

# Supplementation with lipid sources in diets for Jersey cows in the first third of lactation

**Abstract** – The objective of this work was to evaluate the effect of the addition of whole-crude oilseeds of linseed, sunflower, and soybean to the diet of Jersey cows, in the first third of lactation, on the following characteristics: intake, apparent nutrient digestibility, milk production and its variables, energy-corrected milk, feed efficiency, energy balance, milk fatty acid profile, and blood metabolic profile. Eight multiparous Jersey cows were used in a double Latin square design. The treatments were the experimental diets with the oilseeds, and calcium salts of fatty acids were used as the control. Protein digestibility was lower for soybean, whereas that of ether extract was higher in the control, not differing between the evaluated oilseeds. There was no difference between oilseeds for milk production, milk production corrected for fat, milk nutritional content, feed efficiency, energy balance, and blood metabolic profile. The levels of milk monounsaturated fatty acids increased with the use of sunflower. Higher levels of cis-9, trans-11 conjugated linoleic acid (CLA) in milk were observed in the control. The addition of the evaluated whole-crude oilseeds to the diet of Jersey cows in the first third of lactation does not affect milk production variables, feed efficiency, energy balance, and blood biochemical profile, but alters the fatty acid profile.

**Index terms:** alternative feeding, bypass fat, energy balance, fatty acid, nutrient intake.

## Suplementação com fontes lipídicas em dietas para vacas Jersey no terço inicial de lactação

**Resumo** – O objetivo deste trabalho foi avaliar o efeito da adição de grãos integrais das oleaginosas linhaça, girassol e soja na dieta de vacas Jersey, no primeiro terço da lactação, sobre as seguintes características: consumo, digestibilidade aparente dos nutrientes, produção de leite e suas variáveis, leite com correção energética, eficiência alimentar, balanço energético, perfil de ácidos graxos do leite e perfil metabólico sanguíneo. Oito vacas Jersey múltiparas foram utilizadas em delineamento duplo quadrado latino. Os tratamentos foram as dietas experimentais com as oleaginosas, e sais de cálcio de ácidos graxos foram utilizados como o controle. A digestibilidade da proteína foi menor para a soja, enquanto a do extrato etéreo foi maior no controle, sem diferir entre as oleaginosas. Não houve diferença entre as oleaginosas para produção de leite, produção de leite corrigida para gordura, conteúdo nutricional do leite, eficiência alimentar, balanço energético e perfil metabólico sanguíneo. Os teores de ácidos graxos monoinsaturados no leite aumentaram com uso do girassol. Níveis mais altos de ácido linoleico conjugado cis-9 trans-11 no leite foram observados no controle. A adição das oleaginosas integrais avaliadas na dieta de vacas Jersey, no primeiro terço da lactação, não afeta as variáveis de produção de leite, a eficiência alimentar, o balanço energético e o perfil metabólico sanguíneo, mas altera o perfil de ácidos graxos.

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**Termos para indexação:** alimentos alternativos, fonte de gordura naturalmente protegida, balanço energético, ácidos graxos, consumo de nutrientes.

## Introduction

During the first third of lactation, the nutritional status of dairy cows is affected by a significantly reduced dry matter intake and negative energy balance, requiring high-energy diets that can cause a reduction in postpartum productive and reproductive performance, with an increased incidence of metabolic disorders and diseases, as well as changes in the milk fatty acid profile and, consequently, in the quality of dairy products (Diskin et al., 2016).

To raise the energy density of diets without negative effects, protected fat sources have been used in animal feeding to minimize metabolic challenges and improve the quality of dairy products (Palmquist & Jenkins, 2017), without extrapolating the limits of free fat supply in the rumen (Dang Van et al., 2020). Several fat sources are available, particularly as oils readily available to the rumen or as non-degradable rumen sources, which can be artificially or naturally protected, such as calcium salts of fatty acids and oilseeds, respectively (Wanderley et al., 2023).

When supplied as whole-crude grains, the oilseeds of cottonseed, linseed, sunflower, canola, and soybean are the main lipid sources that do not require any chemical or thermal treatments. This occurs because their hull acts as a physical barrier that limits the performance of the ruminal microbiota and the dissolution of the lipid content in the food bolus, reducing the effect of hydrolysis and biohydrogenation, which leads to a possible increase in the absorption of polyunsaturated fatty acids in the intestine and their incorporation into milk fat (Barletta et al., 2016).

