Proximate chemical composition and fatty acid profiles of *Longissimus thoracis* from pasture fed LHRH immunocastrated, castrated and intact *Bos indicus* bulls

M.R. Ruiz[1], M. Matsushita[1], J.V. Visentainer[1], J.A. Hernandez[2], E.L. de A. Ribeiro[3], M. Shimokomaki[4], J.J. Reeves[2] and N.E. de Souza[1#]

[1]Department of Chemistry, Maringa State University, Av. Colombo, 5790, CEP 87020-900, Maringá, Paraná State, Brazil

[2]Department of Animal Sciences, Washington State University, Pullman, WA 99164, USA

[3]Department of Animal Sciences and [4]Department of Food and Drugs Technology, Londrina State University, CEP 86051-970, Londrina, Paraná State, Brazil

Abstract

This study was carried out to evaluate the effect of a luteinizing hormone-releasing hormone (LHRH) fusion protein vaccine *vs*. surgical castration on the chemical composition and fatty acid profile of beef cattle (*Bos indicus*) slaughtered at three years of age. Thirty bulls (Nellore-cross) were divided into three groups: immunized against LHRH fusion protein, castrated or left intact (control). These animals were 24 months old at the initiation of the study and ranged on Brachiaria grass in Mato Grosso, Brazil. Meat of intact bulls presented a lower fat content compared to castrated or surgically castrated animals. For the immunized and surgically castrated animals there were differences in fat and cholesterol concentrations. Although the fatty acid profiles of the three treatments were similar in composition, there were differences in the concentrations of some fatty acids (miristic, C14:0; stearic, C18:0; linolenic, C18:3n-3; and eicosapentaenoic, C22:5n-3). Meat samples of surgically castrated animals had less polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids (8.2 and 37.7%, respectively), higher saturated (SFA) fatty acids (52.2%) and a lower PUFA/SFA ratio (0.16) and were different compared to intact and immunized bulls. The PUFA/SFA ratio increased as the fat content decreased. On the other hand, surgically castrated bulls had a higher lipid and lower cholesterol contents. It is concluded that immunization against LHRH using new recombinant LHRH fusion proteins could be an alternative to physical castration to improve carcass quality in cattle production.

Keywords: Beef bulls, polyunsaturated fatty acids, Nellore, LHRH immunocastration, chemical composition
[#]Corresponding author. E-mail address: nesouza@uem.br

Introduction

In order to obtain meat of a better quality, particularly from animals slaughtered at older ages, the use of castration is an important tool in beef cattle management. Surgical castration is not only painful to the animals but also decreases weight gain and, in extreme situations, can result in losses through death from infection (Zweiacher *et al.*, 1979). In spite of the possible problems related to lean quality of intact bulls, late castration has been recommended as a means to take advantage of greater weight gain and feed efficiency in bulls (Knight *et al*., 1999). To avoid this weight loss, some Brazilian ranchers do not castrate their animals. Intact bulls, however, are difficult to manage, and as a consequence of their increased activity level have a higher tendency to yield dark cutting meat at slaughter (Gregory & Ford, 1983; Restle *et al*., 1996). Even with younger animals some differences can be detected between intact bulls and castrated animals. Morgan *et al*. (1993) observed leaner carcasses with lower quality grades in bulls than in steers at 12 months of age. Meat from bulls also had higher shear force values.

Restle *et al*. (1994a; b) observed that steers castrated at 12 months of age had greater weight gains, were heavier at slaughter and presented heavier carcasses than steers castrated at 1.5 months of age, and did not differ from intact bulls. Castrated animals also had greater subcutaneous fat thickness than bulls. The major difference in weight gain between intact and castrated animals was observed from the 20th to the 25th month of age (Restle *et al*., 1994b).

Beef consumption with both a high saturated fatty acids and cholesterol content has led to a negative image of beef by some consumers (Rule *et al*., 1997). Nevertheless, beef possesses a similar or lower cholesterol level when compared to other meat sources (Feeley *et al*., 1972), and the harmful effect of the total cholesterol and LDL levels is related to the lipid fraction of the meat and not to the lean meat (O'Dea *et al*., 1990). Red meat, however, has the worst reputation in terms of a healthy human diet (Aharoni *et al*., 1995). Beef fat is a significant source of saturated fatty acids in a diet. The diverse effect of saturated fatty acids on the human plasma cholesterol levels makes it important to include fatty acid analysis in the evaluation of the meat composition (Mills *et al*., 1992). Recent observations by our group showed that there was a markedly higher score in meat marbling of immunocastrated animals compared to surgically castrated animals (Ribeiro *et al*., 2004).

