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ANIMAL PRODUCTION

Milk fatty acid composition of cows and ewes supplemented with black wattle tannin extract

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ABSTRACT: This study evaluated the effects of *Acacia mearnsii* tannin extract on milk fatty acid profile of dairy ewes and cows. In experiment 1, twenty-four Lacaune ewes received one of the following dietary treatments: control (no tannin extract added to the diet), T30 (30 g tannin extract/kg concentrate), and T40 (40 g extract/kg concentrate). In experiment 2, thirty Jersey cows received either a control diet (no tannin extract added to the diet) or the same diet containing 40 g tannin extract/kg concentrate (T40). Dry matter intake, milk production and milk solids content of ewes and cows were unaffected by tannin supplementation. The *cis*-9, *trans*-11 conjugated linoleic acid (CLA) content increased linearly by 21% in milk fat from ewes fed tannin extract, while the C18:2 n-6 (linoleic acid) content tended (P = 0.051) to increase by 13% in cows fed TE. The supplementation with black wattle tannin extract improves the nutritional quality of milk fat of ewes and cows to a small extent, with no adverse effects on performance or gross milk composition.
Key words: *Acacia mearnsii*, plant secondary compounds, ruminant diets, dairy fat.

Composição em ácidos graxos no leite de vacas e ovelhas suplementadas com extrato tanífero de acácia negra

RESUMO: O objetivo deste trabalho foi avaliar os efeitos da suplementação com extrato tanífero de acácia negra (*Acacia mearnsii*) sobre o perfil de ácidos graxos no leite de ovelhas e vacas. No experimento 1, vinte e quatro ovelhas Lacaune receberam um dos seguintes tratamentos dietéticos: controle (sem extrato tanífero), T30 (30 g de extrato tanífero/kg concentrado) e T40 (40 g de extrato tanífero/kg concentrado). No experimento 2, trinta vacas Jersey receberam uma dieta controle ou a mesma dieta contendo 40 g de extrato tanífero/kg concentrado (T40). O consumo de matéria seca, a produção de leite e o teor de sólidos do leite das ovelhas e vacas não foram afetados pela suplementação com o extrato tanífero. O teor de ácido linoleico conjugado (CLA) *cis-9, trans-11* aumentou em 21% na gordura do leite de ovelhas alimentadas com o extrato tanífero, enquanto o teor de C18:2 n-6 (ácido linoleico) tendeu (P = 0,051) a aumentar em 13% em vacas suplementadas com o extrato. A suplementação com extrato tanífero de acácia negra melhora em pequena escala a qualidade nutricional da gordura do leite de ovelhas e vacas, sem efeitos adversos no desempenho ou na composição bruta do leite.
Palavras-chave: *Acacia mearnsii*, componentes secundários de plantas, ruminantes, gordura do leite.

INTRODUCTION

The use of condensed tannins (CTs) in ruminant diets has been proposed as a tool to confer nutritional and environmental advantages in livestock systems. From a nutritional point of view, it is well known that CTs decrease ruminal protein degradability, increasing the flow of metabolizable protein to the small intestine (KOZLOSKI et al., 2012). From an environmental point of view, it has been shown that CTs may reduce urinary N excretion, increasing fecal N excretion (AGUERRE et al., 2016; GRIFFITHS et al., 2013; HENKE et al., 2017). As a result, losses of leachate and nitrous oxides (N_2O) to soil water and air may be reduced. Furthermore, CTs have been shown to reduce enteric methane emission in some studies (ALVES et al. 2017a).

All the advantages described above are the results of the interactions between CTs and the rumen microbial population, as tannins can bind to rumen microbes or their enzymes (MIN et al., 2003). Accordingly, some *in vitro* studies have shown that tannins inhibit the growth and activity of bacteria responsible for the ruminal biohydrogenation (BH) of polyunsaturated fatty acids (FAs) (SZCZECHOWIAK et al., 2016), which may in turn improve the FA composition of milk fat. Endogenous synthesis from vaccenic acid escaping from ruminal BH is the major source of rumenic acid (*cis*-9, *trans*-11 conjugated linoleic acid (CLA)) secreted in milk (BAUMAN et

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al., 1999). Hence, inhibiting the last step of ruminal BH, in which vaccenic acid in converted to stearic acid, is an effective dietary strategy to increase the milk fat CLA content.

