Can by-products replace conventional ingredients in concentrate of dairy goat diet?

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ABSTRACT

Intensive dairy goat production in the Mediterranean basin is based on imported conventional ingredients to be included in concentrates. Fourteen Murciano-Granadina goats in the middle of the third lactation were allocated into 2 groups of 7 animals each fed, respectively, a control diet based on alfalfa hay and concentrate in a 40:60 ratio, and a diet in which the concentrate included tomato fruits, citrus pulp, brewer's grain and brewer's yeast (T100CBY) to study the effect of diet on nutrient utilization, ruminal fermentation, purine derivatives excretion in urine, milk yield and composition, and methane emissions. No effect of the diet on total dry matter intake was observed. Digestibility of neutral detergent fiber and acid detergent fiber were higher for T100CBY compared with the control diet. The N in feces and urine was lower and balance and retained N were higher in animals fed T100CBY than the control diet. Milk protein N and energy were similar for both diets. Metabolizable energy per energy intake and metabolizable energy per digestible energy were higher and energy in methane was lower with diet T100CBY than with the control. Milk yield and composition were not affected by diet, with the exception of protein, casein, and total solids, which were higher for diet T100CBY than the control. Diet T100CBY promoted less saturated fatty acids and higher mono- and polyunsaturated fatty acids in milk than the control diet. Diet T100CBY produced less methane and NH₃ concentration in the rumen, higher propionate, and a lower acetate-to-propionate ratio without an effect on the volatile fatty acid concentration. The concentrate with by-products did not affect urinary excretion of total purine derivatives, reduced feeding costs, and

increased profit margin by 14 and 16% compared with the control. The mixture of tomato fruits, citrus pulp, brewer's grain, and brewer's yeast could replace 47% of conventional ingredients (corn, wheat bran, sunflower meal, and soy flour) in the concentrate of the dairy goat diet, reducing feeding cost and methane production, leading to a healthier fatty acids profile in milk without compromising nutrient utilization or milk yield.

Key words: goat, by-product, methane, milk

INTRODUCTION

Dairy goat production is of increasing interest in the Mediterranean basin due to the exponential growth of market demand for milk and dairy products (Haenlein, 2001). Scarcity of pastures, especially during drought periods, have led to more intensive dairy goat production systems and imported conventional ingredients to be included in concentrates. The high and variable prices of conventional ingredients in recent years (FAO, 2011) has driven the attention of ruminant nutritionists toward local alternative resources (Romero-Huelva et al., 2012; Megersa et al., 2013) to reduce production costs (Ben-Salem and Znaidi, 2008) without altering yield and product quality. Additionally, environmental issues associated with both livestock production and by-products accumulation (Greenwood et al., 2012) could be prevented.

In the Mediterranean area, greenhouse horticulture represents about 15% of the world production, Spain being the main producer. This horticulture generates huge amounts of wastes, mainly tomato fruit wastes (about 350,000 t/yr), available during the whole year. About 24% of world production of citrus is concentrated in Mediterranean countries. In addition, most developed countries continuously produce these and other abundant by-products, such as brewer's grain and brewer's yeast. Beer is the fifth most consumed beverage in the world, with an estimated annual world production of about 1.34 billion hectoliters (Bampidis

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and Robinson, 2006). The use of tomato wastes, citrus pulp, brewer's grain, and brewer's yeast for ruminant feeding has been explored (Fegeros et al., 1995; Angulo et al., 2012; Romero-Huelva et al., 2012), but no information exists on the effect of a mixture of those by-products. The replacement of conventional ingredients with a combination of alternative sources of protein and structural carbohydrates and NSC could promote different ruminal fermentation or N and energy balances patterns. The high moisture of by-products is a limitation for their storage, inclusion in diets, and for logistics, which is additionally more expensive. The development of appropriate drying methods could help to overcome that limitation.

Interest has recently increased for dietary strategies that could reduce methane emissions by ruminants (Romero-Huelva et al., 2012; Knapp et al., 2014; Caro et al., 2016). Methane is an important greenhouse gas, which otherwise represents a significant energy loss for the ruminant (2 to 12% of the gross energy intake). Globally, goat milk and meat production are responsible for ~174.5 million tonnes of carbon dioxide equivalents, which are mainly associated with CH₄ and N₂O emissions, respectively, from enteric fermentation and manure management (Zervas and Tsiplakou, 2012; Opio et al., 2013). Goats produce 11.9 million tonnes of milk, of which the associated greenhouse gas emissions are 62.4 million tonnes of CO₂ equivalents (Opio et al., 2013; Marino et al., 2016), representing around the 0.85% of total emissions from domestic ruminants.

Research regarding ruminant nutrition has been focused as well on dietary strategies that could reduce the formation of some SFA and increase the concentration of UFA in ruminant products. As far as we know, few studies have investigated the effect of diets based on the use of by-products on milk fatty acid composition (Molina-Alcaide et al., 2010; Abbeddou et al., 2011, 2015; Romero-Huelva et al., 2012) or methane emissions by ruminants (Moate et al., 2011; Romero-Huelva et al., 2012; Romero-Huelva and Molina-Alcaide, 2013).

Our hypothesis was that a mixture of dried tomato fruits, citrus pulp, brewer's grain, and brewer's yeast could partially replace conventional ingredients in concentrates for dairy goat, decreasing feeding cost and methane emissions and improving the quality of the milk fatty acid profile without compromising ruminal fermentation, nutrients utilization, or milk yield. Thus, the aim of the present work was to study, in dairy goats, the effect of replacing 47% of conventional ingredients in the concentrate with agro-industrial by-products on milk yield and composition, nutrients utilization, rumen fermentation, and methane emissions.

