Ciencia e Investigación AGRARIA

Cien. Inv. Agr. 44(3):320-332. 2017
www.rcia.uc.cl

ANIMAL PRODUCTION

DOI: 10.7764/rcia.v44i3.1861

ESSAY

Meat quality of heifers finished on pasture with tropical grass and supplemented with glycerin

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Abstract

R.R. Silva, L.M.A. M. Facuri, G.G.P. Carvalho, F.F. Silva, J.I. Simionato, C.B. Sampaio, L.S. Bezerra, R.M. Prado, I.N. Prado, A.P.G. Silva, M.L.G.M.L. Araujo, and B.M.A. Carvalho. 2017. Meat quality of heifers finished on pasture with tropical grass and supplemented with glycerin. Cien. Inv. Agr. 44(3): 320-332. Glycerin is an organic compound with an alcoholic function and can be esterified into fatty acids to form triglycerides. Due to the increasing availability of glycerin, studies that determine the best level of its inclusion in diets for ruminants are needed. This study evaluated the effects of glycerin supplementation on the proximate composition and fatty acid profile of the *longissimus lumborum* of heifers fed on *Brachiaria brizantha* pasture. Thirty-six heifers were distributed in a totally randomized design with four treatments (G0.0 = without glycerin, G4.6 = 4.6% glycerin, G9.3 = 9.3% glycerin and G14.3 = 14.3% glycerin). The addition of glycerin decreased the tetradecanoic and octadecanoic fatty acids but increased the pentadecanoic, heptadecanoic, heptadecenoic, eicosanoic, eicosatetraenoic and docosatetraenoic fatty acids. The saturated (SFA), monounsaturated (MUFA) and omega-3 (n-3) fatty acid concentrations were similar across the diets. However, the polyunsaturated (PUFA) concentrations and the PUFA:MUFA and n-6:n-3 ratios increased with the inclusion of glycerin in the diet. Glycerin levels up to 14.3% (corresponding to a substitution of 50.5% of corn for this byproduct as an energy source) did not alter the proximate composition of the meat but improved the fatty acid profile of the *longissimus lumborum* muscle and, consequently, increased the meat quality, potentially providing benefits for human health.

Keywords: Biodiesel, CLA, glycerol, omega-3, polyunsaturated.

Received August 09, 2017. Accepted November 09, 2017.
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Introduction

The rapid expansion of the biodiesel industry over the past decade has increased glycerin availability. For each hundred kg of biodiesel, an average of ten kg of crude glycerin is produced (Johnson & Taconi, 2007) as a byproduct. The increase in the availability of glycerin has driven prices down and contributed to excess production. This byproduct may be used for other purposes, such as for animal feed (Eiras *et al*., 2014, Cruz *et al*., 2014, Moreira *et al*., 2016, San Vito *et al*., 2015, Silva *et al*., 2014, Strada *et al*. 2015).

Glycerin is an organic compound with an alcoholic function, and it can be esterified to fatty acids to form triglycerides. Glycerol is the main component of glycerin, which also contains small amounts of ash, water and methanol (Eiras *et al*., 2014). Glycerol is metabolized by ruminal microorganisms and increases the total volatile fatty acid content in the rumen (El-Nor *et al*., 2010). Because it has gluconeogenic properties (Donkin *et al*., 2009), glycerol could potentially improve carcass and meat quality grades (Eiras *et al*., 2014, Françozo *et al*., 2013).

Glycerin supplementation could increase lipogenesis and thus increase marbling and subcutaneous fat (Parsons *et al*., 2009). Glycerin may also be converted into glucose in the livers of cattle. Thus, the glucose supply in bulls supplemented with glycerin may foster a rise in lipogenesis. However, other studies noted a linear decrease in marbling scores and subcutaneous fat when glycerin was included in the diets of cattle, which could negatively affect the carcass grades (Mach *et al*., 2009, Parsons *et al*., 2009).

As mentioned by Destefanis *et al*. (2000), statistical analysis of many heterogeneous data obtained through classic methods provides information for evaluating and understanding each variable. Although an analytical method, this approach neither reveals the relation between the variables nor allows the grouping of samples when they have homogeneous characteristics. Therefore, Karlsson (1992) suggested the evaluation of meat quality through correlated characteristics using principal component analysis. This study evaluated the effect of glycerin on the proximate composition and fatty acid profile in the *longissimus lumborum* of heifers supplemented while grazing on *Brachiaria brizantha* cultivar Marandu pasture.

