

GRAZING HEIGHT OF XARAÉS GRASS IMPROVE *LONGISSIMUS* MUSCLE FATTY ACIDS PROFILE OF NELLORE CATTLE

ALTURA DE PASTAGEM DE CAPIM XARAÉS MELHORA O PERFIL DE ÁCIDOS GRAXOS DO MÚSCULO *LONGISSIMUS* DE BOVINOS DA RAÇA NELLORE

LA ALTURA DE PASTOREO DEL PASTO XARAÉS MEJORA EL PERFIL DE ÁCIDOS GRASOS DEL MÚSCULO *LONGISSIMUS* DEL GANADO NELLORE

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Abstract

Differences in chemical composition and fatty acid profile of *Longissimus* muscle in Nellore cattle under different grazing heights (15, 30, 45 and 60 cm) of xaraés grass were tested. *Longissimus* muscle sample was collected 24 h after slaughter and the chemical (moisture, mineral residue, protein and total lipids) and fatty acids composition was quantified. Only fatty acid profiles were different on percentages of lauric (12:0), oleic (18:1n9c) and eicosadienoic acid (20:2), being best in height of 30 cm grazing (0.36%, 0.85% 39.7% and 1.83%, respectively); myristic (14:0) and conjugated linoleic acid (C18:2,c9-t11) presented better value for the height of 60 cm (4.04% and 0.23%, respectively). Percentage of omega-3 (n-3) and omega-6 (n-6) were better at 30 cm grazing, with values of 2.61% and 8.32%, respectively. Grazing height between 30 and 45 cm improves the levels of fatty acids, important to human healthy, in *Longissimus* muscle of Nellore cattle.

Keywords: *Brachiariabrizantha*, cattle management, grass, health, meat.

Chemical compounds studied in this article:

Lauric Acid (PubChem CID: 3893); Myristic Acid (PubChem CID: 11005); Conjugated Linoleic Acid (PubChem CID: 74607); Eicosadienoic Acid (PubChem CID: 3208); Oleic Acid (PubChem CID: 445639).

INTRODUCTION

Brazil have 170 million hectares of grasslands, 100 million are cultivated pastures (FONSECA et al., 2010) and 80% of cultivated pastures is formed by the genus *Brachiaria*(VALLE et al., 2001; MACEDO et al., 2014). Grazing height directly influences animal selectivity on pasture and feeding behavior for situations of low grazing (overgrazing) hamper the animal selectivity, reducing the quality of the ingested diet (LALA et al., 2018). High pasture (sub-grazing) disfavor forage quality, because the older the plant, the greater its lignification, and the lower its nutritional value (BARBOSA et al., 2013).

As the plant reaches maturity, there is an increase in fiber content, stem elongation and a decrease in the proportion of leaves, besides the increase in the content of triacylglycerols in the seeds, and the lipid content decreases determining reduction in the fatty acids content, mainly polyunsaturated (DEWHURST et al., 2001). Thus, situations of grazing height with balance between productivity and quality are necessary to sustainable production system.

The way the forage is provided to the animals (fresh, ensiled or baled) is crucial in the proportion of polyunsaturated fatty acids in meat. Fresh forage has a higher concentration of linoleic acid (n-3), while grains are rich in linoleic acid (n-6) (FRENCH et al., 2000; ENSER, 2001; GARCIA et al., 2008; BRESSAN et al., 2011). Fat of ruminant is natural source of these fatty acids and intramuscular fat of grazing animals had a higher concentration of total CLA and CLA isomers cis-9, trans-11 than animals fed concentrate (5.3 compared to 2.5 and 4.1 to 2.3 mg CLA g⁻¹ lipid, respectively) (REALINI et al., 2004).

Some fatty acids, particularly polyunsaturated, are used as feedstock for substances that regulate immunity, contraction of the vessels, blood pressure and hormones production (KUSS et al., 2007). Alteration in fatty acid profile is interesting to human health from the point of view of reducing the risk of coronary heart disease, whereas saturated fatty acids are hypercholesterolemic and the less percentage is better (WILLIAMS, 2000). According to FAO (2008) higher proportions of omega-3 and polyunsaturated fatty acids in the diet of humans is important to prevent the onset of coronary heart disease, autoimmune diseases, breast cancer, prostate and colon cancer and rheumatoid arthritis.

