PAB
Pesquisa Agropecuária Brasileira

Animal Science/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Chemical and fatty acid composition of milk from crossbred cows subjected to feed restriction

Deiyse Alves Silva[1], Vicente Ribeiro Rocha Júnior[1 ✉], José Reinaldo Mendes Ruas[1], Pedro Felipe Santana[1], Luana Alcântara Borges[1], Luciana Albuquerque Caldeira[1], Sidnei Tavares dos Reis[2], Jordana Carvalho de Menezes[1] and Dante Pazzanese Duarte Lanna[3]

[1] Universidade Estadual de Montes Claros, Campus de Janaúba, Avenida Reinaldo Viana, no 2.630, Bico da Pedra, CEP 39440-000 Janaúba, MG, Brazil. E-mail: deysealvess@hotmail.com, vicente.rocha@unimontes.br, jrmruas@gmail.com, pedrofelipesantana@hotmail.com, luanaalcantara.vet@hotmail.com, luciana.caldeira@unimontes.br, jordanakarvalhom@hotmail.com

[2] Universidade Federal de Sergipe, Departamento de Agronomia, Campus do Sertão, Rodovia Engenheiro Jorge Neto, Km 03, s/no, Silos, CEP 49680-000 Nossa Senhora da Glória, SE, Brazil. E-mail: streis@uai.com.br

[3] Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Campus Piracicaba, Avenida Pádua Dias, no 11, Caixa Postal 09, CEP 13418-900 Piracicaba, SP, Brazil. E-mail: dplanna@usp.br

✉ Corresponding author

Received
August 9, 2018

Accepted
November 7, 2018

How to cite
SILVA, D.A.; ROCHA JÚNIOR, V.R.; RUAS, J.R.M.; SANTANA, P.F.; BORGES, L.A.; CALDEIRA, L.A.; REIS, S.T. dos; MENEZES, J.C. de; LANNA, D.P.D. Chemical and fatty acid composition of milk from crossbred cows subjected to feed restriction. **Pesquisa Agropecuária Brasileira**, v.54, e000051, 2019. DOI: https://doi.org/10.1590/S1678-3921.pab2019.v54.00051.

Abstract – The objective of this work was to evaluate the chemical composition and fatty acid profile of milk from F1 Holstein/Zebu cows in different lactation periods, when receiving different levels of dietary supply in percentage of body weight. Sixty cows were evaluated, with five levels of dietary supply and three lactation periods. The levels of dietary supply had no effect on the production of milk corrected to 3.5% fat (12.25 kg per day). There was also no effect of dietary supply levels, in the different lactation periods, on contents of fat (3.34%), protein (3.41%), lactose (4.60%), total solids (12.0%), defatted dry extract (8.80%), and urinary nitrogen (14.5 mg dL^{-1}), nor on somatic cell count (89.98 mL^{-1}). As the dietary supply level was reduced, the sum of saturated fatty acids in milk was decreased in up to 9.15% and that of monounsaturated fatty acids was increased in up to 25.28%. Feed restriction does not alter the chemical composition of milk, but improves its quality of fat by reducing saturated fatty acid content, increasing the concentration of monounsaturated and desirable fatty acids in up to 54%, and increasing the hypo- and hypercholesterolemic fatty acid ratio in up to 168.97%.

Index terms: desirable fatty acids, F1 Holstein/Zebu, lactation period, milk quality.

Composição química e de ácidos graxos do leite de vacas mestiças submetidas à restrição alimentar

Resumo – O objetivo deste trabalho foi avaliar a composição química e o perfil de ácidos graxos do leite de vacas F1 Holandês/Zebu, em diferentes períodos de lactação, ao receber diferentes níveis de fornecimento da dieta em percentagem de peso corporal. Foram utilizadas 60 vacas, com cinco níveis de oferta da dieta e três períodos de lactação. Os níveis de oferta não influenciaram a produção de leite corrigida a 3,5% de gordura (12,25 kg por dia). Também não houve efeito dos níveis de oferta, nos diferentes períodos de lactação, sobre os teores de gordura (3,34%), proteína (3,41%), lactose (4,60%), sólidos totais (12,0%), extrato seco desengordurado (8,80%) e nitrogênio urético (14,5 mg dL^{-1}), nem sobre a contagem de células somáticas (89,98 mL^{-1}). À medida que os níveis de oferta foram reduzidos, o somatório dos ácidos graxos saturados do leite diminuiu em até 9,15% e o dos monoinsaturados aumentou em até 25,28%. A restrição alimentar de vacas F1 Holandês/Zebu não altera a composição química do leite, mas melhora a qualidade da sua gordura, ao reduzir os teores de ácidos graxos saturados, aumentar os dos ácidos graxos monoinsaturados e dos ácidos graxos desejáveis em até 54%, e elevar a relação dos ácidos graxos hipo e hipercolesterolêmicos em até 168,97%.

Termos para indexação: ácidos graxos desejáveis, F1 Holandês/Zebu, período de lactação, qualidade do leite.

Introduction

The animal's nutritional level is among the main factors that alter milk production and quality (Barbosa et al., 2012; Nudda et al., 2014). The nutritional restrictions and/or imbalances generally observed in Brazilian breeding systems, besides altering milk production, also decrease its density (Marques et al., 2010) and protein and casein contents (Barbosa et al., 2012; Gabbi et al., 2016). However, these results vary between experiments, and their comparison is difficult due to the diversity and duration of feed restriction (Gabbi et al., 2016).

Limiting an animal's food supply may imply a reduction in the passage rate of the digesta through the gastrointestinal tract, and this provides a longer retention of food for rumen degradation, which may favor a more efficient nutrient use. However, for an effective feed restriction, it is important to consider its magnitude and duration, as well as the lactation stage in which it is applied (Dessauge et al., 2011; Gross et al., 2011; Bjerre-Harpøth et al., 2012).

In this context, the search for animals that are better adapted and have higher productivity is relevant. The Holstein-Zebu crossbred stands out in Brazilian dairy production due to its high capacity for milk production, rusticity, and adaptation to the tropical environment and to low food availability periods, which may favor the production system (Ribeiro et al., 2017). However, it is still necessary to evaluate the impact of feed restriction on crossbred cows, aiming to increase their potential in milk production systems. According to several studies (Souza et al., 2015; Melo et al., 2017), because they are closely related to the adopted production conditions and feeding strategies, milk components may be used to assess the response of these animals to the offered diet.

