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Sodium monensin or propolis extract in the diet of Nellore bulls finished in feedlot: chemical composition and fatty acid profile of *Longissimus* muscle

Monensina sódica ou extrato de própolis na dieta de bovinos Nelore terminados em confinamento: composição química e perfil de ácidos graxos do músculo *Longissimus*

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Abstract

This work was carried out to evaluate the chemical composition and fatty acid profile of *Longissimus* muscle of 33 Nellore bulls with 27 months old and initial average weight of 402 ± 7.58 kg finished in feedlot. Three treatments (Control – CON, Monensin – MON and Propolis extract – PRO) were evaluated. The animals were kept in feedlot during 84 days and slaughtered with final average weight with 488 ± 24.9 kg. The addition of additives (monensin or propolis extract) did not influence (P > 0.10) the chemical composition of *Longissimus* muscle of bulls. Saturated fatty acid (SFA) did not have difference (P > 0.10) among treatments. Monounsaturated fatty acids (MUFA) have difference (P < 0.01) among treatments. The highest value was observed in CON treatment (47.0%). There is also difference (P < 0.01) among treatments for polyunsaturated fatty acids (PUFA). Bulls of MON (9.92%) treatment obtained highest (P < 0.01) value for PUFA. Bulls of CON (6.74%) and PRO (6.93%) have lowest (P < 0.01) values for PUFA.
Key words: Additives, CLA, cholesterol, meat quality, ruminant

Resumo

Este trabalho foi realizado para avaliar a composição química e a composição de ácidos graxos do músculo *Longissimus* de 33 machos não castrados da raça Nelore com 27 meses de idade e peso médio inicial de 402 ± 7.58 kg terminados em confinamento. Foram usados três tratamentos: Controle – CON, Monensina – MON e Extrato de Própolis – PRO. Os bovinos foram mantidos confinados durante 84 dias e abatidos com peso vivo médio final de 488 ± 24.9 kg. A adição dos aditivos (monensina ou extrato de própolis) não tiveram influência (P > 0,10) na composição química do músculo *Longissimus* dos bovinos. A composição de ácidos graxos saturados (AGS) foi semelhante entre os tratamentos (P > 0,10). A composição de ácidos graxos monoinsaturados foi diferente entre os tratamentos (P < 0,01). Valor superior foi observado nos bovinos da dieta CON (47,0%). Da mesma forma, houve diferença (P

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< 0,01) entre tratamentos para os ácidos graxos poliinsaturados (AGPI). Bovinos do tratamento (9,92%) apresentaram maior (P < 0,01) valor para os AGPI. Os bovinos dos tratamentos CON (6,74%) e PRO (6,93%) apresentaram menores valores (P < 0,01) para os AGPI.
Palavras-chave: Aditivos, CLA, colesterol, qualidade da carne, ruminantes

Introduction

The meat has important nutritional function on human diet by proteins with high quality (WEBB; O'NEIL, 2008), essential fatty acids (MOREIRA et al., 2003; KAZAMA et al., 2008; MACEDO et al., 2008; DUCATTI et al., 2009; MAGGIONI et al., 2009; ROTTA et al., 2009a; 2009b), minerals and complex B vitamins. However, the consumption of red meat has been associated with health problems (cardio diseases, obesity, hypertension and cancers, WOOD et al., 2004) by presence of saturated fatty acids and cholesterol (KAZAMA et al., 2008; PRADO et al., 2009a; 2009b; 2009c). The presence of saturated fatty acid in meat is related to the biohydrogenation process in the rumen (TAMMINGA; DOREAU, 1991).

To increase the animal production is necessary to develop new process and technologies. Additives (ionophores) are used in ruminant feed with a purpose to improve the alimentary efficiency (OLIVEIRA et al., 2006). The sodium monensin has been used in ruminant diets. Sodium monensin act in gram-positives bacteria by alteration on flux of ions in cellular membrane to select the gram-negatives bacteria (DUFFIELD; BAGG, 2000). Ruminal micro flora is modified to alter the production of volatile fatty acids which ultimately improve the animal performance and the alimentary conversion (OLIVEIRA et al., 2006). In Europe the inclusion of sodium monensin is prohibited. The resolution EU 1831/2003 prohibits the use of sodium monensin since January of 2006. Thus, new researches are necessary for the development of alternative products to diets of ruminants.

