Feeding dairy cows with full fat extruded or toasted soybean seeds as replacement of soybean meal and effects on milk yield, fatty acid profile and CLA content

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ABSTRACT

The aim of this study was to evaluate the effects of the replacement of about 70% of soybean meal (SBM) with extruded (ES) or toasted (TS) full-fat soybean seeds in diets for lactating cows on milk quality, fatty acid profile, and conjugated linoleic acid (CLA) content. Eighteen lactating cows were assigned to 3 groups which received a basal diet, supplemented with 1.8, 2.1 and 2.1 kg/head, respectively, of SBM, ES and TS. There was no significant effect on milk yield, calculated as the difference between daily yield during the experimental period and the mean of the last 5 days of adaptation (-1.65, -1.29 and -0.20 kg/d, respectively, for SBM, ES and TS; *P>0.10*) and milk quality parameters (fat, protein, urea and cheese making parameters) among treatments. In the ES group there was a decrease in the short chain FA content (from C4 to C13) in milk fat (9.2 vs 11.0 and 10.8 g/100 g lipids, respectively, for ES, SBM and TS; *P<0.05*). Medium chain FA (from C14 to C17) content in milk fat was lower for ES and TS groups compared with SBM (46.8 and 48.0 vs 54.8 g/100 g lipids respectively; *P<0.01*), while long chain FA (C≥18) concentration in milk fat was lower in the SBM group compared to the others (34.3 vs 44.2 and 41.2 g/100 g lipids, respectively, for SBM, ES and TS; *P<0.001*). The replacement of SBM with ES enhanced oleic and linoleic acid and, particularly, CLA content. Intermediate values were observed for the TS group. CLA content (0.91, 0.62 and 0.56 g/100 g lipids, respectively, for ES, TS and SBM; *P<0.05*) increased throughout the trial in all groups. ES also reduced the proportion of SFA with respect to SBM (65.2, 68.2 and 70.9 g/100 g lipids, respectively, for ES, TS and SBM; *P<0.05*), and increased MUFA (26.9, 24.5 and 23.1 g/100 g lipids in the same order; *P<0.05*) and PUFA (7.4, 6.9 and 5.5 g/100 g lipids in the same order; *P<0.05*) of milk fat, thus improving the health-quality of milk. The various soybean products did not affect either metabolic profile (protein, urea, glucose, cholesterol, NEFA, triglycerides, liver parameters and mineral serum content) or rumen parameters (pH, ammonia and VFAs). The replacement of SBM with ES and TS permitted an improvement in the nutritional properties of milk without negatively affecting animal performances.

Key words: Dairy cows, Soybean sources, Milk, Fatty acid profile, Conjugated linoleic acids (CLA)

RIASSUNTO

SOSTITUZIONE DELLA FARINA DI ESTRAZIONE DI SOIA CON SOIA INTEGRALE ESTRUSA O TOSTATA NELL'ALIMENTAZIONE DI VACCHE IN LATTAZIONE: EFFETTI SULLA PRODUZIONE DI LATTE, SUL PROFILO ACIDICO E SUL CONTENUTO DI CLA

Le sempre maggiori difficoltà a reperire fonti proteiche di qualità che forniscano possibili percorsi di rintracciabilità dei prodotti di origine animale, hanno portato ad una rivalutazione delle materie prime di origine aziendale. Nell'ultimo decennio, inoltre, molte ricerche si sono rivolte allo studio degli effetti nutraceutici degli alimenti destinati all'uomo. In questa prova la farina di estrazione di soia (SBM) della dieta di vacche in lattazione è stata sostituita per circa il 70% con soia integra-

le estrusa (ES) o tostata (TS) per studiarne gli effetti sulle prestazioni produttive e sui parametri qualitativi del latte, con particolare riguardo al contenuto di CLA (isomeri coniugati dell'acido linoleico). Diciotto vacche in lattazione sono state suddivise in tre gruppi e sono state alimentate con una dieta di base (unifeed a secco), integrata con SBM (1.8 kg/d per capo), o con ES o TS (2.1 kg/d per capo). Nessun effetto del trattamento alimentare è stato osservato, né sulla quantità di latte (la produzione è diminuita di 1.65, 1.29 e 0.20 kg/d nelle tesi SBM, ES e TS rispetto alla media del periodo di adattamento; P>0.10*), né sui parametri qualitativi del latte (grasso, proteina e urea) o sui parametri lattodinamografici. Per quanto riguarda il profilo acidico del latte, il trattamento ES ha diminuito il contenuto di acidi grassi a corta catena (da C4 a C13) rispetto agli altri due trattamenti (9.2 vs 11.0 e 10.8 g/100 g di lipidi, risp. per ES, SBM e TS; P<0.05). Le bovine alimentate con ES e TS hanno prodotto un latte con una minor percentuale di acidi grassi a media catena (da C14 a C17; 46.8 e 48.0 vs 54.8 g/100 g di lipidi, risp. per ES, TS e SBM;* P<0.01*) ed hanno aumentato la concentrazione di quelli a lunga catena (C≥18; 44.2 e 41.2 vs 34.3 g/100 g di lipidi, risp. per ES, TS e SBM;* P<0.001*). La dieta con ES ha fornito inoltre un latte con un minore contenuto di acidi grassi saturi ed una maggiore presenza di acidi grassi mono e polinsaturi rispetto alla SBM. Anche il contenuto di CLA è stato maggiore nel latte prodotto dalle bovine dei gruppi ES rispetto alla SBM (0.91 e 0.62 vs 0.56 g/100 g di lipidi, risp. per ES, TS e SBM;* P<0.01*). Nessun effetto del trattamento alimentare è stato osservato sui parametri ematici (proteine, urea, glucosio, colesterolo, NEFA, trigliceridi, funzionalità epatica e profilo minerale) né sulle caratteristiche del liquido ruminale (pH, azoto ammoniacale e AGV). Questi risultati confermano che l'uso di semi di soia integrali nelle diete per vacche da latte può migliorare le caratteristiche qualitative del latte ed in particolare del profilo acidico, senza avere conseguenze negative sulle prestazioni produttive e sullo stato di salute degli animali. La soia integrale estrusa o tostata di produzione aziendale può ridurre il grado di dipendenza dal mercato delle aziende per l'approvvigionamento delle fonti proteiche, riducendo i rischi di contaminazione con prodotti GM, e può quindi agevolare l'introduzione di un sistema di rintracciabilità nell'ambito delle filiere di produzione del latte.*