Materials and methods

Location, animals and experimental diets

This experiment was approved by the Department of Animal Science (approval number 17/2012) of the State University of Bahia (CIOMS/OMS, 1985). The experiment lasted 135 d and was preceded by a 14-d adaptation period during which the animals were adapted to the diets, handling procedures and facilities.

The heifers were grazed on *B. brizantha* cultivar Marandu pasture. The total experimental area used was 18 ha, which was divided into ten paddocks of approximately 1.8 ha each for rotational grazing. Thirty-six 20-month-old crossbred heifers (average initial body weight 264 ± 12 kg) were distributed in a totally randomized design with four treatments and nine replicates as follows: G0.0 = control, G4.6 = 4.6% glycerin, G9.3 = 9.3% glycerin and G14.3 = 14.3% glycerin (based on the dry matter of the total diet); glycerin replaced 16.1%, 33.0% and 50.5% of the corn grain, respectively.

The supplement was provided to the heifers once daily (10:00 h) in plastic troughs, and because the experiment was conducted in a grazing system, the animals were kept in groups as described by Neto *et al*. (2015), Pouzo *et al*. (2016) and Wright *et al*. (2015). Animals in each treatment group were kept in a separate paddock for an occupation period of 7 d. Supplementation with glycerin was provided to the nine animals in each group, and levels were calculated according to the amount ingested per heifer. As with other studies (Barbero

et al., 2015, San Vito *et al*., 2015) for animals kept in groups on pasture, we estimated the nutritional and productive parameters in each animal individually through the supply of LIPE® capsules and the use of titanium dioxide in the supplement according to Titgemeyer *et al*. (1997, 2001).

The glycerin used in this study was supplied by a soy-diesel company (BIOPAR ®-Bioenergia do Paraná LTDA., Paraná State, Brazil) (Table 1). Four levels of glycerin were used to replace the corn grain (Table 2). The diets were formulated to be isonitrogenous and isoenergetic and to meet the nutritional requirements of growing heifers (NRC, 2000). The concentrate was formulated with specific mineral supplements (Table 2) for heifers and was composed of corn, soybean meal and different levels of glycerin.

Table 1. Chemical composition of glycerin used in heifer diets.

Parameters	Results
Water† (mg kg^{-1})	232
Ash (%)	4.76
Glycerol (%)	81.20
Methanol (%)	0.33
Sodium (mg kg^{-1})	11,634.40
Potassium (mg kg^{-1})	79.10
Chloride (mg kg^{-1})	35.80
Magnesium (mg kg^{-1})	16.30
Phosphorus (mg kg^{-1})	239.80
Gross energy (kcal)	3.400

†Water content was determined by the Karl Fischer method at the Institute of Technology of Paraná (TECPAR).

The pasture was evaluated every 28 d (Table 2), and the availability of the dry matter (DM) was determined according to McMeniman (1997). The daily residual biomass (RB) was estimated using the double-sampling method (Wilm *et al*., 1994). Prior to cutting the samples, the DM of the biomass from the sample was estimated visually. The equation proposed by Gardner (1986) was utilized to calculate the forage biomass (expressed in kg ha^{-1}).

The forage, ingredients and concentrate samples were pre-dried in a forced-ventilation oven at 65 °C for 72 h, processed with a Wiley cutting mill (Tecnal, Piracicaba City, São Paulo State, Brazil) with a 1-mm sieve, stored in plastic containers and sealed properly prior to laboratory analysis, which was conducted at the Laboratory of Feed Analyses and Animal Nutrition of the State University of Bahia (Table 2).

The dry matter (DM; Method 930.15), crude protein (CP; Method 976.05) and ether extract (EE, Method 2003.05) levels were determined according to the method of the Association of Official Analytical Chemists - AOAC (2006). The organic matter (OM) content was calculated as the difference between the DM and ash contents by incineration in an oven at 550 °C for 5 h (AOAC, 2006). The methods described by Mertens (2002) were used to determine the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents. Total carbohydrates (TCs) were obtained by the following equation proposed by Sniffen *et al*. (1992): TC = 100 – (% CP + % EE + % MM). Non fiber carbohydrates (NFCs) were determined by the difference between the TC and the NDF. The total digestible nutrient (TDN) content of the diets was obtained using the methodology described by Kearl (1982).

