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Quality of aged meat of young bulls fed crude glycerin associated with different roughage sources

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ABSTRACT. This trial aimed to evaluate the inclusion of 10% of crude glycerin associated with roughage sources on the quality of meat aged for 1, 7 or 14 days *post mortem* of Nellore young bulls. Thirty feedlot animals (n = 10) with initial body weight of 416.70 ± 24.74 kg and 18 months of age were assigned to three treatments: corn silage (CS), sugar cane (SC) and sugar cane bagasse (CB), using a completely randomized design. After 85 days of feeding, animals were slaughtered with 554.51 ± 38.51 kg. Samples of longissimus muscle were collected, after carcass chilling, and vacuum-packed. Diets influenced pH, meat color and subcutaneous fat (SF) ($p > 0.05$). Animals fed CS showed higher values of b* in SF ($p < 0.05$). Differences were not found in the meat fatty acid profile ($p > 0.05$). Aging times influenced pH and shear force of beef ($p < 0.05$). Beef aged for 14 days showed higher pH (5.90) and lower shear force (2.40 kgf). Diets containing 10% crude glycerin in the DM associated with CS, SC or CB had no effect on the fatty acid profile in beef. The aging process for 14 days reduces shear force, improving meat quality.

Keywords: beef cattle, sugar cane bagasse, fatty acids, glycerol, tenderness

Qualidade da carne maturada de tourinhos alimentados com glicerina bruta associada a diferentes fontes de fibra

RESUMO. Objetivou-se avaliar a inclusão de 10% de glicerina bruta (GB) associada a fontes de volumosos sobre a qualidade da carne maturada em 24 horas, sete e 14 dias *post mortem* de tourinhos da raça Nelore. Utilizaram-se 30 animais confinados (n = 10), com peso inicial de 416,70 ± 24,74 kg e 18 meses de idade, sendo submetidos aos três tratamentos: silagem de milho (SM), cana-de-açúcar (CA) ou bagaço de cana (BC), utilizando delineamento inteiramente casualizado. Após 85 dias, os animais foram abatidos com 554,51 ± 38,51 kg. Amostras do longissimus foram coletadas após resfriamento das carcaças e embaladas a vácuo. A dieta influenciou o pH, a coloração da carne e da gordura subcutânea (GS; $p < 0,05$). Animais alimentados com SM apresentaram maior valor de b* na GS ($p < 0,05$). A dieta não modificou o perfil de ácidos graxos (AG) da carne ($p > 0,05$). A maturação influenciou pH e força de cisalhamento da carne (FC; $p < 0,05$). Carnes maturadas aos 14 dias apresentaram maior pH (5,90) e menor FC (2,40 kgf). Dietas contendo 10% GB na MS associada à SM, CA ou BC não modificam os AG da carne. A maturação em 14 dias reduz a FC do longissimus, melhorando a qualidade da carne.

Palavras-chave: bovinos de corte, bagaço de cana, ácidos graxos, glicerol, maciez.

Introduction

The increase in biodiesel production has encouraged the use of crude glycerin in animal feed - an energetic ingredient that can replace corn in up to 20% of the diet DM without affecting the growth of beef feedlot cattle (Bartoš et al., 2013; Cruz et al., 2014; Eiras et al., 2014a, b; Eiras et al., 2013; Françozo et al., 2013; Parsons et al., 2009).

Although the concentrate feed has the key role in reducing the slaughter age of beef cattle and hence improve the meat quality, the roughage fraction is a large portion of the diet for feedlot animals slaughtered in Brazil - around 40% in DM (Fugita et al., 2012; Maggioni et al., 2009). The main forages supplied for Brazilian feedlot beef cattle include sugar cane, corn silage, sorghum silage and sugar cane bagasse (Millen et al., 2009).

Studies reporting the use of crude glycerin in beef cattle diets and its effects on meat quality have used corn silage or hay as roughage, which requires the knowledge of the effects of this byproduct on meat quality when combined with other types of forages commonly used in Brazil. This is important

to be studied, since crude glycerin has been reported to affect the fiber digestibility in ruminant diets (Abo El-Nor et al., 2010; Eiras et al., 2014a; Ramos & Kerley, 2012; Silva et al., 2014) and also to inhibit lipolysis (Krueger et al., 2010), reducing the accumulation of free fatty acids in the rumen, resulting in greater amount of fatty acids available to be incorporated in meat products (Eiras et al., 2014b; Françozo et al., 2013; Lage et al., 2014). In this sense, crude glycerin can possibly change the amount and composition of fatty acids deposited in tissues (Eiras et al., 2014a), as some cellulolytic bacteria participate in the ruminal biohydrogenation process (Tamminga & Doreau, 1991).

