Effect of forage brassicas in dairy cow diets on the fatty acid profile and sensory characteristics of Chanco and Ricotta cheeses

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ABSTRACT

In humid temperate regions, forage turnip (*Brassica rapa* ssp. *rapa*) and forage rape (*Brassica napus* ssp. *biennis*) are common fodder crops used for dairy cattle during the summer season. However, there is little scientific information regarding the use of brassicas and their effect on the fatty acid (FA) profile in blood, milk, and milk products. The aim of this study was to evaluate the effect of forage brassica supplementation in dairy cows on the FA profile of blood plasma, milk, and cheese, and on the sensory characteristics of Chanco and Ricotta cheeses. Twelve multiparous dairy cows (Holstein Friesian) were housed and submitted to a 3 × 3 replicated (n = 3) Latin square design with 3 treatments (control, turnip, or rape) in 3 periods of 21 d each (14 d of diet adaptation and 7 d of measurements). The control diet consisted of 16.20 kg of grass silage (*Lolium perenne*), 2.25 kg of soybean bran, and 2.25 kg of commercial concentrate, all on a dry matter (DM) basis. In the treatments with forage brassicas, 24.15% of the total DM was replaced by turnip or rape; thus, they consisted of 12.25 kg of silage, 2.25 kg of soybean bran, 1.2 kg of concentrate, and 5 kg of turnip or rape (DM basis). A principal components analysis was performed on the results of the cheese sensory evaluation. Supplementation with turnip or rape modified the profile of FA in blood plasma and milk, increasing the saturated fraction, mainly short- and medium-chain FA, and decreasing the mono- and polyunsaturated FA. In the sensory evaluation, diet did not affect any of the 18 attributes evaluated. However, in the principal components analysis, cheeses made with milk from animals

fed turnip and rape were differentiated by increased odor, flavor, spiciness, bitterness, and acidity. Overall, brassicas can be used as an alternative forage source with no negative effects on sensory characteristics of cheeses.

Key words: medium-chain fatty acids, polyunsaturated fatty acids, forage rape, short-chain fatty acids, turnip

INTRODUCTION

In humid temperate regions (such as Chile, Ireland, and New Zealand), the main feed resources for dairy cattle are permanent pastures, which are characterized by comparatively low production costs that ensure the competitiveness and economical sustainability of the region's dairy farms (Pulido et al., 2010). Milk from pasture systems has a higher content of UFA, including rumenic acid (CLA, C18:2 *cis*-9,*trans*-11), compared with milk from cows fed a TMR with a similar dietary lipid content (Schroeder et al., 2003). Thus, pastures have become a valuable tool to increase the concentration of beneficial fatty acids (FA) in milk (Lock and Garnsworthy, 2002) and derived products (McIntosh et al., 2006). However, climatic variation between seasons results in marked differences in pasture production during the year. The nutritional quality decreases in summer as physiological maturity progresses, increasing fiber content and decreasing digestible protein content, which results in a lower DMI (Keim et al., 2015). Therefore, pastures in summer do not satisfy the animals' nutritional requirements and supplementary crops are needed to maintain the profitability of dairy farms (García et al., 2008).

The turnip (*Brassica rapa* ssp. *rapa*) and rape (*Brassica napus* ssp. *biennis*) are common supplementary crops for cattle feeding during the summer (Barry, 2013). The presence of secondary compounds such as

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glucosinolates, *S*-methylcysteine sulfoxide, and nitrates have been reported in *Brassica* species. According to Shankaranarayana et al. (1974), glucosinolates belong to the family of sulfur compounds and are associated with a strong and distinct flavor. Wiedenhoeft and Barton (1995) and Moate et al. (1996) noted that glucosinolates can be transferred to the milk, causing an undesired flavor, and can also affect the manufacture of cheeses. Forage brassicas contain high levels of easily fermentable carbohydrates, which improves DM digestibility and ruminal fermentation (Barry, 2013). Sun et al. (2016) showed that supplementation with turnip or rape reduces the acetate-to-propionate ratio and energy losses in the rumen, mainly methane emissions, resulting in improved feed efficiency.

Few studies have reported on the FA profile of brassica-fed dairy cows and their end products. For example, Thomson et al. (2000) found that the short-chain (SCFA) and medium-chain (MCFA) FA in milk increased significantly and UFA decreased when cows were supplemented with turnip compared with cows on a pasture-based diet. However, to the best of our knowledge, no comparison between turnip and rape has been made on fatty acids of milk and organoleptic characteristics of the resultant cheeses. Thus, the aim of this study was to evaluate the effect of supplementation with forage rape or summer turnip in the diet of dairy cows on the FA profile of blood plasma, milk, and cheese, and the organoleptic characteristics of cheeses. In this study, we selected 2 cheese varieties, Chanco and Ricotta. Chanco is one of the main cheese varieties produced in Chile, and it is produced from rennet coagulation of pasteurized milk, with production of lactic acid by mesophilic starter cultures (Vyhmeister et al., 2019). Ricotta cheese is obtained from induced heat-acid coagulation of whey proteins and is generally produced by farmstead cheese producers as an alternative use of sweet whey.

MATERIALS AND METHODS

Animals and Treatments

Animal care and procedures were carried out according to the guidelines of the Animal Care Committee of the Universidad Austral de Chile (Valdivia, Chile). Twelve multiparous, mid-lactation (150 ± 24 d) dairy cows (Holstein Friesian) were selected based on milk yield (21.37 ± 2.45 L/d). The experiment was carried out in 3 periods of 21 d, which consisted of a 14-d diet adaptation period and a 7-d sampling period, with water offered ad libitum and animals housed in individual stalls.

Table 1 shows the composition of dietary treatments offered. The control diet consisted of 16.20 kg of grass silage, 2.25 kg of soybeans, and 2.25 kg of concentrate, all on a DM basis. In treatments with forage brassicas, 24.15% of the total DM (20.70 kg of DM/d) was replaced by forage turnip or forage rape, resulting in a diet of 12.25 kg of silage, 2.25 kg of soybean meal, 1.20 kg of concentrate, and 5.00 kg of forage turnip or forage rape on a DM basis. In addition, 200 g of mineral salts was offered daily in all of the diets.

