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Rockrose and quebracho condensed tannins have a minor impact on the fatty acid profile of goat milk and cheese without altering animal performance and composition of products

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ABSTRACT

This study was carried out to test the hypothesis that the inclusion of condensed tannins (CT) from rockrose (*Cistus ladanifer* L.) and quebracho (*Schinopsis lorentzii*) in dairy goat diets supplemented with vegetable oil rich in polyunsaturated fatty acids (PUFA) can increase the potentially healthy fatty acids (FA) in milk and cheese fat, without compromising animal performance and milk and cheese composition. A feeding experiment was conducted with 18 Serpentina goats distributed into three dietary treatments: C) Basal diet (Control); R) Basal diet supplemented with CT from rockrose; and Q) Basal diet supplemented with CT from quebracho. Basal diet was composed of forage and concentrate at a ratio of 25:75 and supplemented with 50 g/kg dry matter (DM) of soybean oil. Basal diet was offered at level of 800 g/head and day, and hay was offered *ad libitum*. Individual milk yield, chemical composition and FA profile were analyzed on days 0, 7, 14 and 21 of the experimental period. In the last week of experiment, milk of five consecutive milking from each goat was pooled to make fresh cheese, and cheese was analyzed for chemical composition and FA profile. The inclusion of CT from rockrose and quebracho in goat diets did not affect the milk yield or the milk and cheese chemical composition.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; BCFA, branched-chain fatty acids; BH, biohydrogenation; BI, biohydrogenation intermediates; CLA, conjugated linoleic acid; CT, condensed tannins; DM, dry matter; FA, fatty acids; FAME, fatty acid methyl esters; GC-FID, gas chromatography with flame ionization detection; LC, linear chain; LC-SFA, linear chain satureted fatty acids; MUFA, monounsaturated fatty acids; NDF, neutral detergent fibre; PUFA, polyunsaturated fatty acids; SEM, standard error of the mean; SFA, saturated fatty acids.

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Both CT sources had a minor impact on milk and cheese FA profile, limited to a reduction of some branched-chain FA in milk and cheese and a reduction of 18:1 *trans*-10 in milk. Rockrose CT also reduced the contents of 18:1 *trans*-9 in milk compared to control diet. The basal diet used in present work, containing 250 g/kg DM of dehydrated Lucerne, low-starch ingredient replacing part of the cereals and supplemented with PUFA-rich vegetable oil, resulted in goat milk and cheese with high levels of FA with potential health benefits. However, the rockrose and quebracho CT did not increase PUFA, 18:1 *trans*-11 and 18:2 *cis*-9, *trans*-11 levels in milk and cheese.

1. Introduction

Milk and dairy products are a significant source of several healthy nutrients in the human diet, including vitamins, minerals, peptides, but also lipids such as polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA) isomers, *trans* fatty acids (FA) and branched-chain fatty acids (BCFA).

Rumenic acid (18:2 *cis*-9, *trans*-11) is the major CLA isomer in ruminant fat and shows anti-inflammatory, anti-atherogenic, anti-obese, and anti-carcinogenic properties (Kuhnt et al., 2016; Shokryazdan et al., 2017). Vaccenic acid (18:1 *trans*-11), in addition to having beneficial effects (Vahmani et al., 2020), is the precursor of the endogenous synthesis of 18:2 *cis*-9, *trans*-11, being the main source of the 18:2 *cis*-9, *trans*-11 in goat milk the endogenous conversion of 18:1 *trans*-11 in the mammary gland (Bernard et al., 2010). Moreover, ruminant fat is distinguished from the other fats by its richness in BCFA (Vlaeminck et al., 2006) which have demonstrated bioactivities such as anti-carcinogenic and anti-inflammatory effects and reduction of the incidence of necrotizing enterocolitis (Ran-Ressler et al., 2011; Vahmani et al., 2019; Wongtangtintharn et al., 2004). Despite its richness in several healthy nutrients, consumption of milk and dairy products has been a cause of concern due to their high amounts of saturated FA (SFA), once excessive consumption of SFA is considered a risk factor for the development of chronic diseases (Astrup et al., 2019). These concerns influence consumption and, consequently, stimulated the search for healthier foods. To improve the FA profile of ruminant-derived products several nutritional strategies that can modulate the ruminal biohydrogenation (BH), and thus increase the concentrations of beneficial FA and reduce FA that could have some detrimental effects, have been widely researched.

The inclusion of condensed tannins (CT), polyphenolic compounds resulting from the secondary metabolism of plants, in ruminant diets has been one of the nutritional approaches studied to modulate the ruminal BH. Results from *in vitro* and *in vivo* studies showed that CT-rich plant or extracts are able to change the ruminal BH pattern, increasing the concentration of linolenic acid (18:3*n*-3), linoleic acid (18:2*n*-6), 18:1 *trans*-11 and 18:2 *cis*-9, *trans*-11 in ruminant derived products (Frutos et al., 2020; Vasta and Bessa, 2012).

Table 1
Ingredients, chemical composition, and fatty acid (FA) profile of the experimental diets.