Parole chiave: *Vacca da latte, Soia, Latte, Acidi grassi, Isomeri coniugati dell'acido linoleico (CLA)*

Introduction

The EU market is not self sufficient in supplying protein for animal feeds and this shortage has been made worse by the ban on the use of animal protein in both ruminant and non ruminant feeds. The degree of self-supply of soybean meal in Italy is only about 35% (ASSALZOO, 2003) and the remainder is mainly imported from Argentina (70%), Brazil (20%) and the USA (<10%). In these countries, with a partial exception for Brazil, the soybean is mainly produced from GM varieties (Di Giovannantonio, 2001). Consumers worry about the use of GM feeds in animal diets for the fear of possible consequences on health, but in this context, many doubts remain about the possibility of assuring the absence of GM-free products in animal feeds. On the other hand, there is little doubt that the economic sustainability of milk production in many areas strongly depends on the availability of soybean, which remains the most important source for the quality of its protein. Thus, the promotion of a protein feed production directly on the farms themselves, such as full fat toasted soybean seeds, is important in order to reduce the dependence of farms on the matrket, especially with respect to the organic production system.

Heat-treated full-fat soybean presents an interesting fatty acid profile that can improve the quality of fat in animal products according to consumer demand of healthier foods (Hulshof *et al.*, 1999; Chilliard *et al*., 2000). The health quality of milk fat can be improved by reducing the proportion of saturated FA and, in particular, of lauric (C12:0) myristic (C14:0) and palmitic (C16:0) acids, recognized as risk factors for coronary heart disease (Ashes *et al.*, 1997). It is also desirable to increase the butyric acid (C4:0), oleic acid (C18:1), polyunsaturated FA (especially n-3 FA) and conjugated linoleic acid (CLA) contents, which can have antiatherogenic, antiobesity or anticarcinogenic roles (O'Shea *et al.*, 1998; Williams, 2000; Chilliard *et al.*, 2001; Ramaswamy *et al.*, 2001; Antongiovanni *et al.*, 2003).

The use of home-made soybean products can offer the opportunity of reducing the market dependence of farms for protein supply, thereby minimizing the risks of cross contamination with GM products or other undesirable compounds, but also to increase the nutritional properties of milk. Thus, this study was designed to evaluate the effects of the replacement of soybean meal with extruded or toasted full fat soybeans in diets for lactating cows on the milk quality, fatty acid profile and conjugated linoleic acid (CLA) content.

Material and methods

Experimental design and animal feeding management

Eighteen Italian Friesian cows were blocked in three groups of six animals each, according to the days of lactation (80 ± 41 d), parity (2.3 ± 1.4) and the milk yield (37.0 ± 7.7 kg/d). Each group was fed a basal diet supplemented with either soybean meal (SBM group: 1.8 kg/d of soybean meal + 1.5 kg/d corn meal per head) or full-fat extruded soybean (ES group: 2.1 kg/d of extruded soybean per head) or full-fat cracked toasted soybean (TS group: 2.1 kg/d of toasted soybean per head). The three diets were formulated to be isoenergetic and isonitrogenous and were balanced for minerals and vitamins to meet the nutritional requirements of the cows according to INRA (1988) and NRC (2001). The cows received the basal mixed ration once daily by mixer wagon (7.30 am), and immediately after, the supplementation was added to the diet by top dressing. Ingredients and chemical composition of diets are given in Table 1. The animals were tied up till the whole supplementation dose was ingested. Table 2 gives the chemical composition and fatty acid profile of soybean ingredients. The protein-mineral supplement of the basal mixed diet contained about 15% of soybean meal. The experimental period lasted 57 days. The first 22 days of the trial were used as an adaptation period, while during the following 35 days, experimental controls were performed. The cows were milked twice a day (6:00 a.m. and 5:00 p.m.).

Table 1. Ingredients and chemical composition of the diets.

		Treatment		
		SBM	ES	TS
Ingredients:				
Permanent meadow	% DM	15.9	16.7	16.7
Alfalfa hay	"	13.3	14.0	13.9
Dehydrated alfalfa	"	12.9	13.6	13.6
Cracked corn seeds	"	7.3	7.7	7.7
Corn meal	"	20.3	15.0	15.1
Protein-mineral premix[1]	"	22.9	24.0	24.0
Soybean meal	"	7.4		
Extruded soybean	"		9.0	
Toasted soybean	"			9.0
Forage: concentrate ratio		42:58	44:56	44:56
Chemical composition:				
Dry Matter	%	57.3	56.3	56.3
Crude Protein	% DM	16.2	15.7	15.7
Fat	"	2.9	4.4	4.4
Ash	"	8.0	8.2	8.2
NDF	"	34.5	35.3	35.3
ADF	"	20.5	21.4	21.4
ADL	"	3.2	3.4	3.4
Milk FU	/kg DM	0.97	0.94	0.94

[1] *Contained in kg of feed compound: vit. A 75,000 U, vit. D_3 4500 U, vit. E 45 mg, vit. B_1 7.5 mg, vit. B_{12} 0.0015 mg, vit. PP 450 mg, Mn 150 mg, Fe 150 mg, Cu 3 mg, Co 1.5 mg, I 3 mg, Zn 150 mg, Se 0.45 mg. The protein-mineral premix contained 15% of soybean meal.*

SBM: soybean meal; ES: extruded soybean; TS: toasted soybean.

Table 2. Chemical composition and fatty acid profile (% of total fatty acids or mg/100 mg) of the three soybean supplements.