DMI, Milk Production, and Milk Composition

Orts were measured daily to determine daily DMI for each cow. Cows were milked at 0500 and 1500 h, and milk yield was recorded at each milking using automatic milk meters (MM27BC, DeLaval, Tumba, Sweden). The average for the final week of each period is reported. Representative milk samples for a.m. and p.m. milkings (10 mL total) were collected on 3 d in the last week of each the experimental period for fat, protein, lactose, and MUN analyses by infrared spectrophotometer (Foss 4300 Milkoscan, Foss Electric, Hillerød, Denmark).

Table 1. Diet composition of control, turnip, and rape dietary treatments

Component	Diet ¹		
	Control	Turnip	Rape
Ingredient composition (kg of DM/d)			
Grass silage	16.20	12.25	12.25
Soybean bran	2.25	2.25	2.25
Concentrate	2.25	1.20	1.20
Forage turnip	—	5.00	—
Forage rape	—	—	5.00
Chemical composition (% of DM)			
DM	47.34	38.23	35.94
CP	19.21	20.75	21.31
Ether extract	3.84	3.30	3.44
NDF	37.89	33.56	34.02
ADF	23.58	21.20	21.42
Fatty acids			
C16:0	6.5	10.31	8.87
C18:0	2.6	3.95	20.68
C18:1	79.03	18.33	32.37
C18:2	7.38	4.23	5.87
C18:3	3.89	15.49	20.53
>C20	0.59	47.7	11.68
SFA	9.11	14.26	29.55
MUFA	79.03	18.33	32.37
PUFA	11.86	67.41	38.08

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

Chemical Analyses of Feeds

Dry matter content was determined by weighing before and after drying in a forced-air oven at 60°C for 48 h and thereafter at 105°C for 12 h. For each feed sample, ash and ether extract were analyzed according to AOAC International (1996; methods 942.05 and 920.39, respectively). Nitrogen content was determined by combustion (model FP-428 Nitrogen Determinator, Leco Corp., St Joseph, MI) and used to calculate CP content ($N \times 6.25$); NDF was determined according to Van Soest et al. (1991) using heat-stable amylase (Ankom Technology Corp., Macedon, NY) and ADF according to AOAC International (1996; method 973.18). The sequential fiber analysis with correction for residual ash was conducted because pectins and ash can interfere with detergent fiber analysis of brassica forages (Cassida et al., 2007).

Blood Plasma

In the week of measurement, 10 mL of blood was obtained from each animal from the coccygeal vein using heparinized vacuum tubes (Becton Dickinson Co., Franklin Lakes, NJ). Each sample was mixed gently and centrifuged at $3,200 \times g$ for 10 min at room temperature. Plasma was recovered, transferred to plastic vials, and frozen at -20°C for subsequent analysis of FA.

Cheese Making: Chanco and Ricotta

On the last day of each sampling week, approximately 4 L of milk was collected from each cow, weighting production in the morning (60%; 0700 h) and afternoon (40%; 1630 h) milkings. Later, the milk of the same treatment was mixed and transported at 4°C to the Department of Animal Sciences of the Pontificia Universidad Católica de Chile (Santiago, Chile), where the cheeses were made and subjected to sensory analysis.

Chanco cheese manufacturing was carried out based on the protocol described by Vargas-Bello-Pérez et al. (2018) and Oliveira and Brito (2006). The milk obtained from each treatment was pasteurized at 65°C for 30 min and then left to cool to 31°C . The pH was then reduced to 5.9 with a 25% citric acid solution, and 3 g of calcium chloride was added. After 3 min, 3 g of commercial rennet powder was added (Nievi, Productos Nievi S.L., Erandio, Spain) and allowed to stand for 45 min. Once the firmness of the curd was evaluated, the curd was cut into cubes of 1 cm^3 . The curd was gently stirred for 10 min and then cooked at 38°C for 30 min under constant agitation. The whey

was completely drained out of the cheese vats for 30 min. The obtained curd was milled and salted with 0.3 L of brine solution (18% wt/vol NaCl) and left to rest for 20 min. The curd was transferred to molds of 250 g and the cheeses were pressed overnight. Finally, cheeses were transferred to a maturation room where they remained for 21 d at a temperature of 10°C and a relative humidity of 80%.

To make Ricotta cheese, 12 L of whey was used (obtained during preparation of the Chanco cheese), which was drained and then added to 3 L of skim milk. The temperature of the whey-milk mixture was increased to 90°C and the pH reduced to 5.4 by adding a citric acid solution (25% wt/vol). Once the curd began to float on the surface of the cheese pot, it moved slowly from the outside to the center. This process took 10 to 15 min. Subsequently, the curd was maintained at 90°C for 10 min before being transferred to 250-g molds. Finally, the Ricotta cheese was left to rest overnight at 2 to 5°C .

Sensory Analysis of Cheeses

A descriptive sensory analysis was performed on experimental cheeses. The sensory panel was composed of 14 trained judges who were not provided with any information about the previous treatment of the cheese samples (slices of 1 cm^3 , approximately 10 g). The panelists were given cheese samples in the form of 10-g slices and evaluated the following attributes: general odor, milk odor, mature cheese odor, general flavor, ripe cheese flavor, astringency, homogeneity, hardness, granularity, sound, spiciness before, spiciness after, bitterness, acid, saltiness, holes, adhesion, and humidity. The judges evaluated the cheeses sequentially by rating the attributes on a continuous intensity scale from 0 to 9, where 0 means less intense and 9 more intense, according to the description of Vargas-Bello-Pérez et al. (2015). In contrast to Chanco, Ricotta cheeses were informally evaluated by a panel of 5 cheese graders with extensive knowledge and experience to determine main attributes and defects in appearance, texture, flavor, and aromas of milk and dairy products.