Propolis has been demonstrated several bioactivities with eminence to anti-bacteria action that is related to the flavonoids (MARCUCCI, 1995). However, researches using propolis extract on meat quality in cattle are not found in literature.

This work was carried out to study the chemical composition and the fatty acid profile of *Longissimus* muscle of Nellore bulls finished in feedlot and fed with sodium monensin or propolis extract.

Material and Methods

Animal management and sampling

The committee of Animal Production at the State University of Maringá approved this study (CIOMS/OMS, 1985), which was carried out at the Experimental Farm of the State University of Maringá, Paraná, South Brazil. The diets and meat were analyzed at the laboratory of Feed Analyses and Animal Nutrition and Chemistry Laboratory of Food respectively of State University of Maringá.

Thirty three Nellore bulls were used with an initial average age of 27 months old and initial live weight of 402 kg ± 7.58. Before the experiment, bulls were kept in pasture (*Cynodon* spp. cv. Tifton 85) without supplementation.

During feedlot the bulls were kept separate in individual pens (10 m^2 for each animal) and fed twice a day. Bulls had access to the diet formulated by the requirements for fattening beef cattle (NRC, 1996) with a gain of 1.40 kg/day. The bulls were fed corn silage and water *ad libitum* and concentrate diet with cracked corn, soybean meal, mineral salt, limestone and urea (Table 1).

The bulls were weighed at the beginning of the experiment. Thereafter, they were weighed every 14 days, observing 16-hours fast, accomplished by removing all feed at 4 p.m. on the day prior to weighing. The total experimental period lasted 84 days, during which the animals reached an average final live weight of 485 ± 24.9 kg.

Table 1. Chemical composition of the ingredients and basal diet and percent composition (%/DM) of the diet.

		% DM							
Ingredients	**DM[1]**	**CP[2]**	**OM[3]**	**EE[4]**	**NDF[5]**	**ADF[6]**	**TC[7]**	**NFC[8]**	**Diet %**
Corn silage	32.1	8.15	96.5	2.06	48.4	25.6	86.3	38.0	52.0
Corn cracked	88.9	8.93	99.1	3.50	17.7	4.40	86.6	68.9	42.9
Soybean meal	88.6	49.0	93.7	1.30	13.7	5.97	43.5	29.8	4.30
Mineral salt	99.3								0.32
Limestone	99.3								0.19
Urea	97.5	282							0.32
Diet	41.9	11.1	97.1	2.62	33.3	15.4	83.9	50.6	100

[1]Dry matter, [2]Crude protein, [3]Organic matter, [4]ether Extract, [5]Neutral detergent fiber, [6]Acid detergent fiber, [7]Total carbohydrates, [8]Non fiber carbohydrates.

There were three treatments in the experiment: CON – control (11 bulls), MON – monesin (11 bulls) and PRO – propolis extract (11 bulls). The propolis extract LLOS (C1++) was prepared according Franco e Bueno (1999). Extracts with alcohol were diluted between 50 to 96º GL and with a fix concentration of propolis extract. The extracts were dried using the lyophilization during 24 hours. The samples were stored in closed bottles in a temperature of -5ºC. It is chartered by intellectual patrimonial by number PI 0605768-3. To prepare the concentrates, the extracts were established in ambient temperature and mixed in soybean and corn to form an addictive that was used to complete the concentrate to the animals. The propolis extract nucleus was developed by Pharmacy Laboratory (UEM – State University of Maringa) according Franco e Bueno (1999).

The product LLOSC1++ contains 0.054 mg/g of total flavonoids in chrysin. Monensin is produced by Elanco®.

Monensin and propolis extract nucleus were mixed with concentrate in the same time to feed the animals. These were used 300 mg/animal/day of monensin and 35 g/animal/day of propolis extract nucleus.