	Concentrate	Hay
Ingredients, g/kg DM		
Maize	112	
Wheat	103	
Barley	86	
Beet pulp	162.1	
Soybean meal	170.3	
Soybean oil	50	
Molasses	40	
Dehydrated Lucerne pellets	250	
Sodium bicarbonate	0.20	
Calcium carbonate	17	
Salt	9	
Levucell SC2	0.40	
Chemical composition, g/kg DM		
Dry matter	877	908
Crude protein	181	69.6
Starch	348	-
Sugar	62.1	-
NDF	253	648
ADF	133	438
ADL	14.7	63.8
Ether extract	64.4	13.0
Ash	78.6	53.6
Fatty acid profile, g/kg total fatty acids		
16:0	131	199
18:0	36.4	41.4
18:1 *cis*-9	209	123
18:2*n*-6	500	223
18:3*n*-3	60.5	49.5

On the other hand, feeding lipid sources rich in 18:2*n*-6 and 18:3*n*-3 have been successfully used to improve the nutritional value of lipid fraction of ruminant edible products (Jerónimo and Malcata, 2013; Scollan et al., 2014; Shingfield et al., 2013). Although the PUFA overload in the rumen could increase the amount of dietary unsaturated FA escaping the rumen and the ruminal outflow of biohydrogenation intermediates (BI), the effectiveness of dietary supplementation with lipid sources rich in PUFA to increase the PUFA content of ruminant fat is strongly limited by the extensive ruminal BH of unsaturated FA. Association of PUFA supplementation with dietary factors able to modulate the ruminal BH might be a good strategy to decrease the disappearance of dietary PUFA and/or increase the ruminal production of healthy BI.

So, considering the ability of CT to modulate the BH we hypothesize that CT from rockrose and quebracho can exacerbate the effect of dietary supplementation with PUFA-rich vegetable oils, and produce goat milk and cheese with increased levels of healthy FA without compromising the animal performance and the quality of products. In the present work, it was evaluated the effect of the inclusion of CT-rich extracts from rockrose and quebracho in goat diets supplemented with soybean oil on milk yield and composition and FA profile of milk and cheese.

2. Material and methods

2.1. Animals, diets and sampling

The present study was conducted in the facilities of the Centro Experimental do Baixo Alentejo – Direção Regional de Agricultura e Pescas do Alentejo (CEBA—DRAPAL), Vila Nova de São Bento, Portugal. The animal handling followed European Union Directive 2010/63/UE concerning animal care.

Eighteen multiparous Serpentina goats at 47.4 ± 3.13 days in milking from CEBA—DRAPAL, were randomly allocated into three experimental groups, 6 animals per group, and blocked for number of lactations. Goats were housed in individual pens with access to drinking water and feed. The trial lasts 21 days after 14 days of adaptation to experimental conditions. The goats were fed a basal diet contained 250 g/kg dry matter (DM) of dehydrated Lucerne, 301 g/kg DM of cereals, 162.1 g/kg DM of beet pulp, 170.3 g/kg DM of soybean meal and 50 g/kg DM of soybean oil (Table 1). The dehydrated Lucerne was milled to a final particle size of 1 mm and mixed with the other milled ingredients. Basal diet was offered at level of 800 g/head and day. Moreover, hay from a mixture of triticale and forage pea was offered *ad libitum*. The hay intake was not quantified in this work, as there is a loss of hay to the ground, not allowing precise quantification of consumption. In two experimental groups, extracts rich in CT from rockrose (*Cistus ladanifer* L.) and quebracho (*Schinopsis lorentzii*) were added daily to the basal diet to achieve an intake of 20 g CT/head per day. This led to three experimental treatments: C) Basal diet (Control); R) Basal diet supplemented with CT from rockrose; and Q) Basal diet supplemented with CT from quebracho.

Rockrose extract, which was prepared from aerial part of plant (leaves and soft stem) according to Guerreiro et al. (2020), containing 616.3 g/kg DM of CT. Extract of quebracho was a commercial extract (Angelo Coimbra S. A., Portugal) containing 518.9 g/kg DM of CT. During the experiment, the feeds were daily distributed after the morning milking. Sub-samples of basal diet and hay were collected weekly for chemical analysis. Diet ingredients and chemical composition are presented in Table 1.

The goats were milked twice daily at 06:30 and 14:30 using a milking machine, and individual milk yield was recorded 4 times during the experimental period (days 0, 7, 14 and 21). On same days, individual milk samples from morning and evening milking were collected and pooled according to diet and day of sampling and stored in aliquots for analysis. One aliquot was stored at 4 °C until analysis for fat, protein, lactose, urea and total solids, while other aliquot was stored at − 80 °C until FA analysis.

2.2. Cheese preparation

In the last week of experiment, milk from each goat from five consecutive milking was mixed and stored at 4 °C until cheese manufacture. Cheeses were prepared in the dairy of CEBA—DRAPAL according to the traditional manufacture method. Bulk milk of each goat was filtrated through a cotton cloth, and then heated to 80 °C for 5 min. After cooling the milk to 50 °C, was included the salt (15 g/L of milk) and coagulating agent (extract from *Cynara cardunculus* L.). Aqueous extracts of *C. cardunculus* flower is employed for centuries in French, Italian, Spanish and Portuguese cheesemaking (Conceição et al., 2018). The coagulant agent was prepared following a traditional method, which 3.8 g of dry flower (styles and stigmas) was placed in water infusion (100 mL) and macerated by hand in a mortar. The mixture was filtered through a piece of cotton cloth, and the recovered liquid was added to milk (3.8 mL/L of milk). After coagulation, the coagulum was cut, and the whey drained manually. Then, cheese was shaped in the form of a cylinder (70 mm of diameter) and transported to Laboratory of Tecnologia e Qualidade dos Produtos Regionais of University of Évora, where was maintained to 4 °C during 12 h. At this time, one cheese from each goat was divided into subsamples, vacuum-packed, and frozen at − 20 °C until analysis of chemical and FA composition.