Fatty Acid Profile

Lipids of the samples analyzed (blood plasma, milk, and Chanco and Ricotta cheeses) were extracted according to the method proposed by Bligh and Dyer (1959), and methylation was performed according to the protocol of Christie (1982) with modifications by Chouinard et al. (1999). A gas chromatography system (AOC-20, Shimadzu Scientific Instruments, Columbia, MD) equipped with a 100-m column with the following

chromatographic conditions was used: after injection, the oven temperature was set at 110°C for 4 min and then raised to 160°C at a rate of 5°C/min for 10 min, to 225°C at 3°C/min for 10 min, and finally increased to 240°C at 3°C/min. The temperature of the ionization flame was 260°C, injection volume 2 µL, hydrogen flow 25 mL/min, the air flow 400 mL/min, and the flow of nitrogen that makes up the gas 40 mL/min. The fatty acid peaks in the GC were identified using standardization methyl esters of fatty acids (FAME, 37 Component FAME mix, Supelco, Bellefonte, PA).

Nutritional Evaluation of Fat

The nutritional quality of the FA contained in the milk and cheeses was evaluated as indicated by Ulbricht and Southgate (1991). The atherogenic and thrombogenic indices were determined by the following formulas:

$$\text{Atherogenic index} = [\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}/(\text{PUFA n-6} + \text{PUFA n-3}) + \text{MUFA}] \text{ and}$$

$$\begin{aligned} \text{Thrombogenic index} = & [(\text{C14:0} + \text{C16:0} \\ & + \text{C18:0}/0.5 \times \text{MUFA}) + (0.5 \times \text{PUFA n-6}) \\ & + (3 \times \text{PUFA n-3}) + (\text{PUFA n-3}/\text{PUFA n-6})]. \end{aligned}$$

When the values of these indices are low, fats are likely to have less negative effects on human health (Allred et al., 2006). Additionally, the n-6/n-3 and PUFA/SFA ratios were determined.

Experimental Design and Statistical Analysis

According to production, live weight, fat, protein, and DIM, 4 sub-groups of 3 animals (squares) were constituted and randomized to the treatments control, turnip, and rape. The animals went through each of the treatments in a replicated ($n = 4$) 3×3 Latin square design and balanced for residual effects (3 treatments and 3 periods of 21 d). As a carryover effect was not detected, data were analyzed using the mixed model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) to account for effects of square, period within square, cow within square, and treatment. The dietary treatment was considered a fixed effect; square, period within square, and cow within square were considered random effects.

To determine the FA profile contained in the cheeses (Chanco and Ricotta), a completely randomized block design was used, where the block corresponded to the experimental period.

Before carrying out the ANOVA, the assumptions of normality were checked by the Kolmogorov-Smirnov test and homogeneity of variance with the Levene test. When statistical differences were observed ($P < 0.05$), the means were compared by the Tukey test.

A principal component analysis (PCA) was also carried out on the results of the cheese sensory evaluations by means of the statistical software Minitab 18 (Minitab Inc., State College, PA) to identify groups of data related to the treatments evaluated.

RESULTS AND DISCUSSION

Under the conditions of this study, forage brassicas were found to be an interesting forage alternative during summer and they can be useful for those farmers seeking added value for their dairy products, including Chanco and Ricotta cheeses. Most of the changes described here are explained by the unique FA profile that brassicas have. In this study, the FA profile from diets (Table 1) showed that brassicas have high contents of FA with >20 carbons, which is in agreement with previous research on different brassica species (Sharafi et al., 2015).

Milk Production and Composition

Milk production and composition did not differ between treatments ($P > 0.3$, Table 2). The average milk yield was 24.19 kg/d, with 4.32% fat and 3.36% CP. Differences in DMI, however, were observed ($P < 0.01$): 19 kg/d for control, 17.88 kg/d for turnip, and 18.02 kg/d for rape treatments. There were no differences between brassicas ($P > 0.05$). The reason for the lower DMI could be the low DM content of diets supplemented with brassicas (Table 1). This effect was previously reported in sheep (Sun et al., 2012) and dairy cattle (Moate et al., 1998), in which the DMI decreased 13 and 16%, respectively, when brassicas were included in diets.

Fatty Acids in Blood Plasma

Table 3 shows that the concentration of SFA in blood plasma was higher in cows in the turnip and rape treatments, compared with the control ($P < 0.01$). The FA that contributed to this difference were C14:0, C15:0, C16:0, C17:0, and C18:0. The percentage of MUFA was 2.95 and 2.17% lower in the diet supplemented with forage rape compared with the control and turnip treatments, respectively ($P < 0.01$). Plasma had a lower PUFA content in cows fed forage turnip compared those fed the control and forage rape diets ($P = 0.02$).

Table 2. Production and composition of milk from cows fed control, turnip, and rape diets

Parameter	Diet ¹			SEM	P-value
	Control	Turnip	Rape		
DMI (kg/d)	19.00 ^a	17.88 ^b	18.02 ^b	0.46	<0.01
Milk yield (kg/d)	24.16	24.11	24.31	0.78	0.94
FCM (kg/d)	25.19	25.17	25.76	0.90	0.66
ECM (kg/d)	26.78	26.96	27.37	0.88	0.69
Composition (%)					
Fat	4.28	4.28	4.41	0.14	0.54
Protein	3.31	3.42	3.36	0.08	0.32
Lactose	4.87	4.84	4.88	0.03	0.40

^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

The total content of n-3 fatty acids was 1.99% lower in cows supplemented with brassicas ($P < 0.01$) compared with the control group.