The chemical composition of silage and concentrate ingredients is presented in Table 1. Dry matter (DM), crude protein (CP), organic matter (OM), mineral matter (MM), ether extract EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined using the method described by Silva and Queiroz (2002). Total carbohydrates (TCH) were determined by equation: TCH = 100 – CP(%) + EE(%) + MM(%), described by Sniffen et al. (1992). No fiber carbohydrates (NFC) were obtained by difference between TCH and NDF.

Chemical composition

The animals were slaughtered at a commercial slaughterhouse 10 km away from the Maringá, according to industrial practices in Brazil. Following slaughter, the carcasses were identified and chilling for 24 h at 4ºC. After chilling, the right part of the carcass was used to determine the quantitative characteristics. Twenty-four hours later, *Longissimus* muscle (LM) samples were taken by a complete cross section between the 12th and 13th ribs. The fat thickness was discarded and the muscle portion was frozen at -20ºC for further analyses.

For analyses the samples were thawed at room temperature (20ºC), grounded (cracker mill), homogenized and analyzed in triplicate.

Meat moisture and ash percentage were determined according to AOAC (CUNNIF, 1998). Crude protein percentage was obtained through the Kjeldahl method (CUNNIF, 1998). Total lipids were extracted through the method Bligh and Dyer (1959) with a chloroform/methanol mixture. Fatty acid methyl esters (FAME) were prepared by triacylglycerol methylation, according to the ISO method (1978).

Cholesterol quantification

Cholesterol analysis was carried out by method modified by Rowe et al. (1999). A 60% (w/v) solution of potassium hydroxide was added to the samples in quantities equivalent to 2 mL h^{-1} of sample under 1-h reflux. The residue was dissolved again in 2 mL hexane containing 0.2 mg mL^{-1} 5-α cholestane internal standard (IS) (Sigma, USA).

Cholesterol content was analyzed in a 14-A gas chromatograph (Shimadzu, Japan), equipped with a flame ionization detector and fused silica capillary column (25 m long, 0.25-mm internal diameter, and 0.20 μm Ohio Valley-30). Injector, column, and detector temperatures were 260, 280, and 280ºC, respectively. Ultra-pure gas fluxes (White Martins) of 1.5 mL min^{-1} H_2 as carrier gas, 30 mL min^{-1} N_2 as make-up gas, 300 mL min^{-1} synthetic gas, and 30 mL min^{-1} N_2 for flame were used. The sample injection split mode was: 1:150. Peak integration was carried out with a CG-300 computing integrator (CG Instruments, Brazil) and cholesterol was identified by comparison with standards from Sigma (USA). Sample cholesterol quantification was carried out after verification of method linearity. Standard cholesterol solutions (Sigma, USA) were prepared with concentrations 0.0; 0.4; 0.8; 1.6, and 2.0 mg mL^{-1}, all containing 0.20 mg mL^{-1} 5α-cholestane (Sigma, USA) and analyzed. The ratio of the areas of cholesterol and 5-α cholestane was plotted against the cholesterol concentration for injected volumes of 0.0; 2.0; 3.0; 4.0, and 5.0 μL. The curve obtained was used for cholesterol analysis in mg 100 g^{-1}.

Analysis of fatty acid methyl esters

The fatty acids methyl esters (FAMEs) were analyzed in gas chromatograph (Varian, USA) equipped with flame ionization detector and fused silica capillary column CP-7420 (100 m, 0.25 mm and 0.39 μm o.d., Varian, USA) Select Fame. Column temperature was programmed at 165ºC for 18 min, 180ºC (30ºC min^{-1}) for 22 min, and 240ºC (15ºC min^{-1}) for 30 min, with 45-psi pressure. The injector and detector were kept at 220ºC and 245oC, respectively. The gas fluxes (White Martins) used was: 1.4 mL min^{-1} for the carrier gas (H_2); 30 ml min^{-1} for the make-up gas (N_2) and 30 mL min^{-1} and 300 ml min^{-1} for H_2 and the synthetic flame gas, respectively. Sample injection split mode was 1/80. Fatty acids were identified by comparing the relative retention times of FAME peaks of the samples with fatty acids methyl esters standards from Sigma (USA) by spiking samples with standard. The peak areas were determined by Star software (Varian). The data were expressed as percentages of the normalized area of fatty acids.

