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Sources of polyunsaturated fatty acids from oilseeds in dairy cows diets can alter yield of and fatty acid profile in milk

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Abstract

Our hypothesis was that different sources of unsaturated fatty acids from oilseeds (sunflower, soybeans, and cottonseed) could alter milk yield, fatty acid profile, and the amount of unsaturated fatty acids yielded in milk. It aimed to evaluate the effects of lipid sources on yield and composition and fatty acids in milk of primiparous Girolando cows. Five cows in lactation (35 ± 8 days), with 410 ± 7.15 kg BW were randomly distributed in a 5 x 5 Latin square. Five experimental diets, namely a control diet without an additional source of lipid and four diets with different lipid sources: cottonseed, sunflower seed, whole soybean, or soybean oil, as lipid source, to reach 70 g/kg of ethereal extract dry matter basis were used. Cottonseed and soybean oil addition reduced daily yield by 15 and 22%. Oilseed can alter fatty acid profile in milk. Whole soybean provided greater daily milk and fatty acids yields.

Keywords: biohydrogenation; daily intake; fatty acids; oilseeds.

Practical Application: This research contributes to investigate diets with different lipid sources capable of altering the fatty acid profile of primiparous milk cows.

1 Introduction

Milk has been part of the human diet since animal domestication about 13,000 years ago, being an important source of fats, proteins and minerals (Verruck et al., 2019). The composition of milk, mainly the fat content, varies according to the time of year and rearing system (Bai et al., 2022); breed, age, lactation stage and health of the animal (National Research Council, 2001; Smykov et al., 2021). However, diet and the way the food is processed may be the factors that most change the composition of milk (Costa et al., 2020; Yonjalli et al., 2020). Therefore, dietary intervention is a well-recognized approach to modifying milk production and changes in physicochemical characteristics (Verruck et al., 2019; Yonjalli et al., 2020).

The use of whole oilseeds on lactating cow nutrition has been reported in recent years (Gandra et al., 2016; Rico et al., 2017; Araújo et al., 2018; Muñoz et al., 2019). The use of these lipid sources can have effects on the yield and composition of milk and on the digestive process of cows. As they have natural protection, seeds can be inert in the rumen and therefore, have less digestibility of their nutrients. However, this protection can reduce the deleterious effects of polyunsaturated fatty acids on fiber degradability in diets with a high content of lipids (Palmquist & Mattos, 2011).

Changes in the fatty acid profile of milk have been reported by authors using different sources and lipids in the diet of lactating cows (Mourthé et al., 2015; Kliem et al., 2017; Bayat et al., 2018). However, Palmquist & Mattos (2011) stated that supplementation with readily available sources of unsaturated lipids has deleterious effects on methanogenic bacteria and ruminal protozoa and also increases the biohydrogenation process, reducing the amount of unsaturated fatty acids absorbed in the intestine. Cabiddu et al. (2017) reported disadvantages on lipid supplementation for ruminants, such as reduced daily dry matter intake and total dry matter digestibility, despite bettering milk composition.

The use of lipid sources from oilseeds can reduce deleterious effects on the rumen. Oilseeds have natural protection and can be inert on ruminal bacteria, not interfering with ruminal fermentation processes, especially fiber (Palmquist & Mattos, 2011). Miyaki et al. (2021) evaluated the influence of dietary oilseeds on physic-chemical and sensory parameters of the meat of Nellore steers and found inclusion of oilseeds modified the composition of fatty acids in the meat. Likewise, several studies have shown different benefits effects of adding different oilseeds to the diet of high-yielding lactating cows (Gandra et al., 2016; Rico et al., 2017; Araújo et al., 2018; Muñoz et al., 2019). However, more studies using cow mid-yielding of 19 kg/day of milk and

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11 kg/day for cows in the first lactation (Canaza-Cayo et al., 2018) as Girolando breed (5/8 Holstein × 3/8 Gir) are necessary.

The hypothesis was that different sources of polyunsaturated fatty acids from oilseeds (sunflower, soybeans, and cottonseed) could alter milk yield, fatty acid profile, and the amount of unsaturated fatty acids in the milk of primiparous crossbred cows. Thus, the aim of this study was to evaluate the effects of different additional lipid sources for lactating cows on the production and composition of milk and fatty acids in the milk of Girolando cows.