At the ruminal level, dietary lipids undergo 2 important modifications: lipolysis and ruminal biohydrogena-

tion (**RBH**). Lipolysis is the process in which lipids are hydrolyzed by lipases (i.e., galactolipases and phospholipases) mostly of bacterial origin and, to a lesser extent, protozoal. This results in the release of FA and glycerol, or, in the case of glycolipids, FA and galactose

Table 3. Fatty acids contained in the blood plasma of cows fed control, turnip, and rape diets

Fatty acid (%)	Diet ¹			SEM	P-value
	Control	Turnip	Rape		
C8:0	0.48	0.46	0.52	0.09	0.88
C10:0	1.59	1.30	1.93	0.30	0.34
C14:0	0.50 ^b	0.67 ^a	0.73 ^a	0.04	<0.01
C14:1	0.31 ^a	0.21 ^b	0.21 ^b	0.03	0.04
C15:0	0.56 ^b	0.70 ^a	0.71 ^a	0.04	0.03
C15:1	0.22 ^a	0.17 ^b	0.19 ^b	0.01	0.01
C16:0	15.57 ^b	17.38 ^a	18.36 ^a	0.59	<0.01
C16:1	0.51	0.47	0.54	0.06	0.69
C17:0	0.87 ^b	1.04 ^{ab}	1.14 ^a	0.07	0.05
C18:0	22.78 ^b	26.08 ^a	24.8 ^{ab}	0.67	0.01
C18:1 <i>trans</i> -10	0.71 ^a	0.59 ^b	0.51 ^b	0.03	<0.01
C18:1 <i>trans</i> -11	0.44 ^a	0.34 ^b	0.34 ^b	0.04	0.04
C18:1n-9 <i>cis</i>	4.91 ^a	4.47 ^a	2.34 ^b	0.44	<0.01
C18:2n-6 <i>cis</i>	35.71	34.70	37.31	0.93	0.16
C20:0	0.38	0.38	0.36	0.05	0.92
C18:3n-6	0.54 ^a	0.44 ^{ab}	0.30 ^b	0.05	<0.01
C18:3n-3	6.69 ^a	5.49 ^{ab}	5.21 ^b	0.40	0.04
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.42	0.39	0.36	0.05	0.71
C20:2	0.19	0.13	0.13	0.03	0.22
C22:0	0.20	0.14	0.20	0.03	0.34
C20:3n-6	1.75 ^a	1.35 ^{ab}	1.14 ^b	0.17	0.05
C20:4n-6	2.85 ^a	1.80 ^b	1.63 ^b	0.22	<0.01
C22:2	0.12	0.10	0.10	0.02	0.49
C24:0	0.14	0.11	0.11	0.02	0.33
C20:5n-3	0.92 ^a	0.64 ^b	0.49 ^b	0.08	<0.01
C22:6n-3	0.71 ^a	0.41 ^b	0.38 ^b	0.05	<0.01
Σ SFA	43.08 ^b	48.28 ^a	48.83 ^a	1.15	<0.01
Σ MUFA	7.08 ^a	6.30 ^a	4.13 ^b	0.44	<0.01
Σ PUFA	49.89 ^a	45.44 ^b	47.04 ^a	1.03	0.02
Σ n-3	8.30 ^a	6.54 ^b	6.07 ^b	0.45	<0.01
Σ n-6	40.85	38.28	40.38	0.89	0.11

^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

(Lourengo et al., 2010). Ruminal biohydrogenation is a detoxification mechanism that ruminal microorganisms use to protect themselves from the harmful effects of lipids, principally the bactericidal action of free FA. Molecules that have one or more double bonds have a greater antimicrobial power than SFA (Maia et al., 2007). Therefore, the rate of lipolysis and RBH depends on the type of fat in the diet. Consequently, if the content of UFA from food exceeds the detoxification capacity of ruminal microorganisms, both processes lose efficiency, increasing the amount of FA with double bonds that escape from the rumen (Shingfield et al., 2008).

The more important source of FA contained in the blood plasma is the diet, whereas the rest of the FA can be mobilized from the adipose tissue, especially during the first month of lactation (Halmemies-Beauchet-Filleau et al., 2013). Thomson et al. (2000) showed that the fatty acids contained in the turnip plant differ from those of forage grasses, mainly because the forage turnip has a greater amount of SFA and lower unsaturated content. However, Khan and Chaudhry (2011) reported that the FA profile of the forage rape plant has less linoleic acid (C18:2n-6 *cis*) and linolenic acid (C18:3n-3) than that of grass silage.

As mentioned above, a higher concentration of UFA, as shown in the plasma of cows fed the control diet, can be toxic to fiber-fermenting microorganisms, decreasing their efficiency. In this context, Maia et al. (2010) reported a decrease in cellulolytic bacteria due to the increased concentration of UFA in the rumen. Thus, decreasing the fermentation of the fiber results in the simultaneous decrease of acetate production. Since acetate is the main precursor of de novo synthesis in the mammary gland, the SCFA and MCFA content in milk may also decrease. However, Chilliard et al. (2000) indicate that long-chain (LCFA) and very long chain (VLCFA) FA are powerful inhibitors of acetyl-CoA carboxylase, an enzyme used in de novo synthesis of FA.

Numerous studies have shown an increase in the C18:2 *cis*-9,*trans*-11 and *trans* vaccenic acid (TVA, C18:1 *trans*-11) content of milk when cows are fed diets rich in UFA (Prieto et al., 2016).

Fatty Acids in Milk

Table 4 shows that the concentration of total SFA increased 4.11% in the milk of cows fed turnip and rape compared with milk from cows in the control group ($P < 0.01$). The most abundant SFA were C4:0, C6:0, C8:0, C10:0, and C16:0. The concentrations of MUFA and PUFA decreased in the treatments supple-

mented with brassicas ($P < 0.01$ and $P = 0.04$). The least abundant MUFA were C18:1n-9 *cis* and C20:1n-9, whereas the least abundant PUFA were C18:2n-6 *cis*, C18:2 *cis*-9,*trans*-11, C18:3n-3, and C22:6n-3. The concentration of n-3 FA, mainly C18:3n-3 and C22:6n-3, decreased 0.28% with the supplementation of brassicas ($P < 0.01$).

Nutritional evaluation of FA showed that supplementation with brassicas increased the n-6/n-3 ratio ($P < 0.01$, control: 1.96, turnip: 4.45 and rape: 5.32). Additionally, the PUFA/SFA ratio decreased ($P = 0.02$, control: 0.03, turnip or rape: 0.02). The atherogenic and thrombogenic indices increased (which is less favorable for human health) with the inclusion of brassicas in the diet ($P = 0.01$ and $P < 0.01$, respectively).

