Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
http://dx.doi.org/10.5424/sjar/2012102-341-11

Spanish Journal of Agricultural Research 2012 10(2), 409-418
ISSN: 1695-971-X
eISSN: 2171-9292

Effect of extruded whole soybean dietary concentrate on conjugated linoleic acid concentration in milk in Jersey cows under pasture conditions

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Abstract

Contradictory results has been found on the effects of soybean supplementation and conjugated linoleic acid (CLA) content in milk on feeding systems based on fresh forage The objective of the study was to evaluate the effect of a dietary supplement with different quantities of extruded whole soybean on the production and composition of milk, and CLA concentration or their isomers in Jersey cows under pasture conditions. Twenty-one Jersey cows were randomly assigned into 3 groups of 7 animals each. The cows were supplemented with a dietary concentrate (5 kg d^{-1}), and each group received one of the three next treatments: control without soybean (0-SB), with extruded whole soybean at 0.5 kg d^{-1} (0.5-SB) or at 1 kg d^{-1} (1-SB). The basic diet was a pasture composed of *Lolium perenne* (70%), *Trifolium repens* (25%) and other species. The duration of the study was 75 d. Milk production ($p = 0.706$) and protein production ($p = 0.926$) were not affected by treatments. Fat ($p = 0.015$) and protein ($p = 0.045$) content as well as fat production ($p = 0.010$) were lower in the 1-SB group. There was no effect of the inclusion of extruded soybean on total CLA content ($p = 0.290$) or the content of *cis*-9, *trans*-11 ($p = 0.582$), *trans*-10, *cis*-12 ($p = 0.136$) and *cis*-10, *cis*-12 ($p = 0.288$) isomers. However, concentrations of all isomers were affected by the nutritional quality of the pasture, with low values observed at greater maturity stages of pasture.

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

Resumen

Efecto de la suplementación con concentrado de soja entera extrusionada en vacas Jersey en pastoreo sobre el contenido de ácido linoléico conjugado en la leche

El efecto de la suplementación con soja en sistemas de pastoreo sobre el contenido de ácido linoléico conjugado (CLA) en leche es contradictorio. El objetivo de este estudio fue evaluar en vacas Jersey en pastoreo, el efecto de la suplementación de un concentrado con diferentes cantidades de soja entera extrusionada sobre la producción de leche y su composición, especialmente sobre el contenido en CLA y sus isómeros. 21 vacas Jersey fueron divididas al azar en 3 grupos de 7 animales cada uno. Los animales fueron suplementados con un concentrado (5 kg d^{-1}) y a cada grupo se le asignó uno de los tres siguientes tratamientos: control sin soja (0-SB), con 0.5 kg d^{-1} de soja (0.5-SB) y con 1 kg d^{-1} de soja (1-SB). La base de la alimentación fue el pasto, compuesto mayoritariamente por *Lolium perenne* (70%) y *Trifolium repens* (25%). La duración del estudio fue de 75 días. La producción de leche ($p = 0.706$) y la producción de proteína ($p = 0.926$) no se vieron afectados. Los porcentajes de grasa ($p = 0.015$) y proteína ($p = 0.045$) y la producción de grasa ($p = 0.010$) fueron más bajos en el grupo 1-SB. Las cantidades de soja no modificaron los contenidos de CLA total ($p = 0.290$) y de los isómeros *cis*-9, *trans*-11 ($p = 0.582$), *trans*-10, *cis*-12 ($p = 0.136$) y *cis*-10, *cis*-12 ($p = 0.288$), pero si fueron afectados por la calidad nutritiva del pasto, observándose menores valores al aumentar la madurez del pasto.

Palabras clave adicionales: calidad de leche; CLA; pasto; vacas de leche.

