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5 Nutritive evaluation and milk quality of including of tomato or olive by-

6 products silages with sunflower oil in the diet of dairy goats

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ABSTRACT

There are worldwide a number of by-products with a strong potential for use in animal feeding to cover purposes as farm economic profitability enhancement or searching of functional food ingredients. Probably fruit and vegetable industry (i.e. olive milling wastes) are amongst the most representative sectors. Two experiments were performed with lactating Murciano-Granadina goats. In Experiment 1 the objective was to assess the effect of the partial replacement of the forage in the diet with olive by-products or tomato silages supplemented with sunflower oil (SFO, 20 g/kg DM) on the nutrient utilization, rumen fermentation parameters and microbial abundances, methane production and milk yield and composition. Eighteen goats were distributed in three experimental groups and fed control diet (CD) against diets containing olive by-products silage (OBSD) or tomato surplus silage (TSD). Both OBSD and TSD resulted in higher fat intake and apparent digestibility, higher digestible and metabolizable energy and total purine derivatives excretion. The rest of measured parameters remained unaffected. Diets OBSD and TSD promoted higher milk content in C18:1 trans-11, C20:2 and CLA. Milk fat of goats consuming TSD had the lowest content in C4:0, C6:0 and C8:0, while OBSD produced more total saturated FA (SFA) and less C18:1 n-9 cis and both total mono- and polyunsaturated FA. The acetate concentration was lower in the rumen of animals fed silages, while the butyrate and protozoa concentrations were higher in OBSD. The CH₄ production (L/kg DMI) tended to be lower in goats consuming TSD. Experiment 2 was conducted to test the persistence (over 90 days) and in a larger number of animals (n=24) of the effects observed with TSD diet in experiment 1. The TS diet promoted higher DM intake and body weight and lower acetate:propionate ratio than CD, whereas milk yield and composition were not affected. The FA profile was modified by increases in SFA and reducing monounsaturated FA and CLA. It may be concluded that the strategy of partial replacement of conventional forage by local by-products silage combined

BY-PRODUCTS SILAGES FOR FEEDING DAIRY GOATS

with SFO supplementation is valuable nutritional strategy in dairy goats since it may improve the animal energy balance while ruminal fermentation, nutrients utilization and milk yield are not compromised.

Keywords: by-products, silage, sunflower oil, goat milk, milk fatty acids

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; ANOVA, analysis of variance; BW, body weight; CD, control diet; CD2, control diet in experiment 2; CLA, conjugated linoleic acid; CP, crude protein; DM, dry matter; DMI, dry matter intake; FA, fatty acids; FB, feed-blocks; GC, gas chromatography; LA, linoleic acid; LNA, linolenic acid; MFA, monounsaturated FA; N, nitrogen; NDF, neutral detergent fibre; OBS, olive oil by-products silage; OBSD, containing olive by-products silage diet; OM, organic matter; PD, purine derivatives; PUFA, polyunsaturated FA; RA, rumenic acid; SCC, somatic cells counts; SEM, standard error of the mean; SFA, saturated FA; SFO, sunflower oil; TMR, totally mixed ration; TS, Tomato surplus silage; TSD, containing tomato surplus silage diet; TSD2, tomato surplus silage diet in experiment 2.; UFA, unsaturated FA.; VA, vaccenic acid; VFA, volatile FA

1. Introduction

The inclusion of agro-industrial by-products in ruminants diets may be of interest for reducing feeding costs and diminishing environmental impacts associated with by-products accumulation (Vasta et al., 2008). The Mediterranean countries produce 98% of olive oil worldwide (Sesli et al., 2009) and the resulting by-products (such as olive cake and leaves) account for 80 and 5%, respectively, of the total weight of processed olives (Delgado-Pertíñez et al., 1994). With an estimated worldwide production over 150 million tonnes in 2011 (FAO, 2013), tomato is the product of major importance among fruits and vegetables, which near 10% are wasted only in the distribution stage of the food supply chain (FAO, 2011). The high moisture content in those by-products is one of the greatest challenges for their practical use because it may cause storage problems and affect the environment as a result of their rapid deterioration. Ensiling is an adequate and cost-effective technology to preserve high moisture feedstuffs, that in addition could enhance their nutritional value and increase the palatability (Meneses et al., 2007). However including by-products in the diet could result in lower metabolizable energy intake (Molina-Alcaide et al., 2010). The use of oils as additional source of energy could overcome this problem. Sunflower oil (SFO) is rich in linoleic and linolenic acid and promotes the conjugated linoleic acid (CLA) production (Bu et al., 2007). Despite several studies have investigated the effect of including in various forms olive by-products (Hadjipanayiotou, 1999; Chiofalo et al., 2004; Molina-Alcaide et al., 2010; Abbeddou et al., 2015) or tomato wastes (Ben Salem and Znaidi, 2008; Romero-Huelva et al., 2012 and 2017) in the diet of ruminants, to our knowledge no research has been conducted feeding dairy goats with silages made from a mixture of olive cake and olive leaves or tomatoes in combination with SFO.

Our hypothesis is that silages made with tomato surplus or olive by-products in combination with sunflower oil could be included in the diet of dairy goats' without

compromising rumen fermentation and milk production and potentially improving the fatty acids (FA) profile of milk. Therefore, the objective of the present work was to study the nutrient utilization, ruminal fermentation, microbial abundances, methane emissions, and milk yield and composition in dairy goats fed diets including tomato or olive by-products silages and supplemented with SFO. A second experiment was conducted to evaluate the long-term effects of the use of the most promising by-products on animal performance and milk yield and quality.

2. Materials and methods

Two *in vivo* experiments were performed using lactating Murciano-Granadina goats. Experiment 1 studied the effect of replacing oats hay, as forage constituent of the diet, by either tomato surplus or olive by-products silages, in combination with SFO, on nutrient utilization, rumen fermentation parameters and microbial abundances, methane production and milk yield and composition. On the basis of the results obtained in experiment 1, a longer term (90 days) *in vivo* trial (Experiment 2) was carried out using the most favourable strategy to study whether the observed effects persisted over that period of time. In both experiments goats had permanent access to fresh water and mineral blocks and were fed twice a day (9:00 h and 18:00 h) an amount of experimental diets to allow daily milk production of around to 2 kg per goat. Animals were milked twice a day at 8:00 and 17:00 h in a milking parlour.

Animals were cared and handled in accordance with the Spanish guidelines for experimental animal protection (Royal Decree 53/2013 on the protection of animals used for experimentation or other scientific purposes) in line to corresponding European Directive (2010/63/EU). The corresponding experimental protocol was approved by the Ethics and Animal Welfare Committee of the Estación Experimental del Zaidín (Spanish National

Research Council) and authorized by the Andalusian regional Government (SSA/Sl/tttD/ps, May 2012).

2.1. Experiment 1

2.1.1 Animals, by-products and diets

On day 60 of lactation eighteen lactating goats (50.0 ± 6.6 kg, 2.9 ± 0.4 parity) were placed in individual pens ($1.2 \text{ m} \times 1.2 \text{ m}$).

