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Conjugated linoleic acid content in milk of Chilean Black Friesian cows under pasture conditions and supplemented with canola seed (*Brassica napus*) concentrate

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Abstract

At present, there is limited and contradictory information about the effects of the use of canola (*Brassica napus*) seed as supplement on the contents of conjugated linoleic acid (CLA) in milk of grazing cows. The objective of this study was to evaluate the effect of a dietary supplement with canola seed on the production and composition of milk, and CLA concentration in Chilean Black Friesian cows under pasture conditions. Three experiments were done. Experiment 1: control group was fed 5 kg d^{-1} of commercial concentrate without canola (0-TC1) and treatment group that was fed 3.75 kg of commercial concentrate plus 1.16 kg of whole canola seed (1.16-TC1). Experiment 2: Control group was fed 8 kg d^{-1} commercial concentrate without canola (0-TC2) and treatment group that was fed 6.2 kg of commercial concentrate plus 1.2 kg of ground canola seed (1.2-TC2). Experiment 3: control group was fed 6 kg d^{-1} commercial concentrate without canola (0-TC3) and treatment group was fed 6 kg of commercial concentrate with 20% of whole canola seed (1.2 kg d^{-1}, 1.2-TC3). The duration of each experiment was 60 days. No differences in milk production and quality were observed among the experimental groups in every assay. The CLA isomers *trans*-10, *cis*-12 and *cis*-10, *cis*-12 were higher than those normally found in the scientific literature. There was no effect of the inclusion of canola seed on total CLA content or the content of *cis*-9, *trans*-11, *trans*-10, *cis*-12 and *cis*-10, *cis*-12 isomers.

Additional key words: CLA; dairy cows; grazing; milk quality.

Introduction

Conjugated linoleic acid (CLA) has been indicated as one of the most beneficial fatty acids for human health (Pariza & Park, 2001). Of all possible isomers, only *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have shown an interesting biological activity (Wahle *et al.*, 2004). Studies in animal models and human cell lines have provided evidence that certain isomers of CLA, including *cis*-9, *trans*-11 CLA, exhibit potent antiinflammatory, immunomodulatory, antiobesity, and anticarcinogenic activities; and also improve biomarkers of cardiovascular health (Wahle *et al*., 2004; Shingfield *et al*., 2008).

Levels of CLA in milk and dairy products are of particular nutritional interest, since the main food sources of CLA for humans are dairy and meat products from ruminant animals, with dairy products contributing up to 70% of total CLA intake (Lawson *et al*., 2001; Wahle *et al*., 2004). Diet has a major influence on milk fat CLA content and it has been extensively investigated (Bauman *et al.,* 2001). The content of CLA in fat from ruminant-derived foods is dependent on the ruminal production of both CLA, trans-vaccenic acid (TVA), and on the tissue activity of Δ^9-desaturase. For instance, it has been reported that fresh forage (Khanal *et al*., 2005; Dewhurst *et al*., 2006; Butler *et al*., 2008;

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Abbreviations used: ADF (acid detergent fibre); BCS (body condition score); CLA (conjugated linoleic acid); DIM (days in milk); DM (dry matter); FA (fatty acid); FCM (fat corrected milk production); GLM (general linear model); ME (metabolizable energy); NDF (neutral detergent fibre); TC (treatment canola); TMR (total mixed ration); TVA (trans-vaccenic acid).

Rego *et al*., 2008) and diets supplemented with plant oils, high in linoleic acid (Bauman *et al*., 2001; Stanton *et al*., 2003) increase CLA concentration in milk fat. Within the seeds of oilseeds, rapeseed (*Brassica napus*) has been used in TMR (total mixed ration) diets in dairy cows (Bayourthe *et al.,* 2000, Chouinard *et al.,* 2001; Ward *et al.,* 2002; Chichlowski *et al.,* 2005; Chen *et al*., 2008; Neves *et al*., 2009; Lerch *et al*., 2012b). However, the information about its utilization as a supplement under grazing conditions is limited and contradictory (Stanton *et al*., 1997; Lawless *et al*., 1998; Fearon *et al*., 2004; Lerch *et al*., 2012b).

In Chile, Chilean Black Friesian (Holstein × Black Friesian) is the most important dairy breed and produces almost 80% of cow's milk receptioned on dairy industry (INE, 2007). In addition, most of the Chilean dairy feeding systems are based on grazing due to the low cost of the diet (Balocchi *et al*., 2002). However, the quality of chilean southern pastures does not allow that milk productions being greater than 22 L cow^{-1} d^{-1}. Therefore, cows with high yield potential need to be supplemented with concentrates in the milking parlour. In recent years, canola has acquired a greater importance in the country, especially in the south, when compared with other oils crops such as the soybean. As result, a great proportion of this feedstuffs is being used for animal feeding as whole grain or its byproducts (*e.g.* canola bran) (INE, 2007). This oilseed is an excellent source of oleic and linoleic acids. The fatty acid profile of canola seed contains over 85% of total fatty acids (FA) as C18 unsaturated FA, primarily from 18:1 (> 60% of the total FA) and 18:2 (>20% of the total FA) (Aldrich *et al.,* 1997). Hence, we hypothesized that a diet containing canola seeds would provide linoleic and linolenic acids stimulating TVA and CLA production in the rumen and in the milk. Therefore, the objective of this study was to evaluate the effect of a dietary supplement with canola seeds as (i) whole grain plus commercial concentrate, ii) ground canola grain plus commercial concentrate, and iii) different levels of canola seeds inclusion, on milk yield, CLA isomers content, and the metabolic profile of Chilean Black Friesian cows in grazing systems.