Feeding brassicas resulted in milk with higher percentages of SCFA and MCFA ($P < 0.01$ and $P = 0.01$, respectively); the main FA involved were C4:0, C6:0, C8:0, and C10:0. However, LCFA were 1.25% lower, on average, in milk from cows fed the turnip and rape diets compared with the control ($P < 0.01$). The main LCFA with reduced content were C18:1n-9 *cis*, C18:2n-6 *cis*, C18:2 *cis*-9,*trans*-11, C18:3n-3, and C20:1n-9 (Table 5).

According to Heid and Keenan (2005), the lipids of bovine milk are contained within the fat globules. The FA in the fat globules of bovine milk have 3 main origins (Clegg et al., 2001): FA contained in the lipoproteins circulating in the blood (from the diet and ruminal digestion), the nonesterified fatty acids from body mobilization (bound to albumin), and FA synthesized de novo in the mammary gland.

The SCFA and MCFA are mostly synthesized de novo in the mammary gland, whereas LCFA and VLCFA come from the lipids circulating in the blood and from the fat mobilized from body reserves (Halmemies-Beauchet-Filleau et al., 2013). In this regard, Bauman et al. (2011) noted that acetate is the main carbon source for most FA that are synthesized. Our results agree with those of Thomson et al. (2000), who found that by supplementing the diet of cows with turnip in the summer period, the concentrations of SCFA and MCFA increased, whereas the amount of UFA decreased compared with a pasture-only diet.

The risk of coronary heart disease associated with SFA varies from no association to a significant risk (Houston, 2018). Also, saturated fats have been shown to have beneficial effects on human health, an aspect that is currently being investigated (O'Sullivan et al., 2013). In the current study, the concentrations of SFA in milk increased when feeding the turnip and rape diets, generally due to differences in the SFA derived from de novo synthesis, such as C4:0, C6:0, C8:0, C10:0, and C16:0, which explains greater synthesis with the

turnip and rape diets. In addition, the UFA (MUFA and PUFA) decreased, reflecting characteristics of the foods used in the ration. Chilliard et al. (2000) note that PUFA are not synthesized by tissues of ruminants; therefore, their concentration in milk or derived products depends first on the PUFA contained in the diet and then on the amount of PUFA that escapes RBH.

Thomson et al. (2000) reported lower amounts of UFA in milk, including CLA C18:2 *cis*-9,*trans*-11, when supplementing the diet of dairy cows with forage turnip.

In the mammary gland, the activity of Δ^9 -desaturase plays an important role in the degree of desaturation of FA, especially those of 18 carbons (Chilliard et al., 2001). The most relevant desaturation is that of

Table 4. Fatty acids contained in the milk of cows fed control, turnip and rape diets

Fatty acid (%)	Diet ¹			SEM	P-value
	Control	Turnip	Rape		
C4:0	3.23 ^b	3.60 ^a	3.57 ^a	0.11	<0.01
C6:0	2.06 ^b	2.24 ^a	2.32 ^a	0.07	<0.01
C8:0	1.12 ^b	1.23 ^a	1.29 ^a	0.05	<0.01
C10:0	2.74 ^b	3.03 ^{ab}	3.16 ^a	0.14	0.01
C11:0	0.26	0.35	0.30	0.04	0.27
C12:0	3.11	3.58	3.43	0.15	0.06
C13:0	0.07	0.10	0.08	0.02	0.44
C14:0	12.17	11.46	12.17	0.67	0.68
C14:1	0.82	0.87	0.91	0.06	0.29
C15:0	0.94	0.97	0.94	0.04	0.65
C15:1	0.12	0.14	0.14	0.01	0.17
C16:0	37.46 ^b	40.04 ^a	39.99 ^a	0.78	<0.01
C16:1	1.16	1.15	1.15	0.12	0.99
C17:0	0.37	0.39	0.38	0.02	0.69
C17:1	0.21	0.19	0.17	0.02	0.43
C18:0	8.52	8.70	9.23	0.50	0.60
C18:1 <i>trans</i> -10	0.07	0.02	0.01	0.02	0.16
C18:1 <i>trans</i> -11	0.28	0.05	0.03	0.09	0.09
C18:1n-9 <i>cis</i>	22.70 ^a	20.12 ^b	18.94 ^b	0.65	<0.01
C18:2n-6 <i>trans</i>	0.45 ^b	0.54 ^{ab}	0.62 ^a	0.05	0.03
C18:2n-6 <i>cis</i>	0.11 ^a	0.07 ^{ab}	0.05 ^b	0.01	0.02
C20:0	0.04	0.02	0.02	0.02	0.67
C20:1n-9	0.05 ^a	0.01 ^{ab}	0.00 ^b	0.01	0.03
C18:3n-6	0.05	0.01	0.02	0.01	0.16
C18:3n-3	0.32 ^a	0.10 ^b	0.08 ^b	0.04	<0.01
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.49 ^a	0.39 ^b	0.44 ^{ab}	0.03	0.02
C20:2	0.09	0.05	0.02	0.04	0.18
C22:0	0.12	0.15	0.10	0.06	0.84
C20:3n-3	0.04	0.04	0.02	0.01	0.52
C20:3n-6	0.14	0.07	0.07	0.05	0.57
C22:1n-9	0.09	0.05	0.07	0.05	0.81
C23:0	0.13	0.05	0.05	0.07	0.63
C20:4n-6	0.07	0.03	0.04	0.02	0.48
C22:2	0.15	0.03	0.05	0.05	0.23
C24:0	0.10	0.08	0.07	0.05	0.92
C20:5n-3	0.05	0.03	0.03	0.01	0.32
C24:1n-9	0.04	0.01	0.01	0.01	0.13
C22:6n-3	0.05 ^a	0.04 ^{ab}	0.01 ^b	0.01	0.02
Σ SFA	72.45 ^b	75.99 ^a	77.13 ^a	0.73	<0.01
Σ MUFA	25.53 ^a	22.60 ^b	21.42 ^b	0.64	<0.01
Σ PUFA	2.01 ^a	1.41 ^b	1.45 ^b	0.18	0.04
Σ n-3	0.47 ^a	0.21 ^b	0.16 ^b	0.05	<0.01
Σ n-6	0.81	0.73	0.80	0.08	0.65
Σ n-6/Σ n-3	1.96 ^b	4.45 ^a	5.32 ^a	0.39	<0.01
Σ PUFA/Σ SFA	0.03 ^a	0.02 ^b	0.02 ^b	0.01	0.02
Atherogenic index	3.39 ^b	3.82 ^{ab}	4.17 ^a	0.15	0.01
Thrombogenic index	3.93 ^b	4.82 ^a	5.31 ^a	0.18	<0.01

