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Cheese quality from cows given a tannin extract in 2 different grazing seasons

R. Menci,[1] A. Natalello,[1]* G. Luciano,[1] A. Priolo,[1] B. Valenti,[2] A. Difalco,[3] T. Rapisarda,[3] M. Caccamo,[3] I. Constant,[4] V. Niderkorn,[4] and M. Coppa[5]

[1]Department Di3A, University of Catania, via Valdisavoia 5, 95123 Catania, Italy
[2]Department DSA3, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
[3]Consorzio per la Ricerca nel settore della Filiera Lattiero-Casearia e dell'agroalimentare (CoRFiLaC), Regione Siciliana, 97100 Ragusa, Italy
[4]INRAE, Université Clermont Auvergne, Vetagro Sup, UMRH, 63122, Saint-Genès-Champanelle, France
[5]Independent researcher at INRAE, Université Clermont Auvergne, Vetagro Sup, UMRH, 63122, Saint-Genès-Champanelle, France

ABSTRACT

The aim of the present study was to compare the effect of dietary tannins on cow cheese quality in 2 different grazing seasons in the Mediterranean. Two experiments were performed on 14 dairy cows reared in an extensive system. The first experiment took place in the wet season (WS), and the second experiment took place in the dry season (DS). In the WS and DS experiments, cows freely grazed green pasture or dry stubbles, respectively, and the diet was supplemented with pelleted concentrate and hay. In both experiments, the cows were divided into 2 balanced groups: a control group and a group (TAN) receiving 150 g of tannin extract/head per day. After 23 d of dietary treatment, individual milk was collected, processed into individual cheeses, and aged 25 d. Milk was analyzed for chemical composition, color parameters, and cheesemaking aptitude (laboratory cheese yield and milk coagulation properties). Cheese was analyzed for chemical composition, proteolysis, color parameters, rheological parameters, fatty acid profile, and odor-active volatile compounds. Data from the WS and DS experiments were statistically analyzed separately with an analysis of covariance model. In the WS experiment, dietary tannin supplementation had no effect on milk and cheese parameters except for a reduced concentration of 2-heptanone in cheese. In the DS experiment, TAN milk showed lower urea N, and TAN cheese had lower C18:1 *trans*-10 concentration and n-6:n-3 polyunsaturated fatty acid ratio compared with the control group. These differences are likely due to the effect of tannins on rumen N metabolism and fatty acid biohydrogenation. Dietary tannins may differently affect the quality of cheese from Mediterranean grazing cows according to the grazing season. Indeed, tannin bioactivity on rumen metabolism seems to be enhanced during the dry season, when diet is low in protein and rich in acid detergent fiber and lignin. The supplementation dose used in this study (1% of estimated dry matter intake) had no detrimental effects on cheese yield or cheesemaking parameters. Also, it is unlikely that sensorial characteristics would be affected by this kind of dietary tannin supplementation.
Key words: tannin, cheese quality, dairy cow, grazing season

INTRODUCTION

Tannins are a class of polyphenols found in forages, especially in plant species characterizing marginal areas or dry habitats, and in agricultural by-products (Vasta et al., 2019). Thanks to their antimicrobial and protein-binding activities, tannins are known to modify ruminal biohydrogenation (**BH**) and N metabolism (Patra and Saxena, 2011), with positive consequences on milk and cheese quality. Through the impairing of ruminal BH, dietary tannins are often reported to reduce the SFA content and increase the concentration of PUFA, C18:1 *trans*-11, and C18:2 *cis*-9,*trans*-11 in milk (Frutos et al., 2020). Binding with proteins, tannins can improve the ratio of ruminal protein escape (Waghorn, 2008), increasing both the NAN outflow from the rumen and the EAA concentration of plasma (Min et al., 2002). This could affect cheesemaking properties and cheese quality, as the concentration and type of protein in milk markedly affect cheese yield and its syneretic and rheological characteristics (Guinee, 2003). For instance, dietary chestnut tannin extract was found to increase the casein proportion in ewe milk (Buccioni et al., 2015b) and to delay clotting and firming time (Buccioni et al., 2017).

However, the information available in the literature does not clarify whether and how the effects of dietary

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*Corresponding author: antonio.natalello@unict.it

tannins on cheese quality might vary according to grazing season in extensive farming systems. Indeed, extensive farming systems are characterized by periods with different forage availability during the year because they strictly depend on climatic conditions that allow grazing or not (Ramírez-Rivera et al., 2019). This imbalance in diet during the year has well-known implications for animal performance and product quality. In Mediterranean traditional husbandry, dairy cows have higher milk yield, protein content, and fat content during the green season compared with the dry season (Licitra et al., 1998). In addition, grazing green pasture is reported to increase the content of vitamins and aromatic compounds (Prache et al., 2020) and proportions of PUFA and CLA (Coppa et al., 2019) of milk.

In a recent study, a different response to in vitro rumen BH and fermentation was observed when 2 different tannin extracts were supplemented to a green forage or a hay substrate (Menci et al., 2021). For instance, both the ratio of C18:2 *cis*-9,*trans*-11 to C18:2 *cis*-9,*cis*-12 and valerate concentration were lower when tannin extracts were included in the hay substrate. Therefore, we hypothesized that dietary tannins could exert different effects on cow cheese quality and composition when supplemented to a green herbage-based diet or a dry forage-based diet. Thus, the aim of the present study was to compare, for the first time, the effect of dietary tannins on cow cheese quality in 2 different grazing seasons in the Mediterranean. We chose to fit into on-farm conditions to directly test the practical effects of dietary tannin extract supplementation. Previous to this study, the response of some of the parameters analyzed, such as rheology and aromatic compounds, to dietary tannin supplementation had never been investigated.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

All procedures were approved by the animal welfare committee (OPBA) of the University of Catania (UNCTCLE-0015295). Two experiments were conducted in a commercial extensive farm located in the municipality of Ragusa, Italy (36°57′ N, 14°40′ E; altitude: 670 m; annual rainfall: 560 mm), an upland area of the Mediterranean island of Sicily, Italy. The 2 experiments were carried out in different seasons: the first one was conducted in the wet season (**WS**), and the second one was conducted in the dry season (**DS**). The WS experiment was performed in the period between March and April 2019, with a total rainfall of 48.5 mm and the temperature ranging from 4 to 18°C (average temperature: 10°C). The DS experiment was performed in July 2019, with a total rainfall of 1.25 mm and the temperature ranging from 15° to 37°C (average temperature: 24.5°C).