It should be noted that the milk production system can become more profitable with food restriction due to reductions in food costs by increasing feed efficiency; however, it is important to maintain milk quality for this strategy to work.

The objective of this work was to evaluate the chemical composition and fatty acid profile of milk from F1 Holstein/Zebu cows in different lactation periods, when receiving different levels of dietary supply in percentage of body weight.

Materials and Methods

The study was approved by the ethics committee on animal experimentation and welfare of Universidade Estadual de Montes Claros, located in the state of Minas Gerais, Brazil, under registration number 128/2016. Sixty F1 Holstein/Zebu cows in lactation were evaluated in a completely randomized experimental design, in a 5x3 factorial arrangement, with five dietary supply levels and three lactation periods. The dietary supply levels were: diet supplied ad libitum, allowing 5% leftovers in relation to the amount of dry matter supplied; and diets defined as a percentage (2.75, 2.5, 2.25, and 2.0%) of body weight supplied as dry matter. Twenty cows were used per experimental period, four per dietary supply level.

The mean values of lactation days in each period and their respective standard deviations were: 50.0±12.80 days in the first period; 111.5±11.75 days in the second one; and 183.0±17.5 days in the third. The three experimental periods were 21 days long each, and the first 16 days of each period were for the adaptation of the animals to the dietary level offered, whereas the last 5 days were for data collection and sampling. The experiment lasted 77 days, and, before the first experimental period, all cows received the experimental diet provided ad libitum for 14 days.

The cows were kept in individual 26-m^2 stalls, equipped with troughs and drinking fountains. The diet was prepared with a 75:25 forage:concentrate ratio, on a dry matter basis, and was given to the cows twice daily at 8 a.m. and 3 p.m. in a complete diet system. The forage of the diet was corn silage, and leftovers were weighed daily. The chemical composition of the diet, on a dry matter basis, was 60.85% dry matter, 4.80% mineral matter, 11.00% crude protein, 2.80% ether extract, 32.67% non-fiber carbohydrate, 49.16% neutral detergent fiber crude protein, and 8.65% lignin. The analyses were performed as described in Detmann et al. (2012).

Cows were milked with a mechanical milking machine twice a day, at 7 a.m. and 2 p.m., with the presence of the calf to stimulate the secretion of the milk. Milk samples from each animal were collected twice a day, in the last four days of each period, in proportion to the amount produced in the morning and afternoon. Flasks containing the preservative bronopol received 50 mL of the sample and were sent to the milk clinic at Department of Animal Science of Escola

Superior de Agricultura Luiz de Queiroz (Esalq) of Universidade de São Paulo, located in the municipality of Piracicaba, in the state of São Paulo, Brazil, for analyses of chemical composition and somatic cell count. Milk yields were corrected to 3.5% fat. In order to determine milk chemical characteristics, the following were obtained: percentage of fat, protein content, lactose, defatted dry extract, milk urea nitrogen (MUN), casein by the infrared method, and somatic cell count (SCC) by the flow cytometry method.

Milk samples were sent to the laboratory of animal nutrition and growth of Esalq to determine the profile of fatty acids. Transmethylated samples were analyzed in the Focus GC gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA), with flame ionization detector, CP-Sil 88 capillary column (Agilent Technologies, Santa Clara, CA, USA), 100 μm length, 0.25 μm internal diameter, and 0.20 μm film thickness. The fatty acids were identified by comparing the retention times of the methyl esters of the samples with the fatty acid patterns of butter and were quantified by normalizing the areas of the methyl esters.

The nutritional quality of the milk lipid fraction was evaluated by the fatty acid composition data, using the following equations: atherogenicity index (AI) = {(C12:0+(4×C14:0)+C16:0)}/(Σmonounsaturated fatty acids + Σω6 + Σω3) and thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/{(0.5 × Σmonounsaturated fatty acids) + (0.5 × Σω6 + (3 × Σω3) + (Σω3 /Σω6)}, according to Ulbricth & Southgate (1991); ratio of hypo- to hypercholesterolemic fatty acids (h/H) = (monounsaturated + polyunsaturated)/(C14:0 + C16:0) and desirable fatty acids (DFAs) = (unsaturated + C18:0) (Costa et al., 2008); and ratio of polyunsaturated fatty acids to saturated fatty acids, as the ω6/ω3 ratio (Costa et al., 2008).

The obtained data were tested by the analysis of variance, and, when the F-test was significant, dietary supply levels were subjected to the regression analysis, at 5% probability, and the lactation periods were compared by Tukey's test, also at 5% probability, using the SAS software (SAS Institute Inc., Cary, NC, USA). For the comparison between the control (diet provided ad libitum) and treatments, Dunnett's test was applied, at 5% probability.

Results and Discussion

There was no interaction of dietary supply levels with lactation periods. The dietary supply levels also did not influence the chemical composition and SCC of milk (Table 1), which complied with Normative Instruction 76 (Brasil, 2018) for refrigerated raw milk.

Some studies have shown that, during periods of food shortage or nutritional deficiency, there is a reduction in milk protein content (Zanela et al., 2006; Burke et al., 2010). In addition, energy intake is the primary nutritional factor that influences the percentage and yield of milk protein, depending on the dry matter intake and energy density of the diet (Lean et al., 2013). In this sense, the reduced dietary supply levels feed to the animals possibly maintained the microbial production in the rumen, providing metabolizable protein for protein synthesis in milk, which is satisfactory for the observed level of production. The values obtained for total solids content were similar to those for milk fat and protein (Table 1).

The results for SCC are in alignment with those reported by Zanela et al. (2006), who found no significant difference (393 vs 206 thousand cells per milliliter) between animals with 60 and 100% of their nutritional needs met. The values observed for SCC were within the ranges considered normal for fresh milk; the maximum value recommended is 500 thousand cells per milliliter (Brasil, 2018).

There was also no significant difference for MUN, whose mean was of 14.5 mg dL^{-1} (Table 1). This measure can be an indicator of dietary energy and protein balance (Galvão Júnior et al., 2010), and its mean reference value should be between 12 and 18 mg dL^{-1} (Drudik et al., 2007); mean values above 16 mg dL^{-1}, according to Roseler et al. (1993), would indicate excess protein in the diet, deficiency in the fermentation of non-fiber carbohydrates, and/or imbalance between the availability of energy and nitrogen in the rumen. In the present study, the MUN values were below 16 mg dL^{-1}, which suggests the efficiency of the F1 Holstein/Zebu cows fed different supply levels in the use of dietary nitrogen and, consequently, a lower excretion of nitrogen.

