HOW DOES THE DIETARY GLYCERINE LEVEL AFFECT THE *LONGISSIMUS* MUSCLE FATTY ACID COMPOSITION IN BULLS FINISHED IN FEEDLOT?

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Abstract – The glycerine metabolization have influence on lipogenesis and could potentially improve the meat quality. The aim of this study was to determine the effect of dietary glycerine level (as an energy source) on the *Longissimus* muscle (LM) fatty acids in bulls finished in feedlot. Forty Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) were distributed in a completely randomised design into 4 treatments during 229-d: G00: without glycerine; G06: 6% glycerine; G12: 12% glycerine; G18: 18% glycerine on a DM. The bulls were slaughtered (472 ± 57.3 kg) at commercial slaughterhouse. LM samples were taken between the 12th and 13th ribs after chilling (24-h at 4 ºC) of the carcass. LM saturated FA concentration was reduced in bulls receiving glycerine in their diets. LM mono-unsaturated and poly-unsaturated FA increased in bulls fed diets with glycerine. The *n*-6 and *n*-3 PUFA concentrations increased in bulls fed diets with glycerine. The PUFA:SFA ratio was lower with the diet containing no glycerine. The PUFA *n*-6:PUFA *n*-3 ratio was not significantly different between treatments. Glycerine is an interesting source of dietary energy, which can successfully substitute corn in beef finishing diets and which provides a meat healthier fatty acids profile.

Key Words – biodiesel, co-product, meat quality.

I. INTRODUCTION

Glycerol is an organic compound with an alcohol function which can be esterified to fatty acids to form triglycerides. Glycerine is a commercial product with glycerol as the main compound and also contains small amounts of ash, water and methanol. Glycerol is metabolized by ruminal microorganisms increasing total volatile fatty acids in the rumen [1], it has gluconeogenic properties [2] and could potentially improve carcass and meat quality [3].

The hypothesis of this study was that glycerine supplementation could influence lipogenesis, thus changing the meat fatty acid composition. Previous studies reported a reduction in the acetate to propionate ratio in the rumen, mainly resulting from an increase in rumen molar proportions of propionate, which is a glucose precursor [4]. Furthermore, glycerine might be converted to glucose in the liver. Thus, it was expected that a glucose supply would increase in bulls supplemented with glycerine, inducing a rise in lipogenesis.

The aim of this study was to determine the effect of the level of dietary glycerine (as an energy source) on *Longissimus* muscle fatty acid composition in bulls.

II. MATERIALS AND METHODS

The experiment was conducted at the Experimental Station of the Paraná Agronomic Institute (IAPAR) in Ponta Grossa city, Paraná State, South Brazil. Forty Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) were distributed in a completely randomised design in four treatments with ten replications per treatment: G00: without glycerine; G06: 6% glycerine; G12: 12% glycerine; G18: 18% glycerine on a DM basis (glycerine replacing 18.3; 38.5; and 61.3% of the corn grain). Bulls were individually penned. After an 11-day adaptation period, the bulls were weighed. Average initial BW and age were 209 ± 33.3 kg and 8 ± 0.9 months. Body weight was recorded monthly, and intake of concentrate and corn silage were recorded daily (4.08 kg DM of

concentrate and 3.79 kg DM of corn silage). The bulls weighed 472 ± 57.3 kg at the end of the experimentation.

The diet formulation (corn silage, ground corn and soybean meal) and quantity offered were designed to provide a weight gain of 1.2 kg/day, according to NRC [5] (Table 1). Glycerine with medium purity (glycerol - 81.2%) was used as an energy supplement in order to obtain four iso-energetic diets. All diets were formulated as iso-nitrogenous.

Table 1. Chemical composition of the base diets (g/kg DM) fed to Purunã bulls from 209 to 472 kg BW

Item	G00	G06	G12	G18
DM	540	547	554	562
OM	879	884	880	896
Ash	27.9	30	32.2	34.5
CP	108	108	108	109
EE	36.9	33.6	30.3	27.0
TC	734	689	644	598
NFC	475	438	401	364
NDF	259	251	242	234
ADF	126	125	123	122
TDN	702	701	670	699

DM: Dry matter; OM: Organic matter; CP: Crude protein; EE: Ether extract; TC: Total carbohydrate; NFC: Non fibrous carbohydrate; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; TDN: Total digestible nutrients.