However, a number of studies have shown that *in vitro* and *in vivo* responses to tannin supplementation are dependent on the extract source. One of the main sources of tannin used in *in vivo* studies to improve milk FA profiles in ruminants is the quebracho (BUCCIONI et al., 2015a; BUCCIONI et al., 2017; TORAL et al., 2013). In contrast, tannin from *Acacia mearnsii* was used in previous investigations mostly as a means to increase the intestinal flow of metabolizable protein (KOZLOSKI et al., 2012; ORLANDI et al., 2015), improve animal performance (ALVES, 2017b; GERLACH et al., 2018; GRIFFITHS et al., 2013), or reduce methane emissions (ALVES et al, 2017a). In contrast, much less is known about the effects of tannin from this source on ruminal BH and the milk FA composition of ruminant animals.

Acacia mearnsii is the only temperate *Acacia* species grown commercially on a significant international scale (CHAN et al., 2015). The species is endemic to south-eastern Australia, where it was first recognized as an excellent source of natural tannin in the early 1800s, but nowadays is mainly planted in South Africa and Brazil for tannin production and wood chip exports (GRIFFIN et al., 2011). Tannins are present in practically all organs of *Acacia sp.*, but in the bark their contents can reach 30% of dry weight (SHERRY, 1971). In Brazil, *A. mearnsii* is only cultivated in the state of Rio Grande do Sul, having a current planted area estimated between 150 000 and 170 000 ha (FOELKEL, 2008).

This research evaluated the effects of diet supplementation with tannin extract (TE) from *A. mearnsii* on milk production and composition (with an emphasis on the FA profile) of dairy ewes and cows. It was hypothesized that TE from *A. mearnsii* may improve the milk FA composition of both ruminant species.

MATERIALS AND METHODS

Location, animals, experimental design and treatments

Two experiments were conducted. The first experiment was conducted in dairy ewes from January to May 2013, and the second experiment was conducted in dairy cattle from January to March 2014. Both experiments were performed in the city of Bom Retiro (Santa Catarina State, Brazil; 27°45'S, 49°38'W). The climate of the region is humid subtropical with a mean temperature and annual rainfall of 19 °C and 1400 mm, respectively.

Experiment 1

Twenty-four lactating Lacaune ewes were assigned to three groups balanced for milk production (1.3 ± 0.4 kg/day), days in milk (78.6 ± 9.8), parity (2.5 ± 0.6) and live weight (LW) (52.4 ± 5.3 kg) over two experimental periods of 21 days each (16 days of adaptation and 8 days of sampling). The ewes were treated with CT extracted from black wattle at one of three levels: zero (control), 30 (T30) and 40 (T40) g/kg concentrate feed. The levels of CT included in the treatments were not equal because of a previous experiment conducted using 20 g/kg CT extract concentrate feed, in which no significant result was reported. The CT extract was mixed with the concentrate at 1.2 and 1.5% of the total dry matter intake (DMI).

Each experimental group had access to separated paddocks of a mixed white clover (*Trifolium repens*) and tall fescue (*Festuca arundinacea*) pasture for 8 h/day between morning and afternoon milking. After each milking, the ewes received 300 g concentrate, and after afternoon milking, they received 1 kg corn silage (as-fed basis). The concentrate contained 470 g/kg ground corn, 200 g/kg soybean meal, 280 g/kg soybean hulls and 50 g/kg mineral mixture. Silage and concentrate supplementation were balanced to avoid ruminal energy restriction (Table 1). The pasture was rotationally grazed with a similar pre-grazing sward height, and the animals were moved to a new paddock when the grazing down level reached 50% of the pre-grazing sward pasture height.

Experiment 2

Thirty lactating Jersey cows were separated into two homogeneous groups according to milk yield (16.3 ± 3.1 kg/day) and LW (386 ± 39 kg) and evaluated over two experimental periods lasting 21 days each, with the last five days being used for data collection and sampling. The cows were treated with one of two levels of CT extract from black wattle (*A. mearnsii*): zero (control) and 40 (T40) g CT/kg concentrate. The CT extract was first mixed with the concentrate and then mixed with corn silage to be offered (pTMR) equivalent to 1.5% of the total DMI.

