DOI: 10.5433/1679-0359.2024v45n1p71

Milk fatty acid profile of cows grazing elephant grass BRS Kurumi pasture with and without energy supplementation

Perfil de ácidos graxos do leite de vacas em pastagem de capim-elefante BRS Kurumi com e sem suplementação energética

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Highlights

Cows exclusively grazing BRS Kurumi consume 26% more α-linolenic acid.
Cows exclusively grazing BRS Kurumi produce milk containing 21% more rumenic acid.
Unsupplemented cows produce milk with 7% less pro-atherogenic fatty acids.
Milk from unsupplemented cows has lower atherogenicity and thrombogenicity indices.

Abstract

This study evaluated the effects of energy supplementation on the intake and milk fatty acid composition of cows grazing BRS Kurumi elephant grass pasture during the rainy season. Two treatments (with and without supplementation) were evaluated using a switchback design with six Holstein × Gyr dairy cows after the peak of lactation. The average milk yield, body weight, and days in milk of the cows at the beginning of the study were 18.0±2.89 kg day^{-1}, 560±66 kg, and 99±12, respectively. The evaluations were performed over three grazing cycles, with adaptation periods of 14 days and six days of sampling. In the energy supplementation treatment, each cow received 3 kg day^{-1} of ground corn (as-fed basis), with 2 kg day^{-1} at the morning milking and 1 kg day^{-1} at the afternoon milking. The ground corn presented 87.5% dry matter, 7.3% crude protein, 5.1% ether extract, and 85% of total digestible nutrients. The cows supplemented with ground corn consumed more oleic (+567%) and linoleic (+88%) acids. Unsupplemented cows consumed 26% more α-linolenic acid and produced milk with more oleic (+10%), vaccenic (+23%), and rumenic (+21%) acids, and less (−7%) pro-atherogenic fatty acids (lauric

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Received: Sept. 21, 2023 Approved: Feb. 02, 2023

+ myristic + palmitic acids). Milk fat from unsupplemented cows showed better nutritional quality, with lower atherogenicity and thrombogenicity indices and a higher hypo/hypercholesterolemic fatty acid ratio.

Key words: *Cenchrus purpureus.* Conjugated linoleic acid. *Pennisetum purpureum.* Rumenic acid.

Resumo

Este estudo avaliou os efeitos da suplementação energética sobre o consumo e a composição de ácidos graxos do leite de vacas em pastagem de capim-elefante BRS Kurumi durante o período chuvoso. Dois tratamentos (com e sem suplementação) foram avaliados em delineamento de reversão completa (*switchback*), com seis vacas Holandês × Gir após o pico da lactação. A produção média de leite, o peso corporal e dias em lactação das vacas no início do estudo foram de 18,0±2,89 kg dia^{-1}, 560±66 kg e 99±12, respectivamente. As avaliações foram realizadas durante três ciclos de pastejo, com períodos de adaptação de 14 dias, e seis dias de coletas de amostras. No tratamento com suplementação energética, cada vaca recebeu 3 kg dia^{-1} de milho moído (base da matéria natural), sendo 2 kg dia^{-1} na ordenha da manhã e 1 kg dia^{-1} na ordenha da tarde. O milho moído apresentou 87,5% de matéria seca, 7,3% de proteína bruta, 5,1% de extrato etéreo e 85% de nutrientes digestíveis totais. Maiores consumos dos ácidos oleico (+567%) e linoleico (+88%) foram observados nas vacas suplementadas com milho moído. As vacas não suplementadas consumiram 26% a mais de ácido α-linolênico e produziram leite com maiores teores dos ácidos oleico (+10%), vacênico (+23%) e rumênico (+21%), e menor teor (−7%) de ácidos graxos pró-aterogênicos (ácidos láurico + mirístico + palmítico). A gordura do leite das vacas não suplementadas apresentou melhor qualidade nutricional, com menores índices de aterogenicidade e trombogenicidade e maior relação de ácidos graxos hipo/hipercolesterolêmicos.

