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Short communication

Effect of the inclusion of lemon leaves and rice straw by-products in the diet of dairy goats on the quality characteristics of milk and matured cheeses

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ABSTRACT

The effect of the dietary inclusion of lemon leaves and rice straw by-products on the quality characteristics of goats' milk and cheese was evaluated. Twenty-six Murciano-Granadina goats were used in a crossover design experiment; milk from each experimental group was collected to produce 60-days matured cheeses. Fat and dry matter content was higher in milk and cheeses from the diet containing by-products; medium-chain fatty acids and total free fatty acids were lower. Triangle tests revealed significant differences in the organoleptic characteristics of the cheeses between diets. However, when sensory attributes differentiating cheeses were evaluated individually, differences did not become significant. The inclusion of lemon leaves and rice straw in balanced diets with soya oil could be beneficial, as it does not appear to adversely affect the quality of milk and related mature cheeses, contributing to the reduction of the cost of the diet and the recycling of agricultural by-products.

1. Introduction

The use of crop and food processing industry by-products in the diet of small ruminants has, in recent years, become a common practice, allowing the reduction of feed costs and the environmental problems related to the management of such waste materials.

Citrus and rice crops generate large amounts of by-products as waste, most of which is burned, dumped into landfills or used as compost, leading to wasted resources and the need for costly waste management programs (Gutiérrez, Siles, Diz, Chica, & Martín, 2017). However, recent studies showed that these by-products could be successfully recycled to feed dairy goats (Criscioni & Fernandez, 2016; Fernández et al., 2018; Romero et al., 2020).

It should be noted that dietary composition affects ruminal fermentation and milk composition could be modified to a lesser or greater extent, when by-products are included in the diet (Chilliard, Ferlay, Rouel, & Lamberet, 2003; Romero, Ramírez, Planelles, Gracía, & Molina, 2017). However, the effect of the inclusion of these by-products in the diet on the characteristics of the final products, mainly cheeses, has so far not been studied. Therefore, the aim of this study was to evaluate the inclusion of lemon leaves and rice straw by-products in the diet of dairy goats on the quality characteristics of raw milk and mature cheeses.

2. Material and methods

2.1. Experimental design and animal diets

This study was carried out at the caprine experimental farm of Institute of Animal Science and Technology (ICTA) of Universitat Politècnica de València (UPV, Spain). Experimental procedures were approved (2017/VSC/PEA/00182) by the Committee on Animal Use and Care at UPV.

Twenty-six multiparous Murciano-Granadina goats in mid-lactation were selected and divided into two homogenous groups of 13 goats each based on similar body weight (47.3 ± 0.07 kg) and milk production in the previous lactation (650.3 ± 42 kg of milk per 210 ± 30 d of lactation, on average). Two different mixed rations were evaluated in a cross-design experiment, each goat receiving two treatments in two experimental periods. Each experimental period was initiated two weeks after the adaptation of the animals to the diet supplied, and lasting seven days.

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Table 1
Ingredients and chemical composition of the concentrate used in each of the two experimental diets fed to goats.[a]

Parameter	Concentrate	
	CON	LRS
Ingredients (g kg^{-1} DM)		
Alfalfa hay	370	370
Barley	256	32
Lemon leaves	–	189
Rice straw	–	120
Soy hulls	221	–
Field pea, spring	63	126
Horse beans	63	120
Beet molasses	13	13
Soya oil	–	19
Calcium carbonate	8	6
Sodium chloride	3	2
Dicalcium phosphate	2	2
Premix	3	3
Chemical composition (% of DM)		
Dry matter	93	94
Organic matter	86	83
Ash	7	11
Crude protein (CP)	18	18
Ether extract (EE)	2	3
Neutral detergent fibre (NDF)	42	35
Acid detergent fibre (ADF)	25	22
Acid detergent lignin (ADL)	3	4
Non-fibrous carbohydrate (NFC)	31	33
Starch	18	12
Gross energy (MJ kg^{-1} DM)	17	16