Total lipids were extracted using the Bligh and Dyer [6] method with a chloroform/methanol mixture. Fatty acid methyl esters (FAME) were prepared by triacyl glycerine methylation according to ISO [7] method. FAME were analysed in a gas chromatograph (Varian, USA), equipped with a flame ionisation detector and a fused silica capillary column CP-7420 (100 m, 0.25 mm and 0.39 µmo.d., Varian, USA) Select Fame. The column temperature was programmed at 165°C for 18 minutes, 180°C (30°C min^{-1}) for 22 min, and 240°C (15°C min^{-1}) for 30 minutes with 45-psi pressure. The injector and detector were kept at 220°C and 245°C, respectively. Gas flows (White Martins, Brazil) were 1.4 mL min^{-1} for carrier gas (H_2); 30 ml min^{-1} for make-up gas (N_2); and 30 mL min^{-1} and 300 mL min^{-1} for H_2 and synthetic flame gas, respectively. The sample was injected using a split mode 1/80. Fatty acids were identified by comparing the relative retention time of FAME peaks of the samples with FAME standard 189-19 from Sigma Company, USA by spiking samples with the standard. The peak areas were determined using Star software® (Varian, USA).

Data were analyzed by the regression equations using the MIXED procedure to determine the linear and quadratic effects of glycerine and 0% glycerine *vs.* glycerine treatment. Treatment means were computed with the LSMEANS option using SAS [8].

III. RESULTS AND DISCUSSION

Saturated fatty acids (SFA) concentration were lower (P<0.05) in bulls fed diets with glycerine (Table 2). The mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids concentrations increased (P<0.01) in muscles of bulls fed diets with glycerine. The *n*-6 PUFA and *n*-3 PUFA concentrations increased (P<0.01) with glycerine levels. The PUFA:SFA ratio was lower (P<0.01) for diets with no glycerine. The PUFA *n*-6:PUFA *n*-3 ratio was not significantly different (P=0.11) between diets.

SFA concentration represented approximately 46.4% of total FA in LM of bulls fed glycerine. SFA concentration was lower (10.8%) with diets containing glycerine. LM SFA concentration of bulls fed with glycerine was lower than that evaluated in bulls from different crossbreeding systems finished in feedlot [9].

Mono-unsaturated and PUFA concentration increased by 7.4% and 44.0%, respectively. These values were higher than those reported by Françozo [3] when evaluating beef cattle Nellore fed diets containing glycerine (0, 5 and 12%).

The concentration of *n*-6 PUFA increased by 39.9% when bulls were fed glycerine, which is in line with Rotta [9] who observed that percentage ranged between 3.5 and 9.3%. Likewise, LM *n*-3 PUFA concentration presented an increase of 56.8% in treatments with glycerine.

Table 2. Influence of the dietary glycerine level on *Longissimus* muscle fatty acid composition in Purunã bulls finished in feedlot

Fatty acids, %	Treatment				SEM[5]	P-value		
	G00[1]	G06[2]	G12[3]	G18[4]		L	Q	0 *vs* glycerine
SFA[6]	50.49	45.46	45.47	44.22	0.53	<0.01	<0.01	<0.01
MUFA[7]	44.62	48.50	46.81	48.42	0.43	0.01	0.04	<0.01
PUFA[8]	4.89	6.04	7.72	7.36	0.23	<0.01	<0.01	<0.01
n-6[9]	3.41	4.05	5.27	4.99	0.15	<0.01	<0.01	<0.01
n-3[10]	1.39	1.90	2.36	2.28	0.09	<0.01	<0.01	<0.01
PUFA:SFA[11]	0.10	0.13	0.17	0.17	0.07	<0.01	<0.01	<0.01
n-6:*n*-3[12]	2.45	2.14	2.23	2.18	<0.01	0.27	0.45	0.11