In both experiments, 14 lactating dairy cows (Modicana breed) were used. On the 2 d preceding the beginning of both trials, individual milk was sampled and analyzed. Animals were divided into 2 equivalent groups (n = 7)—control (**CON**) and tannin (**TAN**)—balanced for average milk yield (WS: 11.9 ± 1.5 kg/d; DS: 13.6 ± 2.6 kg/d), protein (WS: 40.2 ± 1.3 g/kg; DS: 33.5 ± 1.6 g/kg), and fat (WS: 39.6 ± 2.4 g/kg; DS: 37.2 ± 3.3 g/kg) contents recorded in these 2 d as well as DIM (WS: 190 ± 38 d; DS: 137 ± 43 d), parity (WS: 4 ± 1; DS: 4.2 ± 1), and BCS (WS: 2.8 ± 0.2; DS: 3.6 ± 0.1).

In WS, the cows were free to graze on 20 ha of spontaneous pasture and had free access to drinking water. Thirty botanical species were identified through botanical surveys of the pasture using the vertical point-quadrat method (Daget and Poissonet, 1971). The main botanical species were *Bromus hordaeceus* L., *Medicago polymorpha* L., *Lolium perenne* L., and *Anthemis arvensis* L. (17, 13, 12, and 11% on ground cover, respectively). In DS, the cows were free to graze on 20 ha of dry stubble (20-cm harvest cut height because of rocky soil) of an annual crop, composed of vetch (40%), oat (40%), and barley (20%). During the DS experiment, no fresh herbage was available. In both experiments, supplemental pelleted concentrate was individually offered to cows in 2 equal meals just before milking at a rate of 6.4 and 9.6 kg/head per day in WS and DS, respectively. Pelleted concentrate was composed of corn grain (420 g/kg), soybean meal CP 48% (250 g/kg), wheat middlings (100 g/kg), corn flakes (66 g/kg), carob germ (60 g/kg), carob pods (30 g/kg), beet pulp (30 g/kg), rumen-protected fat (10 g/kg; Magnapac, Or Sell S.p.a.), Na_2CO_3 (10 g/kg), Ca_2CO_3 (10 g/kg), NaCl (8 g/kg), vitamin and mineral supplement (4 g/kg), and urea (2 g/kg). In addition, in both experiments cows were individually fed supplemental hay (vetch:oat:barley 40:40:20) during milking in 2 equal meals at a rate of 2 kg/head per day. Pelleted concentrate and hay were always completely consumed by all the cows. The chemical composition of feedstuffs is shown in Table 1.

In both the WS and DS experiments, the TAN group received 150 g/head per day of a commercial tannin extract (Silvafeed ByProX, Silvateam), a 60:40 mixture of chestnut (*Castanea sativa* Mill.) and quebracho (*Schinopsis lorentzii* Engl.) tannins, included in pelleted concentrate. Total phenolic compound concentration in tannin extract was 688 g of tannic acid equivalents/kg of DM, with 90.2% of tannins, according to the method

Table 1. Chemical composition of feeds used in the wet season (WS) and dry season (DS) experiments

Item	Concentrate	Hay	Pasture (only WS)	Stubble (only DS)
DM, g/kg	889	833	186	876
Chemical composition, g/kg of DM				
CP	200	79	222	69
Ether extract	36	12	28	11
NDF	179	708	415	672
ADF	80	460	269	472
ADL	22	62	38	76
Ash	51	66	94	67
Phenolic compounds, g of TAeq[1]/kg of DM				
Phenols	5.2	5.2	14.2	5.4
Tannins	3.9	1.6	4.7	1.7
Protein fraction,[2] g/100 g of CP				
A	15.1	37.3	37.1	22.3
B1	7.9	10.8	7.6	18.2
B2	59.6	12.2	21.4	20.3
B3	12.9	29.5	28.2	26.0
C	4.5	10.1	5.8	13.1
Fatty acids, g/100 g of fatty acids				
C16:0	18.2	29.2	14.1	25.1
C18:0	9.2	6.0	2.4	6.2
C18:1 *cis*-9	16.8	9.4	3.0	6.5
C18:2 *cis*-9,*cis*-12	36.2	27.0	12.1	21.1
C18:3 *cis*-9,*cis*-12,*cis*-15	1.8	16.0	52.2	21.6

[1]TAeq = tannic acid equivalents.

[2]A = NPN; B1 = buffer-soluble true protein; B2 = neutral detergent soluble protein; B3 = acid detergent soluble protein; C = acid detergent insoluble protein.

of Makkar et al. (1993). The estimated intake of tannin extract corresponded to 1% of expected DMI based on the potential intake capacity of experimental cows, according to INRA (2018). The feeding trials lasted 23 d.

Feedstuff Sampling and Analyses

During the WS and DS experiments, samples of concentrates, hay, pasture, and dry stubble were collected weekly, vacuum packed, and stored at −20°C. For the analyses, the weekly subsamples were pooled to get a representative sample of each feed.

Ether extract, CP, and ash were determined according to AOAC International (1995) methods 920.39, 976.06, and 942.05, respectively. Protein fractions were calculated according to the Cornell Net Carbohydrate and Protein System, as modified by Licitra et al. (1996). The analyses of NDF, ADF, and ADL were performed following the method of Van Soest et al. (1991). Total phenolic compounds and total tannins were analyzed according to the procedure of Makkar et al. (1993) with some modification, as reported by Luciano et al. (2019). Fatty acid (**FA**) profile of feeds was determined through a 1-step extraction-transesterification with chloroform and sulfuric acid (2% in methanol, vol/vol) as methylation reagent (Valenti et al., 2018). Fatty acid methyl ester separation and quantification was performed using a Thermo Finnigan Trace gas chromatograph equipped with a flame ionization detector (ThermoQuest) and 100-m high-polar fused silica capillary column (SP-2560 fused silica, Supelco; 0.25-mm i.d., 0.25-μm film thickness). Helium was used as carrier gas at a constant flow of 1 mL/min. Oven, injector, and detector were set as described by Natalello et al. (2019). Identification of individual FAME was based on comparison with the retention time of commercially available standard FAME mixtures (Nu-Chek Prep Inc., Larodan Fine Chemicals). Individual FA were expressed as grams per 100 grams of total FA.