2 Material and methods

This study was carried out at School Farm of the Catholic University Dom Bosco and was approved by the Ethics Committee for the use of animals in experiments (protocol nº 011/2016).

2.1 Animals, experimental design and treatments

Five primiparous Girolando (5/8 Holstein × 3/8 Gir) cows were used, with an average of 410.0 ± 7.15 kg BW (average body weight; average standard error) in early lactation 35 ± 8 days (average days after calving; mean standard error), randomly distributed in a 5 x 5 Latin square. Feed was provided for *ad libitum* consumption in the form of mixed total feed and the cows were housed in individual pens with free access to water. The cows were treated with an antiparasitic 30 days before the beginning of the experiment and confirmed negative for mastitis.

The diets were balanced in order to meet the nutritional requirements of primiparous cows with an average production of 18 kg milk/day, being isoprotein and isoenergy (National Research Council, 2001). The treatments consisted of five experimental diets, including a control diet with 32 g/kg of lipids and four diets with 70 g/kg of lipids. Cottonseed, sunflower seed, whole soybean, and soybean oil were used to total mix ration formulated reach 70 g/kg of ethereal extract (Table 1).

2.2 Experimental procedures

The experiment lasted 105 days and was divided into five periods of 21 days each. Before the experimental period, fourteen days pre-experimental period was used to adapt to management and facilities, where the cows received the same diet containing 400 g/kg corn silage and 600 g/kg concentrate without lipid source (control). In the remaining five periods, the cows received the different treatments (diets) and the data related to the study were collected (Table 1). Food was provided twice a day at 6 a.m. and 4 p.m.

Two milking were performed each day, at 5 a.m. and 3 p.m. Milk collection was performed on four consecutive milking in the last three days of each experimental period. The samples for determining the milk components were stored at 4 °C with Bronopol-B2 until the moment of analysis and the samples destined for the determination of fatty acids were stored at −20 °C. To determine the fatty acid profile, the samples were frozen without preservative. Milk production was determined by weighing the milk in the last three days of each experimental period.

2.3 Intake and apparent digestibility

Nutrient intake was estimated as the difference between the amount of nutrient provided daily and the nutrients from the trough food leftovers. The apparent digestibility of the diet was measured as the difference between the amount of nutrient ingested and the amount of nutrient excreted in the feces. Two stool samples (± 500 g) per day were collected during the last three days of each experimental period. To estimate total faecal excretion, the internal marker, acid detergent fiber indigestible (ADFi), was used (Ítavo et al., 2002). Samples of food, leftovers and feces were placed in polypropylene bags and incubated in the rumen for 144 h. After the incubation period, the bags were removed and the ADFi content was evaluated.

Table 1. Ingredients and chemical composition of experimental diets (g/kg DM).

	Diets				
	Control	Soybean oil	Cottonseed	Sunflower	Whole Soybean
Ingredients					
Corn silage	400.0	400.0	400.0	400.0	400.0
Corn ground	383.0	406.7	264.5	209.4	316.0
Soybean meal	167.0	101.3	42.9	97.6	-
Urea extruded	20.0	20.0	20.0	20.0	20.0
Mineral mix[1]	30.0	30.0	30.0	30.0	30.0
Cottonseed	-	-	242.6	-	-
Sunflower seed	-	-	-	243.0	-
Whole Soybean	-	-	-	-	234.0
Soybean oil	-	42.0	-	-	-
Chemical composition					
OM	936	916	902	896	923
CP	180	180	180	180	180
EE	32	70	70	70	70
NDF	305	290	401	362	332
ADF	231	210	292	279	234
Lignin	29.28	28.52	44.25	35.96	31.31
TC	724	666	652	646	673
NFC	419	376	251	284	341
Fatty acids					
C14:0 - myristic acid	0.02	0.01	0.31	0.02	0.02
C16:0 - palmitic acid	3.46	7.49	13.48	5.53	7.81
C16:1 - palmitoleic acid	0.03	0.02	0.2	0.02	0.1
C18:0 - stearic acid	0.66	1.93	1.37	2.26	1.85
C18:1 - oleic acid	7.79	16.28	12.67	19.92	16.12
C18:2 - linoleic acid	17.88	37.97	39.92	40.11	38.74
C18:3 - linolenic acid	0.98	0.79	0.66	0.75	3.68
C:20 - arachidonic acid	0.05	0.05	0.05	0.05	0.06
C20:1 - gadoleic acid	0.04	2.53	0.04	0.04	0.05
C22 - behenic acid	0.04	0.04	0.04	0.52	0.05
Others	1.05	2.89	1.25	0.79	1.52