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Received: 13-07-11. Accepted: 30-04-12

Introduction

Conjugated linoleic acid (CLA) represents between 20 and 28 isomers of linoleic acid C18:2 (Lock & Garnsworthy, 2003) that has been indicated as one of the most beneficial fatty acids for human health (Pariza & Park, 2001). Likewise, ruminant products as milk and meat constitute the principal source of CLA for humans. Of all possible isomers, only *cis*-9, *trans*-11 and *trans*-10, *cis*-12 have shown an interesting biological activity (Wahle *et al.*, 2004). The *cis*-9, *trans*-11 isomer, also known as rumenic acid, has been documented to have an anticarcinogenic (Ha *et al.*, 1987; Visonneau *et al.*, 1997; Aro *et al.*, 2000) and antioxidant effect (Devery *et al.*, 2001), whereas the *trans*-10, *cis*-12 isomer is capable of decreasing body fat and increasing lean body mass.

Diet has a major influence on milk fat CLA and it has been extensively investigated (Bauman *et al.*, 2001). Several nutritional studies have been addressed to increase CLA content in animal products and to improve their nutritional properties. For instance, it has been reported that fresh forage and oil-rich feeds increase CLA concentration (Khanal *et al.*, 2005; Dewhurst *et al.*, 2006). Soybean is widely used in total mixed ration (TMR) in different proportions, and it has been observed that seed treatment (roasting or extrusion) results in a higher increase on CLA content than that observed with intact seed (Chouinard *et al.*, 2001). However, contradictory results has been found on the effects of soybean supplementation and CLA content in milk on feeding systems based on fresh forage. Some studies (Bartolozzo *et al.*, 2003; Khanal *et al.*, 2005) did not found effects on milk CLA concentration in dairy cows, while others (Lawless *et al.*, 1998; Paradis *et al.*, 2008) observed an increase in the CLA using dairy and beef cattle.

On the other hand, the effects of other factors such as breed, lactation and parity on CLA content in milk fat have received less attention. Kelsey *et al.* (2003) reported that breed (Holstein *vs.* Brown Swiss), parity, and days in milk accounted for < 0.1, < 0.3, and < 2.0% of the total variation in CLA concentration in milk fat, respectively. The incorporation of Jersey cattle in dairy farms has been increased in the last decade in Chile due to their high level of total milk solids produced (INE, 2007). Although, it has been documented that concentration of CLA in milk from Jersey cows is 18% lower than that observed in Holstein cows (White *et al.*, 2001), apparently few studies have been conducted regarding CLA content in Jersey cows.

Whilst functional foods have been considered a promising area for human health (Starling, 2002), it has frequently been observed that consumers expect added-value products without substantial extra cost, suggesting that the development of low-cost approaches will be important (Dewhurst *et al.*, 2006). Considering that soybean is an imported feedstuff in the majority of the countries and given the current prices in the market, the objective of this study was to evaluate the effect of a dietary supplement with different quantities of extruded whole soybean (0.5 and 1.0 kg d^{-1}) on milk yield, CLA isomers content and metabolic profile in Jersey cows maintained in pasture-based systems.

Materials and methods

Animals and diets

The experiment was carried out in a farm with a Jersey herd based on grazing plus the use of concentrate, located in the Entre Lagos sector (district of Osorno, Chile), approximately 72° 36’ 24” W 40° 41’ 26” S, 250 m above sea level. The farm lies in the pre-mountain range of the Chilean Andes, Region X, which is characterized by an average rainfall of 2250 mm per year, with an average daily temperature of 21.8°C in January and 3°C in August.

The experiment was developed in compliance with the principles and specific guidelines on animal care and welfare as required by Chilean law (SAG, 2010). The duration of the experiment was 75 d, between 15th November 2005 and 25th January 2006 (spring period). The first 15 d were for adaptation to the experimental diets and the experimental period was from day 15 to day 75. Twenty-one healthy cows between 2 and 7 calvings, with a range from 60 to120 days in milk (92 ± 5 DIM) and a body condition score of 2.75 ± 0.7 were used in the study. An average of 18.4 ± 3.73 kg d^{-1} of milk production was recorded. Cows were selected for the study based on previous milk production in order to make three ho-