Cheesemaking

In both the WS and DS experiments, 2 cheesemaking sessions were performed, one the day before the beginning of the trial and the other after 23 d of dietary treatment. For each cheesemaking session, 1 pressed and cooked curd cheese (Canestrato type) was made with the individual milk of each experimental cow, for a total of 14 cheeses in each session and 28 cheeses in each experiment. At cheesemaking, individual raw whole milk from the morning milking was pooled with the corresponding refrigerated raw whole milk from the previous evening milking based on individual milk yield. Then, 7 kg of individual raw whole milk was heated

in a water bath to reach the optimal temperature for coagulation (38–39°C). A commercial liquid veal rennet [105 international milk clotting units (**IMCU**)/mL, 80% chymosin and 20% pepsin; Biotec Fermenti s.r.l.] was then added to get a final concentration of 37 IMCU/L, following manufacturer recommendation. One hour later, the curd was cut to a size of 5 to 10 mm, and 1.2 L of water at 75°C was added for curd cooking. Curd was precipitated for 10 min, transferred to a basin, and pressed to remove excess whey. After 20 min of forming, with continuous turning of the curd, a second cooking was carried out by diving forms into 5 L of hot water (75–80°C) for 1 h. Cheeses were then immersed in saturated brine (23 °Bé; 300 g/L NaCl) at 10°C for 2 h. After brining, cheeses were aged in a ripening cellar for 25 d at 10°C and 80% relative humidity. Clotting time and pH were monitored during each cheesemaking step. Weight of cheese was recorded before and after brining and weekly during aging.

Milk Sampling and Analyses

Aliquots of the individual milk used for cheesemaking were stored at −20°C pending Ca determination or immediately processed for determination of proximate composition, SCC, color parameters, laboratory cheese yield, and milk coagulation properties. Fat, lactose, and protein contents in milk and MUN were analyzed with a Milkoscan FT 1 (Foss) according to ISO 9622 (ISO, 2013). On the same aliquot, SCC was determined using a BacSomatic (Foss) according to ISO 13366-2 (ISO, 2006). Calcium content in milk was quantified by back titration with EDTA of ashes (Kindstedt and Kosikowski, 1985).

Milk color parameters in the CIE L*a*b space were measured using a Minolta CM-2022 portable spectrophotometer (d/8° geometry; Minolta Co. Ltd.) using illuminant A and 10° standard observer. The reading was performed on a 3-mL milk sample in a 10-mm plastic cuvette. Measured parameters were lightness (L*), redness (a*), yellowness (b*), chroma (C*), hue angle (H*), and the reflectance spectra between 400 and 700 nm. The reflectance spectrum at wavelengths between 450 and 530 nm was used to calculate the integral value $I_{450–530}$ (Priolo et al., 2002).

Laboratory cheese yield was assessed according to the method of Hurtaud et al. (1995). Briefly, 65 μL of the same rennet used for cheesemaking was added to 50 mL of preheated milk in a centrifuge tube and incubated for 1 h at 35°C. After a 5-min pause for syneresis, the coagulum was cut longitudinally using a spatula and centrifuged for 10 min at 2,700 × g at 35°C. The weight of curd after removal of whey was recorded, and laboratory cheese yield was expressed as grams per 100 grams of milk. The individual whey obtained was dried at 100°C, and the laboratory DM cheese yield (LDMCY) was calculated using the following formula (Hurtaud et al., 1995):

$$\text{LDMCY} = 1 - (\text{dry weight of whey}/\text{dry weight of curd and whey}).$$

The milk coagulation properties of milk at 35°C were analyzed using a milk coagulation meter (Maspres) with a recording interval of 15 s, following the method of Zannoni and Annibaldi (1981). Briefly, the same rennet used for cheesemaking was prediluted with water, and 200 μL of the solution was added to 10 mL of milk to obtain a final concentration of 0.046 IMCU/mL. Determined parameters were clotting time (time needed for the beginning of coagulation), firming time (time needed to reach 20 mm of amplitude on the chart), and curd firmness (amplitude of the chart, in mm) after 30 min and after 2 times clotting time.

Cheese Sampling and Analyses

After aging, individual cheeses were sampled for analyses and aliquots were vacuum stored at −20°C. Color parameters of the fresh-cut surface of each cheese were measured before storing, and the average value of 3 nonoverlapping zones was recorded. The color parameters and device used were the same as those described for milk analyses.

Total N and DM were measured according to ISO 5534 (ISO, 2004) and ISO 8968-1 (ISO, 2014), respectively. Water-soluble N and phosphotungstic acid-soluble N were measured using the method proposed by Ardö (1999). Calcium content in cheese was quantified by back titration with EDTA of ashes (Kindstedt and Kosikowski, 1985). Rheological properties of cheese were assessed by uniaxial compression at constant displacement rate (1 mm/s), as reported by Coppa et al. (2011).

Extraction and quantification of fat followed the Röse-Gottlieb method (AOAC, 1990), as modified by Secchiari et al. (2003). Fatty acid profile of cheese was then determined through transesterification using a combined basic and acid methylation, as proposed by Cruz-Hernandez et al. (2004). Briefly, the dry lipid extract was dissolved in hexane to get a final concentration of 30 mg/mL. Then, 0.5 mL of lipid extract was incubated at 50°C for 15 min with 1.5 mL of sodium methoxide in methanol (0.5 M). After cooling to room temperature, 1 mL of 5% methanolic HCl was added

and the mixture was incubated at 50°C for 30 min. Then, 1 mL of 6% aqueous K_2CO_3 was added, and a triple centrifugation with 3 mL of hexane at 1,500 × *g* for 10 min at 4°C was performed. The supernatants collected after each centrifugation were pooled and evaporated under N_2 flow at 37°C and then dissolved in 1 mL of GC-grade hexane. The GC setting for FAME identification was the same as described for feedstuff analysis. Moreover, the separation of C18:1 isomers was achieved by isothermal analysis at 165°C. Individual FA were expressed as grams per 100 grams of total FA.

