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Sodium monensin or propolis extract in the diet of crossbred (½ Red Angus vs. ½ Nellore) bulls finished in feedlot: chemical composition and fatty acid profile of the *Longissimus* muscle

Monensina sódica ou extrato de própolis em dietas de bovinos mestiços (½ Red Angus vs. ½ Nelore) terminados em confinamento: composição química e ácidos graxos do músculo *Longissimus*

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Abstract

This work was carried out to evaluate the chemical composition and the fatty acid profile of the *Longissimus* muscle of crossbred bulls (F1 – ½ Red Angus *x* ½ Nellore) with an initial average age of 20 months old and an initial average weight of 393 ± 24 kg finished in feedlot. Three treatments (Control – CON, Sodium monensin – MON and Propolis extract – PRO) were evaluated. The animals were kept in feedlot for 70 days and slaughtered at an average weight of 498 ± 25 kg. They were fed with corn silage (roughage), cracked corn, soybean meal, urea, limestone and mineral salt. The roughage:concentrate ratio was 50:50. The bulls were fed twice daily, at 8 a.m. and 4 p.m. The chemical composition and the fatty acid profile of the *Longissimus* muscle was measured between the 12th and 13th ribs. The inclusion of additives (sodium monensin or propolis extract) did not influence ($P > 0.05$) the chemical composition (moisture, crude protein, ashes, total lipids and total cholesterol) of the animals' *Longissimus* muscle. Therefore, diet has little effect on fatty acid composition of the *Longissimus* muscle of bulls. However, the percentage of C18:2 n-6 fatty acid was lower ($P < 0.05$) as a percentage of total fatty acids in the CON diet in comparison to the MON and PRO diets. On the contrary, the percentage of C22:6 *n* – 3 fatty acid was higher ($P < 0.05$) as a percentage of total fatty acids in the CON diet in comparison to the PRO diet. However, diet did not influence ($P > 0.05$) polyunsaturated, monounsaturated, saturated fatty, *n*-6, or *n*-3 fatty acids or the ratio of PUFA/SFA and *n*-6/*n*-3 fatty acids.

Key words: CLA, cholesterol, fatty acid, ionophores, meat quality

Resumo

Este trabalho foi realizado para estudar o efeito da adição de monensina sódica ou produto à base de própolis sobre as características de carcaça e composição química do músculo *Longissimus* de bovinos

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mestiços não castrados terminados em confinamento. Foram usados 24 bovinos com peso vivo médio de 393,3 ± 24 kg e 20 meses de idade. Os bovinos foram divididos em três tratamentos: 1. Controle (CON), 2. Monensina sódica (MON) ou 3. Produto à base de própolis (PRO). Os bovinos foram mantidos em confinamento durante 70 dias e alimentados com silagem de milho (volumoso) e milho moído, farelo de soja, ureia, calcário e sal mineral (concentrado). A relação volumoso: concentrado foi de 50:50. Os bovinos foram alimentados duas vezes ao dia (8 e 16 horas). Foram determinadas as características de carcaça e composição química do músculo *Longissimus*. As características quantitativas (peso de carcaça quente, rendimento de carcaça quente, espessura de gordura de cobertura, área de olho de lombo e a percentagem de músculo, gordura e osso) e qualitativas (marmorização, textura e cor) das carcaças não foram alteradas (P > 0,05) pelas dietas. A composição química do músculo *Longissimus* (umidade, cinzas, proteína bruta, lipídeos totais e colesterol total) foi semelhante (P > 0,05) nos bovinos das três dietas. Da mesma forma, a composição de ácidos graxos saturados, monoinsaturados, poliinsaturados, *n*-6 e *n*-3 não foram alterados (P > 0,05) pelas dietas, assim como as razões AGPI/AGS e *n*-6/*n*-3.
Palavras-chave: CLA, colesterol, ácidos graxos, ionóforos, qualidade da carne

Introduction

To increase animal production, it is necessary to develop new processes and technologies. Additives (ionophores) are used in ruminant feed with the purpose of improving alimentary efficiency (OLIVEIRA et al., 2006). The use of sodium monensin, a polyether antibiotic, is one of the most common methods for modulating ruminal fermentation (DUFFIELD; BAGG, 2000). When used in ruminant diets, sodium monensin acts on gram-positive bacteria by altering the flux of ions in the cellular membrane to select gram-negative bacteria (DUFFIELD; BAGG, 2000). The primary action of the monensin appears to take place in the rumen. Monensin's modes of action rely on the selective growth inhibition of gram-positive bacteria (VAN NEVEL; DEMEYER, 1988), reducing lactic acid production (DENNIS; NAGARAJA; BARTLEY, 1981) and methanogenesis in the rumen (GOODRICH et al., 1984) and increasing the molar proportion of propionate (GOODRICH et al., 1984) and N retention. The positive production responses of livestock to dietary monensin supplementation have been well-documented, e.g., the improvement of feed conversion, particularly in high-grain diets (BOUCQUÉ et al., 1982; OLIVEIRA et al., 2006).