Palavras-chave: Ácido linoleico conjugado. Ácido rumênico. *Cenchrus purpureus. Pennisetum purpureum.*

Introduction

Changes in consumption habits, motivated by a growing appreciation for a healthy and sustainable lifestyle, have fostered the search for foods that promote health and well-being (R. A. Rego, 2017). Hence, consumers of milk and dairy products are increasingly interested in products naturally enriched (biofortified) with bioactive compounds that positively affect human health and prevent disease (Alves et al., 2017), as well as in products of pasture-based systems. Grazing is perceived as more natural than feedlot systems, where diets are based mainly on roughage preserved as silage and hay (Elgersma, 2015; Rivero & Lee, 2022). In addition, products originating from pasture-based production systems are also perceived by consumers as positively associated with animal freedom, health and welfare, and environmental sustainability (Conner & Oppenheim, 2008; Joubran et al., 2021; Rivero & Lee, 2022).

Among the many physiologically bioactive compounds present in milk, we highlight rumenic acid (*cis*-9, *trans*-11 CLA), the main isomer of CLA (conjugated linoleic acid) in ruminant milk fat. This fatty acid (FA) presents anticarcinogenic, antidiabetogenic (type 2 diabetes), anti-atherogenic, and immunomodulatory properties (Yang et al., 2015; Alves et al., 2017). In addition, cows under grazing produce milk fat with higher levels of rumenic acid, as well as other FAs beneficial to human health, such as oleic acid (*cis*-9 C18:1) and α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3) compared to those fed silage-based diets (O. A. Rego et al., 2016; Frétin et al., 2019).

α-Linolenic acid is the main FA in forage and the most useful for enhancing the nutritional quality of milk fat (Glasser et al., 2013). Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.)] pastures contain high levels of α-linolenic acid (Batistel et al., 2017; Dias et al., 2017), which is the main substrate for vaccenic acid (*trans*-11 C18:1) production by ruminal microorganisms, together with linoleic acid (*cis*-9, *cis*-12 C18:2). Vaccenic acid is the precursor for the synthesis in the mammary gland of 86.8% (±2.8) of the rumenic acid secreted in bovine milk (Prado et al., 2019).

In 2014, as part of an elephant grass breeding program started in 1991 to address the development of improved cultivars for use in feeding ruminants, Embrapa Dairy Cattle (Juiz de Fora, MG, Brazil) launched the BRS Kurumi cultivar (Pereira et al., 2021), which is small in size (dwarf), has a high leaf:stem ratio, and produces forage with high nutritional value. It is adapted for intensive grazing with high stocking rates. Due to the high crude protein (CP) content (18%–20% dry matter (DM)) in BRS Kurumi pastures, energy supplementation is recommended only during the rainy season to enable greater production of milk.

In the studies carried out in elephant grass pastures (cultivars Cameroon and Pioneiro) to evaluate the milk FA profile, medium to high rumenic acid levels (0.66–2.18 g 100 g^{-1} FA) were obtained (Macedo et al., 2016; Batistel et al., 2017; Souza et al., 2017; Dias et al., 2019). No study has evaluated the milk FA profile in cows grazing BRS Kurumi pastures.

This study aimed to evaluate supplementation with 3 kg day^{-1} of ground corn (as-fed basis) on the intake and milk FA composition of cows grazing BRS Kurumi elephant grass pasture during the rainy season.

Materials and Methods

Location, animals, treatments, experimental design, and diets

The study was conducted from February to April 2019 at the Campo Experimental José Henrique Bruschi, belonging to Embrapa Dairy Cattle, located in Coronel Pacheco, Minas Gerais, Brazil. The experimental procedures were approved by the Ethics Commission on Animal Use of Embrapa Dairy Cattle (Protocol n. 2636081118).

Two treatments (with and without energy supplementation) were evaluated using a switchback design with six 1/2 to

7/8 Holstein × Gyr dairy cows after the peak of lactation. The average milk yield, body weight, and days in milk of the cows at the beginning of the study were 18.0±2.89 kg day^{-1}, 560±66 kg, and 99±12, respectively.

The evaluations were performed over three grazing cycles, with adaptation periods of 14 days and six days of sample collection.

The energy supplement evaluated was ground corn, which presented 87.5% DM, 7.3% CP, 5.1% ether extract (EE), and 85% of total digestible nutrients. In the treatment with energy supplementation, each cow received 3 kg day^{-1} of ground corn (as-fed basis): 2 kg day^{-1} in the morning milking and 1 kg day^{-1} in the afternoon milking.