			Treatment		
			SBM	ES	TS
Chemical composition:					
Dry Matter		%	88.25	89.47	88.04
Crude Protein		% DM	45.09	39.42	39.68
Fat		"	1.61	19.15	18.43
Ash		"	7.04	5.76	5.25
Crude fiber		"	5.91	5.63	9.36
Fatty acid (FA) profile:					
$C_{14:0}$		% of the total FA	0.16	0.07	0.07
$C_{15:0}$		"	0.04	0.00	0.01
$C_{16:0}$		"	15.18	11.75	11.64
$C_{16:1}$		"	0.10	0.08	0.08
$C_{17:0}$		"	0.12	0.10	0.09
$C_{18:0}$		"	3.83	3.90	3.89
$C_{18:1}$	n-9	"	14.05	20.23	20.15
$C_{18:1}$	n-7	"	1.52	1.55	1.63
$C_{18:2}$	n-6	"	52.43	54.17	53.84
$C_{18:3}$	n-3	"	8.58	7.18	7.29
$C_{20:0}$		"	0.19	0.21	0.22
$C_{20:1}$	n-9	"	0.15	0.14	0.16
$C_{20:2}$	n-6	"	0.01	0.01	0.02
$C_{22:0}$		"	0.27	0.18	0.21
$C_{22:5}$	n-3	"	0.00	0.02	0.03
$C_{23:0}$		"	0.16	0.02	0.04
$C_{24:0}$		"	0.00	0.00	0.04
Others		"	3.22	0.39	0.59
SFA		"	19.94	16.23	16.20
MUFA		"	15.82	22.00	22.02
PUFA		"	61.02	61.38	61.18
SFA/(MUFA+PUFA)			0.26	0.19	0.19
n-6		"	52.44	54.18	53.86
n-3		"	8.58	7.20	7.32
n-6/n-3			6.10	7.50	7.40

BCS and feed analysis

Each cow was evaluated for its nutritional status by Body Condition Score (BCS) at the beginning of the adaptation period and at the end of the experimental period (scores from 1 to 5; Edmonson *et al.*, 1989). Animal health was detected daily throughout the trial.

To evaluate DM daily intake, the basal mixed ration residues of each experimental group were weighted weekly. A sample of each feed compound of the diets was collected at the beginning of the adaptation period and at the end of the trial or at every batch change. Concentrate samples were ground through a mill with a 1 mm screen (Retsch ZNC) while forage samples were previously ground with a 2 mm screen (Fritsch Pulverinet

15). Each feed sample was analyzed for dry matter, crude protein, fat, ash and for NDF, ADF and ADL fractions (Martillotti *et al.*, 1987; Van Soest *et al.*, 1991). The soybean supplements (ES, TS; SBM) were analyzed for their *in situ* rumen degradability of protein as suggested by ASPA (1994), using two rumen cannulated cows. Potential and effective degradability were calculated as suggested by Ørskov and McDonald (1979) using the value of 6%/h for the rate of rumen passage (INRA, 1988). Soybean supplements were also analyzed for their fatty acids profile. The samples were previously ground through a 1 mm screen. Lipid extraction of soybean fat was carried out according to Martillotti *et al.* (1987) and Folch *et al.* (1957), using a chloroform/methanol solution (2:1 v/v) followed by a NaCl solution at 2%. The residues, previously dried and weighed, were then trans-esterified according to the Christie (1982) and Chouinard *et al.* (1999) procedure, and fatty acid profile was determined as described later.

Rumen fluid analysis

Rumen fluid samples were collected from each cow at the end of the experimental period, 3 hours after feeding and through an esophageal vacuum pump. The pH values were immediately measured and then, after adding 20% of metaphosphoric acid, the rumen fluid samples were stored at -18°C. Ammonia was measured by a pH-meter specific electrode, according to official methods of analyses (AOAC, 2000). The volatile fatty acids (VFA) were evaluated by means of the gas chromatographic method as described by Osl (1988).

Metabolic profiles

Individual blood samples, collected at the beginning and the end of the experimental period, were taken from the jugular vein before the morning feeding and after milking using evacuated tubes containing lithium-heparin. Plasma was immediately separated and analyses were performed at the Istituto Zooprofilattico Sperimentale delle Venezie. On each sample protein fraction (total proteins, albumin), urea, glucose, total cholesterol, triglycerides, NEFA, AST, GGT, CK, Ca, P and Mg content were determined. All plasma traits were determined automatically by biochemical analyzer (Hitachi 911, Roche, Milano, Italy) at 37°C, except for the NEFA content, which was analyzed by manual procedure (FA 115, Randox Lab., UK).

Milk yield and quality

Individual daily milk yield was automatically recorded and the average milk yield of the adaptation period was used to calculate the differences compared to the daily milk yield observed during the experimental period. Individual milk samples were collected at the beginning and the end of the adaptation period and then weekly throughout the trial. Samples from evening and following morning milkings were collected, split into two portions for further analysis, and refrigerated either at 4°C for quality parameters or -18°C to evaluate fatty acid composition. Milk samples were analyzed for fat, total protein and lactose by infrared analysis (Bentley 2000, Bentley Instruments Inc., Minnesota, USA). Urea content was analyzed automatically by the conductimetric-enzymatic method (CL 10 micro analyzer, Eurochem, Roma, Italy). cheese making properties (renneting clotting time, r; rate of curd firming, K_{20}; and curd firmness after 30 min, A_{30}) were measured according to the method of Zannoni *et al.* (1981) on a Formagraph apparatus (CRM 48, Polo Trade, Padova, Italy).

Milk fatty acid composition

Lipid extraction of milk fat was performed according to Nourooz-Zadeh and Appelqvist (1988), using an hexane/isopropanol solution (3:2 v/v) followed by a 0.47M sodium sulfate solution. The residues previously dried and weighed were then trans-esterified according to the Christie (1982) and Chouinard *et al.* (1999) procedure. A solution (1:1) of hexane (2 mL) and internal standard (nonadecanoic acid methyl ester C19:0) was added to 40 mg of butter oil followed by 40 μL of methyl acetate. After that, the mixture was vortexed and 40 μL of methylation reagent (1M methoxy in methanol) was added and allowed to react for 10 min. Then, 60 mL of termination reagent (1 g oxalic acid/ 30 mL ethyl ether) were added. The sample was centrifuged for 5 min at 2400 x g at 5°C leaving a clear layer of hexane; an

aliquot of hexane was taken and used directly for chromatographic determination. Fatty acid methyl esters (FAME) were separated through a capillary column Omegawax 250 (30 m x 0.25 mm, Supelco Bellefonte, PA, USA). Hydrogen was used as the carrier gas with a flow of 1.60 ml/min and linear speed of 40.20 cm/sec. The oven temperature was programmed from 140 to 220°C at 4°C/min. Injector and detector were kept at 250°C.

Statistical analysis

The data were processed using the General Linear Models procedure of the SAS-STAT (1996) according to a split-plot model in which the treatment (i.e. diet) was the main plot (on three levels: SBM, ES or TS) and the sampling-time and the interaction treatment x sampling-time were the sub-plots. Contrasts were used to test differences among treatments.