Aging is a practice used to improve some aspects of meat quality, such as tenderness (Campo et al., 1999). However, in products with a higher proportion of unsaturated fat, this technique need to be assessed, because depending on the storage time under 0-2°C, it may occur oxidation of unsaturated fat, affecting the quality of the meat.

Therefore, we hypothesized that crude glycerin associated with roughage sources of low quality fibers (sugar cane and sugar cane bagasse) can be supplied to beef cattle feedlot diet without impairing the quality of aged meat, given fixed amounts of forage NDF in diet.

This study was conducted to evaluate the quality of meat aged for 1, 7 or 14 days after the slaughter of young Nellore bulls fed crude glycerin and different sources of roughage during the finishing phase.

Material and methods

The experiment was conducted in the Sector of Food and Digestibility in the Department of Animal Science, FCAV/Unesp, Jaboticabal, São Paulo State, Brazil.

Thirty Nellore bulls with initial body weight of 416.7 ± 24.7 kg and about 18 months of age were housed in individual pens of 8 m^2 (4 x 2 m), containing feeders and drinkers.

Cattle were introduced to the feedlot, where they were adapted for 21 days and distributed in a completely randomized design with three treatments and ten replications.

The experiment was conducted to evaluate the inclusion of 10% crude glycerin (80.3% glycerol, 1.6% ether extract, 5.0% mineral and 12.0% of water) in the diet dry matter, replacing corn, comparing three roughage sources: corn silage, fresh chopped sugar cane or sugar cane bagasse. Diets were formulated in accordance with the requirements of the animals and fixed in NDF of the forage at 15%; thus, it is possible to compare the association of crude glycerin with roughage in the same concentration of forage NDF. Diets were isonitrogenous and isocaloric (Table 1).

Table 1. Ingredients and chemical composition of the experimental diets.

	Diets		
	CS[1]	SC[2]	CB[3]
Ingredients (% DM)			
Corn silage	28.85	-	-
Sugar cane	-	27.54	-
Sugar cane bagasse	-	-	17.27
Corn	35.66	33.66	45.11
Soybean meal	22.49	25.8	24.62
Crude glycerin	10.00	10.00	10.00
Mineral supplement	3.00	3.00	3.00
Chemical composition (% DM)			
Crude protein	15.91	15.91	15.91
Neutral detergent fiber	23.58	23.93	26.3
Forage neutral detergent fiber	15.00	15.00	15.00
Total digestible nutrients	71.93	69.91	70.82
Non-fiber carbohydrates	50.19	51.95	47.61
Ether extract	2.75	2.33	2.53

[1]CS = corn silage; [2]SC = sugar cane; [3]CB = sugar cane bagasse.

Table 2 shows the content of fatty acids in ingredients used in experimental diets, according to the methodology of Hartman and Lago (1973).

Table 2. Fatty acid composition (%) of corn silage, sugar cane, sugar cane bagasse, corn, soybean meal and crude glycerin.

Fatty acids	CS	SC	CB	Co	SM	CG
SFA	20.34	33.29	44.85	16.84	21.7	27.14
UFA	79.66	64.80	51.69	83.16	78.30	72.86
MUFA	31.97	19.95	31.70	36.02	20.72	38.11
PUFA	47.69	44.85	19.99	47.14	57.58	34.75

CS = Corn silage; SC = Sugar cane; CB = Sugar cane bagasse; Co = Corn; SM = Soybean meal; CG = Crude glycerin; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

After 85 days, cattle were weighed after solid fasting for 16h, and transported to a commercial slaughterhouse with an average weight of 554.5 ± 38.5 kg.

Carcasses were divided into half-carcasses and chilled in a cold chamber at 4°C for 24 hours. After chilling, *longissimus* muscle samples were obtained from the 11st and 13rd ribs (left half carcass) for meat quality analysis. From each carcass was collected three samples corresponding to the time one, seven and 14 days *post mortem*, including three steaks standardized at 2.5 cm to evaluate each meat quality variable at the respective time, totaling nine samples per animal. In this sense, we used different steaks to evaluate a given variable at each aging time. Samples were individually vacuum-packed. Samples referring to the day one were frozen at -20°C for further analysis of meat quality. Samples of the times seven and 14 days were aged in cold chamber (no light) between 0 and 2°C and then stored at -20°C until quality analyses.

In the time of 24 hours *post mortem*, we evaluated: water holding capacity, pH, beef and subcutaneous

fat color, cooking losses, shear force, TBARS, fatty acid profile and chemical composition (intramuscular fat).