Odor-active volatile compounds (**OAC**) were extracted using static solid-phase microextraction as reported by Carpino et al. (2004), with some modifications. A divinylbenzene/carboxen/polydimethylsiloxane coated fiber (50/30 μm; Supelco) was used to adsorb OAC from the headspace of samples. Samples were prepared by conditioning 10 g of cheese at 40°C for 40 min. An additional 40 min was required for the fiber to establish volatile compound equilibrium between the sample headspace and the fiber solid phase. The fiber was conditioned for 1 h at 225°C before the initial use and for 5 min between each analysis. For the GC-MS analysis and the identification of OAC, a 7890A Series GC system (Agilent Technologies) coupled with an Agilent 5975C Mass Selective Detector (triple axis) was used. An HP-5 capillary column (30 m × 0.25-mm i.d. × 0.25-μm film thickness; Agilent Technologies) was used to separate the volatile components. The chromatographic conditions were as follows: splitless injector at 220°C; oven program conditions: 35°C for 3 min, 6°C/min to 200°C, and 30°C/min to 240°C for 3 min. Carrier gas (helium) pressure and flow were set at 93.77 MPa and 1.0 mL/min, respectively. The mass selective detector operated in scan mode (5.15 scans/s) with 70 eV ionization energy. Peaks were identified by comparison of mass spectra with the bibliographic data of the Wiley 175 library (Wiley and Sons Inc.) and with the linear retention indices of authentic standards (Sigma-Aldrich), calculated by running a paraffin series (from C5 to C20) under the same working conditions. The OAC data were expressed as arbitrary units of chromatograph area.

For the GC olfactometry analysis, an HP 6890 Series GC system (Agilent Technologies) GC coupled with an olfactometer was used. Column, injection, and oven setting were the same as reported for GC-MS. A trained human nose (sniffer) was used as a final detector simultaneously with the mass detector (Rapisarda et al., 2014). The eluted compounds were mixed with humidified air, and the sniffer was continuously exposed to this source for 30 min. During the olfactometric analysis, the sniffer described the perceptions and duration of odors. The OAC recognition was performed using the single sniff method (Marin et al., 1988). The sniffer was trained with reference chemicals consisting of a group of 8 compounds used to evaluate olfactory acuity. The sniffer had no specific anosmia for these standards.

Calculations and Statistics

The index of atherogenicity (**IA**) and the index of thrombogenicity (**IT**) were calculated according to Ulbricht and Southgate (1991):

$$\mathrm{IA} = \frac{\mathrm{C12{:}0 + C14{:}0 + C16{:}0}}{\Sigma\,\mathrm{MUFA} + \Sigma\ \mathrm{PUFA}};$$

$$\mathrm{IT} = \frac{\mathrm{C14{:}0 + C16{:}0 + C18{:}0}}{\begin{array}{c}\Sigma\,\mathrm{MUFA} \times 0.5 + \Sigma\ \text{n-6 PUFA} \times 0.5 \\ +\Sigma\ \text{n-3 PUFA} + \left(\dfrac{\Sigma\ \text{n-3 PUFA}}{\Sigma\ \text{n-6 PUFA}}\right)\end{array}}.$$

The hypocholesterolemic to hypercholesterolemic ratio (**h:H**) was calculated according to Mierliță (2018):

$$\mathrm{h{:}H} = \frac{\mathrm{C18{:}1}\ cis\text{-}9 + \Sigma\ \mathrm{PUFA}}{\mathrm{C12{:}0 + C14{:}0 + C16{:}0}}.$$

Before statistical analysis, SCC data were transformed to $\log_{10}$ per milliliter, and OAC data were transformed to ln(x + 1) to obtain normalized distribution.

All data from the WS and DS experiments were analyzed separately using an analysis of covariance (ANCOVA) model of SPSS Statistics 21 (IBM), figuring the fixed effect of dietary treatment (CON, TAN). Individual milk and cheese samples were used as statistical unit. Statistical elaboration was adjusted for a covariate composed by (1) the average data of the 2 d before the beginning of the feeding trials for milk or (2) the data of the day preceding the beginning of the feeding trials for cheese. Differences between means were considered significant at $P \leq 0.05$ and a trend toward significance at $P \leq 0.10$.

RESULTS

WS Experiment

Table 2 shows the results for milk composition, color parameters, and cheesemaking aptitude, and Table 3 presents data from measurements during cheesemaking,

Table 2. Effect of dietary tannin extract supplementation on cow milk yield, chemical composition, color properties, and cheesemaking aptitude in the wet season (WS) and dry season (DS) experiments

Item	WS				DS			
	Treatment[1]				Treatment			
	CON	TAN	SEM	*P*-value	CON	TAN	SEM	*P*-value
Milk yield, kg/d	10.3	11.1	0.55	0.479	12.3	12.3	0.20	0.969
Protein, g/kg	39.2	39.9	0.83	0.703	33.2	32.2	0.28	0.137
Fat, g/kg	39.3	39.5	1.26	0.942	34.8	38.4	1.70	0.318
Lactose, g/kg	45.8	46.1	0.62	0.816	46.6	46.1	0.32	0.422
Calcium, g/kg	1.43	1.71	0.130	0.136	1.43	1.00	0.170	0.100
SCC, $\log_{10}$/mL	2.85	2.97	0.050	0.284	2.80	2.97	0.048	0.119
MUN, mg/dL	33.2	34.2	0.74	0.533	29.6	25.4	1.09	0.031
Color parameter[2]								
L*	70.9	71.5	0.33	0.377	68.8	69.7	0.33	0.198
a*	−1.44	−1.39	0.090	0.752	−2.00	−1.80	0.114	0.405
b*	3.80	3.81	0.329	0.994	−0.149	0.659	0.211	0.091
C*	4.10	4.15	0.278	0.928	2.74	2.83	0.178	0.827
H*	113	113	2.9	0.984	165	159	5.0	0.554
$I_{450–530}$	−289	−258	20.4	0.347	−48.9	−92.7	19.21	0.144
Cheesemaking aptitude[3]								
LCY, g/100 g	26.0	26.0	0.01	0.973	23.7	25.0	0.01	0.347
LDMCY, g/100 g	8.60	8.70	0.002	0.750	7.20	7.80	0.002	0.238
R, min:s	22:45	24:14	1:18	0.590	20:49	21:43	2:04	0.842
K20, min:s	6:04	6:01	0:54	0.984	4:53	4:04	0:13	0.133
A30, mm	39.2	27.1	5.00	0.283	32.2	24.6	4.53	0.467
A2R, mm	47.0	45.7	2.73	0.825	37.3	37.3	1.07	0.966

[1]CON = control group; TAN = group receiving 150 g of tannin extract/head per day.