Although the use of whole-crude oilseeds in cow diets has been previously evaluated (Schroeder et al., 2014; Kliem et al., 2017), few studies present factors linked to animal productive performance and milk fatty acid profile (Barletta et al., 2016; Meignan et al., 2017). Kliem et al. (2017), for example, found that supplementing dairy cow diets with whole-crude oilseed grains is an effective way of replacing saturated fatty acids with unsaturated fatty acids, whereas Loften et al. (2014) concluded that whole-crude oilseeds can have beneficial effects on milk composition, particularly regarding the palmitic (C16:0) and stearic (C18:0) saturated fatty acids.

Despite these results presented in the literature, lipid sources may also negatively affect feed intake, fiber digestion, ruminal function, milk fat content, and animal productivity in general (Hristov et al., 2013; Bionaz et al., 2020). However, in the initial third of lactation, these sources can provide substantial improvements in milk production without causing metabolic damage to the animals, whose energy demand is high, especially due to the aforementioned negative energy balance (Rennó et al., 2014).

The objective of this work was to evaluate the effect of the addition of whole-crude oilseeds of linseed, sunflower, and soybean to the diet of Jersey cows, in the first third of lactation, on the following characteristics: intake, apparent digestibility of nutrients, milk production and its variables, energy-corrected milk, feed efficiency, energy balance, milk fat acid profile, and blood metabolic profile.

## Materials and Methods

The experiment was carried out at the experimental farm of Estação Experimental Terras Baixas of Embrapa Clima Temperado, located in the municipality of Pelotas, in the state of Rio Grande do Sul, Brazil (31°52'20"S, 52°21'24"W, at an altitude of 21 m). The study was approved by the committee on animal research and experimentation of Universidade Federal de Pelotas (case number 6850).

Eight lactating multiparous Jersey cows, with 40±5 days in milk, 40±17 kg body weight, and 20±2.2 kg per day milk yield, were selected from a herd of approximately 80 animals, based on age, weight, and lactation order (between the second and fourth lactation).

The experimental design was a 4×4 double Latin square, consisting of four experimental diets and four sampling periods. The treatments were the experimental diets with whole-crude grains of linseed, sunflower, and soybean; Megalac-E calcium salts of fatty acids (Química Geral do Nordeste S/A, Nova Ponte, MG, Brazil) were used as the control. The experimental period totaled 17 days, of which 13 were for diet adaptation and 4 for sample collection. The cows were considered the experimental units and distributed in the square according to their previous milk yield and parity order.

All diets (Table 1) were formulated to reach the same levels of energy, crude protein, and neutral detergent fiber (NDF) according to the Nutrient requirements of dairy cattle published by National Research Council (NRC, 2001). Corn silage was used as roughage feed, provided twice a day and adjusted to 100 g per kilogram of refusals. The concentrate ingredients of the experimental diets, excluding Megalac-E and oilseed grains, were mixed prior to the beginning of the experiment. For the addition of whole-crude oilseeds, the content corresponding to each treatment was manually weighed every two days and mixed with the previously formulated concentrate. To ensure total intake, the mix with the whole-crude oilseed and the concentrate was provided to each animal three times a day.

Feed intake and refusals were measured and recorded weekly for the evaluation of dry matter mass intake (DMI) and apparent nutrient digestibility, as well as for the chemical compound analysis. For nutrient digestibility, fecal production was estimated using an external marker by supplying 5.0 g chromium (Cr<sub>2</sub>O<sub>3</sub>) twice a day after milking (Hopper et al., 1978). During the days of data collection, feces samples were obtained directly from the rectum or by voluntary evacuation, twice a day, before milking, and placed into plastic bags.

Feed, refusal, and feces samples were pre-dried in a 55°C forced-air oven for 72 hours and ground to pass through a 1.0 mm sieve of a Wiley-type mill. Dry matter, organic matter, and crude protein were determined using methods 967.03, 942.05, and 954.05, respectively, of Association of Official Analytical Chemists (AOAC) (Cuniff, 1996). Ether extract was obtained by the Filter Bags Technology (Ankom Technology, Macedon, NY, USA). Neutral detergent

fiber correct for ash (aNDF) and acid detergent fiber contents were determined according to Van Soest et al. (1991), whereas the acid detergent lignin content was estimated by method 973.18 of AOAC (Cuniff, 1998).