Results and discussion

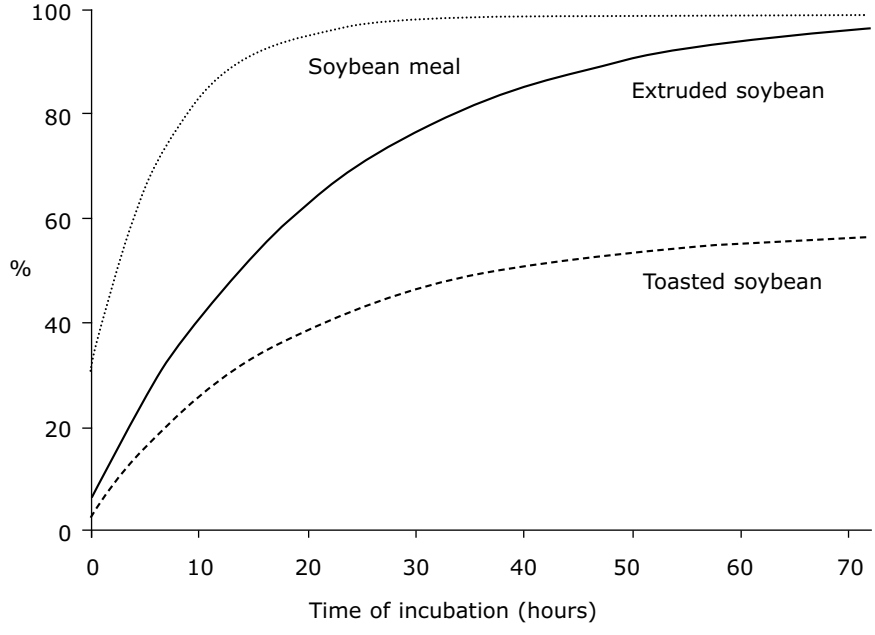
Characteristics of the soybean products used

Figure 1 shows the *in situ* protein degradability of the three soybean sources used in the trial. The results are in agreement with previous findings (Ramanzin *et al.*, 1991; Cozzi *et al.*, 1992; Martillotti *et al.*, 1996; Abu Ghazaleh *et al.*, 2002).

It's well known that toasted soybean exhibits more available protein for the post-ruminal tract than other sources of processed soybeans. Compared to the other sources, TS showed the lowest value of immediately degradable protein, i.e. 1.8%, with 55.2% of slowly degradable fraction and a protein effective degradability of 11.0% (k=6%/h). ES exhibited an intermediate trend with an immediately degradable protein fraction of 6.0%, and an effective degradability of 21.6% (k=6%/h), but with the highest potential degradability (94.0%). SBM had the highest immediately degradable fraction (29.4%) compared to the other two protein sources, and an effective degradability (k=6%/h) of 40.9%. Based on these results, TS and, in second order ES, are potentially able to supply higher amount of bypass protein, with a lower degradation rate (an average 5%/h) compared to SBM (15%/h).

Fatty acid profiles of ES and TS were very similar (Table 2), with 54 g/100 g lipids of linoleic acid (the most representative among PUFA) and 20 g/100 g lipids of oleic acid (the most representative among MUFA). SBM had a lower level of oleic acid (C18:1; 14 g/100 g lipids) and higher level of palmitic acid (C16:0; 15 g/100 g lipids) compared to the other two protein sources (Martillotti *et al.*,

Figure 1. *In situ* protein degradability of the soybean sources.



1996; Secchiari *et al.*, 2003). The α-linolenic acid (C18:3 n-3) content, the precursor of n-3 acids, was similar among the three supplements. Soybean meal showed the highest level of saturated fatty acids (SFA; 20 g/100 g lipids) and the lowest content of MUFA (16 g/100 g lipids) resulting in slightly higher SFA/(MUFA+PUFA) ratio (0.26) compared to extruded and toasted soybean (on average 0.19).

BCS, rumen fluid characteristics and metabolic profile

As expected, no differences were observed across all treatments on BCS of the cows either at the beginning of the adaptation period or at the end of the experimental period (Figure 2), since the diets were formulated to be isoenergetic and isonitrogenous. Nor were changes observed within treatments throughout the experimental periods, as the experimental period was of short duration (35 d). Volatile fatty acids, pH and ammonia contents of the rumen fluid analyzed at the end of the experiment were not altered by treatments (Table 3) and they were close to the expected levels (Cozzi *et al.*, 1993; Solomon *et al.* 2000).

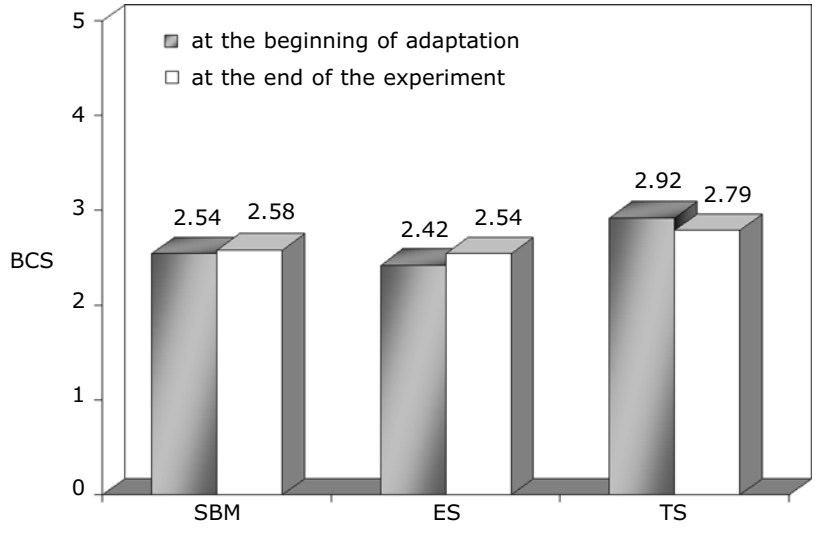
Throughout the trial the cows were in good health and did not show any relevant pathology. The various soybean products did not affect the metabolic parameters analyzed (Table 4) and the metabolic profile was in agreement with the values related to healthy lactating dairy cow conditions (Bertoni and Piccioli Cappelli, 1999). There were no differences among groups for the plasma urea concentration and the haematic glucose levels were slightly lower than those reported by Bertoni and Piccioli Cappelli (1999). Triglycerides, cholesterol, NEFA, hepatic enzymes and plasma mineral profile were within the ranges indicated by Bertoni and Piccioli Cappelli (1999).

Daily DMI and milk yield

Daily DMI were similar among different experimental groups throughout the trial, with a mean of 20.3 kg/d per head.