Material and methods

Animals and diets

Three experiments were conducted in three representative farms of southern Chile, characterized by different feed systems according to their technological level. All the experiments were done following the principles and specific guidelines on animal care and welfare as required by Chilean law (SAG, 2010). The genotype of dairy cows utilized in the experiments was Chilean Black Friesian. The supplements were formulated according to the animal requirements established by NRC (2001), and the ration supplements were made isoenergetic. Visual observation of feed intake indicated that cows consumed all concentrate offered. The duration of each experiment was 60 d, the first 15 d were for adaptation to the experimental diets and the experimental period was from day 15 to day 60. Cows were selected for the study based on previous milk production in order to make homogenous groups.

Experiment 1. This experiment was conducted in a farm with a herd of 122 dairy cows, located at Vilcún (38° 39' 0" S and 72° 14' 0" W), during months of November and December (end of spring-beginning of the summer). The farm lies in the pre-mountain range of the Chilean Andes, which is characterized by an average rainfall of 1,400 mm per year, with an average daily temperature of 23.6°C in January and 1.5°C in August. The altitude is 950 m asl. A total of 14 healthy multiparous cows (3.6 ± 0.8 calvings), with 45 ± 11 day in milk (DIM), and a body condition score (BCS) of 2.50 ± 1.0, were used in the study. Average milk production of cows was 13.8 ± 1.20 kg d^{-1}. Cows selected for this study were randomly assigned (n = 7 per group) into two groups (Table 1). The control group was fed with 5 kg $head^{-1}$ d^{-1} of commercial concentrate without addition of canola seeds (0-TC1). The treatment group was fed with 3.75 kg of commercial concentrate plus the addition of 1.16 kg of whole canola seeds (1.16-TC1). The supplement (commercial concentrate + canola seeds) was supplied twice per day at the milking parlour during the milking time, at 06:00 h in the morning and at 14:30 h in the afternoon. The basic diet of both groups was natural —composed of *Trifolium pratense, Dactylis glomerata, Bromus* spp*., Lolium multiflorum, Taraxacum officinale* and *Plantago lanceolata*— and improved pastures —composed of *Lolium perenne* and *Trifolium repens*—. In addition, each animal received 6 kg DM d^{-1} of pasture silage —composed of *Lolium perenne* and *Avena sativa*— (Table 1). The cows grazed an area of 57 ha with 8 paddocks under continuous modality (1 paddock of 8 ha, 5 paddocks of 6 ha, and 2 paddocks of 9.5 ha), the criterium to switch cows among paddocks was the canopy height (5 cm).

Table 1. Ingredients and chemical composition of the supplemented dietary concentrates[1] and forages during experiment 1

	0-TC1	1.16-TC1	Canola sed	Natural pasture	Improved pasture	Silage
Ingredients (kg d^{-1})						
Whole canola seed		1.16				
Commercial concentrate[2]	5.00	3.75				
Chemical composition (%, DM basis)						
DM (%)	86.7	87.3	88.1	33.7	17.7	17.3
Crude protein	18.1	19.1	21.1	10.1	20.2	11.0
Ether extract	3.4	7.8	22.9	0.1	0.8	0.6
Neutral detergent fibre	40.0	37.8	30.7	43.1	50.7	54.7
Acid detergent fibre	17.0	18.7	20.5	23.8	29.6	35.3
Crude fibre	14.0	21.9	48.4	26.5	16.5	38.9
Ash	5.6	5.0	3.3	4.0	4.4	1.1
Metabolizable energy (Mcal kg^{-1})[3]	2.80	2.85	3.02	2.42	2.61	2.27

[1] 0-TC1: Control group to which commercial concentrate without canola (5 kg d^{-1}) was provided; 1.16-TC1: treatment group to which 3.75 kg d^{-1} of commercial concentrate plus 1.16 kg d^{-1} of whole canola seed were provided. [2] Corn (45%), wheat bran (25%), gluten meal (15%), gluten feed (13%), premix vitamins and minerals (2%). [3] Estimated according to NRC (2001).

Experiment 2. This experiment was carried out in a farm with a herd of 230 dairy cows, located at San Pablo sector (40° 24' 0" S and 73° 1' 0" W), between months of October and November (spring period). The farm lies in the central valley of southern Chile, which is characterized by an average rainfall of 1380 mm per year, with an average daily temperature of 23.4°C in January and 4.2°C in August. The altitude is 65 m asl. A total of 20 healthy multiparous cows (2.2 ± 0.8 calvings), with 44 ± 11 DIM, and a BCS of 2.75 ± 0.5 were selected for this study and randomly assigned into two groups (n = 10 per group). Average milk production of cows was 29.5 ± 5.84 kg d^{-1}. The control group was fed with 8 kg d^{-1} per animal of a commercial concentrate without adding canola seeds (0-TC2). The treatment group was fed with 6.2 kg of commercial concentrate plus 1.2 kg of ground canola seeds (1.2-TC2). Supplements were supplied twice per day at the milking parlour, at 04:30 h in the morning and at 15:30 h during the afternoon. The basic diet of all groups was an improved pasture composed of *L. perenne* and *T. repens* (Table 2). The animals grazed 10 paddocks of 0.5 ha each, that was handled under a rotational strip-grazing systems with an electric fencing. The animals were moved from one strip to the next one every 12 or 24 h.