England Department of Health (HMSO, 1994) recommends that the quantity consumed must be less than four parts of omega-6 to omega-3 for human consumption. However, there are few studies available that allow characterizing the beef produced on pasture and the link between grazing heights

and fatty acids profile of meat. The aim of this study was to identify the grazing height that improves the profile of *Longissimus* muscle fatty acids in Nellore cattle, from the perspective of human health.

MATERIAL AND METHODS

Local, animals and management

The experiment was lead in CidadeGaúcha city, Paraná, southern Brazil, Cfa climate (KÖPPEN; GEIGER, 1928), sandstone Caiuá(Embrapa, 2006), using the *Longissimus* muscle of 72 Nellore cattle with an average weight of 483 ± 34.14 kg and slaughter age of 23 ± 0.9 months, which were assigned to four treatments (15, 30, 45 and 60 cm grazing heights of *Brachiariabrizantha* cv. xaraés – xaraés grass).

We utilized three paddocks of 1.0 ha, for each treatment. In every treatment were 9 animals (repetitions) total. The experiment was conducted over 9 months with no effect of time neither location within the paddock, since the animals had free access for the 3 hectares. Animals height regulators were also used (MOTT; LUCAS, 1952). Average weight of the animals at the beginning of the experiment was 335 ± 25.4 kg and age of 15 ± 0.9 months. Animals were weighed at the beginning of the experiment and on the day of slaughter, after 12 hours of solid fasting.

Slaughter and analysis

The slaughter was preceded by stunning with air gun penetration, and bled immediately after stunning by cutting the great vessels, following the standards of humane slaughter (BRASIL, 2000) in commercial slaughterhouse, 4.5 km far from the farm. After slaughter, five samples were taken from each animal, representing replicates within each experimental unit, totaling 45 replicates per treatment. Each replicate was analyzed in triplicate, totalizing, in this way, 135 analyzes of *Longissimus* muscle per treatment. Chemical composition analysis for the determination of moisture, mineral residue and protein of samples was performed according to the methodology of AOAC (AOAC, 2012).

Extraction of total lipids was carried out using the cold technique described by Bligh and Dyer (1959). Samples were submitted to transesterification of triglycerides by the technique of (HARTMAN; LAGO, 1973). The fatty acid methyl esters were analyzed by gas chromatography (Trace GC Ultra, Thermo Scientific, EUA) auto sampler equipped with a flame ionization detector at 240°C and fused silica capillary column (100 m long, 0.25 mm intern diameter and $0,20\ \mu\text{m}$, Restek 2560). Identification of the fatty acid sample was performed by comparison with the retention time of methyl esters of fatty acids patterns of samples (Sigma, F.A.M.E. Mix, C4-C24) and the calculation of peak areas determined

by Chromquest 5.0 Clarity Lite software version 2.4.1.91. The quantification of these fatty acids in g 100g⁻¹ of total lipids was performed in relation to the internal standard, methyl tricosanoate (Sigma).

The data were evaluated using the REG procedure (SAS Inst. Inc., Cary, NC, USA). The results were expressed as mean value and standard deviation.

RESULTS

There was no effect ($P < 0.05$) of grazing heights for mass forage production and chemical composition of *Brachiariabrizantha* cv. xaraés (Tab 1) and moisture, protein, mineral residue and amount of total lipids in meat (Tab 2).

Table 1. Mass forage production (kg DM ha⁻¹ year) and chemical composition (%) of *Brachiariabrizantha* cv. xaraés at different grazing height.

	Grazing Heights (cm)				P value
	15	30	45	60	
MFP	4892.08±408.98 ¹	7300.1±610.29	9053.37±756.86	9704.55±911.30	0.0933
Dry matter	27.83±4.22	28.78±4.36	25.83±3.91	26.95±4.08	0.2387
Mineral residue	8.02±0.88	8.64±0.95	8.01±0.88	7.00±0.77	0.1274
Protein	15.43±2.64	17.21±2.94	15.33±2.62	15.02±2.57	0.2563
NDF	51.67±2.68	50.8±2.64	54.73±2.84	53.11±2.76	0.0863
ADF	28.42±2.71	27.25±2.59	30.32±2.89	29.98±2.85	0.7486

¹means±standard error. MFP = mass forage production; NDF = neutral detergent fiber; ADF = acid detergent fiber. $P < 0.05$.

Table 2. Humidity, Protein, Mineral residue and Total Lipids (g/100g) of *Longissimus* muscle, and Fat thickness (mm) and Carcass Weight (kg) of Nellore cattle on grazing heights of *Brachiariabrizantha* cv. xaraés.