[1]Diet without glycerine; [2]6% of glycerine on DM basis; [3]12% of glycerine on DM basis; [4]18% of glycerine on DM basis;[5]Standard error of mean; [6]Saturated fatty acids - $\hat{Y}=50.277-0.980x+0.035x^2$ ($r^2=0.61$); [7]Mono-unsaturated fatty acids - $\hat{Y}=48.988-0.336x$ ($r^2=0.13$); [8]Poly-unsaturated fatty acids - $\hat{Y}=4.778+0.312x-0.009x^2$ ($r^2=0.48$); [9]*n*-6 PUFA - $\hat{Y}=3.317+0.195x-0.005x^2$ ($r^2=0.47$), [10]*n*-3 PUFA - $\hat{Y}=1.373+0.115x-0.003x^2$ ($r^2=0.36$), [11]PUFA:SFA - $\hat{Y}=0.105+0.003x$ ($r^2=0.51$); [12]*n*-6 PUFA/*n*-3 PUFA ratio.

Glycerine increased the PUFA:SFA ratio from 0.10 (G00) to 0.17 (G18). This ratio is below the recommended value of 0.42 [10], which is considered beneficial to human health.

The *n*-6 PUFA:*n*-3 PUFA averaged 2.25, which is considered healthy, as it is below the value of 4 recommended by the HMSO [10]. This low ratio may be explained by the biohydrogenation process under gone by dietary unsaturated FAs in the rumen by microorganisms [4].

In fact, glycerine with higher quality (medium purity) could be more metabolized when compared to crude glycerine. Françozo [3] observed higher PUFA *n*-6:PUFA *n*-3 ratios (2.63, 2.70 and 2.61%) which evaluated beef cattle Nellore fed on diets with glycerine in feedlot.

IV. CONCLUSION

Results from this study demonstrated that diet with up to 18% glycerine can affect the fatty acid composition of *Longissimus* muscle. The effects of glycerine metabolism on LM fatty acid composition might require further research.

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REFERENCES

1. Abo El-Nor, S., AbuGhazaleh, A. A., Potu, R. B., Hastings, D., &Khattab, M. S. A. (2010). Effects of differing levels of glycerol on rumen fermentation and bacteria. Animal Feed Science and Technology, 162: 99-105.
2. Donkin, S. S., Koser, S. L., White, H. M., Doane, P. H., &Cecava, M. J. (2009). Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. Journal of Dairy Science, 92: 5111-5119.
3. Françozo, M. C., Prado, I. N., Cecato, U., Valero, M. V., Zawadzki, F., Ribeiro, O. L., Prado, R. M., & Visentainer, J. V. (2013). Growth performance, carcass characteristics and meat quality of finishing bulls fed crude glycerine-supplemented diets. Brazilian Archives of Biology and Technology, 56: 327-336.
4. Kijora, C., Bergner, H., Götz, K. P., Bartelt, J., Szakacs, J., & Sommer, A. (1998). Investigation on the metabolism of glycerol in the rumen of bulls. Archiv fur Tierernahrung, 51:, 341-348.
5. NRC. (2000). Nutrient Requirements of Beef Cattle (7th ed.). Washington, DC, USA: National Academy Press.
6. Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal Biochemistry and Physiology, 37: 911-917.
7. ISO. (1978). Animal and Vegetable Fats and Oils – Preparation of Methyl Esters of Fatty Acids. Method ISO 5509. Geneva, Switzerland: International Organization for Standardization.
8. SAS. (2004). SAS/STAT User guide, Version 9.1.2. Cary, NC, USA: SAS Institute Inc.
9. Rotta, P. P., Prado, R. M., Prado, I. N., Valero, M. V., Visentainer, J. V., & Silva, R. R. (2009). The effects of genetic groups, nutrition, finishing

systems and gender of Brazilian cattle on carcass characteristics and beef composition and appearance: a review. Asian-Australasian Journal of Animal Science, 22: 1718-1734.
10. HMSO. (1994). England Department of Health Nutritional. Aspects of cardiovascular disease. Report on Health and Social Subjects, 46: 37-46.