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*Abbreviations used: ADF (acid detergent fibre); CLA (conjugated linoleic acid); DIM (days in milk); FCM (fat corrected milk production); GLM (general linear model); IVDMD (*in vitro* dry matter digestibility); ME (metabolizable energy); NDF (neutral detergent fibre); SB (soybean); TMR (total mixed ration).

mogenous groups and they were randomly assigned (n=7/per group) to receive a dietary concentrate or treatment with different quantities of extruded whole soybean: T1= control diet without supplementation (0-SB), T2 = 0.5 kg d^{-1} (0.5-SB) and T3 = 1 kg d^{-1} (1-SB). Each animal was fed with 5 kg d^{-1} of isoenergetic dietary concentrate (Table 1), distributed in two visits to the milking parlour, at 06:00 h in the morning and in the afternoon at 16:00 h. Visual observation of feed intake indicated that cows consumed all concentrate offered. The animals grazed in two paddocks of 18 hectares each, and managed with rotational strip-grazing and electric fencing. The pasture was an improved natural pasture with grasses being the predominant species (70% *Lolium perenne*, 25% *Trifolium repens*, 3% *Bromus* sp). The animals were transferred from one strip to the next every 24 h. The diets were formulated according to the animal requirements established by NRC (2001).

Chemical composition and nutritional value of feedstuff

Samples of the pasture and concentrates were taken every 10 days to determine their chemical and nutritional composition (Tables 1 and 2). Representative samples of pasture forage were collected from the paddock before grazing at a height of 8 cm above the ground, using a 1-m^2 quadrant. Dry matter contents of the pasture were determined by forced air oven at 60°C for 48 h. Samples of pasture and concentrates were ground to pass a 1-mm screen in a Willey mill before analysis. Dry matter (method 934.01), ash (method

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

	0-SB	0.5-SB	1-SB
Ingredients (kg d^{-1})			
Extruded whole soybean	–	0.50	1.00
Triticale grain	0.95	0.30	0.30
Wheat meal	1.15	2.50	2.90
Maize grain	2.50	1.50	0.70
Rapeseed meal	0.35	0.15	–
Tricalcium phosphate	–	0.05	0.05
Sodium bicarbonate	0.05	–	0.05
Chemical composition (%, DM basis)			
Crude protein	16.7	19.0	21.0
Crude fibre	13.6	8.5	9.0
Neutral detergent fibre	32.1	31.3	32.3
Ether extract	2.6	3.4	4.5
Metabolizable energy (Mcal kg^{-1})	3.01	3.03	3.04

[1] Control without extruded whole soybean (0-SB), with extruded whole soybean at 0.5 kg d^{-1} (0.5-SB), and with extruded whole soybean at 1 kg d^{-1} (1-SB).

Table 2. Nutritional composition of the pasture during the experiment

	Sample days					
	1	15	30	45	60	75
Dry matter (DM, %)	17.6	14.3	18.8	19.7	22.6	30.6
Ash (% DM)	10.6	11.6	11.9	12.9	10.1	11.2
Crude protein (% DM)	24.8	21.1	18.0	19.8	15.0	11.4
Crude fibre (% DM)	17.7	24.5	22.0	24.6	27.7	26.4
Neutral detergent fibre (% DM)	43.7	56.4	50.2	51.3	62.0	59.3
Acid detergent fibre (% DM)	22.5	30.7	30.0	30.5	33.8	35.2
IVDMD[1] (%)	84.2	73.2	70.8	73.2	61.9	60.3
ME[2] (Mcal kg^{-1})	2.75	2.41	2.33	2.36	2.06	2.07

[1] IVDMD: *in vitro* DM digestibility. [2] ME: metabolizable energy.

942.05), ether extract (method 920.39), N (method 984.13) and crude fiber (method 978.10) were determined according to AOAC (2005) methods. The N values determined by the Kjeldahl procedure, and converted to crude protein by multiplying by a factor of 6.25. 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 exclusive of residual ash. All fiber fractions were analyzed on a Fibertec 1030 Hot Extractor (Tecator, Sweden). The fat content was measured by extraction with petroleum ether (boiling point, 40 to 60°C) on a Soxtec System 1040 Extraction Unit (FOSS Tecator AB, Sweden).