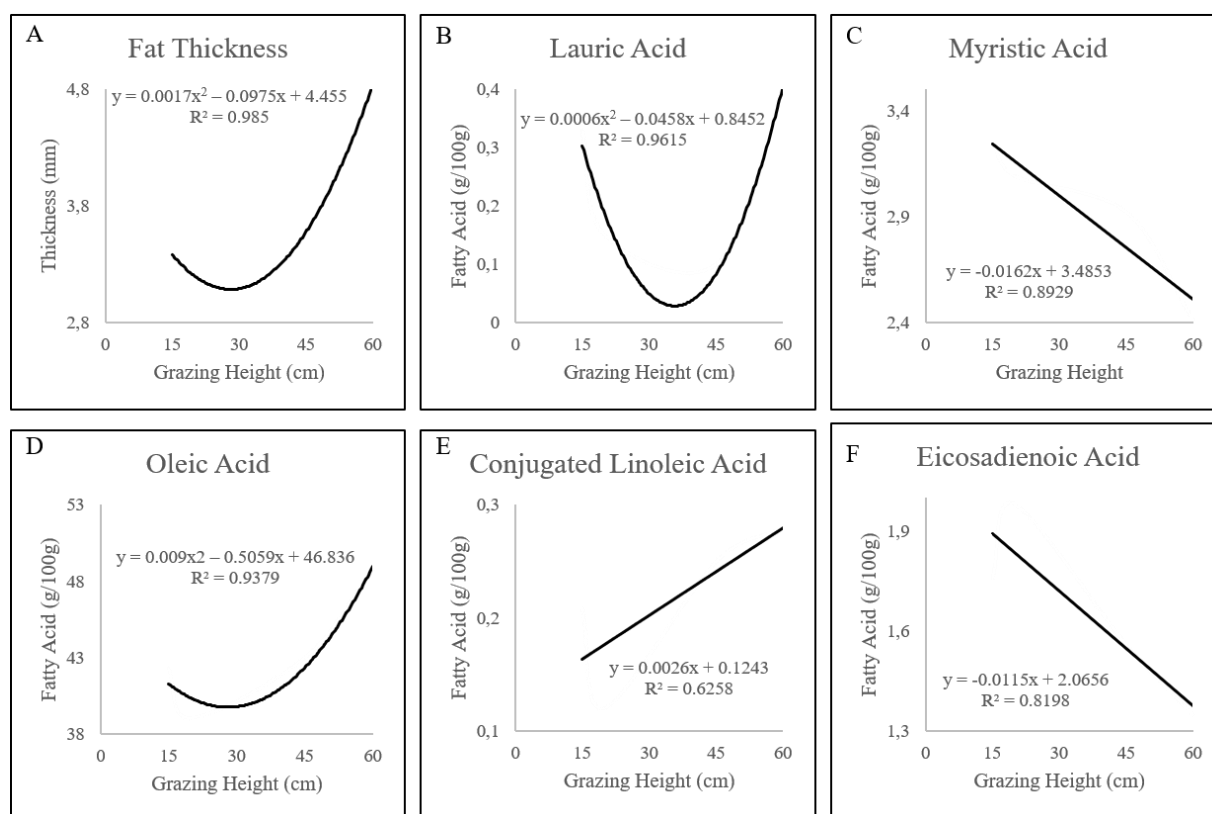
	Grazing Heights (cm)				P value
	15	30	45	60	
Humidity	73.86±0.44 ¹	73.22±0.44	74.00±0.44	73.17±0.44	0.4722
Protein	21.79±0.08	22.12±0.08	21.95±0.02	21.72±0.08	0.4440
Mineral residue	1.03±0.02	0.98±0.02	1.02±0.02	0.99±0.02	0.7499
Total Lipids	1.59±0.23	1.66±0.32	1.70±0.26	1.89±0.23	0.7453

Fat thickness	3.42±0.22	2.98±0.21	3.69±0.16	4.81±0.12	0.0057 ²
Carcass weight	252.40±43.00	271.70±45.01	260.13±55.00	267.19±58.01	0.0970

¹means±standard error. ² $\hat{Y}=0.0017x^2-0.0975x+4.455^2$. ($P<0.05$).