Monensin inhibits lipolysis and, to some extent, fatty acid biohydrogenation (VAN NEVEL; DEMEYER, 1995), so it may also affect the production of fatty acids in the rumen. However, Köhler, Karch and Schmidt (2000) reported that certain antibacterials used as growth promoters in animal feeding might increase the horizontal transfer of virulence genes between bacteria, though the effect was not observed in monensin *in vitro*. The use of in-feed antibiotics is subject to critique and raises concerns because of its potential involvement in the presence of antibiotic residues in animal products and the development of antibiotic-resistant pathogenic microbes. In Europe, the inclusion of sodium monensin in ruminant diets is prohibited (EPC, 2003). New research is thus necessary for the development of alternative products for ruminant diets, and antimicrobial feed additives must be replaced by specific feed additives (MARTIN et al., 1999).

Propolis has several demonstrated bioactivities, especially antibacterial action related to flavonoids (MARCUCCI, 1995). However, studies of the effect of propolis extract on meat quality in cattle are not present in the literature.

The present study was carried out to investigate the chemical composition and the fatty acid profile of the *Longissimus* muscle of ½ Red Angus *x* ½ 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, which was carried out at the Experimental Farm of the State University of Maringá, Paraná, Southern Brazil, and followed the guiding principles of biomedical research with animals (CIOMS, 1985). The analyses of diets were performed at the Laboratory of Feed Analyses and Animal Nutrition at the State University of Maringá.

Thirty-three bulls (½ Red Angus *x* ½ Nellore) at an initial average age of 20 months and an initial average live weight of 394 kg ± 24.0 were used. Before the experiment, the bulls were kept in pasture (*Brachiaria decumbens*) without supplementation.

The bulls were weighed at the beginning of the experiment. Thereafter, they were weighed every 28 days after observing a 16-hour fast, which was accomplished by removing all feed at 4 p.m. the day prior to weighing. The total experimental period lasted 70 days, during which the animals reached an average final live weight of 499 ± 24.9 kg.

The bulls were kept in separate, individual pens (10 m^2 for each animal) and fed twice daily. The bulls had access to a diet formulated to meet requirements for the fattening of beef cattle (NRC, 1996) at a gain of 1.50 kg/day. They were fed with 50% corn silage, 43.2% cracked corn, 5.41% soybean meal, 0.47% urea, 0.47% limestone and 0.47% mineral salt (Table 1). The dry matter (DM), crude protein (CP), organic matter (OM), mineral matter (MM), extract ether (EE) and neutral fiber detergent (NFD) were determined using the method described by AOAC (1998).

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 carbohydrates fiber.

Three treatments were used in this study: CON – control (8 bulls), MON – sodium monensin (8 bulls) and PRO – propolis extract (7 bulls). The propolis extract LLOS (C1++) was prepared according to Franco e Bueno (1999). Extracts with alcohol were diluted between 50 to 96º GL with a fixed concentration of propolis extract. The extracts were dried using lyophilization during 24 hours. The samples were stored in closed bottles at a temperature of -5ºC (this method was chartered under intellectual property number PI 0605768-3). To prepare the concentrates, the extracts were

established at an ambient temperature and mixed with soybean and corn to form an additive used to provide the concentrate to the animals. The product LLOSC1++ contains 0.054 mg/g of total flavonoids in chrysin. Monensin is produced by Elanco®.

At the same time, monensin and propolis extract nucleus were mixed with concentrate to feed the animals (300 mg/animal/day of monensin and 35 g/animal/day of propolis extract nucleus).

Chemical composition

The animals were slaughtered at a commercial slaughterhouse 20 km away from the Maringá city according to Brazilian industrial practices. Following slaughter, the carcasses were identified and chilled for 24 h at 4ºC. After chilling, the appropriate section of the carcass was used to determine 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 was frozen at -20ºC for further analysis.