The metabolizable energy (ME) of the supplemented dietary concentrates was estimated according to NRC (2001). The *in vitro* dry matter digestibility (IVDMD) of the pasture was determined according to the procedure described by Tilley & Terry (1963) modified by Van Soest (1991) and the ME was estimated according to the equation (Garrido, 1981):

$$ME = 0.279 + 0.0325 \times IVDMD.$$

Milk yield and quality

Cow milk production was determined using a Waikato® measuring equipment, on days 1, 15, 30, 45, 60 and 75. At each control, a milk sample of 30 ml was taken (to which was added 0.03 g of potassium dichromate at 0.1% as a preservative), and the contents of fat, protein and urea were determined automatically using an infrared spectrophotometer (Foss 4200 Milko-scan; Foss Electric, Denmark).

CLA content and composition

At each milk control, milk samples of 100 mL were taken 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 methylation of the fatty acids of the samples was done using the method described by Morrison & Smith (1964).

Fatty acid 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, Pennsylvania, 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 per min to 100°C (held for 3 min), increased by 10°C per min to 175°C (held for 40 min), and then increased by 5°C per min 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 fatty acids identified.

Metabolic profile

At the beginning and at the end of the experiment blood samples were taken (5 mL animal^{-1}) by coccygeal venipuncture flow and placed in tubes with sodium heparin. The samples were then centrifuged for 3 min at 3000 rpm and the plasma was aliquoted and frozen (–18°C) in microtubes of 1.5 mL. For each sample, the following plasma traits were determined: cholesterol (cholesterol-oxidase method, Cholesterol Liquicolor 10028 Human), albumin (Albumin Liquicolor Method BCG-Bromo Cresol), total protein (Total Protein Liquicolor-Biuret Method), calcium (Arsenazo III AA), Mg (Mg-color AA), phosphorus (Fosfataria UV AA), aspartate aminotransferase (IFCC Mod. LiquiUV test) and urea (ureasa/NADH method, UREA LiquiUV 10521 Human). All plasma traits were determined automatically by biochemical analyser (SelectraVitalab, Merk, Darmstdt, Germany).

Statistical analysis

Data of milk production, milk's constituents and metabolic profile were analysed as repeated measures, 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} = observations for dependent variables; μ = overall mean; T_i = fixed effect of treatment group or dietary concentrate; A_{ij} = random effect of animal j for the i treatment; W_k = fixed effect of the k week of lactation; T × W = interactions among these factors for the i treatment and k week of lactation, and ε_{ijk} = random effect of residual. Pairwise comparisons of means were carried out, where appropriate, using Tukey's honest significant difference tests. The level of significance for the analyses was 5%. The Pearson correlation coefficient between the milk fat concentration and the content of *trans*-10, *cis*-12 isomer was also determined.

Results

Milk yield and quality

In the initial day no differences in milk production ($p = 0.390$) and quality were observed among the three experimental groups (data not shown). In the experimental period (from day 15 to day 75) milk production ($p = 0.706$) and fat corrected milk production (FCM, kg d^{-1}) ($p = 0.241$) were similar among groups (Table 3). The amounts of milk fat (kg d^{-1}) ($p = 0.010$), as well as protein ($p = 0.045$) and milk fat ($p = 0.015$) concentrations were lower in the 1-SB treatment, while the quantities of protein ($p = 0.926$) and urea ($p = 145$) were similar among all treatments.

The patterns of milk production and basic composition throughout lactation were affected by the lactation day for all the components (Table 3). Milk yield significantly decreased as a function of the week and for the chemical composition, the highest values for these components were found in the last weeks (data not shown).