In all aging times, we evaluated water holding capacity, beef color, pH, cooking losses, shear force and chemical composition (intramuscular fat). All assessments in all aging times were made in steaks thawed in a refrigerator with temperature controlled between 0 and 2°C.

pH measurements were performed directly in all samples, using pHmeter (SG2 - ELK, Seven Go™, Mettler Toledo International Inc.), with penetration electrode, inserting it into the cuts.

Beef color was evaluated in the same steaks used for analysis of water holding capacity, previously thawed. For fat color analysis, we removed a portion of subcutaneous fat between the 12nd and 13rd ribs. Meat color was determined as described by Houben et al. (2000), using a colorimeter (CR 300, Minolta Camera Co. Ltd., Osaka, Japan), evaluating the lightness (L★) 0 = black; 100 = white, the intensity of the red color (a★) and the intensity of the yellow color (b★). Thirty minutes before the evaluations at different points of the meat sample, a cross section of the muscle was made to expose the myoglobin to oxygen. The instrument calibration was performed, before the reading of the samples, with a standard white and black in different points of each steak or portion of subcutaneous fat. After 30 minutes of exposure to air, L★, a★, b★ values were determined according to the Cielab system. Values of L★, a★, b★ were obtained from five readings taken at different points in each steak or portion of subcutaneous fat.

Water holding capacity was obtained by the difference between the weights of the meat samples of approximately 2 g, which were under pressure of 10 kg for 5 minutes.

Meat shear force was determined as recommended by AMSA (1995). For this analysis, we used a steak with 2.5 cm thick. Each sample was roasted in a preheated oven at 180°C (Layr, Luxo Inox); its internal temperature was monitored with the aid of thermocouples (Omega Engineering, Stamford, CT). After the internal temperature reached 70°C, samples were removed from the oven and chilled in a refrigerator for 24 hours at 2 to 5°C. From each steak, we removed six homogeneous cylinders, 1.3 cm diameter, parallel to the muscle fibers, preventing connective tissue and fat, using a stainless steel sampler. The cylindrical samples were sheared perpendicularly to the direction of muscle fibers, using a texturometer (G-R Manufacturing Company, Manhattan, KS, USA). Cooking losses were evaluated in the same samples used for shear force measurements. Total cooking loss was calculated as the difference between the weight of steaks before and after cooking.

The chemical composition analysis was performed to obtain the ether extract content of the longissimus muscle, according to the AOAC (1995).

Lipid oxidation of the meat was carried out by the methodology described by Vyncke (1970), in which is obtained the content of mg malonaldehyde kg^{-1} meat. First, meat was weighed in a homogenizer cup (20 g crushed sample) using an analytical balance (AUY 220, Shimadzu). We added 60 mL of 7.5% TCA solution, and homogenized in Sorwall/Omni mixer for 2 minutes. The mixture was then filtered through filter paper, similar to Whatman 1. We pipetted 5 mL of the distillate into a test tube with screw cap, added 5 mL TBA reagent, stirred the tubes and immersed in water bath for 45 minutes. After chiling in an ice water bath for 10 minutes, the value was obtained by reading the sample absorbance at 538 nm against a blank (BioSpectrophotometro basic, UV, Eppendorf).

To determine the fatty acid composition of the fresh meat, samples of the transversal section were collected from the longissimus muscle, freeze-dried, and frozen for lipid extraction and methylation. The fatty material was extracted using a mixture of chloroform–methanol, as reported by Bligh and Dyer (1959) and the fatty acid methyl esters (FAME) were obtained by ISO-R-5509 (1978) method. Qualitative and quantitative measurements of fatty acid content were performed by gas chromatography using a chromatograph (Shimadzu, Kyoto, Japan - Model GC-14B with a Communication Bus Module - CBM 102) with a flame ionization detector (FID) and fused silica capillary column (Omegawax 250), which was 30 m in length and 0.25 mm in diameter and had a film thickness of 0.25 µm (Supelco SP-24136). Helium was used as a carrier gas at a flowof 1 mL min.$^{-1}$. A 1-µL aliquot of the samplewas injected into a "split" at a division ratio of 1 100^{-1} and a temperature of 250°C. The temperature of the oven was programmed to remain at 100°C for 2 min. and then increased to 220°C at 4°C min.$^{-1}$ for 25 min., while the detector was at 280°C. Identification and quantification of the methyl esters of the fatty acidswere achieved by comparisonwith the retention times and concentrations of methyl esters of standard fatty acids.

Data of fatty acid profile, TBARS and subcutaneous fat color were analyzed using the PROC Mixed of SAS (2004) using the animal as an experimental unit. The model tested the fixed effect of diet. The analysis of meat color, shear force, cooking loss, water holding capacity, pH and chemical composition were analyzed using PROC Mixed.