Figure 3 shows the pattern of the daily milk variation, calculated as the difference between daily yield during the experimental period and the mean of the last 5 days of adaptation (on average 37.2, 36.9 and 34.3 kg/d, respectively, for SBM, ES and TS). No significant differences on milk variation were observed among treatments, although milk yield decreased throughout the trial in all the experimental groups (-1.65, -1.28 and -0.20 kg/d, respectively, for SBM, ES and TS groups; *P>0.10*).

Figure 2. Mean BCS values at the beginning of the adaptation period and the end of the experimental period.



The different soybean source did not significantly affect milk yield during the experimental period, which averaged 35 kg/d. These results are in agreement with other findings (Van Dijk *et al.*, 1983; Bernard, 1990; Chouinard *et al.*, 1997).

Figure 3. Pattern of daily milk variation, calculated as difference between daily yield during the experimental period and the mean of the last 5 days of adaptation (kg).

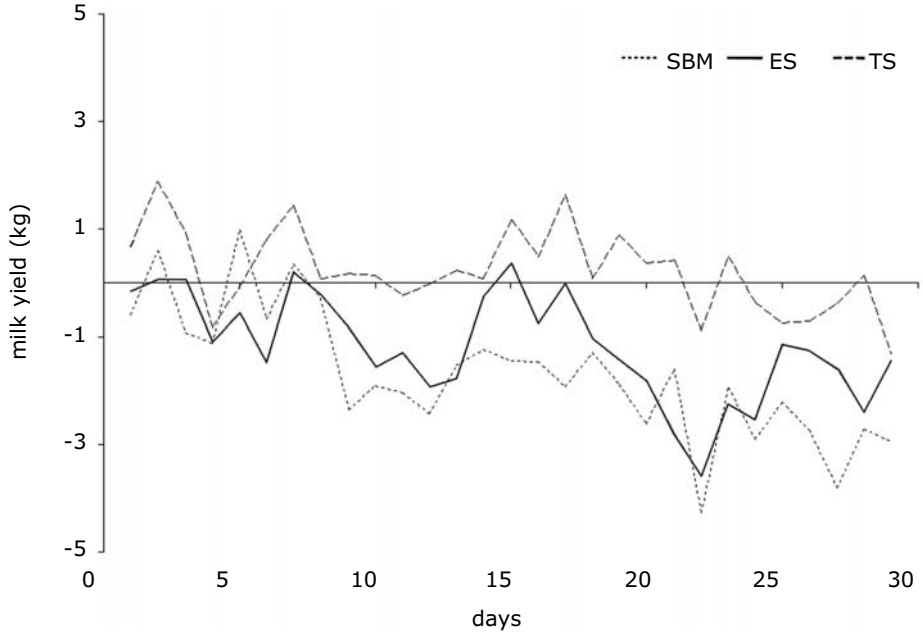


Table 3. Rumen parameters.

		Treatment			RSD	P
		SBM	ES	TS		
pH		6.54	7.01	6.64	0.20	ns
Ammonia	mmol/l	10.24	10.58	11.69	4.02	ns
VFA	molar %					
- acetate	"	70.03	70.37	68.25	3.29	ns
- propionate	"	25.74	24.30	26.68	3.32	ns
- iso-butyrate	"	0.62	1.03	0.72	0.19	ns
- n-butyrate	"	1.21	1.11	1.22	0.20	ns
- 2-meta-butyrate	"	0.42	0.56	0.69	0.29	ns
- total butyrate	"	2.16	2.70	2.63	0.47	ns
- iso-valerianate	"	0.40	0.39	0.48	0.43	ns
- n-valerianate	"	1.67	2.24	1.95	0.46	ns
- total valerianate	"	2.07	2.63	2.44	0.33	ns
C2/C3		2.75	3.06	2.57	0.43	ns
(C2+C4)/C3		2.84	3.18	2.67	0.45	ns

ns: not significant

Table 4. Plasma metabolic profile.

		Treatment			RSD	P
		SBM	ES	TS		
Total protein	g/l	81.75	82.58	84.00	3.55	ns
Albumin	"	35.41	34.67	34.92	1.12	ns
Urea	mmol/l	6.70	6.86	7.07	0.91	ns
Glucose	"	3.42	3.43	3.32	0.18	ns
Total cholesterol	"	5.23	5.87	5.44	0.59	ns
Triglycerides	"	0.10	0.10	0.08	0.02	ns
NEFA	"	0.16	0.16	0.10	0.10	ns
AST	U/l	91.50	83.17	92.58	11.22	ns
GGT	"	26.91	26.08	26.33	2.35	ns
CK	"	212.33	209.00	155.66	132.60	ns
Ca	mmol/l	2.41	2.47	2.50	0.19	ns
P	"	1.62	1.68	1.65	0.23	ns
Mg	"	1.02	0.97	0.97	0.06	ns

ns: not significant

Milk composition

Milk quality parameters were not affected by treatments (Table 5). In agreement with previous findings (Scott *et al.*, 1991; Chouinard *et al.*, 1997), no differences due to the treatment were observed for milk protein and urea content. Urea values (on average 34.05 mg/100 ml) were higher than those reported by Solomon *et al.* (2000) and Bertoni (1996). These results may indicate that for all dietary treatments there was a slight excess in rumen degradable protein compared to the energy substrate available for microbes. Milk cheese making properties (r, K_{20} and A_{30}) were not affected by soybean supplements and showed good making-cheese characteristics (Table 5).

The ES group showed the lower milk fat content although the difference with the other groups was not significant. These results are in agreement with previous experimental data by Dhiman *et al.*, 1999, in which the milk fat content from

Table 5. Milk composition.

		Treatment			RSD	P
		SBM	ES	TS		
FCM(*)	kg/d	30.60	29.80	29.05	2.69	ns
Fat content	%	3.10	2.88	3.09	0.39	ns
Protein content	"	3.13	3.07	3.07	0.06	ns
Lactose	"	5.16	5.12	5.14	0.32	ns
Urea	mg/100 ml	33.74	34.35	34.08	2.33	ns
Clotting parameters:						
r(**)	min	16.08	16.73	14.83	5.85	ns
K_{20}(**)	"	2.71	3.78	3.91	2.71	ns
A_{30}(**)	mm	25.63	36.60	23.87	12.25	ns

(*) FCM = 3.5% fat corrected milk
(**) r =renneting clotting time in samples that clotted within 45 min; K_{20} = rate of curd firming in samples that clotted within 45 min; A_{30} = curd firmness after 30 min

Table 6. Fatty acid composition (g/100 g lipids) of milk.