^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

Table 5. Fatty acid profile of milk classified according to the number of carbon atoms

Fatty acid ¹ (%)	Diet ²			SEM	P-value
	Control	Turnip	Rape		
SCFA (3–7 C)	5.29 ^b	5.84 ^a	5.90 ^a	0.17	<0.01
MCFA (8–13 C)	7.29 ^b	8.29 ^a	8.26 ^a	0.33	0.01
LCFA (14–20 C)	86.73 ^a	85.47 ^b	85.48 ^b	0.45	<0.01
VLCFA (>21 C)	0.68	0.41	0.36	0.24	0.61

^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

¹SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; VLCFA = very long chain fatty acids.

²Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

TVA, which results in the formation of CLA C18:2 *cis*-9,*trans*-11 (Pariza et al., 2001). The concentration of CLA C18:2 *cis*-9,*trans*-11 was higher with the control diet than with the turnip diet. This could be due to the greater amount of TVA present in blood plasma of cows fed the control diet.

Regarding the nutritional evaluation of the milk FA, we observed that the n-6/n-3 ratio was higher for milk from the turnip and rape treatments. The recommended value for this ratio is <4 (British Department of Health, 1994), a recommendation that was met only in control milk. However, the PUFA/SFA ratio in milk from all of the diets was below the recommended amount (~0.45).

Ulbricht and Southgate (1991) define the atherogenic index as the ability of fats to produce a plaque in the endothelium of blood vessels and the thrombogenic index as the ability of fats to produce thrombosis. Therefore, the milk of cows fed the control treatment had better nutritional quality than milk from the treatments supplemented with brassicas, as indicated by the lower n-3/n-6 ratio, atherogenic index, and thrombogenic index.

Fatty Acids in Chanco Cheese

The percentages of SFA, MUFA, PUFA, and n-6 FA were not affected by treatment ($P > 0.05$, Table 6) and neither were the ratios of n-6/n-3 and PUFA/SFA ($P > 0.05$). However, concentrations of C14:0 and C16:0 were higher in Chanco cheese from cows supplemented with brassicas ($P < 0.05$). Additionally, supplementation with turnip or rape reduced the concentration of n-3 FA by 0.12% ($P < 0.01$), and the same diets showed higher atherogenic and thrombogenic indices ($P = 0.02$ and $P < 0.01$, respectively).

The quality and composition of cheese are strongly influenced by factors attributable to the initial milk quality (Lobos-Ortega et al., 2012). Falchero et al. (2010) reported similar values between the FA profile

of milk and cheese. Scerra et al. (2016) pointed out that the processing of milk does not, to a great extent, alter the composition of FA contained in the cheeses. Although the cheeses of the control diet had a lower SFA fraction than cheeses of the turnip and rape diets (2.33 and 3.89% respectively), there were no major statistical differences (as in the FA profile of the milk), perhaps due to the number of replicates. However, the concentration of palmitic acid (C16:0) was higher in both milk and cheeses from cows fed forage brassicas.

Fatty Acids in Ricotta Cheese

Table 7 shows that the n-3 FA content was lower in Ricotta cheese from the rape treatment ($P < 0.01$). In addition, the atherogenic and thrombogenic indices were higher in cheese from cows supplemented with forage brassicas ($P = 0.03$ and $P = 0.04$, respectively). However, SFA, MUFA, PUFA, n-6 FA, n-6/n-3 ratio, and PUFA/SFA ratio did not differ between the evaluated diets ($P > 0.05$). The exceptions were the SFA C14:0 and C16:0; the former was higher in the rape diet ($P = 0.03$) and the latter was higher in the turnip and rape diets ($P = 0.02$). In contrast, the concentration of C20:5n-3 (eicosapentaenoic acid) decreased in the cheese of cows fed brassicas ($P = 0.04$).

The FA profile of Ricotta cheese was similar to that of Chanco cheese, mainly because Ricotta cheese was prepared from whey obtained during the preparation of the Chanco cheese. Similarly, in cows grazing summer highland pastures, Bergamaschi and Bittante (2017) found that milk, fresh cheese, and Ricotta cheese had similar contents of SFA and UFA. Nudda et al. (2005) found that in sheep dairy products, the FA concentrations in fresh cheese and Ricotta cheese depended mainly on the FA content of unprocessed raw milk, in agreement with results of Lobos-Ortega et al. (2012).

Our results indicated that the nutritional quality (atherogenic and thrombogenic indices) of the cheeses

depended mainly on the nutritional quality of the unprocessed milk. Currently, we are not aware of any other studies related to the use of forages or to the use of diets based on grass silage on the FA profile in Chanco and Ricotta cheeses. Due to the importance of FA in human health, more research is needed in this area.