[a] Abbreviations are: CON, control diet; LRS, lemon leaves and rice straw diet; DM, dry matter. Premix was provided by NACOOP S.A. (Spain), composition (ppm or UI per kilogram of premix): Se, 40; I, 250; Co, 80; Cu, 3000; Fe, 6000; Zn, 23400; Mn, 29,000; S, 60,000; Mg, 60,000; vitamin A, 2,000,000 UI; vitamin D3, 400,000; vitamin E, 2000 ppm; nicotinic acid, 10,000; choline, 20,300. NFC calculated as 100 − (NDF + ash + CP + EE).

Goats were fed daily with 1 kg alfalfa hay forage and 1.7 kg concentrate (37/63 forage to concentrate ratio). The different composition of the concentrate used in the two experimental diets (CON: control diet; LRS: diet including by-products) is presented in Table 1. Soybean oil was added in the LRS diet to make both mixed rations isoenergetic.

Raw milk from the two groups of animals (CON and LRS) was stored separately in refrigerated tanks during a 3-day period to produce cheeses. Two batches of CON and LRS cheeses were produced in each experimental period following the traditional process for ripened Tronchón goat cheese (Quintanilla, Beltrán, Molina, Escriche, & Molina, 2019). Chemical and sensory characteristics of the cheeses were evaluated after 60 days of maturation. Cheese yield was calculated by weighing milk and related ripened cheeses, being expressed as kg of cheese 100 kg^{-1} milk.

2.2. Milk and cheese analysis

Bulk milk was analysed for gross composition using MilkoScan 6000 (Foss, Hillerød, Denmark), somatic cell count (SCC) by Fossomatic 5000 (Foss), and total bacterial count (TBC) using Bactoscan FC (Foss). For cheese composition a FoodScan analyser (Foss) was employed. The fatty acid (FA) profile of the milk and cheese samples was analysed according to Nudda, Mcguire, Battacone, and Pulina (2005) using a focus gas chromatograph (Thermo, Milán, Italia) equipped with a split/splitless injector and a flame ionisation detector. The FAs were classified as short-chain FAs (C4–C8), medium-chain FAs (C10–C16) and long-chain FAs (>C16). The total content of free fatty acids (FFAs) in the cheeses (meq FFA 100 g^{-1} fat) was analysed according to Nuñez, García, Rodríguez, Medina, and Gaya (1986).

Sensory evaluation of the mature cheeses was carried by 12 experienced tasters belonging to the ICTA staff (UPV). Four triangle tests (ISO 4120; ISO, 2004) were carried out on different days to detect differences in the cheeses between diets, as well as to identify the attributes found by tasters in the samples perceived as different. In addition, CON and LRS cheeses were individually evaluated (two sets of samples by each experimental period), and the sensory attributes differentiating cheeses scored on an unstructured 10 cm straight line (ISO 4121; ISO, 2003).

2.3. Statistical analysis

A two-way ANOVA test using Statgraphics Centurion XVII.II software (StatPoint Technologies, Warrenton, VA, USA) was applied to evaluate the effect of the diet (D: CON and LRS) and the experimental period (P: one and two) on the quality characteristics of the goats' milk and cheeses.

3. Results and discussion

As shown in Table 2, significantly higher contents of fat and dry matter were observed in milk from goats fed the LRS diet, which could be related to the elevated lipid content of the lemon leaves (around 3% ether extract, according to Fernández et al., 2018), and the inclusion of soybean oil to balance the energetic value of the two experimental mixed rations. Milk composition was unaffected by the experimental period ($P > 0.05$) and no significant interactions were found in any case. As for milk, cheeses made from the milk from goats fed the LRS diet (Table 3) had significantly higher concentrations of fat and dry matter than CON cheeses. A lower content of protein was also detected in LRS cheeses in agreement with that observed by Sánchez-Macías et al. (2010) when making cheeses from goats' milk containing different fat levels. However, there were no significant differences for cheese