Experiment 3. This experiment was conducted in a farm with herd of 342 dairy cows located at Vilcún sector (IX Region of Chile), between June and July (during the winter period). Twenty multiparous healthy cows (3.3 ± 0.5 calvings), with 32 ± 8 DIM, and a BCS of 2.75 ± 0.2 were selected for this study and randomly assigned into two groups (n = 10 per group). Average milk production of the cows was 15.4 ± 2.63 kg d^{-1}. The control group was fed with 6 kg d^{-1} per animal of commercial concentrate without the addition of canola seeds (0-TC3). The treatment group was fed with 6 kg d^{-1} of commercial concentrate with 20% of whole canola seed (1.2-TC3). Supplements were supplied twice per day at the milking parlour, at 05:30 h in the morning and at 15:30 h in the afternoon. The basic diet of both groups was a sown pasture composed of *A. sativa*, *L. perenne,* and *T. repens* (Table 3). In addition, each animal received 6 kg DM d^{-1} of silage (composed of *A. sativa*, *L. perenne,* and *T. repens*), and 1.5 kg DM d^{-1} of fodder cabbage (*Brassica oleracea*) (Table 3). The animals grazed 20 paddocks of 1-ha each, managed with a rotational system with the use of an electric fencing. The animals were moved from one strip to the next one every 12 h.

Laboratory analyses

A bromatological analysis, previous to the beginning of each experiment, was conducted by mean of a sampling of pastures and concentrates in order to determine their chemical and nutritional composition (Tables 1, 2 and 3). Representative samples of pasture were collected from each paddock before grazing with

Table 2. Ingredients and chemical composition of the supplemented dietary concentrates[1] and pasture during the experiment 2

	0-TC2	1.2-TC2	Canola seed	Improved pasture
Ingredients (kg d^{-1})				
Ground canola seed		1.2		
Commercial concentrate[2]	8.0	6.2		
Chemical composition (%, DM basis)				
DM (%)	89.1	88.4	88.1	13.9
Crude protein	14.0	15.1	21.1	23.9
Ether extract	4.0	7.0	22.9	1.6
Neutral detergent fibre	38.5	37.2	30.7	48.6
Acid detergent fibre	16.2	14.6	20.5	24.5
Crude fibre	7.9	14.4	48.4	25.2
Ash	3.2	3.2	3.3	7.0
Metabolizable energy (Mcal kg^{-1})[3]	3.00	3.02	3.02	2.34

[1] O-TC2: Control group to which commercial concentrate without canola (8 kg d^{-1}) was provided; 1.2-TC2: treatment group to which 6.2 kg d^{-1} of commercial concentrate plus 1.2 kg d^{-1} of ground canola seed were provided. [2] Corn (47%), oily brans (23%), comushroom (15%), molasses (9%), flours (4%), minerals and vitamins (2%). [3] Estimated according to NRC (2001).

a canopy height of 8 cm above the ground, using a 1-m^2 quadrant. Depending of the paddock size, 6 to 12 subsamples were taken in a diagonal pattern, forming an X. Subsequently, one composed sample was obtained from these subsamples. All the samples (pasture and concentrates) were ground to pass a 1-mm screen in a Willey mill before analysis. Dry matter (method 934.01), ash (method 942.05), ether extract (method

Table 3. Ingredients and chemical composition of the supplemented dietary concentrates[1] and forages during the experiment 3

	0-TC3	1.2-TC3	Artificial pasture	Fodder cabbage	Silage
Ingredients of concentrate (kg d^{-1})					
Whole canola seed		1.2			
Grain triticale	2.8	0.6			
Bran wheat	0.6	2.0			
Grain maize	0.3	0.6			
Lupins	2.3	0.6			
Barley bud		1.0			
Chemical composition (%, DM basis)					
DM (%)	88.6	87.2	17.4	15.2	24.3
Crude protein	16.9	17.4	24.2	22.6	10.5
Ether extract	4.4	7.7	1.5	1.8	1.7
Crude fibre	9.7	10.6	18.3	13.2	34.4
Neutral detergent fibre	44.3	40.4	25.0	36.5	52.3
Acid detergent fibre	18.2	16.7	42.0	26.6	42.1
Ash	6.3	6.5	9.0	11.5	6.4
Metabolizable energy (Mcal kg^{-1})[2]	3.01	3.02	2.43	2.47	2.17

[1] O-TC3: Control group to which commercial concentrate without canola (6 kg d^{-1}) was provided; 1.2-TC3: treatment group to which 6 kg d^{-1} of commercial concentrate with 20% of whole canola seed were provided (1.2 kg d^{-1}). [2] Estimated according to NRC (2001).

920.39), N (method 984.13), and crude fiber (method 978.10) were determined according to AOAC (2005) methods as previously described by Avilez *et al*. (2012). The analyses of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were carried out according to Van Soest *et al*. (1991), and both NDF and ADF were expressed as exclusive of residual ash. The metabolizable energy (ME) was estimated according to NRC (2001).