OM: organic matter; CP: crude protein; EE: ethereal extract; NDF: amylase-treated neutral detergent fibre; ADF: acid detergent fibre; TC: total carbohydrates; NFC: non-fibrous carbohydrates. [1]Levels per 1,000 g of product: Na: 100 g/kg; P: 88 g/kg; Ca: 188 g/kg; S: 22 g/kg; Mg: 8000 mg/kg; Zn: 3000 mg/kg; Cu: 1000 mg/kg; Co: 80 mg/kg; I: 60 mg/kg; Se: 20 mg/kg; F: 880 mg/kg.

2.4 Chemical analysis

The samples of food and feces were subjected to laboratory analysis to evaluate the contents of dry matter (DM), mineral matter (MM), crude protein (CP) and ethereal extract (EE) according to methods 930.15, 942.05, 976.05 and 920.39 (Association of Official Analytical Chemists, 2000). The neutral detergent fiber content (NDF) according to Mertens (2002) using alpha-amylase (Termamyl 120 L®) and acid detergent fiber (ADF) content by the method of Van Soest et al. (1991).

2.5 Analysis of milk components

The milk samples were analyzed at the Central Laboratory of the Paranaense Association of Holstein Cattle Breeders. The levels of protein, fat, lactose and solids were evaluated using the infrared method (International IDF Standard 141C; 2000). To analyze the composition of milk fatty acids, the lipids were esterified according to Hartman & Lago (1973). The fatty acid esters were analyzed by a Thermal Finnegan gas chromatograph, equipped with a fused silica capillary column (Supelco, Sigma-Aldrich) (100 m long x 0.25 mm inner diameter x 0.2 µm film thickness) and detector flame ionization (DIF). The column was heated to 35 °C for 2 min and increased by 10 °C per minute until a temperature of 150 °C was reached, remaining for 2 min. Then, it was increased by 2 °C per minute until reaching 200 °C, remaining for 2 min and again it was increased by 2 °C per minute until reaching 220 °C, remaining for 21 min, with a run total of 73.5 min. Nitrogen was used as the carrier gas at 0.9 mL/min. The volume of injected sample (split mode) was 1µL. The temperature used for the detector was 280 °C. The injections were performed in triplicate. The identification of fatty acids was performed by comparing the retention times of patterns of methyl esters of fatty acids (Sigma-Aldrich).

2.6 Calculations and statistical analysis

The daily intake of each nutrient was estimated as the difference between the nutrient supplied and the leftover nutrient in the diets (National Research Council, 2001). Faecal production was estimated by dividing the content of ingested ADFi (food – leftovers) by the ADFi contained in the feces. Apparent digestibility was determined by subtracting the total of each nutrient ingested from the total of the same nutrient excreted in the feces.

The total carbohydrate (TC) contents were determined by the equation: TC = {100 – [CP (%DM) + EE (%DM) + MM (%DM)]} and the total digestible nutrients (TDN) calculated from the equation: TDN (g/day) = {(CP ingested – CP feces) + (TC ingested – TC feces) + [2.25 * (EE ingested – EE feces)]}}, proposed by Sniffen et al. (1992). Non-fibrous carbohydrates (NFC) were estimated according to the equation proposed by Hall (2000), where NFC = {100 – [[CP (%DM) – % urea derived + % urea] + NDF (%DM) + EE (%DM) + MM (%DM)]}.

The daily milk yield was calculated by adding the individual production to each milking, with an average of the last three days of each experimental period. Correction of milk to 4% fat was performed using the equation of National Research Council (2001) (Equation 1):

$$FCM = 0.4 \times milk\ yield + 15 \times Fat/100 \times milk\ yield \quad (1)$$

Where: FCM = milk yield corrected to 4% fat in kg/d; milk yield = milk yield in kg/d; Fat = percentage of milk fat.