The areas of peak were determined by software Data Station advanced DataApex Clarity Litr (v.2.4.1.9.1, 2003) the identification of total cholesterol was effectuated by comparison Sigma (EUA).

Experimental design and statistical analysis

The experimental design consisted of 3 treatments: CON (control), MON (sodium monesin) and PRO (propolis extract). The data were submitted to analysis of variance and the means were compared using Tukey test at 5% of significance levels, by SAS statistical software (2000).

Results and Discussion

Chemical composition

The addition of additives (sodium monensin and propolis extract) did not influenced (P>0.10) the chemical composition of *Longissimus* muscle of bulls (moisture, ash, crude protein, total lipids and total cholesterol) (Table 2).

Table 2. Chemical composition (means) of the *Longissimus* muscle of Nellore bulls finished in feedlot.

	Treatments				
Items	**CON[1]**	**MON[2]**	**PRO[3]**	**SE[4]**	**P<F**
n	11	11	11		
Moisture, %	72.0	72.8	71.3	0.87	NS
Ash, %	1.07	1.08	1.10	0.02	NS
Crude protein, %	24.0	23.3	23.8	0.25	NS
Total lipids, %	1.16	1.30	1.30	0.09	NS
Total cholesterol[5]	34.3	34.7	34.2	0.32	NS

[1]Control, [2]Monensin, [3]Propolis extract, [4]Standart error, [5]mg/100 g of muscle, NS – non – significant.

The percentage of moisture in *Longissimus* muscle (72.0%) was near of the values observed in bulls finished in similar conditions of diet (ABRAHÃO et al., 2005; ROTTA et al., 2009a). Moisture percentage depends of total lipids in *Longissimus* muscle, because fat is poor in water.

In the same way, ash percentage (1.10%) in *Longissimus* muscle is near of results obtained by some authors (ABRAHÃO et al., 2005; PRADO et al., 2008b; 2008c; 2008d; PRADO et al., 2009a; 2009b; 2009c). Ash percentage change little in function of diets (ROTTA et al., 2009b).

The percentage of crude protein in *Longissimus* muscle (23.70%) was similar to some researches that used bulls finished in feedlot (ARICETTI et al., 2008; PRADO et al., 2008b; 2008c; 2008d). In general, crude protein percentage presents low variation due diet.

Total lipids percentage (1.20%) was considered low to bulls finished in feedlot receiving high energy in the diet. This could be explained by the age of these animals (29 months), breed (*Bos taurus* indicus) and physiological condition (bulls). In general, animals slaughtered younger show less total lipids percentage in *Longissimus* muscle (PADRE et al., 2006; PADRE et al., 2007; ARICETTI et al., 2008; KAZAMA et al., 2008). In the same way, bulls of zebu breed show low percentage of total lipids in *Longissimus* muscle in function of less selection to this characteristic (PRADO et al., 2008c). So, bulls finished in pasture system or feedlot show less percentage of total lipids in function of hormonal effects (testosterone) that act to high deposition of muscle tissue (LEE et al., 1990).

The average of total cholesterol for the treatments was 34.4 mg/100 g of muscle. These levels of total cholesterol can be related with the age of slaughter (27 months) and the breed of the animals (*Bos taurus* indicus). In general, animal slaughtered with 30 months or less show a variation between 30.0 to 45.0 mg/100 g of muscle (ARICETTI et al., 2008; PRADO et al., 2009a; 2009b; ROTTA et al., 2009b).

Fatty acid profile

The percentage of fatty acid in LM intramuscular

fat is shown on Table 3. Fatty acid diversity is partly explained by biohydrogenation that occurs in the rumen (TAMMINGA; DOREAU, 1991).

The percentage of fatty acids C14:0, C14:1 *n*-7, C15:1 *n*-9, C16:0, C16:1 *n*-7, C16:1 *n*-5, C18:0, C22:0 and C22: *n*-3 (DHA) did not differ (P>0.05) among treatments.

Table 3. Fatty acid profile (means) of the *Longissimus* muscle of Nellore bulls finished in feedlot.