Cow's milk production was determined using a Waikato® measuring equipment, on days 1, 15, 30, 45 and 60. At each control, a milk sample of 30 mL was collected (to which was added 0.03 g of potassium dichromate at 0.1% as a preservative). The contents of fat, protein, and urea were determined automatically as previously described by Avilez *et al*. (2012). Somatic cells counts were measured by flow cytometry, using a Fossomatic Electronic Cell Counter (Fossomatic 5000, Hillerod, Denmark). Additionally, another sample of 100 mL was also collected and sent to the laboratory in thermally insulated containers at 4°C for analysis of CLA isomers (*cis*-9, *trans*-11; *trans*-10, *cis*-12; *cis*-10, *cis*-12). Total lipids were extracted by the method of Folch *et al.* (1957), using a mixture of chloroform and methanol (2:1, v:v). The fatty acids (FA) contents were analyzed after transesterification of FA to FA methyl esters (Morrison & Smith, 1964), as previously described by Avilez *et al*. (2012). Briefly, FA methyl esters were analyzed by gas chromatography (HP 6890, Hewlett Packard, Surrey, UK), Flame Ionization Detector (FID), a capillary column SP-2560 (100 m, 0.25 mm i.d. with 0.20 µm thickness in the stationary phase; Supelco Inc., Bellefonte, PA, USA) using He as the tracer gas. Gas chromatography conditions were as follow: the injection volume was 0.5 µL, a split injection was used (70:1, v:v); ultrapure hydrogen was the carrier gas; and the injector and detector temperatures were 250 and 300°C, respectively. The initial temperature was 70°C (held for 1 min), increased by 5°C min^{-1} to 100°C (held for 3 min), increased by 10°C min^{-1} to 175°C (held for 40 min), and then increased by 5°C min^{-1} to 220°C (held for 19 min) for a total run time of 86.5 min. Data were then quantified using the HPCHEM Stations software, and expressed as a percentage of area according to the total FA identified.

On the other hand, at the beginning and at the end of the each experiment, blood samples were also collected (5 mL $animal^{-1}$) by coccygeal venipuncture flow and placed in tubes with sodium heparin. The samples were then centrifuged for 3 min at 3,000 rpm and the plasma was aliquoted and frozen (–18°C) in microtubes of 1.5 mL. For each sample, the metabolic profile was determined as previously described by Avilez *et al*. (2012).

Statistical analysis

Data of milk production, milk's constituents, and metabolic profile of each experiment were analyzed with the repeated measures procedure, using the general linear model (GLM) of the SPSS for Windows 18.0 package (SPSS Inc., Chicago, IL, USA). The linear model used for each parameter was as follows:

$$Y_{ijk} = \mu + T_i + A_{ij} + W_k + (T \times W)_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} corresponds to each of the response variable; μ = overall mean; T_i = fixed effect of the *i* treatment group; A_{ij} = random effect of animal *j* for the *i* treatment; W_k= fixed effect of the *k* week of lactation; $T \times W$ = interactions among these factors for the *i* treatment and *k* week of lactation, and ε_{ijk} = random effect of the residuals. Pairwise comparisons of means were carried out, where appropriate, using Tukey's significant difference tests. The level of significance for the analyses was 5%.

Results

Milk yield and quality

There was no difference in none of the experiments between treatment and control groups for milk production or milk composition prior to the start of the experimental periods (data not shown). In the experimental period (from day 15 to day 60), there was no significant interaction effects (treatment × week) or main effects (Table 4). In this respect, no differences in milk production ($p = 0.542$ in exp. 1; $p = 0.622$ in exp. 2; $p = 0.454$ in exp. 3), fat corrected milk production (FCM, kg d^{-1}) ($p = 0.361$ in exp. 1; $p = 0.742$ in exp. 2; $p = 0.677$ in exp. 3), fat content (%) ($p = 0.891$ in exp. 1; $p = 0.772$ in exp. 2; $p = 0.214$ in exp. 3), fat yield (kg d^{-1}) ($p = 0.243$ in exp. 1; $p = 0.901$ in exp. 2; $p = 0.910$ in exp. 3), protein content (%) ($p = 0.793$ in exp. 1; $p = 0.886$ in exp. 2; $p = 0.642$ in exp. 3), protein yield (kg d^{-1}) ($p = 0.161$ in exp. 1; $p = 0.254$ in exp. 2; $p = 0.153$ in exp. 3), and urea ($p = 0.254$ in exp. 1; $p = 0.091$ in exp. 2;

Table 4. Production and chemical composition (mean values) of the milk of Chilean Black Friesian cows supplemented with canola seeds

	Treatments[1]						SEM[2]	Effect (*p*-values)[3]		
	Experiment 1		Experiment 2		Experiment 3			Exp. 1	Exp. 2	Exp. 3
	0-TC1	1.16-TC1	0-TC2	1.2-TC2	0-TC3	1.2-TC3		W	W	W
Milk yield (kg d^{-1})	14.8	12.8	27.6	30.5	14.3	16.2	2.01	0.010	0.000	0.020
4% FCM (kg d^{-1})	13.8	11.9	26.7	27.5	13.9	15.5	1.73	0.002	0.030	0.000
Fat (%)	3.60	3.52	3.42	3.34	3.76	3.41	0.275	0.023	0.045	0.001
Fat (kg d^{-1})	0.53	0.45	1.04	1.02	0.54	0.55	0.192	0.004	0.010	0.007
Protein (%)	3.10	3.00	3.10	3.15	3.10	3.12	0.062	0.024	0.003	0.009
Protein (kg d^{-1})	0.46	0.38	0.44	0.59	0.44	0.59	0.034	0.012	0.056	0.056
Urea (mg/100 mL)	0.025	0.028	0.023	0.059	0.023	0.031	0.0224	0.344	0.548	0.274
Somatic cells[4] ($\times 10^3$ mL^{-1})	0.015	0.022	0.023	0.030	0.023	0.031	0.0056	0.756	0.822	0.620
CLA (g/100 g FA)										
Total CLA	0.91	1.08	1.50	1.41	0.92	1.03	0.036	0.023	0.002	0.004
CLA *cis*-9, *trans*-11	0.43	0.56	0.63	0.43	0.50	0.45	0.029	0.006	0.005	0.000
CLA *trans*-10, *cis*-12	0.48	0.52	0.46	0.45	0.34	0.31	0.028	0.000	0.000	0.000
CLA *cis*-10, *cis*-12	0.00	0.00	0.41	0.53	0.23	0.17	0.024	0.000	0.000	0.000

[1] See Tables 1, 2 and 3. [2] Standard error of the mean. [3] W: week. In every experiment, no significant treatment main effects or treatment × week interactions were noted ($p > 0.05$). [4] For the statistical analysis, the values were transformed to logarithmic scale, base 10.