2.7 Statistical analysis

For statistical analysis, the data were processed using the Exp.Des.pt package of the R software (R Development Core Team, 2018). The Shapiro-Wilk test was performed to determine the normality of the data and the Bartlett test to determine homogeneity. After the assumptions were met, an analysis of variance was performed in a 5 x 5 Latin square. The means were compared by Tukey test with 5% significance according to the mathematical model Yijk = μ + Ci + Pj + Tk (i, j) + eijk, where: Yijk = dependent variable value; μ = overall average; Ci = cow effect; Pj = period effect; Tk (i, j) = effect of treatment within each cow and period; eijk = experimental error.

3 Results

The daily dry matter intake (DMI) was not influenced by the lipid content of the diet (P = 0.070), with the diet containing cottonseed reducing the DMI. This reduction was also observed in the intake as a percentage of live weight and in g/kg of metabolic weight. The daily intake of organic matter (OMI) was higher in cows that received sunflower and whole soybean. The supply of the whole soybean in the diet promoted a higher daily intake of crude protein (CPI). The daily intake of ethereal extract (EEI) was higher when the diets lipid-rich. The use of soybean oil as a lipid source promoted greater EE intake than the other sources. The daily intake of NDF (NDFI) was lower for cows receiving diets with soybean oil. The diet lipid-rich (70 g/kg EE) promoted an increase in daily intake of ADF (ADFI); however, the use of soybean oil in the diet reduced the ADF intake compared to the use of grains. The daily intake of total carbohydrates (TCI) and non-fibrous carbohydrates (NFC) were higher in cows receiving the control diet. TDN intake was higher in cows receiving sunflower grain and whole soybean (Table 2).

Apparent dry matter digestibility (DMD) was higher in cows consuming the control diet. The same behavior was observed in relation to the apparent organic matter digestibility (OMD), where the control diet was superior to diets lipid-rich. The supply of sunflower grain resulted in a higher CP digestibility (CPD) than cottonseed and whole soybean. The use of a readily available lipid source with soybean oil resulted in a higher EE digestibility than the use of grains. Cottonseed reduced the NDF digestibility in comparison to other treatments.

The control diet showed a higher apparent digestibility of total carbohydrates (TCD). Cows consuming soybean oil showed higher TCD than cows fed oilseeds. Diets lipid-rich reduced the NFC digestibility. The supply of cottonseed reduced the TDN of the diet; consequently, there was a reduction in digestible energy, metabolizable energy, and net energy of lactation (Table 2).

There was an effect of treatment on milk yield, milk yield corrected to 4% fat (FCM), and yield efficiency (YE). Cows fed the whole soybean were higher averages than other oilseeds and control treatments. Cows fed soybean oil had a decrease in the fat content of milk. The use of diet lipid-rich did not alter milk protein content, lactose, or total solids. The use of soybean oil in the diet decreased the total solids in milk (Table 3).

There was an effect of treatment on saturated acids: C6:0, C12:0, C14:0, C15:0, C16:0, C17:0, C22:0, and C24:0. Likewise, there was an effect of treatment on unsaturated acids: C18:1, C18:2, C18:3. Cows fed control treatment (without lipid source) presented higher averages of C6:0, C16:0, C18:0, and C24:0 content in milk. Cows fed cottonseed and sunflower presented higher averages of C18:1 and C18:2, and C22:0. There was an effect on the sum of the percentage of fatty acids. The milk of cows fed cottonseed and sunflower had the lower averages of the sum of the fatty acids with 4 to 16 carbons, and higher averages of the sum of C17 - C24 fatty acids (Table 4).

The production of milk fatty acids (g/day) according to the size of the carbon chain or saturation was altered by the lipid content of the diet. Among the sources used to reach 70 g/kg of lipids, the soybean oil reduced and whole soybean increased the production of short-chain, medium-chain, long-chain, saturated, monounsaturated and polyunsaturated fatty acids (Table 5).

Table 2. Nutrient intake (kg/day) and digestibility (g/100 g) of nutrients in crossbred cows fed different lipid sources.