CLA content and composition

In the initial day no differences in total CLA ($p = 0.791$) and of each of its isomers were observed among the three experimental groups (data not shown). In the experimental period, there was no effect of the inclusion of extruded soybean on total CLA content ($p = 0.290$) or the content of *cis*-9, *trans*-11 ($p = 0.582$), *trans*-10, *cis*-12 ($p = 0.136$) and *cis*-10, *cis*-12 ($p = 0.288$) isomers (Table 3). Although the highest values were found for the *cis*-9, *trans*-11 isomer (53-59% of total CLA), the *trans*-10, *cis*-12 and *cis*-10, *cis*-12 isomers presented higher values (17-23% and 20-25% of total CLA, respectively) than normally reported in the literature.

The pattern of fatty acid composition throughout lactation was affected by the lactation day for all components (Table 3; Fig. 1). For the content of total CLA and of each of its isomers, a similar trend is observed in all the treatments. The lowest CLA values were obtained in the lasted weeks, when the herbage presented the poorest nutritional quality (see Table 2). The *cis*-10, *cis*-12 isomer was the only one that diminished from day 1 to day 45, and increased after day 60.

Table 3. Production and chemical composition (mean values) of the milk of Jersey cows supplemented with dietary concentrates during the experimental period

	Treatments[1] (dietary concentrates supplemented)			SEM[2]	Effects[3] (p =)		
	0-SB	0.5-SB	1-SB		T	W	T × W
Milk yield (kg d^{-1})	18.7	18.3	19.7	0.33	0.706	0.000	0.447
4% FCM[4] (kg d^{-1})	21.5	20.8	20.2	0.33	0.241	0.000	0.476
Fat (%)	5.08 a	4.91 a	4.17 b	0.073	0.015	0.005	0.375
Fat (kg d^{-1})	0.93 a	0.90 a	0.82 b	0.015	0.010	0.004	0.126
Protein (%)	3.75 a	3.70 a	3.50 b	0.026	0.045	0.000	0.217
Protein (kg d^{-1})	0.70	0.68	0.69	0.011	0.926	0.000	0.328
Urea (mg/100 mL)	0.047	0.044	0.052	0.0013	0.145	0.000	0.540
CLA[5] (g/100 g fatty acids)							
Total CLA	1.21	1.28	1.44	0.040	0.290	0.007	0.391
CLA *cis*-9, *trans*-11	0.70	0.73	0.75	0.023	0.582	0.022	0.393
CLA *trans*-10, *cis*-12	0.21	0.29	0.29	0.023	0.136	0.000	0.909
CLA *cis*-10, *cis*-12	0.30	0.27	0.39	0.038	0.288	0.000	0.202

[1] See Table 1. [a, b]: mean values within a row with different superscripts are different ($p < 0.05$). [2] SEM: standard error of mean. [3] T: Treatment; W: Week; T × W: Treatment × Week interaction. [4] FCM: fat corrected milk production. [5] CLA: conjugated linoleic acid.