Thus, the aim of the present study was to examine the effects of castration (surgical or immunological) on the chemical characteristics (moisture, ash, fat, protein and cholesterol) and fatty acid profiles of Nellore crossbred cattle.

Materials and Methods

The study was carried out on a ranch in the state of Mato Grosso, Brazil. The 24 month old animals were kept on a *Brachiaria brizantha* pasture during the whole experimental period. A total of 30 Nellore-cross (*Bos indicus*) bulls were randomly allocated to one of the following treatments: T1 – immunization against luteinizing hormone-releasing hormone (LHRH) fusion protein (Aissat *et al*., 2002), T2 – surgical castration and T3 – intact bulls (controls).

The animals in the T1 group received three injections; the first or primary immunization was given when the bulls were approximately two years of age, followed by two booster immunizations at 144 day intervals. The surgical castration was performed using a Brazilian traditional castration method on day 141 of the study. The control animals were intact males. Ninety six days after the last immunization, all the bulls were sent to a commercial slaughter plant 400 km from the ranch, where they were killed the next day, as described in Ribeiro *et al.* (2004). The *Longissimus thoracis* muscle between the 12^{th} and 13^{th} rib without subcutaneous fat was excised and 1.5 cm thick steaks were cut, kept frozen (-18 °C) for four weeks until the chemical analyses were performed.

At the beginning of each analysis the samples were allowed to equilibrate to room temperature, triturated and homogenized. Moisture, ash and protein contents were determined according to AOAC methods (Cunniff, 1998). Lipids were extracted from muscle tissue using the method of Folch *et al.* (1957). Meat samples (20.0 ± 0.01 g) were homogenized with 90 mL of chloroform-methanol (2:1 v/v) solution for 2 min. The homogenate was filtered (using a Whatman nº 1 paper filter and a Buchner funnel), and the filtrate collected in a beaker and transferred to a separation funnel containing 50 mL of NaCl 0.9% (m/v). After allowing the filtrate to separate into two layers, the lipid content was determined by weighing after evaporation of the chloroform phase.

The extraction and quantification of cholesterol were carried out by the method of Al-Hasani *et al.* (1993), with modifications (Rowe *et al*., 1999). Meat samples (5-10 g) were placed in a 250 mL flat-bottom flask and dispersed in an ethanol-methanol-isopropanol (90:5:5 v/v/v) solution in an amount equivalent to 4 mL/g of sample where-after 1 mL of 60% KOH/g sample was added. The flask containing this mixture was connected to a water-cooled condenser and refluxed for 1 h. After cooling to room temperature, 100 mL of hexane was added and the mixture was stirred for 10 min and finally 25 mL of deionized water was added and the mixture was stirred for a further 15 min. Layers were then separated and the hexane layer was collected in a flask. An aliquot of 25 mL of the hexane layer was evaporated to dryness under nitrogen. The residue was dissolved in 2 mL of hexane containing 0.2 mg of 5α-cholestane internal standard/mL and transferred to a sample vial. Approximately 3 μL were injected into a gas chromatograph. A Shimadzu 14A instrument GC (Japan) fitted with a flame ionization detector (FID, 300 °C) and a split/splitless injector (260 °C, split 1:150) was used for the analysis of cholesterol. Separation was carried out in a fused silica capillary column at 300 °C (25 m x 0.25 mm i.d.), coated with SE-30 (0.25 μm phase thickness) (Quadrex, U.S.A.). The carrier gas was hydrogen (1.5 mL/min) and the make-up gas was nitrogen (25 mL/min). Cholesterol identification was made by comparing the relative retention time of peaks from samples with standards from SIGMA (U.S.A.). For peak integration a CG 300 computing integrator (CG Instruments, Brazil) was used.