The energy content of the diet was calculated using the equations of NRC (2001) for digestible energy, metabolizable energy, and net energy of lactation (NEL); apparent digestibility was considered for energy

**Table 1.** Ingredients and chemical composition of the lipid sources offered to Jersey cows in the first third of lactation.

Ingredient (g kg <sup>-1</sup> DM)	Treatment <sup>(1)</sup>			
	CS	Linseed	Sunflower	Soybean
	Diet composition			
Corn silage	533.2	538.1	526.3	503.8
Concentrate	466.8	461.9	473.7	496.2
Calcium salts	29.7	-	-	-
Linseed	-	74.7	-	-
Sunflower	-	-	127.4	-
Soybean	-	-	-	145.3
Corn grain	144.2	124.4	127.1	151.6
Soybean meal	173.8	155.4	177.8	85.4
Wheat bran	96.3	75.2	9.3	82.0
Mineral-vitamin <sup>(2)</sup>	11.9	14.9	15.3	15.2
Limestone	1.8	8.0	7.5	7.5
Alcamix <sup>(3)</sup>	9.1	9.1	9.3	9.3
	Chemical composition			
Dry matter (g kg <sup>-1</sup> WM)	400.1	398.6	402.4	416.3
Organic matter (g kg <sup>-1</sup> DM)	931.8	929.1	932.1	931.3
Crude protein (g kg <sup>-1</sup> OM)	204.0	209.6	212.7	211.6
Ether extract (g kg <sup>-1</sup> DM)	61.1	62.9	63.6	62.3
aNDF (g kg <sup>-1</sup> DM)	333.5	333.4	333.3	333.5
NFC (g kg <sup>-1</sup> DM)	377.8	365.8	363.2	365.3
NEL (MJ kg <sup>-1</sup> OM)	8.89	8.91	8.81	8.78
C18:2n6c	24.98	12.93	7.71	52.88
SFA	44.46	12.66	6.43	19.76
MUFA	23.19	22.26	83.52	20.45
PUFA	32.35	65.08	10.05	59.79

<sup>(1)</sup>CS, Megalac-E calcium salts of fatty acids (Química Geral do Nordeste S/A, Nova Ponte, MG, Brazil); and Linseed, Sunflower, and Soybean, whole-crude grains. <sup>(2)</sup>Minimum composition per kilogram: 229 g Ca, 95 g P, 1.1 g Mg, 60 g Na, 12 g S, 120.000 UI vitamin A, 30.000 UI vitamin D<sub>3</sub>, 1,200 UI vitamin E, 20 g Se, 3.0 g Zn, and 1,000 mg lasalocid. <sup>(3)</sup>Composition: 349.4 g kg<sup>-1</sup> calcium carbonate, 160 g kg<sup>-1</sup> magnesium oxide, and 49 g kg<sup>-1</sup> sodium bicarbonate. DM, dry matter; WM, wet matter mass; OM, organic matter; aNDF, insoluble fiber in neutral detergent; NFC, non-fibrous carbohydrates; NEL, net energy of lactation; C18:2n6c, linoleic acid in proportion to total fatty acids; SFA, saturated fatty acid in proportion to total fatty acids; MUFA, monounsaturated fatty acid in proportion to total fatty acids; and PUFA, polyunsaturated fatty acid in proportion to total fatty acids.

calculations. Energy balance was also determined using NRC (2001) equations. Total fecal production (TFP) for the different treatments was estimated considering the concentrations of fecal chromium in dry matter mass (DM), using the following equation:

$$\text{TFP} = (\text{g Cr} / \text{day}) / (\text{g Cr} / \text{g fecal DM})$$

The cows were mechanically milked twice a day in a double 4×4 piped milking machine equipped with an automatic milk meter, with results expressed in kilogram of milk per milking, and with an automatic set extractor, with a 12-hour interval between morning (6:30 a.m.) and afternoon (6:30 p.m.) milking. Milk samples of two consecutive milking (morning and afternoon) of all experimental animals were collected on the sixteenth and seventeenth experimental days and mixed according to milk production. The samples were placed in tubes containing bronopol (2-bromo-2-nitro-1,3-propanediol), refrigerated, and sent to the laboratory within 24 hours for fat, protein, lactose, and total solids analyses.