Fat thickness (Figure 1A) showed quadratic effect (minimum point, 3.06 mm at 28.68 cm grazing height) and lower value at 30 cm grazing height.

Figure 1. Fat thickness (A) and fatty acids in Nellore *Longissimus* muscle on grazing heights of xaraés grass with equation of regression and R² value. (B) Lauric Acid; (C) Myristic Acid; (D) Oleic Acid; (E) Conjugates Linoleic Acid; (F) Eicosadienoic Acid.



No effects of heights managements were found on composition of fatty acids, except for percentages of lauric acid (12:0), myristic acid (14:0), oleic acid (18:1n9c), conjugated linoleic acid (C18:2c9t11) and eicosadienoic acid (C20:2n-6) (Tab 3).

Table 3.Fatty acids (FA) profile (g/100g of muscle) of *Longissimus* muscle of Nellore cattle on different grazing heights of *Brachiaria brizantha* cv. xaraés.

SFA ¹	Nomenclature of FA*	Grazing Heights (cm)				SEM	P-value
		15	30	45	60		
10:0	Capric	0.02	0.02	0.02	0.01	0.003	0.1275
12:0	Lauric	0.33	0.15	0.10	0.40	0.037	0.0455 ⁴
14:0	Myristic	3.25	3.09	2.92	2.41	0.183	0.0461 ⁵
16:0	Palmitic	25.01	23.97	24.70	25.56	0.280	0.3836
17:0	Margaric	1.51	1.44	1.31	1.23	0.053	0.3323
18:0	Stearic	16.01	20.60	18.18	16.75	0.141	0.1072
20:0	Arachidic	0.25	0.25	0.29	0.27	0.013	0.2348
22:0	Behenic	0.52	0.42	0.05	0.28	0.034	0.2301
23:0	Tricosylic	0.74	1.29	0.83	0.21	0.076	0.3495
24:0	Lignoceric	0.03	0.05	0.02	0.04	0.004	0.2343
TOTAL SFA		47.67	51.29	48.43	43.64	1.660	0.0560
MFA²							
14:1 (n-5)	Myristoleic	0.58	0.55	0.51	0.45	0.040	0.2920
16:1 (n-7)	Palmitoleic	4.08	3.54	3.57	3.21	0.116	0.2995
tr 18:1 (n-9)	Elaidic	1.45	1.57	1.11	1.04	0.056	0.2407
18:1 (n-9)	Oleic	42.25	39.02	42.94	48.75	0.211	0.0494 ⁶
20:1 (n-9)	Gondoic	0.13	0.13	0.12	0.08	0.032	0.2897
22:1 (n-9)	Eurucic	0.17	0.25	0.22	0.12	0.016	0.3467
24:1 (n-9)	Nervonic	0.02	0.04	0.01	0.03	0.011	0.2461
TOTAL MFA		48.68	45.10	48.48	53.69	2.601	0.0614
PUFA³							
18:2c9t11	Conjugated linoleic	0.21	0.12	0.25	0.28	0.012	0.0349 ⁷
tr 18:2 (n-6)	trans-Linoleic	0.20	0.19	0.19	0.18	0.010	0.2314
18:2 (n-6)	Linoleic	0.26	0.25	0.25	0.21	0.016	0.2315
18:3 (n-3)	a-linolenic	0.11	0.12	0.11	0.10	0.005	0.2081
18:3 (n-6)	g-linolenic	0.17	0.18	0.16	0.14	0.011	0.3481

20:2	Eicosadienoic	1.76	1.98	1.56	1.35	0.172	0.0213 ⁸
20:3 (n-3)	2-homo- α -linolenic	0.04	0.03	0.02	0.02	0.060	0.3550
20:3 (n-6)	Eicosatrienoic	0.21	0.19	0.21	0.16	0.035	0.2905
20:4 (n-6)	Arachidonic	0.02	0.03	0.03	0.03	0.003	0.8637
20:5 (n-3)	Eicosapentaenoic (EPA)	0.12	0.13	0.11	0.06	0.006	0.4576
22:2	Clupanodonic	0.13	0.21	0.14	0.11	0.027	0.3582
22:6 (n-3)	Docosahexaenoic (DHA)	0.40	0.19	0.07	0.20	0.044	0.4460
TOTAL PUFA		3.65	3.61	3.09	2.67		0.1054