Methyl esters were prepared by transmethylation according to the procedure of the ISO (1978), using 2 mol/L KOH in methanol and n-heptane. Fatty acids methyl esters (FAME) were analyzed using a Shimadzu 14A (Japan) gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (50 m x 0.25 mm) with 0.20 μm of Carbowax 20M (Quadrex, U.S.A.). The column temperature was programmed at 10 °C/min from 150 to 240 °C. The injection port and detector were maintained at 220 and 245 °C, respectively. Carrier gas was hydrogen (1.2 mL/min) and the make-up gas was nitrogen (30 mL/min). The split used was 1/100. Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from SIGMA (U.S.A.). The peak areas were determined by the CG-300 computing integrator (CG Instruments, Brazil). Data were calculated as normalized area percentages of fatty acids identified.

Data were analyzed by analysis of variance for a completely randomized design using a fixed linear model with the GLM procedure using Statistica 5.0 software (StatSoft, USA, 1996). The model included the effect of treatment. Mean comparisons when effect was significant ($P < 0.05$), were done by t-test.

Results and Discussion

Proximate chemical composition of *Longissimus thoracis* samples as related to the respective treatments are presented in Table 1. No difference in water, protein and ash contents were observed among the treatments ($P > 0.05$), which ranged from 74.7 to 76.2%, 19.9 to 21.1% and 0.9 to 1.0%, respectively. Fat concentrations ranged from 1.2% for intact bulls (T3) to 2.2% for surgically castrated animals (T2), and cholesterol concentrations ranged ($P < 0.05$) from 49.0 mg/100 g for T2 to 51.8 mg/100 g for immunized animals (T1). Values obtained for muscle protein concentration were lower than those reported by VanKoevering *et al*. (1995), whose values varied from 22.1 to 22.7% for heifers. A low fat value of 1.71% was observed by Abularach *et al*. (1998) in their studies with young Nelore bulls. The cholesterol concentrations in this study were lower than those found by Bohac & Rhee (1988) for beef and pork (56.00 mg/100 g and 55.90 mg/100 g, respectively). None-the-less, these values were similar to those found by VanKoevering *et al*. (1995) (49.4 mg/100 g) for beef.

Table 1 Proximate chemical composition and cholesterol concentrations (mean ± s.d.) of 10 samples per treatment analyzed in triplicate of *Longissimus thoracis* of pasture fed LHRH immunocastrated, castrated and intact bulls

	Treatment		
	Immunization	Surgical	Intact
Moisture (g/100 g)	75.7 ± 0.78	74.7 ± 0.45	76.2 ± 1.65
Ash (g/100 g)	0.94 ± 0.05	0.93 ± 0.09	0.97 ± 0.06
Protein (g/100 g)	21.1 ± 1.23	20.9 ± 1.57	19.9 ± 1.39
Fat (g/100 g)	1.61 ± 0.52^{b}	2.17 ± 0.53^{a}	1.23 ± 0.35^{c}
Cholesterol (mg/100 g)	51.8 ± 1.63^{a}	49.0 ± 1.35^{b}	50.0 ± 1.48^{ab}

[a,b]Means in the same row with different superscripts differ significantly at $P < 0.05$

Palmitic (C16:0), stearic (C18:0) and oleic (C18:1n-9) acids comprised the greatest proportion of the fatty acids in the *Longissimus* muscle (Table 2). After castration, higher levels of stearic acid were found, 22.0% for surgical and 20.2% for immunized animals, which differed ($P < 0.05$) from that in the intact animals. Linoleic acid (C18:2n-6) concentration was lower ($P < 0.05$) for the surgically castrated group (3.5%) than for the intact animals (5.1%). The same fatty acids were identified in the three treatments, however, with significant differences in the concentrations of some of them, namely mirystic (C14:0), oleic (C18:0), linolenic (C18:3n-3) and eicosapentaenoic (C22:5n-3). These fatty acid profiles were similar to those found by Enser *et al*. (1998) for beef and lamb. There was no effect of castration and immunization on the percentage of the major trans-fatty acid, elaidic acid (C18:1-9 t).