MATERIALS AND METHODS

Animals and Diets

Fourteen Murciano-Granadina dairy goats in the middle of the third lactation were selected and divided into 2 homogeneous groups of 7 goats each based on BW (44.5 ± 3.39 kg of BW), average voluntary feed intake (88.7 g of DM/kg^{0.75}), milk production in previous lactations (471.5 ± 23 kg of milk per 210 ± 30 d of lactation), and milk yield at the beginning of the experiment ($1,472 \pm 123.1$ g of milk/d). Animals were placed in individual boxes.

Each group was randomly assigned to 1 of the following diets, with composition shown in Table 1. The control diet was based on alfalfa hay and a commercial concentrate in a 40:60 ratio; the second diet (**T100CBY**) was based on a 40:60 ratio of alfalfa hay and a concentrate in which 47% of a mixture of corn, wheat bran, sunflower meal, and soy flour (250, 50.0, 120, and 50.1 g/kg of DM, respectively) was replaced with a mixture of tomato fruits, citrus pulp, brewer's grain, and brewer's yeast (250, 100, 70, and 50 g/kg of DM, respectively). Diets were formulated to be isoen-ergetic and isoproteic. By-products were provided by the company Aspero S.A. (Sevilla, Spain). Tomato fruits were cut and dried at 65°C in a convection oven under controlled conditions (ZoitechLab SL, Madrid, Spain). All the other by-products were also dried at 65°C and ground to be included in concentrate pellets.

The specific N and energy requirements of Murciano-Granadina goat breed (Aguilera et al., 1990; Prieto et al., 1990) were considered in the diets formulation. The amount of alfalfa hay and concentrate supplied to the animals were sufficient to allow daily milk production of up to 2 kg/goat. The total amount supplied during the adaptation period was 110.7 g of DM/kg of metabolic BW (BW^{0.75}), with refusals representing 20 to 25% of distributed feeds. The amount supplied when animals were placed in metabolism crates was 106.3 g of DM/kg of BW^{0.75} and the average intake was 88.5 ± 3.02 g of DM/kg of BW^{0.75}.

Animals were cared and handled in accordance with the Spanish guidelines for experimental animal protection (Government of Spain, 2013) in line with the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (European Commission, 2012). All procedures were approved by the Ethic Committee for Animal Experimentation of the Spanish Research Council and Junta de Andalucía (24/05/2016/091 and 22/06/2016/115).

Experimental Procedure

The animals were individually allocated in boxes, fed once a day at 0900 h, had free access to water, and were milked once a day in the morning in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain). After 25 d of adaptation to the corresponding experimental diet, animals were moved to individual metabolism crates. After 3 d of adaptation to metabolism crates, total feces

and urine were collected for 5 d. Individual intakes of diet ingredients were registered throughout the whole trial, calculated from feeds supplied and corresponding refusals. During the sampling period, alfalfa hay and concentrate refusals from each animal were collected and weighted daily, pooled, and stored at −20°C. Urine was collected in buckets with 10% HCl (vol/vol) to maintain pH below 3 and avoid N losses. Aliquots (100 mL) of urine were daily taken for density measurement

Table 1. Ingredients and average chemical composition (g/kg of DM) and price of diet ingredients

Item	Alfalfa hay	Concentrate	Concentrate T100CBY ¹
Ingredient			
Corn	—	250	—
Wheat bran	—	211	161.1
Sunflower meal	—	120	—
Soy hull	—	100	100
Sorghum	—	100	100
Soy flour	—	109	58.9
Wheat	—	52.2	52.2
Tomato	—	—	250
Citrus pulp	—	—	100
Brewer's grains	—	—	70.0
Brewer's yeast	—	—	50.0
Calcium carbonate	—	10.2	10.2
Beet molasses	—	10.0	10.0
Sepiolite ²	—	10.0	10.0
Palm soap	—	12.6	12.6
Sodium chloride	—	5.00	5.00
Vitamin-mineral mixture	—	10.0	10.0
Chemical composition			
DM, g/kg fresh	948	962	956
OM	894	946	929
Ash	106	54	71
CP	161	180	174
NDF	506	299	269
ADF	317	122	125
ADL	69.4	18.5	18.4
Ether extract	13.0	31.1	35.5
Gross energy, MJ/kg of DM	15.8	17.3	15.8
ME, MJ/kg of DM	7.70	10.6	10.4
Fatty acids, g/100 g of identified fatty acids			
C12:0	0.476	ND ³	ND
C14:0	1.29	0.210	0.396
C14:1 <i>cis</i> -9	ND	0.210	0.396
C16:0	32.2	29.0	31.3
C16:1 <i>cis</i> -9	3.42	0.531	1.31
C18:0	5.21	3.77	3.48
C18:1 <i>trans</i> -9	0.401	ND	0.215
C18:1 <i>trans</i> -11	ND	1.04	ND
C18:1 <i>cis</i> -9	2.31	28.2	25.8
C18:1 <i>cis</i> -11	0.625	ND	1.23
C18:2 <i>trans</i> -9, <i>trans</i> -12	ND	0.314	0.273
C18:2 <i>cis</i> -9, <i>cis</i> -12	15.9	36.0	33.1
C20:0	0.987	0.324	0.522
C18:3 <i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12	0.304	ND	0.296
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	33.2	0.329	0.255
C22:0	1.02	ND	0.200
C24:0	1.703	ND	0.234
C26:0	0.904	ND	1.04
Price, €/t	216	222	172

¹T100CBY = diet in which the concentrate included tomato, citrus pulp, and brewer's grain and yeast.

²SEPIOLSA (Polígono Miralcampo Crta. Azuqueca de Henares, Guadalajara, Spain).

