R. Bras. Zootec., 48:e20190088, 2019
https://doi.org/10.1590/rbz4820190088

Ruminants
Short communication

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
ISSN 1806-9290
www.rbz.org.br

Milk fatty acid composition of unsupplemented dairy cows grazing on a tropical pasture

Kamila Maciel Dias[1], Marco Antônio Sundfeld da Gama[2], Daniel Schmitt[1], André Fischer Sbrissia[1*]

[1] Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Lages, SC, Brasil.
[2] Embrapa Gado de Leite, Juiz de Fora, MG, Brasil.

***Corresponding author:**
andre.sbrissia@udesc.br

Received: April 25, 2019
Accepted: August 25, 2019

How to cite: Dias, K. M.; Gama, M. A. S.; Schmitt, D. and Sbrissia, A. F. 2019. Milk fatty acid composition of unsupplemented dairy cows grazing on a tropical pasture. Revista Brasileira de Zootecnia 48:e20190088.
https://doi.org/10.1590/rbz4820190088

ABSTRACT - This study aimed to evaluate the milk fatty acid (FA) composition of unsupplemented dairy cows grazing on elephantgrass (*Pennisetum purpureum* Schum. cv. Pioneiro) pastures under three grazing management strategies: pre-grazing height of 120 cm with defoliation intensities of 50 and 70% of the initial height (treatments 120/50 and 120/70) and pre-grazing height of 90 cm with defoliation intensity of 70% (treatment 90/70). Treatments were repeated three times in a complete randomized block design. Tester animals were six multiparous Holsteins cows grouped into pairs according to days in milk and milk yield and allocated to blocks. Individual milk samples were collected after the first and second days of grazing (two-day occupation period) and analyzed for FA composition. Milk fat from 120/50 and 90/70 treatments resulted in higher concentrations of C4:0, C6:0, C17:0 anteiso, rumenic acid (RA), vacenic acid (VA), *trans*-12 C18:1, *trans*-13/14 C18:1, *cis*-11 C18:1, *cis*-12 C18:1, *cis*-9, *trans*-12 C18:2, *trans*-11, *cis*-15 C18:2, and total polyunsaturated fatty acids. Additionally, both SCD_{14} (*cis*-9 14:1/14:0 + *cis*-9 14:1) and SCD_{RA} (RA/VA + RA) desaturase indices were reduced in milk fat from cows subjected to 120/50 and 90/70 treatments. On average, the milk fat levels of RA and VA observed in our study were greater than those reported in most studies with tropical grass-based diets and similar to levels usually found in cows grazing on temperate pastures. Overall, our results showed that milk with a desirable fatty acid composition is produced by cows grazing on a tropical grass (elephantgrass cv. Pioneiro), with the combination of a pre-grazing height of 120 cm and a defoliation intensity of 50% of the initial height, being a practical management strategy to achieve this goal.

Keywords: dairy fat, elephantgrass, functional foods, human health, *Pennisetum purpureum*, rumenic acid

Introduction

Over the last 40 years, the medical community and public health agencies have recommended a limited consumption of animal fats based on the purported association of saturated fat intake with an increased risk of cardiovascular diseases (Givens and Shingfield, 2004; Kliem and Givens, 2011). As ruminant milk fat is the major dietary source of saturated fatty acids (FA) in most of developed countries (Kliem and Shingfield, 2016), this recommendation has been translated into an avoidance of full-fat dairy products as a means of reducing saturated fat intake. However, recent studies have indicated that dairy fat intake does not increase the risk of cardiovascular diseases and is associated with a lower risk of obesity and type 2 diabetes (Kratz et al., 2013; Mozaffarian, 2014). Moreover, ruminant milk fat has been shown to be a natural source of a number of potentially beneficial FA (Kratz et al., 2013).