	Diets					CV	P-value
	Control	Soybean oil	Cottonseed	Sunflower	Whole Soybean		
Nutrient intake							
DM (kg/day)	13.95a	13.02a	11.98b	13.55a	13.51a	7.08	0.042
DM (% BW)	3.39a	3.30a	2.94b	3.42a	3.21a	9.04	0.029
DM (g/kg MW)	152.68a	148.69a	132.09b	152.37a	145.24a	8.21	0.003
OM (kg/day)	13.32a	12.11a	11.01b	12.78a	12.85a	8.55	0.039
CP (kg/day)	1.96b	1.85b	1.79b	1.92b	2.39a	11.63	0.011
EE (kg/day)	0.46c	0.93a	0.72b	0.86a	0.77b	13.62	< 0.001
NDF (kg/day)	5.61ab	5.29b	5.75ab	6.26a	5.96ab	5.95	0.009
ADF (kg/day)	3.40bc	3.21c	3.89a	4.06a	3.78ab	5.91	< 0.001
TC (kg/day)	10.90a	9.33b	8.92c	10.00b	9.69b	7.96	0.005
NFC (kg/day)	5.29a	4.04b	3.17c	3.74b	3.73b	12.96	< 0.001
TDN (kg/day)	10.59a	10.11a	7.36b	9.82a	9.90a	13.07	0.012
NEL (MCal/kg)	25.93a	24.78a	18.02b	24.06a	24.25a	13.07	0.012
Apparent digestibility							
DM (%)	74.93a	72.01a	57.08b	70.52a	68.59a	6.63	< 0.001
OM (%)	76.95a	74.47a	60.07b	72.47a	71.04a	6.13	< 0.001
CP (%)	68.64ab	71.62ab	62.05b	77.20a	69.72ab	8.11	0.029
EE (%)	84.25b	90.95a	74.44d	77.86cd	81.13bc	2.50	< 0.001
NDF (%)	67.59a	63.39a	50.93b	62.91a	59.94ab	9.27	0.007
ADF (%)	51.91	49.51	34.66	47.04	46.20	23.89	0.186
TC (%)	76.71a	72.69a	56.27b	68.19a	70.25a	7.03	< 0.001
NFC (%)	87.79a	85.61a	68.00b	78.80a	87.60a	8.81	0.004
TDN (%)	75.89a	73.57a	61.19b	72.34a	73.07a	9.86	0.046
DE (MCal/kg)	3.35a	3.24a	2.70b	3.19a	3.22a	9.87	0.046
ME (MCal/kg)	2.93a	2.83a	2.27b	2.77a	2.80a	11.17	0.045
NEL (MCal/kg)	1.86a	1.80a	1.50b	1.77a	1.79a	9.85	0.047

DM: Dry matter. OM: organic matter. CP: crude protein. EE: ethereal extract. NDF: amylase-treated neutral detergent fibre. ADF: acid detergent fibre. TC: total carbohydrates. NFC: non-fibrous carbohydrates. BW: body weight. MW: metabolic weight. DE: digestible energy. ME: metabolisable energy. NEL: net energy of lactation. CV: Coefficient of variation. [a, b] Means followed by different letters differ according to Tukey test ($P \leq 0.05$).

Table 3. Milk yield and composition from crossbred cows fed different lipid sources.

	Diets					CV	P-value
	Control	Soybean oil	Cottonseed	Sunflower	Whole Soybean		
Milk yield (kg/day)	13.3b	11.5c	11.4c	13.4b	14.6a	11.86	0.029
FCM (kg/day)	10.3b	8.0d	9.0c	10.7b	11.9a	17.02	0.037
YE	0.73bc	0.64c	0.75bc	0.80b	0.89a	20.86	0.042
Fat (g/kg)	24.6a	19.6b	25.4a	26.5a	27.6a	16.27	0.021
Protein (g/kg)	32.4	30.2	31.1	33.5	31.2	5.89	0.104
Lactose (g/kg)	47.0	47.2	45.3	46.4	47.9	4.91	0.501
TS (g/kg)	114.0a	107.0b	113.0a	117.0a	117.0a	4.18	0.036

Milk yield: milk yield (kg/day); FCM: milk corrected for 4% fat (kg/day); YE: yield efficiency; TS: total solids. [a, b] Means followed by different letters differ according to Tukey test ($P \leq 0.05$)

Table 4. Fatty acid profile of milk from crossbred cows fed different lipid sources (g/100 g).