Slaughter and sample collection

At the end of confinement and after resting and a 14-h period of fasting from solids, the heifers were slaughtered in a commercial slaughterhouse (Confrigo Frigorífico Ltd., Vitória da Conquista, BA, Brazil) by concussion stunning (air gun), followed by bleeding, skinning and evisceration. The slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, Ministério Pecuária e Abastecimento, 2000).

After dressing and evisceration, the carcasses were split into two identical longitudinal halves

Table 2. Ingredient composition (% of DM) of the concentrate and chemical composition of *Brachiaria brizantha* cv. Marandu, including the total availability of the dry and residual biomass, allotment space, accumulation rate and forage availability and the chemical compositions of the concentrates.

Ingredient (% DM)		Level of replacement (% DM)			
		0†	16.12‡	32.99§	50.49¶
Soybean meal		20.57	23.69	26.94	30.30
Ground corn		77.65	61.94	45.60	28.63
Glycerin		0.00	12.52	25.62	39.21
Mineral salt#		1.77	1.80	1.83	1.88
Ingredients	*Brachiaria brizantha*	Glycerin levels (% DM)			
		G0.0†	G4.6‡	G9.3§	G14.3¶
Dry matter (%)	35.97	91.7	92.4	91.8	92.2
Crude protein††	5.62	21.1	22.1	23.3	24.6
Ether extract††	1.20	2.47	2.72	2.79	2.82
Total carbohydrates††	84.3	72.8	71.0	68.9	66.7
Non fiber carbohydrates††	19.1	58.9	57.3	55.5	53.4
Gross energy (kcal kg^{-1})‡‡	4.22	4.30	4.24	4.25	4.19
Neutral detergent fiber††	66.5	13.9	13.7	13.4	13.3
Acid detergent fiber††	40.5	3.59	3.79	3.92	4.17
Mineral matter††	8.87	3.66	4.15	4.99	5.82
Total digestible nutrients††	51.0	63.7	60.6	61.4	61.4
Total availability of DM (kg ha^{-1})	3103	-	-	-	-
Residual biomass (kg DM ha^{-1} day^{-1})	111	-	-	-	-
Allotment rate (AU ha^{-1})	1.33	-	-	-	-
Accumulation rate (kg DM ha^{-1} day^{-1})	27.7	-	-	-	-
Forage supply (kg DM 100 kg^{-1} LW^{-1} day^{-1})	23.2	-	-	-	-

†Without glycerin; ‡4.6% glycerin (% DM); §9.3% glycerin (% DM); ¶14.3% glycerin (% DM). #Supplied per kilogram of product: calcium – 175 g; phosphorus – 60 g; sodium – 107 g; sulfur – 12 g; magnesium – 5 g; cobalt – 107 mg; copper – 1.30 g; iodine – 70 mg; manganese – 1000 mg; selenium – 18 mg; zinc – 4.0 g; iron – 1.4 g. ††Values represent a percentage of the dry matter; ‡‡The gross energy was determined using a bomb calorimeter.

through the sternum and spine. The half carcasses were identified, transported to a cold chamber and maintained at 2 °C for 24 h. The section between the 10th and 13th ribs on the right half of the carcass was removed according to the methodology described by Hankins & Howe (1946) and adapted by Muller (1987).

Proximate composition analysis and fatty acid profile

Analyses of the *longissimus lumborum* samples were performed two months after sampling using samples from the right side of the carcass after a cross-section cut was made between the 12th and 13th ribs. The *longissimus lumborum* samples were ground, homogenized and analyzed in triplicate.

The proximate compositions of the *longissimus lumborum* samples in terms of moisture, ash and crude protein were determined according to the AOAC (2006) methods. Total lipids were extracted by the Bligh and Dyer (1959) method.