Studies have shown the possibility of enriching the milk fat through dietary manipulation (Dewhurst et al., 2006). Rumenic acid (RA) is the main isomer of conjugated linoleic acid (CLA) in milk and is formed during the incomplete ruminal biohydrogenation of *cis*-9, *cis*-12 C18:2 (Linoleic acid: LA) or, to a greater extent, by stearoyl-CoA desaturase (Δ9-desaturase) activity from VA in mammary gland (Bauman et al., 2003). The major FA found in fresh forages are linolenic acid (LNA), LA, and C16:0, with LNA being the most abundant FA (50-75% of total FA) in most grasses (Bauchart et al., 1984; Elgersma, 2015). As LNA is the major VA precursor in forage-based diets, the use of fresh forage as the main feed source increases the concentration of beneficial milk FA (Palladino et al., 2009; Kratz et al., 2013).

It is well known that including tropical forage species (Souza, 2014; Macedo, 2012) can beneficially affect the milk FA composition. However, the effect of grazing management strategies of tropical pastures on milk FA composition of cows not receiving supplementation is unknown. Grazing management strategies could be an important tool to increase the beneficial FA in milk, because they modulate forage chemical composition and FA concentrations. Dias et al. (2017) reported higher proportions of desirable FA when pastures are managed to create leafier structures. Thus, the objective of our study was to evaluate the milk FA composition of unsupplemented cows grazing on elephantgrass pastures.

Material and Methods

All procedures involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee guidelines and approved under case no. 01.19.14. The experiment was conducted in Lages, Santa Catarina, Brazil (27°47' S, 50°18' W, 913 m altitude), between January and June 2011. The experimental area was a 0.8-ha elephantgrass (*Pennisetum purpureum* Schum. cv Pioneiro) pasture, divided into three blocks containing three plots of 650 m^2 each plus an adjacent area for animal adaptation. Pastures received a total of 200 kg N ha^{-1} during the experimental period.

The treatments were the combination of grazing heights: 90/70, 120/70, and 120/50, in which 90 and 120 cm were the pre-grazing heights, and 50 and 70 corresponded to the proportion (%) of removal of initial canopy height (defoliation intensity). The choice of a pre-grazing height of 90 cm was based on data from two other elephantgrass cultivars, Cameroon and Napier, which also have large leaf masses at heights of 100 and 85 cm (Voltolini et al., 2010; Pereira et al., 2014). The 120 cm height was chosen to test management strategies that promote taller canopies with higher dry matter production, which generally results in stemmy structures. The defoliation intensities were chosen to compare the milk FA contents from cows grazing pastures with lower herbage mass but greater leaf proportion (50%) with cows allowed to graze pastures with greater herbage mass but lower leaf proportion (higher grazing efficiency; 70%). The average pre- and post-grazing heights were measured using a ruler in a "zig-zag" pattern at 60 randomly selected points in each plot. Canopy height was measured from ground level to the top of the leaf horizon.

Six multiparous Holsteins cows in late lactation were divided into three homogeneous groups, according to days in milk and milk yield. Each pair of cows was fixed allocated to one block. Cows were milked twice-daily at approximately 08.00 h (morning milking) and 18.00 h (afternoon milking). Individual milk samples (pooled morning and afternoon milking) were collected during two consecutive days after 24 h grazing. Stocking rate was adjusted by put-and-take (Allen et al., 2011), allocating nonlactating cows besides the fixed pair of cows to ensure that grazing depletion targets were reached within two days. On the first day, animals were allocated to plots after morning milking and then moved out to the adaptation area following the afternoon milking on the second day, when the plots achieved the post-grazing height target. Mineral salt was provided *ad libitum*, but no concentrate feeds were provided throughout the study.