³ND = not detected.

and analyses of purine derivative (**PD**) and creatinine concentrations. Aliquots representing 10 and 20% of daily feces and urine, respectively, were taken daily and stored at -20°C for chemical analyses. Every day during the sampling period, milk yield was recorded before feeding. Milk density was measured and aliquots were stored at -30°C without preservatives for the analyses of fat, protein, casein, and TS. Aliquots of milk taken in d 1, 3, and 5 during the sampling period were used for fatty acids (**FA**) content analysis. On d 34, approximately 20 mL of rumen content was collected from each animal before feeding, using a stomach tube attached to a vacuum pump and strained through 2 layers of cheesecloth. The pH was measured and aliquots were taken for $\text{NH}_3\text{-N}$ and VFA analyses.

From d 34, four goats from each dietary treatment were sequentially placed in 4 individual open circuit respiration chambers for 3 d to measure methane emissions. A battery of 4 chambers was used, each measuring 1.8 m wide $\times$ 1.8 m deep $\times$ 1.5 m tall and made of metal frame and polycarbonate. Chamber air temperature was maintained between 10 and 20°C . Methane measurement interruptions occurred daily at 0900 h for diet supply, milking, and chamber floor cleaning. These interruptions had little effect on daily methane emissions, as they lasted for around 10 min for each chamber and occurred when the corresponding exhaust duct was not being sampled. Fluxes were calculated 3 times a day and then averaged to derive the 24-h emission value.

Chemical Analyses

Dry matter (method 934.01), ash (method 942.05), ether extract (method 920.39), and N (method 984.13) content in samples of supplied and refused diet ingredients and feces were analyzed according to AOAC International (2005). Urine was analyzed for N and energy contents. The N values of feeds, refusals, feces, and urine, determined by the Kjeldahl procedure (AOAC International, 2005), were converted to CP multiplying by 6.25. The analyses of NDF and ADF were carried out according to Van Soest et al. (1991) using an Ankom220 Fiber Analyzer unit (Ankom Technology Corp., Macedon, NY), with α -amylase for NDF analysis in concentrate samples and both NDF and ADF were expressed exclusive of residual ash. Lignin was determined by solubilization of cellulose with 72% sulfuric acid. The total ether extract content in feedstuffs and feces was measured by extraction with petroleum ether (boiling point, 40 to 60°C). Energy content of samples was determined by using an adiabatic calorimeter (model 1356, Parr Instruments Co., Moline, IL). Rumen content samples were also analyzed for total and individual

VFA by GC following the method described by Isac et al. (1994). The $\text{NH}_3\text{-N}$ concentration was determined by a colorimetric method (Weatherburn, 1967).

Milk TS content was determined by lyophilization of milk samples. The total N content in milk was determined by the Kjeldahl procedure. Nonprotein N was analyzed in samples of milk filtrates after precipitation with 12% (wt/vol) trichloroacetic acid solution. Noncasein N was determined in milk filtrates after precipitation with 10% (wt/vol) acetic acid at a pH of 4.1 (Recio et al., 1997). Values of N in milk were converted to the corresponding protein by multiplying by 6.38. The milk fat content was measured following the Gerber method (Pearson, 1976).

For methane measurements, air stream in each duct was subsampled, and methane concentration was measured continuously using a Gas analyzer ADC MGA3000 (Spurling Works, Herts, UK).

The concentration of creatinine and PD in urine were determined according to Balcells et al. (1992) using an HPLC, which consisted of a multisolvent delivery system (model 510; Waters, Milford, MA), an injector (Waters 717 plus Autosampler, Waters), a multiwavelength detector (model 481; Lambda-Max, LC Spectrophotometer, Waters, set to 205 nm), and a double 4.0×250 mm C18 ODS-2 analytical column (Waters Spherisorb). The PD were quantified by peak integration using the Waters HPLC systems software Millennium32.

Extraction of total FA in samples of feedstuffs was performed following the recommendations of Devillard et al. (2006). The FAME were prepared according to a standard procedure described previously (Wasowska et al., 2006). For milk FA composition analysis, FA were extracted and transesterified to FAME as described by Abecia et al. (2012). Milk fat methyl esters were separated and quantified using a gas chromatograph (Model Focus GC, Thermo Scientific, Milan, Italy) equipped with a flame-ionization detector and a 100-m fused silica capillary column (0.25 mm i.d., 0.2- μm film thickness; SP-Supelco, Bellefonte, PA) and helium as the carrier gas. Total FAME profile in a 1- μL sample volume at a split ratio of 1:50 was determined using a temperature gradient program (Shingfield et al., 2003). Peaks were routinely identified based on retention time comparisons with authentic FAME standards when available (47885-U, N5252, and O5632: Sigma Aldrich, Madrid, Spain; 20-1813-9: Larodan Fine Chemicals, Malmö, Sweden) and chromatograms reported in the literature (e.g., Shingfield et al., 2003). Identification was validated based on GC-MS analysis of FAME. The mass spectrometer (ITQ 900, Thermo Scientific, Austin, TX) was operated at 225°C in the electron impact ionization mode, and mass spectra were recorded with

70 eV ionization energy using helium as the carrier gas and the same temperature gradient used for routine analysis of FAME (Shingfield et al., 2003). Although the mass spectra of FAME provides limited information concerning FA structure, comparisons with an online reference library of electron impact ionization spectra (<http://lipidlibrary.aocs.org/Analysis/content.cfm?ItemNumber=39469>) and chromatographic retention data allowed the identification of >94% of FAME in all samples.