*Köfeler, 2016. ¹Saturated Fatty Acids. ²Monounsaturated Fatty Acids. ³Polyunsaturated Fatty Acids. ⁴ $\hat{Y}=0.0006x^2-0.0458x+0.8452$. ⁵ $\hat{Y}=-0.0162x+3.4853$. ⁶ $\hat{Y}=0.009x^2-0.5059x+46.836$. ⁷ $\hat{Y}=0.0026x+0.1243$. ⁸ $\hat{Y}=-0.0115x+2.0656$

Lauric acid (Figure 1B) showed a quadratic effect (minimum point, 0.03% at 38.17 cm grazing height) and myristic acid (Figure 1C) had negative linear effect. Oleic acid (C18:1n9) (Figure 1D) had a higher percentage than other fatty acids (general medium of 43.24%) and showed quadratic effect (minimum point, 39.73% at 28.11 cm grazing height) highest values occurred in the treatment with 28.11 cm of grazing height. Conjugated linoleic acid (C18:2c9t11) (Figure 1E) showed positive linear effect and eicosadienoic acid (C22:2n6) (Figure 1F) had a negative linear effect.

There was no difference between the treatments to medium chain fatty acids and long chain fatty acids (Tab 4). To the heights, values of omega-3 and omega-6 have difference and ratio n-6:n-3 did not differ. Between treatments the values to saturated fatty acid (SFA), monounsaturated fatty acid (MFA) and polyunsaturated fatty acid (PUFA) did not differ and PUFA:SFA ratio were similar.

Table 4. Sum of fatty acids (g / 100 g): saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), ômega-3 fatty acids (n-3) and ômega-6 fatty acids (n-6), and ratios n-6:n-3 and PUFA:SFA of *Longissimus* muscle of Nelore cattle on different grazing heights of *Brachiariabrizantha* cv. xaraés.

Fatty Acids	Grazing Heights (cm)				P-value
	15	30	45	60	
SFA	45.2±1.73 ¹	50.61±1.53	50.61±1.74	50.55±1.64	0.1486
MFA	51.95±2.55	47.22±2.44	46.4±2.53	47.04±2.88	0.1317
PUFA	2.85±0.53	3.16±0.58	2.99±0.57	2.41±0.65	0.9317
n-3	0.85±0.16	0.91±0.19	0.85±0.15	0.72±0.19	0.0027
n-6	0.62±0.26	0.60±0.78	0.61±0.54	0.65±0.66	0.2473
n-6:n-3	0.729±0.65	0.659±0.49	0.718±0.68	0.903±0.47	0.3987
PUFA:SFA	0.029±0.03	0.033±0.04	0.031±0.06	0.025±0.07	0.8359

¹means±standard error. *P*<0.05.

DISCUSSION

Values found for moisture, protein, mineral residue and total lipids (Tab 2) corroborate that evaluated animals finished on pastures (EIRAS et al. 2014). According to Food Advisory Committee (1990) food containing up to 5% of fat may be considered feed with low fat content, which ensures the consumption of meat evaluated for consumers seeking a healthier diet.

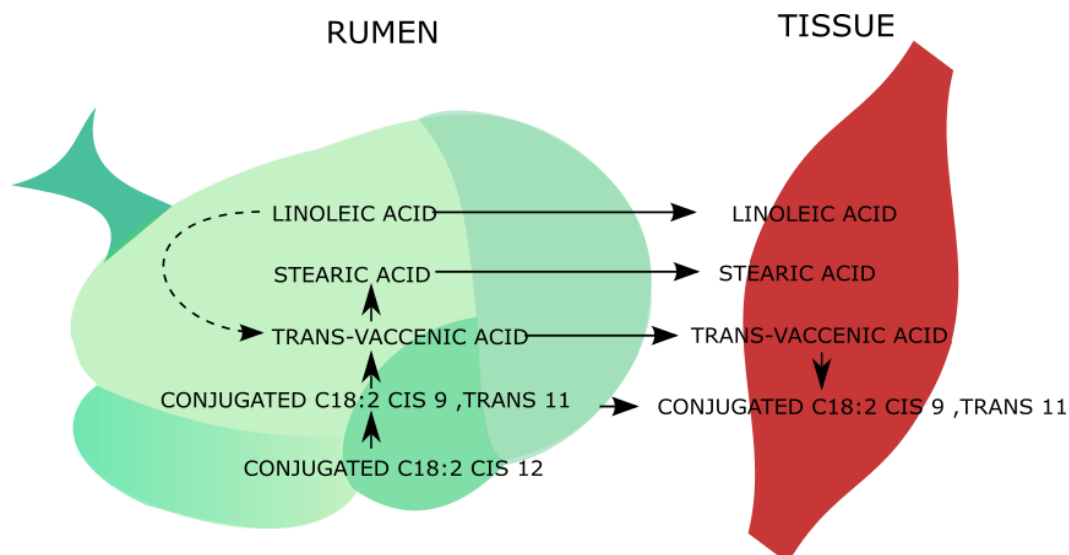
Data show that for the percentage of fatty acids, height 30 cm was more efficient, showing the best values for lauric acid (C12:0) (0.36%). The myristic acid (C14:0) was lower in height of 60 (4.04%) and