Milk samples were thawed at room temperature, and a volume of 1 mL was used for lipid extraction using a mixture of diethyl ether and hexane according to a reference procedure (method 989.05; AOAC, 1990). The organic phase containing the milk fat (~20 mg) was evaporated to dryness at 40 °C under oxygen-free nitrogen. Fatty acid methyl esters (FAME) were obtained by base-catalyzed transmethylation using a freshly prepared solution of sodium methoxide as described in detail elsewhere (Baldin et al., 2013). The FAME were quantified by a gas chromatograph (model 7820-A, Agilent Technologies) fitted with a flame-ionization detector and equipped with a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm × 0.2 µm film thickness; Varian Inc.). Operating conditions were performed as described by Cruz-Hernandez et al. (2007). The FAME were identified by comparing retention times with commercial FAME standards, and minor *trans*/*cis*-C18:1 isomers were identified according to their order of elution as reported under the same GC conditions (Cruz-Hernandez et al., 2007). Milk FA composition was expressed as a weight percentage of total FA using theoretical relative response factors (Wolff et al., 1995). Stearoyl-CoA desaturase (SCD) indices were calculated for four pairs of FA by expressing each product as a proportion of the precursor plus product (i.e. SCD_{14} = *cis*-9 14:1/14:0 + *cis*-9 14:1; SCD_{16} = *cis*-9 16:1/16:0 + *cis*-9 16:1; SCD_{18} = *cis*-9 18:1/18:0 + *cis*-9 18:1; and SCD_{RA}= RA/VA + RA) (Kelsey et al., 2003).

Data were analyzed using the MIXED procedure (mixed models) of SAS (Statistical Analysis System, version 9.1 for Windows), according to the following model:

$$Y_{ij} = \mu + T_i + B_j + e_{ij},$$

in which Y_{ij} is the dependent variable, μ is the overall mean, T_i is the effect of the *i*-th treatment, B_j is the effect of the *j*-th block, and e_{ij} is the residual error assumed ij. Animal effect was not included in the model, since the plot was the experimental unit. Grazing cycle was considered as a repeated measurement, since it was not the main objective to detect possible variations over time. Covariance matrix was chosen using Akaike's Information Criterion (AIC). Tukey's test was used to compare the means with a significance level of 5%.

Results

Milk FA composition was affected by grazing management strategies (Table 1). In general, the concentrations of most milk FA from the 120/50 treatment were similar to those observed in the 90/70 treatment, while both treatments differed from 120/70 treatment for a number of FA. Specifically, milk fat from cows in the 120/50 and 90/70 treatments had higher concentrations of 4:0, 6:0, 17:0 anteiso, VA, *trans*-12 C18:1, *trans*-13+14 18:1, *cis*-11 C18:1, *cis*-12 18:1, *cis*-9, *trans*-12 18:2, *trans*-11, *cis*-15 18:2, RA, and ΣPUFA and lower concentrations of *trans*-C18:1 isomers with double bounds at carbon ≤10, *cis*-9 12:1, *cis*-9 14:1, 17:0, 17:0 iso, 20:0, *cis*-9 20:1, 20:3 n-6, 20:4 n-6, 24:0, SCD_{14}, and SCD_{RA} than milk fat from cows in the 120/70 treatment.

Table 1 - Effects of different grazing management strategies on milk fatty acid (FA) composition (g/100 g of total FA) in cows grazing on elephantgrass cultivar Pioneiro (*Pennisetum purpureum* Schum.)

Fatty acid	Treatment[1]			SE
	120/50	120/70	90/70	
Selected individual FA				
4:0	2.82a	2.03b	2.87a	0.051
6:0	1.48a	1.35b	1.50a	0.033
8:0	0.74	0.76	0.76	0.026
10:0	1.43	1.58	1.47	0.066
12:0	1.67	1.93	1.71	0.075
cis-9 12:1	0.04b	0.06a	0.04b	0.002
13:0	0.07	0.07	0.07	0.005
14:0	7.41	8.21	7.57	0.225
cis-9 14:1	0.81b	1.11a	0.76b	0.050
15:0	1.59a	1.39b	1.49ab	0.046
15:0 iso	0.44	0.46	0.41	0.020
15:0 anteiso	0.79ab	0.84a	0.74b	0.023
16:0	24.4b	26.1a	25.9ab	0.362
16:0 iso	0.36a	0.39a	0.33b	0.010
cis-9 16:1	1.63	1.70	1.56	0.070
17:0	0.63b	0.82a	0.61b	0.018

Continues...