Dietary supply levels did not cause any changes in casein content and percentage in relation to total milk protein. The fact that this milk protein fraction remained stable between treatments indicates that the used diets provided adequate amino acid values

to the milk secreting tissue, since the mean values and percentage of casein in relation to crude protein content are in agreement with those described in the literature, ranging from 2.25 to 2.40% and from 75 to 80%, respectively (Gandra et al., 2010). During lactation periods, casein percentages varied, being higher in the third one, compared with the first (Table 2), which may be justified by physiological changes in the animal metabolism, with a reduction in milk production (Oliveira et al., 2010).

The different levels of dietary supply in relation to the percentage of body weight did not influence milk production corrected to 3.5% fat (p=0.147) (Table 1). This shows that, even under moderate feed restriction, F1 Holstein/Zebus cows are able to maintain the production of milk with a similar chemical quality to that when they are fed ad libitum. A significant effect was observed on milk yield in relation to lactation periods (Table 2). The animals presented greater milk production in the first period (p=0.000), for which a mean value of 13.27 kg milk corrected to 3.5% fat per day was obtained, indicating a superior production to that of the other studied periods. The first period also coincides with the one closest to the peak of lactation for crossbred cows, which, according to Glória et al. (2010), is earlier in F1 Holstein/Zebu.

There was a decreasing linear effect for the sum of saturated fatty acids (SFAs) as the dietary supply levels decreased (Table 3), with a reduction of 9.15% for 2.0% body weight in relation to the diet provided

Table 1. Physical and chemical composition, somatic cell count (SCC), and production of milk from F1 Holstein/Zebu cows fed different dietary supply levels, with respective coefficients of variation (CV) and real p-values (Pr> Fc).

Variable[1]	Dietary supply level (% body weight)					Coefficient of variation (%)	Mean	Pr>Fc
	Ad libitum[2]	2.75	2.50	2.25	2.00			
Fat (%)	3.34	3.01	3.64	3.23	3.37	19.77	Ŷ=3.32	0.140
Protein (%)	3.41	2.98	3.04	3.15	3.21	9.53	Ŷ=3.15	0.252
Lactose (%)	4.69	4.57	4.58	4.54	4.56	4.94	Ŷ=4.60	0.847
Total solids (%)	12.42	11.92	11.81	12.42	11.76	7.33	Ŷ=12.0	0.252
DDE (%)	8.96	8.72	8.90	8.69	8.76	4.88	Ŷ=8.80	0.441
SCC (SC mL^{-1})	101.16	82.33	52.92	39.58	173.91	23.63	Ŷ=1.60	0.128
MUN (mg dL^{-1})	13.51	15.53	14.08	14.77	13.83	13.38	Ŷ=14.5	0.091
Casein (% m m^{-1})	2.61	2.23	2.23	2.42	2.43	11.42	Ŷ=2.30	0.154
PCAS	76.67	74.61	74.44	74.79	75.45	2.82	Ŷ=74.8	0.316
PLCG (kg)	13.02	11.00	11.30	10.72	9.57	17.90	Ŷ=12.3	0.147

[1]DDE, defatted dry extract; MUN, milk urea nitrogen; PCAS, percentage of casein in total protein; and PLCG, milk production corrected to 3.5% fat. [2]Average dry matter intake as a percentage of body weight equal to 3.39%. *Significant differences between mean values and the control (diet supplied ad libitum) by Dunnett's test, at 5% probability.

Table 2. Physical and chemical composition, somatic cell count (SCC), and production of milk from F1 Holstein/Zebu cows in different lactation periods, with respective means, coefficients of variation (CV), and real p-values (Pr> Fc)[1].

Variable[2]	Lactation period (days)[3]			Coefficient of variation (%)	Pr>Fc
	First	Second	Third		
Fat (%)	3.17	3.58	3.20	19.77	0.235
Protein (%)	3.00	3.21	3.27	9.53	0.054
Lactose (%)	4.69	4.61	4.52	4.94	0.173
Total solids (%)	11.83	12.42	11.95	7.33	0.165
DDE (%)	8.66	8.96	8.63	4.88	0.409
SCC (SC mL^{-1})	68.81	73.06	119.68	23.63	0.561
MUN (mg dL^{-1})	15.04a	15.86a	12.12b	13.38	0.000
Casein (%m m^{-1})	2.23b	2.42ab	2.49a	11.42	0.002
PCAS	74.23b	75.37ab	76.42a	2.82	0.016
PLCG (kg)	13.27a	10.97b	9.10b	17.90	0.000

[1]Means followed by different letters in the same row differ significantly by Tukey's test, at 5% probability. [2]DDE, defatted dry extract; MUN, milk urea nitrogen; PCAS, percentage of casein in total protein; and PLCG, milk production corrected to 3.5% fat. [3]Lactation periods: first, 50±12.80 days; second, 111.5±11.75 days; and third, 183±17.25 days.

Table 3. Fatty acid content of milk fat of F1 Holstein/Zebu cows fed different dietary supply levels, with respective regression equations (RE), coefficients of variation (CV), and real p-values (Pr> Fc).