As presented in a companion paper by Moraes et al. (2021), the average chemical compositions of the pastures of the treatments with and without energy supplementation were, respectively, 12.3% and 12.4% DM, 19.1% and 19.0% CP, 58.3% and 58.8% neutral detergent fiber (NDF), 2.6% and 2.5% lignin, and 83.2% and 81.6% *in vitro* DM digestibility (IVDMD). The average EE content in the pastures of both treatments was 3.7%.

Experimental management and procedures

The cows were mechanically milked (07h00 and 14h00), and milk samples (30 mL; 2/3 at morning milking + 1/3 at afternoon milking) were collected in bottles without preservatives on the 19th day of each switchback period. Then, the samples were frozen, subsequently thawed at room temperature, and analyzed for FA composition at the Laboratory of Chromatography of Embrapa Dairy Cattle (Juiz de Fora, MG), according to the procedures described by Gama et al. (2021).

The results of milk production and composition have been presented in the companion paper (Moraes et al., 2021).

Twenty-nine paddocks (~900 m²) were used (ten paddocks/treatment + nine reserve paddocks). The grazing method was rotational stocking with two days of paddock occupation. The resting period was 18 days, corresponding to the time for the canopy to reach a pre-grazing height of 80 cm. The average residual height of the pasture was 40 cm.

The pasture DM intake was estimated in the last six days of each switchback period using the external marker titanium dioxide (TiO_2) associated with the IVDMD of the pasture and ground corn. In addition to the formula used to estimate pasture DM intake, the procedures for marker administering, sampling, and processing and analyzing the feces have been presented in the companion paper (Moraes et al., 2021). IVDMD was determined according to the method of Silva and Queiroz (2002).

To determine the FA composition of the pasture, sampling was performed in two or three paddocks/treatment in each switchback period (*n* = 8 samples/treatment) on the day before the cows entered the pasture. The simulated grazing technique was used to obtain a representative sample of the fraction potentially ingested by the cow, collected at 50% of the height in the pre-grazing period. Ground corn samples were collected weekly and transformed into composite samples for each switchback period.

The food samples were freeze-dried (model L120, Liotop, Liobras, São Carlos, Brazil), ground, and analyzed by gas chromatography to obtain the FA profile at the Laboratory of Chromatography of Embrapa Dairy Cattle, according to the procedures described by Gama et al. (2021).

The oleic, linoleic, and α-linolenic acid intakes were calculated as the product of the estimated pasture DM intake + ground corn intake from the contents (% DM) of these FAs in these foods.

The nutritional quality of the milk fat was evaluated by the atherogenicity (AI) and thrombogenicity (TI) indices and by the relationship between the hypo- and hypercholesterolemic FAs (h/H FA ratio), according to the equations described by Chen and Liu (2020). The ratio of Σ omega-6 FA/Σ omega-3 FA (ω-6/ω-3 FA ratio) was also calculated. The indices of stearoyl-CoA desaturase-1 enzyme (SCD1) activity were calculated for five pairs of FAs by expressing each product as a proportion of the precursor plus the product (Kelsey et al., 2003).

Statistical analyses

The data were analyzed using the mixed model procedure of Statistical Analysis System – SAS (version 9.0) (SAS Institute Inc., Cary, NC, USA). The supplementation level (0 and 3 kg cow^{-1} day^{-1} of ground corn), period, and sequence were considered fixed effects, while the repetition (cow) nested to sequence and the experimental error were considered random effects.

All results are reported as least squares means, and significance was declared at $P \leq 0.05$.

The Pearson correlations between specific variables were obtained using the CORR procedure of SAS.

Results and Discussion

The BRS Kurumi pastures used for both treatments had similar levels of the main FAs (Table 1) and, on average, the amount of α-linolenic, palmitic, and linoleic acids was greater than the other FAs, accounting for approximately 93.4% of the total FAs.

In the companion paper (Moraes et al., 2021), the cows that consumed ground corn showed a 23.4% reduction in pasture DM intake, corresponding to 2.96 kg day^{-1} less of pasture DM intake compared to the cows that did not receive energy supplementation. Thus, as a consequence of the differences in the amounts of pasture and ground corn consumed (Moraes et al., 2021) and their FA profiles (Table 1), there were significant differences in the oleic, linoleic, and α-linolenic acid intakes between treatments (Table 2), with important impacts on the FA composition (Tables 3 to 5) and nutritional quality of the corresponding milk fat (Table 6).