Sensory Analysis of Chanco and Ricotta Cheeses

In Chanco cheese, diet did not affect any of the 18 attributes considered: homogeneity, holes, general smell, smell of ripe cheese, smell of milk, salty, sour, bitter, spiciness before swallowing, spiciness after swallowing, general flavor, ripe cheese flavor, astringency, hardness,

Table 6. Fatty acids contained in Chanco cheese from milk of cows fed control, turnip, and rape diets

Fatty acid (%)	Diet ¹			SEM	P-value
	Control	Turnip	Rape		
C4:0	3.89	3.93	4.18	0.07	0.66
C6:0	2.56	2.15	2.68	0.03	0.49
C8:0	1.29	1.35	1.43	0.04	0.86
C10:0	2.75	2.39	3.23	0.11	0.81
C11:0	0.29	0.31	0.33	0.02	0.72
C12:0	3.21	3.73	3.67	0.14	0.87
C13:0	0.08	0.07	0.07	0.01	1.00
C14:0	11.63 ^b	12.29 ^a	12.25 ^a	0.03	0.04
C14:1	0.81	0.96	1.02	0.07	0.93
C15:0	0.97	0.98	0.97	0.06	0.64
C15:1	0.12	0.23	0.12	0.01	0.11
C16:0	37.12 ^b	39.01 ^a	38.43 ^a	0.49	0.01
C16:1	1.21	1.33	1.24	0.08	0.84
C17:0	0.36	0.42	0.41	0.02	0.53
C17:1	0.22	0.21	0.21	0.01	0.82
C18:0	8.92	9.55	10.03	0.81	0.63
C18:1 <i>trans</i> -10	0.04	0.04	0.02	0.01	0.26
C18:1 <i>trans</i> -11	0.06	0.08	0.03	0.01	0.11
C18:1n-9 <i>cis</i>	20.75	18.94	18.05	1.24	0.42
C18:2n-6 <i>trans</i>	0.74	0.70	0.67	0.01	0.70
C18:2n-6 <i>cis</i>	0.07	0.04	0.04	0.01	0.96
C20:0	0.01	0.05	0.08	0.03	0.41
C20:1n-9	0.02	0.01	ND ²	0.01	0.53
C18:3n-6	0.01	0.01	ND	0.01	0.69
C18:3n-3	0.34	0.32	0.17	0.05	0.09
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.41	0.36	0.43	0.03	0.09
C20:2	0.01	0.02	0.01	0.01	0.76
C22:0	0.05	0.05	0.07	0.02	0.57
C20:3n-3	0.01	0.05	0.01	0.01	0.55
C20:3n-6	0.03	0.11	0.02	0.01	0.59
C22:1n-9	0.05	0.11	0.02	0.01	0.50
C23:0	0.02	0.03	0.05	0.01	0.37
C20:4n-6	0.05	0.12	0.01	0.01	0.05
C22:2	0.02	ND	0.01	0.01	0.11
C24:0	0.02	0.02	0.01	0.01	0.61
C20:5n-3	0.03	0.01	0.02	0.01	0.63
C24:1n-9	0.04	0.03	0.03	0.01	0.95
C22:6n-3	0.01	0.01	0.01	0.01	0.26
Σ SFA	73.99	76.32	77.88	1.30	0.11
Σ MUFA	24.28	21.93	20.73	1.18	0.22
Σ PUFA	1.73	1.75	1.39	0.57	0.17
Σ n-3	0.40 ^a	0.36 ^b	0.21 ^b	0.04	<0.01
Σ n-6	0.90	0.98	0.73	0.16	0.40
Σ n-6/Σ n-3	2.31	2.54	3.59	0.36	0.12
Σ PUFA/Σ SFA	0.02	0.02	0.02	0.02	0.44
Atherogenic index	3.50 ^b	3.94 ^a	4.32 ^a	0.18	0.02
Thrombogenic index	4.10 ^b	4.68 ^a	5.37 ^a	0.17	<0.01

^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

²Not detected.

granularity, sound, humidity, or adhesion ($P > 0.05$, Table 8). However, in the PCA, 2 components were selected. The main component 1 (PC1) explained 59.1% of the total variability and differentiated the rape cheese from the control and turnip cheeses; the rape cheese showed greater intensity of general flavor, humidity, ripe cheese flavor, salty, smell of mature cheese, general

smell, and spiciness after swallowing. The rape cheese was also characterized as a cheese with less hardness, astringency, sound, and granularity. In contrast, main component 2 (PC2) explained 40.9% of the total variability and differentiated the control cheese from the turnip and rape cheeses; the control cheese had greater homogeneity, whereas the turnip and rape cheeses were

Table 7. Fatty acids contained in Ricotta cheese from milk of cows fed control, turnip and rape diets

Fatty acid (%)	Diet ¹			SEM	P-value
	Control	Turnip	Rape		
C4:0	3.57	4.20	3.86	0.13	0.18
C6:0	2.14	2.13	2.29	0.05	0.45
C8:0	1.18	1.20	1.30	0.03	0.38
C10:0	2.76	2.63	3.05	0.14	0.46
C11:0	0.28	0.27	0.33	0.02	0.06
C12:0	3.21	3.43	3.53	0.11	0.42
C13:0	0.08	0.08	0.07	0.01	0.16
C14:0	11.55 ^b	11.63 ^b	12.52 ^a	0.45	0.03
C14:1	0.81	0.74	0.95	0.05	0.12
C15:0	0.98	0.99	0.97	0.02	0.72
C15:1	0.09	0.24	0.09	0.04	0.46
C16:0	37.11 ^b	40.88 ^a	40.64 ^a	1.24	0.02
C16:1	1.21	0.91	1.26	0.08	0.16
C17:0	0.31	0.45	0.40	0.05	0.56
C17:1	0.13	0.11	0.18	0.03	0.34
C18:0	8.98	8.31	7.55	0.39	0.44
C18:1 <i>trans</i> -10	0.52	0.01	0.01	0.16	0.40
C18:1 <i>trans</i> -11	0.68	0.02	0.02	0.21	0.41
C18:1n-9 <i>cis</i>	21.10	18.35	18.88	1.10	0.21
C18:2n-6 <i>trans</i>	0.65	0.57	0.67	0.03	0.51
C18:2n-6 <i>cis</i>	0.13	0.01	0.03	0.03	0.34
C20:0	0.12	ND ²	0.02	0.03	0.45
C20:1n-9	0.11	ND	ND	0.03	0.17
C18:3n-6	0.02	ND	ND	0.01	0.44
C18:3n-3	0.42	0.39	0.26	0.05	0.08
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.63	0.49	0.49	0.06	0.42
C20:2	0.20	0.01	0.06	0.06	0.45
C22:0	ND	0.19	0.12	0.06	0.29
C20:3n-3	0.02	0.09	ND	0.03	0.47
C20:3n-6	0.09	0.15	0.08	0.04	0.49
C22:1n-9	0.42	0.23	0.02	0.13	0.33
C23:0	0.01	0.38	0.03	0.09	0.25
C20:4n-6	0.11	0.12	0.11	0.05	0.93
C22:2	ND	0.28	ND	0.09	0.39
C24:0	0.02	0.36	0.10	0.10	0.31
C20:5n-3	0.08 ^a	0.03 ^b	0.07 ^{ab}	0.02	0.04
C24:1n-9	0.24	0.12	0.03	0.08	0.43
C22:6n-3	0.05	0.01	0.05	0.01	0.25
Σ SFA	72.28	77.12	76.76	1.64	0.09
Σ MUFA	25.32	20.72	21.43	1.42	0.11
Σ PUFA	2.40	2.16	1.81	0.29	0.19
Σ n-3	0.58 ^a	0.52 ^a	0.37 ^b	0.08	<0.01
Σ n-6	1.00	0.85	0.88	0.08	0.22
Σ n-6/Σ n-3	1.75	1.73	2.80	0.25	0.11
Σ PUFA/Σ SFA	0.03	0.03	0.02	0.01	0.44
Atherogenic index	3.39 ^b	4.17 ^a	4.34 ^a	0.36	0.03
Thrombogenic index	3.92 ^b	4.79 ^a	5.05 ^a	0.11	0.04