Variable	Dietary supply level (% body weight)					CV	RE	R^2	Pr>Fc
	Ad libitum[1]	2.75	2.50	2.25	2.00	(%)		(%)	
Σ AGS[2]	73.1	72.8	71.7	68.9*	66.4*	4.80	Ŷ=49,01-8,80x	97.4	0.000
C4:0	2.84	2.78	2.78	2.80	2.81	12.5	Ŷ=2.80		0.997
C6:0	1.81	1.78	1.7	1.69	1.57*	8.53	Ŷ=1.1-0.25x	90.6	0.010
C8:0	1.31	1.17	1.21	1.10*	1.05*	11.2	Ŷ=0.68-0.19x	73.1	0.028
C10:0	3.36	2.86	2.82	2.59	2.48	22.7	Ŷ=2.80		0.379
C11:0	0.70	0.07	0.06	0.06	0.05*	26.2	Ŷ=0.01-0.02x	65.5	0.018
C12:0	4.31	3.55	3.99	3.15	3.01*	18.4	Ŷ=-11.68-11.88x-2.29x²	66.6	0.002
C13:0 iso	0.03	0.03	0.03	0.03	0.03	14.5	Ŷ=0.03		0.061
C13:0 anteiso	0.09	0.09	0.08	0.07	0.09*	23.4	Ŷ=0.020-0.025x	68.5	0.030
C13:0	0.14	0.12	0.11*	0.10*	0.90*	19.2	Ŷ=0.04-0.02x	39.9	0.016
C14:0 iso	0.11	0.09	0.09	0.09	0.11	24.9	Ŷ=0.09		0.060
C14:0	12.4	11.0	11	12.4	11.6	10.3	Ŷ=11.7		0.069
C15:0 iso	0.23	0.23	0.23	0.23	0.24	12.2	Ŷ=0.23		0.060
C15:0 anteiso	0.47	0.48	0.45	0.43	0.48	14.0	Ŷ=0.46		0.135
C15:0	0.89	0.89	0.94	0.83	0.87	10.7	Ŷ=0.80-0.02x	82.1	0.048
C16:0 iso	0.36	0.35	0.37	0.36	0.42	23.6	Ŷ=0.37		0.248
C16:0	33.6	36.8	35.1	34.5*	31.7*	10.3	Ŷ=27.34-2.92x	94.3	0.011
C17:0 iso	0.31	0.33	0.36	0.36*	0.40*	12.4	Ŷ=0.54-0.07x	85.6	0.008
C17:0	0.63	0.60*	0.62	0.60*	0.67*	5.76	Ŷ=1.83-0.96x+0.19x²	75.2	0.001
C18:0	9.73	8.75	8.04	8.73	9.19	22.2	Ŷ=8.88		0.541
C20:0	0.12	0.11	0.11	0.11	0.12	26.4	Ŷ=0.12		0.724
C22:0	0.03	0.03	0.03	0.03	0.03	46.4	Ŷ=0.03		0.542
C21:0	0.01	0.00	0.01	0.00	0.01	29.2	Ŷ=0.01		0.356
C23:0	0.01	0.01	0.01	0.01	0.01	48.9	Ŷ=0.01		0.815
C24:0	0.02	0.02	0.02	0.02	0.02	52.4	Ŷ=0.02		0.496
Σ AGMI[3]	24.2	24.5	25.2	28.1*	30.3*	12.1	Ŷ=46.53+8.22x	95.5	0.002
C10:1	0.34	0.35	0.4	0.38	0.35	23.2	Ŷ =0.36		0.535
C12:1	0.08	0.08	0.08	0.08	0.9	45.6	0.08		0.016
C14:1 c9	1.28	1.34	1.34	1.36	1.27	23.6	Ŷ=1.32		0.901
C16:1 c9	2.01	2.28	2.28	2.39	2.29	17.4	Ŷ= 2.25		0.893
C18:1 trans	1.15	1.26	1.26	1.32	1.4	20.4	Ŷ=1.28		0.597
C18:1 c9	16.1	16.1	16.1	18.7	20.8*	14.1	Ŷ=33.85+6.67x	92.9	0.001
C18:1 c11	2.07	1.90	1.90	1.52*	1.48*	15.9	Ŷ=0.63-0.42x	99.6	0.000
C18:1 c12	0.89	0.83	0.83	0.67*	0.66*	17.2	Ŷ=0.31-0.17x	96.6	0.000
C18:1 c13	0.50	0.46	0.46	0.42*	0.38*	13.6	Ŷ=0.24-0.07x	96.3	0.005
C18:1 t16	0.26	0.22	0.22	0.22	0.24	19.5	Ŷ=0.23		0.530
C18:1 c15	0.07	0.06*	0.06*	0.07	0.06*	27.4	Ŷ=0.13+0.02x	78.9	0.024
C20:1	0.06	0.07	0.07	0.06	0.06	29.2	Ŷ=0.06		0.413
C22:1 n9	0.00	0.00	0.00	0.01	0.00	77.3	Ŷ=0.00		0.274
C24:1	0.01	0.01	0.01	0.01	0.01	62.4	Ŷ=0.01		0.682
Σ AGPI[4]	1.83	1.91	1.91	2.11	2.19	15.2	Ŷ=1.9		0.073
C18:2 c9 c12	1.37	1.26	1.32	1.24	1.26	15.8	Ŷ=1.2		0.449
C18:3 n6	0.01	0.01	0.01	0.01	0.01	37.7	Ŷ=0.01		0.840
C18:3 n3	0.15	0.15	0.15	0.18	0.18*	19.2	Ŷ=0.29+0.05x	82.5	0.008
C18:2 c9 t11 (CLA)[5]	0.25	0.44	0.44	0.34	0.34	15.9	Ŷ=0.36		0.059
C18:2 t10c12	0.00	0.00	0.00	0.00	0.00	693	Ŷ=0.00		0.404
C20:2	0.00	0.00	0.00	0.00	0.00	142	Ŷ=0.00		0.353
C20:3 n6	0.03	0.03	0.03	0.04	0.04	33.8	Ŷ=0.03		0.631
C20:3 n3	0.05	0.07	0.06	0.06	0.07	21.7	Ŷ=0.06		0.044
C20:4 n6	0.01	0.02	0.02	0.02	0.02	29.9	Ŷ=0.02		0.105
C22:2	0.00	0.00	0.00	0.00	0.00	311	Ŷ=0.00		0.393
C20:5 n3	0.01	0.01	0.01	0.01	0.01	47.8	Ŷ=0.01		0.069
C22:5	0.00	0.00	0.00	0.00	0.00	30.7	Ŷ=0.00		0.065
C22:6 n3	0.00	0.00	0.00	0.00	0.00	123	Ŷ=0.00		0.058

[1]Average dry matter intake as a percentage of body weight equal to 3.39%. [2]Saturated fatty acids. [3]Monounsaturated fatty acids. [4]Polyunsaturated fatty acids. [5]CLA, conjugated linoleic acid. *Significant differences between mean values and the control (diet supplied ad libitum) by Dunnett's test, at 5% probability. R^2, coefficient of determination.

ad libitum. The sum of SFAs was higher for the diet provided ad libitum, when compared with the levels of 2.0 and 2.25% body weight. The highest percentages of SFAs were found for palmitic acid (C16:0) and myristic acid (C14:0). It was observed that the concentrations of the SFAs C6:0, C8:0, C11:0, C13:0, and C16:0 had a decreasing linear effect, with a reduction of 13.25, 19.84, 28.57, 35.71, and 5.68%, respectively, for 2.0% body weight dietary supply in relation to the diet provided ad libitum. The SFA C17:0 iso increased with the reduction of supply levels, whereas C12:0 and C17:0 presented a quadratic effect, with minimum and maximum points of 2.59 and 2.56%, respectively (Table 3). Mourthé et al. (2015) verified a decrease in the sum of odd- and branched-chain fatty acids in the milk of cows receiving diets with increasing levels of soybean [*Glycine max* (L.) Merr.] grain. According to Vlaeminck et al. (2006), these fatty acids are mainly derived from the ruminal microorganisms that synthesize them after changes in dietary lipid biosynthesis and may be an indicative of modifications in the ruminal microbiota population. The obtained results show that the reduction in the dietary supply levels decreased the rumen passage rate and the microbial profile of the rumen, and, consequently, the content that reaches the small intestine.