Calculations and Statistical Analyses

Nutrient apparent digestibilities were calculated from corresponding nutrient intake and losses in feces. Nitrogen and energy balances were obtained taking into account N and energy losses in urine, feces, and milk and energy lost in methane (0.890 MJ/mol of methane; Newbold et al., 2005). The amounts of N and energy excreted into milk were also considered to obtain N and energy balances. Digestible, balance, and retained N were calculated as intake N – fecal N, digestible N – urine N, and N balance – milk total N, respectively.

Milk protein N content was calculated as the difference between total N and NPN in milk. The casein N in milk was calculated as the difference between total N and NCN. The whey N in milk was calculated as the difference between milk protein and casein N. Milk lactose was calculated as the difference between the amount of TS and that of protein, fat, and total ash in milk.

The flux of methane for each chamber was calculated for the last 2 d of the measurement period from the chamber fresh-air intake and exhaust concentrations and means air flow (L/min).

We used SPSS for Windows, version 19.0 (2010; SPSS Inc., Chicago, IL) for data entry and statistical analysis. Data on milk yield and composition were analyzed by the GLM using the repeated-measures ANOVA procedure. The statistical model considered diet as fixed effect, and time and animal as random effects. When a significant effect of diet was found, post hoc comparison of means was made using the least significant difference test: $\alpha_c = 1 - (1 - \alpha)^k$, where α_c is the comparison-wise error rate, α is the significance level, and k is the number of comparisons performed. Covariance structure was the compound symmetric established from the information criteria. One-way ANOVA by using the ANOVA procedure was applied to N and energy balance, apparent digestibility, intake, rumen fermentation, and urine metabolites excretion data. Animal was nested within diet and used as the error term to contrast effects of including by-products in goat diet. When a significant effect of diet was found,

post hoc comparison of means was made using the Tukey test. Differences were considered significant at $P < 0.05$, and $P < 0.10$ values were declared as trends and discussed.

RESULTS AND DISCUSSION

Feed Composition

The concentrate in diet T100CBY had lower OM, CP, NDF, and gross energy but higher ether extract content compared with the control concentrate (Table 1). Differences in diets composition reflected those in the respective concentrates. The most abundant FA in diet ingredients was palmitic acid (32.2, 29.0, and 31.3 g/100 g of identified FA in alfalfa hay, control, and T100CBY concentrates, respectively). Oleic and linoleic acids were also abundant in both concentrates (28.2 and 25.8 and 36.0 and 33.1 g/100 g of identified FA in control and T100CBY concentrates, respectively). The α -linolenic acid (ALNA) was abundant in alfalfa hay (33.2 g/100 g of identified FA).

Intake, Nutrient Apparent Digestibility, and Energy and N Utilization

No differences ($P \geq 0.12$) in total DM and concentrate intakes were observed between diets (Table 2), but a trend ($P = 0.07$) for higher alfalfa hay intake was observed in animals fed diet including by-products. Although DMI was not different ($P = 0.40$) between diets, intakes of OM and NDF were lower ($P < 0.001$) with diet T100CBY compared with control, which is strongly associated with the chemical composition of the offered concentrates. Fat intake was about 7% higher ($P = 0.02$) for T100CBY than for control. The average intakes of diets were in line with those found by Molina-Alcaide et al. (2010) and Romero-Huelva et al. (2012) in Murciano-Granadina dairy goats fed diets including feed blocks containing olive cake and tomato or cucumber fruits, respectively. However, the level of by-products inclusion on a DM basis was higher in the present study, which means higher daily intakes of by-products per animal compared with previous works. Dietary intakes calculated (data not shown) for ALNA, linoleic (LA), oleic, and stearic acids (2.98, 12.0, 8.50, and 1.57 mg/animal per day, on average, respectively) were not affected ($P \geq 0.157$) by diet.

Whereas the apparent digestibility of DM and fat was unaffected ($P \geq 0.09$) by diet, the inclusion of by-products in diet improved ($P \leq 0.001$) the average digestibility of NDF and ADF. Digestibility of NDF and ADF was greater for diets including by-products compared with the control, probably due to the differ-

ent sources of carbohydrates included in both diets. The fiber of by-products has different physical and chemical properties from that of forage; in particular, particles are smaller and higher density (Firkins, 1997). The use of different types of protein sources in the control and T100CBY could promote changes in the synchronization of energy and N supply as well, which is strongly correlated with fiber digestibility and VFA profile. A shift in the site of OM digestion from the rumen to the intestine could also occur. Moreover, animals fed diet T100CBY showed a trend ($P \geq 0.08$) toward higher OM and CP digestibility compared with the control diet. Indeed, it has been demonstrated that supplementation with more than 4 g/head per day of yeast in diets containing tomato pulp could improve OM and CP digestibility (Paryad and Rashidi, 2009). Values obtained in the present work for CP digestibility of diet T100CBY were higher than those reported in previous works of the group (0.81 vs. 0.76) in goats fed diets including multinutrient blocks containing tomato and cucumber fruit wastes (Romero-Huelva et al., 2012).