		Treatment			RSD	P
		SBM	ES	TS		
$C_{4:0}$		2.46	2.80	2.75	0.33	ns
$C_{6:0}$		1.21	1.31	1.26	0.65	ns
$C_{8:0}$		0.68	0.48	0.51	0.41	ns
$C_{10:0}$		2.33^{Ab}	1.47^{Bc}	2.81^{Aa}	0.65	<0.001
$C_{10:1}$		0.11^{A}	0.02^{B}	0.08^{A}	0.07	<0.01
$C_{11:0}$		0.06^{Aa}	0.02^{ABb}	0.01^{Bb}	0.03	<0.05
$C_{12:0}$		3.96^{Aa}	2.95^{Bb}	3.32^{ABb}	0.28	<0.01
$C_{12:1}$	n-9	0.04^{Aa}	0.02^{ABb}	0.02^{Bb}	0.02	<0.05
$C_{13:0}$		0.14^{a}	0.07^{b}	0.07^{b}	0.03	<0.05
Short chain[(1)]		10.98^{a}	9.15^{b}	10.83^{a}	1.40	<0.05
$C_{14:0}$		13.11^{a}	11.73^{b}	12.30^{ab}	0.79	<0.05
$C_{14:1}$		1.11^{a}	0.85^{ab}	0.75^{b}	0.16	ns
$C_{15:0}$		2.51^{Aa}	2.00^{Bb}	2.07^{ABb}	0.17	<0.05
$C_{15:1}$		0.01	0.00	0.01	0.01	ns
$C_{16:0}$		34.25^{Aa}	29.15^{Bb}	30.12^{ABb}	1.65	<0.05
$C_{16:1}$		1.72^{a}	1.23^{ab}	0.97^{b}	0.45	<0.05
$C_{17:0}$		1.64^{a}	1.44^{b}	1.49^{b}	0.11	ns
$C_{17:1}$		0.36^{a}	0.29^{ab}	0.25^{b}	0.06	0.05
Medium chain[(1)]		54.79^{A}	46.77^{B}	48.01^{B}	2.70	<0.01
$C_{18:0}$		8.31^{b}	11.55^{a}	11.25^{a}	1.16	<0.05
$C_{18:1}$	n-7	2.11^{b}	3.06^{a}	2.22^{ab}	0.85	ns
$C_{18:1}$	n-9	17.76^{Bb}	21.62^{Aa}	20.32^{ABa}	0.94	<0.01
$C_{18:2}$	n-6	3.33^{B}	4.81^{A}	4.34^{A}	0.21	<0.05
$C_{18:3}$	n-6	0.34	0.33	0.46	0.23	ns
$C_{18:3}$	n-3	0.62^{b}	0.80^{a}	0.79^{a}	0.08	<0.05
CLA		0.56^{Bb}	0.91^{Aa}	0.62^{ABb}	0.11	<0.05
$C_{20:0}$		0.15^{Bb}	0.19^{Aa}	0.18^{ABa}	0.02	<0.05
$C_{20:1}$	n-9	0.05	0.06	0.05	0.01	ns
$C_{20:2}$	n-6	0.04	0.04	0.04	0.01	ns
$C_{20:3}$	n-6	0.15^{ab}	0.13^{b}	0.16^{a}	0.02	ns
$C_{20:3}$	n-3	0.01	0.02	0.02	0.01	ns
$C_{20:4}$	n-6	0.28^{a}	0.22^{b}	0.28^{a}	0.05	<0.05
$C_{20:5}$	n-3	0.05	0.05	0.06	0.01	ns
$C_{21:0}$		0.01^{B}	0.03^{A}	0.02^{AB}	0.02	<0.05
$C_{22:0}$		0.05^{b}	0.06^{ab}	0.06^{a}	0.01	<0.05
$C_{22:1}$	n-9	0.02	0.01	0.02	0.02	ns
$C_{22:2}$	n-6	0.00^{ab}	0.01^{a}	0.00^{b}	0.01	ns
$C_{22:5}$	n-3	0.10	0.08	0.09	0.02	ns
$C_{22:6}$	n-3	0.01^{ABa}	0.01^{Aa}	0.01^{Bb}	0.01	<0.05
$C_{23:0}$		0.07	0.04	0.06	0.05	ns
$C_{24:0}$		0.02	0.02	0.03	0.02	ns
$C_{24:1}$	n-9	0.27^{a}	0.11^{b}	0.14^{ab}	0.23	ns
Long chain[(1)]		34.32^{B}	44.16^{A}	41.23^{A}	2.49	<0.001

[(1)] short chain: from C_4 to C_{13}; medium chain: from C_{14} to C_{17}; long chain: ≥C_{18}
Values within a row with different letters differ significantly (lower-case letter: P<0.05; capital letter: P<0.01)

cows fed extruded soybean was lower than the milk fat produced by cows fed soybean meal and extruded cottonseed (*P=0.09*). These Authors ascribed their results to a higher rumen availability of fat and in particular of C18:2 from extruded oilseeds, due to the processing method. It is known that one of the dietary fats exposed to biohydrogenation in the rumen is the C18:2 and that production of trans isomers C18:1 mainly depends from the availability of this dietary FA. An increase of the trans isomers C18:1 in the milk was associated with a decrease in milk fat content and, moreover, these compounds are thought to be responsible forf the inhibition of *de novo* FA mammary synthesis (Dhiman *et al.*, 1999; Chilliard *et al.*, 2002; Secchiari *et al.*, 2003). However, over the last 20 years variable results on milk fat percentage from cows fed differently processed soybean products (extruded, raw, toasted or soybean meal) have been found (Chouinard *et al.*, 1997). In the present trial, the C18:2 dietary content was close to about 2.4% DM in ES and TS groups and 1.5% DM in the SBM group. These levels were probably too small to significantly affect milk yield and fat content.

Short chain fatty acids

In Table 6 the milk fatty acid composition of experimental groups is given. Important differences were observed among the fatty acid profiles of milk fat.