[2]L* = lightness; a* = redness; b* = yellowness; C* = chroma; H* = hue angle; $I_{450–530}$ = integral value of the absorbance spectrum between 450 and 530 nm.

[3]LCY = laboratory cheese yield; LDMCY = laboratory DM cheese yield; R = clotting time; K20 = firming time; A30 = curd firmness after 30 min; A2R = curd firmness after 2 times R.

cheese composition, and cheese physical characteristics. Dietary treatment did not affect ($P > 0.05$) any of these parameters in the WS experiment.

Fatty acids proportion in cheese is reported in Table 4. In the WS experiment, cheese from the TAN group did not differ ($P > 0.05$) from cheese from the CON group, except that it had a higher ($P < 0.001$) proportion of C20:3 n-6. Supplementation with tannin extract did not affect ($P > 0.05$) BH and healthfulness indices after 23 d of dietary treatment. A more detailed FA profile is given in Supplemental Table S1 (http://dx.doi.org/10.17632/bb4xjj3fbt.1).

We detected up to 61 different OAC in cheeses from the WS experiment, belonging to the following chemical classes: acids (8), alcohols (13), aldehydes (4), aromatic hydrocarbons (7), esters (9), ketones (8), sulfurs (5), and terpenes (7). However, not all of these OAC were detected in every cheese sample. Therefore, Table 5 shows only the OAC for which statistical analysis could be performed. 2-Nonanone (ketones), ethyl hexanoate (ester), and hexanoic and octanoic acid were the main OAC in WS cheeses. 2-Heptanone was the most abundant compound in CON cheese, but dietary tannin extract decreased ($P = 0.018$) its concentration.

DS Experiment

Concerning milk characteristics (Table 2), only MUN differed ($P = 0.031$) between treatments, being lower in the TAN group than in the CON group. Tannin supplementation did not exert any effect ($P > 0.05$) on cheesemaking parameters, cheese composition, and physical characteristics (Table 3) in the DS experiment. The color of cheeses only slightly differed for luminosity, with the TAN group showing a tendency for a higher ($P = 0.057$) L* value than CON.

Concerning FA profile (Table 4), dietary tannin extract decreased ($P = 0.010$) C18:1 *trans*-10 concentration and tended to increase ($P = 0.054$) C18:3 *cis*-9,*cis*-12,*cis*-15 concentration. Consequently, we found higher ($P = 0.033$) C18:1 *trans*-11 to C18:1 *trans*-10 ratio and lower ($P = 0.031$) n-6 PUFA to n-3 PUFA ratio (**n-6:n-3**) in cheeses from the TAN group. A more detailed FA profile is given in Supplemental Table S1.

We detected up to 41 different OAC in cheeses from the DS experiment, belonging to the following chemical classes: acids (5), alcohols (7), aldehydes (2), aromatic hydrocarbons (5), esters (9), ketones (7), sulfurs (2), and terpenes (4). However, not all of these OAC were

detected in every cheese sample. Therefore, Table 5 shows only the OAC for which statistical analysis could be performed. Ketones were the most abundant compounds in all the cheeses, especially 2-heptanone and 2-nonanone, followed by ethyl butanoate (ester) and hexanoic acid. We found no differences ($P > 0.05$) in the OAC composition between the cheeses of the 2 dietary groups.

DISCUSSION

Effect on Parameters Related to N Metabolism

One of the starting hypotheses of this study was that tannins, thanks to their well-known protein-binding and antimicrobial activity (Patra and Saxena, 2011), could affect N metabolism in vivo and consequently modify some of the cheese parameters related to protein content and composition. However, this occurred only in the DS experiment and was limited to MUN, with no consequences on protein content, proteolysis, or clotting and rheology parameters.

The reduction of MUN in ruminants eating tannins from either extracts or forages is reported in several studies on dairy cows (Broderick et al., 2017; Zhang et al., 2019; Aguerre et al., 2020) and ewes (Buccioni et al., 2015b; Maamouri et al., 2019). This phenomenon is often combined with a lower ureic N concentration in urine because it is due to an impaired protein ruminal degradation that decreases the concentration of ammonia in the rumen (Patra and Saxena, 2011). As a consequence, ammonia conversion to urea in the liver and subsequent ureic emission from the ruminant are reduced as well as the nitrous oxide emissions from manure, with positive implications for the environment (Naumann et al., 2017). This effect is desirable in the WS, when young green herbage is rich in degradable protein, which may cause a surplus of soluble N in the

Table 3. Effect of dietary tannin extract supplementation on cow cheese composition, color properties, rheological properties, and cheesemaking measurements in the wet season (WS) and dry season (DS) experiments