The cows supplemented with ground corn, which has high oleic and linoleic acid contents (Table 1), consumed 567% and 88% more of these FAs, respectively, than the unsupplemented cows (Table 2). However, the cows that did not receive ground corn and consumed 2.96 kg day^{-1} more of BRS Kurumi pasture DM (Moraes et al., 2021), which has a high content of α-linolenic acid (Table 1), consumed 26% more of this FA than the supplemented cows (Table 2).

Table 1
Concentrations of the major fatty acids in ground corn and BRS Kurumi pastures

Fatty acid (FA) (mean ± SD)	Ground corn (n = 3 samples)	BRS Kurumi pasture Without supplementation (n = 8 samples)	BRS Kurumi pasture With supplementation (n = 8 samples)
(% dry matter)			
Palmitic acid	0.607 ± 0.093	0.491 ± 0.073	0.508 ± 0.069
Stearic acid	0.067 ± 0.012	0.026 ± 0.003	0.027 ± 0.003
Oleic acid	1.270 ± 0.258	0.045 ± 0.008	0.047 ± 0.008
Linoleic acid	1.762 ± 0.378	0.356 ± 0.064	0.371 ± 0.078
α-Linolenic acid	0.032 ± 0.006	0.846 ± 0.128	0.865 ± 0.157
Σ total fatty acids[a]	3.805 ± 0.758	1.811 ± 0.241	1.866± 0.294
g 100 g^{-1} of total FA			
Palmitic acid	16.07 ± 0.96	27.19 ± 2.46	27.40 ± 2.44
Stearic acid	1.75 ± 0.07	1.44 ± 0.07	1.45 ± 0.11
Oleic acid	33.35 ± 0.16	2.52 ± 0.38	2.51 ± 0.35
Linoleic acid	46.22 ± 1.01	19.54 ± 1.25	19.75 ± 1.34
α-Linolenic acid	0.85 ± 0.02	46.67 ± 3.23	46.22 ± 3.47

[a] The following fatty acids were also identified: C12:0, C14:0, C15:0, *cis*-9 C16:1, C17:0, *cis*-11 C18:1, C20:0, C22:0 and C24:0. In ground corn, C12:0 and C15:0 were not found.

Table 2
Fatty acid intake by cows grazing BRS Kurumi, with and without ground corn supplementation

Fatty acid intake (g cow^{-1} day^{-1})	Ground corn (kg cow^{-1} day^{-1}) 0	Ground corn (kg cow^{-1} day^{-1}) 3	SEM	*P*-value
Oleic acid intake from pasture	5.8	4.5	0.1930	0.0006
Linoleic acid intake from pasture	44.2	35.5	1.4059	0.0009
α-Linolenic acid intake from pasture	105.9	83.0	3.7325	0.0010
Linoleic + α-linolenic acid intake from pasture	150.1	118.4	5.1276	0.0010
Total oleic acid intake	5.8	38.7	1.0784	<0.0001
Total linoleic acid intake	44.2	83.1	1.6809	<0.0001
Total α-linolenic acid intake	105.9	83.9	3.7210	0.0013
Total linoleic + α-linolenic acid intake	150.1	166.9	4.5091	0.0234

The cows supplemented with ground corn showed 11.2% higher intake of linoleic + α-linolenic acids; however, their intake of α-linolenic acid corresponded to 50% of this total, compared to 70% in the unsupplemented cows (Table 2). Linoleic and α-linolenic acids are the main substrates for ruminal production of vaccenic acid, which is the precursor for the synthesis of 86.8% (±2.8) of the rumenic acid secreted in bovine milk (Prado et al., 2019). Furthermore, α-linolenic acid is the most useful FA for enhancing the milk fat nutritional quality (Glasser et al., 2013). Approximately 79% of the production of rumenic acid in bovine milk has been explained by the intake of α-linolenic acid (Mohammed et al., 2009). According to Coppa et al. (2013), the proportion of fresh forage in the diet is the main independent variable for predicting the rumenic and vaccenic acid contents of milk. In addition, the extent of ruminal biohydrogenation of α-linolenic acid (94.7%) is greater than that of linoleic acid (89.8%), as estimated *in vivo* by Baldin et al. (2018).