Table 3. Mean and SEM for pH, meat color, water holding capacity, shear force and cooking loss of meat of Nellore young bulls fed crude glycerin associated with different roughage sources

	Diet (D)					Aging time (AT)					
ITEM	CS	SC	CB	SEM	P*	1	7	14	SEM	P*	D x AT
pH	5.44[b]	6.08[a]	5.62[b]	0.13	<0.01	5.59[b]	5.66[b]	5.90[a]	0.09	<0.01	0.92
Color ,											
L*	42.74[a]	35.90[b]	39.83[ab]	1.66	0.02	38.94	39.97	39.57	1.13	0.61	0.97
a*	17.26	16.77	17.26	0.28	0.37	16.89	16.88	17.53	0.28	0.18	0.96
b*	15.11	12.61	14.39	0.9	0.16	13.4	14.28	14.44	0.61	0.13	0.96
WHC, %	74.24	73.45	73.45	0.72	0.68	73.7	73.37	74.07	0.79	0.72	0.90
SF, kgf	3.03	2.47	3.15	0.25	0.15	3.42[a]	2.83[b]	2.40[c]	0.17	<0.01	0.78
CKL, %	24.38	22.49	22.89	2.62	0.88	24.58[a]	24.44[a]	20.75[b]	1.75	0.02	0.55

CS = corn silage, SC = sugar cane; CB = sugar cane bagasse; WHC = water holding capacity, SF = shear force, CKL = cooking loss, SEM = standard error of the mean.*probability ($p < 0.05$).

The model tested the fixed effects of diet, aging time and interactions. The animal was included in the model as a random effect. When the interaction was not significant, it was removed from the model. The least squares means were generated for main effects and interactions were considered significant using tukey's test ($p < 0.05$).

Results and discussion

There was no interaction between diets and aging times ($p > 0.05$) on variables, so the results were presented separately.

Animals fed sugarcane exhibited higher pH compared to animals fed corn silage or sugarcane bagasse associated with crude glycerin (Tabela 3; $p < 0.05$). Meat with pH above 6.0 is considered DFD meat (dark, firm and dry), tougher and darker, which is undesirable by consumers. In non-stressed animals with large reserves of muscle glycogen, the muscle pH usually decreases from an initial value of 7.0 to 7.2, after slaughter, to final values between 5.4 and 5.8 within 48 hours post-mortem (Young et al., 2004). Low production of lactic acid is due to lack of glycogen stores at the time of slaughter, so the higher pH in animals fed sugar cane may be due to some stress of animals before slaughter, as the mixture of lots in the slaughterhouse.

Animals fed sugar cane had lower lightness of the meat compared to animals fed corn silage ($p < 0.05$), which is directly associated with the pH value, since the muscle pH exerts effects on meat lightness, because at pH above 6.0, there is a higher holding of water and less penetration of oxygen, thereby decreasing the lightness of the product.

Aging times influenced the pH, shear force and cooking loss ($p < 0.05$). The pH was higher in meat aged for 14 days, this occurs due to enzymatic attack during aging, which increases the osmotic pressure of the medium as a result of degradation of protein to smaller molecules and intermolecular rearrangement of these proteins that determine changes in electric charges (Lawrie, 1977). This effect was more significant for the time 14 days, where the enzymatic attack was possibly greater than other times, which resulted in a higher pH.

The values of shear force were different ($p < 0.05$) among treatments, in which the longer the aging the more tender the meat (lower shear force). Shear force is a very important characteristic for the consumer and is greatly influenced by aging process, and the longer the aging, the lower the shear force. During aging, protein denaturates, muscle fibers disaggregate, directly affecting the shear force, improving the tenderness of the meat (French et al., 2001).

In this study, meat was more tender when subjected to 14 days of aging, which is evidenced by a lower cooking loss in that aging time ($p < 0.05$). It is important to emphasize that the meat with lower cooking loss tend to have higher juiciness, preventing dryness and contributing to a lower shear force.

Table 4. Mean and SEM for the ether extract content, malonaldehyde and fat color of longissimus muscle of Nellore young bulls fed crude glycerin associated with different roughage sources

	Diets				
Parameters	CS	SC	CB	SEM	p*
EE, %	3.90	3.52	4.53	0.21	0.17
MDA, mg kg^{-1}	0.49	0.44	0.49	0.02	0.39
Fat					
L*	67.71	67.7	69.12	0.63	0.59
a*	10.02	9.70	8.20	0.39	0.12
b*	17.78[a]	16.68[ab]	15.52[b]	0.35	0.03

CS = corn silage; SC = sugar cane; CB = sugar cane bagasse; EE = ether extract; MDA = malonaldehyde; SEM = standard error of the mean; *probability ($p < 0.05$).