Table 1 (Continued)

Fatty acid	Treatment[1]			SE
	120/50	120/70	90/70	
Selected individual FA				
cis-9 17:1	0.35	0.38	0.33	0.013
17:0 iso	0.45b	0.55a	0.44b	0.013
17:0 anteiso	0.71a	0.52b	0.69a	0.026
18:0	11.1	10.6	11.0	0.256
trans-4 18:1	0.003b	0.011a	0.001b	0.001
trans-6+7+8 18:1	0.18b	0.28a	0.19b	0.013
trans-9 18:1	0.21b	0.24a	0.20b	0.007
trans-10 18:1	0.18b	0.27a	0.17b	0.010
trans-11 18:1	3.90a	2.86b	3.84a	0.102
trans-12 18:1	0.40a	0.20b	0.37a	0.011
trans-13+14 18:1	0.39a	0.25b	0.37a	0.015
cis-9+*trans*-15 18:1	25.1	25.2	24.8	0.670
cis-11 18:1	1.27a	0.45b	1.25a	0.055
cis-12 18:1	0.25a	0.08b	0.24a	0.012
cis-13 18:1	0.07	0.06	0.06	0.005
trans-16 18:1	0.27	0.24	0.25	0.010
cis-9, *trans*-12 18:2	0.10a	0.06b	0.09a	0.004
trans-11, cis-15 18:2[2]	0.46a	0.32b	0.44a	0.021
18:2 n-6	1.13	1.19	1.17	0.037
18:3 n-6	0.008	0.007	0.011	0.001
18:3 n-3 (LNA)	0.43	0.44	0.41	0.012
cis-9, *trans*-11 CLA	2.18a	1.84b	2.07a	0.061
20:0	0.12b	0.19a	0.12b	0.006
cis-9 20:1	0.13b	0.17a	0.12b	0.010
cis-11 20:1	0.04	0.07	0.05	0.006
20:2 n-6	0.05	0.05	0.04	0.004
20:3 n-6	0.04b	0.09a	0.04b	0.003
20:4 n-6	0.09b	0.17a	0.10b	0.007
20:5 n-3	0.05	0.04	0.05	0.004
22:0	0.08	0.08	0.07	0.006
23:0	0.04	0.03	0.03	0.005
24:0	0.04b	0.07a	0.03b	0.005
Σ unidentified	3.58	3.81	3.59	0.128
Summation by saturation				
ΣSFA[3]	56.3	58.0	57.0	0.627
ΣMUFA[4]	35.2	33.7	34.6	0.670
ΣPUFA[5]	4.66a	4.22b	4.54a	0.083
ΣOBCFA[6]	5.41	5.44	5.13	0.106
SFA:UFA ratio	1.42	1.54	1.47	0.083
n-6:n-3 ratio[7]	2.78	3.07	3.03	0.177
SCD index[8]				
SCD_{14}	0.09b	0.12a	0.09b	0.014
SCD_{16}	0.06	0.06	0.06	0.006
SCD_{18}	0.69	0.70	0.69	0.014
SCD_{RA}	0.36b	0.39a	0.35b	0.018

[1] 90/70, 120/50, and 120/70: pre-grazing heights (90 or 120 cm) and grazing levels (50 or 70%) based on pre-grazing heights. Only milk samples from the second grazing cycle were collected in 120/70 treatments, as there was not a 3rd grazing cycle for this treatment.
[2] Co-eluted with *trans*-9, *cis*-12 C18:2.
[3] Saturated FA.
[4] Monounsaturated FA.
[5] Polyunsaturated FA.
[6] Sum of odd- and branched-chain FA.
[7] Calculated as (18:2 n-6 + 18:3 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6)/(C18:3 n-3 + C20:5 n-3).
[8] Calculated according to Kelsey et al. (2003).
a,b,c - Indicate significant differences within a row (P<0.05).