Table 3

Linear even-chain saturated fatty acids and major odd- and branched-chain fatty acids in milk fat of cows grazing BRS Kurumi, receiving 0 or 3 kg day^{-1} of ground corn

Fatty acid (FA) (g 100 g^{-1} of total FA)	Ground corn (kg cow^{-1} day^{-1})		SEM	*P*-value
	0	3		
C4:0 + C6:0 + C8:0 + C10:0	7.74	8.54	0.2939	0.0035
C12:0	2.11	2.46	0.1893	0.0036
C14:0	7.91	8.97	0.4272	0.0009
C16:0	22.93	23.96	0.6969	0.0454
C12:0 + C14:0 + C16:0	32.95	35.38	0.6538	0.0013
C18:0	11.83	12.41	0.5351	0.2111
C18:0 + C20:0 + C22:0 + C24:0	12.09	12.69	0.5421	0.2573
Σ C5:0 + C7:0 + C9:0 + C11:0 + C13:0	0.21	0.26	0.0182	0.1033
iso C15:0	0.39	0.41	0.0249	0.5622
anteiso C15:0	0.62	0.66	0.0286	0.2382
C15:0	1.36	1.35	0.0402	0.8000
C17:0	0.84	0.77	0.0124	0.0012
cis-9 C17:1	0.39	0.30	0.0272	0.0014
Odd- and branched-chain FA[a]	5.23	5.02	0.1205	0.1045

[a] C5:0 + C7:0 + C9:0 + C11:0 + C13:0 + C15:0 + C17:0 + *cis*-9 C17:1 + *iso* C14:0 + *iso* C15:0 + *iso* C16:0 + *iso* C17:0 + *iso* C18:0 + *anteiso* C15:0 + C21:0 + C23:0.

Thus, the 22.8% higher vaccenic acid content of milk fat from cows under exclusive grazing (i.e., unsupplemented) (Table 4) can be mainly attributed to their relatively higher α-linolenic acid intake (Table 2). This result agrees with the results of Dias et al. (2019) in pastures of elephant grass cv. Pioneiro. Furthermore, this increase in the vaccenic acid content of milk fat to the detriment of the other *trans*-C18:1 FAs (Table 3) demonstrates that the *trans*-11 pathway, the main route of α-linolenic acid biohydrogenation (BH) (Dewanckele et al., 2020), is preferentially used by the rumen microbiota of unsupplemented cows. However, secondary routes of C18 unsaturated FAs BH were also active, given the increase in the content of *cis*-11 C18:1 in the milk fat (Dewanckele et al., 2020) and *cis*-13 C18:1 (Gama et al., 2008) in cows under exclusive grazing (Table 4).

The absence of the treatment effect on SCD1 activity for the rumenic/vaccenic FA pair (Table 6) indicates that the 21% higher rumenic acid content of milk fat from cows under exclusive grazing (Table 5) can be attributed to the higher α-linolenic acid intake by these cows, as also noted by Dias et al. (2019) (Table 2). More vaccenic acid was available in the rumen and, therefore, in the bloodstream, for mammary gland uptake and desaturation of rumenic acid (Prado et al., 2019).

Table 4

***cis*- and *trans*-monounsaturated fatty acids in milk fat of cows grazing BRS Kurumi, receiving 0 or 3 kg day^{-1} of ground corn**

Fatty acid (FA) (g 100 g^{-1} of total FA)	Ground corn (kg cow^{-1} day^{-1}) 0	3	SEM	*P*-value
cis-9 C14:1	0.77	0.85	0.0706	0.0753
cis-9 C16:1[a]	1.94	1.72	0.1215	0.0128
cis-9 C18:1	25.47	23.00	0.9215	0.0180
cis-11 C18:1	0.91	0.78	0.0477	0.0080
cis-12 C18:1	0.17	0.17	0.0061	0.8688
cis-13 C18:1	0.104	0.085	0.0076	0.0070
trans-4 C18:1	0.035	0.034	0.0020	0.9107
trans-5 C18:1	0.022	0.022	0.0017	0.8071
trans-6–8 C18:1	0.21	0.20	0.0050	0.2742
trans-9 C18:1	0.23	0.23	0.0068	0.7582
trans-10 C18:1	0.46	0.38	0.0494	0.1607
trans-11 C18:1	2.85	2.32	0.1377	0.0023
trans-12 C18:1	0.27	0.25	0.0114	0.1978
trans-13–14 C18:1	0.24	0.26	0.0227	0.5739
trans-16 C18:1	0.34	0.31	0.0151	0.1663

[a]Co-elutes with *anteiso* C17:0.