The N intake per kilogram of BW^{0.75} (Table 3) was lower ($P < 0.001$) for animals fed T100CBY than the control. Milk total and protein N remain unaffected ($P \geq 0.50$) by diet. For animals fed diet T100CBY, digestible N was lower ($P = 0.02$) and retained and N balance were higher ($P < 0.001$) than for animals fed the control diet. The N balances observed in the present study were in the range of values found by others in dairy goats (Ramos-Morales et al., 2010), but different to those found by Molina-Alcaide et al. (2010) and Romero-Huelva et al. (2012) in goats fed diets including olive cake and tomato and cucumber

fruits, respectively. The higher values obtained in the present study for both balance and retained N might be due to an improvement of N utilization when different by-products were combined in diet T100CBY. The replacement of conventional ingredients with a mixture of by-products in the concentrate lead to lower N excretion in urine, thus promoting lower ($P = 0.01$) percentage of milk protein N in relation to the N balance and higher N balance:digestible N, milk protein N:digestible N, and milk protein N:intake N ratios ($P \leq 0.004$). In agreement with these findings, Bequette et al. (1999) stated that the AA profile of different protein sources may have an influence on body retention and milk yield of N. Overall, the inclusion of agro-industrial by-products in the concentrate of the goat diet improved N utilization, promoting lower ($P < 0.01$) N excretion in feces and urine compared with animals fed the control diet, which has important environmental implications (Vasta et al., 2008). These results are in line with those obtained by Paryad and Rashidi (2009), who observed that the use of yeast in the diet of sheep had a positive effect on N utilization of diets including tomato pomace. The inclusion of brewer's yeast in T100CBY diet might improve N utilization not only of tomato fruits, but also of citrus pulp and brewer's grains.

The energy intake was higher ($P < 0.001$) for animals fed the control diet than for those receiving diet T100CBY. Fecal and urine energy were also lower ($P \leq 0.02$) in goats fed the T100CBY diet compared with the control. Digestible energy and energy in methane were higher ($P < 0.05$) for control than the T100CBY diet. Overall, goats fed a diet including by-products had lower energy intakes but also lower losses of energy

Table 2. Average values of intake (g/d) and apparent digestibility (g/g) of nutrients in dairy goats fed the experimental diets

Item	Diet		SEM	P-value
	Control	T100CBY ¹		
Intake				
DM	1,537	1,512	32.6	0.40
Alfalfa hay	626	645	11.7	0.07
Concentrate	911	867	31.8	0.12
OM	1,350	1,263	33.2	<0.001
Fat	36.6	38.9	1.13	0.02
CP	263	254	6.10	0.10
NDF	594	559	12.2	<0.001
ADF	315	316	4.10	0.90
Apparent digestibility				
DM	0.77	0.79	0.015	0.13
OM	0.79	0.81	0.014	0.09
Fat	0.82	0.82	0.013	0.76
CP	0.79	0.81	0.014	0.08
NDF	0.68	0.74	0.021	<0.001
ADF	0.63	0.70	0.027	0.001

¹T100CBY diet in which the concentrate includes tomato, citrus pulp, and brewer's grain and yeast.

in feces, urine, and CH₄, thus promoting a trend ($P = 0.08$) for lower ME values. Consequently, the percentage of ME in relation to both energy intake and digestible energy were affected ($P \leq 0.02$) by diet, the lowest values for the control diet in comparison with T100CBY. In many feed systems, ME efficiency for milk synthesis is positively related to the diet ME-to-DE ratio, but our results do not agree, possibly because of lower losses of energy in urine and methane promoted by diet T100CBY in comparison with the control.

Milk Yield and Composition

Milk yield and composition (Table 4) were not affected ($P \geq 0.17$) by diet, with the exception of protein, casein yields, and TS contents values being higher ($P \leq 0.03$) for T100CBY diet than for the control. Al-

though increased milk fat content is common when dietary fiber intake increases (Chilliard et al., 2003), no differences were found in our study due to the diet, in agreement with the observations of Ibáñez et al. (2016). Higher NDF content in the control diet could be compensated with the higher NDF digestibility found for diet T100CBY. In contrast to our results, Molina-Alcaide et al. (2010) found lower values in milk production of goats fed blocks containing olive cake, without any differences in milk composition. Those differences may rely upon the lower N content and purine derivatives excretion in urine promoted by diets used by Molina-Alcaide et al. (2010). The efficiency of feed utilization was higher ($P = 0.05$) with diet T100CBY compared with the control, showing the potential of by-products included in the problem concentrate to successfully replace conventional ingredients. The suc-

Table 3. Average values of BW, nitrogen, and energy utilization in dairy goats fed the experimental diets

Item	Diet		SEM	P-value
	Control	T100CBY ¹		
BW, kg	43.3	45.7	1.09	0.34
BW ^{0.75}	16.9	17.6	0.33	0.34
g/kg of BW ^{0.75}				
N intake	2.49	2.31	0.056	<0.001
Fecal N	0.519	0.434	0.037	0.01
Urine N	0.951	0.578	0.099	<0.001
Milk total N	0.444	0.462	0.011	0.50
Milk protein N	0.416	0.431	0.011	0.57
Digestible N ²	1.97	1.87	0.048	0.02
N balance ³	1.02	1.30	0.096	<0.001
Retained N ⁴	0.577	0.835	0.095	<0.001
N utilization, %				
Digestible N/intake N	79.1	81.3	1.40	0.08
N balance/digestible N	51.6	69.1	4.96	<0.001
Milk protein N/digestible N	21.2	23.0	0.73	0.004
Milk protein N/N balance	42.9	33.6	3.97	0.01
Milk protein N/milk total N	93.6	93.2	0.41	0.70
Milk protein N/intake N	16.7	18.7	0.65	<0.001
MJ/kg of BW ^{0.75}				
Energy intake	1.52	1.36	0.040	<0.001
Fecal energy	0.359	0.298	0.025	0.005
Urine energy	0.057	0.048	0.004	0.02
Milk energy	0.264	0.250	0.009	0.47
Digestible energy ⁵	1.16	1.06	0.037	0.002
Methane energy ⁶	0.123	0.086	0.009	0.01
ME ⁷	0.984	0.930	0.033	0.08
Energy utilization, %				
Digestible energy/energy intake	76.3	78.2	1.61	0.19
Milk energy/digestible energy	22.8	23.5	0.80	0.34
Milk energy/ME	27.1	26.9	0.98	0.87
ME/energy intake	64.5	68.4	1.89	0.02
ME/digestible energy	84.4	87.4	0.86	<0.001

¹T100CBY diet in which the concentrate includes tomato, citrus pulp, and brewer's grain and yeast.