Discussion

Regardless of treatments, the highlight of the milk FA results of our study was the high VA and RA content. These results were unexpected, because cows were not receiving supplements and were grazing on a tropical pasture, which usually has smaller LNA content than temperate pastures. The LNA is the major VA precursor, which is used to synthesize RA from Δ9-desaturase activity in mammary gland. The milk fat concentrations of RA and VA observed in our study were, respectively, around 2.0 and 3.5 g/100 g of total FA (Table 1). As shown in previous studies, more than 91% of RA secreted in milk from cows fed fresh pasture is derived from endogenous synthesis from VA escaping the rumen (Kay et al., 2004), which is consistent with the close association between the milk fat concentrations of VA and RA observed in our study. The milk RA and VA contents observed in our study were, respectively, 57 and 69% higher than in milk from cows fed a total mixed ration composed of chopped elephantgrass plus concentrate (Lopes et al., 2009); 176-243 and 55-212% higher than in milk from cows grazing on elephantgrass cultivar Cameron and fed concentrates containing or not lipid supplements (soybean oil, palm oil, or Megalag-E®) (Souza, 2014; Macedo, 2012); and 99 and 88% higher than in milk from cows grazing on *Brachiaria brizantha* or *Panicum maximum* supplemented with two levels of concentrate (Lopes et al., 2011). Overall, the RA and VA contents found in the present study were similar or even superior to those reported in milk fat from cows feeding fresh or conserved temperate grasses (Schwendel et al., 2015; Mendoza et al., 2016). Likewise, the average milk fat CLA content observed in our study was generally higher than those reported in milk from cows fed plant oil supplements and similar to those found in milk from cows fed fish oil (Kliem and Shingfield, 2016).

The increased milk fat VA and RA contents observed in cows from the 120/50 and 90/70 treatments were probably the result of greater forage LNA intake, since forage potentially consumed in those treatments had a higher proportion of leaves (Dias et al., 2017; Schmitt et al., 2019). No treatment differences in milk fat RA concentrations were observed in previous studies in which cows grazed on high-quality pasture. For instance, no differences in milk fat RA content were found by Palladino et al. (2009) when they evaluated the effects of herbage mass (2400 or 1700 kg DM/ha) and daily herbage allowance (20 or 16 kg DM/d/cow) of ryegrass pasture on milk FA composition of dairy cows. Even when pasture allowances were 20 or 70 kg DM/d/cow (Wales et al., 1999) and 25 or 50 kg DM/d/cow (Stockdale et al., 2003), no differences in milk fat RA concentrations were found (1.4 and 1.05 g/100 g FA, respectively).

Regarding milk fat *cis*-9 C18:1 (also known as oleic acid), another important bioactive FA found in milk fat, the levels observed in our study (~25 g/100g total FA) were higher than values of Mendoza et al. (2016) (19-21.1 g/100 g total FA) and Lerch et al. (2012) (18.2-23.6 g/100 g total FA). Most *cis*-9 C18:1 secreted in milk is derived either from endogenous synthesis by Stearoyl-CoA enzyme 1 (SCD-1, also called Δ-9 desaturase) from C18:0 formed during rumen biohydrogenation of dietary PUFA or from blood plasma non-esterified FA released during periods of negative energy balance (Palmquist, 2006). The main biological role of Δ9-desaturase in the mammary gland is to maintain fluidity of milk by conversion of C18:0 to *cis*-9 C18:1, although this enzyme is also responsible for the synthesis of other milk UFA such as *cis*-9 C14:1, *cis*-9 C16:1, and RA (Garnsworthy et al., 2010). Mammary synthesis via Δ-9 desaturase action from C18:0 derived from rumen biohydrogenation of forage PUFA (especially C18:3 n-3) was probably the main source of milk fat *cis*-9 C18:1 in the present study. However, as the body condition score of the animals was reduced throughout the experimental period (visual observation; no data recording), it is reasonable to assume that body fat mobilization may have contributed to increase the milk fat *cis*-9 C18:1 content in the present study.