Tomato (*Solanum lycopersium* L.) fruits, as rejected unmarketable pieces, were collected at the horticulture vegetables distributor company Fulgencio Spa S.L. (Motril, Spain). Olive oil by-products (olive leaves and crude olive cake) were obtained from the olive oil secondary extraction factory Sierra Sur S.A. (Granada, Spain). Silages were prepared by weighing and mixing the ingredients and then were pressed and wrapped with four to six layers of "bale wrap plastic" ($25 \mu\text{m}$ stretch film). This was performed using a bale wrapper machine with front-loader (Vicon RF 135 Balepack 3D Opticut 23, Brazil). The cylindrical bales were of 1.2 m tall $\times$ 0.90 m diameter and weighed approximately 850 kg . Tomato surplus silage (TS) contained (fresh matter) 850 g/kg tomato surplus and 150 g/kg barley straw. Olive oil by-products silage (OBS) contained olive leaves, crude olive cake (from olive oil extraction process) and barley flour, in the proportion 2:1:1 (fresh matter). Formic acid was added (0.45% of fresh matter) to both silages to enhance the drop in pH. Bales were opened after 70 days of ensiling and pH was measured. The TS pH was 3.91 ± 0.76 and OBS pH was 5.23 ± 0.1 . When opened, the colour, aroma and the lack of mould were checked as indication of the quality of the ensiling process. The dry matter contents were 315 ± 15 and $508 \pm 25 \text{ g/kg}$ of fresh matter for tomato and olive by-products silage, respectively.

Three experimental diets were formulated as follows (Table 1): **Control** diet: Total Mixed Ration (TMR), based on grains mixture plus chopped alfalfa (*Medicago sativa*) hay,

oats hay (*Avena sativa*) and glycerin (58:20:20:2, CD); olive by-products diet (**OBSD**): equivalent to CD but oats hay was replaced by OBS and glycerine by SFO; tomato surplus diet (**TSD**): oats hay was replaced by TS and glycerine by SFO. Proportions are expressed in dry matter basis.

2.1.2. Experimental procedure

Goats were assigned to three homogeneous experimental groups (n=6 each) according to the feed intake and the milk yield exhibited previously to the experiment start. After a 21 days adaptation period, on days 22 to 25 (Fig 1), feed intake and milk production were daily recorded. Milk samples were collected in the morning and afternoon milking throughout the 4-days period. A first aliquot (40 mL) for milk component analysis was collected, preserved with azidiol and stored at 4°C until analysis the following day, and a second aliquot (50 mL) was frozen and stored at -20°C for FA analyses. Feed refusals were also sampled, homogenised and stored at -20°C until the corresponding analysis to calculate the daily feed nutrients intake. On day 25, goats were allocated in metabolic cages for 4 days (days 25-29). Daily refusals and milk production from each animal were weighed and 30% and 5%, respectively, were collected, frozen and stored at -20°C until subsequent chemical composition analysis for N and energy balance calculations. Urine was collected in buckets with 10 % H₂SO₄ (vol/vol) and then frozen and stored at -20°C. Urine aliquots (100 mL) were taken for density measurement and analysis of purine derivatives (PD) and creatinine concentrations. Aliquots representing 10% and 20% of daily faeces and urine production, respectively, were mixed and stored at -20°C for chemical analyses. On day 29, approximately 50 mL of rumen content was collected from each animal before the morning feeding, using an oral probe attached to a vacuum pump and strained through a nylon membrane (400 µm; Fisher Scientific S.L., Madrid, Spain) as described in Ramos-Morales et

al. (2014). The pH was measured and aliquots were taken and stored at -20°C for volatile fatty acids (VFA) or at -80 °C for molecular analysis.

From day 29 to 31 animals were allocated in individual open-circuit respiration chambers for enteric methane emission measurement following the procedure described by Martínez-Fernández et al. (2014). During periods when the animals were placed either in metabolic cages or in respiration chambers they were milked using a mobile milking unit (28010, Flaco -Geräte GmbH, Gütersloh, Germany).

2.2. Experiment 2

2.2.1. Animals, by-products and diets

Twenty four lactating goats (44.8±10.7 kg; 3.5±0.3 parity), 30 days in milk, were distributed in two groups according to body weight (BW) and milk production (n=12, for each group) and fed two experimental TMR diets (Table 6): A control diet similar to CD in Experiment 1 but with a different mixture of grains (**CD2**); and a tomato silage based diet (**TSD2**) equivalent to CD2 but where oats hay was substituted by TS. Both CD2 and TSD2 resulted in a 58:19:3 proportion of concentrate:forage:glycerine.

2.2.2. Experimental procedure

During the 55 days adaptation period (Fig 2) both animal groups were kept separated from each other in 15 m × 4 m pens. During two periods of five days (from day 56 to 60 and 86 to 90) animals were individually housed and BW, feed intake and milk yield were daily recorded. Aliquots of milk, feed refusals and diets were collected for chemical analyses as indicated for experiment 1. During the period in between measurements (from day 61 to 85) animals were fed and handled in the same manner as in the adaptation period. On day 90, approximately 50 mL of rumen content was collected from each animal before morning feeding, using an oral probe as for Experiment 1. The pH was measured and aliquots were taken and stored at -20°C for VFA analysis.

2.3. *Chemical analyses*

Dry matter (DM) (method 934.01) and ash (OM) (method 942.05) in samples of supplied and refused diet, faeces, urine and milk were determined according to AOAC (2005). The nitrogen (N) values of feeds, refusals, freeze-dried faeces (AOAC method 990.03), and urine (AOAC 993.13) were determined by Dumas method (Leco TruSpec CN®, St. Joseph, Michigan, USA) and, when required, converted to crude protein (CP) by multiplying by 6.25. The diets, refusals and freeze-dried faeces analyses of neutral detergent fiber (aNDFom-NDF) and acid detergent fiber (aADFom-ADF) were carried out according to Van Soest et al. (1991) using an Ankom 220® fiber analyser unit (Ankom Technology Corp., Macedon, New York, USA), with α -amylase for NDF analysis in concentrate samples, and both NDF and ADF were expressed exclusive of residual ash. Lignin (ADL) was determined by solubilisation of cellulose with 72% sulfuric acid. The fat content in feedstuffs and freeze-dried faeces was measured by extraction with petroleum ether (AOAC method 920.39). The gross energy content of diets, refusals, faeces and freeze-dried urine and milk samples was determined by using an adiabatic calorimeter (Parr Instruments Co. model 1356, Moline, Illinois, USA).

The total N content in freeze dried milk samples was also determined by Dumas method (AOAC method 990.03). Non-protein N was analysed in samples of milk filtrates after precipitation with 15 % (wt/vol) trichloroacetic acid solution. Values of N in milk were converted to the corresponding protein by multiplying by 6.38. Milk samples treated with Azidiol were analysed for milk fat, protein, lactose, total solids and somatic cells counts (SCC) by near infrared analysis, using the CombiScope™ FTIR 600 Dairy analyzer (Delta Instruments, Drachten, Netherlands) in the Laboratory of Animal Production and Health of the Junta de Andalucía (Andalusian regional govern, Spain). Additional unpreserved milk samples were stored at -20°C prior FA analysis.