Table 2
Effect of the inclusion of lemon leaves and rice straw in the diet of Murciano-Granadina goats on the milk quality parameters.[a]

Parameter	Diet			*P*-value
	CON	LRS	SEM	
Fat (%)	4.56	5.86	0.063	0.0001
Protein (%)	3.92	3.92	0.034	0.9213
Lactose (%)	4.64	4.61	0.016	0.2458
Dry matter (%)	13.87	15.21	0.059	0.0001
Log SCC (cells mL^{-1})	6.19	6.16	0.056	0.6907
Log TBC (cfu mL^{-1})	4.82	4.61	0.185	0.4682

[a] Abbreviations are: CON, control diet; LRS, lemon leaves and rice straw diet; Log SCC, logarithm of somatic cell count; Log TBC, logarithm of total bacterial count.

Table 3
Effect of the inclusion of lemon leaves and rice straw in the diet of Murciano-Granadina goats on cheese yield and chemical composition of 60-day matured cheeses.[a]

Parameters	Diet			*P*-value
	CON	LRS	SEM	
pH	5.32	5.34	0.036	0.7967
Fat (%)	32.52	37.06	0.256	<0.0001
Protein (%)	25.42	22.56	0.335	0.0001
Dry matter (%)	62.69	64.35	0.251	0.0005
NaCl (%)	2.17	2.01	0.031	0.0034
FFA (meq 100 g^{-1} of fat)	5.23	2.83	0.240	<0.0001
Cheese yield (kg cheese 100 kg^{-1} milk)	13.51	13.69	0.274	0.6545

[a] Abbreviations are: CON, control diet; LRS, lemon leaves and rice straw diet; FFA, free fatty acids content.

Table 4
Effect of the inclusion of lemon leaves and rice straw in the diet of Murciano-Granadina goats on the fatty acids (mg 100 mg^{-1} fat) of milk and 60-day matured cheeses.[a]

Fatty acid	Goats' milk				Cheese			
	Diet			*P*-value	Diet			*P*-value
	CON	LRS	SEM		CON	LRS	SEM	
C4:0	1.10	1.03	0.064	0.533	1.09	0.96	0.081	0.277
C6:0	2.06	1.91	0.113	0.388	1.92	1.83	0.113	0.591
C8:0	2.50	2.32	0.134	0.405	2.26	2.22	0.121	0.821
C10:0	9.66	8.89	0.543	0.373	8.87	8.22	0.357	0.221
C11:0	0.29	0.27	0.021	0.591	0.28	0.24	0.014	0.048
C12:0	5.10	5.02	0.313	0.873	5.08	4.12	0.269	0.026
C14:0	9.20	8.83	0.576	0.674	8.87	7.62	0.354	0.027
C14:1c9	0.14	0.14	0.011	0.676	0.17	0.13	0.012	0.029
C15:0	0.83	0.84	0.073	0.962	0.89	0.76	0.055	0.099
C16:0	27.45	24.72	1.835	0.352	25.39	21.50	1.054	0.023
C16:1	0.54	0.53	0.064	0.939	0.70	0.54	0.046	0.031
C17:0	0.53	0.50	0.058	0.795	0.44	0.55	0.054	0.170
C17:1	0.15	0.17	0.016	0.419	0.18	0.16	0.012	0.383
C18:0	5.21	5.42	0.399	0.732	4.37	5.50	0.516	0.147
C18:1n9c	12.20	12.29	0.686	0.925	10.70	12.30	0.708	0.137
C18:1 n9t	1.68	1.66	0.117	0.898	1.28	2.11	0.295	0.069
C18:2 n6t	0.23	0.21	0.020	0.570	0.19	0.24	0.024	0.173
C18:2 n6c	3.03	3.09	0.110	0.723	2.70	2.88	0.148	0.419
C18:3n3	0.52	0.55	0.027	0.503	0.48	0.52	0.026	0.335
CLA9c11t	0.85	0.85	0.029	0.934	0.64	0.92	0.103	0.075
CLA9c11c	0.07	0.07	0.007	0.876	0.06	0.07	0.005	0.169
C20:4n6	0.17	0.18	0.010	0.568	0.16	0.15	0.006	0.158
Total FAs	83.51	79.50	4.805	0.434	76.74	73.54	3.086	0.478
Short-chain FAs	5.65	5.26	0.309	0.419	5.27	5.01	0.309	0.565
Medium-chain FAs	53.21	49.24	3.258	0.437	50.26	43.12	1.985	0.026
Long-chain FAs	24.64	24.99	1.360	0.863	21.21	25.41	1.810	0.127
SFAs	63.92	59.76	3.851	0.487	59.47	53.52	2.225	0.083
MUFAs	14.71	14.79	0.819	0.949	13.02	15.24	0.964	0.130
PUFAs	4.87	4.95	0.188	0.794	4.24	4.78	0.293	0.214