$p = 0.119$ in exp. 3) were observed among the experimental groups.

The patterns of milk production and milk composition throughout lactation were affected by the lactation day (Tables 4 and 5). Differences in milk production, fat corrected milk production, fat content, fat yield, protein content and protein yield (this parameter only in exp. 1) were observed among the experimental weeks. Nevertheless, there were no differences in urea content and somatic cells.

CLA content and composition

In the initial day no differences in total CLA and of each of its isomers were observed among the experimental groups in every experiment (data not shown). In the experimental period, was no effect of interaction. Likewise, the inclusion of rapeseed on total CLA content was no significant ($p = 0.121$ in exp. 1; $p = 0.232$ in exp. 2; $p = 0.961$ in exp. 3) or the content of *cis*-9, *trans*-11 ($p = 0.242$ in exp. 1; $p = 0.166$ in exp. 2;

Table 5. Production and chemical composition (mean values) of the milk of Chilean Black Friesian cows supplemented with canola seeds during the weeks of lactation

	Experiment 1 (days)[1]				Experiment 2 (days)				Experiment 3 (days)			
	15	30	45	60	15	30	45	60	15	30	45	60
Milk yield (kg d^{-1})	13.0^b	12.5^b	15.5^a	14.0^a	30.2^a	29.1^a	28.7^b	28.4^b	14.2^b	14.1^b	16.5^a	16.4^a
4% FCM (kg d^{-1})	12.3^b	12.0^b	14.7^a	12.4^b	26.5^a	26.7^a	26.3^a	25.8^a	13.6^b	13.5^b	15.8^a	14.4^b
Fat (%)	3.62^a	3.83^a	3.72^a	3.32^b	3.23^b	3.51^a	3.43^a	3.44^a	3.75^a	3.78^a	3.77^a	3.58^b
Fat (kg d^{-1})	0.47^b	0.47^b	0.57^a	0.45^b	0.97^a	1.00^a	0.99^a	0.97^a	0.53^b	0.52^b	0.61^a	0.57^a
Protein (%)	3.01^b	3.14^a	3.08^b	3.14^a	3.23^a	3.02^b	3.25^a	3.16^a	3.01^b	3.10^b	3.15^a	3.19^a
Protein (kg d^{-1})	0.38^c	0.39^c	0.48^a	0.43^b	0.95^a	0.87^a	0.90^a	0.88^a	0.49^a	0.49^a	0.51^a	0.51^a
Urea (mg/100 mL)	0.027^a	0.028^a	0.026^a	0.025^a	0.043^a	0.046^a	0.036^a	0.040^a	0.022^a	0.029^a	0.028^a	0.026^a
Somatic cells ($\times 10^3$ mL^{-1})	0.020^a	0.018^a	0.018^a	0.019^a	0.025^a	0.023^a	0.031^a	0.027^a	0.028^a	0.029^a	0.027^a	0.026^a

[1] Experiments explained in Tables 1, 2 and 3. ^{a,b,c}: means with different superscript within a row differ significantly.

$p = 0.125$ in exp. 3), *trans*-10, *cis*-12 ($p = 0.263$ in exp. 1; $p = 0.082$ in exp. 2; $p = 0.161$ in exp. 3) and *cis*-10, *cis*-12 ($p = 0.233$ in exp. 2; $p = 0.143$ in exp. 3) isomers (Table 4). However, the highest values found for the *cis*-9, *trans*-11 isomer (49-51%, 31-42%, and 42-46% of total CLA in exp. 1, 2 and 3, respectively), for the *trans*-10, *cis*-12 (49-51%, 31-32%, and 36-42% in exp. 1, 2, and 3, respectively), and for the *cis*-10, *cis*-12 isomers (not detected in exp. 1; 27-37% and 16-20% in exp. 2 and 3, respectively), were greater than values normally reported in the literature.

The pattern of FA composition throughout lactation was affected by the lactation day for all components (Table 4 and Fig. 1). The total content of CLA and its isomers, showed a similar trend than those observed in all the treatments. In the experiment 2, the highest values of CLA isomers were obtained in the later weeks, except for the *trans*-10, *cis*-12 isomer. In the experiment 1, the lowest CLA values were obtained in the later weeks, while in the experiment 3 the first week had the highest CLA isomers concentration, except for the *trans*-10, *cis*-12 isomer.