	Diets					CV	P-value
	Control	Soybean oil	Cottonseed	Sunflower	Whole Soybean		
C4:0	1.58	1.59	1.59	1.57	1.58	1.35	0.111
C6:0	1.70a	1.68ab	1.66b	1.67ab	1.68ab	1.67	0.015
C8:0	2.93	2.96	2.84	2.92	2.89	3.02	0.140
C10:0	6.61	6.61	6.78	6.74	6.69	2.81	0.682
C12:0	4.18b	4.25a	4.20b	4.23a	4.19b	0.62	< 0.001
C14:0	10.16b	10.77ab	11.04a	10.78ab	10.47b	2.21	0.001
C14:1	0.050	0.050	0.053	0.050	0.053	4.90	0.084
C15:0	1.41c	1.47a	1.51a	1.50b	1.44c	2.28	< 0.001
C15:1	0.19	0.20	0.20	0.19	0.19	4.26	0.288
C16:0	28.79a	28.42a	27.04b	27.25b	28.72a	3.76	0.012
C16:1	0.96	0.94	0.96	0.99	0.93	2.89	0.446
C17:0	0.16ab	0.17ab	0.19a	0.19ab	0.16b	5.04	0.024
C17:1	0.45	0.46	0.43	0.42	0.46	6.51	0.163
C18:0	14.55a	14.27c	14.30c	14.20c	14.42b	0.52	< 0.001
trans-11 C18:1	7.11b	7.02b	7.27a	7.33a	7.04b	3.46	0.013
cis-9 C18:1	13.10b	13.12b	13.66a	13.50a	13.11b	2.69	0.047
C18:2 ω6	1.57b	1.59b	1.88a	1.99a	1.58b	14.92	0.019
cis-9. trans-11 C18:2	0.87a	0.85ab	0.86a	0.85b	0.86ab	1.67	0.002
C18:3 ω 3	1.61b	1.57b	1.57b	1.65a	1.58b	2.63	0.040
C20:0	0.91	0.94	0.89	0.95	0.91	4.77	0.215
C22:0	0.84b	0.86ab	0.87a	0.87a	0.83b	2.50	0.021
C24:0	0.24a	0.22ab	0.20ab	0.18b	0.21ab	12.15	< 0.001
Saturated	74.05a	74.20a	73.12b	73.03b	74.20a	1.18	0.048
Monounsaturated	21.89b	21.78b	22.58a	22.49a	21.79b	2.77	0.044
Polyunsaturated	4.05c	4.01c	4.31b	4.48a	4.01c	6.71	0.015
S:U ratio	2.85ab	2.88a	2.72b	2.72b	2.88a	4.15	0.040
Short chain	3.28	3.26	3.25	3.23	3.26	1.36	0.140
Medium chain	13.72	13.82	13.82	13.90	13.77	1.20	0.920
Long chain	83.00	82.92	82.93	82.87	82.97	0.23	0.998
Σ C4 – C16	58.56a	58.93a	57.89b	57.88b	58.83a	1.36	< 0.001
Σ C17 – C24	41.44b	41.07b	42.11a	42.12a	41.17b	1.93	< 0.001

Σ C4 – C16: Sum of the percentages of fatty acids with chains of 4-16 carbons; Σ C17 – C24: Sum of the percentages of fatty acids with U ratio – saturated: unsaturated ratio. [a, b] Means followed by different letters differ according to Tukey test ($P \leq 0.05$)

Table 5. Production of fatty acids (g/day) from the milk of crossbred cows fed different lipid sources.