The decrease in dietary supply levels also reduced linearly the intake of dry matter and nutrients; for dry matter intake, the mean values were 16.8, 12.94, 11.63, 10.16, and 8.75 kg per day for 2.75, 2.5, 2.25, and 2.0% body weight, respectively. A lower feed intake due to a quantitative restriction in food supply altered the energy balance and, therefore, reduced the content of SFAs in milk. It should be highlighted that studies have sought to decrease the levels of saturated medium-chain fatty acids in milk (Santos et al., 2013; Lanier & Corl, 2015) because they are related to an increased risk of cardiovascular diseases. Between the first and second lactation periods, the SFA content did not differ. The last lactation period showed a mean value similar to that of the second one but higher than that of the first (Table 4). The SFAs C10:0, C13:0 iso, C15:0 anteiso, C16:0, C17:0, and C21:0 had higher levels in the first two lactation periods (Table 4). At the beginning of lactation, an imbalance in energy flow may cause the mobilization of fat reserves, strongly influencing the fatty acid profile (Lanier & Corl, 2015). The lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids, as well as trans fatty acids, have been epidemiologically associated with cardiovascular diseases because they induce increased blood cholesterol (Santos et al., 2013). The contents found for these acids in the present study – except for the fatty acid C14:0, which was maintained – were reduced linearly with the decrease in dietary supply.

Unlike SFAs, long-chain, mono-, and polyunsaturated fatty acids contribute to the increase of high-density blood cholesterol (Santos et al., 2013). Regarding beneficial effects, stand out the oleic unsaturated fatty acid (C18:1 *cis*-9) and the conjugated linoleic acid isomers, related to cholesterol reduction and anticarcinogenic effects, respectively (Haug et al., 2007).

The sum of the monounsaturated fatty acids (MUFAs) showed an increasing linear effect with reducing levels of dietary supply. The levels of 2.0 and 2.5% body weight differed from the ad libitum diet (Table 3); there was a 25.28% increase for 2.0% body weight in relation to the control treatment. C18:1 *cis*-9, one of the isomers of oleic acid, contributed the most to the total of MUFAs among levels and presented an increasing linear effect with the reduction of the level of dietary supply. Anti-cholesterol effects are attributed to oleic acid (C18:1 c9), which makes it important under the nutritional view of milk (Lanier & Corl, 2015). As for lactation periods, the first and second did not differ regarding the sum of MUFAs; the exception was the last one, with a lower value (Table 4). The MUFAs C18:1 c9, C18:1 c11, C18:1 c12, and C18:1 c13 showed an increasing linear effect with the reduction of supply levels, with an increase of 54.66, 28.50, 25.0, and 24%, respectively, for 2.0% body weight in relation to the diet provided ad libitum (Table 3).

Among the polyunsaturated fatty acids (PUFAs) of milk, only C18:3 n-3 varied according to the level of dietary supply ($p>0.05$) (Table 3), showing a linear increase with the decrease in supply levels and differing between 2.0 and 2.25% body weight and the diet provided ad libitum. The PUFAs present in milk fat are derived from fatty acids in the blood plasma, which originate from free fatty acids from the mobilization of body fat and dietary fatty acids transported as triglycerides by very low-density

Table 4. Fatty acid profile of milk fat of Holstein/Zebu cows in different lactation periods, with respective means, coefficients of variation, and real p-values (Pr>Fc)[1].

Fatty	Lactation period (days)[2]			Coefficient of variation (%)	Pr>Fc
	First	Second	Third		
Σ AGS[3]	67.85b	70.45ab	71.50a	4.88	0.011
C4:0	2.83	2.78	2.76	12.49	0.847
C6:0	1.75	1.67	1.75	8.53	0.064
C8:0	1.13	1.19	1.08	11.21	0.074
C10:0	2.42b	3.00a	2.65ab	22.68	0.035
C11:0	0.06	0.06	0.06	26.5	0.588
C12:0	3.24	3.33	3.70	18.36	0.100
C13:0 iso	0.02b	0.03a	0.03a	14.49	0.001
C13:0 anteiso	0.08	0.08	0.08	23.39	0.900
C13:0	0.10	0.10	0.10	19.24	0.360
C14:0 iso	0.10	0.09	0.10	24.99	0.340
C14:0	11.05	11.4	12.04	10.32	0.072
C15:0 iso	0.23	0.24	0.24	12.25	0.512
C15:0 anteiso	0.42b	0.49a	0.48a	14.00	0.007
C15:0	0.85	0.88	0.91	10.70	0.238
C16:0 iso	0.34	0.39	0.39	23.59	0.211
C16:0	33.01b	34.19ab	36.42a	10.32	0.031
C17:0 iso	0.38	0.36	0.35	12.45	0.195
C17:0	0.60b	0.64a	0.63a	5.76	0.011
C18:0	8.29	9.04	8.71	22.19	0.538
C20:0	0.10	0.11	0.12	26.40	0.226
C22:0	0.03	0.03	0.03	46.42	0.404
C21:0	0.00b	0.01a	0.01a	29.24	0.002
C23:0	0.10	0.10	0.10	48.90	0.092
C24:0	0.10	0.10	0.10	52.36	0.041
Σ AGMI[4]	28.70a	26.61ab	25.69b	12.09	0.040
C10:1	0.34	0.38	0.38	23.2	0.361
C12:1	0.09	0.09	0.09	45.64	0.073
C14:1 c9	1.17	1.37	1.44	23.61	0.06
C16:1c9	2.20	2.37	2.36	17.38	0.394
C18:1 trans	1.47a	1.18b	1.28ab	20.4	0.012
C18:1 c9	19.35a	17.85ab	16.82b	14.06	0.020
C18:1 c11	2.06a	1.64b	1.65b	15.98	0.000
C18:1 c12	0.90a	0.72b	0.69b	17.16	0.000
C18:1 c13	0.50a	0.42b	0.40b	13.57	0.000
C18:1 t16	0.23	0.22	0.22	19.52	0.800
C18:1 c15	0.07	0.07	0.07	27.36	0.720
C20:1	0.06b	0.08a	0.06b	29.24	0.000
C22:1n9	0.00b	0.00b	0.10a	77.28	0.000
C24:1	0.10	0.10	0.10	62.38	0.060
Σ AGPI[5]	2.12	2.02	1.95	15.16	0.270
C18:2 c9 c12	1.35	1.33	1.22	15.79	0.150
C18:3 n6	0.01	0.01	0.02	37.75	0.060
C18:3 n3	0.15	0.17	0.17	19.2	0.130
C18:2 c9 t11	0.44a	0.33b	0.39ab	15.98	0.029
C18:2 t10c12	0.00	0.00	0.00	692.82	0.370
C20:2	0.00	0.00	0.00	142.15	0.420
C20:3 n6	0.3	0.4	0.4	33.85	0.890
C20:3 n3	0.06b	0.07a	0.07a	21.71	0.250
C20:4 n6	0.01b	0.02a	0.02a	29.96	0.000
C22:2	0.06b	0.07a	0.07a	311.26	0.010
C20:5 n3	0.01	0.10	0.10	47.78	0.060
C22:5	0.04	0.04	0.03	30.69	0.110
C22:6 n3	0.00	0.00	0.00	123.23	0.700