Table 5
Long-chain polyunsaturated fatty acids in milk fat of cows grazing BRS Kurumi, receiving 0 or 3 kg day^{-1} of ground corn

Fatty acid (FA) (g 100 g^{-1} of total FA)	Ground corn (kg cow^{-1} day^{-1})			
	0	3	SEM	*P*-value
Σ non conjugated isomers of linoleic acid[a]	0.12	0.11	0.0053	0.0553
cis-9, *trans-11* CLA	1.44	1.19	0.0721	0.0095
trans-9, *cis-11* CLA	0.039	0.036	0.0028	0.1814
trans-10, *cis*-12 CLA	0.006	0.008	0.0016	0.2729
cis-9, *cis*-12 C18:2 (ω-6)	0.72	0.75	0.0300	0.2562
cis-6, *cis*-9, *cis*-12 γ-C18:3 (ω-6)	0.012	0.014	0.0010	0.2311
cis-9, *cis*-12, *cis*-15 C18:3 (ω-3)	0.46	0.40	0.0289	0.0737
cis-11, *cis*-14 C20:2 (ω-6)	0.037	0.040	0.0020	0.3773
cis-8, *cis*-11, *cis*-14 C20:3 (ω-6)	0.050	0.049	0.0029	0.8927
cis-5, *cis*-8, *cis*-11, *cis*-14 C20:4 (ω-6)	0.070	0.071	0.0045	0.6939
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5 (ω-3)	0.042	0.045	0.0025	0.4608
cis-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:5 (ω-3)	0.065	0.061	0.0020	0.1731
Σ *cis* ω-6 FA	0.89	0.92	0.0326	0.1563
Σ *cis* ω-3 FA	0.56	0.50	0.0279	0.0567

[a] *trans*-9, *trans*-12 C18:2 + *cis*-9, *trans*-12 C18:2 + *trans*-9, *cis*-12 C18:2.

The absence of effect between treatments on the linoleic and α-linolenic acid contents of milk fat (Table 5), despite the significant differences in the respective intakes (Table 2), indicates that there was extensive BH of these FAs in the rumen of the cows receiving both treatments, confirmed by the presence of several intermediate FAs of their ruminal BH (Dewanckele et al., 2020), *e.g.*, *trans*-9 to *trans*-16 C18:1; *cis*-11 and *cis*-12 C18:1; *trans*-9, *cis*-11 CLA; *trans*-10, *cis*-12 CLA; and *trans*-9, *cis*-12 C18:2 (Tables 4 and 5).

Table 6

Indices of milk fat nutritional quality and stearoyl-CoA desaturase-1 enzyme activity in cows grazing BRS Kurumi, receiving 0 or 3 kg day^{-1} of ground corn

Indice	Ground corn (kg cow^{-1} day^{-1})		SEM	*P*-value
	0	3		
Stearoyl-CoA desaturase-1 indices[a]				
cis-9 C14:1/(*cis*-9 C14:1 + C14:0)	0.089	0.087	0.0087	0.6065
cis-9 C16:1/(*cis*-9 C16:1 + C16:0)	0.078	0.067	0.0048	0.0050
cis-9 C17:1/(*cis*-9 C17:1 + C17:0)	0.312	0.278	0.0153	0.0091
cis-9 C18:1/(*cis*-9 C18:1 + C18:0)	0.682	0.649	0.0160	0.0222
cis-9, *trans*-11 CLA/(*cis*-9, *trans*-11 CLA + *trans*-11 C18:1)	0.335	0.340	0.0090	0.6343
Milk fat nutritional quality indices				
Atherogenicity index[b]	2.14	2.58	0.1261	0.0018
Thrombogenicity index[b]	2.78	3.25	0.1278	0.0015
Hypo/hypercholesterolemic fatty acid ratio[b]	0.80	0.67	0.0390	0.0077
ω-6/ω-3 fatty acid ratio	1.64	1.84	0.1196	0.0394

[a] Calculated according to the formula described by Kelsey et al. (2003).
[b] Calculated according to the equations described by Chen and Liu (2020).