	Diets					CV	P-value
	Control	Soybean oil	Cottonseed	Sunflower	Whole Soybean		
Short chain (g/day)	10.66c	7.34d	9.65c	11.63b	12.79a	25.52	0.040
Medium chain (g/day)	45.63c	31.26e	40.37d	49.26b	53.92a	25.45	0.041
Long chain (g/day)	274.22c	187.51e	244.34d	295.47b	324.88a	25.92	0.047
Saturated (g/day)	242.49c	165.07e	218.12d	264.33b	290.65a	26.24	0.043
Monounsaturated (g/day)	73.52b	51.20d	64.31c	77.73b	85.21a	25.13	0.048
Polyunsaturated (g/day)	14.50b	9.83c	11.92c	14.30b	15.72a	24.17	0.041

[a, b] Means followed by different letters differ according to Tukey test ($P \leq 0.05$)

4 Discussion

Except from the diet containing cottonseed, the addition of lipid sources did not alter voluntary intake, suggesting there was no connection between the lipid content of the diet and food intake using lipid content up to 70 g/kg DM. It is likely that the fiber content of the diet was a limiting factor for consumption since the diet containing cottonseed had a higher NDF and ADF than the others, as well as a lower NFC content. Van Soest (1994) indicated fiber as a limiting component of intake because the NDF and FDA are fractions of slow ruminal degradability, thus remaining inside the digestive tract for a longer time, causing physical filling and consequently, reducing intake.

The highest CP intake was observed in the diet containing whole soybean. Because diets were isoprotein and isoenergy, so higher consumption of CP observed in the diet containing whole soybean can be associated with higher DM intake. Almeida et al. (2017) observed greater CP intake in diets with whole soybean and a control diet compared to a diet containing cottonseed, and attributed this increase to a reduction in DM intake of the diet containing cottonseed.

The reduction in TC intake in diets lipid-rich was closely associated with lower DM intake in the cottonseed diet and was also responsible for the reduction in TC intake since the other lipid sources showed similar values to the control diet. Among the foods supplied, the concentrate was the one with the highest NFC content, and its participation was lower in diets with grains as a source of lipids, which explains the reduction in the consumption of this nutrient. According to Cabiddu et al. (2017) the reduction in the NFC intake in animals receiving lipid sources may also be related to lower food intake.

The apparent digestibility of DM, OM and CP in the diets was reduced with the use of cottonseed as a lipid source. The higher fiber content of this grain led to an increase in the ADF intake, a component with less digestibility in the diet, resulting in less apparent digestibility. The apparent digestibility of EE was higher in the diet containing soybean oil. The greater amount ingested and the greater availability of EE in this source of lipid resulted in this increase, since, in the control diet, EE was exclusively from concentrate and silage, and in diets containing grains, it was protected.

There was a reduction in the apparent digestibility of NDF in the diet with cottonseed as an additional lipid source, as well as in the digestibility of TC and NFC. The higher NDF and ADF content in this diet were probably limiting to the nutrient digestibility. Rico et al. (2017) also reported lower nutrient digestibility in diets containing cottonseed compared to diets without grain inclusion. Freitas et al. (2018) did not observe a reduction in the digestibility of diets with grains or soybean oil, however, the consumption of DM, NDF, ADF and TC was not influenced by the diet, which reinforces the theory that the reduction of digestibility occurs due to the higher consumption of fibrous fractions of the diet.

The reduction in apparent digestibility of most nutrients in the diet also resulted in a lower TDN in the diet containing cottonseed, demonstrating that, probably due to its more fibrous constitution, cottonseed limits the amount of energy available to cows in lactation.

The results for milk production, fatty acid profile and yield were associated with the intake of nutrients and their utilization. It was possible to observe that the diet containing cottonseed reduced the use of nutrients when compared, mainly, with the control diet. The amount of nutrients used by cows consuming this diet was approximately 56% less than the control diet in NFC and 35% less in DM. This reduction resulted in a lower amount of net energy of lactation (NEL), which explains the lower production. The other lipid sources showed values similar to the control diet for these parameters, which also explains the similarity in the production of milk and its fatty acids.

The inclusion of lipid sources in the diet of lactating cows did alter daily milk production. The highest production was observed in cows consuming whole soybean and the lowest in cows that received cottonseed in the diet. Although the DM intake of cows consuming whole soybean was not the highest observed, the consumption of CP was higher than the other diets, which, associated with a higher DTC, may explain the greater milk production. Naves et al. (2016) reported similar values in the milk production of cows consuming whole soybean and a diet without added lipids.