ES significantly reduced the proportions of short-chain fatty acids (C4:0-C13:0) in milk fat compared to SBM and TS (9.15, 10.83 and 10.98 g/100 g lipids respectively for ES. TS and SBM; *P<0.05*). This reduction particularly involved the caprinic (C10:0) and lauric (C12:0) acids (*P<0.01*). According to expectations, the use of ES could have inhibited the *de novo* fatty acid synthesis (from C4:0 to C16:0) by the mammary gland cells (Offer *et al.* 1999). These result are in agreement with the high level of trans C18:1 isomers observed in the milk of cows fed with ES (see Table 6) compared to the other treatments. Similar results were observed by Chouinard *et al.* (1997) who found a higher proportion of trans C18:1 isomers in blood and milk of cows fed with ES compared to raw ground, micronized and roasted soybean supplementation. The extrusion processes imply the rupture of plant cells and the consequent leak of oil from the seed, which adheres to the surface of the feed ES particles (Chouinard *et al.*, 1997). Such free oil (rich in PUFA) inhibits the activity of rumen microorganisms, responsible for the last step of bio-hydrogenation. The consequent increase in the trans bio-hydrogenation intermediates reaching the mammary gland can, in turn, inhibit the *de novo* short FA synthesis.

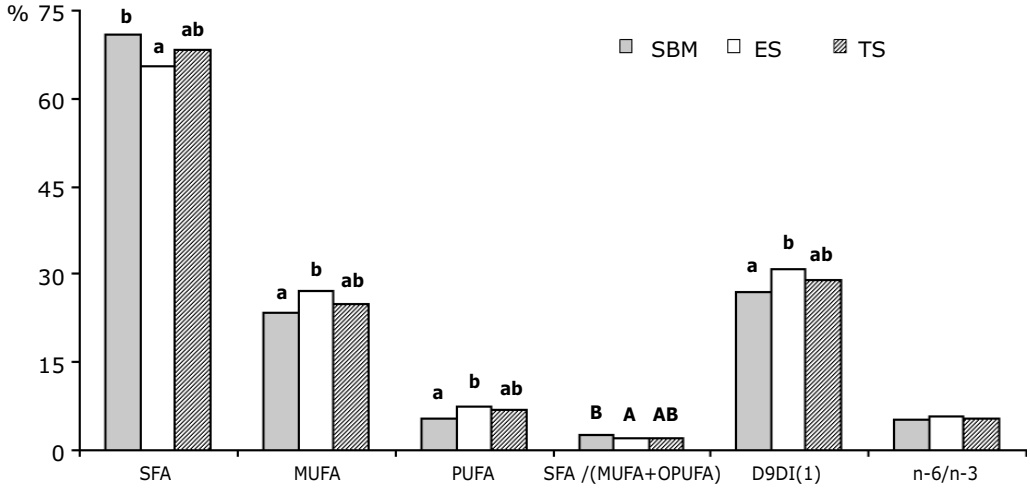
Medium chain fatty acids

The concentrations in milk fat of medium chain FA (C14:0 - C17:1) were lower (*P<0.001*) for cows fed ES and TS than for SBM (46.77, 48.01 and 54.79 mg/100 mg lipids respectively for group ES, TS and SBM). This inhibitory effect observed for ES and TS supplementation (especially on C14:0 and C16:0) might have been due to either a higher proportion of trans isomers resulting from the incomplete bio-hydrogenation of dietary fat at rumen level or to a direct inhibitory effect of PUFA on ACC activity (acetyl-CoA carboxylase, which catalyzes the synthesis of malonyl-CoA starting from acetate at the mammary gland level) (Chilliard *et al.*, 2000). From the human health point of view, these results are important since C12:0 (lauric acid), C14:0 (myristic acid) and C16:0 (palmitic acid) have been indicated as the main fatty acids responsible for increasing plasma total and LDL cholesterol concentrations (Antongiovanni *et al.*, 2003).

Long chain fatty acids

As expected from previous studies, ES and TS showed higher proportions of long-chain fatty acids (C >18) compared to SBM (34.32, 41.23 and 44.16 g/100 g lipids, respectively, for group SBM, TS and ES; *P<0.001*). Full fat extruded and toasted soybean significantly increased stearic, oleic, linoleic and α-linolenic acids compared with soybean meal supplementation. As discussed previously, the higher proportion of long chain FA and, particularly of these four long chain FA, was probably due to a higher rumen flow of PUFA and biohydrogenation intermediates available for mammary gland uptake. In the mammary gland the higher availability of stearic acid and of C18:1 iso-

Figure 4. Acidic composition of milk.



(1) D9DI = Δ9 desaturation index =100x[($C_{14:1}$ + $C_{16:1}$ + $C_{18:1}$ + CLA)/($C_{14:1}$ + $C_{16:1}$ + $C_{18:1}$ + CLA + $C_{14:0}$ + $C_{16:0}$ + $C_{18:0}$ + trans-11 $C_{18:1}$)]

mers like vaccenic acid enhances the Δ^9-desaturase activity. In this study the Δ^9-desaturase index was significantly different among treatments (from 26.9 to 29.0 and to 30.8 in SBM, ES and TS group respectively; *P<0.05*) (Figure 4) to produce oleic acid and CLA, with results similar to those observed by Secchiari *et al.* (2003) who compared toasted full fat soybean with other protein supplements.

Saturated fatty acids

The content of SFA (Figure 4) was higher for SBM group, intermediate for the TS group and lower for the ES group, (71.0, 68.3 and 65.3 g/100 g lipids, respectively, for SBM, TS and ES groups; *P<0.05*). By feeding the cows with ES and TS, the palmitic acid (C16:0) content decreased, while the stearic acid (C18:0) increased (Table 6). This response is positive for human health since palmitic acid has been reported to be one of the SFA responsible for increasing plasma total and LDL cholesterol concentrations, while stearic acid has been shown not to increase total or LDL cholesterol content (Chilliard *et al.*, 2000; Antongiovanni *et al.*, 2003).