[1]Mean values followed by different letters in the same row differ significantly by Tukey's test, at 5% probability. [2]Lactation periods: first, 50±12.80 days; second, 111.5±11.75 days; and third, 183±17.25 days. [3]Saturated fatty acids. [4]Monounsaturated fatty acids. [5]Polyunsaturated fatty acids.

lipoproteins (O'Donnell-Megaro et al., 2011; Lanier & Corl, 2015). As PUFAs are not synthesized by ruminant tissues, their concentration in milk is determined by the amount that reaches the duodenum (Chilliard et al., 2007).

Table 5 shows the indices indicating the nutritional quality of the lipid profile related to human health. The AI and TI indices presented a linear decrease as a function of the reduction in supply levels, decreasing in 37.81 and 75.83% for the level of 2% body weight in relation to the diet provided ad libitum. Dietary supply levels of 2.25 and 2.0% body weight for AI and all levels for TI differed from the diet provided ad libitum. The highest TI values coincide with the first and second lactation periods (Table 6). It should be noted that lower AI and TI values express a fatty acid ratio more favorable to human health.

The ratio of hypo- to hypercholesterolemic fatty acids presented an increasing linear effect with the reduction in dietary supply levels. Levels of 2.50, 2.25, and 2.0% body weight differed from the control treatment. According to the obtained results, the concentration of DFAs in milk increased linearly with the reduction of dietary supply levels (Table 5). The levels of 2.25 and 2.0% body weight differed from the diet provided ad libitum. The PUFAs/SFAs ratio in milk was not altered by dietary supply levels; however, all studied levels differed from the diet ad libitum (Table 5). The ω6/ω3 ratio was also not affected by dietary supply levels, with a mean of 0.28, but was influenced by lactation periods; as lactation progressed, it increased (Table 6), but with values below 4.0%, suggesting desirable amounts in the diet for the prevention of cardiovascular diseases (Haug et al., 2007).

Table 5. Atherogenicity index (AI), thrombogenicity index (TI), hypo- and hypercholesterolemic ratio (h/H), desirable fatty acids (DFAs), polyunsaturated fatty acids/saturated fatty acids ratio (PUFAs/SFAs), and ω6/ω3 ratio of milk from F1 Holstein/Zebu cows fed different dietary supply levels, with their respective regression equations (RE), coefficients of variation (CV), and real p-values (Pr>Fc).

Variable	Dietary supply level (% body weight)					CV (%)	RE	R^2 (%)	Pr>Fc
	Ad libitum[1]	2.75	2.50	2.25	2.00				
AI	4.76	3.6	3.56	2.96*	2.63*	20.05	Ŷ=-0.142-1.40x	91.93	0.001
TI	2.62	2.04*	1.91*	1.71*	1.49*	21.00	Ŷ=0.038-0.73x	98.88	0.006
h/H ratio	0.29	0.55	0.58*	0.68*	0.78*	18.95	Ŷ=1.395+0.31x	95.07	0.000
DFAs	27.09	35.11	35.13	38.89*	41.72*	10.90	Ŷ=60.13+9.44x	90.08	0.001
PUFAs/SFAs ratio	0.02	0.03*	0.03*	0.03*	0.03*	18.05	Ŷ=0.052+0.009x	91.97	0.008
ω6/ω3 ratio	0.29	0.30	0.28	0.27	0.26	30.82	Ŷ=0.28	-	0.732

[1]Average dry matter intake as a percentage of body weight equal to 3.39%. *Significant difference between mean values and the control (diet supplied ad libitum) by Dunnett's test, at 5% probability. R^2, coefficient of determination.

Table 6. Atherogenicity index (AI), thrombogenicity index (TI), hypo- and hypercholesterolemic ratio (h/H), desirable fatty acids (DFAs), polyunsaturated fatty acids/saturated fatty acids ratio (PUFAs/SFAs), and ω6/ω3 ratio of milk from F1 Holstein/Zebu cows in different lactation periods, with their respective means, coefficients of variation, and real p-values (Pr>Fc)[1].

Variable	Lactation period (days)[2]			Coefficient of variation (%)	Pr>Fc
	First	Second	Third		
AI	2.93	3.28	3.35	20.05	0.150
TI	1.62b	1.78ab	1.97a	21.00	0.039
h/H ratio	0.72	0.63	0.59	18.95	0.009
DFAs	47.28a	37.67b	40.20ab	10.90	0.040
PUFAs/SFAs ratio	0.30	0.30	0.30	18.05	0.079
ω6/ω3 ratio	0.22 b	0.27ab	0.34a	30.82	0.010

[1]Mean values followed by different letters in the same row differ significantly by Tukey's test, at 5% probability. [2]Lactation periods: first, 50±12.80 days; second, 111.5±11.75 days; and third, 183±17.25 days.

Conclusions

1. The feed restriction of F1 Holstein/Zebu cows, with a dry matter supply level of 2% body weight, regardless of the lactation period, may be a nutritional strategy, since it does not modify the chemical composition of milk, but improves its quality of fat, reducing saturated fatty acid content and increasing monounsaturated fatty acids, desirable fatty acids, and the ratio of hypo- to hypercholesterolemic fatty acids.