Metabolic profile

Throughout each experiment, cows presented a good health status, and did not show any relevant pathology. All the metabolites evaluated, except blood urea at the

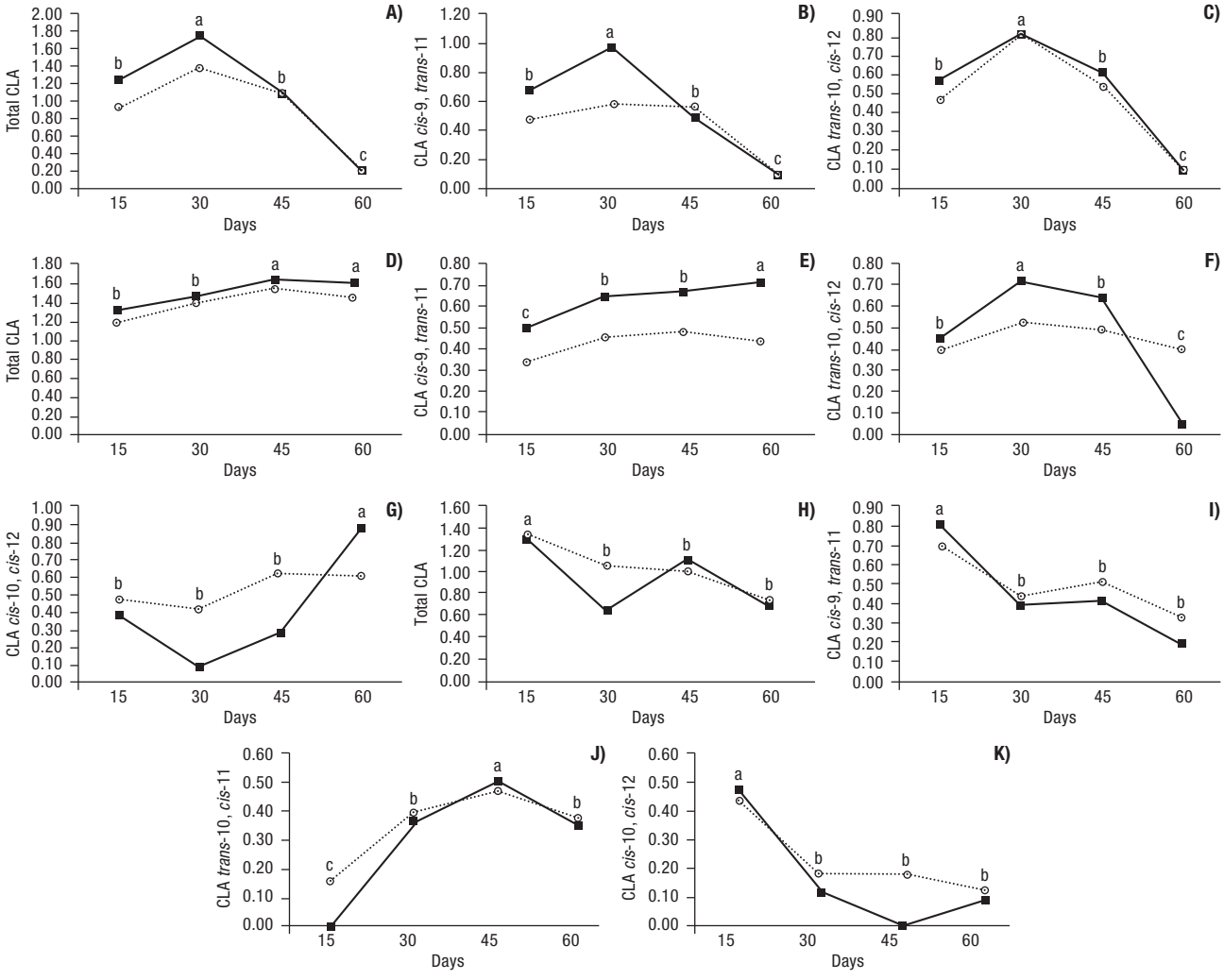

Figure 1. Temporal patterns of the content of conjugated linoleic acid (CLA) isomers (g/100 g total fatty acids) in the milk during the experiments 1 (A, B and C), 2 (D, E, F and G) and 3 (H, I, J and K) (experiments explained in Tables 1, 2 and 3). Control group without canola seed (·····); treatment group with canola seed (——). The mean values for each date were compared, and those with the same letter (a,b,c) do not differ ($p > 0.05$).

Table 6. Plasma metabolic profile of Chilean Black Friesian cows supplemented with canola seeds at the beginning and the end of every experiment

Metabolites	Experiment 1[1]				Experiment 2				Experiment 3			
	0TC1		1.16TC1		0TC2		1.2TC2		0TC3		1.2TC3	
	Day 15	Day 60	Day 15	Day 60	Day 15	Day 60	Day 15	Day 60	Day 15	Day 60	Day 15	Day 60
Cholesterol (mmol L^{-1})	4.46	4.13	3.72	3.77	4.00	5.67	4.21	5.59	6.01	5.46	4.24	5.67
Albumin (g L^{-1})	32.3	34.6	35.9	49.1	34.5	42.8	36.0	39.1	49.5	45.2	42.4	42.8
Total protein (g L^{-1})	62.4	58.6	55.6	60.3	65.3	67.0	62.2	70.4	81.5	75.3	73.9	74.4
Calcium (mmol L^{-1})	1.96	2.01	1.82	2.22	1.17	1.13	2.14	1.84	3.84	3.74	3.22	3.33
Mg (mmol L^{-1})	1.10	1.61	0.73	0.31	1.13	1.55	0.91	1.02	1.71	1.22	1.22	1.82
Phosphorus (mmol L^{-1})	0.94	1.03	0.86	1.22	1.18	1.23	1.01	1.13	3.83	2.92	3.23	3.54
AST[2] (U L^{-1})	56.0	98.1	57.2	102.2	106.4	122.6	118.4	119.9	158.0	121.3	132.5	145.2
Urea (mmol L^{-1})	8.01	9.22	8.23	8.73	8.26	10.09	9.32	11.05	5.88	6.27	5.24	4.65

[1] Experiments explained in Tables 1, 2 and 3. For all the metabolites evaluated no significant differences were observed among treatments ($p > 0.05$). [2] Aspartate aminotransferase.

end of each experiment, were found to be within the normal range, with no significant differences observed between treatments (Table 6).