	WS				DS			
	Treatment[1]				Treatment			
Item	CON	TAN	SEM	*P*-value	CON	TAN	SEM	*P*-value
Cheesemaking measurement								
Cheese yield, g/kg	112.5	112.8	3.47	0.978	83.9	83.5	2.36	0.931
Milk pH	6.68	6.68	0.015	0.885	6.54	6.56	0.009	0.216
pH after first curd cooking	6.65	6.60	0.022	0.231	6.50	6.51	0.010	0.670
pH after second curd cooking	6.54	6.55	0.025	0.852	6.39	6.37	0.027	0.657
Weight after first curd cooking, g	1,505	1,513	70.7	0.960	1,218	1,100	29.4	0.075
Weight after second curd cooking, g	1,031	1,083	46.5	0.591	874	786	29.4	0.166
Weight before brining, g	949	952	32.7	0.968	741	680	19.4	0.164
Weight after brining, g	950	958	33.1	0.907	743	683	19.4	0.167
Composition[2]								
DM, g/100 g	55.4	56.2	0.47	0.358	63.3	62.4	0.95	0.643
Fat, g/100 g	17.8	18.4	0.80	0.688	19.7	20.5	0.92	0.691
Fat, g/100 g of DM	31.8	32.7	1.15	0.711	30.7	32.9	1.36	0.438
TN, g/100 g of DM	6.89	6.63	0.085	0.159	6.75	6.41	0.151	0.289
WSN, g/100 g of DM	0.802	0.745	0.055	0.617	0.601	0.735	0.051	0.223
PTASN, g/100 g of DM	0.546	0.515	0.051	0.766	0.471	0.542	0.048	0.492
Calcium, g/100 g	0.845	0.913	0.050	0.369	1.08	1.01	0.027	0.135
Color parameter[3]								
L*	83.2	83.8	0.89	0.722	77.0	79.9	0.94	0.057
a*	3.76	3.70	0.111	0.783	1.70	1.93	0.106	0.154
b*	19.9	20.4	0.60	0.679	9.57	10.21	0.420	0.306
C*	20.2	20.8	0.61	0.698	9.72	10.40	0.425	0.288
H*	79.4	79.6	0.11	0.406	79.6	79.7	0.28	0.757
$I_{450–530}$	−1,152	−1,211	37.7	0.493	−438	−509	32.4	0.156
Rheology, N/cm^2								
Strength to 20% deformation	2.18	2.60	0.236	0.234	6.53	5.58	1.003	0.523
Strength to 40% deformation	6.02	6.89	0.614	0.339	15.6	12.8	1.88	0.324
Strength to 60% deformation	8.29	9.47	0.670	0.243	20.8	16.6	2.29	0.231
Young modulus (undeformability)	25.0	27.7	2.00	0.358	63.6	47.9	11.90	0.372

[1]CON = control group; TAN = group receiving 150 g of tannin extract/head per day.

[2]TN = total N; WSN = water-soluble N; PTASN = phosphotungstic acid-soluble N.

[3]L* = lightness; a* = redness; b* = yellowness; C* = chroma; H* = hue angle; $I_{450–530}$ = integral value of the absorbance spectrum between 450 and 530 nm.

rumen (Kingston-Smith and Theodorou, 2000). In the WS experiment in the present study, the lack of reduction in MUN in the TAN group may be due to the high level of CP intake from green pasture, which hid the effect of tannin extract supplementation at the dosage used here. A higher proportion of tannins in DMI likely could have resulted in a significant effect in the WS experiment, as the effect of tannins on rumen fermentation is often dose dependent (Toral et al., 2018). However, a high intake of tannins could have detrimental consequences on animal performance (Aguerre et al., 2016) and could be economically impractical on a commercial farm.

On the other hand, the literature lacks studies assessing the effect of dietary tannins on the physical properties of cow cheese or the cheesemaking aptitude of cow milk. Kälber et al. (2013) found that milk of cows eating 6.1 g/d of condensed tannins from buckwheat (*Fagopyrum esculentum* Moench) forage had a shorter clotting time compared with milk of cows eating 2.2 g/d of condensed tannins from chicory (*Cichorium intybus* L.) or ryegrass (*Lolium multiflorum* Lam.) forage. However, the authors did not observe any differences in milk composition that could explain that positive result. In another study, a trained panel noted moderate differences in hardness and adhesiveness of Gruyère cheese from Holstein cows eating 691 g/head per day of condensed tannins from sainfoin (*Onobrychis viciifolia* Scop.) pellets, but no difference in protein or casein content was observed in the milk used for cheesemaking (Girard et al., 2016). Additional information can be collected from experiments on small ruminants,

Table 4. Effect of dietary tannin extract supplementation on cow cheese fatty acid (FA) profile (g/100 g of total FA) in the wet season (WS) and dry season (DS) experiments

	WS				DS			
	Treatment[2]				Treatment			
Item[1]	CON	TAN	SEM	*P*-value	CON	TAN	SEM	*P*-value
Σ de novo FA	19.65	18.29	1.130	0.413	15.75	14.87	0.871	0.484
C16:0	26.86	28.90	0.751	0.210	29.65	29.90	0.408	0.799
C16:1 *cis*-9	1.30	1.26	0.061	0.715	1.34	1.43	0.056	0.509
C18:0	10.95	11.06	0.379	0.884	11.58	11.15	0.491	0.780
C18:1 *trans*-9	0.32	0.31	0.024	0.801	0.36	0.38	0.022	0.656
C18:1 *trans*-10	0.29	0.31	0.017	0.694	0.35	0.27	0.012	0.010
C18:1 *trans*-11	3.61	3.67	0.156	0.853	2.08	2.23	0.042	0.133
C18:1 *cis*-9	21.25	20.62	0.769	0.687	24.19	25.49	0.374	0.128
C18:1 *cis*-11	1.68	1.70	0.090	0.935	2.24	2.22	0.099	0.925
C18:2 *cis*-9,*cis*-12 (LA)	1.92	2.02	0.082	0.558	2.48	2.44	0.084	0.793
C18:2 *cis*-9,*trans*-11 (RA)	1.37	1.33	0.099	0.870	0.66	0.68	0.048	0.872
C18:3 *cis*-9,*cis*-12,*cis*-15	1.04	0.92	0.082	0.481	0.28	0.39	0.026	0.054
C20:0	0.12	0.12	0.010	0.949	0.16	0.17	0.014	0.589
C20:5 n-3	0.06	0.07	0.011	0.428	0.05	0.06	0.012	0.883
C22:0	0.11	0.18	0.021	0.116	0.09	0.08	0.009	0.818
C22:5 n-3	0.04	0.07	0.008	0.118	0.06	0.06	0.015	0.805
C24:0	0.04	0.04	0.008	0.893	0.03	0.03	0.008	0.991
Σ SFA	57.38	58.95	1.104	0.495	57.10	56.39	0.498	0.511
Σ MUFA	31.73	30.88	0.897	0.646	33.52	34.68	0.391	0.187
Σ PUFA	5.18	5.18	0.243	0.992	3.89	4.12	0.155	0.488
Σ OBCFA	5.59	5.30	0.172	0.542	5.33	5.20	0.138	0.659
SFA/PUFA	11.3	11.8	0.654	0.709	14.94	14.10	0.647	0.549
Σ n-6 PUFA	2.09	2.25	0.085	0.369	2.727	2.720	0.090	0.970
Σ n-3 PUFA	1.13	1.08	0.081	0.744	0.381	0.501	0.031	0.092
n-6:n-3 PUFA	1.86	2.52	0.305	0.304	7.49	5.64	0.370	0.031
C18:1 *trans*-11/*trans*-10	13.0	12.0	0.792	0.546	6.06	8.39	0.461	0.033
BHI	9.99	9.73	0.412	0.758	7.79	7.92	0.176	0.753
RA/LA	0.728	0.657	0.044	0.443	0.272	0.288	0.024	0.749
DSI C14	0.066	0.056	0.005	0.325	0.060	0.061	0.003	0.924
IA	2.24	2.35	0.169	0.634	2.06	1.92	0.105	0.373
IT	2.43	2.65	0.153	0.337	2.71	2.55	0.090	0.241
h:H	0.625	0.576	0.054	0.531	0.655	0.699	0.030	0.347