Triacylglycerols (TAGs) were transesterified to obtain fatty acid methyl esters (FAMEs) via triacylglycerol methylation according to the ISO

(1978) method. The FAMEs were analyzed in a gas chromatograph (Varian, USA) equipped with a flame ionization detector and a fused silica capillary Select FAME column (CP-7420, 100 m, 0.25 mm and 0.39 µm o.d. Varian, USA). The column temperature was programmed for 165 °C for 18 min, 180 °C (30 °C min^{-1}) for 22 min, and 240 °C (15 °C min^{-1}) for 30 min, with 45 psi of pressure. The injector and detector were kept at 220 °C and 245 °C, respectively. The gas flows (White Martins, São Paulo, Brazil) were 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. The sample was injected using a split mode 1/80. Fatty acids (FAs) were identified by comparing the relative retention times of the FAME peaks of samples spiked with FAME standard 189–19 (Sigma Company, St. Louis, MO, USA). Peak areas were determined using Star software (Varian, Walnut Creek, CA, USA), as described by Simionato *et al*. (2010). Data were expressed as percentages of the normalized fatty acid area (Table 3).

Statistical analysis

The experimental design was completely randomized, with four treatments and eight replicates using the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} = the observed value of the dependent variable, μ = the overall mean, T_i = the effect of treatment i (i = 1 to 5), and e_{ij} = the experimental error. The univariate statistical analysis was performed with SAS (2009), and the characteristics in this study were tested for normality. Data that showed normal distributions were analyzed by regression equations following the MIXED procedure to determine the linear and quadratic effects of glycerin. The treatment means were computed with the LSMEANS option. The treatment means were declared significant at $P<0.01$.

The values of parameters obtained from the fatty acid profile analysis in heifers fed diets with glycerin consisted of multivariate data sets arranged in a matrix (36 × 36) and interpreted using principal component analysis. The analysis was performed with the Statistical Analysis System (SAS 9.0®, 2009) using the data-centric average.

Results

The moisture, ash, crude protein and total lipid concentration averages were 74.9, 1.10, 22.7 and 1.16%, respectively (Table 4). A linear reduction of C14:0- tetradecanoic acid (24.4%) was observed

Table 3. Fatty acid profiles of glycerin, roughage, forage and concentrate.

Fatty acid	*B. brizantha*	Glycerin	Glycerin levels (% DM)			
			G0.0†	G4.6‡	G9.3§	G14.3¶
C16:0	30.25	12.24	13.18	13.99	14.34	15.38
C17:0	5.59	4.97	4.48	3.34	3.68	4.29
C18:0	11.77	25.77	39.13	39.03	40.49	32.11
C18:1n9	22.81	51.62	39.92	41.36	39.12	45.14
C18:2n6	25.60	4.06	2.07	1.62	1.50	2.47
C 21:0	2.87	1.33	0.70	0.24	0.34	0.25
C 22:2n6	1.11	-	0.52	0.41	0.53	0.36
C 22:6n3	1.09	-	-	-	-	-

†Without glycerin; ‡4.6% glycerin (% DM); §9.3% glycerin (% DM); ¶14.3% glycerin (% DM). 16:0 – Hexadecanoic acid; 17:0 – Heptadecanoic acid; 18:0 – Octadecanoic acid; 18:1n9 – Octadecenoic acid; 18:2n6 – octadecadienoic acid; 21:0 – Heneicosanoic acid; 20:5n3 Eicosapentaenoic acid (EPA), 22:2n6 – Docosadienoic acid 13, 16; 22:6n3 – Docosahexaenoic acid (DHA).

Table 4. Proximate composition of the *longissimus lumborum* muscle from heifers finished in the pasture system and supplemented with glycerin in their diets.

Item (% NM)	Glycerin levels (% DM)				SEM[#]	P Values	
	G0.0[†]	G4.6[‡]	G9.3[§]	G14.3[¶]		Linear	Quadratic
Moisture	74.5	74.8	75.0	75.4	0.26	0.22	0.48
Ash	1.13	1.09	1.10	1.11	0.09	0.25	0.33
Crude protein	22.8	22.9	22.9	22.5	0.24	0.71	0.86
Total lipids	1.13	1.24	1.20	1.08	0.08	0.34	0.60

[†]Without glycerin; [‡]4.6% glycerin (% DM); [§]9.3% glycerin (% DM); [¶]14.3% glycerin (% DM). [#]Standard error of the mean.

with the inclusion of glycerin in the diet. However, increasing levels of glycerin increased the C15:0 (pentadecanoic acid) fatty acid concentration (Table 5), linearly increased the C17:0 (heptadecanoic acid) and C17:1 (heptadecenoic acid) fatty acid concentrations, and linearly decreased the C18:0 (octadecanoic acid) fatty acid concentration in the *longissimus lumborum*.