After morning milking, each experimental group had access to separated paddocks of a pasture of predominantly tall fescue. After afternoon milking, the cows received 10 kg/day of a pTMR containing 4 kg concentrate feed and 6 kg corn silage (as-fed basis). The concentrate contained 470 g/kg ground corn, 200

Table 1 - Chemical composition (g/kg dry matter (DM)) and energetic value of pasture and supplemental feeds (corn silage and concentrate) fed to ewes and cows in experiments 1 and 2, respectively.

Parameter	Pasture[1]	Corn silage	Concentrate[2]
----------Dairy ewes----------			
Dry matter (g/kg fresh)	191	337	900
Organic matter	916	963	920
Crude protein	258	79	219
Neutral detergent fiber	454	410	337
Acid detergent fiber	223	205	144
NE_L (MJ/kg DM)[3]	6.8	7.4	7.3
----------Dairy cows----------			
Dry matter (g/kg fresh)	212	338	912
Organic matter	888	966	922
Crude protein	170	84	204
Neutral detergent fiber	539	359	286
Acid detergent fiber	282	187	134
NE_L (MJ/kg DM)[3]	6.6	8.3	8.4

[1]Tall fescue (*Festuca arundinacea*)- and white clover (*Trifolium repens*)-predominant.
[2]470 g/kg ground corn, 200 g/kg soybean meal, 280 g/kg soybean hulls and 50 g/kg mineral mixture.
[3]Net energy for lactation.

g/kg soybean meal, 280 g/kg soybean hulls and 50 g/kg mineral mixture. The grazing method was rotational grazing, with the post-grazing herbage height no lower than 50% of the pre-grazing herbage height.

Sample collection and measurements

In both experiments, animals were milked twice a day (at 0600 and 1530 hours), and individual milk yields were recorded daily for two days before the start of each experiment (day zero) and during the sampling period. The milk fat, protein and milk urea N concentrations were measured every other day during the sampling period (on days 17, 19 and 21) by infrared spectrophotometry (International IDF Standard 141C:2000). For FA analyses, milk samples were collected from each animal from morning and afternoon milking on the last day of each period. These samples were mixed constituting a composite sample by animal per period. The LW was measured once a week after morning milking.

The pasture intake (PI) was estimated by the relationship between metabolic energy (ME) requirements (for lactation and maintenance) and ME supplied by supplements and pasture. The ME for maintenance (ME_m) was calculated from the LW, and the ME for lactation (ME_l) was calculated from milk production and milk composition according to the methods of (INRA, 2018). The energetic value of the pasture, corn silage and concentrate were estimated by methods proposed by the National Research Council (NRC, 2001). The PI was calculated as the difference between ME requirements and ME consumption from supplements, as proposed by.

Chemical analyses

The dry matter (DM) concentration was determined by drying at 105 °C for 24 hours. The ash content was determined by combustion in a muffle furnace at 550 °C for 4 hours, and the organic matter (OM) content was determined by mass difference. The total N was determined using the Kjeldahl method (Method 988.05; AOAC, 2019). Neutral detergent fiber (NDF) analyses were performed according to the methods of Mertens (MERTENS, 2002), except the samples were weighed into filter bags and treated with neutral detergent in ANKOM equipment (ANKOM Technology, Macedon NY, USA). This analysis included alpha-amylase but not sodium sulfite. The concentrations of acid detergent fiber (ADF) and sulfuric acid detergent lignin (ADL) were analyzed according to method 973.18 of AOAC (AOAC, 2019).

Milk samples were thawed at room temperature, and 1 mL of milk was used for lipid extraction using a mixture of diethyl ether and hexane according to a reference procedure (AOAC Official Method 989.05). The organic phase containing milk