Rumen content samples were analysed for total and individual VFA as follows. One mL of strained rumen fluid was added to 1 mL of an acidic solution (0.5 N HCl, 20 % wt/vol metaphosphoric acid) containing crotonic acid (0.8 g/L, internal standard) and, after standing for 30 min on ice, centrifuged at 2700xg for 20 min. The supernatant was stored at -20°C in gas chromatography (GC) vials. The concentration of individual VFA was determined by a GC system coupled with a Flame Ionization Detector (Autosystem Perkin-Elmer Cor., Norwalk, Connecticut, USA) using a cross-linked 100 % polyethylene glycol column (TRB-FFAP 30 m × 0.53 mm i.d. × 1 µm film thickness, Teknokroma, Barcelona, Spain). One µL of sample was injected, in split mode (20 mL/min). Nitrogen (1 mL/min) was used as carrier gas, and the injector and the detector temperature were 260°C and 275 °C, respectively. The column temperature was held at 100 °C for 1 minute, increased at 15 °C/min up to 160 °C, remaining at this point 1 minute. Identification of VFA peaks was based on retention time of external standards, using crotonic acid as internal standard for quantification. Standard curves were prepared by dilution (1:1) of standard's mixture in the same solution in which the rumen content samples were added.

The PD and creatinine concentration in urine were determined according to Balcells et al. (1992) using a HPLC system, which consisted of a multisolvent delivery system (model 5110; Hitachi Chromaster, Japan), equipped with autosampler (model 5210). A double 4.0 × 250 mm C18 ODS-2 analytical column (Waters Spherisorb®, Ireland) was used for separation, and detection was made with a Diode Array Detector (model 5430, Hitachi Chromaster, Japan), in a wavelength range from 190 to 250 nm, set at 205 nm for quantification. The software EZChrom Elite (Agilent Technologies, version 3.3.2) was used for system control and for identification and quantification of PD, performing calibration curves (area vs amount) for each derivative and using Allopurinol (50 µM) as internal standard.

Feed samples and a freeze-dried aliquot of each milk sample stored at -20°C were used for gas GC of FA in the Research, Technology and Innovation Centre of the University of Seville (Spain). Separation, identification and quantification of FA methyl esters were performed as described by Delgado-Pertíñez et al. (2013). Identification of the CLA isomers was done by comparing retention times with those of another authenticated standard mix (Sigma Chemical Co. Ltd., Pool, UK). Fatty acids content was expressed as the percentage of total methyl esters identified.

Samples of rumen content for molecular analyses were freeze-dried, the DNA extraction was performed from 50 mg following Yu and Morrison (2004) method. DNA was used as a template to quantify the copy numbers of 16S rRNA gene (for bacteria), the mcrA gene (for methanogenic archaea) and 18S rRNA gene (for protozoa) by real-time PCR as described by Abecia et al. (2012).

2.4. Calculations and statistical analyses

Nutrient apparent digestibility was determined from corresponding intakes and losses in faeces. Nitrogen and energy balances were obtained, taking into account N and gross energy losses in urine, faeces, and milk, and energy lost in methane (0.890 MJ/mol of methane) (Newbold et al., 2007). Milk protein N content was calculated as the difference between total and non-protein N in milk.

The SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, New York, USA) was used for statistical analysis. In Experiment 1, the effect of diet on intake and milk yield and composition was analysed by general linear model (GLM) repeated measures. The statistical model included the fixed effects of diet, time, their interaction, and the corresponding values of parameter for each animal measured previously to the start of the experiment (covariate, data not shown). The BW data of animals during their metabolic cages housing period was analysed by a univariate model using as a

covariate initial record before the experiment start (data not shown). Apparent digestibility of nutrients, N and energy utilization, feed efficiency and urine excretion of PD, methane emissions, average values for pH, total and individual VFA, and microbial abundance in the rumen data were analysed by 1-way ANOVA.

Effect of diet on BW, intake, milk production and composition in Experiment 2 was analysed by a repeated measures including the fixed effect of diet, the random effect of sampling period (days 56 to 60 and 86 to 90), their interaction, and corresponding data recorded before the start of the experiment (covariate, data not shown). The values for pH, total concentration and individual proportions of VFA in the rumen were analysed by 1-way ANOVA.

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. Differences were considered significant at $P < 0.05$, and $P < 0.10$ values were declared as trends.

3. Results

3.1. Experiment 1

3.1.1. Feed composition

Ingredients and chemical composition of the experimental diets are shown in Table 1. The DM content in diets OBSD and TSD was lower than in CD due to the contribution of silages. Compared to CD, diets containing by-products had higher ether extract, due to the SFO addition, and lower crude protein, while the OBSD had the highest content of lignin. The gross energy content was rather similar among experimental diets.

3.1.2. Intake, nutrient apparent digestibility, and milk yield and composition

After the adaptation period, dry matter intake (DMI) (Table 2) did not differ among groups ($P = 0.772$). Only fat digestibility differed among diets ($P < 0.001$), being greater for OBSD and TSD than for CD.

Apart from the lower fat and total solids content in milk with CD ($P = 0.024$ and $P = 0.049$), no significant differences resulted for milk yield and milk nutrients composition. No differences in the SCC were observed among diets, ranging from 1,913 to $3,131 \times 10^3 \text{ mL}^{-1}$, respectively, for OBSD and CD.

The most abundant individual milk FA (Table 3) was palmitic followed by oleic, stearic, capric, myristic, and lauric acids. The milk FA profile was modified in TSD and OBSD, increasing significantly the proportions of C18:1 trans11 (VA, +188%), C18:2 cis9,trans11 (RA, +32%), C18:2 trans-9,cis-11, C20:2 and total CLA sum (+38%). The C16:0 proportion showed a trend ($P = 0.05$) to be higher with those diets. In the case of OBSD the total percentage of milk saturated FA was greater and that of monounsaturated and specially C18:1 n-9 cis were lower ($P < 0.01$) than with CD or TSD. A significantly lower $\Delta 9\text{C}18$ index was detected in OBSD milk. In relation to changes induced by TSD compared to other diets it was noticeable the reduction ($P \leq 0.017$) in C4:0, C6:0 and C8:0 short chain FA and C18:2 cis9,cis11 CLA. Total polyunsaturated FA proportion in milk remained unchanged by using TSD and OBSD.

3.1.3. Energy and nitrogen utilization

Animals fed OBSD exhibited lower ($P = 0.005$) percentage of digestible N in relation to N intake than the other two groups (Table 4), while efficiency of feed utilization was similar for all diets ($P = 0.954$). The inclusion of silages and SFO increased the digestible and metabolizable energy contents ($P = 0.010$ and $P = 0.008$ respectively) of the diets.

3.1.4. Ruminal fermentation and methane production

No statistical differences were observed on pH, total VFA concentration (Table 5) or acetate to propionate ratio. Acetic acid decreased ($P = 0.005$) and butyrate increased significantly ($P = 0.029$) when by-products silage and SFO were included in the diet. A trend ($P = 0.082$) was observed towards lower methane emissions with TSD addition.