Discussion

Milk yield and quality

Milk urea values were within the normal range, based on the report of Schüler *et al.* (1990). Similarly, somatic cells counts remained within a optimal range according to San Martin *et al*. (2002). Based on milk production and milk urea content, in every experiment, the diet contributed with an adequate level of protein and energy, in accordance with NRC (2001) recommendations.

Many of the studies with canola seeds refer to its use in diets as TMR, whereas that reports of its use on grazing systems are limited. Often fat supplements can result in decreased milk production, which may be due to inhibition of rumen fermentation or the low digestibility of FA in the rumen (NRC, 2001). Murphy *et al*. (1995) observed that cows on pasture and supplemented with concentrate containing 1.65 kg or 0.82 kg cow^{-1} d^{-1} of whole fat canola seeds, compared with cow's fed only with pasture, increased significantly milk yield. Nevertheless, as noted in this work in all experiments, in TMR [Delbecchi *et al*. (2001): using crushed canola seed well protected by pass or unprotected, 1 kg d^{-1}; Chichlowski *et al*. (2005): ground canola seed compared with canola meal mechanically extracted, 1.20 kg d^{-1}; Neves *et al*. (2009): feeding extruded *versus* non-extruded canola seed, 2.0 kg d^{-1}, treated with, or without, 50 g kg^{-1} lignosulfonate; Lerch *et al*. (2012a): cows received treatments for 2 consecutive lactations (treatments comprised the basal diet with no additional lipid, or supplements of extruded canola seeds, cold-pressed fat-rich canola seed meal, or whole unprocessed canola seeds to provide 2.5 to 3.0% of additional oil in diet dry matter; cows were housed during indoor periods, whereas cows were at pasture during outdoor periods)] and grazing diets [Lawless *et al*. (1998): cows on pasture and supplemented with 3.1 kg d^{-1} of unmolassed beet pulp or 3.0 kg d^{-1} of canola seed concentrate containing 1.65 kg of whole ground canola seed d^{-1}; Fearon *et al*. (2004): animals on pasture and supplemented with four levels of canola seed oil inclusion, incorporated as whole canola seeds and offered as part of a dairy concentrate ration (control 0 g oil d^{-1}; 200 g oil d^{-1}or 0.4 kg of canola seed d^{-1}; 400 g oil d^{-1}or 0.8 kg of canola seed d^{-1}; 600 g oil d^{-1}or 1.3 kg of canola seed d^{-1}); Lerch *et al*. (2012a)], milk production were similar between treatments.

In relation to milk components, it is well known that low fiber diets, high rich concentrates diets, and/or high vegetable oils diets can cause a reduction in milk fat content in dairy cattle. Likewise, supplementation of dietary fat can decreasing the percentage of milk protein. Hence, presentation of the canola seeds as whole oilseeds is employed to minimise adverse effects of unsaturated dietary lipid on rumen microbial activity and possibly provide some measure of protection for the unsaturated oilseed fatty acids from ruminal biohydrogenation (Fearon *et al*., 2004). In this respect, Murphy *et al.* (1990) observed that feeding unground,

intact canola seeds in an unpelleted concentrate to dairy cows resulted in the passage of a proportion of unbroken seeds through the digestive tract into the faeces and that, in this form, the oilseeds had less impact on milk fat composition than ground canola seeds. However, in the three experiments herein, the percentages and amounts of fat and milk protein were not different between the groups, despite the higher percentage of fat in the diet supplements in the groups with canola (Tables 1 and 3). This may be due to similar input fiber and energy of the total ration in each of the experiments, as was suggested by Murphy *et al*. (2008). In other study that compared the use of canola seeds treated with alkaline peroxide *vs.* ground canola grain (2.4 kg d^{-1}) in TMR diets, there was no differences on the percentage of milk fat (Aldrich *et al.,* 1997), while the use of ground canola seed compared to canola meal extracted, decreased the percentage of fat (Chichlowski *et al*., 2005; Neves *et al*., 2009). Lerch *et al*. (2012a) observed higher milk fat contents in cows receiving cold-pressed fat-rich canola seed meal and whole unprocessed canola seeds compared with other treatments. On the other hand, in grazing studies, the inclusion of whole fat canola seeds resulted in a decreasing on milk fat content (Murphy *et al*., 1995; Lawless *et al*., 1998; Fearon *et al*., 2004), while the study reported by Lerch *et al*. (2012a) showed that whole unprocessed canola seeds increased milk fat content when compared with the control treatment. Regarding to the protein content, some of these authors (Lawless *et al*., 1998; Chichlowski *et al*., 2005; Lerch *et al*., 2012a) reported a decreasing in the percentage of milk protein with the use of canola seeds. This reduction in the percentage of protein appears to be associated with negative effects on the growth of ruminal micro-organisms and the production of microbial protein (Solomon *et al*., 2000).

The differences in feeding (mainly due to ingestion and nutritional composition of the herbage) and lactational effects can explain the changes on milk production and components across the weeks of the study. However, given that ingestion and nutritional composition of the herbage during the grazing period were not monitored in this study, this will have to be tested in future studies.