²Digestible N = intake N – fecal N.

³N balance = digestible N – urine N.

⁴Retained N = N balance – milk total N.

⁵Digestible energy = energy intake – fecal energy.

⁶Methane energy = 0.890 MJ/mol of CH₄ (Newbold et al., 2007).

⁷ME = digestible energy – urine energy – methane energy.

Table 4. Average values of milk production and composition, feed efficiency and cell count in dairy goats fed the experimental diets

Item	Diet		SEM	P-value
	Control	T100CBY ¹		
Milk yield, g/d				
Milk	1,314	1,286	15.9	0.44
Fat	68.5	67.8	0.99	0.74
Protein	44.7	48.2	0.94	0.03
Casein	37.4	42.0	1.10	0.005
Whey protein	7.27	6.22	0.37	0.18
Lactose	59.6	57.7	0.83	0.29
TS	180	180	2.13	0.87
Gross energy, MJ/d	4.44	4.40	0.065	0.76
Milk composition, g/kg				
Fat	52.2	52.7	0.35	0.51
Protein	34.0	37.5	0.80	<0.001
Casein	28.5	32.7	0.97	<0.001
Whey protein	5.53	4.83	0.25	0.17
Lactose	45.4	44.9	0.24	0.36
TS	137	140	0.88	0.01
Gross energy, MJ/kg	3.38	3.42	0.021	0.40
Feed efficiency ²	17.4	18.4	0.60	0.05

¹T100CBY diet in which the concentrate includes tomato, citrus pulp and brewer's grain and yeast.

²Efficiency of feed utilization; feed efficiency = (Milk energy/energy intake) × 100.

cess of the utilization of alternative protein sources in dairy goat feeding depends on the ability to formulate diets balanced in EAA. The results obtained in the present work concerning milk casein and protein composition could be associated with the AA (Mabjeesh et al., 2002) composition of by-products used, specifically that of brewer's yeast.

The effect of diets including by-products on milk FA profile depends on energy value, FA composition, and fiber content in by-products (Vasta et al., 2008). Nevertheless, few studies have investigated the potential of individual by-products used in the current study, such as tomato fruits (Romero-Huelva et al., 2012), citrus pulp (Solomon et al., 2000), and brewer's grain (Miyazawa et al., 2007) and brewer's yeast on milk FA composition in ruminants.

Total SFA were lower ($P = 0.030$) in milk from goats fed diet T100CBY compared with the control (Table 5). The same effect of diet was observed for the stearic acid ($P < 0.001$). A decrease of SFA and *trans*-FA in the human diet has been recommended (Lovegrove and Hobbs, 2016; Wang et al., 2016), although information on the association between SFA and coronary heart disease is contradictory (Parodi, 2009). Among SFA in milk, the branched-chain FA (BCFA) C15:0 *iso* and C17:0 *iso* were not affected ($P \geq 0.732$) by diet, but C18:0 *anteiso* was higher ($P = 0.021$) in milk from goats fed diet T100CBY than the control. The BCFA have been suggested as potential biomarkers of duodenal flow of microbial biomass (Vlaeminck et al., 2015). In this sense the results found in the present

study regarding the lack of effect of the diet on both BCFA and the urinary excretion of PD, an index of the synthesis of microbial protein in the rumen, seem to support that fact.

Total MUFA and PUFA were higher ($P \leq 0.044$) in milk from animals receiving diet T100CBY in comparison with the control. Regarding individual MUFA, the effect of diet was only found for oleic ($P = 0.010$) and vaccenic ($P = 0.022$) acids, which were higher with diet T100CBY compared with the control. Among PUFA a clear effect of diet was found for gamma LNA and ALNA, which were higher ($P < 0.001$) in milk from goats fed diet T100CBY compared with the control. Although differences were not significant quantitatively higher amount of rumenic acid (0.432 and 0.585 g/100 g of identified FA) was observed in milk from animals fed the control and diet including by-products, respectively.

Identified *trans* isomers of C18:1 (C18:1 *trans*-9 and C18:1 *trans*-11) were not affected ($P = 0.771$ and 0.552, respectively) by diet. On the contrary, *cis* isomers (C18:1 *cis*-9 and C18:1 *cis*-11) were higher ($P = 0.010$ and 0.022, respectively) in milk from goats fed diet T100CBY compared with the control. The ALNA-to-LA ratio was 0.05 and 0.134, respectively, for milk from goats fed diets control and T100BCY. Taken into account the origin of FA in milk, only the *de novo* synthesis with diet control was higher ($P = 0.023$) than with diet T100CBY. The $\Sigma n3$ -to- $\Sigma n6$ ratio, which is considered to be of great importance for human health (Simopoulos, 2002), was higher ($P = 0.047$) in milk

from goats fed diet T100CBY than with the control. The atherogenicity index was lower ($P = 0.003$) in milk from animals fed diet T100CBY compared with the control.

In addition to the FA supply, other factors such as energy supply (Leiber et al., 2005) or the presence of plant secondary compounds (Vasta et al., 2009) could determine dietary effects on milk FA profile. As the FA supply seems to be very similar with both diets, and energy intake was slightly lower with diet T100CBY than with the control, the effect on milk FA composition might be due to both, the inhibition of biohydrogenation by dietary components and different energy availability. The higher accumulation of LA and ALNA in the milk of goats fed the diet T100CBY could be associated with changes in the ruminal ecosystem due

to energy availability or to the presence of specific secondary plant compounds in the by-products included (Leiber et al., 2005). It could be speculated that tomato seeds were partially crushed during the manufacture of the concentrate, making a fraction of its FA accessible to biohydrogenating bacteria, leading to accumulation of biohydrogenation intermediates, whereas another FA fraction could remain protected, making higher amounts of PUFA from diet available in the small intestine for absorption.