As for microbial abundances, a higher ($P = 0.037$) concentration of protozoa gene copies was observed for OBSD.

Total PD urinary excretion resulted higher ($P = 0.020$) for animals fed OBSD and TSD compared to CD, while the excretion of creatinine was similar ($P = 0.988$) for all diets and ranged from 386 to 394 $\mu\text{mol/kg BW}^{0.75}$.

3.2. Experiment 2

3.2.1. Feed composition.

Ingredients and chemical composition of the experimental diets are shown in Table 6. Diets had analogous content in fat, protein and fiber and similar FA profile.

3.2.2. Intake, milk yield and composition

Intake of DM ($P < 0.001$) and BW ($P = 0.032$) of animals fed TSD2 were higher compared to the CD2, and this did not result in milk yield ($P = 0.736$) and composition ($P \geq 0.164$) being affected (Table 7).

The proportion of the milk FA (Table 8) was modified with TSD2. The most relevant decreases were observed for oleic acid ($P = 0.036$), RA ($P = 0.003$), MFA ($P = 0.006$), total CLA ($P = 0.005$), while C8:0 ($P = 0.026$), total SFA ($P = 0.004$) and AI ($P = 0.048$) were significantly increased.

3.2.3. Ruminal fermentation

The average pH in rumen of goats in experiment 2 tended to be lower ($P = 0.060$) in animals fed TSD2 (Table 9). Furthermore, no statistical differences were observed on total VFA concentration, while the acetic acid proportion decreased ($P = 0.017$), propionate

increased ($P = 0.007$) and consequently the acetate-to-propionate ratio decreased ($P=0.008$) when TS was included in the diet.

4. Discussion

4.1. Experiment 1

4.1.1. Feed composition

Despite SFO supplementation, not many differences were found in diets' FA profile apart from the higher percentages of PUFA and linoleic acid (LA; C18:2 n-6) detected in the TSD, that could have been caused by the richness in those FA in tomato seeds (Romano et al., 2010). The reasons for having a high content in LA and linolenic acid (LNA; C18:3 n-3) in CD could be ascribed to the oats hay because oats grains, that come mixed in the hay, are rich in LA (Elke and Zannini, 2013) while a high content in LNA has been reported in oats stems (Jusaitis et al., 1982).

4.1.2. Intake, milk yield and composition, and nutrient apparent digestibility

Molina-Alcaide et al. (2010) and Romero-Huelva et al. (2012) found no effect on DMI when dairy goats were fed feed-blocks (FB) containing olive cake and tomato wastes (12 % and 12.5 % as-fed basis, respectively for olive cake and tomatoes). However, Molina-Alcaide et al. (2010), in contrast to our results with OBSD, observed lower milk yield in goats fed FB containing olive cake, and no differences in milk composition. Abdollahzadeh et al. (2010) observed that substitution (15 %) of alfalfa hay by a mixture of tomato and apple silage in the diet of lactating cows increased milk production and the digestibility of DM and OM without affecting milk composition.

The variable effects reported in the literature of including silage made with agro-industrial by-products on intake and performance necessarily need to be explained by the variation in the level of inclusion in the diet and the nutritive quality of the replaced ingredient.

The values of milk SCC detected in our experiments were within the range (1,700 - 5,000 × 10³ cells/mL) observed by Jiménez-Granado et al. (2014) in 65 % of goat herds as part of a meta-analysis from 200 farms. Otherwise, increases in the SCC in goat milk have been associated with unbalanced ration (N, energy or minerals) (Sánchez et al., 2007) or metabolic disorders (acidosis, alkalosis, etc.) (Lerondelle et al., 1992; Fedele et al., 1996). In our study, the lack of effect of the diet on this parameter suggests the absence of disorders induced by the dietary use of by-products.

As for SFO addition, Toral et al. (2010) did not find changes in DMI, milk yield nor milk composition when supplemented dairy sheep diet with 25 g of SFO/kg DM. Similar results were observed by Chilliard et al. (2003) and Bernard et al. (2009) on the effects of 34 and 55 g of SFO/kg DM on goats' milk composition. In agreement with our results, Roy et al. (2013) observed that fat digestibility increased when wethers' diets were supplemented with SFO. It has been demonstrated (Chilliard et al., 2007; Bernard et al., 2009) that the SFO supplementation (over 40 g SFO/kg DM) of goats diet may cause, to different extents, increases of LA, VA and RA, and a decrease in C18:0 in milk, due to the altered biohydrogenation of LA and the higher availability in the rumen of LA and LNA. In our study the lack of effect on C18:0 or LA, could be attributed to a much lower SFO supplementation (20 g SFO/kg DM) than the one used by those authors or to the similar content in LA and LNA in CD and diets supplemented with SFO. Nevertheless, Chilliard et al. (2007) reported that VA and RA seem to be more sensitive to low SFO additions and this could be another reason for the increase in those FA observed in our animals. Furthermore, Romero-Huelva et al. (2012) detected a similar increase in RA in the milk of goats that consumed FB including tomato and Abbeddou et al. (2011) and Romano et al. (2010) reported higher contents of CLA (43.3 and 19.8%, respectively) in the milk of sheep supplemented with tomato by-products compared to a standard diet. Molina-Alcaide et al.

(2010) also described increases in VA and RA in the milk of goats fed olive cake containing FB but lower than that promoted by OSBD.

Organoleptic properties of goat milk are related to its content in short chain FA (C4:0 to C10:0) (Alonso et al., 1999; Chilliard and Lambert, 2001). The total content of these FA in CD and OSBD corresponded to common values described for milk goat by Sanz-Ceballos et al. (2009), while the values obtained for TSD were much lower and nearer to the values described for cow milk by those authors. This might indicate a likely impact of TSD on the milk flavour, which was not assessed in this study. On the other hand, contribution of short chain FA to milk fat has been also ascribed to the mobilization of body fat, especially in case of a negative energy balance (Chilliard et al., 2007) that could be, to some extent, minimized by the use of TSD. The lower $\Delta^9\text{C}_{18}$ index detected in OSBD milk could be due to its lower LA and LNA content or also to lower content of C18:1 cis in the mammary gland.

4.1.3. Energy and nitrogen utilization

In contrast to our results, Molina-Alcaide et al. (2010) and Romero-Huelva et al. (2012) reported lower energy intake and consequently lower urine energy and milk energy excretions, and digestible energy when included olive by-products and tomato fruits in FB, respectively. Such differences could be ascribed to the improvement induced in our case by the oil supplementation, which represents an extra energy source for the animal. This affirmation is supported by the data on urinary excretion of total PD that resulted higher for animals fed silages compared to CD, indicating that microbial protein synthesis in the rumen was enhanced by the strategy tested in our study.