CLA content and composition

In the present study, CLA values obtained in milk were higher (0.995, 1.450 and 0.880% of total FA, in exp. 1, 2 y 3, respectively) than those found in TMR rations, but similar to others obtained in grazing studies. It is well established that milk coming from grazing cows contains higher concentrations of total CLA when compared with milk coming from cows fed with diets containing high proportions of grass silage or dried grass (Mohammed *et al*., 2009; Lerch *et al*., 2012b), corn silage (Couvreur *et al*., 2006), or concentrates (Kraft *et al*., 2003; Butler *et al*., 2008; Rego *et al*., 2008). Likewise, Lerch *et al.* (2012b) observed that the increase in milk CLA concentration from pasture is not solely explained by marginally higher dietary 18:2n-6 and 18:3n-3 concentration during outdoor than indoor periods. The underlying reasons for the higher enrichment of conjugated FA in milk from pasture *vs.* those coming from conserved forages are not fully understood, but may be related to the relatively high amount of rapidly fermentable sugars, soluble fiber, or possibly certain metabolites such as polyphenols and terpenes in fresh grass, higher rumen passage rate and fluid dilution rate in cows fed fresh grass, or possibly due to feeding behavior (meal size, feeding frequency and rumination; Kraft *et al*., 2003; Mohammed *et al*., 2009).

The use of canola seeds in dairy systems based on grazing, like in the present study, has not increased the CLA contents in milk. In TMR diets, comparing ground canola seed and canola meal mechanically extracted, the differences were not significant in the contents of total milk CLA and its *cis*-9, *trans*-11 isomer (Chichlowski *et al*., 2005; Neves *et al*., 2009), while cows received extruded canola seeds or cold-pressed fat-rich canola seed meal increased both parameters to a similar extent compared with the control treatment with no additional lipid (Lerch *et al*., 2012b). At pasture conditions, extruded canola seeds and whole unprocessed canola seeds in particular, decreased milk total CLA content and *trans*-10, *cis*-12 CLA concentrations, while all of the canola seed treatments, whole unprocessed canola seeds in particular, lowered milk fat *cis*-9, *trans*-11 CLA concentration (Lerch *et al*., 2012b). The responses to canola seeds supplementation in that study, and in the present experiments, differ from previous studies that reported that supplements of ground or pelleted canola seeds increased milk *cis*-9, *trans*-11 CLA content in grazing cows (Stanton *et al*., 1997; Lawless *et al*., 1998; Fearon *et al*., 2004). The different CLA values found in the experiments conducted in this study, and between this and earlier experiments, may be related to differences in the amount and

quality of the pasture and forages ingested, derived from the different botanical and agronomic characteristics of the herbage used (Dewhurst *et al*., 2006), or by the level of fermentable starch in the diet (Lerch *et al*., 2012b). Diets rich in PUFA and starch are known to stimulate the accumulation of *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA, and *trans* 18:1 isomers in the rumen, alterations in ruminal biohydrogenation that are often accompanied by a shift from *trans*-11 18:1 toward *trans*-10 18:1 as the major intermediate leaving the rumen (Griinari & Bauman, 1999; Palmquist *et al.,* 2005).

In the present study, in agreement with the results found in a previous work by Avilez *et al*. (2012), the *trans*-10, *cis*-12 and *cis*-10, *cis*-12 isomers presented higher values than normally reported in the literature. The regulation of isomer balance is largely unknown. Nevertheless, the *cis*-9, *trans*-11 isomer is mainly generated from vaccenic acid in the mammary gland (Palmquist *et al*., 2005; Mosley *et al*., 2006), while the *trans*-10, *cis*-12 is a minor intermediate of rumen biohydrogenation (Walker *et al*., 2004), and is relatively unaffected by changes in the diet, except at very high levels of concentrate feeding (Chilliard *et al*., 2007). Therefore, future studies are necessary to determine its biological function and metabolic production routes.

The pattern of FA composition throughout lactation (Fig. 1) may be related to differences in the nutritional composition of the herbage, which has also been shown to affect the FA composition of milk (Dewhurst *et al*., 2006). In this respect, lower CLA contents in milk have been observed with more mature pasture. This effect has been attributed to the declining quality and quantity of the herbage (Lock & Garnsworthy, 2003; Ward *et al*., 2003; Avilez *et al*., 2012). In exp. 2, the highest CLA values were obtained in the later weeks, while in exp. 1 and 3 the lowest values were obtained in the later weeks. These results could be explained because the experiments 1 and 3 were done during the period in which the quality and quantity of the herbage was decreasing, while that experiment 2 was done in the period of major quality and quantity (Ruiz, 1996).

Metabolic profile

All the metabolites evaluated, except blood urea at the end of each experiment, were found to be within the normal range, in agreement with the values for healthy lactating dairy cows (Bertoni & Piccioli, 1999). Previous studies (Pulido, 2009; Avilez *et al*., 2012) have shown an increase in blood urea when diets present high levels of degradable protein, which is the case with animals fed to pasture on grass (*L. perenne*).

Conclusions

The addition of canola (*B. napus*) seed in whole grain or in ground grain to a supplement concentrate, considering quantities of 1.2 kg d^{-1}, fed to Chilean Black Friesian (Holstein × Overo Negro) cows, on pasture-based systems, did not influence milk production or total CLA contents or its *cis*-9, *trans*-11, *trans*-10, *cis*-12 and *cis*-10, *cis*-12 isomers. In the present study, high quantities of the *trans*-10, *cis*-12 and *cis*-10, *cis*-12 isomers were obtained in comparison to those normally found. The *cis*-10, *cis*-12 isomer does not appear in the scientific literature, therefore future studies are necessary to determine its biological function and metabolic production routes. The reasons of why the addition of canola seeds into the commercial supplement fed to Chilean Black Frison cows did not increase the concentration of *cis*-9, *trans*-11 CLA in milk fat, require further investigation.

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