Fatty acids	**Treatments**				
	CON[1]	**MON[2]**	**PRO[3]**	**SE[4]**	**P<F**
N	11	11	11		
14:0	3.02	2.96	3.23	0.14	NS
14:1 *n*-7	0.66	0.65	0.67	0.07	NS
15:0	0.32a	0.28ab	0.26b	0.01	0.03
15:1 *n*-9	0.11	0.10	0.10	0.01	NS
16:0	27.9	27.8	28.6	0.39	NS
16:1 *n*-9	0.15ab	0.17a	0.12b	0.01	0.01
16:1 *n*-7	3.24	3.13	3.08	0.17	NS
16:1 *n*-5	0.41	0.40	0.37	0.01	NS
17:0	0.86a	0.73b	0.72b	0.03	0.01
17:1 *n*-9	0.64a	0.56b	0.53b	0.01	0.01
18:0	14.2	13.8	14.6	0.58	NS
18:1 *n*-9	40.2a	37.0b	39.7ab	0.65	0.01
18:1 *n*-7	1.06a	1.05a	0.92b	0.04	0.04
18:1 *t*-11	0.50a	0.46ab	0.39b	0.02	0.01
18:2 *n*-6	4.21b	6.36a	4.30b	0.38	0.01
18:3 *n*-6	0.05 b	0.08a	0.04b	0.01	0.01
18:2 *cis* 9, *trans* 11	0.25ab	0.28a	0.20b	0.02	0.02
18:3 *n*-3	0.43b	0.57a	0.42b	0.03	0.01
20:4 *n*-6	0.99b	1.45a	1.10b	0.10	0.01
20:5 *n*-3 (EPA)	0.24b	0.35a	0.26b	0.02	0.01
22:0	0.05	0.24	0.05	0.10	NS
22:5 *n*-3 (DPA)	0.50b	0.73a	0.54b	0.05	0.01
22:6 *n*-3 (DHA)	0.04	0.06	0.05	0.01	NS

[1]Control, [2]Sodium monensin, [3]Propolis extract, [4]Standart error, NS - non – significant.

The percentages of C15:0 (0.32%), C18:1 n-9 (40.2%) and C18:1 *t*-11 (0.50%) was higher (P<0.03) to CON treatment. However, the percentages of these fatty acids are similar to MON treatment (0.28; 37.7 and 0.46%, respectively) in comparison to PRO treatment

(0.26; 39.7 and 0.39%, respectively), but this percentage did not differ between MON and PRO treatments.

The percentage of C16:1 *n*-9 and C18:2 cis 9 trans 11 (CLA) were higher (P<0.02) to MON treatment (0.17 and 0.28%, respectively) in comparison to PRO treatments (0.12 and 0.20%, respectively). However, these fatty acids did not differ between MON and CON (0.15 and 0.25%) treatments. PRO treatment (0.12 and 0.20%, respectively) presented lower (P<0.02) values to these fatty acids but did not differ between CON.

The percentages of C17:0 and C17:1 *n*-9 were higher (P<0.01) to CON treatment (0.86 and 0.64%, respectively) in comparison with MON (0.73 and 0.56%, respectively) and PRO treatments (0.72 and 0.53%, respectively).

The percentage of C18:1 *n*-7 were higher (P<0.04) in CON (1.06%) and MON (1.05%) treatments, but similar between them. The treatment PRO has the lower (P<0.04) value for this fatty acid (0.92%).

The percentages of C18:3 *n*-6, C18:3 n-6, C18:3 n-3, C20:4 n-6 and C22:5 n-3 were higher (P<0.01) to MON treatment.

The percentage of C20:5 *n*-3 (EPA) were higher (P<0.01) for MON treatment, but there is no difference between this treatment with PRO treatment.

The higher (P<0.08) value obtained for C22:6 *n*-3 (DHA) was observed for MON treatment, but there is no difference between this treatment with PRO treatment.

As ruminant diets contain low fat concentration, the majority of the adipose tissue is synthesized from lipogenesis. Fatty acids are elongated up to C18:0 and are converted into C18:1 by unsaturation (RULE; MACNEIL; SHORT, 1997). As the adipose tissue increases, the deposition of C18:1 content also increases and C18:2 is reduced.