4.1.4. Ruminal fermentation and methane production

The change in the molar proportion of some VFAs (reduced acetate and increased butyrate and isobutyrate) may be explained by the different substrate provided for microbial fermentation. The CD contained glycerol to balance the energy addition in the experimental

diet diets by SFO. Glycerol is converted through triose phosphate to pyruvic acid, and acetic acid is the major end product formed by the decarboxylation of pyruvic acid (Wright 1969). This explains the greater acetate content in CD. Also, the inclusion of both silages in the diet of goats promoted higher protozoa counts and protozoa have been associated with greater butyrate production in the rumen (Brossard et al., 2004).

Regardless the impact of the diets on the abundances of different microbial groups, the urinary excretion of PD indicated a consistently greater synthesis of microbial protein outflow to the duodenum. The values obtained for creatinine excretion agree with previous works conducted in goats (Mota et al., 2008) and show that the total collection of urine was accomplished. Although OBSD and TSD diets contained 14 and 10 % less protein than CD, the nutrients included in tomato and olive by-products enhanced the microbial activity and compensated this lower supply, as overall N balance in animals was not affected by the experimental treatment.

In the last decade there has been an increased interest on the development of dietary strategies that could reduce CH₄ emission by ruminants (Martin et al., 2010). Methane is an important greenhouse gas, which represents a significant energy loss for the animal (2 to 12% of the gross energy intake) (Johnson and Johnson, 1995). Recent studies have demonstrated the ability of including tomato surplus in goat diet to reduce CH₄ production (Romero-Huelva et al., 2012; Romero-Huelva and Molina-Alcaide, 2013; Arco-Pérez et al., 2013). When TSD was fed to the animals, a tendency was found for methane production per kilogram of ingested DM to be reduced 15%. Previous *in vitro* experiments (Arco-Pérez et al., 2013; Romero-Huelva et al., 2013) also showed a decrease in CH₄ production when tomato surplus was incubated in buffered goats rumen liquor. The anti-methanogenic mechanisms of tomato ingredients remain yet unknown and deserve further research in the future.

4.2. Experiment 2

The objective of this second experiment was to confirm the findings from the first trial using long-term application of the most successful dietary strategy from experiment 1. The tendency to decrease CH₄ emissions without changes in nutrient digestibility, the slight modifications in rumen fermentation patterns and the improvement of milk quality that promoted the partial replacement of forage with TS plus SFO, suggested that this feeding strategy could be the most promising.

Due to the satisfactory values for protein digestibility that were obtained in experiment 1, in experiment 2 the grain mixture composition was rather selected in order to meet the energy requirements of the animal, so that the protein content in CD2 and TSD2 resulted lower than CD and TSD, respectively.

In this second experiment, the differences between diets composition can only be ascribed to the replacement of oat hay by TS and not to the SFO supplementation that was identical for both experimental diets. The fat content of sunflower seeds used was 47.5 g/100g DM, so that together with the inclusion of SFO (3.06 g/kg), the amount of total sunflower lipids included in CD2 and TSD2 was approximately 11 g/kg DM, which was around half than that in experiment 1.

The higher intake of TSD2 along the 90 days resulted in a greater final BW in this group that could indicate that the extra energy intake was transformed into body reserves rather than into milk production. This is important for the recovery of animals' body condition score that put them in better shape of health and fertility to face the next pregnancy (Kusina et al., 2001).

The fat content in milk from animals consuming CD2 or CD (experiment 1) was similar, while TSD2 promoted milk fat content under the range observed in milk of goats fed by-products silage in experiment 1.

Increased SFA proportion and decreased MFA were observed for TSD2, while PUFA remained in the same proportion than in CD2. In line with these results, Nogalski et al.

(2012) stated that mobilization of body fat reserves led to an increase in the concentrations of MFA (mostly C18:1 cis-9, followed by C18:1 trans-11), a decrease in the levels of SFA, and a drop in the content of LA, arachidonic and docosahexaenoic acids in cow milk fat, and the improvement of the energy balance contributed to a decrease in the concentrations of UFA. Such findings seem to support the idea that TSD2 was better than CD2 to facilitate the deposition of body reserves during mid to late lactation.

Increased concentration of CLA found in experiment 1 with TSD did not occurred in this second experiment, this is probably explained by the slight differences in FA content between CD2 and TSD2 were insufficient to promote such an effect. Contrarily to TSD effect in experiment 1 the RA concentration in milk fat of goats fed TSD2 over 90 days was lower than with CD2. However it is worth mentioning that RA content in CD was much lower than CD2 in experiment 2 (0.225 and 0.530 %, respectively), resulting in opposed significant effects when CD2 and TSD2 were compared, despite to the also higher RA content in TSD2 (0.383 %) than in TSD (0.297 %). As a consequence of such results changes in RA in experiment 1 could probably assigned to the SFO supplementation rather than to the presence of by-products in the diets.

5. Conclusions

The dietary strategy of replacing oats hay in the diet of lactating goats with silages made of tomatoes or olive oil by-products together with SFO supplementation (20 g/kg of DM) improved milk quality without affecting animal efficiency. Furthermore, the long-term use of tomato silage in dairy goats fosters voluntary intake, which resulted in higher BW gain, without compromising the milk production and composition. Such findings suggest that using silages made with tomato surplus in dairy goats is a feasible dietary strategy in areas where the by-product is available.

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BY-PRODUCTS SILAGES FOR FEEDING DAIRY GOATS

Table 1
Ingredient and chemical composition of the experimental diets¹ (experiment 1)

Item	CD	OBSD	TSD
Ingredients (g/kg DM)			
Oat hay	202		
Olive by-products silage		202	
Tomato surplus silage			202
Alfalfa hay	194	194	194
Whole corn grain	145	145	145
Soya 44	99	99	99
Sugar beet pulp	68	68	68
Whole barley grain	59	59	59
Pea seeds	59	59	59
Dry distillers corn grains soluble	51	51	51
Whole oats grain	47	47	47
Sugarcane molasses	40	40	40
Glycerine	20		
Sunflower oil		20	20
Caprimix N30 ²	16	16	16
Nutrients (g/kg DM)			
Dry matter (g/kg fresh matter)	926	801	668
Organic matter	937	895	935
Ether extract	30.0	52.6	52.4
Crude protein	196	169	177
NDF	330	292	353
ADF	152	144	172
ADL	25.4	39.9	33.2
Gross energy (MJ/kg DM)	18.8	18.7	19.3
FA (% of total fat) ³			
C12:0	0.189	0.128	0.111
C14:0	0.726	0.650	0.608
C16:0	33.6	32.2	29.6
C16:1	0.145	0.147	0.191
C18:0	20.3	22.4	19.2
C18:1 n-9	15.2	17.1	16.0
C18:2 n-6	25.0	23.5	29.5
C18:3 n-3	2.65	1.40	2.03
SFA ³	56.1	56.5	50.7
MFA ³	15.6	17.5	16.4
PUFA ³	28.3	25.9	32.9
UFA ³	43.9	43.5	49.3

¹ CD = control diet; OBSD = olive oil by-products silage diet; TSD = tomato surplus silage diet.