45 cm (4.16%). Palmitic acid (C16:0) do not show difference. Lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) are undesirable for the reason that they induce the increase of cholesterol (MAGGIONI et al., 2010), being appointed as the main responsible for the hypercholesterolemic effect of saturated fatty acids and increased low density lipoprotein (LDL, or bad cholesterol) which are responsible for coronary heart disease.

To oleic acid (C18:1n9), the highest values were 30 cm in the treatment of pasture height (39.71%) and for conjugated linoleic acid (CLA) (C18: 2c9t11), the best times were 15 (0.20%) and 60 cm (0.23%). Animal selectivity contributed to the higher quality diet, since leaves have greater amounts of fatty acids and lipids are predominantly in fodder leaves (HARFOOT; HAZLEWOOD, 1997; FARRUGGIA et al., 2008). Grasses have around 50% of linolenic and linoleic fatty acids in lipid profile and, as the plant reaches maturity, fat content decreases determining drop in fatty acid profile mainly polyunsaturated (DEWHURST et al., 2001).

In the rumen, dietary lipids are modified by hydrolysis and biohydrogenation (Figure 2). Hydrolysis occurs rapidly by the extracellular enzymes of ruminal microorganisms releasing fatty acids, glycerol and other molecules, according to their origin. Biohydrogenation is a protective mechanism of ruminal microorganisms against the toxic effect of unsaturated fatty acids, they do not use lipids as energy source, they only incorporate fatty acids in the cell membrane (NELSON; COX, 2014; ARRIGONI et al. 2016).

Figure 2. Biohydrogenation process. Conjugated C18:2 cis-12 undergoes the isomerization action of group A bacteria, whereas *Butyrivibrio fibrisolvens* is responsible for the transformation into Conjugate C18:2 cis-9, trans-11 (CLA), the same group responsible for hydrogenation for formation of trans-vaccenic acid. In the next step of biohydrogenation, the B group of bacteria hydrogenate the trans-vaccenic acid, transforming it into stearic acid. Some ruminal conditions may favor the direct passage of some acids to be absorbed post rumen, thus increasing their deposition in the tissues. The forage maturity may influence this passage, as it presents a low quality in the mass produced and low levels of nitrogen, which affects the fermenting bacteria of non - structural carbohydrates that also act in the biohydrogenation. With the passage of trans-vaccenic acid to the tissues the $\Delta 9$ -desaturase enzyme acts on the same fatty acid, transforming it also into CLA, an alternative for the deposition of this fatty acid in the tissue. Also, linoleic acid can be converted to transvaccenic acid, or else be directly absorbed post-rumen and deposited into the muscle (NELSON; COX, 2014; ARRIGONI et al., 2016).



Pasture management influenced the fat thickness (Figure 1), which had a reduction in height of 30 cm and a subsequent increase in height of 45 cm and favors the values found for saturated fatty acids, suggesting that the lower fat thickness, the lower will be the amount of saturated fatty acids present in meat. Thus, for saturated fatty acids (Tab 3) there was a reduction in the proportion of the saturated fatty acids lauric and myristic, indicating less incorporation of them in muscle tissue, which are undesirable for human health, which suggest the best health for the animal.

The reduction shown in Figure 1 for the saturated fatty acids and the increase in polyunsaturated fatty acid content demonstrated the improvement in fatty acid profile to 30 cm of grazing height, since polyunsaturated fatty acids are desirable for human consumption and saturated fat acids, as C12:0 and C14:0, blocks the coronary artery and worsens ulcerative colitis (BARNES et al., 2017; SPEZIALI et al., 2016).