[1]de novo FA = C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C14:0; OBCFA = odd- and branched-chain fatty acids; BHI = biohydrogenation intermediates; DSI C14 = desaturation index, calculated as C14:1 *cis*-9/(C14:0 + C14:1 *cis*-9); IA = index of atherogenicity (Ulbricht and Southgate, 1991); IT = index of thrombogenicity (Ulbricht and Southgate, 1991); h:H = hypocholesterolemic:hypercolesterolemic ratio (Mierliță, 2018); LA = linoleic acid; RA = rumenic acid.

[2]CON = control group; TAN = group receiving 150 g of tannin extract/head per day.

although caution is needed when knowledge is extrapolated from different ruminant species, as it is a relevant source of variation. Some articles reported no effect of dietary quebracho or chestnut tannin extracts on ewe milk clotting parameters (Buccioni et al., 2015a,b) or even longer clotting and firming time (Buccioni et al., 2017), probably related to the interference with caseins or rennin of some bioactive monomers derived from tannin biodegradation. Bonanno et al. (2016) observed a positive effect on ewe milk and cheese protein content when animals grazed on sulla (*Sulla coronaria* Medik.) compared with ryegrass. These results were attributed to the presence of condensed tannins in sulla; however, animals grazing ryegrass had a significantly lower CP daily intake (−53%). In summary, a recent meta-analysis by Herremans et al. (2020) concluded that dietary tannins do not have any effect on N use efficiency in dairy cattle, except for the reduction of urea emissions, which is beneficial for the environment.

Effect on FA Profile

The significant reduction of n-6:n-3 in 25-d-old cheeses from cows ingesting tannin extract in the DS experiment is most likely related to the effect of tannins on rumen BH. Indeed, the milk FA synthesized de novo in animal tissues are mainly SFA, or they result from the activity of desaturase and elongase enzymes (Palmquist, 2006). Milk C18:2 *cis*-9,*cis*-12 and C18:3 *cis*-9,*cis*-12,*cis*-15, the most relevant n-6 and n-3 PUFA, respectively, are mainly of dietary origin, and their concentration can be modified by modulating BH (Chilliard et al., 2007). A lower dietary n-6:n-3 could reduce cardiovascular disease risk in human, especially if achieved as a result of an increase in C18:3 *cis*-9,*cis*-12,*cis*-15 concentration (Harris, 2006), as occurred in our study. Likewise, Girard et al. (2016) reported an increase of 17% of C18:3 *cis*-9,*cis*-12,*cis*-15 proportion in cheese from cows fed sainfoin pellets (691

Table 5. Effect of dietary tannin extract supplementation on proportion of cow cheese odor-active volatile compounds[1] (OAC) in wet season (WS) and dry season (DS) experiments (expressed as arbitrary units of chromatograph area)

Item	Odor perception	LRI[2]	WS				DS			
			Treatment[3]				Treatment			
			CON	TAN	SEM	*P*-value	CON	TAN	SEM	*P*-value
Acids, total			17.4	16.9	0.27	0.345	16.3	16.3	0.80	0.895
Butanoic acid	Cheese	820	15.4	13.9	0.77	0.342	12.5	10.6	1.12	0.413
3-Methylbutanoic acid	Rancid, cheese	877	14.5	14.5	0.92	0.934	—	—	—	—
Hexanoic acid	Sweat	1,019	17.0	16.5	0.73	0.470	14.6	15.6	1.29	0.126
Octanoic acid	Cheese	1,279	16.0	15.5	0.65	0.127	13.7	13.8	0.94	0.984
Decanoic acid	Rancid	1,373	14.3	11.5	1.04	0.198	11.5	10.9	1.28	0.803
Alcohols, total			13.1	13.9	0.87	0.665	13.4	13.6	0.89	0.875
3-Methylacetatebutan-1-ol	Banana	876	—	—	—	—	10.8	11.9	1.06	0.630
2-Ethylhexan-1-ol	Rose, green	1,032	11.0	11.3	1.11	0.889	11.3	12.8	0.85	0.401
Aldehydes, total			10.5	10.5	1.02	0.981	8.9	10.3	0.99	0.498
Nonanal	Fresh, green	1,104	10.5	10.5	1.02	0.997	8.8	10.3	0.99	0.464
Aromatic hydrocarbons, total			15.9	16.3	0.23	0.526	14.3	14.3	0.63	0.924
Benzeneacetaldehyde	Honey	1,049	10.6	10.5	0.92	0.971	8.5	10.1	0.90	0.392
Phenylethyl alcohol	Rose, honey, orange	1,118	13.8	14.1	0.21	0.465	13.1	11.7	0.79	0.388
Toluene	Paint	773	15.5	15.6	0.38	0.969	—	—	—	—
Esters, total			16.2	16.3	0.34	0.860	14.6	15.0	1.16	0.856
Ethyl butanoate	Apple	804	12.7	13.4	1.06	0.781	15.0	15.8	1.08	0.228
Ethyl hexanoate	Orange	1,000	15.9	16.4	0.71	0.295	14.3	14.7	1.10	0.884
Ethyl octanoate	Wine	1,198	14.9	14.8	0.60	0.969	11.5	13.6	1.06	0.348
Ethyl decanoate	Grape	1,398	12.4	10.3	0.77	0.200	10.6	12.1	0.84	0.421
Ketones, total			18.2	16.7	0.35	0.078	18.0	18.0	0.43	0.990
2-Heptanone	Soap, fruit	895	18.2	14.0	0.66	0.018	18.2	17.8	0.47	0.534
2-Octanone	Solvent	999	12.6	12.4	0.90	0.924	14.4	13.8	0.95	0.246
2-Nonanone	Hot milk	1,093	17.0	15.9	0.43	0.267	17.1	17.2	0.41	0.836
2-Undecanone	Orange	1,296	—	—	—	—	13.1	13.0	0.88	0.944
Terpenes, total			9.9	11.4	0.96	0.441	—	—	—	—
α-Pinene	Fresh	939	9.0	9.7	0.95	0.726	—	—	—	—

[1]All OAC reported in this table were identified using both an Agilent 5975C Mass Selective Detector and the bibliographic data of the Wiley 175 library (Wiley and Sons Inc.).