^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

²Not detected.

Table 8. Scores of the attributes considered in the sensory evaluation of Chanco cheese

Attribute	Diet ¹			SEM	P-value
	Control	Turnip	Rape		
Homogeneity	7.00	6.36	5.82	0.23	0.10
Holes	3.46	4.76	3.72	0.50	0.59
General smell	3.97	4.40	4.86	0.25	0.40
Smell of mature cheese	3.33	3.56	3.88	0.19	0.55
Smell of milk	3.50	3.93	3.68	0.12	0.39
Salty	5.46	5.42	6.68	0.33	0.22
Acid	3.12	3.72	3.33	0.22	0.60
Bitter	2.45	2.98	3.25	0.21	0.34
Spiciness before swallowing	2.86	3.32	3.22	0.14	0.46
Spiciness after swallowing	2.46	2.69	2.91	0.14	0.48
General flavor	5.33	4.98	5.67	0.17	0.25
Mature cheese flavor	3.67	3.61	4.43	0.17	0.07
Astringency	3.74	3.78	3.52	0.13	0.74
Hardness	4.56	4.55	3.81	0.19	0.19
Granularity	4.14	4.37	4.12	0.21	0.89
Sound	3.84	4.18	3.41	0.28	0.61
Humidity	3.90	3.71	4.29	0.16	0.36
Adherence	3.36	3.58	3.42	0.07	0.48

¹Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg (DM basis) of forage turnip; Rape = diet supplemented with 5 kg (DM basis) of forage rape.

more bitter and had more holes, greater adherence, and greater intensity of acidity, milk odor, and spiciness before swallowing (Figure 1).

According to the results obtained from the PCA, we observed that cheeses from the turnip and rape treatments presented greater intensity of aroma and flavor, and characteristics of feeding brassicas were also reflected in sensations of bitterness, spiciness, and acidity (Fenwick et al., 1983). This could be explained by the fact that the secondary compounds that derive from the forages can be easily transferred to milk or cheese (Wiedenhoeft and Barton, 1995).

McSweeney (2004) noted that during ripening of cheese, several biochemical processes occur, such as glycolysis, lipolysis, and proteolysis, the processes that degrade lactose, lipids, and proteins, respectively. This gives rise to important changes in the texture and sensory characteristic of cheeses. The metabolism of lactose and the hydrolysis of fats are of critical importance in the cheese industry, because both processes result in aroma and flavor properties in cheeses. In contrast, proteolysis brings about changes in texture by breaking down the protein network (McSweeney, 2004).

Milk contains lipoprotein lipase, a powerful enzyme that participates in the hydrolysis of triglycerides (Fox et al., 2000). Chilliard et al. (2003) concluded that a cow diet rich in unsaturated fats resulted in the decreased lipolytic enzymes in milk, leading to a lower intensity in the taste of milk and fresh cheese, as in the control diet in our experiment. This is in agreement with Chen et al. (2004), who showed a softer texture of cheeses made from milk with a higher content of UFA.

Therefore, we can infer that in the mammary gland, physiological regulation occurs between FA and the synthesis of lipoprotein lipase (Chilliard et al., 2003).

Ricotta cheese was evaluated through an informal sensory panel. The Ricotta cheeses from the brassica treatments could be distinguished by their greater bitterness, spiciness, and acidity.

CONCLUSIONS

Feeding cows forage brassicas substantially modified the FA profile in plasma, increasing the percentage of SFA and decreasing UFA, including some n-3 and n-6 fatty acids. The SFA, primarily SCFA and MCFA, were increased in milk from cows fed brassicas, whereas UFA, including C18:2 *cis*-9,*trans*-11 and fatty acids belonging to the n-3 family, decreased. Additionally, the atherogenic and thrombogenic indices and n-6/n-3 ratio increased. Supplementation with brassicas resulted in an increased percentage of FA C14:0 and C16:0 in Chanco cheese and a decrease in C20:5n-3 in Ricotta cheese. Feeding forage brassicas did not significantly modify the sensory characteristics of the cheeses. However, principal components analysis showed that the turnip and rape diets produced a greater intensity of flavor, aroma, spiciness, bitterness, and acidity.

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Figure 1. Principal components (PC) analysis for all 18 sensory attributes of all dietary treatments. Control = no forage brassica supplemented; Turnip = diet supplemented with 5 kg of forage turnip; Rape = diet supplemented with 5 kg of forage rape (DM basis).

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