No effect was observed on the unsaturated fatty acid concentrations in the C18 series. Thus, no difference was detected in the c9,c12 octadecadienoic acid concentration of the meat (C18:2) with the increasing dietary crude glycerin. Additionally, no difference was observed in the c9,t11-octadecadienoic acid concentration of the meat (C18:2) with increasing dietary crude glycerin.

The levels of the C20:0 (eicosanoic acid), C20:4n-6 (eicosatetraenoic acid) and C22:4n-6 (docosatetraenoic acid) fatty acids in the *longissimus lumborum* increased linearly with the addition of increasing amounts of glycerin to the heifer diets. The saturated fatty acid (SFA) and MUFA concentrations were similar (Table 5). The PUFA concentrations in the *longissimus lumborum* increased linearly. The concentration of the sum of the n-6 fatty acid series increased 45%. The n6:n3 ratio in the *longissimus lumborum* increased linearly from 3.6 to 5.37.

Figure 1 and Table 6, respectively, show the PCA scores and estimates of the eigenvalues associated with the studied variables that are associated with the fatty acid profiles in the muscle samples of *longissimus lumborum* in the heifers fed diets supplemented with glycerin.

The principal component analysis showed that 92.57% of the variation of the results was explained by the first and second main components. Thus, contributions of the first (61.85%) and second (30.72%) principal components were observed.

Table 6. Principal component (PC), eigenvalues (λ_i), and percentage of variance explained by the component (% VCP) of the fatty acid studied in the *longissimus lumborum* of heifers supplemented with glycerin in the diet.

I	Principal component	λ_i	% VCP	% VCP (accumulated)
1	PC_1	2.6165	61.85	61.85
2	PC_2	1.2995	30.72	92.57
3	PC_3	0.3144	7.43	100.00
4	PC_4	0	0.000	100.00
5	PC_5	0	0.000	100.00
6	PC_6	0	0.000	100.00
7	PC_7	0	0.000	100.00
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
35	PC_{35}	0	0.000	100.00

To evaluate the discrimination of the fatty acid profile, the two main components (the first principal component (PC1) and PC2) were plotted (Figure 1). The score portion of the PC1 compared

Table 5. Fatty acid percentage and sum of the *longissimus lumborum* in heifers finished in the pasture system and supplemented with glycerin in their diets.