2.3. Analytical methods

2.3.1. Feed analysis

Feeds were analysed for DM content (ISO, 6496, 1999), ash (ISO, 5984, 2002), crude protein (CP) (ISO, 5983, 1997) and ether extract (ISO, 6492, 1999). Sugar and starch were determined according to Clegg (1956). Neutral detergent fiber (NDF), assayed with sodium sulphite without α-amylase and expressed with residual ash, and acid detergent fiber (ADF) was determined according to Van Soest et al. (1991). The analysis of CT content of the rockrose and quebracho extracts was carried out as described by Guerreiro et al.

(2020) and using the purified CT from rockrose and quebracho as a standard, respectively. Condensed tannins from rockrose and quebracho were purified using a Sephadex LH-20 (GE Healthcare Bio-Science, Uppsala, Sweden) chromatographic column, according to Strumeyer and Malin (1975). Fatty acid methyl esters (FAME) from feeds were prepared by one-step extraction transesterification with toluene and nonadecanoic acid (C19:0, 1 mg/mL) as internal standard, according to Sukhija and Palmquist (1988).

2.3.2. Milk and cheese analysis

Milk was analysed in triplicate for total solids, protein, fat, lactose and urea content using a MilkoScan FT 6000 (Hillerød, Denmark) according to the reference method (ISO 1, 2329, 2020). Samples of fresh cheese were analyzed in triplicate for moisture (ISO, 5534, 2004), protein (ISO 1, 1489, 2002) and fat content (ISO, 3432, 2008).

Fatty acid analysis was performed in lyophilized milk fat and cheese samples. The FAME from milk fat samples were prepared as described by Santos-Silva et al. (2016) and from cheese samples were prepared as described by Sant'Ana et al. (2019). Then, FAME were analysed by gas chromatography coupled with flame ionization detection (GC-FID) (Shimadzu GC-2010 Plus, Kyoto, Japan), using a SP-2560 capillary column (100 m, 0.25 mm i.d., 0.20 μm film thickness; Supelco Inc., Bellefont, PA, USA). The chromatographic conditions were described by Sant'Ana et al. (2019). Nonadecanoic acid (19:0, 1 mg/mL) was used as internal standard for FA quantification. Identification of FAME was achieved by comparison of retention times with those of a commercial standard mixture (FAME mix 37 components from Supelco Inc.) and by electron impact mass spectrometry using a Shimadzu GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) with a SP-2560 column.

2.4. Statistical analysis

Data on milk yield and composition were analyzed for repeated measures using MIXED procedure of SAS (SAS Institute Inc., Cary NC) considering the diet (D), time (T) and their interactions (D x T) as fixed effects and block as a random effect. Covariance structures were tested and selected using both Akaike corrected and Bayesian information criteria after adjusting models using the AR(1), ARH(1), ANTE(1), TOEP, TOEPH, CS, CSH and UN covariance structures. The experimental unit was the animal.

Data on cheese composition was analyzed using MIXED procedure of SAS (SAS Institute Inc., Cary NC) and considering the diet (D) as fixed effect and block as a random effect. The cheese was prepared individually from the milk collected from each goat; therefore, the individual cheese sample was the experimental unit.

The variance homogeneity was tested at a level of $P = 0.001$, and when significant, the variance heterogeneity was accommodated in the model. Regarding to milk data, as the interaction D x T was not significant in most cases, only the means for the principal effects are present in the tables. Results on time effect are presents in supplementary material. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Animal performance and milk and cheese composition

Independently of the dietary treatment, the amount of basal diet offered daily (800 g/head) was completely consumed by the animals. Milk yield was not affected by the inclusion of rockrose and quebracho CT in the diets supplemented with soybean oil ($P = 0.186$), averaging 700 g/d (Table 2). Dietary treatments had no effect on milk components yield and composition. Milk yield was lower at the end of the experiment compared to day 0 ($P = 0.017$, 752 and 672 g/d at the days 0 and 21, respectively, Table 1 supplementary material). The milk yield of the days 7 and 14 was similar to other days. Regarding to milk composition, protein and urea contents varied significantly over the experimental period, while milk contents of total solids, fat, and lactose contents remained constants.

The gross fresh cheese composition (Table 3) was not affected by the inclusion of rockrose and quebracho CT in the diets ($P > 0.05$).

Table 2
Effect of dietary rockrose (R) and quebracho (Q) condensed tannin extracts on milk yield and composition.

	Diets			SEM	*P value*	
	C	R	Q		Diet (D)	Time (T)
Yield, g/d						
Milk	617.5	678.8	804.4	72.35	0.186	0.017
Total solids	102.2	106.5	127.7	11.20	0.234	0.014
Fat	43.05	42.84	51.39	4.759	0.356	0.026
Protein	25.30	27.80	33.97	3.919	0.106	0.033
Urea, mg/d	463.8	446.5	465.4	12.11	0.488	< 0.001
Lactose	36.80	35.58	35.91	0.553	0.297	0.084
Composition, g/100 g						
Total solids	16.50	15.65	16.01	0.280	0.132	0.297
Fat	6.92	6.29	6.46	0.197	0.072	0.312
Protein	4.13	4.10	4.26	0.092	0.435	0.013
Urea, mg/100 g	61.03	58.75	61.24	1.593	0.488	< 0.001
Lactose	4.84	4.68	4.73	0.073	0.297	0.084

Values are means ± standard error of the mean. Means within a row with different letters are significantly different ($P < 0.05$).