Table 2 Fatty acid profile (as percentage of the total fatty acids identified) of the *Longissimus thoracis* muscle derived from pasture fed LHRH immunocastrated, castrated and intact bulls

Fatty acid	Treatment		
	Immunization	Surgical	Intact
C14:0	1.83 ± 0.08[a]	1.92 ± 0.17[ab]	2.03 ± 0.10[b]
C14:1n-7	0.20 ± 0.02	0.20 ± 0.01	0.19 ± 0.01
C14:1n-5	0.22 ± 0.03[a]	0.24 ± 0.02[a]	0.28 ± 0.03[b]
C15:0	0.58 ± 0.05	0.66 ± 0.06	0.62 ± 0.05
C15:1n-10	0.19 ± 0.02	0.19 ± 0.01	0.17 ± 0.01
C16:0	25.32 ± 1.23	26.58 ± 1.13	25.13 ± 0.99
C16:1n-9	0.87 ± 0.06	0.85 ± 0.05	0.81 ± 0.07
C16:1n-7	1.60 ± 0.15	1.63 ± 0.15	1.66 ± 0.18
C17:0	1.08 ± 0.11	1.13 ± 0.15	1.04 ± 0.06
C17:1n-9	0.55 ± 0.07	0.52 ± 0.05	0.52 ± 0.04
C18:0	20.19 ± 2.51[a]	21.95 ± 1.98[a]	18.45 ± 1.26[b]
C18:1n-9t	1.52 ± 0.27	1.62 ± 0.18	1.79 ± 0.17
C18:1n-9	35.68 ± 1.97	34.04 ± 1.46	35.17 ± 2.84
C18:2n-6t,t	0.20 ± 0.07[a]	0.20 ± 0.03[a]	0.13 ± 0.01[b]
C18:2n-6	4.45 ± 0.94[a]	3.47 ± 0.35[b]	5.05 ± 0.52[a]
C18:3n-3	0.98 ± 0.08[a]	0.83 ± 0.09[b]	1.80 ± 0.06[c]
C20:2n-6	0.37 ± 0.04	0.36 ± 0.03	0.34 ± 0.02
C20:2n-3	0.22 ± 0.03	0.18 ± 0.02	0.18 ± 0.02
C20:3n-6	0.26 ± 0.04	0.24 ± 0.04	0.29 ± 0.02
C20:4n-6	1.41 ± 0.14	1.61 ± 0.17	1.59 ± 0.15
C20:5n-3	0.12 ± 0.01[a]	0.14 ± 0.02[a]	0.19 ± 0.01[b]
C22:4n-6	0.60 ± 0.07[a]	0.39 ± 0.03[b]	0.73 ± 0.07[c,a]
C22:5n-6	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
C22:5n-3	1.06 ± 0.04[a]	0.67 ± 0.08[b]	1.56 ± 0.14[c]
C22:6n-3	0.12 ± 0.02	0.12 ± 0.01	0.11 ± 0.01
PUFA[1]	9.73 ± 0.92[b]	8.16 ± 0.61[c]	11.98 ± 0.80[a]
MUFA[2]	39.31 ± 2.27	37.67 ± 2.47	38.80 ± 2.19
SFA[3]	49.00 ± 1.73[a]	52.24 ± 2.62[a]	47.27 ± 2.79[b]
n-6[4]	7.23 ± 0.28[a]	6.22 ±.0.27[b]	8.14 ± 0.17[a]
n-3[5]	2.50 ± 0.21[a]	1.94 ± 0.14[a]	3.84 ± 0.23[b]
PUFA:SFA[6]	0.20 ± 0.02[a,b]	0.16 ± 0.04[b]	0.25 ± 0.02[a]
n-6: n-3[7]	2.89 ± 0.13[a]	3.20 ± 0.09[a]	2.12 ± 0.15[b]

[a,b,c]Means in the same row with different superscripts differ significantly at $P < 0.05$
[1]Polyunsaturated fatty acids. [2]Monounsaturated fatty acids. [3]Saturated fatty acids. [4]omega-6 fatty acids. [5]omega-3 fatty acids. [6]Ratio of polyunsaturated fatty acids and saturated fatty acids. [7]Ratio of omega-6 and omega-3 fatty acids

Muscle samples from the surgically castrated animals contained less polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids (8.2% and 37.7%, respectively) and more saturated fatty acids (SFA) (52.2%) resulting in a smaller PUFA/SFA ratio (0.16) than immunized and intact bulls ($P < 0.05$). Overall, the PUFA/SFA ratio increased as the fat content decreased. This is due to the fact that at low levels of fat the

contribution made by phospholipids is proportionately greater and these are more unsaturated than the triacylglycerols which themselves increased in proportion as total lipid increased (Marmer *et al*., 1984).

When the n-6/n-3 ratio is compared to the recommended value (4.0) it is noticeable that all treatments presented lower values: 3.2 for castrated, 2.9 for immunized and 2.1 for intact bulls than the recommended and more beneficial (HMSO, 1994).