To estimate the energy-corrected milk, milk production was adjusted for energy using the equation described by Sjaunja et al. (1990):

$$\text{Milk yield} = (\text{kg milk} \times (((383 \times \text{fat}\% + (242 \times \text{protein}\%)) + (165.4 \times \text{lactose}\%) + 20.7) / 3,140)$$

The milk variables fat, protein, lactose, and total solids were determined by infrared spectroscopy using method 972.16 of AOAC (Cuniff, 1996). Somatic cell count (SCC) was obtained through the following equation of Shook (1993):

$$\text{Somatic cell score} = [\log_2 (\text{SCC}/100)] + 3$$

The fatty acid profile was determined according to Simionato et al. (2010). The identification of fatty acid methyl esters (FAME) was performed by comparing the retention times of the sample constituents, using a mixture consisting of 37 Component FAME Mix (Supelco, Sigma-Aldrich, San Luisa, MO, USA) and trans-vaccenic acid methyl ester standard (18:1n7 t11) (46905U, trans1octadecenoic methyl ester), with a mixture of isomers of the rumenic acid methyl ester (18:2n7 c9, t11), a conjugated linolenic fatty acid (CLA); the CLA t-10, c-12-octadecadienoic acid (18:2n6 – t10, c12); and linoleic acid, a conjugated methyl ester (O5632).

Blood samples were taken from the fifteenth to seventeenth day of the experimental period,

immediately after morning milking, by venipuncture of the jugular vein. The samples were kept at rest for 10 min, centrifuged immediately after, at 7,871 RCF for 10 min, placed in an isothermal box, and sent for analysis at a commercial laboratory. The evaluated blood parameters were: glucose, triglycerides, and cholesterol, using the automated colorimetric enzymatic method; blood urea, through automated kinetic methods; non-esterified free fatty acid (NEFA), by the spectrophotometric enzyme assay; and gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST), with the automated kinetic enzymatic method.

The Glimmix procedure of the SAS software (SAS Institute Inc., Cary, NC, USA) was used, following the general linear mixed model method, with the choice of distribution that would best fit the data. This decision was made using the corrected Akaike value. The analysis of variance followed the mathematical model:

$$Y_{ijkl} = \mu + \alpha_i + a_j + p_k + s_l (\alpha \times p)_{ik} + \varepsilon_{ijkl}$$

where  $Y_{ijkl}$  is the observation concerning the  $i$ -th treatment ( $\alpha_i$ ) in the  $j$ -th animal ( $a_j$ ) of the  $k$ -th period ( $p_k$ ) in the  $l$ -th square ( $s_l$ ). Treatments were considered as the fixed effect, and periods and interactions with treatments, as random effects. Data were subjected to the analysis of variance, and the treatment effect was evaluated by the F-test, at 5% probability of error. When significant, means were compared by Tukey-Kramer's test, at 5% probability.

## Results and Discussion

Lipid supplementation had no effect on dry matter as nutrient intake (kilogram per day) or related to body weight (gram per kilogram of body weight). The treatments with oilseeds did not affect the apparent digestibility of dry matter, organic matter, and aNDF. Comparing treatments, crude protein digestibility was lower in the soybean diet due to a higher inclusion of protein from this grain, whereas fat content and ether extract digestibility were higher in the control, and the apparent digestibility of non-fibrous carbohydrates was higher in soybean and lower in the sunflower diet (Table 2). Dias Júnior et al. (2017) reported a similar result when comparing whole-crude and roasted soybean grains, suggesting that the way fat is arranged in whole-crude grains may be a limiting factor due to the presence of fibrous exosperms in the

grain or to the association of fat with the protein grain matrix, which can affect negatively both the access of ruminal microflora and the digestive enzymes in the small intestine (Miyaki et al., 2022). Although supplementation with vegetable oil in free form (rich in unsaturated fatty acids) could be a challenge to ruminal fermentation, the slower fat released by oilseeds reduces such disorders, without losses on nutrient digestibility (Palmquist & Jenkins, 2017), as confirmed in the present study.