[a] Abbreviations are: CON, control diet; LRS, lemon leaves and rice straw diet; CLA, conjugated linoleic acid; FAs, fatty acids; short-chain FAs, fatty acids from C4 to C8; medium-chain FAs, fatty acids from C10 to C16; long-chain FAs, fatty acids > C16; SFAs, saturated fatty acids; MUFAs, mono-unsaturated fatty acids; PUFAs, poly-unsaturated fatty acids.

yield between diets or between periods. Regarding lipolysis, a lower FFA concentration (Table 3) was also observed in LRS cheeses, especially in the second experimental period (D × P, $P < 0.05$), suggesting a reduced lipolytic activity during maturation.

The milk FA composition (Table 4) was unaffected by the inclusion of lemon leaves and rice straw in mixed diet balanced with soybean oil, although significant interactions were detected for some FA, including C12:0 ($P = 0.0135$), C18:1n9c ($P = 0.0434$), C18:1n9t ($P = 0.0242$), C18:2n6t ($P = 0.0004$), and CLA 9c11t ($P = 0.0002$), which do not allow a clear determination of the effect of the diet on the final concentration of these compounds in the milk. However, the concentration of medium-chain FA (C10 to C16) was significantly lower ($P < 0.05$) in cheeses from goats fed the LRS diet (Table 4). The experimental period did not affect the FA composition of the cheeses and there were no significant interactions.

Higher fat content and lower concentration of medium-chain FAs were also obtained by Schettino-Bermúdez et al. (2020) in cheeses from goats fed diets including high concentration of chia seeds, containing 25–40% oil and polyunsaturated FAs (PUFAs), which could explain these differences. It should be noted that the trans-fatty acids produced in the rumen from biohydrogenation of PUFAs may inhibit the de novo synthesis of FAs (Chilliard, Ferlay, & Doreau, 2001). This inhibition could affect the final concentrations of FAs with 16 or fewer carbon atoms that are mainly derived from de novo synthesis (Chilliard et al., 2003). In our study, the higher fat content in the mature cheeses from LRS diet (Table 3) might have been due to the higher PUFA content in the LRS diet plus lipolysis. Thus, PUFAs from lemon leaves and soybean oil might have inhibited microbial synthesis and biohydrogenation, as reported by Chillard et al. (2003), leading to reduced contents of medium-chain FAs and increased levels of PUFAs in the mature LRS cheeses.

Significant differences ($P < 0.001$) between CON and LRS cheeses were detected through triangle tests performed, which were mainly related to the texture in the mouth and the basic taste descriptors. Thus, when the sensory attributes differentiating cheeses were rated on an unstructured scale, LRS cheeses were perceived as less hard and more adherent to the palate and teeth than CON cheeses, as well as less acidic, bitter and spicy. However, the differences become non-significant ($P > 0.05$) regardless of the diet and the experimental period considered. No significant interactions were found in any case.