Fatty acid	Glycerin levels (% DM)				SEM[#]	P Value		R^2
	G0.0[†]	G4.6[‡]	G9.3[§]	G14.3[¶]		Linear	Quadratic	
C 14:0 -Tetradecanoic	2.99	2.75	2.40	2.26	0.09	0.01	0.92	0.55
C 14:1 -Tetradecenoic	0.50	0.40	0.45	0.48	0.02	0.70	0.25	-
C 15:0 -Pentadecanoic	0.37	0.46	0.46	0.53	0.02	0.01	0.74	0.89
C 15:1- Pentadecenoic	0.21	0.21	0.17	0.19	0.01	0.10	0.65	-
C 16:0 -Hexadecanoic	25.0	24.8	25.8	25.6	0.39	0.29	0.83	-
C 16:1 -Hexadecenoic	2.71	2.64	2.68	2.87	0.07	0.45	0.36	-
C 17:0 -Heptadecanoic	1.04	1.18	1.32	1.69	0.05	0.001	0.07	0.99
C 17:1 -Heptadecenoic	0.89	0.93	1.15	1.47	0.05	0.001	0.06	0.91
C 18:0 -Octadecanoic	17.9	18.4	16.4	15.6	0.41	0.01	0.39	0.78
C 18:1-*n*7 - cis- 11 Octadecenoic	1.67	1.92	1.74	1.73	0.06	1.00	0.28	-
C 18:1-*n*9c -Octadecenoic	40.2	39.9	39.9	39.6	0.40	0.07	0.91	-
C 18:1-*n*11t - Trans-Octadecenoic	1.78	1.54	1.75	1.88	0.08	0.30	0.20	-
C 18:2-*n*6 - c9,c12 Octadecadienoic	2.06	2.07	2.87	2.69	0.10	0.12	0.06	-
C 18:2-c9t11 - c9,t 11-Octadecadienoic	0.16	0.14	0.15	0.11	0.25	0.03	0.09	-
C 18:3-*n*3 - c9,c12,c15-Octadecatrienoic	0.49	0.54	0.45	0.46	0.01	0.26	0.61	-
C 18:3-*n*6 - c6,c9,c12-Octadecatrienoic	0.10	0.11	0.09	0.10	0.00	0.60	0.46	-
C 20:0 Eicosanoic	0.33	0.37	0.35	0.49	0.02	0.01	0.17	0.68
C 20:2-*n*6 – Eicosadienoic	0.10	0.13	0.11	0.11	0.01	0.16	0.12	
C 20:4-*n*6 -Eicosatetraenoic	0.55	0.66	0.75	0.99	0.05	0.01	0.50	0.95
C 20:5-*n*3 - Eicosapentaenoic	0.25	0.19	0.31	0.28	0.01	0.07	0.54	-
C 22:0 - Docosanoic	0.16	0.17	0.16	0.17	0.02	0.23	0.35	-
C 22:4-*n*6 – Docosatetraenoic	0.19	0.19	0.22	0.46	0.01	0.01	0.53	0.99
C 22:6-*n*3 - Docosahexaenoic	0.27	0.27	0.26	0.28	0.01	0.58	0.12	-
SFA[††]	47.8	48.1	46.8	46.3	0.46	0.34	0.73	-
MUFA[‡‡]	48.0	47.5	47.9	48.8	0.46	0.85	0.33	-
PUFA[§§]	4.24	4.36	5.28	5.55	0.15	0.01	0.10	0.61
n-6[¶¶]	3.00	3.16	4.04	4.35	0.12	0.01	0.18	0.55
n-3[##]	0.83	0.87	0.78	0.81	0.03	0.31	0.10	-
PUFA:MUFA[†††]	0.09	0.09	0.11	0.12	0.01	0.03	0.08	0.59
n-6:*n*-3[‡‡‡]	3.61	3.63	5.18	5.37	0.14	0.01	0.08	0.81

[†]Without glycerin; [‡]4.6% glycerin (% DM); [§]9.3% glycerin (% DM); [¶]14.3% glycerin (% DM). [#]Standard error of the mean; [††]Saturated fatty acids; [‡‡]Monounsaturated fatty acids; [§§]Polyunsaturated fatty acids; [¶¶]Fatty acids *n*-6; [##]Fatty acids *n*-3; [†††]PUFA:MUFA ratio; [‡‡‡]*n*-6:*n*-3 ratio.

to that of PC2, with a cumulative contribution of 92.57%, was the easiest method for viewing the main trends defined in the different treatment samples. A tendency existed for separation between the samples in accordance with the increased levels of glycerin in the diet. The increasing addition of glycerin was distinguished into three different groups (Figure 1).

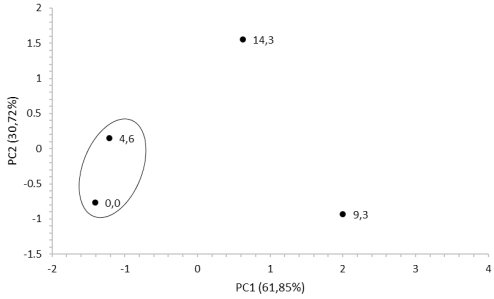

Figure 1. The first two principal components of the fatty acid profile analysis in the samples from the *longissimus lumborum* in heifers fed diets supplemented with glycerin (0.0; 4.6; 9.3 and 14.3).

Discussion

The moisture, ash and crude protein levels were similar to those reported in the literature (Correia *et al*., 2016, Françozo *et al*., 2013, Rotta *et al*., 2009a, Rotta *et al*., 2009b). Rivaroli *et al*. (2016) investigated the effect of the different doses of an essential oil blend on meat quality and observed that the meat proximate compositions (moisture, ash, crude protein and total lipids) were unaffected by the addition of essential oils to the diet.