There was no effect of diet on the ether extract content (Table 4) in *Longissimus* muscle and MDA (mg kg^{-1} meat; $p > 0.05$). Unsaturated fatty acids and lipid oxidation of meat is directly related, as for the occurrence of lipid oxidation, it is necessary the presence of unsaturated fatty acids in meat. Therefore, Table 5 shows that there was no difference in the deposition of fatty acids in meat of animals fed the different diets ($p > 0.05$).

The inclusion of 10% crude glycerin to different roughage sources in the diet DM did not affect the values of L* and a* of the subcutaneous fat ($p > 0.05$); however, animals fed corn silage had higher value of b* ($p < 0.05$) compared to subcutaneous fat of animals fed sugar cane bagasse or sugar cane. These results are related to the amount of carotenoid in the corn silage, since this silage has green leaves in its structure, thus leading to a greater percentage of carotenoids in the diet, influencing the value of b*.

There were no statistical effects ($p > 0.05$) of diets on the contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and unsaturated fatty acid (UFA).

The content of fatty acids in the roughages used in diets is shown in Table 2. Although corn silage had a lower content of SFA (20.3%) compared with sugarcane bagasse (44.8%), the levels found in meat did not differ ($p > 0.05$).

Table 5. Mean and SEM for the main fatty acids (%) found in the longissimus muscle of Nellore young bulls fed crude glycerin associated with different roughage sources.

	Diet			SEM	p*
Fatty acids	CS	SC	CB		
SFA	43.719	40.887	40.627	0.83	0.75
MUFA	44.857	46.081	43.217	1.01	0.52
PUFA	11.044	11.962	14.395	0.66	0.81
UFA	56.281	59.113	59.378	0.83	0.75
UFA/SFA	1.302	1.453	1.439	0.04	0.71

CS = corn silage; SC = sugar cane; CB = sugar cane bagasse; SEM = standard error of the mean; SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; *probability ($p < 0.05$).

This is related to the amount supplied of each food, since the diet had a fixed content of NDF, then the amount of sugar cane bagasse in the diet was nearly the half (17.3%) of silage corn (28.8%) due to the NDF of each roughage. The nutritional value of sugar cane bagasse is low, due to the bonds between cellulose, hemicellulose and lignin, in cell wall. Fibers of sugar cane bagasse contain, as main components, about 40% cellulose, 35% hemicellulose and 15% lignin, this latter is responsible for its low use in animal feed. This justifies its lower amount in the diet, as corn silage has a larger amount of available carbohydrates and a lower NDF content.

The levels of UFA and the PUFA of sugar cane bagasse were lower (51.7 and 20.0%, respectively) compared to corn silage (79.7 and 47.7%, respectively), and sugar cane (64.8 and 44.8%, respectively); however, animals fed sugar cane bagasse showed levels of UFA and PUFA similar to meat of animals fed other diets. This is because the interaction of low quality fiber with crude glycerin may have affected more strongly bacteria responsible for biohydrogenation, which led to a greater passage of unsaturated and polyunsaturated fatty acids in the diet containing sugar cane bagasse as roughage. Crude glycerin can inhibit lipolysis, causing a greater passage of unsaturated fatty acids by rumen (Krueger et al., 2010), being important to note that the ruminal biohydrogenation is only possible after the occurrence of lipolysis, as the enzymes responsible for this process act only on free fatty acids. In this way, these same authors investigated the effect of glycerol at 2 to 20% on ruminal fermentation by incubation and observed that lipolysis was inhibited by 48 and 77%, depending on the glycerol content. This lower biohydrogenation in the diet containing lower quality fiber, can also be explained by the greater content of concentrate in the diet containing sugar cane bagasse as roughage, in comparison with other treatments, as the NDF content of sugar cane bagasse is higher than that in corn silage and sugar cane. In consequence, the amount of concentrate in the diet containing sugar cane bagasse was proportionately higher compared to the other treatments, which may have created an unfavorable environment for cellulolytic bacteria acting in the ruminal biohydrogenation.

Conclusion

Diets containing 10% crude glycerin in the DM associated with corn silage, sugar cane or sugar cane bagasse as roughage had no effect on the meat fatty acid profile. However, animals fed corn silage have yellowness fat compared to animals fed sugar cane bagasse.

The aging for 14 days reduces cooking loss and shear force of the longissimus muscle of Nellore young bulls, and should be adopted for improving the meat quality.

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