4. Conclusions

The results presented here indicate that the inclusion of lemon leaves and rice straw by-products in combination with soybean oil to reach balanced mixed diets for dairy goats increases the fat content in milk and related cheeses. Moreover, no negative effects on cheese yield and the organoleptic characteristics of the cheeses were detected. Therefore, the use of such crop residues could be an interesting strategy to reduce feeding costs in producing areas contributing to the sustainable management of agricultural waste.

Declaration of competing interest

The authors have not stated any conflicts of interest.

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References

Chilliard, Y., Ferlay, A., & Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science, 70*, 31–48.

Chilliard, Y., Ferlay, A., Rouel, J., & Lamberet, G. (2003). A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science, 86*, 1751–1770.

Criscioni, P., & Fernandez, C. (2016). Effect of rice bran as a replacement for oat grain in energy and nitrogen balance, methane emissions, and milk performance of Murciano-Granadina goats. *Journal of Dairy Science, 99*, 280–290.

Fernández, C., Martí, J. V., Pérez, I., Palomares, J. L., Ibáñez, C., & Segarra, J. V. (2018). Effect of lemon leaves on energy and C–N balances, methane emission, and milk performance in Murciano-Granadina dairy goats. *Journal of Animal Science, 96*, 1508–1518.

Gutiérrez, M. C., Siles, J. A., Diz, J., Chica, A. F., & Martín, M. A. (2017). Modelling of composting process of different organic waste at pilot scale: Biodegradability and odor emissions. *Waste Management, 59*, 48–58.

ISO. (2003). *Sensory analysis. Evaluation of food products by methods using scales*. Geneva, Switzerland: International Organisation for Standardisation. ISO 4121.

ISO. (2004). *Sensory analysis*. Geneva, Switzerland: International Organisation for Standardisation. Triangle test. ISO 4120.

Nudda, A., Mcguire, M. A., Battacone, G., & Pulina, G. (2005). Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. *Journal of Dairy Science, 88*, 1311–1319.

Nuñez, M., García, C., Rodríguez, M. A., Medina, M., & Gaya, P. (1986). The effect of ripening and cooking temperatures on proteolysis and lipolysis in Manchego cheese. *Food Chemistry, 21*, 115–123.

Quintanilla, P., Beltrán, M. C., Molina, A., Escriche, I., & Molina, M. P. (2019). Characteristics of ripened Tronchón cheese from raw goat milk containing legally admissible amounts of antibiotics. *Journal of Dairy Science, 102*, 2941–2953.

Romero, T., Pérez, I., Larsen, T., Gomis, J., Loor, J. J., & Fernández, C. (2020). Inclusion of lemon leaves and rice straw into the compound feed and its effect on nutrient balance, milk yield and methane emissions in dairy goats. *Journal of Dairy Science, 103*, 6178–6189.

Romero, M., Ramírez, M. A., Planelles, R., García, P., & Molina, E. (2017). Can by-products replace conventional ingredients in concentrate of dairy goat diet? *Journal of Dairy Science, 100*, 4500–4512.

Sánchez-Macías, D., Fresno, M., Moreno, I., Castro, N., Morales, A., Álvarez, S., et al. (2010). Physicochemical analysis of full-fat, reduced-fat, and low-fat artisan-style goat cheese. *Journal of Dairy Science, 93*, 3950–3956.

Schettino-Bermúdez, B., Vega y León, S., Gutiérrez-Tolentino, R., Pérez-Gonzáles, J. J., Escobar, A., Gonzales-Ronquillo, M., et al. (2020). Effect of dietary inclusion of chia seed (*Salvia hispánica* L.) on goat cheese fatty acids profile and conjugated linoleic acid isomers. *International Dairy Journal, 105*. Article 104664.