fat was evaporated to dryness at 40 °C under oxygen-free nitrogen. FA methyl esters (FAMEs) were obtained by base-catalyzed transmethylation using a freshly prepared sodium methoxide solution as described in detail by Baldin (BALDIN et al., 2013). FAMEs were separated and quantified by a gas chromatograph (model 7820-A, Agilent Technologies) fitted with a flame-ionization detector and equipped with a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm × 0.2 µm film thickness; Varian Inc). Gas chromatography (GC) operating conditions included injector and detector temperatures both at 250 °C, H_2 as carrier gas (1 mL/min), and for the flame-ionization detector (35 mL/min), N_2 as makeup gas (30 mL/min), and purified air (286 mL/min). The initial temperature was 45 °C and held for 4 min, increased by 13 °C/min to 175 °C and held for 27 min, and increased by 4 °C/min to 215 °C and held for 35 min.The FAMEs were identified by comparison of their retention times with those of commercial FAME standards (Sigma Aldrich®, Nu-Chek Prep, Inc.) and Luta-CLA® 60 (BASF), and minor *trans*/*cis*-C18:1 isomers were identified by their orders of elution reported under the same GC conditions. Milk FA composition was expressed as a weight percentage of the total FAs using theoretical relative response factors (WOLFF et al., 1995). Stearoyl-CoA desaturase (SCD) indices for four pairs of FAs were calculated by expressing each product as a proportion of the precursor plus the product (i.e., SCD_{14} = *cis*-9 14:1/14:0 + *cis*-9 14:1; SCD_{16} = *cis*-9 16:1/16:0 + *cis*-9 16:1; SCD_{18} = *cis*-9 18:1/18:0 + *cis*-9 18:1; and SCD_{RA} = RA/VA + RA), as described in (KELSEY et al., 2003).

Statistical analyses

Statistical analysis was performed as repeated measures in time using PROC MIXED of SAS software (1999, version 9.3, SAS Institute, Cary, NC) considering the model:

$$Y_{ijk} = \mu + period_i + treatment_k + animal_{j(i)} + e_{ijk}$$

where Y_{ijk}, $\boldsymbol{\mu}$, $period_i$ + $treatment_k$ + $animal_{j(i)}$ + e_{ijk} represent the analyzed variable, the overall mean, the random effect of period, the fixed effects of treatment, the random effect of animal nested in period and the residual error, respectively.

Akaike's Information Criterion was used to choose the variance-covariance matrix. In the first experiment, the linear and quadratic effects of supplementation level were tested using polynomial orthogonal contrasts, in which the quadratic component was equivalent to the lack-of-fit sum of squares for linearity. Each *F* value was a ratio of the contrast mean square to the residual (experimental error) mean square. In the second experiment, the differences between means were determined by the means procedure using Student`s t-test at a 5% significance level, and *P*-values between 0.05 and 0.10 indicated a tendency.

RESULTS AND DISCUSSION

Milk production and gross composition

The total DMI, milk production and milk fat and protein concentrations were no different in dairy ewes receiving TE supplementation compared to those receiving the control treatment and averaged 1.3 kg/day, 1.2 kg/day, 5.8% and 5.2%, respectively (Table 2). Similarly, the total DMI, milk production and milk fat and protein concentrations were similar between dairy cows that did or did not receive TE supplementation and averaged 9.3 kg/day, 13.3 kg/day, 4.1% and 3.5%, respectively (Table 3).

The lack of a difference in milk production and composition in dairy ewes and dairy cows that did or did not receive TE supplementation showed that CTs have low potential to improve milk yield in dairy cows or ewes. Although, TE from *A. mearnsii* can increase the duodenal flow of metabolizable protein (ÁVILA et al., 2015; KOZLOSKI et al., 2012) and change the amino acid profile in the small intestine (ORLANDI et al., 2015) of ruminants, a possible increase in duodenal flow may not be reflected by increased milk protein synthesis and milk yield because either the circulating amino acids exceeded the productive capacity of milk synthesis, or the total N digestibility was reduced. According to TORAL et al. (2013), CTs may have a negative effect on nutrient absorption in the small intestine due to their interaction with the intestinal mucosa, reducing N digestibility.

Indeed, our results are in agreement with those of many other authors who reported no effect of condensed tannin extract for dairy ewes in proportions ranging from 10 to 50 g/kg of the DM in different diet conditions on milk production or composition (BUCCIONI et al., 2015b; BUCCIONI et al., 2017; DALLASTRA et al., 2018). In the same way, supplementation with condensed tannin extract did not effectively improve milk production or composition in dairy cows receiving a total mixed ration (FOCANT et al., 2019; GERLACH et al., 2018) or grazing tropical (ALVES et al., 2017a) or temperate pastures (GRIFFITHS et al., 2013; ALVES, T.P. et al., 2017b) when the inclusion level of the extract was less than 3% of the DMI. However, reductions in DMI and milk production were observed when greater inclusion levels of condensed tannin extract were used (AGUERRE et al., 2016; HENKE; et al., 2017).