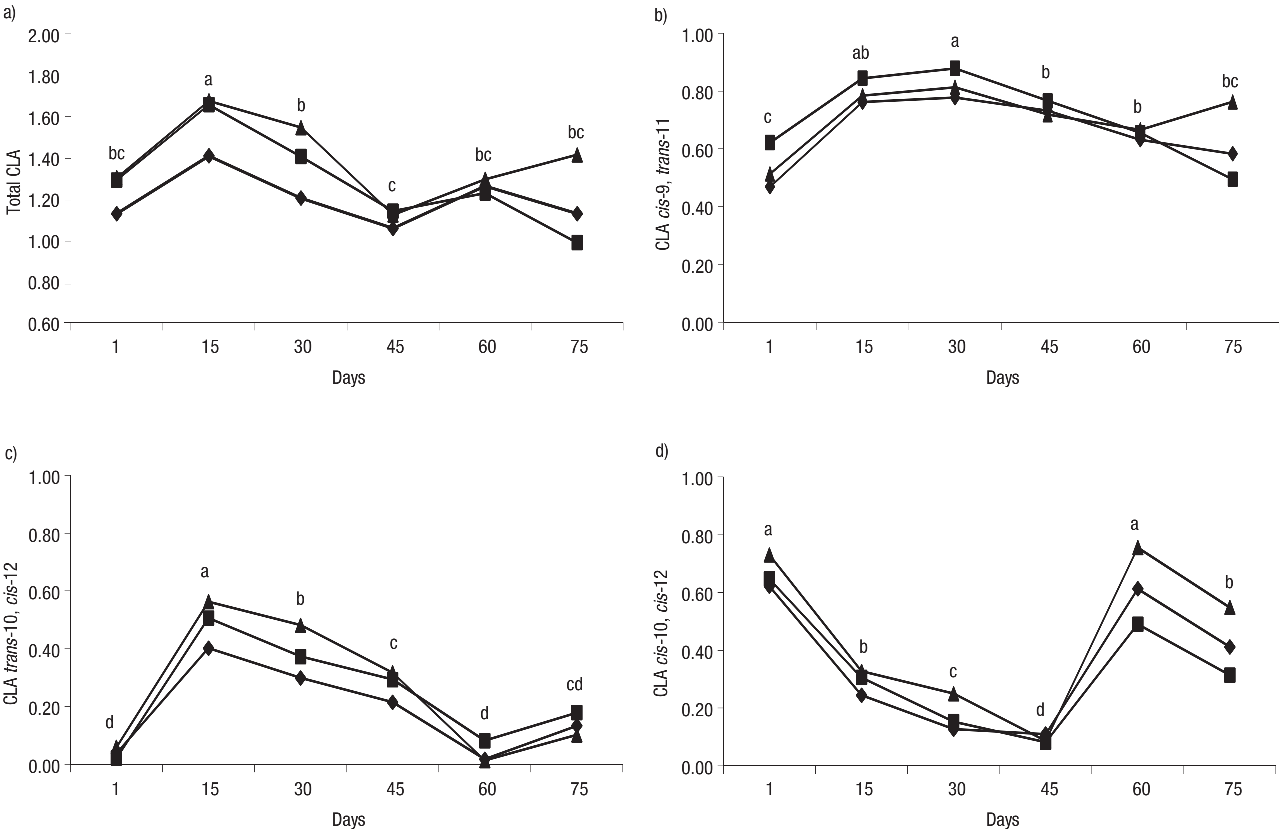

Figure 1. Temporal patterns of the content of conjugated linoleic acid (CLA) isomers (g/100g total fatty acids) in the milk of Jersey cows supplemented with different dietary concentrates: a) total CLA; b) CLA *cis*-9, *trans*-11; c) CLA *trans*-10, *cis*-12; d) CLA *cis*-10, *cis*-12. Control without extruded whole soybean (—◆—), with extruded whole soybean at 0.5 kg d^{-1} (—■—), and with extruded whole soybean at 1 kg d^{-1} (—▲—). Initial measurements (d 1) were made when animals were fed with the pasture on the multi-species paddock. The mean values for each date were compared, and those with the same letter do not differ ($p > 0.05$).

Metabolic profile

Throughout the trial the cows were in good health and did not show any relevant pathology. All the metabolites evaluated, except blood urea at the end of the trial, were found to be within the normal range, with no significant differences observed between treatments (Table 4).

Table 4. Plasma metabolic profile of Jersey dairy cows supplemented with dietary concentrates[1] at the beginning and at the end of the experiment

Metabolites	**0-SB**		**0.5-SB**		**1-SB**		**SEM[2]**	**Effects (p =)**	
	Beginning	**End**	**Beginning**	**End**	**Beginning**	**End**		**Beginning**	**End**
Cholesterol (mmol L^{-1})	4.14	5.00	4.86	5.71	4.86	5.14	0.177	0.509	0.161
Albumin (g L^{-1})	33.5	37.4	33.8	39.3	32.3	36.1	0.76	0.991	0.065
Total protein (g L^{-1})	62.6	68.2	65.0	72.1	65.7	68.8	1.43	0.874	0.349
Calcium (mmol L^{-1})	2.20	2.87	2.20	3.05	2.25	2.90	0.075	0.724	0.094
Mg (mmol L^{-1})	0.80	0.90	0.83	0.97	0.82	0.87	0.020	0.889	0.270
Phosphorus (mmol L^{-1})	1.54	2.02	1.53	2.16	1.68	2.90	0.053	0.309	0.408
AST[3] (U L^{-1})	84.0	94.4	101.1	106.7	98.9	101.1	12.01	0.580	0.295
Urea (mmol L^{-1})	7.00	10.93	5.91	9.77	6.60	10.63	1.348	0.322	0.420