The average milk production of 12.82 kg/day observed was lower than expected by the diet formulation (18 kg/day), however, data published by Alvim et al. (2019) demonstrated that the average production of primiparous Girolando (5/8 Holstein × 3/8 Gir) cows were 9.57 kg/day lower than observed in this study. The National Research Council (2001) indicates that factors such as basal diet, lactation stage, and energy balance, type of lipid source, and level of inclusion of these sources in the diet can influence the response of milk production to lipid supplementation. The diet containing soybean oil showed lower milk yield because of the lower fat content observed in the milk of cows consuming this diet. The effects of the addition of lipids on the fat content of milk are quite varied and may vary according to the source of lipid used, the saturation of the lipids supplied, the basal diet provided, and the type of processing of these lipid sources.

The lower amount of NDF in the diet containing soybean oil or the fact that the lipids supplied in the form of oil are not protected can be pointed out as causes of the reduction in the fat content of the cows receiving soybean oil. The reduction in the NDF intake can reduce the production of acetate in the rumen, leading to a drop in the fat content of milk (Bauman & Griinari, 2003). Short-chain fatty acids during rumen fermentation are produced, and transported to the mammary glands through the bloodstream, where are absorbed and used as precursors in the lipid synthesis. Approximately, 17-45% of milk fat originates from acetate and 8-25% from butyrate (Santos & Fonseca, 2007). Milk fat is the main component of total solids, so the reduction in fat content resulted in a lower percentage of total solids in the milk of cows consuming soybean oil. Murta et al. (2016) reported a reduction in total milk content solids using soybean oil in the diet of lactating cows. Oliveira et al. (2007) in a study testing high and low level of concentrate and lipid, reported that the addition of lipids reduced both fat and total milk solids.

There was a change in the some short, medium and long-chain fatty acids content, it was possible to observe that the general sum for each chain size was affected by the diet offered. In addition, it was possible to verify a reduction of fatty acids from the new synthesis that occurred in the diet containing cottonseed. The theory of intermediate fatty acids from biohydrogenation seems to be the one that best explains the results, since the milk of cows consuming cottonseed showed a lower proportion of fatty acids from lipid synthesis and a higher concentration of oleic and linoleic fatty acids. Griinari et al. (1998) cited that supplementation with polyunsaturated fatty acids in diets with low forage content reduces lipid synthesis in lactating cows.

As in our study, this reduction is associated with a higher proportion of oleic and linoleic acids, demonstrating the deleterious effect of these fatty acids on lipid synthesis. Costa et al. (2018) reported a linear reduction of fatty acids from lipid synthesis in an experiment testing levels of inclusion of 0-24% of cottonseed in the diet, however, contrary to the current study, there was no increase in the proportion of oleic acid and linoleic.

Changes in the percentages of stearic, oleic, linoleic, and linolenic fatty acids varied according to the lipid source used. The lipid sources used were able to increase the content of all these fatty acids in milk, demonstrating that there is a difference in the lipid biohydrogenation of each of them. According to Rabiee et al. (2012) the extent of biohydrogenation from different sources and lipids is quite heterogeneous. Although biohydrogenation can be high, reaching 90%, the characteristics of the lipid source, the retention time of this source in the rumen and the characteristics of the ruminal microbial population can interfere with the amount of lipid to be biohydrogenated. Generally, animals that receive oilseed sources in their diets have higher concentrations of unsaturated fatty acids in their milk, especially polyunsaturated fatty acids and total unsaturated fatty acids (Yonjalli et al., 2020).

Sunflower and Cottonseed were able to significantly increase the content of 18-carbon fatty acids in the milk. The source of unprotected lipid (soybean oil) did not cause changes in the profile of these fatty acids compared to the control diet. The lipid sources used do appear to have undergone ruminal biohydrogenation or this biohydrogenation may have occurred in small quantities.

Amounts of milk fatty acids (g/kg) were altered by the inclusion of lipid sources in the diet. The diets with the inclusion of lipid sources did differ from the control diet. However, a reduction in the amounts in the diet containing soybean oil in comparison to the diets containing whole soybeans and sunflower grain occurred in most cases. This change was associated with a reduction in the fat content of milk presented by the soybean oil diet.

5 Conclusion

Whole soybean in the diet of lactating primiparous crossbred cows is recommended. Soybean oil decreases the percentage of saturated and unsaturated fatty acids in the milk. Oilseeds provide greater production in kg/day of these fatty acids and can alter milk production and fatty acids profile in milk of Girolando cows.

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