Table 2 - Feed intake, milk production, gross milk composition and plasma nonesterified fatty acid (NEFA) concentration in dairy ewes grazing tall fescue (*Festuca arundinacea*)- and white clover (*Trifolium repens*)-predominant pasture supplemented with corn silage plus a concentrate mixture with or without black wattle tannin extract.

Parameter	----------Treatment[1]----------			SEM	P-value	
	Control	T30	T40		Linear	Quadratic
----------DM intake (kg/day)----------						
Pasture	0.54	0.64	0.58	0.081	0.755	0.440
Corn silage	0.30[a]	0.24[b]	0.29[a]	0.014	0.651	<0.001
Concentrate	0.52[a]	0.38[b]	0.47[a]	0.023	0.153	<0.001
Total	1.35	1.26	1.34	0.091	0.948	0.407
Milk production (kg/day)	1.30	1.10	1.20	0.080	0.531	0.075
6.5% FCM production (kg/day)[2]	1.20	1.00	1.10	0.082	0.402	0.176
Milk fat concentration (%)	5.93	5.83	5.75	0.270	0.658	0.970
Milk protein concentration (%)	5.27	5.21	5.12	0.161	0.539	0.955
Milk fat production (g/day)	76.2	65.1	68.8	5.53	0.354	0.292
Milk protein production (g/day)	63.6	56.1	60.9	4.43	0.678	0.266
NEFA (mg/dL)	5.87[b]	7.01[a]	5.19[b]	0.325	0.3370	0.020

[1]Treatments: C = control (without supplementation with tannin extract); T30 and T40 = supplemented with tannin extract as a proportion of 30 and 40 g/kg concentrate, respectively.
[2]6.5% fat-corrected milk production.

Milk FA composition

The milk fat cis-9 C18:1 content was on average 11% higher (quadratic effect: $P < 0.01$), and the *cis*-9, *trans*-11 CLA was 21% higher (linear effect: $P < 0.05$) in dairy ewes receiving TE supplementation than in dairy ewes without supplementation (Table 4). The proportions of total saturated and monounsaturated FAs did not differ between the treatment groups, aver-

Table 3 - Feed intake, milk production, gross milk composition and plasma nonesterified fatty acid (NEFA) concentration in dairy cows grazing tall fescue (*Festuca arundinacea*)- and white clover (*Trifolium repens*)-predominant pasture supplemented with corn silage plus a concentrate mixture with or without black wattle tannin extract.

Parameter	----------Treatments[1]----------		SEM	P-value
	Control	T40		
----------DM intake (kg/day)----------				
Pasture	3.69	3.50	0.29	0.656
pTMR[2]	5.77	5.72	0.02	0.114
Total	9.49	9.20	0.29	0.511
Milk production (kg/day)	13.4	13.1	0.35	0.658
4.0% FCM production (kg/day)[3]	13.7	14.0	0.38	0.606
Milk fat concentration (%)	4.05	4.21	0.10	0.264
Milk protein concentration (%)	3.51	3.58	0.05	0.328
Milk fat production (g/day)	0.55	0.57	0.01	0.434
Milk protein production (g/day)	0.47	0.48	0.01	0.659
NEFA (mg/dL)	7.99	7.59	0.35	0.435

[1]Treatments: C= control (without supplementation with tannin extract): T40 = supplemented with a tannin extract as a proportion of 40 g/kg concentrate.
[2]Partial total mixed ration: 60:40 corn silage: concentrate mixture; concentrate composition: 470 g/kg ground corn, 200 g/kg soybean meal, 280 g/kg soybean hulls and 50 g/kg mineral mixture
[3]4.0% fat-corrected milk production.

aging 69.9 and 23.3% of the total FAs, respectively. Palmitic acid represented 34% of the total saturated FAs, and *cis*-9 18:1 was 64% of the total monounsaturated fatty acids (MUFAs).