Because LA, ALNA, and CLA are considered healthy for consumers (Kromhout et al., 2010), the inclusion of tomato, citrus pulp, brewer's grain, and brewer's yeast in the goat diet could be an option to improve the nutritional quality of goats milk and dairy products. Although those changes would not have an effect on

Table 5. Average values of fatty acids (FA) composition (g/100 g of identified FA) of milk fat from dairy goats fed the experimental diets

Item	Diet		SEM	P-value
	Control	T100CBY ¹		
C4:0	1.94	1.77	0.091	0.370
C6:0	1.46	1.97	0.150	0.100
C8:0	2.61	2.91	0.092	0.100
C10:0	13.0	10.8	0.603	0.081
C12:0	4.70	4.44	0.091	0.162
C14:0	11.3	10.9	0.260	0.213
C15:0 <i>iso</i>	0.166	0.162	0.011	0.732
C16:0	33.5	33.5	0.971	0.990
C17:0 <i>iso</i>	0.231	0.264	0.021	0.532
C18:0	8.04	6.61	0.270	<0.001
C18:0 <i>anteiso</i>	0.130	0.224	0.031	0.021
C20:0	0.06	0.101	0.023	0.221
Total SFA	77.1	73.6	0.842	0.030
C14:1 <i>cis</i> -9	0.212	0.196	0.010	0.373
C16:1 <i>cis</i> -9	0.609	0.749	0.121	0.892
C16:1 <i>trans</i> -9 <i>iso</i>	0.377	0.399	0.024	0.560
C18:1 <i>cis</i> -9 ²	17.1	20.1	0.62	0.010
C18:1 <i>trans</i> -9	0.314	0.303	0.032	0.771
C18:1 <i>cis</i> -11	0.023	0.145	0.032	0.022
C18:1 <i>trans</i> -11	1.03	0.866	0.131	0.552
Total MUFA	19.7	22.8	0.743	0.044
C18:2 <i>cis</i> -9, <i>cis</i> -12	2.06	2.32	0.080	0.123
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.432	0.585	0.053	0.190
C18:3 <i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12	ND ³	0.083	0.032	0.025
C18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.118	0.232	0.043	<0.001
C20:4 <i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -9, <i>cis</i> -14	0.152	0.194	0.020	0.243
C20:5 <i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17	0.150	0.190	0.012	0.324
Total PUFA	2.91	3.60	0.13	<0.001
According to the origin ⁴				
<16 carbon	35.4	33.1	0.532	0.023
16 carbon	34.5	34.6	0.943	0.982
>16 carbon	29.8	32.2	0.762	0.123
$\Sigma n-3/\Sigma n-6$	0.121	0.162	0.0210	0.047
Atherogenicity index ⁵	3.71	3.09	0.191	0.100

¹T100CBY diet in which the concentrate includes tomato, citrus pulp and brewer's grain.

²Contains C18:1 *trans*-12 and C18:1 *cis*-10 as minor components.

³ND = not detected.

⁴<16-carbon FA represent de novo synthesized FA, >16-carbon FA represent preformed FA taken up from circulation, and 16-carbon FA are derived from both sources.

⁵Calculated as $C12:0 + 4 \times C14:0 + C16:0/UFA$.

Table 6. Average values of methane emissions, pH, NH₃-N, and total and VFA concentrations, individual VFA molar proportions, VFA, and acetate-to-propionate ratios in the rumen of dairy goats fed the experimental diets

Item	Diet		SEM	P-value
	Control	T100CBY ¹		
Methane emissions				
CH ₄ , L/d	52.5	38.3	3.68	0.03
CH ₄ , L/kg of DMI	34.5	25.5	2.38	0.03
CH ₄ , L/kg of BW ^{0.75}	3.11	2.18	0.23	0.01
pH	7.27	7.17	0.14	0.77
NH ₃ -N, mg/100 mL	39.2	24.4	3.44	0.002
Total VFA, mmol/L	75.4	78.3	3.96	0.76
mmol/100 mmol				
Acetate	66.4	69.4	0.84	0.07
Propionate	10.4	13.1	0.66	0.01
Isobutyrate	2.49	2.21	0.20	0.56
Butyrate	15.7	11.6	1.15	0.07
Isovalerate	3.46	2.32	0.33	0.07
Valerate	1.48	1.33	0.06	0.27
Acetate:propionate	6.36	5.31	0.28	0.03

¹T100CBY diet in which the concentrate includes tomato, citrus pulp, and brewer's grain and yeast.

human health given average daily intake, data are still lacking to establish human requirements for CLA and other healthy FA (Siurana and Calsamiglia, 2016). Furthermore, the increasing demand of high-quality and healthy products for human consumption could give an added value to the milk of goats fed diet T100CBY due to their healthier FA profile. Milk from organic farms or with healthy benefits could raise prices up to 30% higher than regular milk in the current market.