² Caprimix N30 (Nutricor SL, Lerida, Spain), nutritional supplement with vitamin (A, D₃, E), mineral and plant extract in alcohol solution. Base ingredients: Soy (400 g/kg), rapeseed cake (200 g/kg), maize flour (120 g/kg), wheat sharp (40 g/kg), sugarcane molasses (25 g/kg), D-L Methionine (10 g/kg), calcium carbonate (8 g/kg), Di-calcium phosphate anhydrous (6 g/kg), NaCl (5 g/kg).

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651 ³ SFA = saturated fatty acids; MFA = monounsaturated fatty acids; PUFA= polyunsaturated
652 fatty acids; UFA = unsaturated fatty acids.
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Table 2
Nutrients intake, apparent digestibility and milk yield and composition in dairy goats fed the experimental diets (n=6, for each diet) (experiment 1)

Item	Diets ¹			SEM	P-value
	CD	OBSD	TSD		
Intake (g/day)					
DM	1391	1456	1491	104	0.772
OM	1304	1394	1302	96.0	0.741
Fat	41.8 ^b	78.1 ^a	76.6 ^a	4.55	0.001
CP	273	246	264	19.5	0.536
NDF	404	394	482	30.8	0.113
GE	26.3	27.1	28.9	2.00	0.624
Apparent digestibility (g/kg)					
DM	773	747	780	5.15	0.062
OM	783	776	793	5.12	0.411
Fat	822 ^b	919 ^a	905 ^a	2.90	0.000
CP	837	818	821	9.17	0.671
NDF	531	574	616	14.7	0.113
Milk yield (g/day)	1347	1230	1307	107	0.733
Fat	62.5	72.3	68.6	4.70	0.558
CP	48.9	49.0	45.7	4.20	0.872
Lactose	61.2	56.3	59.1	5.40	0.743
TS	183	188	184	14.0	0.992
Composition (g/kg milk)					
Fat	46.4 ^b	58.8 ^a	52.5 ^a	1.70	0.024
CP	36.3	39.8	35.0	0.97	0.149
Lactose	45.4	45.8	45.2	0.58	0.909
Total solids	136 ^b	153 ^a	141 ^a	2.7	0.049
SCC ²	6.32	6.16	6.15	0.09	0.705

¹ CD = control diet; OBSD = olive oil by-products silage diet; TSD = tomato surplus silage diet.

² SCC = Log₁₀ somatic cell counts per mL.

^{a,b} Within a row, means not bearing a common superscript letter differ (P<0.05).

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Table 3
Fatty acids composition (g/100 g of identified FA) of milk fat of dairy goats fed the experimental diets (n=6, for each diet) (experiment 1)

Item	Diets ¹			SEM	P-value
	CD	OBSD	TSD		
Saturated FA					
C4:0	2.05 ^a	2.16 ^a	1.50 ^b	0.08	0.017
C6:0	2.35 ^a	2.47 ^a	1.72 ^b	0.10	0.017
C8:0	2.98 ^a	3.13 ^a	2.18 ^b	0.12	0.017
C10:0	12.3	13.5	8.39	0.89	0.092
C11:0	0.096	0.098	0.065	0.007	0.130
C12:0	4.45	4.25	3.33	0.27	0.216
C13:0	0.075	0.061	0.062	0.005	0.392
C14:0	6.95	6.75	7.85	0.28	0.267
C15:0	0.523	0.474	0.591	0.029	0.300
C16:0	23.9	25.8	26.4	0.44	0.080
C17:0	0.477	0.369	0.416	0.016	0.063
C18:0	14.7	16.3	15.0	0.64	0.569
C20:0	0.231 ^b	0.287 ^a	0.240 ^b	0.007	0.021
C21:0	0.031	0.039	0.040	0.002	0.153
C22:0	0.118	0.115	0.127	0.003	0.329
C23:0	0.037	0.039	0.039	0.002	0.890
C24:0	0.029	0.028	0.015	0.003	0.086
Monounsaturated FA					
C14:1	0.161	0.139	0.193	0.014	0.352
C15:1	0.058	0.060	0.031	0.006	0.129
C16:1 cis-9	0.771	0.561	0.737	0.032	0.053
C17:1	0.312 ^a	0.142 ^b	0.251 ^a	0.020	0.016
C18:1 trans-9	0.334	0.844	0.815	0.136	0.263
C18:1 trans-11 (vaccenic acid)	0.995 ^b	2.64 ^a	2.87 ^a	0.181	0.002
C18:1 cis-9	22.4 ^a	15.5 ^b	23.2 ^a	0.78	0.005
C20:1n-9	0.038	0.055	0.038	0.003	0.101
C22:1n-9	0.068 ^{ab}	0.057 ^b	0.071 ^a	0.002	0.035
C24:1	0.027	0.024	0.011	0.003	0.139
PUFA					
C18:2n-6 trans	0.211 ^a	0.144 ^b	0.113 ^b	0.015	0.050
C18:2n-6 cis (linoleic acid)	2.22	2.54	2.44	0.09	0.370
C18:2 cis-9,trans-11 (CLA, rumenic acid)	0.225 ^b	0.279 ^a	0.297 ^a	0.009	0.016
C18:2 trans-9,trans-11 (CLA)	0.023	0.035	0.020	0.003	0.183
C18:2 trans-10,cis-12 (CLA)	0.049	0.060	0.050	0.004	0.527
C18:2 cis-9,cis-11(CLA)	0.033 ^a	0.032 ^a	0.018 ^b	0.002	0.002
C18:2 trans-9,cis-11(CLA)	0.069 ^b	0.145 ^a	0.136 ^a	0.007	0.001
C20:2	0.056 ^b	0.096 ^a	0.086 ^a	0.004	0.005
C22:2	0.053	0.058	0.038	0.005	0.319
C18:3n-6	0.079	0.063	0.076	0.007	0.666
C18:3n-3	0.181	0.174	0.159	0.008	0.478
C20:3n-6	0.047	0.059	0.040	0.004	0.146
C20:3n-3	0.035	0.025	0.013	0.004	0.109
C20:4n-6 (ARA)	0.210	0.204	0.211	0.006	0.861
C20:5n-3 (EPA)	0.052	0.057	0.052	0.001	0.091
C22:5n-3 (DPA)	0.099	0.080	0.069	0.005	0.074
C22:6n-3 (DHA)	0.043	0.046	0.040	0.005	0.885
Summary					
SFA ²	71.2 ^b	75.9 ^a	67.9 ^b	0.83	0.010
MFA ²	25.1 ^a	20.0 ^b	28.2 ^a	0.78	0.005
PUFA ²	3.68	4.10	3.86	0.09	0.216
UFA ²	28.8 ^a	24.1 ^b	32.1 ^a	0.83	0.010
n3	0.410 ^a	0.382 ^{ab}	0.333 ^b	0.010	0.018

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n6	2.77	3.01	2.88	0.09	0.576
Σ CLA	0.399 ^b	0.551 ^a	0.521 ^a	0.012	0.001
Δ9C14 ³	0.023	0.020	0.024	0.002	0.666
Δ9C16 ³	0.032	0.021	0.027	0.002	0.067
Δ9C18 ³	0.603 ^a	0.450 ^b	0.606 ^a	0.012	0.003
CLA index ³	0.212 ^a	0.100 ^b	0.099 ^b	0.017	0.025
AI ³	2.16	2.17	1.92	0.10	0.510
TI ³	2.19	2.16	1.96	0.08	0.448

¹ CD = control diet; OBSD = olive oil by-products silage diet; TSD = tomato surplus silage diet.