The results indicated the marked effect of tannin supplementation on dairy ewes, which led to increased contents of the ruminal BH intermediates *cis*-9, *trans*-11 CLA, *trans*-10 18:1 and *cis*-9 18:1 and decreased levels of *cis*-9, *trans*-12 18:2. It is well known that *Butyrivibrio* sp., the main bacteria responsible for ruminal BH, can convert linoleic acid to rumenic acid and rumenic acid to vaccenic acid, while *B. proteoclasticus* (previously classified as *Clostridium proteoclasticum*) hydrogenates vaccenic acid to stearic acid (PAILARD et al., 2007). In this way, when the *B. fibrisolvens* population increased and the *B. proteoclasticus* population decreased, milk rumenic acid and vaccenic acid levels increased in dairy ewes receiving CTs from quebracho or chestnut TEs (BUCCIONI et al., 2015b). However, there was no increase in the milk fat vaccenic acid content in our study, and as the TE was mixed with the concentrate

Table 4 - Effect of tannin supplementation on milk fatty acid (FA) composition of (g/100 g total FAs) of dairy ewes.

	----------Treatment[1]----------				----------P-value----------	
Fatty acid	C	T30	T40	SEM	Linear	Quadratic
4:0	3.82	3.88	3.81	0.046	0.941	0.483
6:0	3.12	3.11	2.98	0.042	0.207	0.510
8:0	3.03	2.94	2.78	0.057	0.100	0.796
10:0	9.75^{a}	9.28^{ab}	8.93^{b}	0.165	0.059	0.872
12:0	5.60^{a}	5.20^{ab}	5.04^{b}	0.125	0.083	0.648
cis-9 12:1[2]	13.19^{b}	14.71^{a}	13.30^{b}	0.004	0.848	0.007
14:0	11.74	11.18	11.57	0.202	0.736	0.271
15:0	1.25^{a}	1.09^{b}	1.25^{a}	0.025	0.979	0.008
16:0	23.72	22.83	24.88	0.457	0.317	0.142
cis-9 16:1[3]	1.11	1.03	1.15	0.029	0.574	0.128
trans-9 16:1[4]	0.46^{b}	0.53^{a}	0.49^{a}	0.010	0.174	0.017
17:0	0.47^{b}	0.51^{a}	0.50^{a}	0.008	0.099	0.213
cis-9 17:1	0.17^{b}	0.17^{b}	0.20^{a}	0.004	0.021	0.118
18:0	6.29^{b}	7.86^{a}	6.33^{b}	0.234	0.944	0.005
cis-9 18:1	13.19^{b}	14.71^{ab}	13.30^{a}	0.232	0.848	0.007
trans-10 18:1	0.22^{b}	0.30^{a}	0.31^{a}	0.014	0.028	0.354
trans-11 18:1	1.76	1.84	1.96	0.052	0.135	0.837
cis-9, *trans*-12 18:2	0.11^{a}	0.08^{b}	0.07^{b}	0.004	<0.001	0.145
18:2 n-6	1.74	1.68	1.58	0.050	0.199	0.827
cis-9, *trans*-11 CLA	1.07^{b}	1.17^{ab}	1.30^{a}	0.034	0.014	0.821
trans-10, *cis*-12 CLA	0.00	0.00	0.01	0.001	0.507	0.481
18:3 n-3	0.80	0.79	0.75	0.034	0.492	0.867
20:0	0.18	0.18	0.18	0.003	0.901	0.807
SFA[5]	70.47	69.54	69.73	0.423	0.491	0.537
MUFA[6]	20.78	22.09	21.07	0.289	0.608	0.774
PUFA[7]	4.27	4.33	4.21	0.103	0.821	0.665
ΣOBCFA[8]	3.34	3.22	3.38	0.047	0.743	0.169

[1]Treatments: C= control (without supplementation with tannin extract), T30 and T40 = supplemented with a tannin extract as a proportion of 30 or 40 g/kg concentrate;
[2]Coeluted with 13:0;
[3]Coeluted with 17:0 anteiso;
[4]Coeluted with 17:0 iso;
[5]Saturated FAs;
[6]Monounsaturated FAs;
[7]Polyunsaturated FAs;
[8]Odd- and branched-chain FAs.

(the source of linoleic acid), the increased milk rumenic acid content was probably because of higher ruminal bypass of rumenic acid produced from the decreased BH of linoleic acid rather than from the accumulation of linolenic acid intermediates such as vaccenic acid. As for a possible explanation for the contemporaneous effect on *trans* 10 C18:1, vacenic acid numerically increased from 1.76 to 1.96 g/100 of total FA in response to tannin supplementation, so it is possible that the greater variation in vacenic as compared to that of *trans*-10 C18:1 may have precluded us from detecting statistical differences for the former.