Ruminal Fermentation and Methane Production

No effect of diet ($P \geq 0.76$) on either pH or total VFA concentration was observed (Table 6). Total VFA concentration in the rumen of goats was lower than values previously reported for Murciano-Granadina goats fed diets based on alfalfa hay, tomato, and cucumber by-products (Romero-Huelva et al., 2012). Lower VFA and higher pH values have been found in goats when rumen content was obtained using stomach tubing, as in the present work, compared with values obtained from rumen-cannulated animals (Ramos-Morales et al., 2014). Whereas the molar proportion of propionate was lower, the acetate-to-propionate ratio was higher ($P \leq 0.03$) in animals fed the control diet compared with T100CBY. The control diet tended to decrease ($P = 0.07$) the molar proportion of acetate and to increase isovalerate molar proportions. Although higher fiber digestibility found in animals fed diet T100CBY could increase H₂, acetate proportion and methane production in the rumen depend on other factors such as plant secondary compounds. The plant secondary compounds present in by-products may promote less methane and increased propionate molar proportion, which can be

a sink of H₂ acting as natural safe antimethanogenic agent, as suggested by Patra and Saxena (2010). Goats fed diet T100CBY had lower ($P = 0.002$) rumen NH₃-N concentration than those fed the control diet, which could promote a lower synthesis of microbial protein in the rumen as indicated by the lower urinary excretion of PD found in goats fed diet T100CBY compared with the control. On the other hand, the control diet promoted higher ($P \leq 0.002$) energy losses in urine than T100CBY diet, indicating that increased amount of NH₃-N in rumen could increase the energy losses in urine. These ruminal NH₃-N values were within the range of those found in previous studies with goats fed similar diets (Molina-Alcaide et al., 2000).

International markets increasingly require certification of the carbon footprint of imported dairy products. Thus, practical dietary strategies that reduce methane production in the rumen of dairy goats could contribute to farmers achieving such certification. Little information exists regarding the effect of diets based on agro-industrial by-products on methane emissions in ruminants. In the present study the inclusion of by-products in dairy goat diets had an antimethanogenic effect. Thus, compared with the control diet, methane emissions expressed per day, kilogram of DMI, or BW^{0.75} decreased ($P \leq 0.03$) by about 27, 26, and 29%, respectively, in animals receiving T100CBY. The reduction of methane was associated with increased ($P = 0.01$) propionate molar proportions in the rumen, as propionate is a sink of H₂ alternative to methane (McAllister and Newbold, 2008). This effect was also observed with diets including other by-products (Romero-Huelva et al., 2012) and may rely upon chemical composition of the diet. Secondary compounds present in T100CBY

Table 7. Average values of creatinine and purine derivatives in urine and profitability in dairy goats fed the experimental diets

Item	Diet		SEM	P-value
	Control	T100CBY ¹		
Creatinine, $\mu\text{mol/kg}^{0.75}$	261	387	35.2	<0.001
Purine derivatives in urine, $\mu\text{mol/kg}^{0.75}$				
Allantoin	649	584	47.2	0.13
Xanthine	5.05	4.31	0.99	0.41
Hypoxanthine	22.1	37.1	5.20	0.001
Uric acid	154	166	13.0	0.28
Total	829	792	58.0	0.48
Profitability				
Milk value, €/d	0.73	0.75	0.010	0.40
Diet cost, €/d	0.35	0.30	0.011	0.001
Benefit, €/d	0.38	0.45	0.017	0.03

¹T100CBY diet in which the concentrate includes tomato, citrus pulp, and brewer's grain and yeast.

diet including by-products might act as a natural safe antimethanogenic agents, alternative to the chemical agents suggested by Patra and Saxena (2010). Similar values to those observed in the present work for methane emissions using respiration chambers were also found in goats fed brewer's grain (23.7 L/kg of DMI) with proven antimethanogenic activity (Moate et al., 2011). An additional advantage of the strategy used in the present study to decrease methane production, replacing 47% of conventional ingredients in the concentrate with tomato and by-products from the juice and beer industry, over other antimethanogenic strategies is the lack of detrimental effect on DMI observed in the present study (Beauchemin et al., 2008).

Goats fed diet T100CBY showed higher ($P < 0.001$) creatinine and hypoxanthine in urine (Table 7). The urinary excretion of total PD was similar ($P = 0.72$) for both diets, with values being in the range of those obtained by Mota et al. (2008) in Murciano-Granadina goats fed alfalfa plus concentrate (745 μmol of PD/kg of $\text{BW}^{0.75}$). Urinary excretion of PD relies not only upon the synchronized supply of nitrogen and energy to rumen microorganisms (Bach et al., 2005), but also on other factors such as dietary energy and nitrogen quality, which could influence digestibility, N, and energy utilization. Although low availability of protein and nutritive value have been reported for tomatoes by Ventura et al. (2009), other authors (Paryad and Rashidi, 2009) showed that *Saccharomyces cerevisiae* added at 4 g/head per day improved digestibility and nitrogen retention in rams fed tomato pomace, possibly due to an improved supply of N to the rumen microorganisms.

Considering the relative amounts of cereals and other conventional ingredients included in the control and T100CBY concentrates, as well as the average intakes and the current cost of diet ingredients in Spain (Table

1), a reduction of about 14.3% in feeding ($P = 0.001$) cost may be achieved by using diet T100CBY, which is in agreement with other authors (Molina-Alcaide et al., 2010; Romero-Huelva et al., 2012). The absence of changes in milk cost ($P = 0.40$) together with reduced feeding cost made T100CBY diet a very profitable choice for farmers, being about 16% more profitable ($P = 0.03$) than the control diet.

CONCLUSIONS

The replacement of 47% of conventional ingredients in a concentrate for lactating goats with a mixture of tomato fruits, citrus pulp, brewer's grain, and brewer's yeast reduced animal feeding cost and methane emissions and improved the quality of FA profile in milk. Ruminal fermentation, nutrient digestibility, and milk yield were not compromised by the inclusion of by-products in the diet. In addition, the use of by-products in dairy goats can contribute to minimize the negative environmental impact associated with N in urine and feces from animals and to increase the farm profitability.

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