² SFA = saturated fatty acids; MFA = monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; UFA = unsaturated fatty acids.

³ Δ9C14 = (C14:1)/(C14:1 + C14:0); Δ9C16 = (C16:1)/(C16:1 + C16:0); Δ9C18 = (C18:1)/(C18:1 + C18:0); CLA index (rumenic acid/vaccenic acid + CLA); AI (atherogenicity index) = (C12:0 + 4 × 14:0 + C16:0)/(MUFA + PUFA); TI (thrombogenicity index)= (C14:0 + C16:0 + C18:0)/(0.5 × MUFA + 0.5 × n-6-PUFA + 3 × n-3-PUFA + n-3-PUFA/n-6-PUFA).

^{a,b} Within a row, means not bearing a common superscript letter differ (P<0.05).

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Table 4
BW, nitrogen and energy utilization, feed efficiency in dairy goats fed the experimental diets
(n=6, for each diet) (experiment 1)

Item	Diets ¹			SEM	P-value
	CD	OBSD	TSD		
BW ^{0.75} (kg)	20.6	17.9	17.7	0.58	0.165
g/kg of BW ^{0.75}					
Digestible N ²	1.25	1.56	1.62	0.09	0.273
N balance ³	0.430	0.520	0.495	0.055	0.811
Retained N ⁴	0.128	0.134	0.102	0.026	0.864
N utilization (%)					
Digestible N/intake N	80.5 ^a	72.6 ^b	77.5 ^a	0.62	0.005
N balance/ digestible N	34.4	33.4	30.6	3.09	0.866
Milk protein N/ digestible N	23.8	24.2	23.8	2.46	0.951
Milk protein N/N balance	69.2	72.0	80.3	3.71	0.465
Milk protein N/milk total N	95.5	98.0	98.1	1.31	0.677
Milk protein N/intake N	19.1	17.5	18.4	1.84	0.951
MJ/kg of BW ^{0.75}					
Digestible energy ⁵	0.665 ^b	1.10 ^a	1.12 ^a	0.052	0.010
Methane energy ⁶	0.049	0.057	0.056	0.010	0.935
Metabolizable energy (ME ⁷)	0.601 ^b	1.00 ^a	1.01 ^a	0.046	0.008
Gross energy (GE) utilization (%)					
Digestible GE/GE intake	76.8	76.2	78.3	0.74	0.529
Milk energy/digestible GE	49.8	38.7	39.5	4.59	0.569
Milk energy/ME	54.9	42.7	43.6	5.00	0.563
ME/ GE intake	69.4	69.3	70.9	0.93	0.742
ME/ digestible GE	90.5	90.9	90.6	0.74	0.971
Feed efficiency ⁸	11.3	11.7	12.1	2.61	0.954

¹ CD = control diet; OBSD = olive oil by-products silage diet; TSD = tomato surplus silage diet.

² Digestible N = intake N - fecal N.

³ N balance = digestible N - urine N.

⁴ Retained N = N balance – milk total N.

⁵ Digestible energy = GE intake –fecal GE.

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

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

⁸ Efficiency of feed utilization = (milk energy/energy intake) × 100.

^{a,b} Within a row, means not bearing a common superscript letter differ (P<0.05).

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Table 5
Methane emissions, average values for pH, total and individual VFA, rumen microbial abundance and urine excretion of purine derivatives in dairy goats fed the experimental diets (n=6, for each diet) (experiment 1)

Item	Diets ¹			SEM	P-value
	CD	OBSD	TSD		
pH	7.07	7.16	7.33	0.13	0.161
Total VFA (mmol/L)	77.2	60.0	56.6	6.92	0.459
mol/100 mol					
Acetate	67.0 ^a	64.8 ^b	63.0 ^b	0.36	0.005
Propionate	12.7	12.1	13.6	0.53	0.528
Isobutyrate	2.55	2.80	3.59	0.15	0.052
Butyrate	14.2 ^b	16.7 ^a	16.0 ^{ab}	0.34	0.029
Isovalerate	2.33	2.15	2.61	0.11	0.290
Valerate	1.23	1.34	1.22	0.09	0.297
Acetate/Propionate	5.27	5.36	4.63	0.23	0.413
Methane emissions					
CH ₄ (L/day)	20.1	27.5	24.1	2.88	0.657
CH ₄ (L/kg DMI)	21.9	19.3	18.7	0.79	0.082
Microbial densities (gene copies/g of fresh matter)					
Total bacteria	11.7	12.1	11.8	0.25	0.316
Methanogens	9.13	9.19	9.15	0.45	0.992
Protozoa	7.50 ^b	9.28 ^a	7.99 ^{ab}	0.49	0.037
Total purine derivatives in urine (μmol/kg BW ^{0.75})	506 ^b	790 ^a	788 ^a	92.2	0.020
Creatinine (μmol/kg BW ^{0.75})	386	394	388	54.6	0.988

¹ CD = control diet; OBSD = olive oil by-products silage diet; TSD = tomato surplus silage diet.

^{a,b} Within a row, means not bearing a common superscript letter differ (P<0.05).

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Table 6

Ingredient and chemical composition of the experimental diets¹ (experiment 2)

Item	CD2	TSD2
Ingredients (g/kg DM)		
Oats hay	194	
Tomato surplus silage		194
Alfalfa hay	194	194
Whole oats grain	161	161
Whole corn grain	153	153
Sugar beet pulp	67.3	67.3
Lupinus	52.0	52.0
Cotton seeds	42.8	42.8
Dry distillers corn grains soluble	36.7	36.7
Pea seeds	30.6	30.6
Caprimix N30 ²	18.3	18.3
Sunflower seeds	16.5	16.5
Sunflower oil	3.06	3.06
Glycerine	31.0	31.0
Nutrients (g/kg DM)		
Dry Matter, g/kg fresh matter	913	564
Organic Matter	938	943
Ether extract	60.7	64.7
Crude Protein	148	157
NDF	329	323
ADF	175	179
ADL	41.1	45.1
Gross energy, MJ/kg	19.4	19.5
Price, €/ton DM	232	215
FA (% of total fat) ³		
C12:0	0.152	0.126
C14:0	0.552	0.487
C16:0	20.4	21.8
C16:1	0.472	0.354
C18:0	7.62	9.55
C18:1 n-9	25.8	22.4
C18:2 n-6	40.2	40.1
C18:3 n-3	2.79	2.85
SFA ³	29.3	32.4
MFA ³	26.9	23.3
PUFA ³	43.8	44.3
UFA ³	70.7	67.6

¹CD2 = control diet in experiment 2; TSD2 = tomato surplus silage diet in experiment 2.

² See Table 1.

³ SFA = saturated fatty acids; MFA = monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; UFA = unsaturated fatty acids.