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

Meat moisture and ash percentage were determined according AOAC (CUNNIF, 1998). Crude protein percentage was obtained by the Kjeldahl method (CUNNIF, 1998). Total lipids were extracted through the Bligh and Dyer method (1959) using a chloroform/methanol mixture. Fatty acid methyl esters (FAME) were prepared by triacylglycerol methylation, according 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 14-A gas chromatograph (Shimadzu, Japan), equipped with a flame ionization detector and a fused silica capillary column (25 m long, 0.25-mm internal diameter and 0.20 μm Ohio Valley-30). The injector, column and detector temperatures were 260, 280 and 280ºC, respectively. Ultra-pure gas fluxes (White Martins) were used in the following quantities: 1.5 mL min^{-1} H_2 as carrier gas, 30 mL min^{-1} N_2 as make-up gas, 300 mL min^{-1} as synthetic gas and 30 mL min^{-1} N_2 for the flame. 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 linearity method. 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); the solutions were then 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 a 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 were 1.4 mL min^{-1} for the carrier gas (H_2), 30 ml min^{-1} for the make-up gas (N2), 30 mL min^{-1} for H_2

and 300 ml min^{-1} the synthetic flame gas. Sample injection split mode was 1/80. Fatty acids were identified by comparing the relative retention times of the samples' FAME peaks with fatty acids methyl esters standards from Sigma (USA). The samples were spiked with the standard. The peak areas were determined with Star software (Varian). The data were expressed as percentages of the normalized area of fatty acids.

The peak areas were determined by Data Station Advanced DataApex Clarity Litr. Software (v.2.4.1.9.1, 2003) and the identification of total cholesterol was effectuated by comparison with Sigma (USA).

Experimental design and statistical analysis

The experimental design consisted of three treatments: CON (control), MON (sodium monensin) and PRO (propolis extract). The data were submitted to variance analysis and the means were compared using a Tukey test performed with SAS statistical software (2000).

Results and Discussion

Chemical composition

The presence of additives (sodium monensin and propolis extract) did not influence (P>0.05) the chemical composition (moisture, ash, crude protein, total lipids and total cholesterol) of the *Longissimus* muscle of crossbred bulls finished in feedlot (Table 2).

Table 2. Chemical composition (means) of the *Longissimus* muscle of ½ Red Angus x Nellore bulls finished in feedlot.

	Diets				
Items	**CON[1]**	**MON[2]**	**PRO[3]**	**SE[4]**	**P<F**
n	**8**	**8**	**8**		
Moisture, %	74.6	74.8	74.4	0.87	NS
Ash, %	1.06	1.06	1.05	0.02	NS
Crude protein, %	22.9	21.8	22.8	0.25	NS
Total lipids, %	1.60	1.45	1.65	0.09	NS
Total cholesterol[5]	36.8	36.4	37.4	0.32	NS

[1]control, [2]sodium monensin, [3]propolis extract, [4]standart error, [5]mg/100 g of muscle, NS – non significant.

The percentage of moisture of the *Longissimus* muscle (74.6%) is near to the values observed in bulls finished in feedlot conditions (KAZAMA et al., 2008; ROTTA et al., 2009a; 2009b). Moisture percentage depends of total lipids of the *Longissimus* muscle because fat is poor in water.

Similarly, ash percentage (1.06%) of the *Longissimus* muscle resembles results obtained by some authors (PRADO et al., 2008b; 2008c; 2008d; DUCATTI et al., 2009; PRADO et al., 2009a; 2009b; 2009c). Ash percentage changes little as a result of the presence of additives.

The percentage of crude protein of the *Longissimus* muscle (22.7%) is similar to some studies that used bulls finished in feedlot (PRADO et al., 2008b; 2008c; 2008d). In general, crude protein percentage varies little due to diet.

Total lipid percentage (1.56%) is considered low in comparison to bulls finished in feedlot that received high energy diets. This could be explained by the animals' age (24 months), breed (½ Red Angus x ½ Nellore) and physiological condition (bulls). In general, animals slaughtered at younger age show less total lipid percentage in the *Longissimus* muscle (PADRE et al., 2006; PADRE et al., 2007; MACEDO et al., 2008; ROTTA et al., 2009b). Similarly, bulls crossbred from the zebu breed show low percentage of total lipids of the *Longissimus* muscle as a function of lesser selection of this characteristic (PRADO et al., 2008c). Accordingly, bulls finished in feedlot show less total lipid percentage as a function of hormonal effects (testosterone) that act to high deposition of muscle tissue (LEE et al., 1990).

The total average cholesterol for the treatments was 36.8 mg/100 g of muscle. These total cholesterol levels can be related to the age of slaughter (24 months) and the breed of the animals (½ Red Angus *x* ½ Nellore). In general, animals slaughtered at an age of 24 months or less show variation between 30.0 to 45.0 mg/100 g of muscle (PRADO et al., 2009a; 2009b; ROTTA et al., 2009b).

Fatty acid profile

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

Diet has little effect on the fatty acid composition of the *Longissimus* muscle of bulls. The percentage of C18:2 *n*-6 fatty acid was similar (P>0.05) between the MON (6.32%) and PRO (6.28%) diets and superior (P<0.05%) to the PRO diets (4.94%). However, the CON diet presented a higher (P<0.05) percentage of C18:6 *n*-3 fatty acid (2.18%) in comparison to the PRO diet (1.42%).