The oilseed treatments also did not affect the intake of dry matter, organic matter, crude protein, ether extract, aNDF, and energy, which can be explained by the fact that the diets were formulated to be similar in protein, energy, and fiber levels, avoiding non-fiber carbohydrates to refrain acidosis in high-producing animals (Palmquist & Jenkins, 2017). Other authors, however, observed a decrease in the DMI, NDF, and crude protein intake of lactating cows due to the high energy input of an oilseed diet (Schroeder et al., 2013; Barletta et al., 2016; Prado et al., 2016).

Regarding the inclusion of fat in the form of calcium salts in the diet of dairy cattle, no negative effects were observed. However, in the literature, depressive effects

were reported on voluntary intake (Oyebade et al., 2020), associated with a low palatability, unsaturation, and shorter fatty acid chain length (Kliem et al., 2017).

Considering that no differences were observed in the digestibility of dry matter, organic matter, and aNDF, the lipid sources evaluated in the present work did not cause negative effects on the digestive process. However, Schroeder et al. (2014) found that processed linseed sources (rolled or ground) resulted in a better digestibility and ruminal fermentation than whole grains, mainly due to the amount of lipids and how they were offered (as free oil or seed). Here, the lack of significant differences can be explained by the fact that the fat from linseed, sunflower, and soybean was added at an intermediate level, lower than 35 g kg<sup>-1</sup> DMI, maintaining a low ruminal availability.

The used dietary fat from oilseed sources did not modify milk production, milk production corrected for fat, milk composition variables, feed efficiency, and energy balance (Table 3), in alignment with Schroeder et al. (2013), who evaluated whole-crude canola and sunflower seeds fed to Holstein cows in early lactation. Other authors, however, found that whole-crude oilseeds in diets can improve milk production

**Table 2.** Effect of lipid sources on voluntary intake and nutrient digestibility of Jersey cows in the first third of lactation<sup>(1)</sup>.

Variable <sup>(2)</sup>	Treatment <sup>(3)</sup>				SEM	p-value
	CS	Linseed	Sunflower	Soybean		
Voluntary intake						
Dry matter (kg per day)	16.15	16.08	15.74	15.81	0.45	0.8974
Dry matter (g kg <sup>-1</sup> BW)	40.20	40.09	39.09	39.36	1.48	0.9392
Organic matter (kg per day)	15.05	14.94	14.67	14.73	0.31	0.9159
Organic matter (g kg <sup>-1</sup> BW)	37.47	37.25	36.44	36.67	0.07	0.9488
aNDF (kg per day)	5.03	5.00	4.90	4.93	0.1875	0.9685
aNDF (g kg <sup>-1</sup> BW)	12.54	12.42	12.15	12.25	0.0461	0.9621
Crude protein (kg per day)	3.06	3.12	3.11	3.11	0.0440	0.8709
Ether extract (kg per day)	0.91	0.94	0.93	0.91	0.0166	0.6179
NFC (kg per day)	5.68	5.46	5.32	5.37	0.1012	0.4392
NEL (MJ per day)	150.63	149.59	148.31	147.82	2.37	0.7373
Digestibility (g kg <sup>-1</sup> )						
Dry matter	780.70	773.80	760.19	763.06	10.45	0.4185
Organic matter	793.38	785.84	770.43	774.32	10.05	0.3170
Protein	842.70a	837.74a	852.70a	806.98b	9.08	0.0014
Ether extract	791.64a	702.42ab	671.79b	663.09b	29.89	0.0053
aNDF	611.51	623.01	593.38	599.06	20.30	0.7347
NFC	914.21a	900.31ab	883.79b	916.15a	1.30	0.0435

<sup>(1)</sup>Means followed by equal letters do not differ by Tukey-Kramer's test, at 5% probability. <sup>(2)</sup>BW, body weight; aNDF, insoluble fiber in neutral detergent; NFC, non-fibrous carbohydrates; and NEL, net energy of lactation. <sup>(3)</sup>CS, Megalac-E calcium salts of fatty acids (Química Geral do Nordeste S/A, Nova Ponte, MG, Brazil); and Linseed, Sunflower, and Soybean, whole-crude grains. SEM, standard error of the mean.

corrected for 4% fat, incrementing milk fat (Schroeder et al., 2014; Dias Júnior et al., 2017; Oyebade et al., 2020).