Oleic acid (C18:1) increases human HDL-Cholesterol (High Density Lipoprotein) and decreases LDL-cholesterol (Low Density Lipoprotein) concentration in blood (KATAN; ZOCK; MENSINK, 1994). Studies demonstrated a strong relationship between LDL-cholesterol levels and human cardiovascular diseases, and that HDL-cholesterol has an inverse relation with the risk of cardiovascular diseases (KWITEROVICH, 1997).

Saturated fatty acid (SFA) did not have difference (P>0.10) among treatments (Table 4). The average for this parameter was 46.5%. SFA is present in meat in high concentration due biohydrogenation that occur in rumen that change the double bonds of unsaturated fatty acids.

Monounsaturated fatty acids (MUFA) have difference (P<0.01) among treatments. The higher value was observed in CON treatment (47.0%).

There was difference (P<0.01) among treatments for polyunsaturated fatty acids (PUFA). Animals of MON (9.92%) treatment obtained higher (P<0.01) value for this characteristic. CON (6.74%) and PRO (6.93%) have lower values for PUFA. The percentage of PUFA was lower (P<0.01) than SFA and MUFA due the biohidrogenation in rumen, that transform PUFA in MUFA or SFA (TAMMINGA; DOREAU, 1991).

n-3 was higher (P<0.01) in MON treatment (1.73%) in comparison with CON (1.22%) and PRO (1.27%). The percentage of this class of fatty acids was low in relation with *n*-6. This is because the predominant polyunsaturated fatty acid in meat is C18:2 n-6 that appear in high concentration in comparison with C18:3 *n*-3.

Table 4. Proportion (%) (means) of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty (PUFA), fatty acids *n*-6, fatty acids *n*-3, PUFA/SFA and *n*-6/*n*-3 ratio of the *Longissimus* muscle of Nellore bulls finished in feedlot.

Fatty acids	Treatments				
	CON[1]	MON[2]	PRO[3]	SE[4]	P<F
n	11	11	11		
SFA	46.3	45.9	47.4	0.76	NS
MUFA	47.0a	44.2b	45.9b	0.81	0.01
PUFA	6.74b	9.92a	6.93b	0.57	0.01
n-3	1.22b	1.73a	1.27b	0.11	0.01
n-6	5.26b	7.90a	5.44b	0.47	0.01
PUFA/SFA	0.14b	0.22a	0.15b	0.01	0.01
n-6/*n*-3	4.32	4.63	4.33	0.17	NS

[1]Control, [2]Sodium monensin, [3]Propolis extract, [4]Standart error, NS - non – significant.

MON treatment (7.90%) presented higher (P<0.01) values of *n*-6. CON (5.26%) and PRO (5.44%) had lower (P<0.01) values for this parameter. This shows that animals fed with addition of sodium monensin in diet had higher values for essential fatty acids that CON and PRO treatments.

PUFA/SFA ratio was higher (P<0.01) for MON treatment (0.22%) in comparison with CON (0.14%) and PRO (0.15%). However, no treatment has recommended values for this ratio, that should be 0.40 or higher values. PUFA/SFA ideal ratio plays important roles in reducing the risk of coronary heart disease; however, the optimal balance between these two classes of fatty acids is still a matter of debate (HU, 2001).

No difference (P>0.10) was observed for *n*-6/*n*-3 ratio among different treatments. This ratio must be inferior to 4 (ENGLAND, 1994). In this study, the average found for *n*-6/*n*-3 was 4.44, so this value is close that recommended by English Department of Health (1994).

Conclusions

The addition of sodium monensin or propolis extract did not alter the chemical composition of *Longissimus* muscle of bulls finished in feedlot. However, the fatty acid profile is changed by addition of sodium monensin or propolis extract in the diets. Essential fatty acids (C18:2 *n*-6 and C18:3 *n*-3) are found in more concentration in *Longissimus* muscle of animals that received sodium monensin. The meat of animals fed with addition of sodium monensin is better in relation of fatty acid profile in comparison with control and propolis extract treatment. However, with the prohibition of monensin sodium in animal feed, propolis extract can be an alternative because did not damage the meat quality in comparison with control treatment and could reduce the greenhouse effect.

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