Due ruminant diets contain a low fat concentration, the majority of the adipose tissue is synthesized from lipogenesis. The ionophore sodium monensin inhibits lipolysis and to some extent unsaturated fatty acid biohydrogenation (VAN NEVEL; DEMEYER, 1995), so monensin may also affect the production of fatty acids in the rumen. 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. The effects of nutrition on meat quality are more significant in terms of carcass characteristics and chemical composition (ABRAHÃO et al., 2005; PRADO et al., 2008a; MAGGIONI et al., 2009; ROTTA et al., 2009b). On the other hand, the effects of nutrition on fatty profile are generally small but significant; they are often very important in terms of nutritive value, color of the product and quality or fat consistency (WEBB; O'NEIL, 2008).

The additives (sodium monensin or propolis extract) did not alter (P>0.05) to saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids on *Longissimus* muscle of bulls finished in feedlot. The majority of fatty acids found on *Longissimus* muscle were SFA (44.8%), followed by MUFA (42.7%) and PUFA (12.5%). Likewise, Aricetti et al. (2008) and Prado et al. (2008a; 2008b) observed similar percentages of SFA, MUFA and PUFA in bulls finished under similar diets and handling conditions as in this experiment. Thus, SFA, MUFA and PUFA fatty acids percentages vary little as a function of diet.

The percentage of *n*-3 fatty acids was low in comparison with *n*-6. This was because the predominant polyunsaturated fatty acid in meat is C18:2 *n*-6, which appears in high concentration in comparison with C18:3 *n*-3.

Table 3. Fatty acid profile (means) of the *Longissimus* muscle of ½ Red Angus *x* ½ Nellore bulls finished in feedlot.

Fatty acids	Diets				
	CON[1]	MON[2]	PRO[3]	SE[4]	P<F
n	8	8	8		
14:0	1.99	2.06	1.87	0.14	NS
14:1 *n*-7	0.17	0.19	0.20	0.02	NS
15:0	0.31	0.28	0.31	0.05	NS
16:0	25.4	25.9	25.4	0.55	NS
16:1 *n*-9	0.22	0.20	0.24	0.02	NS
16:1 *n*-7	2.64	2.57	2.57	0.14	NS
17:0	0.98	0.92	0.85	0.05	NS
17:1 *n*-9	0.65	0.69	0.59	0.04	NS
18:0	16.5	16.0	16.2	0.65	NS
18:1 *n*-9	38.6	37.7	38.8	0.70	NS
18:1 *n*-7	0.65	0.60	0.65	0.04	NS
18:2 *n*-6	4.94b	6.32a	6.28a	0.42	0.05
18:2 *cis* 9, *trans* 11	0.16	0.16	0.17	0.01	NS
18:3 *n*-6	0.72	0.75	0.78	0.06	NS
18:3 *n*-3	0.21	0.17	0.17	0.02	NS
20:0	0.19	0.17	0.20	0.02	NS
20:4 *n*-6	1.53	1.63	1.54	0.16	NS
20:5 *n*-3 (EPA)	1.01	1.10	1.22	0.11	NS
22:4 *n*-6	0.72	0.59	0.82	0.09	NS
22:6 *n*-3 (DHA)	2.18a	1.73ab	1.42b	0.17	0.01
PUFA	13.1	12.4	11.9	1.33	NS
MUFA	42.5	42.3	43.3	0.89	NS
SFA	44.4	45.2	44.8	0.78	NS
n-6	8.68	8.69	8.10	1.07	NS
n-3	4.00	3.42	3.12	0.36	NS
PUFA/SFA	0.30	0.28	0.27	0.04	NS
n-6/*n*-3	2.64	3.16	3.21	0.22	NS

[1]control, [2]sodium monensin, [3]propolis extract, [4]standart error, NS - non significant, means followed by the different letters differ by Tukey test.

The PUFA/SFA ratio was low for all diets (0.28). Thus, no diet had recommended values of 0.4 or higher for this ratio (ENGLAND, 1994). The ideal PUFA/SFA ratio plays an important role 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 the *n*-6/*n*-3 ratio among different diets. This ratio must be less than 4.0 (ENGLAND, 1994). In this study, the average found for *n*-6/*n*-3 was 3.0, a value lower than that recommended by the English Department of Health (ENGLAND, 1994).

Conclusions

The addition of sodium monensin or propolis extract did not alter the chemical composition of the *Longissimus* muscle of bulls finished in feedlot. However, the fatty acid profile was changed by the addition of sodium monensin or propolis extract in the diets. In consideration of the prohibition of monensin sodium in animal feed, propolis extract could provide an alternative because it did not damage meat quality in comparison with the control treatment, and it could help reduce the greenhouse effect.

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