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Table 7
Intake, milk yield and composition in dairy goats fed the experimental diets (n=12, for each diet) (experiment 2)

Item	Diets ¹		SEM	P-value
	CD2	TSD2		
Intake (g DM/day)	1380	1729	81.7	0.000
BW (kg)	45.0	48.1	1.31	0.032
Milk yield (g/day)	1707	1734	79.2	0.736
Fat	80.4	81.2	6.70	0.450
CP	57.2	58.6	3.30	0.591
Lactose	79.0	79.1	4.0	0.770
Total solids	229	231	13.0	0.724
Composition (g/kg milk)				
Fat	47.1	46.8	1.79	0.886
CP	33.5	33.8	0.68	0.676
Lactose	46.3	45.6	0.49	0.164
Total solids	134	133	2.44	0.929
SCC ²	6.03	6.22	0.08	0.104

¹ CD2 = control diet in experiment 2; TSD2 = tomato surplus silage diet in experiment 2.

² SCC = Log₁₀ somatic cell counts per mL.

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Table 8
Fatty acid composition (g/100g of identify FA) of milk fat of dairy goats fed experimental diets (n=12, for each diet) (experiment 2)

Item	Diets ¹		SEM	P-Value
	CD2	TSD2		
Saturated FA				
C4:0	1.57	1.73	0.04	0.071
C6:0	1.72	1.82	0.05	0.317
C8:0	2.09	2.39	0.06	0.026
C10:0	9.45	9.54	0.18	0.799
C11:0	0.093	0.103	0.01	0.407
C12:0	4.03	4.36	0.14	0.256
C13:0	0.085	0.087	0.00	0.737
C14:0	8.52	8.29	0.16	0.502
C15:0	0.732	0.643	0.02	0.026
C16:0	25.7	26.9	0.35	0.096
C17:0	0.476	0.388	0.01	0.001
C18:0	15.1	16.7	0.34	0.029
C20:0	0.230	0.204	0.01	0.159
C21:0	0.045	0.020	0.00	0.000
C22:0	0.057	0.098	0.00	0.000
C23:0	0.013	0.012	0.00	0.866
C24:0	0.017	0.018	0.00	0.564
Monounsaturated FA				
C14:1	0.311	0.327	0.01	0.326
C15:1	0.037	0.034	0.00	0.522
C16:1 cis-9	0.866	0.767	0.02	0.009
C17:1	0.169	0.142	0.01	0.219
C18:1 trans-9	0.663	0.579	0.02	0.050
C18:1 trans-11 (vaccenic acid)	1.526	1.058	0.13	0.093
C18:1 cis-9	22.7	19.9	0.61	0.036
C20:1n-9	0.071	0.045	0.00	0.000
C22:1n-9	0.033	0.022	0.00	0.026
C24:1	0.015	0.013	0.00	0.369
PUFA				
C18:2n-6 trans	0.275	0.279	0.02	0.884
C18:2n-6 cis (linoleic acid)	2.13	2.29	0.07	0.299
C18:2 cis-9,trans-11 (CLA, rumenic acid)	0.530	0.383	0.02	0.003
C18:2 trans-9,trans-11 (CLA)	0.015	0.015	0.00	0.653
C18:2 trans-10,cis-12 (CLA)	0.023	0.016	0.00	0.043
C18:2 cis-9,cis-11(CLA)	0.018	0.037	0.00	0.000
C18:2 trans-9,cis-11(CLA)	0.013	0.009	0.00	0.009
C20:2	0.033	0.031	0.00	0.704
C22:2	0.015	0.013	0.00	0.080
C18:3n-6	0.059	0.054	0.00	0.402
C18:3n-3	0.217	0.271	0.02	0.120
C20:3n-6	0.034	0.036	0.00	0.379
C20:3n-3	0.020	0.016	0.00	0.006
C20:4n-6 (ARA)	0.197	0.218	0.01	0.113
C20:5n-3 (EPA)	0.042	0.029	0.00	0.004
C22:5n-3 (DPA)	0.067	0.052	0.00	0.013
C22:6n-3 (DHA)	0.030	0.015	0.00	0.004
Summary				
SFA ²	69.9	73.3	0.53	0.004
MFA ²	26.3	22.9	0.56	0.006
PUFA ²	3.72	3.77	0.10	0.829
UFA ²	30.1	26.7	0.52	0.001
n3	0.377	0.383	0.02	0.851

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n6	2.70	2.88	0.09	0.301
Σ CLA	0.599	0.461	0.02	0.005
$\Delta 9C14^3$	0.036	0.038	0.001	0.202
$\Delta 9C16^3$	0.033	0.028	0.001	0.005
$\Delta 9C18^3$	0.596	0.544	0.007	0.002
CLA index ³	0.283	0.266	0.011	0.433
AI ³	2.15	2.43	0.07	0.048
TI ³	1.73	1.86	0.04	0.137

¹ CD2 = control diet in experiment 2; TSD2 = tomato surplus silage diet in experiment 2.

² SFA = saturated fatty acids; MFA = monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; UFA = unsaturated fatty acids.

³ See Table 3.

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Table 9
Average values for pH and total and individual VFA in the rumen of dairy goats fed the experimental diets (n=12, for each diet) (experiment 2)

Item	diets ¹		SEM ²	P-value
	CD2	TSD2		
pH	6.58	6.30	0.070	0.060
Total VFA (mmol/L)	59.6	60.2	3.40	0.923
mol/100 mol				
Acetate	66.5	63.6	0.559	0.017
Propionate	15.6	19.0	0.582	0.007
Isobutirate	1.13	0.925	0.076	0.199
Butirate	14.3	14.2	0.369	0.898
Isovalerate	1.55	1.20	0.105	0.117
Valerate	1.00	1.08	0.042	0.334
Acetate/Propionate	4.36	3.46	0.153	0.008

¹ CD2 = control diet in experiment 2; TSD2 = tomato surplus silage diet in experiment 2.

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Fig 1. Experiment 1 outline

	Pre-adaptation ¹	Adaptation			Sampling ²	MC ³	RC ⁵		
day	-10	0	21	22	25	26	29	30	31
			Rumen content sampling ⁴ ↑						

Rumen content sampling⁴↑

¹ Adaptation period.

² Sampling 1: Nutrients intake and milk yield and composition (Tables 2 and 3).

³ MC = Metabolic cages for digestibility, N and energy utilization and feed efficiency (Table 4) and digestibility (Table 2).

⁴ Rumen content sampling: Fermentation parameters and microbiology (Table 5).

⁵ RC = Respiration chamber for methane emissions measurements (Table 5).

Fig 2. Experiment 2 outline

	Adaptation ¹	S ²	Transition ¹	S ²
day0		56 60		86 90

Rumen content sampling³↑

¹ Adaptation and transition periods: Collective housing of animals divided in two experimental groups: control diet and tomato silage diet.

² Sampling 1 and 2: Individual housing for measurements of body weight, nutrients intake and milk yield and composition (Tables 7 and 8).

³ Rumen content sampling: pH and VFA in the rumen (Table 9).