[2]LRI = linear retention index.

[3]CON = control group; TAN = group receiving 150 g of tannin extract/head per day.

g of condensed tannins/head per day) compared with the control group (alfalfa, *Medicago sativa* L.). Also, dietary tannins were suggested to be responsible for the increase in C18:3 *cis*-9,*cis*-12,*cis*-15 in 60-d-old cheese from ewes fed fresh sulla forage, although tannin content in diets was not investigated (Addis et al., 2005). Unfortunately, the tendentially higher n-3 PUFA concentration found in TAN in the present study cheese was not enough to improve the healthfulness indices (i.e., IA, IT, and h:H).

When the effects of dietary tannins are investigated in vivo, a shift in milk C18:3 *cis*-9,*cis*-12,*cis*-15 concentration is generally combined with changes in the concentration of other FA involved in BH, such as C18:2 *cis*-9,*cis*-12, C18:0, C18:1 *trans*-11, and C18:2 *cis*-9,*trans*-11 (Cabiddu et al., 2009; Buccioni et al., 2015a,b). However, Frutos et al. (2020) summarized in a recent review that although dietary tannins are commonly found to increase C18:3 *cis*-9,*cis*-12,*cis*-15 concentration in milk, regardless of the tannin source, the effects on C18:2 *cis*-9,*cis*-12 and C18:0 are less consistent. In the DS experiment, dietary tannin extract exerted no effect on these protagonists of BH, but we observed a significant decrease in C18:1 *trans*-10 concentration. As the microbial conversion of C18:3 *cis*-9,*cis*-12,*cis*-15 to C18:1 *trans*-10 may occur in the rumen (Bessa et al., 2015), dietary tannin extract may have affected this particular pathway in the DS experiment. An increase in C18:3 *cis*-9,*cis*-12,*cis*-15 concentration and a reduction of C18:1 *trans*-10 concentration in milk due to tannin ingestion (26.5 g of condensed tannins/kg of DMI) was also reported by Cabiddu et al. (2009) in ewes eating fresh sulla, but this effect was also combined with changes in other FA concentrations. Other studies on dietary tannin supplementation to dairy cows reported no effect on C18:1 *trans*-10 concentration in cow milk (Dschaak et al., 2011; Henke et al., 2017), but it should be emphasized that this FA is often not reported in scientific articles or its concentration is summed with that of C18:1 *trans*-11, as they easily coelute in GC.

Conversely, it seems that dietary tannin supplementation did not affect ruminal BH in the WS experiment according to cheese FA profile. In a study comparing the effect of 2 different tannin extracts (quebracho vs. chestnut and quebracho) on FA profile of in vitro rumen fermentation with different forage substrates, hay was found to be more susceptible than green herbage to tannin bioactivity (Menci et al., 2021). Because diet is likely the major factor affecting rumen microbiota composition (Ellison et al., 2017), the low protein content or the high structural carbohydrate content of dry forages or both may select a particular microbiota that is more sensitive to the effects of tannin. The results of the present study confirm this phenomenon in vivo with cows, considering that ruminal microorganisms are among the main factors responsible for FA profile of milk and dairy (Palmquist, 2006). Indeed, the diet in the DS experiment was poorer in CP and richer in ADL compared with the WS experiment, as the chemical composition of the stubble grazed by cows was comparable with that of hay. Further studies on rumen microbiota involving different tannin sources and types are needed to confirm our hypothesis.

Effect on OAC

Dietary tannin extract supplementation did not exert any effect on the aroma of DS cheeses, but it did affect ketone concentration in the WS experiment, particularly 2-heptanone concentration. Methyl ketones originate from the β-oxidation of lipolyzed FA by microorganisms (McSweeney and Sousa, 2000); therefore, they are known to characterize the aroma of blue and surface-mold ripened cheeses. Their presence is also reported in different kinds of cheese (Curioni and Bosset, 2002). Interestingly, herd management affected the presence of ketones in cheese. Valdivielso et al. (2016) observed a significant increase of 2-heptanone and 2-nonanone in cheese from ewes grazing mountain pastures, and Carpino et al. (2004) found acetoin and 2-nonanone only in cheese from grazing cows even though none of these compounds were detected in pasture. At the moment, it is not clear how dietary tannins affected ketone concentration or whether they acted against lipolysis or β-oxidation enzymes. Further research should investigate this aspect, as the literature is devoid of studies on the effect of dietary tannins on aromatic compounds in milk and cheese. In summary, the slight differences in OAC concentrations of cow cheese induced by dietary tannin extract at the dose used in this study would likely not affect the consumer sensory experience.

CONCLUSIONS

The inclusion of tannin extract in the diet of dairy cows at a rate of 1% of estimated DMI for 23 d slightly affected the composition and OAC of cow cheese. The results of the present study indicated that this rate of tannin supplementation has no detrimental effects on cheese yield or other cheesemaking parameters regardless of the variation in cow diet induced by forage availability according to the season of the Mediterranean climate. The bioactivity of dietary tannins appears to

be more efficient during the DS, when the diet is low in CP and rich in ADF and ADL. Further studies are needed to investigate the effects of longer supplementations or different doses and tannin sources.

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ORCIDS

R. Menci https://orcid.org/0000-0001-9967-8986
A. Natalello https://orcid.org/0000-0002-9802-2501
M. Caccamo https://orcid.org/0000-0003-3500-0724
V. Niderkorn https://orcid.org/0000-0002-4631-7623
M. Coppa https://orcid.org/0000-0003-2073-0599