TE supplementation did not affect the milk FA composition in dairy cows, except for the C18:2 n-6 (linoleic acid) content, which tended (P=0.051) to increase by 13% (Table 5). The *cis*-9 18:1 and the *cis*-9, *trans*-11 CLA levels were on average 17.3 and 1.06% of the total milk FA, respectively. The proportions of total saturated FAs and MUFAs were on average 65.9 and 26% of total milk FAs, respectively, and palmitic (16:0) and vaccenic (*cis*-9 18:1) FAs were

Table 5 - Effect of tannin supplementation on milk fatty acid (FA) composition (g/100 g total FAs) of dairy cows.

Fatty acid	----------Treatment[1]----------		SEM	P-value
	Control	T40		
4:0	4.23	4.26	0.104	0.903
6:0	2.56	2.53	0.062	0.770
8:0	1.42	1.40	0.034	0.860
10:0	2.96	2.93	0.075	0.873
12:0	3.15	3.20	0.079	0.757
cis-9 12:1[2]	0.15	0.16	0.004	0.694
14:0	10.59	10.46	0.133	0.628
15:0	0.99	1.04	0.020	0.194
16:0	27.95	26.71	0.474	0.208
cis-9 16:1[3]	1.49	1.50	0.033	0.854
trans-9 16:1[4]	0.47	0.49	0.009	0.241
17:0	0.57	0.59	0.008	0.215
cis-9 17:1	0.20	0.20	0.005	0.837
18:0	10.36	10.65	0.151	0.351
cis-9 18:1	17.04	17.45	0.375	0.586
trans-10 18:1	0.34	0.32	0.012	0.431
trans-11 18:1	2.38	2.49	0.068	0.455
cis-9, *trans*-12 18:2	0.03	0.03	0.001	0.435
18:2 n-6	1.38[b]	1.56[a]	0.042	0.051
cis-9, *trans*-11 CLA	1.05	1.08	0.040	0.632
trans-10, *cis*-12 CLA	0.01	0.01	0.001	1.000
18:3 n-3	0.55	0.58	0.011	0.175
20:0	0.13[b]	0.15[a]	0.003	0.014
SFA[5]	66.3	65.4	0.518	0.385
MUFA[6]	25.66	26.31	0.448	0.482
PUFA[7]	3.46	3.72	0.082	0.128
ΣOBCFA[8]	3.12	3.26	0.058	0.231

[1]Treatments: C= control (without supplementation with tannin extract): T40 = supplemented with a tannin extract as a proportion of 40 g/kg concentrate;
[2]Coeluted with 13:0;
[3]Coeluted with 17:0 anteiso;
[4]Coeluted with 17:0 iso;
[5]Saturated FAs;
[6]Monounsaturated FAs;
[7]Polyunsaturated FAs;
[8]Odd- and branched-chain FAs.

41% of the total saturated FAs and 66% of the MUFAs, respectively.

The change in the milk FA profile of dairy ewes and the lack of this response in dairy cattle suggested that the ewes were more responsive to black wattle supplementation than the cows. These results may be explained, at least partially, by the greater effect of TE on the microbial population of ewes than cows. FRUTOS et al. (2004) observed variations in the susceptibility of microbial populations to the effects of TEs from quebracho in different ruminant species, and divergent FA compositions of milk from different ruminant species are also expected. These findings demonstrated that CTs have limited potential to improve the FA milk profile in dairy cows.

CONCLUSION

Diet supplementation with A. mearnsii TE slightly improves the nutritional quality of milk fat without reducing the milk production of dairy ewes and cows. However, dairy cows are apparently less responsive than dairy ewes to the effects of A. mearnsii TE supplementation on milk FA profile.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The study was performed in accordance with the Ethics Committee for Animal Experimentation (CETEA; protocol number: 01.19.14) from the Agroveterinary Science Center (CAV) at Universidade do Estado de Santa Catarina (UDESC) and according to current legislation and ethical principles published by the Brazilian College of Animal Experimentation (COBEA).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Kamila Maciel Dias: conceptualization, investigation, methodology, formal analysis and writing – original & draft; Marco Antônio Sundfeld da Gama: investigation, resources and writing – review & editing; Henrique M.N. Ribeiro-Filho: conceptualization, resources, supervision and writing – review & editing.

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