These parameters did not differ in the present study despite the different sources of energy supplementation evaluated. This can be attributed to the fact that the diets were formulated to be isoproteic and isoenergetic, as well as to the similar nutritional composition of the used grains as to contents of dry matter, organic matter, crude protein, and ether extract, differing only for saturated fatty acid (SFA), monosaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) profiles in proportion to total fatty acids (Table 1).

Therefore, all evaluated oilseeds affected the fatty acid profile. In ascending order, the main fatty acids found in milk were: C16:0, oleic acid (C18:1n9c), and C18:0; CLA (C18:2c9t11) was superior in the control treatment (Table 4). Calcium salts only affected elaidic acid (C18:1n9t), C18:2c9t11, C18:2t10c12, vaccenic acid (C18:1n11t), and linoleic acid (C:182n6c).

In the milk fatty acid profile, C16:0 and arachidic acid (C:20:0) were lower in the sunflower and linseed diets, respectively, whereas C18:0 and C18:1n9c were higher in the sunflower diet (Table 4). There was no difference for SFAs and PUFAs, but MUFAs were

higher for sunflower. In addition,  $\alpha$ -linolenic acid (C18:3n3) content increased in the fatty acid profile when linseed was included in the diet.

Milk production variables did not differ significantly among the evaluated oilseeds (Table 3), which could be related to the fact that all diets were effective to input high energy densities. Moreover, the absence of changes in milk fat, lactose, and protein content is an indicative of the natural protection of whole grains, as well as of the uniformity of the diets, since these milk components usually vary due to dietary lipids or microbial growth, either because of the lack of fermentable carbohydrates or deficiency in protein intake (Johnson et al., 2002).

In the rumen, several factors influence biohydrogenation and can change the amount and composition of unsaturated fatty acids, either destined for deposition in the adipose tissue or for secretion in milk. According to Acosta Balcazar et al. (2022), biohydrogenation can occur completely or with the formation of intermediate products, such as C18:2c9t11, mostly absorbed in the small intestine and in the bloodstream, but also by the mammary gland, being incorporated into the milk. The frequency of this acid indicates that the desaturation of C18:1n11t occurred

**Table 3.** Effect of lipid sources on milk production variables and energy balance of Jersey cows in the first third of lactation.

Variable	Treatment <sup>(1)</sup>				SE	p-value
	CS	Linseed	Sunflower	Soybean		
Milk production (kg per day)	26.51	25.57	24.99	24.66	0.96	0.5616
Milk production corrected by fat <sup>(2)</sup>	26.93	26.39	25.55	25.67	0.63	0.5323
Feed efficiency (kg milk per kg DM)	1.67	1.65	1.63	1.63	0.05	0.8858
Energy balance (MJ per day)	20.82	21.83	19.67	16.44	4.45	0.7849
Milk urea nitrogen (g kg <sup>-1</sup> )	145.75	161.67	166.33	165.17	11.90	0.5612
Somatic cell count (1,000 mL <sup>-1</sup> )	101.75	58.81	149.62	36.31	32.54	0.0930
	Milk fat					
g kg <sup>-1</sup>	42.07	42.20	42.40	43.65	1.67	0.8962
kg per day	1.11	1.08	1.05	1.07	0.04	0.7023
	Milk protein					
g kg <sup>-1</sup>	32.35	33.81	32.88	32.72	0.09	0.5630
kg per day	0.86	0.86	0.82	0.81	0.03	0.5867
	Milk lactose					
g kg <sup>-1</sup>	47.72	47.86	47.67	48.10	0.03	0.7002
kg per day	1.26	1.224	1.19	1.18	0.05	0.5831
	Milk total solids					
g kg <sup>-1</sup>	131.44	134.01	132.53	134.20	1.87	0.6958
kg per day	3.47	3.42	3.30	3.30	0.11	0.5727

<sup>(1)</sup>CS, Megalac-E calcium salts of fatty acids (Química Geral do Nordeste S/A, Nova Ponte, MG, Brazil); and Linseed, Sunflower, and Soybean, whole-crude grains. <sup>(2)</sup>kg milk  $\times$  ((383 Fat% + 242 Protein% + 165.4 Lactose% + 20.7) / 3140) according to Sjaunja et al. (1990). SE, standard error; and DM, dry matter.

in the mammary gland due to the biohydrogenation process. Higher levels of rumenic acid (cis-9, trans-11 CLA, the predominant isomer of CLA in dairy products with Megalac-E) were found, as well as of cis-12, trans-10 CLA in isomerized form, despite its lower content (Table 4).

