58	3.05	0.103	0.045
Plasma metabolites				
Urea, m*M*	6.96	8.74	0.348	0.006

Glucose, m*M*	2.94	2.80	0.120	0.557
BHB, m*M*	0.33	0.37	0.029	0.488
NEFA[3], mEq/L	395	705	71.1	0.025

[1] NH_3-N = ammonia nitrogen.

[2] CON = Control; ORG = orange leaves and rice straw.

[3]NEFA = non-esterified fatty acids.

Table 4. 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[2]			
Item[1]	CON	ORG	SEM	*P*-value
GEI	2189	2217	27.5	0.586
E_{feces}	687	842	15.7	0.001
E_{urine}	61	101	3.6	0.001
E_{CH4}	96	88	1.3	0.001
MEI	1345	1187	20.7	0.001
E_{milk}	446	476	13.4	0.001
HP	736	733	8.9	0.114
RE_{body}	163	-12	24.1	0.001
kls	0.65	0.63	0.012	0.037
MJ/kg of DM				
GE	17	17	0.2	0.78
DE	12	10	0.3	0.04
ME	11	9	0.3	0.03

[1] GEI = gross energy intake; E_{feces} = energy losses in feces; E_{urine} = energy losses in urine; E_{CH4} = energy losses in methane; MEI = metabolizable energy intake; E_{milk} = recovered energy in milk; HP = heat production; RE_{body} = recovered energy in tissue (RE_{body} = MEI − HP − E_{milk}); kls = ME efficiency for milk production according to INRA (2018); DE = digestible energy; ME = metabolizable energy.

[2] CON = Control; ORG = orange leaves and rice straw.

Table 5. Heat production (kJ/kg of $BW^{0.75}$) from oxidation of nutrients (kJ/kg of $BW^{0.75}$) and their contribution to the heat production (%) of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	ORG	SEM	*P*-value
HPx, kJ/kg of $BW^{0.75}$	713	702	8.7	0.117
HPf, kJ/kg of $BW^{0.75}$	24	21	0.3	0.001
OXP, kJ/kg of $BW^{0.75}$	101	136	5.5	0.001
OXCHO, kJ/kg of $BW^{0.75}$	405	287	14.0	0.001
OXF, kJ/kg of $BW^{0.75}$	206	278	9.8	0.001
OXP/HPx, %	14	19	0.9	0.001
OXCHO/HPx, %	56	41	1.6	0.001
OXF/HPx, %	30	40	1.6	0.001
RQnpx	0.90	0.83	0.005	0.001

[1]HPx = heat production from oxidation of nutrients; HPf = heat production of fermentation [HPf = HP − HPx (Brouwer. 1958)]; OXP = heat production associated with the oxidation of protein; OXCHO = heat production associated with the oxidation of carbohydrates; OXF = heat production associated with the oxidation of fat; RQnpx = nonprotein respiratory quotient (unitless) from oxidation of nutrients $\{[CO_{2x} - (N_{urine} \times 6.25 \times 0.774)]/[O_2 - (N_{urine} \times 6.25 \times 0.957)]$, where CO_2 = CO_2 production from oxidation and Nurine = N in urine$\}$.

[2] CON = Control; ORG = orange leaves and rice straw.

Table 6. Carbon and nitrogen balance (g/kg of $BW^{0.75}$) of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	ORG	SEM	*P*-value
C_{intake}	52.75	53.87	0.674	0.089
C_{feces}	17.98	22.83	0.443	0.001
C_{urine}	1.51	2.39	0.866	0.001
C_{CO2}	19.57	18.00	0.256	0.001
C_{CH4}	1.31	1.19	0.017	0.001
C excretion	40.37	44.40	0.540	0.001
C_{milk}	8.89	9.46	0.267	0.334
$C_{body\ retained}$	3.50	0.01	0.541	0.018
N_{intake}	3.67	3.94	0.056	0.009
N_{feces}	1.90	1.84	0.040	0.408
N_{urine}	0.88	1.18	0.047	0.001
N excretion	2.79	3.03	0.060	0.023
N_{milk}	0.73	0.70	0.021	0.001
$N_{body\ retained}$[3]	0.16	0.22	0.056	0.001
$R_{protein}$, g/d per goat	17	24	6.1	0.535
R_{fat}, g/d per goat	68	-16	10.3	0.003

[1] 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} = recovered C in milk; $C_{body\ retained}$ = recovered C in tissue; N_{intake} = N intake; N_{feces} = N losses in feces; N_{urine} = N losses in urine; N_{milk} = recovered N in milk; $N_{body\ retained}$ = recovered N in tissue; R = recovered protein or fat.

[2] CON = Control; ORG = orange leaves and rice straw.

[3] $N_{body\ retained}$ = is apparently retained.