The concentrations of odd- and branched-chain FA (OBCFA) in milk fat manly reflect rumen function. These FA originate from the membrane lipids of ruminal bacterial populations, especially cellulolytic bacteria (Dewhurst et al., 2006). Patel et al. (2013) observed increases in C15:0, C15:0 iso, C17:0, and the total OBCFA contents in milk fat when cows were fed larger proportions of grass silage in their diets. The same authors also found that the concentration of total OBCFA in milk was positively correlated with the dietary content of neutral detergent fiber. In our study, we observed no differences in total OBCFA contents between treatments. Moreover, the OBCFA contents observed in our study were higher than those of other previously published investigations (Vlaeminck et al., 2006), which was probably related to the lower fiber content of the temperate forages used in most studies.

Conclusions

Cows grazing on a tropical grass (elephantgrass cv. Pioneiro) produce milk with a desirable fatty acid composition, with the combination of a pre-grazing height of 120 cm and a defoliation intensity of 50% of the initial height, being a practical grazing management strategy to achieve this goal.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: K.M. Dias, D. Schmitt and A.F. Sbrissia. Data curation: K.M. Dias, M.A.S. Gama and A.F. Sbrissia. Formal analysis: K.M. Dias, M.A.S. Gama and A.F. Sbrissia. Investigation: M.A.S. Gama, D. Schmitt and A.F. Sbrissia. Methodology: K.M. Dias. Supervision: A.F. Sbrissia. Writing-original draft: K.M. Dias, M.A.S. Gama, D. Schmitt and A.F. Sbrissia. Writing-review & editing: K.M. Dias, M.A.S. Gama, D. Schmitt and A.F. Sbrissia.

Acknowledgments

The authors would like to thank Coordenação de Aperfeiçomento de Pessoal de Nível Superior (CAPES) for financial support through the scholarship and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC), for funding this research (grant numbers 483533/2011-4 and 2019 TR-584, respectively).

References

AOAC - Association of Official Analytical Chemistry. 1990. Official methods of analysis. 15th ed. AOAC International, Arlington, VA.

Allen, V. G.; Batello, C.; Berretta, E. J.; Hodgson, J.; Kothmann, M.; Li, X.; McIvor, J.; Milne, J.; Morris, C.; Peeters, A. and Sanderson, M. 2011. An international terminology for grazing lands and grazing animals. Grass and Forage Science 66:2-28. https://doi.org/10.1111/j.1365-2494.2010.00780.x

Baldin, M.; Gama, M. A. S.; Dresch, R.; Harvatine, K. J. and Oliveira, D. E. 2013. A rumen unprotected conjugated linoleic acid supplement inhibits milk fat synthesis and improves energy balance in lactating goats. Journal of Animal Science 91:3305-3314. https://doi.org/10.2527/jas.2012-5766

Bauchart, D.; Verite, R. and Remond, B. 1984. Long-chain fatty acid digestion in lactating cows fed fresh grass from spring to autumn. Canadian Journal of Animal Science 64:330-331. https://doi.org/10.4141/cjas84-285

Bauman, D. E.; Corl, B. A. and Peterson, D. G. 2003. The biology of conjugated linoleic acid in ruminants. p.146-173. In: Advances in conjugated linoleic acid research. Sébédio, J. L.; Christie, W. W. and Adlof, R., eds. AOCS Press, Champaign.

Cruz-Hernandez, C.; Kramer, J. K. G.; Kennelly, J. J.; Glimm, D. R.; Sorensen, B. M.; Okine, E. K.; Goonewardene, L. A. and Weselake, R. J. 2007. Evaluating the conjugated linoleic acid and *trans* 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. Journal of Dairy Science 90:3786-3801. https://doi.org/10.3168/jds.2006-698

Dewhurst, R. J.; Shingfield, K. J.; Lee, M. R. F. and Scollan, N. D. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Animal Feed Science and Technology 131:168-206. https://doi.org/10.1016/j.anifeedsci.2006.04.016

Dias, K. M.; Schmitt, D.; Rodolfo, G. R.; Deschamps, F. C.; Camargo, G. N.; Pereira, R. S. and Sbrissia, A. F. 2017. Fatty acid profile in vertical strata of elephant grass subjected to intermittent stocking. Anais da Academia Brasileira de Ciências 89:1707-1718. https://doi.org/10.1590/0001-3765201720150272