3.2. Milk and cheese fatty acid composition

The effect of inclusion of rockrose and quebracho CT in the goat diets on FA composition in the milk fat and cheese is reported in Tables 4 and 5, respectively. The supplementary material, Table 2, reports the milk FA composition over the experimental period. The inclusion of CT-rich extracts from rockrose and quebracho in the goat diets did not modify the concentration of the major classes of milk FA, with average of 643, 273 and 54 mg/g of total FA for linear chain saturated fatty acids (LC-SFA), monounsaturated fatty acids (MUFA) and PUFA, respectively. Major classes of FA in fresh cheese were also not affected by dietary treatments, averaging 654, 373 and 51 mg/g of total FA for LC-SFA, MUFA and PUFA, respectively.

In both milk and cheese, the effect of the inclusion of CT-rich extracts in diets was limited. The milk contents of *iso*-17:0 and *anteiso*-17:0 decrease in response to inclusion of both CT-rich extracts in diets. Lower content of *anteiso*-15:0 was observed in milk from goats supplemented with CT from rockrose when compared to milk from goats fed other diets (Table 4). Cheese from goats that received rockrose CT had lower contents of *anteiso*-15:0 and *anteiso*-17:0 when compared to cheese from goats fed control diet, while cheese from goats fed quebracho CT had similar levels to both diets. *Iso*-18:0 was only found in cheese from goats fed CT sources (Table 5).

Only two BI in milk were affected by the inclusion of CT-rich extracts in the goat diets. The milk content of 18:1 *trans*-10 decreased in response to the inclusion of both CT sources in diets ($P < 0.007$, 8.19 *vs.* 6.88 mg/g of total FA in control and CT supplemented diets, respectively) (Table 4). Rockrose CT reduced the contents of 18:1 *trans*-9 in milk ($P < 0.004$, 4.39 mg/g of total FA) compared to control diet (5.04 mg/g of total FA), while milk from goats fed quebracho CT had similar levels to both diets.

4. Discussion

Serpentine goat is an autochthonous breed of the Alentejo region, Portugal, farmed mostly as a dual-purpose breed (meat and milk). In Portugal there is no tradition of consumption of raw goat's milk, and practically all the goat milk is used for cheese production, both fresh and ripened. This study was carried out to test the hypothesis that the inclusion of CT from rockrose and quebracho in dairy goat diets supplemented with PUFA-rich vegetable oil would increase the healthy FA in milk fat and cheese, without compromising animal performance and milk and cheese composition.

The effects of dietary CT on feed efficiency, animal health and performance and on the quality of their products have been extensively investigated in ruminants (Jerónimo et al., 2016; Makkar, 2003; Min et al., 2003; Patra and Saxena, 2011; Pilluza et al., 2014). There is consensus that CT can induce detrimental, innocuous, or beneficial effects depending on several factors, including CT chemical structure, amount ingested, basal diet, and animal species (Makkar, 2003; Patra and Saxena, 2011). In the present work, the intake of basal diet and milk production and composition were not affected by the inclusion of both CT sources in diets. However, the milk fat content tends to decrease with the inclusion of rockrose and quebracho CT in diets ($P = 0.072$). Higher values of milk yield were found in the CT diets, particularly in the diet with quebracho CT, suggesting that the fat decrease is due to the dilution effect. However, the dilution effect is not clear once the milk yield was not significantly affected by the dietary treatment. The effect of CT from rockrose on milk composition was evaluated for the first time in the present study. On the contrary, the impact of dietary quebracho CT on ruminant milk composition is better known, although as far as we know there are no reports on the effect of dietary quebracho on goat milk composition. Henke et al. (2017a) reported a reduction in milk fat yield in cows supplemented with 30 g/kg DM of quebracho, although the milk of these cows had a higher fat content compared with the CT unsupplemented diet. However, in other studies with sheep and cows that received CT from quebracho, were not observed changes in the milk fat yield and content, even for the highest levels of quebracho extract inclusion in the diets (Buccioni et al., 2017, 2015; Dschaak et al., 2011; Toral et al., 2013).

Condensed tannins from different sources, including rockrose and quebracho, have been successfully applied to protect the dietary protein against extensive ruminal degradation, improving the protein utilization efficiency with economic and environmental gains (Dentinho et al., 2020, 2014, 2007; Dschaak et al., 2011; Frutos et al., 2000). However, dietary CT can negatively impact milk protein content, as reported by Avila et al. (2020) when dairy cows' diets were supplemented with increasing levels of CT extract from *Acacia mearnsii*. Moreover, the milk urea content reduction is also reported when different CT sources were incorporated into dairy cow diets (Dschaak et al., 2011; Girard et al., 2016; Henke et al., 2017a; Menci et al., 2021; Zhang et al., 2019). The lack of effect of dietary CT supplementation on protein and urea contents in milk observed in the present work suggests that the levels of CT from rockrose and quebracho used did not affect the ruminal N metabolism.

Goat is a livestock species with high importance in several world regions, such as in the Mediterranean area, producing a variety of high-quality dairy products. Despite the contribution of goat milk and dairy products to the human diet in developing and developed countries, the modulation of ruminal BH in this species and the FA composition improvement of its milk were less explored than in

Table 3
Effect of dietary rockrose (R) and quebracho (Q) condensed tannin extracts on fresh cheese composition.

	Diets			SEM	*P value*
	C	R	Q		
Composition					
Moisture, g/100 g	64.6	66.2	68.0	1.09	0.106
Fat, g/100 g DM	45.6	48.1	49.3	3.44	0.690
Protein, g/100 g DM	40.1	40.0	40.8	1.77	0.938

Values are means ± standard error of the mean. Means within a row with different letters are significantly different ($P < 0.05$).