The lipid rates in the *longissimus lumborum* were low. We expected that the glucose produced by glycerin would increase the muscle lipids in the heifers due to an increase in the blood insulin concentration and lipogenesis. However, Eiras *et al*. (2014) reported a total lipid decrease in the *longissimus lumborum* of bulls fed glycerin (0%, 6%, 12% and 18% of the DM). The low total lipid levels may be explained by the age of the heifers (young animals), which generally present lower total lipids and cholesterol in the meat (Rotta *et al*., 2009a).

The linear reduction of C14:0 acid (24.4%) as glycerin increases in the diet is beneficial to human health (Webb and O'Neill, 2008) because this fatty acid is considered hypercholesterolemic (Scollan *et al*., 2006). Similarly, Eiras *et al*. (2014) reported a reduction in C14:0 in meat from bulls fed increasing levels of glycerin in the diet.

Fatty acids with an odd chain are present at low levels in mammals (Rotta *et al*., 2009b). In ruminants, this acid is formed by *de novo* synthesis from propionic acid produced in the rumen by the fermentation process (Trabue *et al*., 2007). Thus, the increase in C15:0 in the *longissimus lumborum* may be attributed to the formation of propionic acid with the addition of glycerin in the diet (Lee *et al*., 2011, Trabue *et al*., 2007).

Variations in the C17:0 and C17:1 fatty acid levels may be attributed to the ruminal fermentation time. According to Trabue *et al*. (2007), 80% of glycerin is completely metabolized in the rumen 24 h after feeding and is principally transformed into propionic acid, which may be used to form heptadecanoic acid in the meat.

The linear decrease of the C18:0 fatty acid concentration is thought to have a neutral effect on a hypercholesterolemic diet in humans (Webb and O'Neill, 2008). Moreover, this fatty acid represents 10 to 20% of the fats produced by ruminants (Valsta, Tapanainen, & Männistö, 2005). High levels of octadecadienoic acid are present in corn, and therefore, its replacement resulted in lower octadecadienoic acid intake by the animals. The increasing dietary crude glycerin was in agreement with previous studies (Carvalho *et al*., 2014, Carvalho *et al*., 2015). This apparent contradiction may result from lower ruminal biohydrogenation, which results in greater absorption of fatty acids in the duodenum.

SFAs represented approximately 47% of the total fatty acids in the *longissimus lumborum* in heifers, whereas monounsaturated fatty acids (MUFA) represented 48% of the total fatty acids. The inclusion of glycerin in the ruminant diet had a low effect on the fatty acid composition in the *longissimus lumborum* (Eiras *et al*., 2014, Françozo *et al*., 2013). Indeed, the SFAs and the MUFAs of the *longissimus lumborum* bulls fed glycerin were lower than those evaluated in bulls from different crossbreeding systems finished in a feedlot (Ducatti *et al*., 2009, Rotta *et al*., 2009a). The values were similar for the SFAs, although the MUFAs were present at higher concentra-

tions than reported by Françozo *et al*. (2013), who evaluated beef from Nellore cattle fed diets containing glycerin (0%, 5% and 12% of the DM).

The PUFA concentrations ranged between 5 and 10% in the cattle finished in a feedlot (Rotta *et al*., 2009b). In some cases, the concentrations were greater than 10% (Maggioni *et al*., 2009). From the perspective of human health, an increase in the PUFAs in the meat of ruminants is desirable because it is associated with increases in the n-3 and n-6 series of FAs (HMSO, 1994, Webb and O'Neill, 2008, Wood *et al*., 2008). As noted, the replacement of corn with glycerin in the diet of bulls finished in a feedlot significantly increased the PUFA percentage in the *longissimus lumborum* (Eiras *et al*., 2014, Zawadzki *et al*., 2013).

Increasing the n-6 fatty acid concentration corroborated the results of Eiras *et al*. (2014), who found that the n-6 percentage ranged between 3.4% and 5.0% with an increase of 46% due to glycerin inclusion in the diet of bulls finished in a feedlot. Diets with glycerin inclusion that present a n6:n3 ratio greater than 4 are considered beneficial to human health. Human diets are typically close to a ratio of 4. Similarly, Eiras *et al*. (2014) observed an increase in the n6:n3 ratio levels when 0 to 18% glycerin (%DM) was included in the diet of cattle. Thus, the inclusion of glycerin in the diet could be a useful tool for improving the n6:n3 ratio in meat from ruminants finished in a feedlot.