Despite the 567% higher oleic acid intake of the cows supplemented with ground corn (Table 2), the content of this FA in the milk fat of cows under exclusive grazing was 10.3% higher (Table 4). Indeed, oleic acid was the main FA (~25% of total FAs) in the milk of cows under exclusive grazing. According to Prado et al. (2019), 83.8% (±0.75) of the oleic acid in bovine milk fat originates from the desaturation of stearic acid by SCD1 in the mammary gland. As there was no treatment effect on the stearic acid content of the milk fat (Table 3), the 5.1% higher SCD1 activity for the oleic/stearic FA pair in cows under exclusive grazing (Table 6) partially explains the higher oleic acid content in their milk fat (Table 3). In the cows supplemented with ground corn, the higher oleic acid intake (Table 2) suggests that there may have been greater availability of this FA in the bloodstream, allowing for greater uptake in the mammary gland, but reducing by the SCD1 activity for the oleic/stearic FA pair by 4.8% due to a feedback–inhibition mechanism, as discussed by Jayan and Herbein (2000). The negative correlation between SCD1 activity for the oleic/stearic FA pair *versus* total oleic acid intake (r = −0.49; P=0.0370) confirms this result, at least in part.

Endogenously formed oleic acid plays a pivotal role in triglyceride synthesis and maintaining the melting point and fluidity suitable for milk fat secretion. Short-chain saturated FAs (C4:0 to C10:0) have relatively low melting points and may compete with oleic acid at position *sn*-3 as regulators of milk fluidity during the final step of triglyceride synthesis (Gama et al., 2008). However, compared with *cis* double bonds, *trans* double bonds have a more rigid structure, are less fluid, and have a higher melting point (Jayan & Herbein, 2000; Gama et al., 2008). Thus, it is possible that the incorporation of excess vaccenic acid (Table 3) increases the melting point and reduces the fluidity of milk fat. Thus, the esterification of oleic acid in the triglycerides of milk fat from cows in both treatments, particularly in unsupplemented cows, may be a cellular response by the mammary gland to ensure an adequate melting point to counteract the concomitant high esterification of vaccenic acid.

As also noted by Prado et al. (2019), the selective and preferential incorporation of C18 FAs, notably oleic and vaccenic acids, in milk fat (Tables 4 and 5) to the detriment of those FAs with short and medium saturated chains may explain the reduction in *de novo*-synthesized FAs in the milk of the unsupplemented cows (Table 3). Oleic and vaccenic acids have been shown to inhibit the activities of enzymes associated with FA synthesis (Jayan & Herbein, 2000). The negative correlations between milk fat *de novo*-synthetized FA (C4:0 to C16:0) contents *versus* oleic ($r = -0.86$; $P<0.0001$) and vaccenic acid ($r = -0.55$; $P=0.02$), in addition to other FAs (*cis*-11 C18:1, *cis*-13 C18:1 and rumenic acid; $r = -0.48$ to -0.71; $P<0.05$), which are also present in the milk fat of the unsupplemented cows (Tables 4 and 5), could also explain, at least in part, the reduction in the *de novo*-synthesized FA contents in the milk of these cows.

Lower availability of the lipogenic subtracts (acetate and butyrate) in the mammary gland could also explain the lower *de novo*-synthesized FA contents in the milk of the unsupplemented cows (Prado et al., 2019). However, this is likely to be of minor importance, given that unsupplemented cows consumed 1.39 kg day^{-1} more NDF from pasture, as reported in the companion paper (Moraes et al., 2021). In addition, the similar contents of most of the odd- and branched-chain FAs in the milk fat (Table 3) can be considered indicative that the rumen environment of cows from both treatments was favorable to NDF fermentation because these FAs have been used as indirect noninvasive markers of rumen function (Zhang et al., 2017). Specifically, heptadecanoic acid (C17:0) positively correlates with rumen acetate concentration and might be used to predict populations of fibrolytic bacteria (Zhang et al., 2017). The heptadecanoic acid content was ~9% higher in the milk of unsupplemented cows (Table 3), partially indicating that the availability of lipogenic substrates did not limit *de novo* FA synthesis in the mammary gland of these cows. The 30% higher content of *cis*-9 C17:1 in the milk fat of unsupplemented cows (Table 3) is related to the higher availability of C17:0 for desaturation and the ~12% higher SCD1 activity (Table 6) for this pair of FAs in the mammary gland of these cows.