Table 4
Effect of dietary rockrose (R) and quebracho (Q) condensed tannin extracts on fatty acid composition of milk (mg/g of total fatty acids).

	Diets			SEM	*P value*	
	C	R	Q		Diet (D)	Time (T)
Linear chain saturated fatty acids (LC-SFA)						
4:0	19.7	19.4	19.9	0.85	0.837	< 0.001
6:0	26.3	26.5	26.3	0.79	0.970	< 0.001
8:0	33.5	34.9	33.5	1.25	0.630	< 0.001
10:0	108	112	104	5.4	0.417	0.006
11:0	3.31	3.52	3.49	0.300	0.711	< 0.001
12:0	41.3	44.9	41.1	3.86	0.583	0.006
13:0	0.58	0.61	0.56	0.058	0.657	0.681
14:0	81.1	86.5	78.6	3.68	0.310	0.023
15:0	7.31	6.99	7.26	0.198	0.467	0.023
16:0	204	198	197	7.1	0.558	< 0.001
17:0	4.31	4.16	4.24	0.164	0.730	0.899
18:0	102	103	105	6.7	0.953	0.083
20:0	6.33	7.22	6.90	0.411	0.315	< 0.001
21:0	0.63	0.67	0.68	0.047	0.746	< 0.001
22:0	3.86	3.86	4.09	0.344	0.865	< 0.001
23:0	0.65	0.66	0.68	0.043	0.881	< 0.001
24:0	0.82	0.80	0.90	0.068	0.581	< 0.001
Sum LC-SFA	643	653	634	6.8	0.148	< 0.001
Sum LC $\leq$ 14	313	328	306	10.5	0.361	0.005
Sum LC > 14	330	325	327	5.8	0.844	0.648
Branched-chain fatty acids (BCFA)						
iso-14:0	0.78	0.74	0.83	0.057	0.381	0.006
iso-15:0	1.86	1.77	1.73	0.097	0.443	0.042
anteiso-15:0	3.22[b]	2.73[a]	3.15[b]	0.104	0.008	0.202
iso-16:0	2.32	2.40	2.33	0.144	0.893	0.027
iso-17:0	3.33[b]	2.81[a]	2.84[a]	0.143	< 0.001	0.067
anteiso-17:0	3.74[b]	2.92[a]	3.16[a]	0.173	< 0.001	0.160
iso-18:0	0.34	0.32	0.32	0.040	0.911	0.142
Sum *iso*-BCFA	8.63	8.04	8.08	0.416	0.234	0.006
Sum *anteiso*-BCFA	6.96[b]	5.65[a]	6.30[ab]	0.296	< 0.001	0.171
Sum BCFA	15.6[b]	13.7[a]	14.4[a]	0.66	0.014	0.012
Monounsaturated fatty acids (MUFA)						
14:1 *cis*-9	1.39	1.46	1.36	0.174	0.794	0.224
16:1 *trans*-9	1.89	1.61	1.83	0.160	0.423	< 0.001
16:1 *cis*-7	2.27	2.26	2.48	0.127	0.141	< 0.001
16:1 *cis*-9	4.61	4.10	4.22	0.298	0.079	< 0.001
17:1 *cis*-9	1.83	1.65	1.64	0.091	0.289	0.128
18:1 *trans*-4	0.30	0.23	0.25	0.025	0.181	0.265
18:1 *trans*-5	0.29	0.26	0.28	0.026	0.534	0.761
18:1 *trans*-6/7/8	4.21	3.75	4.10	0.224	0.091	< 0.001
18:1 *trans*-9	5.04[b]	4.39[a]	4.76[ab]	0.194	0.004	< 0.001
18:1 *trans*-10	8.19[b]	6.79[a]	6.97[a]	0.402	0.007	0.004
18:1 *trans*-11	29.0	23.9	25.5	2.90	0.209	< 0.001
18:1 *trans*-12	6.56	6.22	6.62	0.317	0.525	< 0.001
18:1 *cis*-9[a]	196	197	209	5.3	0.189	0.071
18:1 *trans*-15	2.67	2.68	2.92	0.164	0.420	< 0.001
18:1 *cis*-11	3.40	3.09	3.26	0.142	0.155	0.071
18:1 *cis*-12	5.74	4.98	5.72	0.358	0.192	< 0.001
18:1 *cis*-13	0.33	0.32	0.30	0.025	0.655	0.708
18:1 *trans*-16/*cis*-14	3.29	3.45	3.63	0.186	0.467	< 0.001
18:1 *cis*-15	0.45	0.41	0.47	0.031	0.388	0.098
18:1 *cis*-16	0.78	0.76	0.73	0.053	0.793	0.965
Sum	274	265	281	5.7	0.175	0.034
Polyunsaturated fatty acids (PUFA)						
18:2 isomers						
18:2 *cis*-9, *trans*-13[b]	3.53	3.65	4.04	0.169	0.133	< 0.001
18:2 *cis*-9, *trans*-15[c]	1.34	1.47	1.58	0.076	0.108	0.238
18:2 *cis*-9, *trans*-12	0.80	0.75	0.84	0.056	0.259	< 0.001
18:2 *trans*-9, *cis*-12	0.50	0.43	0.43	0.032	0.220	0.416
18:2 *trans*-11, *cis*-15[d]	0.84	0.69	0.75	0.057	0.134	< 0.001
18:2 *cis*-9, *trans*-11[e]	17.0	14.0	15.5	1.05	0.138	< 0.001
Sum	24.5	21.5	23.7	1.05	0.122	< 0.001
n-6 polyunsaturated fatty acids (*n*-6 PUFA)						
18:2*n*-6	18.7	21.1	21.2	1.08	0.198	< 0.001
20:3*n*-6	0.14	0.16	0.17	0.028	0.666	0.041
20:4*n*-6	1.60	1.55	1.52	0.064	0.685	0.874