Elgersma, A. 2015. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges and future perspectives. European Journal of Lipid Science and Technology 117:1345-1369. https://doi.org/10.1002/ejlt.201400469

Garnsworthy, P. C.; Feng, S.; Lock, A. L. and Royal, M. D. 2010. Short communication: Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. Journal of Dairy Science 93:1743-1748. https://doi.org/10.3168/jds.2009-2695

Givens, D. I. and Shingfield, K. J. 2004. Foods derived from animals: the impact of animal nutrition on their nutritive value and ability to sustain long-term health. Nutrition Bulletin 29:325-332. https://doi.org/10.1111/j.1467-3010.2004.00444.x

Kay, J. K.; Mackle, T. R.; Auldist, M. J.; Thomson, N. A. and Bauman, D. E. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. Journal of Dairy Science 87:369-378. https://doi.org/10.3168/jds.S0022-0302(04)73176-8

Kelsey, J. A.; Corl, B. A.; Collier, R. J. and Bauman, D. E. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. Journal of Dairy Science 86:2588-2597. https://doi.org/10.3168/jds.S0022-0302(03)73854-5

Kliem, K. E. and Givens, D. I. 2011. Dairy products in the food chain: their impact on health. Annual Review of Food Science and Technology 2:21-36. https://doi.org/10.1146/annurev-food-022510-133734

Kliem, K. E. and Shingfield, K. J. 2016. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. European Journal of Lipid Science and Technology 118:1661-1683. https://doi.org/10.1002/ejlt.201400543

Kratz, M.; Baars, T. and Guyenet, S. 2013. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. European Journal of Nutrition 52:1-24. https://doi.org/10.1007/s00394-012-0418-1

Lerch, S.; Ferlay, A.; Shingfield, K. J.; Martin, B.; Pomiès, D. and Chilliard, Y. 2012. Rapeseed or linseed supplements in grass-based diets: Effects on milk fatty acid composition of Holstein cows over two consecutive lactations. Journal of Dairy Science 95:5221-5241. https://doi.org/10.3168/jds.2012-5337

Lopes, F. C. F.; Ribeiro, C. G. S.; Ribeiro, M. T.; Rodriguez, N. M.; Filho, H. G. B.; Castro, R. J. C.; Barros, P. A. V. and Gama, M. A. S. 2009. Milk fatty acid profile from dairy cows fed increasing levels of soybean oil in diets based on tropical forage. p.588-589. In: Ruminant physiology: digestion, metabolism, and effects of nutrition on reproduction and welfare. Chilliard, Y.; Glasser, F.; Faulconnier, Y.; Bocquier, F.; Veissier, I. and Doreau, M., eds. Wageningen Academic Publisher, Wageningen.

Lopes, F. C. F.; Barros, P. A. V.; Bruschi, J. H.; Silva, P. H. F.; Peixoto, M. G. C. D.; Gomide, C. A. M.; Duque, A. C. A. and Gama, M. A. S. 2011. Perfil de ácidos graxos no leite de vacas Holandês em pastagens tropicais suplementadas com dois níveis de concentrado. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 63:518-521. https://doi.org/10.1590/S0102-09352011000200037

Macedo, F. L. 2012. Doses de concentrado com ou sem gordura inerte na dieta de vacas em lactação mantidas em pastagens tropicais. Dissertação (M.Sc.). Universidade de São Paulo, Piracicaba.