[1] See Table 1. [2] SEM: standard error of mean. [3] AST: aspartate aminotransferase.

Discussion

Milk yield and quality

Although crude protein of supplemented concentrate ranged between 17 and 21%, in all groups the pasture contributed an adequate level of protein and energy in accordance with NRC (2001) recommendations based on milk production and milk urea content (see Table 3).

The reduction in the percentage and amount of fat (kg d^{-1}) in the group fed with a higher quantity of soybean (1 kg d^{-1}) may result from the extrusion process, which breaks up the micelles of fat in the seed, allowing a rapid release of the lipids in the rumen and reducing milk fat content (Mohamed *et al.*, 1988; DePeters & Cant, 1992). Low milk fat syndrome has been recognized for many years, but the exact mechanism is still unclear. Data from several studies revived the theory of *trans* fatty acids, coming from ruminal biohydrogenations and from desaturation by the mammary gland, as the central mechanism of milk fat depression (Griinari & Bauman, 2003; Loor *et al.*, 2005). In particular, the increase of C18:1 *trans*-10 and CLA *trans*-10, *cis*-12 isomers in the mammary gland has been associated with a reduction in the *de novo* synthesis of short and medium chain fatty acids (Banks *et al.*, 1980; Grummer, 1991; Baumgard *et al.*, 2000). The CLA *trans*-10, *cis*-12 isomer was found in the highest quantity (though such difference was not significant) in the 1-SB treatment. Also, in the present study there were an inverse linear relation ($R^2 = 0.11$, $p = 0.04$) between milk fat concentration and the content of this isomer.

The milk protein content was lower in the 1-SB diet when compared with 0-SB and 0.5-SB diets. Although the dietary fat and protein were highest in the 1-SB group, Theurer *et al.* (1995) suggested that increasing the amount of dietary protein within a constant dietary energy level has little effect on milk protein synthesis and whenever dietary protein level increases milk protein yield, the effect seems to be associated with an increase in milk yield. However, in this study no differences between groups were found both in milk protein yield and in milk yield. The decrease in milk protein content might have been due to an increased availability of fat in the rumen in the 1-SB diet (Chouinard *et al.*, 1997). The reduction in the percentage of protein observed in most of studies in animals fed with diets with a high fat content appears to be associated with negative effects on the growth of ruminal microorganisms and the production of microbial protein (Solomon *et al.*, 2000). In addition, when cows are fed fat, the energetic efficiency of milk synthesis is increased. Cows fed high fat diets required less liters of blood flowing to the mammary gland per kg of milk produced (Cant *et al.*, 1993). Because mammary uptake of amino acids is dependent upon amino acid concentration in the blood and blood flow to the mammary gland, these data suggest that the decrease in blood flow per volume of milk produced would limit the uptake of amino acids for milk protein synthesis. However, there are studies that did not found any effect (Guillaume *et al.*, 1991) or even others that found an increase of protein concentration in milk (Block *et al.*, 1981).

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, since total forage ingestion was not monitored in this study this will have to be tested in future studies.