(*continued on next page*)

Table 4 (*continued*)

	Diets			SEM	*P value*	
	C	R	Q		Diet (D)	Time (T)
Sum	20.4	22.8	22.9	1.11	0.225	< 0.001
n-3 polyunsaturated fatty acids (*n*-3 PUFA)						
18:3*n*-3	3.54	3.87	3.98	0.174	0.148	< 0.001
20:3*n*-3	3.02	3.42	3.66	0.397	0.519	< 0.001
20:5*n*-3	0.25	0.27	0.30	0.035	0.549	< 0.001
22:5*n*-3	1.55	1.43	1.44	0.081	0.547	< 0.001
Sum	8.36	8.99	9.37	0.568	0.447	< 0.001
Total PUFA	53.3	53.3	56.0	1.87	0.491	< 0.001
Sums and ratios						
Total BI[f]	91.4	79.6	86.0	5.05	0.103	< 0.001
Total BI 18:1	66.8	58.1	62.4	4.02	0.128	< 0.001
Total BI 18:2	24.5	21.5	23.7	1.04	0.117	< 0.001
SCDi-17[g]	29.7	28.4	27.7	0.93	0.332	0.220

Values are means ± standard error of the mean. Means within a row with different letters are significantly different ($P < 0.05$).

[a] Coelutes with minor quantities of 18:1*trans*-13/*trans*-14.

[b] Coelutes with 18:2 *cis*-9, *trans*-14, 18:2 *trans*-8, *cis*-12 and 11-cyclohexyl-11:0.

[c] Coelutes with 18:2 *trans*-8, *cis*-13.

[d] Coelutes with 18:2 *trans*-10, *cis*-15.

[e] Coelutes with 18:2 *trans*-7, *cis*-9 and 18:2 *trans*-8, *cis*-10.

[f] Biohydrogenation intermediates.

[g] Stearoyl-CoA activity index computed as [17:1 *cis*-9/(17:1 *cis*-9 + 17:0)] x 100.

dairy cows and ewes. Several nutritional approaches have been explored to modulate the ruminal BH and, consequently, the FA composition of ruminant fat. In the last decade, utilization of tannins, either condensed as hydrolyzed tannins, is one of the most studied strategies by ruminant nutritionists, and despite the apparent inconsistencies, tannins show ability to induce beneficial changes in the ruminal BH increasing the rumen outflow of dietary PUFA and/or healthy BI (Frutos et al., 2020; Vasta and Bessa, 2012). Contrary to our expectations, supplementation of goat diets with CT from rockrose and quebracho did not increase the levels of healthy FA in milk and cheese. However, both CT sources reduced the proportion of 18:1 *trans*-10 in milk fat, and the lowest proportion of 18:1 *trans*-9 was found in milk from goats fed rockrose CT, suggesting that CT from rockrose and quebracho affect the ruminal BH, although without the expected beneficial effects on milk and cheese FA composition.

Quebracho is one of the most studied tannin sources as a ruminal BH modulator. Increase of the 18:2*n*-6 and/or 18:3*n*-3 concentration in the ruminal digesta (Carreño et al., 2015; Vasta et al., 2008), lamb intramuscular fat (Vasta et al., 2009) and in cow and ewe milk (Buccioni et al., 2015; Dschaak et al., 2011; Henke et al., 2017b) in response to quebracho extracts was found in various *in vitro* and *in vivo* trials, suggesting that quebracho CT inhibit the initial BH step of the dietary PUFA. On the other hand, other reports showed that quebracho extract increased the 18:1 *trans*-11 levels in ruminal digesta (Vasta et al., 2008), lamb meat (Vasta et al., 2009) and ewe milk (Buccioni et al., 2015), suggesting the ability of quebracho tannins to inhibit the last step of BH. However, the quebracho doses used in the present work seem not to be enough to affect BH extension and the BH pathways associated to the production of healthy BI in dairy goats, which is also in agreement with other reports. Benchaar and Chouinard (2009) and Toral et al. (2011) reported that diet supplementation with 6.7 and 10 g/kg DM of quebracho CT extract did not affect the FA composition of bovine and ewe milk, respectively. Moreover, in agreement with our find, supplementation of dairy ewe diets with 20 g of quebracho extract/kg of diet DM modified the milk FA composition, but without inducing a persistent increase of the beneficial FA (Toral et al., 2013).

The available literature on the ability of CT from rockrose to modulate the ruminal BH is mainly focused on the utilization of soft stems and leaves of rockrose, as a source of CT, in lamb diets, while the impact of CT-rich extract from rockrose on ruminal BH only was evaluated in two *in vitro* studies and in one *in vivo* experiment with lambs. Inclusion of soft stems and leaves of rockrose in lamb diets supplemented with vegetable oils seems to affect ruminal BH by inhibiting the last reductive step, increasing the ruminal production of 18:1 *trans* BI (Alves et al., 2017; Jerónimo et al., 2010). *In vitro* studies showed that CT-rich extract from rockrose (100 g/kg DM) can change the ruminal BH, increasing the disappearance of dietary PUFA and the accumulation of 18:1 *trans*-11 and 18:1 *cis*-9, *trans*-11 in ruminal fluid, without reduction in the 18:0 production suggesting that rockrose CT promote the initial BH steps (Costa et al., 2017; Guerreiro et al., 2016). Conversely, when CT-rich extract from rockrose (12.5 and 25 g/kg DM of CT) was applied in lamb diets composed of dehydrated Lucerne supplemented with soybean oil, the ruminal production of 18:1 *trans*-11 did not increase (Guerreiro et al., 2022). Factors such as tannin chemical structure and levels in diets, the composition of basal diet, and ruminant species can help to explain the inconsistent findings on the impact of CT-rich extract from quebracho and rockrose on ruminal BH and FA composition of ruminant fat.