The CLA value was low because it was considered intermediate fatty acid in the ruminalbiohydrogenation process of linoleic acid (EIFERT et al., 2008), not reaching high values such as eicosadienoic acid. Although CLA is a minor component of the diet, there is already a great deal of interest in it, which has promoted intensive research because it may benefit human health (FUKE; NOMBERG, 2017; KIM et al., 2016; MENEZES; AUGUSTO, 2016; RIBEIRO et al., 2016).

In this study, fatty acids with the highest percentage was oleic acid ($38.00\% \pm 1.51$), supporting (RODRIGUES et al., 2004) and (MENEZES et al., 2009). Diets with high percentage of oleic acid provide reduction in the percentage of LDL, the ratio LDL:HDL and total plasma cholesterol levels, showing a positive effect for human health (BONANOME; GRUNDY, 1988). Consumption by humans

of oleic acid has anticarcinogenic effect, reduces atherosclerosis in restoration of insulin sensitivity, modulation of the immune response and inhibition of tumor growth (PARIZA et al., 2001; FAO, 2008). For eicosadienoic acid (20:2) the height of 30 cm was more efficient (1.83%) compared to other treatments. According to (SIMOPOULOS et al., 1999) some fatty acids, especially polyunsaturated, serve as raw material for substances that regulate immunity, blood clotting, contraction of the vessels and blood pressure. Ruminant fat is some natural source of these fatty acids (FRENCH et al., 2000).

Adequate intake of polyunsaturated fatty acids should be between 6% and 11% of total calories (FAO, 2008) and in 2000 kcal diets, this percentage corresponds to about 130 mg to 240 mg of polyunsaturated fats in total consumption. Percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MFA), polyunsaturated fatty acids (PUFA), ratio n6: n3 and PUFA:SFA were not modified by xaraés grass grazing height. The mean values were 51.53% of SFA, 41.03% of MFA, and 7.19% of PUFA. In general, SFA and MFA values are between 45 and 50% (Maggioni et al., 2010; Fugita et al., 2012) and PUFA between 5 and 10% (ROTTA et al., 2009; MAGGIONI et al., 2010). The daily recommendation of SFA is 200 mg considering a diet for humans with four or more years of age (SILVA; MURA, 2007), but PUFA levels as CLA and eicosadienoic acid, reduce serum LDL (FUENTES, 1998).

Ratio observed of PUFA:SFA were 0,15. This ratio has played an important role in reducing the risk of heart problems (WOOD et al., 2004), however, according to HMSO (1994), the recommended ratio PUFA:SFA is greater than 0.4. The higher n-3 (2.61%) and n-6 (8.32%) content occurred in grazing height of 30 cm certainly for the best balance between productivity and the fractions that make up the forage mass, associated to the stocking rate.

Diet of the animals is a determining factor in levels of n-3 present in beef cattle, as it increases proportion of concentrated in diet the n-3 content tends to decrease, becoming less healthy meat, for food and human health. Specific amounts of n-6 and n-3, considering the recommendation of FAO (2008) on a diet of 2000 kcal (2.5 up to 9.0% and 0.5 up to 2.0% of the caloric value total, respectively), are 6000 to 20000 mg of n-6, and 1000 to 4000 mg of n-3 (MOZAFFARIAN et al., 2006; KRIS-ETHERTON et al., 2010).

Average ratio of n-6:n-3 was 3.27. According to the England Department of Health (HMSO, 1994), this ratio should be less than four parts of n-6 to n-3 aside for human consumption. This ratio is important because of the risks of cancer and coronary heart disease that an unbalanced diet provides. Increased n-3 in human nutrition is associated with many benefits such as reducing bad cholesterol

(LDL) and increasing good cholesterol (HDL) and decrease the risk of cardiovascular diseases, arthritis and type II diabetes (COSTA et al., 2002).

Management of pasture appears to provide additional advantages to productive systems based on pasture, considering that the use of pasture is interesting to minimize production costs, higher production stability, reduced dependency on external factors, greater use of natural conditions of climate and soil, what promotes a sustainable production system and healthy product to human consumption.

CONCLUSIONS

Grazing height does not influence the moisture, protein, mineral residue and total lipids in meat of Nelore cattle.

Management in heights between 30 and 45 cm positively alter the levels of fatty acids, adequate healthy recommendable for human diet, in *Longissimus* muscle of Nelore cattle, such as acids: lauric, myristic, oleic, conjugated linoleic and eicosadienoic, and the levels of n-3 and n-6.

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