CLA content and composition

As in our study, Khanal *et al.* (2005) did not found effects of a mixed supplement containing 2.4 kg d^{-1} of extruded soybean on CLA concentration in the milk of Holstein cows on pasture (1.63 and 1.69% of total FA for groups fed on pasture alone and pasture + supplement mixed with extruded soybean, respectively; these values include the *cis*-9, *trans*-11 isomer). The values obtained by these authors are somewhat higher than those found herein for the three CLA isomers together. Bartolozzo *et al.* (2003), in Friesian cows fed on pasture and fed a mixed supplement containing 2.6 kg of raw soybean, also obtained a high quantity of CLA (0.96%) as compared to TMR diets with raw or extruded soybean (0.52%), which is in agreement with the results of White *et al.* (2001). All these results indicate a greater influence of the pasture than that of the source or level of soybean incorporated into the diet on the CLA milk content. The different CLA values found in the literature may be related to differences in the nutritional composition of the pasture derived from the different botanical and agronomic characteristics of the herbage used in the various studies (Dewhurst *et al.*, 2006) and, to a lesser degree, to the influence of other factors such as the breed (White *et al.*, 2001; Kelsey *et al.*, 2003). In this respect, White *et al.* (2001) observed 18%

less CLA in milk from Jersey cows compared with milk from Holsteins. On the contrary, other authors observed an increase in the CLA milk content as compared to the control groups, with values up to 2.2% for all the CLA isomers in dairy cows on pasture supplemented with 3.1 kg d^{-1} roasted soybean (Lawless *et al.*, 1998), and 2.4% for only the *cis*-9, *trans*-11 isomer in beef cattle on pasture supplemented with 2 kg d^{-1} extruded soybean (Paradis *et al.*, 2008). However, the exact influence of breed related to dietary supply, and possible interactions need to be determined in further studies.

Rumenic acid is typically the most abundant CLA isomer, with values greater than 80% of total CLA (Palmquist *et al.*, 2005). The *cis*-10, *cis*-12 isomer, on the other hand, was found in very low quantities and has no known physiological function (Khanal & Olson, 2004). In the present study, 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 (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.

In the temporal pattern (Fig. 1) for the content of total CLA and of each of its isomers, a similar trend is observed in all the treatments, which would indicate that the influence of the herbage on the CLA content of the milk is greater than that of the different dietary concentrates supplemented. This may be related to differences in the nutritional composition of the herbage, which has also been shown to affect the fatty acid composition of milk (Dewhurst *et al.*, 2006). In this respect, lower CLA contents in milk have been observed with more mature pasture, and this effect has been attributed to the declining quality and quantity of the herbage (Lock & Garnsworthy, 2003; Ward *et al.*, 2003). This is in agreement with the present work, in which the lowest CLA values were obtained in late December and January, when the herbage presented the poorest nutritional quality (see Table 2). The *cis*-10, *cis*-12 isomer was the only one that diminished from day 1 to day 45, and increased after day 60. At present, it is difficult to explain both the higher quantity and the evolution of this isomer, as observed in the present study.

Metabolic profile

All the metabolites evaluated, except blood urea at the end of the trial, 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) 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*). Under this conditions, highly soluble protein is associated with low levels of NDF and high leaf/stalk proportions at the beginning of spring (Van Vuuren *et al.*, 1991), resulting in incomplete use of the nitrogen in the rumen and high levels of blood urea during the spring and early summer (Wittwer *et al.*, 1993). These levels may exceed the normal range, especially at the beginning of spring, and this is considered normal in Chile (Wittwer *et al.*, 1993).

Conclusions

The dietary concentrate with different quantities of extruded soybean (0.5 and 1.0 kg d^{-1}) fed to Jersey cows, on pasture-based systems, did not influence milk production or total CLA content or its *cis*-9, *trans*-11, *trans*-10, *cis*-12 and *cis*-10, *cis*-12 isomers. However, CLA contents were affected by the nutritional quality of the pasture, with lower values observed at greater maturity stages of pasture. 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 failure of the dietary concentrate with different quantities of extruded whole soybean supplemented in Jersey cows on pasture to increase the concentration of CLA *cis*-9, *trans*-11 in milk fat requires further investigation.

Acknowledgments

The authors thank CONICYT (ComisiónNacional de Ciencia y Tecnología) and Nestle S.A-Chile for funding this research (FONDEF Project N° D02I1135 “Development of products with high content of functional active principles from cows’ milk and its derivatives”).

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