Differences in the sensitivity of the ruminal ecosystem to CT among ruminant species might contribute to the distinct effect of CT on ruminal BH. Different ruminant species seem to differ in their sensitivity and tolerance to CT (Frutos et al., 2004; Lamy et al., 2011). Goats are predominantly browsers, consuming large amounts of tannins-rich feeds without adverse effects (Silanikove et al., 1996b, 1996a). Superior tolerance of goats to tannins-rich feeds may limit the effectiveness of CT extracts to modulate the ruminal ecosystem and BH, and higher levels of CT may be necessary to modify BH in these species.

The most expressive effect of both CT sources on milk FA composition was the reduction of 18:1 *trans*-10 content. Reduction of the

Table 5
Effect of dietary rockrose (R) and quebracho (Q) condensed tannin extracts fatty acid composition of cheese (mg/g of total fatty acids).

	Diets			SEM	*P value*
	C	R	Q		
Linear chain saturated fatty acids (LC-SFA)					
4:0	20.7	20.5	21.0	1.04	0.914
6:0	27.3	28.1	27.5	1.18	0.770
8:0	35.1	37.2	34.7	1.68	0.492
10:0	110	116	106	4.7	0.394
11:0	3.99	4.22	3.78	0.281	0.479
12:0	45.8	48.8	45.2	3.68	0.765
13:0	0.54	0.60	0.63	0.070	0.680
14:0	85.4	91.1	81.7	3.64	0.145
15:0	7.65	7.05	7.38	0.344	0.758
16:0	208	202	205	8.0	0.775
17:0	4.58	4.24	4.38	0.207	0.531
18:0	97.9	91.5	106	5.29	0.187
20:0	3.46	4.02	3.72	0.211	0.136
21:0	0.64	0.53	0.64	0.059	0.241
22:0	1.57	1.64	1.73	0.150	0.693
23:0	0.29	0.32	0.32	0.044	0.882
24:0	0.44	0.38	0.43	0.090	0.782
Sum LC-SFA	654	658	650	11.2	0.825
Branched-chain fatty acids (BCFA)					
iso-14:0	0.76	0.72	0.84	0.113	0.693
iso-15:0	2.08	1.77	1.84	0.159	0.090
anteiso-15:0	3.57[b]	2.71[a]	3.24[ab]	0.198	0.019
iso-16:0	2.47	2.47	2.43	0.273	0.993
iso-17:0	3.37	3.07	3.04	0.197	0.368
anteiso-17:0	3.89[b]	3.07[a]	3.35[ab]	0.189	0.017
iso-18:0	0[a]	0.39[b]	0.33[b]	0.047	< 0.001
Sum-BCFA	16.1	14.2	15.2	0.823	0.141
Monounsaturated fatty acids (MUFA)					
14:1 *cis*-9	1.48	1.62	1.44	0.199	0.758
16:1 *trans*-9	1.60	1.52	1.59	0.131	0.877
16:1 *cis*-7	2.46	2.42	2.18	0.168	0.459
16:1 *cis*-9	5.24	4.96	5.34	0.393	0.225
17:1 *cis*-9	2.05	1.89	1.75	0.143	0.343
18:1 *trans*-4	0.20	024	0.15	0.052	0.487
18:1 *trans*-5	0.16	0.18	0.13	0.035	0.401
18:1 *trans*-6/7/8	3.56	3.66	3.70	0.221	0.872
18:1 *trans*-9	4.61	4.38	4.41	0.231	0.499
18:1 *trans*-10	8.00	7.43	6.74	0.623	0.234
18:1 *trans*-11	24.0	21.2	20.6	2.31	0.440
18:1 *trans*-12	5.90	6.18	6.13	0.286	0.570
18:1 *cis*-9[a]	197	196	207	7.4	0.535
18:1 *trans*-15	2.47	2.48	2.69	0.160	0.542
18:1 *cis*-11	3.34	3.12	3.16	0.211	0.516
18:1 *cis*-12	5.19	5.05	5.06	0.453	0.968
18:1 *cis*-13	0.32	0.53	0.35	0.075	0.006
18:1 *trans*-16/*cis*-14	3.15	3.48	3.59	0.193	0.277
18:1 *cis*-15	0.46	0.59	0.50	0.096	0.619
18:1 *cis*-16	0.70	0.77	0.88	0.156	0.318
Sum	372	361	385	10.3	0.246
Polyunsaturated fatty acids (PUFA)					
18:2 isomers					
18:2 *cis*-9, *trans*-13[b]	3.52	3.79	3.72	0.305	0.559
18:2 *cis*-9, *trans*-15[c]	1.48	1.59	1.52	0.108	0.766
18:2 *cis*-9, *trans*-12	0.79	0.90	0.88	0.091	0.318
18:2 *trans*-9, *cis*-12	0.59	0.40	0.56	0.092	0.065
18:2 *trans*-11, *cis*-15[d]	0.63	0.54	0.47	0.067	0.239
18:2 *cis*-9, *trans*-11[e]	15.9	14.2	13.3	1.46	0.411
Sum	23.3	22.1	21.1	1.75	0.569
n-6 polyunsaturated fatty acids (*n*-6 PUFA)					
18:2*n*-6	21.2	23.7	23.2	1.24	0.329
20:3*n*-6	0.14	nd	0.32	0.071	0.171
20:4*n*-6	1.59	1.62	1.53	0.097	0.794
Sum	22.8	25.3	24.9	1.28	0.354
n-3 polyunsaturated fatty acids (*n*-3 PUFA)					
18:3*n*-3	2.79	3.03	2.84	0.169	0.360
20:3*n*-3	0.53	0.67	0.60	0.139	0.680