2. With increased lactation in F1 Holstein/Zebu cows, there is an increase in the sum of saturated fatty acids and a decrease in monounsaturated fatty acids in milk, as well as a decrease in urea nitrogen and an increase in casein contents.

Acknowledgments

To Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), for financial support; to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), and to Fazenda Experimental de Felixlândia (FEFX) of Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig), for scholarships; and to Instituto Nacional de Ciência e Tecnologia – Ciência Animal (INCT-CA), for support.

References

BARBOSA, R.S.; FISCHER, V.; RIBEIRO, M.E.R.; ZANELA, M.B.; STUMPF, M.T.; KOLLING, G.J.; SCHAFHÄUSER JÚNIOR, J.; BARROS, L.E.; EGITO, A.S. do. Caracterização eletroforética de proteínas e estabilidade do leite em vacas submetidas à restrição alimentar. **Pesquisa Agropecuária Brasileira**, v.47, p.621-628, 2012. DOI: https://doi.org/10.1590/S0100-204X2012000400019.

BJERRE-HARPØTH, V.; FRIGGENS, N.C.; THORUP, V.M.; LARSEN, T.; DAMGAARD, B.M.; INGVARTSEN, K.L.; MOYES, K.M. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. **Journal of Dairy Science**, v.95, p.2362-2380, 2012. DOI: https://doi.org/10.3168/jds.2011-4419.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 76, de 26 de novembro de 2018. [Aprova os Regulamentos Técnicos que fixam a identidade e as características de qualidade que devem apresentar o leite cru refrigerado, o leite pasteurizado e o leite pasteurizado tipo A]. **Diário Oficial da União**, 30 nov. 2018. Seção 1. p.9-13.

BURKE, C.R.; WILLIAMS, Y.J.; HOFMANN, L.; KAY, J.K.; PHYN, C.V.C.; MEIER, S. Effects of an acute feed restriction at the onset of the seasonal breeding period on reproductive performance and milk production in pasture-grazed dairy cows. **Journal of Dairy Science**, v.93, p.1116-1125, 2010. DOI: https://doi.org/10.3168/jds.2009-2562.

CHILLIARD, Y.; GLASSER, F.; FERLAY, A.; BERNARD, L.; ROUEL, J.; DOREAU, M. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. **European Journal of Lipid Science and Technology**, v.109, p.828-855, 2007. DOI: https://doi.org/10.1002/ejlt.200700080.

COSTA, R.G.; MESQUITA, I.V.U.; QUEIROGA, R. de C.R. do E.; MEDEIROS, A.N. de; CARVALHO, F.F.R. de; BELTRÃO FILHO, E.M. Características químicas e sensoriais do leite de cabras Moxotó alimentadas com silagem de maniçoba. **Revista Brasileira de Zootecnia**, v.37, p.694-702, 2008. DOI: https://doi.org/10.1590/S1516-35982008000400016.

DESSAUGE, F.; LOLLIVIER, V.; PONCHON, B.; BRUCKMAIER, R.; FINOT, L.; WIART, S.; CUTULLIC, E.; DISENHAUS, C.; BARBEY, S.; BOUTINAUD, M. Effects of nutrient restriction on mammary cell turnover and mammary gland remodeling in lactating dairy cows. **Journal of Dairy Science**, v.94, p.4623-4635, 2011. DOI: https://doi.org/10.3168/jds.2010-4012.

DETMANN, E.; SOUZA, M.A. de; VALADARES FILHO, S. de C.; QUEIROZ, A.C. de; BERCHIELLI, T.T.; SALIBA, E. de O.S.; CABRAL, L. da S.; PINA, D. dos S.; LADEIRA, M.M.; AZEVEDO, J.A.G. (Ed.). **Métodos para análise de alimentos**. Visconde do Rio Branco: Suprema, 2012. 214p.

DRUDIK, D.; KEOWN, J.F.; KONONOFF, P.J. **Milk urea nitrogen testing**. Lincoln: IANR, University of Nebraska, 2007.

GABBI, A.M.; MCMANUS, C.M.; ZANELA, M.B.; STUMPF, M.T.; BARBOSA, R.S.; FRUSCALSO, V.; THALER NETO, A.; SCHMIDT, F.A.; FISCHER, V. Milk traits of lactating cows submitted to feed restriction. **Tropical Animal Health and Production**, v.48, p.37-43, 2016. DOI: https://doi.org/10.1007/s11250-015-0916-2.

GALVÃO JÚNIOR, J.G.B.; RANGEL, A.H. do N.; MEDEIROS, H.R. de; SILVA, J.B.A. da; AGUIAR, E.M. de; MADRUGA, R.C.; LIMA JÚNIOR, D.M. de. Efeito da produção diária e da ordem de parto na composição físico-química do leite de vacas de raças zebuínas. **Acta Veterinaria Brasilica**, v.4, p.25-30, 2010. DOI: https://doi.org/10.21708/avb.2010.4.1.1452.

GANDRA, J.R.; RENNÓ, F.P.; FREITAS JÚNIOR, J.E. de; SANTOS, M.V. dos; SILVA, L.F.P. e; ARAÚJO, A.P.C. de. Productive performance and milk protein fraction composition of dairy cows supplemented with sodium monensin. **Revista Brasileira Zootecnia**, v.39, p.1810-1817, 2010. DOI: https://doi.org/10.1590/S1516-35982010000800025.

GLÓRIA, J.R. da; BERGMANN, J.A.G.; QUIRINO, C.R.; RUAS, J.R.M.; MATOS, C.R.A. de; PEREIRA, J.C.C. Curvas de lactação de quatro grupos genéticos de mestiças Holandês-Zebu. **Revista Brasileira de Zootecnia**, v.39, p.2160-2165, 2010. DOI: https://doi.org/10.1590/S1516-35982010001000009.

GROSS, J.; VAN DORLAND, H.A.; BRUCKMAIER, R.M.; SCHWARZ, F.J. Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. **Journal of Dairy Science**, v.94, p.1820-1830, 2011. DOI: https://doi.org/10.3168/jds.2010-3707.

HAUG, A.; HØSTMARK, A.T.; HARSTAD, O.M. Bovine milk in human nutrition – a review. **Lipids in Health and Disease**, v.6, art.25, 2007. DOI: https://doi.org/10.1186/1476-511X-6-25.