The numbers represented by component analysis demonstrated significant contributions of individual fatty acid variables to the total variability explained by the generated PCs. Hernandez *et al*. (2000) reported that the principal component analysis (PCA) is a multivariate statistical tool for obtaining a restricted group of measures that explain most of the variability of the measurements. According to the authors, the PCA also helps to evaluate the relations between variables and to compare the differences between groups of animals.

According to Cruz *et al*. (2012), the principal component analysis is determined by components that involve at least 80% of the total variation, thereby showing the importance of each variable studied and its effect on the total variation between observations.

PC1 could be discriminated by the glycerin levels in the diet, with samples with higher glycerin levels in the diet located at the negative end of PC1. Thus, three groups were formed with lower, intermediate and higher glycerin values. Pereira *et al*. (2016) used principal component analysis to evaluate the nutritional quality of meat from lambs fed diets containing cotton cake from biodiesel production. The authors found that two principal components explained 83.03% of the variance of the data according to the set of data analyzed. The increasing addition of cotton cake to the diets of the sheep affected the fatty acid compositions.

Similarly, Soliman *et al*. (2016) evaluated two different feed regimens for cattle to determine whether a significant difference existed in the fatty acid profiles across conventional grain-fed and grass-fed beef and observed that the variances of PC1 and PC2 were 51.3 and 28.6%, respectively. The cumulative proportion from PC1 to PC4 was 95.6%, and all fatty acids contributed to PC1. Additionally, all fatty acids except C18:3n-6 contributed to PC2.

However, Bednárová *et al*. (2013) evaluated differences in the fatty acid composition and elemental composition of beef originating from grassland-based production based on animal age and sex and observed that the first two PCs calculated accounted for 55.8% of the total data variance.

Therefore, the main conclusion is that glycerin levels up to 14.3%, which corresponded to the substitution of 50.5% of the corn for this byproduct as an energy source, did not alter the proximate composition of the meat but improved the fatty acid profile of the *longissimus lumborum* muscle and, consequently, increased the meat quality, with benefits for human health.

Resumen

R.R. Silva, L.M.A. M. Facuri, G.G.P. Carvalho, F.F. Silva, J.I. Simionato, C.B. Sampaio, L.S. Bezerra, R.M. Prado, I.N. Prado, A.P.G. Silva, M.L.G.M.L. Araujo, y B.M.A. Carvalho. 2017. Calidad de la carne de vaquillas terminadas en sistema de pastoreo tropical y suplementadas con glicerina. Cien. Inv. Agr. 44(3): 320-332. La glicerina es un compuesto orgánico con una función alcohólica que puede esterificarse en ácidos grasos para formar triglicéridos. Debido a su disponibilidad cada vez mayor, se necesitan estudios que indiquen el mejor nivel de inclusión en las dietas para los rumiantes. Este estudio evaluó los efectos de la glicerina sobre la composición próxima y el perfil de ácidos grasos de *Longissimus lumborum* de novillas suplementadas con pasto de *Brachiaria brizantha*. Se distribuyeron 36 vaquillas en un diseño totalmente aleatorizado con cuatro tratamientos (G0.0 = sin glicerina, G4.6 = 4,6% de glicerina, G9.3 = 9.3% de glicerina y G14.3 = 14.3% de glicerina). La adición de glicerina disminuyó los ácidos grasos tetradecanoico y octadecanoico, pero aumentó los ácidos grasos pentadecanoico, heptadecanoico, heptadecenoico, eicosanoico, eicosatetraenoico y docosatetraenoico. Las concentraciones de ácidos grasos saturados (SFA), monoinsaturados (MUFA) y omega-3 (n-3) fueron similares en todas las dietas. Sin embargo, las concentraciones de poliinsaturados (PUFA) y los niveles de PUFA: MUFA y n-6: n-3 aumentaron con la inclusión de glicerina en las dietas. Los niveles de glicerina hasta 14.3% (que corresponden a una sustitución del 50.5% de maíz por este subproducto como fuente de energía) no alteraron la composición proximal de la carne pero mejoraron el perfil de ácidos grasos del músculo *Longissimus lumborum* y, en consecuencia, aumentaron la carne calidad, potencialmente proporcionando beneficios para la salud humana.

Palabras clave: Biodiesel, CLA, glicerol, omega-3, poliinsaturados.

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