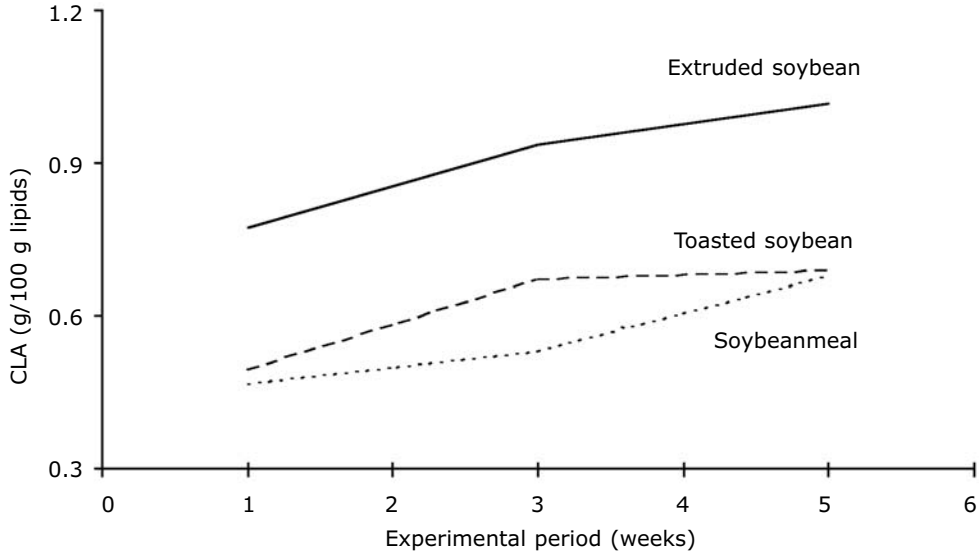
Mono-unsaturated fatty acids

The MUFA content (Figure 4) was higher for the ES group, intermediate for the TS group and lower for the SBM group; the higher amounts of MUFA were mainly due to an increase in C18:1 ω7 (vaccenic acid) and C18:1 ω9 (oleic acid) concentrations in milk fat. This also reflects a higher level of rumen biohydrogenation of dietary fat from the extruded soybean source (Table 6) compared to the other sources. Indeed, it has been hypothesized that about 52% of the total milk fat oleic acid originates from the desaturation at mammary gland level of stearic acid formed in the rumen during biohydrogenation (Enjalbert *et al.*, 1998).

Poly-unsaturated fatty acids

The PUFA content in milk fat (Figure 4) was higher for the ES group, intermediate for the TS group and lower for SBM group (7.4, 6.9 and 5.5 g/100 g lipids; *P<0.05*). Among these fatty acids (Table 6) the ES group showed the higher content of C18:2 ω6 (linoleic acid) compared to the other groups. Chouinard *et al.* (1997) found lower concentration of PUFA (C18:2 and C18:3) in milk fat from cows fed extruded soybean compared to milk

Figure 5. CLA content (g/100 g lipids) in milk of the experimental groups during the trial.



fat from cows fed micronized soybeans and roasted soybeans. The Authors supposed a higher level of rumen biohydrogenation for dietary fat from this source of soybean due to the ruptures of seed by the extrusion process, which releases oil from the cells. Indeed, the micronization and the roasting process were applied on whole seeds that were flaked and rolled immediately after the heat treatment by these Authors. Therefore, they explained the higher PUFA content from extruded soybean with a possible higher physically protected proportion of PUFA from ruminal hydrogenation in micronized and roasted soybean. Thus, also in our study the higher content of linolenic and α-linolenic acids in the ES and TS groups could be reflected by the higher dietary content of these acids as respect to SBM.

According to Secchiari *et al.* (2003) the use of extruded or toasted soybean supplementation compared to soybean meal clearly improved the SFA/(MUFA+PUFA) ratio in milk fat. In addition, the n-6/n-3 ratio decreased linearly from ES to TS and to SBM (5.77, 5.48 and 5.14, respectively).

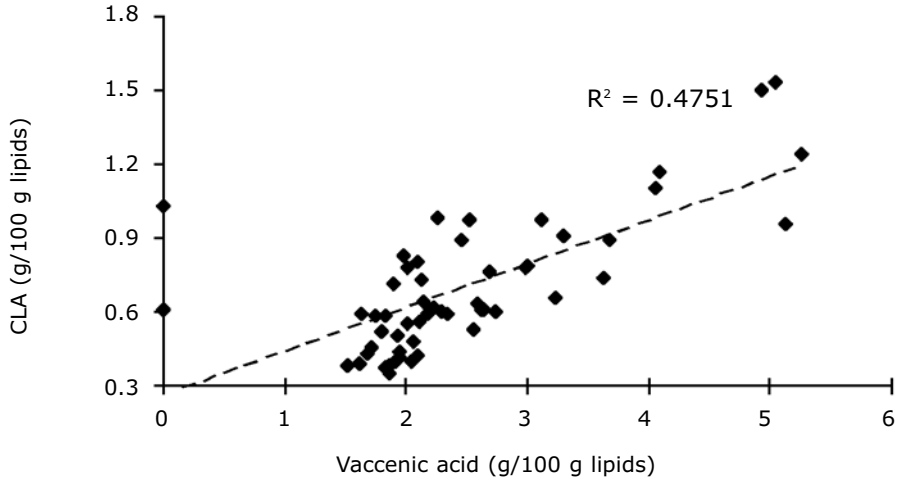
Conjugated linoleic acids (CLA)

The milk CLA content (Figure 5) was higher (*P<0.05*) in the ES, intermediate in the TS and lower in the SBM groups (0.91, 0.62 and 0.56 g/100 g lipids, respectively) in agreement with results obtained by Dhiman *et al.* (1999; 2000) and Solomon *et al.* (2000). However, CLA milk fat content increased throughout the trial in all experimental groups (Figure 5). Among the experimental groups, the average CLA content in milk fat was 0.623 g/100 g lipids, close to the value reported by Chouinard *et al.* (2001). According to the data reported by Solomon *et al.* (2000) there was a correlation between concentrations of vaccenic acid and CLA, reflecting the endogenous synthesis of CLA (Figure 6). However, this correlation was less pronounced as expected on the basis of Solomon *et al.* (2000) data. Perhaps, the lower R^2 of the regression equation we observed could be explained by the use of vaccenic acid (trans-11 C18:1) in replacement of the total concentration of trans C18:1 isomers.

Conclusions

In conclusion, ES and TS are confirmed to be interesting protein sources for dairy cows. They can replace a substantial part, if not completely, of the soybean meal in the diet without negative effects on milk yield, rumen fermentation and metabolic profile in dairy cows. The ES and TS can be used to modify the milk fat quality by increas-

Figure 6. Relationship between concentration of conjugated linoleic acid (CLA) and vaccenic acid in the milk fat of SBM, ES and TS groups.



ing the proportions of fatty acids with beneficial effects on human health. Moreover, when these seeds are home-produced they also make it possible to reduce the market dependence of the farm for protein supply, thereby minimizing the risks of cross contamination with GM products or other undesirable compounds. Thus, their use can be seen as a means to develop a traceability item in the milk production process at the farm level.

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