The highest levels of C18:1n11t in milk were observed in the control treatment, with no difference between the used whole-grain oilseeds (Table 4). Furthermore, higher levels of C18:0 (end product of biohydrogenation) were found in the sunflower treatment and the control. Both of these findings indicate that the biohydrogenation process was more efficient in calcium salt supplementation than in the evaluated whole grains. However, long-chain fatty acids (>17 carbon), taken by the mammary gland from the blood, were increased in the calcium salt and sunflower treatments.

A large portion of  $\alpha$ -linolenic acid is biohydrogenated in the rumen (Meignan et al., 2017), which is important to consider since the presence of this acid is desirable

in milk due to the potential benefits for human feeding related to the prevention of cardiovascular diseases (Turner et al., 2015). The content of this acid in the whole-grain oilseeds was higher in relation to that of the control, especially in linseed, showing that fat protection was efficient, which may be related to the presence of fibrous exosperms.

MUFA values were higher in the soybean diet (Table 4), representing 83.52% of the total fatty acids, which were only 20% in the other treatments. This high content of MUFA in the composition of soybean and in the final composition of milk in this diet is an indicative that, for the total efficiency of biohydrogenation, a balance is needed between the fatty acid compounds present in the feed. Contrastingly, Morsy et al. (2015) and Kliem et al. (2017) did not find that whole-grain sunflower was very effective in increasing MUFA or PUFA in milk composition.

The biochemical blood profile did not differ among treatments (Table 5), confirming the similarity of the diets and the potential of the lipid sources used. The

**Table 4.** Effect of lipid sources on the milk fatty acid profile of Jersey cows, in proportion to total fatty acids, in the first third of lactation<sup>(1)</sup>.

Fatty acid <sup>(2)</sup>	Treatment <sup>(3)</sup>				SE	p-value
	CS	Linseed	Sunflower	Soybean		
C16:0	296.22a	321.48a	262.66b	320.69a	12.7	0.0001
C16:1n7	9.18	10.36	10.71	10.28	0.65	0.3720
C18:0	171.46b	150.00b	200.61a	166.28b	7.19	0.0003
C18:1n9t	4.46a	2.50b	3.65a	2.59b	0.25	0.0001
C18:2c9t11	7.07a	3.43b	4.09b	3.15b	0.40	0.0001
C18:2t10c12	0.47a	0.33b	0.35b	0.30b	0.02	0.0022
C18:3n6	0.89	0.79	1.01	0.77	0.98	0.2515
C18:1n11t	33.46a	10.45b	11.87b	9.63b	2.13	0.0001
C18:1n9c	197.60b	209.85ab	258.78a	202.76b	14.55	0.0084
C18:2n6c	24.65a	20.57ab	15.27b	24.32a	2.16	0.0024
C18:3n3	2.42b	7.54a	1.99b	2.76b	0.87	0.0004
C20:4n6	1.27	1.28	1.17	1.18	0.13	0.9167
C20:0	2.03a	1.63b	1.94a	2.00a	0.08	0.0017
≤16 C <sup>(4)</sup>	519.55a	560.15a	468.21b	551.72a	22.52	0.0020
≥17 C <sup>(5)</sup>	215.50a	172.12b	223.18a	187.07b	7.50	0.0001
SFA	735.58	732.53	691.65	739.11	19.45	0.1297
MUFA	218.39b	228.79ab	277.42a	221.70b	16.54	0.0172
PUFA	44.69	42.13	29.84	39.62	4.47	0.0537
Unsaturated	263.02	271.13	308.35	261.46	20.92	0.2314

<sup>(1)</sup>Means followed by equal letters do not differ by Tukey-Kramer's test, at 5% probability. <sup>(2)</sup>C16:0, palmitic acid; C16:1n7, palmitoleic acid; C18:0, stearic acid; C18:1n9t, elaidic acid; C18:2c9t11, rumenic acid, a cis-9, trans-11 conjugated linolenic fatty acid (CLA); C18:2t10c12, CLA isomer; C18:3n6,  $\nu$ -linolenic acid; C18:1n11t, vaccenic acid; C18:1n9c, oleic acid; C18:2n6c, linoleic acid; C18:3n3,  $\alpha$ -linolenic acid; C20:4n6, araquidonic acid; C20:0, eicosanoic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; and PUFA, polyunsaturated fatty acid. <sup>(3)</sup>CS, Megalac-E calcium salts of fatty acids (Quimica Geral do Nordeste S/A, Nova Ponte, MG, Brazil); and Linseed, Sunflower, and Soybean, whole-crude grains. <sup>(4)</sup> $\Sigma$  Fatty acids with less than 16 carbons. <sup>(5)</sup> $\Sigma$  Fatty acids with more than 17 carbons. SE, standard error.