(*continued on next page*)

Table 5 (*continued*)

	Diets			SEM	*P value*
	C	R	Q		
20:5*n*-3	0.37	0.38	0.28	0.052	0.309
22:5*n*-3	0.86	0.76	0.74	0.091	0.558
Sum	4.31	4.58	4.37	0.331	0.681
Total PUFA	50.4	52.0	50.2	2.59	0.842

Values are means ± standard error of the mean. Means within a row with different letters are significantly different ($P < 0.05$). nd - not detected
[a] Coelutes with minor quantities of 18:1*trans*-13/*trans*-14.
[b] Coelutes with 18:2 *cis*-9, *trans*-14, 18:2 *trans*-8, *cis*-12 and 11-cyclohexyl-11:0.
[c] Coelutes with 18:2 *trans*-8, *cis*-13.
[d] Coelutes with 18:2 *trans*-10, *cis*-15.
[e] Coelutes with 18:2 *trans*-7, *cis*-9 and 18:2 *trans*-8, *cis*-10.

18:1 *trans*-10 due to different CT sources was observed in digesta and milk as reviewed by Frutos et al. (2020). It is generally assumed that a specific bacterial strain is involved in each BH pathway (Bessa et al., 2015). So, the reduction of 18:1 *trans*-10 content in milk fat in animals that received CT-rich extracts without effect on 18:1 *trans*-11 level suggests that CT from quebracho and rockrose affect the bacterial involved in 18:1 *trans*-10 BH pathway but not those conducting the mainstream 18:1 *trans*-11 BH pathways. The effect of CT-rich extracts on milk content and pattern of BCFA, which are markers of the rumen microbial population (Vlaeminck et al., 2006), is consistent with the interaction of CT with ruminal microbime.

The supplementation of diets with PUFA sources has shown to be a valuable strategy to obtain milk and cheese with better nutritional value, increasing the 18:1 *trans*-11 and 18:2 *cis*-9, *trans*-11 contents (Chilliard and Ferlay, 2004; Jerónimo and Malcata, 2013). However, in certain conditions, such as when using a low-fiber and high-starch basal diet, the dietary supplementation with PUFA is associated with a shift in the rumen BH pathways with the production of 18:1 *trans*-10 to the detriment of 18:1 *trans*-11 (Aldai et al., 2013). This change in the rumen BH pathways is not desirable due to detrimental effects 18:1 *trans*-10 (Mapiye et al., 2015; Wang et al., 2012) and to low levels of CLA isomers in fat, once endogenous conversion of 18:1 *trans*-10 into CLA isomers is not possible (Bessa et al., 2015). However, the basal diet used in the present work, which contains 250 g/kg DM of dehydrated Lucerne and part of the high-starch ingredients (cereal) were replaced by a low-starch ingredient (beet pulp), resulted in milk fat and cheese with levels of 18:1 *trans*-11 about 3-fold higher than 18:1 *trans*-10.

In the present study, the 18:1 *trans*-11 level averaged 26.1 and 21.9 mg/g of total FA in milk fat and cheese, respectively, and levels of 18:2 *cis*-9, *trans*-11 averaged 15.5 and 14.5 mg/g of total FA in milk fat and cheese, respectively. Levels of 18:1 *trans*-11 and 18:2 *cis*-9, *trans*-11 observed in milk fat of the present study were higher than those found in animals fed PUFA unsupplemented diets, as reviewed by Chilliard and Ferlay (2004). The milk fat of goats fed PUFA unsupplemented diets showed values of 4.5–12.7 mg/g of total FA of 18:1 *trans*-11 and 3–7 mg/g of total FA of 18:2 *cis*-9, *trans*-11 (Chilliard and Ferlay, 2004).

5. Conclusions

Utilization of a basal diet composed of 250 g/kg DM of dehydrated Lucerne, with partial replacement of cereals by a low-starch ingredient and supplemented with 50 g/kg DM of soybean oil resulted in goat milk and cheese with high levels of healthy FA. Incorporating CT from rockrose and quebracho in goat diets did not increase PUFA, 18:1 *trans*-11 and 18:2 *cis*-9, *trans*-11 levels in milk and cheese. However, both CT extracts reduced the levels of 18:1 *trans*-10 in milk, although this reduction may not have a relevant impact on human health. Condensed tannin sources and level, as well as the high tolerance of goats to dietary tannins can help to explain the lack of effect on the beneficial FA. Research on the use of CT as modulators of the ruminal BH in goats is still limited, and other sources and levels CT should be tested.

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Investigation; Writing – review & editing. **Rui J. B. Bessa:** Conceptualization; Writing – review & editing. **José Santos-Silva**: Conceptualization, Writing – review & editing. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2023.115654.

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