LANIER, J.S.; CORL, B.A. Challenges in enriching milk fat with polyunsaturated fatty acids. **Journal of Animal Science and Biotechnology**, v.6, art.26, 2015. DOI: https://doi.org/10.1186/s40104-015-0025-0.

LEAN, I.J.; VAN SAUN, R.; DEGARIS, P.J. Energy and protein nutrition management of transition dairy cows. **Veterinary Clinics of North America: Food Animal Practice**, v.29, p.337-366, 2013. DOI: https://doi.org/10.1016/j.cvfa.2013.03.005.

MARQUES, L.T.; FISCHER, V.; ZANELA, M.B.; RIBEIRO, M.E.R.; STUMPF JUNIOR, W.; MANZKE, N. Fornecimento de suplementos com diferentes níveis de energia e proteína para vacas Jersey e seus efeitos sobre a instabilidade do leite. **Revista Brasileira de Zootecnia**, v.39, p.2724-2730, 2010. DOI: https://doi.org/10.1590/S1516-35982010001200024.

MELO, M.T.P. de; ROCHA JÚNIOR, V.R.; CALDEIRA, L.A.; PIMENTEL, P.R.S.; REIS, S.T. dos; JESUS, D.L.S. de. Cheese and milk quality of F1 Holstein x Zebu cows fed different levels of banana peel. **Acta Scientiarum. Animal Sciences**, v.39, p.181-187, 2017. DOI: https://doi.org/10.4025/actascianimsci.v39i2.33883.

MOURTHÉ, M.H.F.; REIS, R.B.; GAMA, M.A.S.; BARROS, P.A.V.; ANTONIASSI, R.; BIZZO, H.R.; LOPES, F.C.F. Perfil de ácidos graxos do leite de vacas Holandês x Gir em pastagem de capim-marandu suplementado com quantidades crescentes de grão de soja tostado. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.67, p.1150-1158, 2015. DOI: https://doi.org/10.1590/1678-4162-7489.

NUDDA, A.; BATTACONE, G.; BOAVENTURA NETO, O.; CANNAS, A.; FRACESCONI, A.H.D.; ATZORI, A.S.; PULINA, G. Feeding strategies to design the fatty acid profile of sheep milk and cheese. **Revista Brasileira de Zootecnia**, v.43, p.445-456, 2014. DOI: https://doi.org/10.1590/S1516-35982014000800008.

O'DONNELL-MEGARO, A.M.; BARBANO, D.M.; BAUMAN, D.E. Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. **Journal of Dairy Science**, v.94, p.59-65, 2011. DOI: https://doi.org/10.3168/jds.2010-3571.

OLIVEIRA, E.N.A. de; SANTOS, D. da C.; OLIVEIRA, A. da S.; SOUSA, F.C. de. Composição físico-química de leites em diferentes fases de lactação. **Revista Acadêmica: Ciências Agrárias e Ambientais**, v.8, p.409-415, 2010. DOI: https://doi.org/10.7213/cienciaanimal.v8i4.10982.

RIBEIRO, L.S.; GOES, T.J.F.; TORRES FILHO, R.A.; ARAÚJO, C.V.; REIS, R.B.; SATURNINO, H.M. Desempenhos produtivo e reprodutivo de um rebanho F1 Holandês x Gir em Minas Gerais. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.69, p.1624-1634, 2017. DOI: https://doi.org/10.1590/1678-4162-9076.

ROSELER, D.K.; FERGUSON, J.D.; SNIFFEN, C.J.; HERREMA, J. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. **Journal of Dairy Science**, v.76, p.525-534, 1993. DOI: https://doi.org/10.3168/jds.S0022-0302(93)77372-5.

SANTOS, R.D.; GAGLIARDI, A.C.M.; XAVIER, H.T.; MAGNONI, C.D.; CASSANI, R.; LOTTENBERG, A.M.P; CASELLA FILHO, A.; ARAÚJO, D.B.; CESENA, F.Y.; ALVES, R.J.; FENELON, G.; NISHIOKA, S.A.D.; FALUDI, A.A.; GELONEZE, B.; SCHERR, C.; KOVACS, C.; TOMAZZELA, C.; CARLA, C.; BARRERA-ARELLANO, D.; CINTRA, D.; QUINTÃO, E.; NAKANDAKARE, E.R.; FONSECA, F.A.H.; PIMENTEL, I.; SANTOS, J.E.; BERTOLAMI, M.C.; ROGERO, M.; IZAR, M.C.O.; NAKASATO, M.; DAMASCENO, N.R.T.; MARANHÃO, R.; CASSANI, R.S.L.; PERIM, R.; RAMOS, S. I diretriz sobre o consumo de gorduras e saúde cardiovascular. **Arquivos Brasileiros de Cardiologia**, v.100, p.1-40, 2013. Suplemento 3. DOI: https://doi.org/10.5935/abc.2013S003.

SOUZA, V.M. de; ROCHA JÚNIOR, V.R.; CALDEIRA, L.A.; ANTUNES, A.P. da S.; RUAS, J.R.M.; SOUZA, C.F.; AGUIAR, A.C.R. de; LANNA, D.P.D. Physicochemical composition and fatty acid profile of milk from F1 Holstein x Zebu cows fed with increasing concentrations of urea. **Semina: Ciências Agrárias**, v.36, p.4435-4446, 2015. Suplemento 2. DOI: https://doi.org/10.5433/1679-0359.2015v36n6Supl2p4435.

ULBRICHT, T.L.V.; SOUTHGATE, D.A.T. Coronary heart disease: seven dietary factors. **The Lancet**, v.338, p.985-992, 1991. DOI: https://doi.org/10.1016/0140-6736(91)91846-M.

VLAEMINCK, B.; FIEVEZ, V.; TAMMINGA, S.; DEWHURST, R.J.; VAN VUUREN, A.; DE BRABANDER, D.; DEMEYER, D. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. **Journal of Dairy Science**, v.89, p.3954-3964, 2006. DOI: https://doi.org/10.3168/jds.S0022-0302(06)72437-7.

ZANELA, M.B.; FISCHER, V.; RIBEIRO, M.E.R.; BARBOSA, R.S.; MARQUES, L.T.; STUMPF JUNIOR, W.; ZANELA, C. Leite instável não-ácido e composição do leite de vacas Jersey sob restrição alimentar. **Pesquisa Agropecuária Brasileira**, v.41, p.835-840, 2006. DOI: https://doi.org/10.1590/S0100-204X2006000500016.