Table 7. Daily milk production, composition and metabolites of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet

	Diet[1]			
Item	CON	ORG	SEM	*P*-value
Milk yield, kg/day per goat	2.32	2.06	0.062	0.0278
FCM[2], kg/day per goat	2.82	2.81	0.051	0.3256
Feed efficiency				
Milk yield/DMI	1.05	0.91	0.100	0.2055
FCM/DMI	1.28	1.25	0.025	0.3561
Chemical composition, %				
Fat	5.5	6.5	0.12	0.001
Protein	4.1	4.2	0.05	0.584
Lactose	4.6	4.7	0.03	0.342
Non-fat dry extract	9.5	9.6	0.06	0.212
Cheese extract[3]	9.6	10.7	0.16	0.001
Milk metabolites				
Glutamate, μ*M*	174	146	19.7	0.499
Free amino groups, mEq/L	1.86	1.65	0.070	0.136
Urea, m*M*	6.8	8.3	0.28	0.006
Uric acid, m*M*	30	30	7.7	0.983
Creatinine, m*M*	309	290	21.8	0.668
Glucose 6P, m*M*	192	186	10.6	0.786
Glucose, m*M*	68	54	4.1	0.033

Malate, m*M*	71	53	8.2	0.267
Isocitrate, m*M*	103	140	10.5	0.047
BHB, µM	74	77	1.2	0.043

[1] CON = Control; ORG = orange leaves and rice straw.

[2] FCM = fat corrected milk.

[3] Cheese extract = milk fat + milk protein.

Table 8. Fatty acid (FA) composition (g/100 g of milk fat) of milk fat for goats fed the experimental diets (n=10)

	Diet[2]			
Item[1]	CON	ORG	SEM	*P*-value
C4:0	0.619	0.767	0.0376	0.0462
C6:0	1.507	1.968	0.0970	0.0130
C8:0	2.041	2.705	0.1314	0.0074
C10:0	8.649	9.756	0.3314	0.0954
C11:0	0.338	0.307	0.0164	0.3536
C12:0	5.628	4.687	0.2391	0.0457
C14:0	9.373	9.018	0.3173	0.5900
C14:1	0.217	0.156	0.0150	0.0374
C15:0	0.850	0.645	0.0605	0.0891
C16:0	25.869	23.763	0.5461	0.0222
C16:1	0.764	0.580	0.0500	0.0639
C17:0	0.520	0.317	0.0332	0.0005
C17:1	0.182	0.166	0.0072	0.2719
C18:0	3.199	6.103	0.4004	0.0001
C18:1n9t	0.708	2.380	0.2579	0.0002
C18:1n9c	9.452	14.680	0.7716	0.0001
C18:1n7	0.183	0.531	0.0533	0.0001
C18:2n6t	0.184	0.301	0.0166	0.0001
C18:2n6c	2.537	3.457	0.1894	0.0107
C20:0	0.081	0.139	0.0078	0.0001
C18:3n6	0.014	0.009	0.0019	0.1948
C20:1	0.030	0.032	0.0028	0.7804

C18:3n3	0.430	0.585	0.0320	0.0110
CLA 9c11t	0.501	0.997	0.0742	0.0001
CLA 9t11c	0.051	0.085	0.0050	0.0001
CLA 10t12c	0.003	0.009	0.0008	0.0001
CLA 9c11c	0.008	0.013	0.0009	0.0001
CLA 9t11t	0.062	0.099	0.0069	0.0041
C20:2	0.009	0.011	0.0060	0.3842
C22:0	0.018	0.060	0.0056	0.0001
C20:3n6	0.000	0.004	0.0015	0.1700
C22:1n9	0.008	0.019	0.0081	0.5434
C20:3n3	0.000	0.001	0.0005	0.3084
C20:4n6	0.157	0.130	0.0123	0.2722
C24:0	0.002	0.010	0.0015	0.0040
C20:5n3 EPA	0.025	0.026	0.0029	0.8760
Short-chain FA	4.17	5.44	0.264	0.0115
Medium-chain FA	14.61	14.75	0.455	0.8873
Long-chain FA	55.44	64.33	2.045	0.0253
FA with 16C or fewer[3]	29.22	30.01	0.784	0.7043
FA with 18C or higher	17.66	29.68	2.335	0.0384
Saturated FA	58.70	60.25	1.762	0.6725
Monounsaturated FA	11.55	18.54	1.029	0.0001
Polyunsaturated FA	3.99	5.75	0.307	0.0017
n-6	2.89	3.90	0.202	0.0081
n-3	0.46	0.61	0.032	0.0101
n-6 / n-3 ratio	6.55	6.44	0.312	0.8581
Thrombogenic index	2.60	2.01	0.292	0.0050
Atherogenicity index	4.78	2.85	0.112	0.0001

[1]CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; Thrombogenic index = (C14:0 + C16:0 + C18:0)/[0.5 x mono-unsaturated + 0.5 x n-6 + 3 x n-3 + (n-6/n-3)]; Atherogenicity index = C12:0 + 4 × C14:0 + C16:0/unsaturated fatty acids (Ulbricht and Southgate. 1991).

[2] CON = Control; ORG = orange leaves and rice straw.

[3] According to Chilliard et al. (2003).

Table 9. Methane emission of Murciano-Granadina goats (n = 10) during mid-lactation according to the type of diet

	Diet[2]			
Item[1]	CON	ORG	SEM	*P*-value
CH_4, g/d	30.2	27.2	0.35	0.001
CH_4 /CO_2 in breath	0.07	0.07	0.001	0.638
Ym, %	4.4	4.0	0.07	0.002
CH_4/DMI, g/kg	13.8	12.1	0.22	0.001
CH_4/cheese extract, g/kg	143	130	3.63	0.047
CH_4/milk, g/kg	13.6	14.3	0.48	0.437

[1]Ym = methane energy/gross energy intake.

[2] CON = Control; ORG = orange leaves and rice straw.