Mendoza, A.; Cajarville, C. and Repetto, J. L. 2016. Short communication: Intake, milk production, and milk fatty acid profile of dairy cows fed diets combining fresh forage with a total mixed ration. Journal of Dairy Science 99:1938-1944. https://doi.org/10.3168/jds.2015-10257

Mozaffarian, D. 2014. Saturated fatty acids and type 2 diabetes: more evidence to re-invent dietary guidelines. Lancet Diabetes Endocrinology 2:770-772. https://doi.org/10.1016/S2213-8587(14)70166-4

Palmquist, D. L. 2006. Milk fat: origin of fatty acids and influence of nutritional factors thereon. p.43-92. In: Advanced dairy chemistry. Volume 2: Lipids. 3rd ed. Fox, P. F. and McSweeney, P. L. H., eds. Springer, Boston. https://doi.org/10.1007/0-387-28813-9_2

Palladino, R. A.; O'Donovan, M.; Murphy, J. J.; McEvoy, M.; Callan, J.; Boland, T. M. and Kenny, D. A. 2009. Fatty acid intake and milk fatty acid composition of Holstein dairy cows under different grazing strategies: herbage mass and daily herbage allowance. Journal of Dairy Science 92:5212-5223. https://doi.org/10.3168/jds.2009-2404

Patel, M.; Wredle, E. and Bertilsson J. 2013. Effect of dietary proportion of grass silage on milk fat with emphasis on odd- and branched-chain fatty acids in dairy cows. Journal of Dairy Science 96:390-397. https://doi.org/10.3168/jds.2012-5441

Pereira, L. E. T.; Paiva, A. J.; Geremia, E. V. and Da Silva, S. C. 2014. Components of herbage accumulation in elephant grass cvar Napier subjected to strategies of intermittent stocking management. Journal of Agricultural Science 152:954-966. https://doi.org/10.1017/S0021859613000695

Schwendel, B. H.; Morel, P. C. H.; Wester, T. J.; Tavendale, M. H.; Deadman, C.; Fong, B.; Shadbolt, N. M.; Thatcher, A. and Otter, D. E. 2015. Fatty acid profile differs between organic and conventionally produced cow milk independent of season or milking time. Journal of Dairy Science 98:1411-1425. https://doi.org/10.3168/jds.2014-8322

Schmitt, D.; Padilha, D. A.; Dias, K. M. D.; Santos, G. T.; Rodolfo, G. R.; Zanini, G. D. and Sbrissia, A. F. 2019. Chemical composition of two warm-season perennial grasses subjected to proportions of defoliation. Grassland Science 65:171-178. https://doi.org/10.1111/grs.12236

Souza, J. 2014. Fontes de gordura alteram o desempenho e metabolismo de vacas mantidas em pastagens tropicais. Dissertação (M.Sc.). Universidade de São Paulo, Piracicaba.

Stockdale, C. R.; Walker, G. P.; Wales, W. J.; Dalley, D. E.; Birkett, A.; Shen, Z. and Doyle, P. T. 2003. Influence of pasture and concentrates in the diet of grazing dairy cows on the fatty acid composition of milk. Journal of Dairy Research 70:267-276. https://doi.org/10.1017/S0022029903006009

Vlaeminck, B.; Fievez, V.; Cabrita, A. R. J.; Fonseca, A. J. M. and Dewhurst, R. J. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Animal Feed Science and Technology 131:389-417. https://doi.org/10.1016/j.anifeedsci.2006.06.017

Voltolini, T. V.; Santos, F. A. P.; Martinez, J. C.; Imaizumi, H.; Clarindo, R. L. and Penati, M. A. 2010. Produção e composição do leite de vacas mantidas em pastagens de capim-elefante submetidas a duas frequências de pastejo. Revista Brasileira de Zootecnia 39:121-127. https://doi.org/10.1590/S1516-35982010000100016

Wales, W. J.; Doyle, P. T.; Stockdale, C. R. and Dellow, D. W. 1999. Effects of variations in herbage mass, allowance, and level of supplement on nutrient intake and milk production of dairy cows in spring and summer. Australian Journal of Experimental Agriculture 39:119-130. https://doi.org/10.1071/EA98151

Wolff, R. L.; Bayard, C. C. and Fabien, R. J. 1995. Evaluation of sequential methods for the determination of butterfat fatty acid composition with emphasis on *trans*-18:1 acids. Application to the study of seasonal variations in French butters. Journal of the American Oil Chemists' Society 72:1471-1483. https://doi.org/10.1007/BF02577840