**Table 5.** Effect of lipid sources on blood parameters of Jersey cows.

Blood parameter <sup>(1)</sup>	Treatment <sup>(2)</sup>				SE	p-value
	CS	Linseed	Sunflower	Soybean		
Glucose (mg dL <sup>-1</sup> )	61.81	63.06	62.00	61.50	2.27	0.9658
Triglycerides (mg dL <sup>-1</sup> )	3.57	2.85	3.51	2.63	1.322	0.6732
Blood urea (mg dL <sup>-1</sup> )	36.17	38.987	43.06	38.87	2.371	0.3592
Cholesterol (mg dL <sup>-1</sup> )	184.83	170.68	160.40	173.22	7.911	0.3460
NEFA (mmol L <sup>-1</sup> )	0.27	0.28	0.35	0.29	0.047	0.3066
GGT (U L <sup>-1</sup> )	35.06	37.62	35.06	37.56	1.75	0.5671
AST	89.25	85.68	89.81	84.44	3.69	0.2287

<sup>(1)</sup>NEFA, non-esterified fatty acids; GGT, gamma-glutamyl transferase; and AST, aspartate aminotransferase. <sup>(2)</sup>CS, Megalac-E calcium salts of fatty acids (Química Geral do Nordeste S/A, Nova Ponte, MG, Brazil); and Linseed, Sunflower, and Soybean, whole-crude grains. SE, standard error.

values of 62.1 and 3.34 mg dL<sup>-1</sup> obtained for blood glucose and triglycerides were consistent with those described by Kaneko et al. (2008). However, the concentration of 39.3 mg dL<sup>-1</sup> blood urea was lower than that from 42.8 to 64.3 mg dL<sup>-1</sup> reported by the same authors. Conversely, Cozzi et al. (2011) concluded that the average blood urea for Holstein dairy cows should be around 27.62 mg dL<sup>-1</sup>, which is lower than that observed in the present study. Serum cholesterol, although not reflecting treatment differences, was higher than 120 mg dL<sup>-1</sup>, as also found by these authors. Bionaz et al. (2020) added that elevated cholesterol levels in diets with oil may be related to a greater need for the transportation of long-chain fatty acids.

NEFA, AST, and GGT levels did not differ between the treatments (Table 5). At the beginning of lactation, when the energy in the diet is not enough to meet the high demand of the cows, physiological mobilization occurs as a form of compensation, releasing NEFA, which can be used by the mammary gland and also in the liver (Hanuš et al., 2018). The NEFA value of 0.30 mmol L<sup>-1</sup>, also found by Kaneko et al. (2008), combined with energy balance data (Table 3), shows that there was no negative energy balance during the experiment due the effectiveness of the diets. The absence of effects on AST and GGT concentrations confirms that the used fat sources did not damage the liver. Prado et al. (2016) concluded that the fatty liver syndrome is one of the main problems of using oilseeds to increase the energy concentration in the diet of cows in early lactation, especially when the maximum recommendation of total dietary lipid content (6.0–8.0% of DM) is surpassed (NRC, 2001).

The findings of the present study show that the three used whole-crude grains, at the evaluated levels, can replace efficiently calcium salts of fatty acids as

a natural source of lipid supplementation for cows in early lactation, without damaging animal health and production, while promoting a greater flexibility in the choice of foods for formulating diets.

## Conclusions

1. Adding whole-crude grains of linseed, sunflower, and soybean to the diet of Jersey cows in the first third of lactation does not affect milk production variables, feed efficiency, energy balance, and blood biochemical profile, but alters the fatty acid profile.

2. The inclusion of whole-crude sunflower grains results in higher levels of monounsaturated fatty acids and oleic acid in milk, whereas the addition of whole-crude linseed increases  $\alpha$ -linolenic acid content.

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