The absence of treatment effect on the *trans*-10 C18:1, *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA contents of the milk fats (Table 5), which are associated with inhibition of lipogenesis in the mammary gland (Dewanckele et al., 2020), is partially explained by the similarity (P>0.05) in milk fat contents between treatments, as presented in the companion paper (Moraes et al., 2021), and by the absence of milk fat depression

(MFD) because the milk fat content in both treatments was always higher than 3.0% (Moraes et al., 2021), the minimum value for the raw milk established by Brazilian legislation (Instrução Normativa nº 76, 2018). MFD is characterized by a reduction in milk fat content and yield with concomitant changes in ruminal BH pathways (Dewanckele et al., 2020).

The commonly used nutritional indices for assessing FAs have limitations; therefore, they should be used cautiously. However, these indices are useful for comparing research objects (Chen & Liu, 2020). The milk fat from unsupplemented cows showed better nutritional quality, as the atherogenicity and thrombogenicity indices, as well as the ω-6/ω-3 FAs ratio (Table 6), decreased (Chen & Liu, 2020). Furthermore, the milk from unsupplemented cows showed a higher hypo/hypercholesterolemic FA ratio (Table 6), which is good for human health (Chen & Liu, 2020). These results can be attributed mainly to the 7% lower (Table 3) sum of lauric, myristic and palmitic acid contents of the milk fat, considered pro-atherogenic (Chen & Liu, 2020), and to the concomitant higher contents of FAs beneficial to human health in the milk fat of unsupplemented cows, such as oleic acid (+10.3%; Table 4) and those from the ω-3 family (+12%; Table 5), such as eicosapentaenoic acid (EPA; *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5) and α-linolenic acid. Milk fat with high oleic and α-linolenic acid contents is desirable, as these FAs improve the immune response (anti-inflammatory effect), in addition to having anti-cancer and anti-atherogenic properties, and have a positive effect on cholesterol levels. In addition, EPA has anti-cancer, anti-hypertensive, and anti-inflammatory properties (Hanuš et al., 2018). The milk fat from unsupplemented cows showed higher vaccenic and rumenic acid contents, which are not considered in any indices of nutritional quality (Table 6), although several beneficial effects on human health have been attributed to them (Yang et al., 2015; Alves et al., 2017; Hanuš et al., 2018).

The results obtained demonstrate that the milk produced by cows grazing BRS Kurumi pastures supplemented with ground corn presented oleic, vaccenic, rumenic, and α-linolenic acid contents in their milk fat 4%, 61%, 47%, and 5% higher, respectively, than those reported in cows grazing elephant grass cv. Cameroon and receiving 3 kg day^{-1} of a non-lipid concentrate with ~80% ground corn (Macedo et al., 2016). In contrast, compared with the results presented by Dias et al. (2019) in cows under exclusive grazing of elephant grass cv. Pioneiro, higher contents of oleic (+2%) and α-linolenic (+7%) acids and lower contents of vaccenic (−27%) and rumenic (−34%) acids were recorded from the milk fat of the unsupplemented cows. The high vaccenic and rumenic acid contents in milk fats in Dias et al. (2019) were attributed to the specific subtropical climate of the experimental site. Lower temperatures principally favored a high content of α-linolenic acid (Dias et al., 2017) in the pastures. The pro-atherogenic FAs (lauric + myristic + palmitic acids) were 19% lower in the milk of the cows supplemented with ground corn than in those in the study of Macedo et al. (2016) and 2% lower in the milk of the unsupplemented cows than in those in the study of Dias et al. (2019).

Conclusions

Cows grazing BRS Kurumi elephant grass pasture exclusively compared with those receiving 3 kg day^{-1} of ground corn consumed 26% more α-linolenic acid and produced milk with higher levels of oleic, vaccenic, and rumenic acids and lower levels of pro-atherogenic fatty acids.

Acknowledgments

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) for financial support of this study (SEG n. 03.16.04.024.00.00) and Embrapa Gado de Leite for providing the infrastructure and staff necessary for this research.

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for scholarships.

The technicians Ernando Ferreira Motta and Hernani Guilherme Barbosa Filho, who performed the analyses of fatty acid composition at the Laboratory of Chromatography of Embrapa Gado de Leite.

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