Conclusion

Immunocastration was effective to produce meat with lower intramuscular fat but with a higher cholesterol content. However, the resultant meat contained a higher PUFA and a PUFA:SFA ratio and lower n-6/n-3 ratio when compared to the animals that were surgically castrated. Therefore, it is concluded that to immunize cattle against LHRH using new recombinant LHRH fusion proteins could be an alternative to physical castration to improve carcass quality in cattle production.

Acknowledgements

The authors are grateful to the Washington Technology Centre and Amplicon Express, Pullman, Washington for financially supporting the field studies and CAPES for financing research fellowships.

References

Abularach, M.L.S., Rocha, C.E. & Felicio, P.E., 1998. Características de qualidade do contrafilé (m. L. dorsi) de touros jovens da raça Nelore. Ci. Tecnol. Alim. 18, 205-210,

Aharoni, Y., Nachtomi, E., Holstein, P., Brosh, A., Holzer, Z. & Nitsan, Z., 1995. Dietary effects on fat deposition and fatty acid profiles in muscle and fat depots of Friesian bull calves. J. Anim. Sci. 73, 2712-2720.

Aissat, D., Sosa, J. M., de Avila, D.M., Bertrand, K.P. & Reeves, J.P., 2002. Endocrine, growth, and carcass characteristic of bulls immunized against luteinizing hormone releasing hormone fusion proteins. J. Anim. Sci. 80, 2209-2213.

Al-Hasani, S.M., Hlavac, J. & Carpenter, M.W., 1993. Rapid determination of cholesterol in single and multicomponent prepared foods. J. AOAC, Int. 76, 902-906.

Bohac, C.E. & Rhee, K.S., 1988. Influence of animal diet and muscle location on cholesterol content of beef and pork muscles. Meat Sci. 23, 71-75.

Cunniff, P.A. (Ed.), 1998. Official methods of analysis of the AOAC International, 16th ed. Association of Official Analytical Chemists International, Arlington, Virginia, USA. CD-ROM.

Enser, M., Hallett, K.G., Hewett, B., Fursey, A.J., Wood, J.D. & Harrington, G., 1998. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. Meat Sci. 49, 329-341.

Feeley, R.M., Criner, P.E. & Watt, B.K., 1972. Cholesterol content of foods. J. Am. Diet. Assoc. 61, 134-138.

Folch, J., Lees, M. & Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Gregory, K.E. & Ford, J.J., 1983. Effects of late castration, zeranol and breed group on growth, feed efficiency and carcass characteristics of late maturing bovine males. J. Anim. Sci. 56, 771-780.

HMSO, UK, 1994. Department of Health. Nutritional aspects of cardiovascular disease. London, (Report on Health and Social Subjects, 46). pp. 37-46.

ISO, 1978. International Organization for Standardization: Animal and vegetable fats and oils. Preparation of Methyl Esters of Fatty Acids. ISO 5509, pp. 01-06.

Knight, T.W., Cosgrove, G.P., Lambert, M.G. & Death, A.F., 1999. Effects of method and age at castration on growth rate and meat quality of bulls. N. Z. J. Agric. Res. 42, 255-268.

Marmer, W.N., Maxwell, R.J. & Williams, J.E., 1984. Effects of dietary regimen and tissue site on bovine fatty acid profiles. J. Anim. Sci. 59,109-121.

Mills, E.W., Comerford, J.W., Hollender, R., Harpster, H.W., House, B. & Henning, W.R., 1992. Meat composition and palatability of Holstein and beef steers as influenced by forage type and protein source. J. Anim. Sci. 70, 2446-2451.

Morgan, J.B., Wheeler, T.L., Koohmaraie, M., Savell, J.W. & Crouse, J.D., 1993. Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. J. Anim. Sci. 71, 1471-1476.

O'Dea, K., Traianedes, K., Clisholm, K., Leyden, H. & Sinclair, A.J., 1990. Cholesterol – lowering effect of a low fat diet containing lean beef is reversed by the addition of beef fat. Am. J. Clin. Nutr. 52, 491-496.

Restle, J., Grassi, C. & Feijo, G.L.D., 1994a. Carcasss characteristics of beef cattle males intact or castrated at different ages. Pesq. Agropec. Bras. 29, 1603-1607.

Restle, J., Grassi, C. & Feijo, G.L.D., 1994b. Weight evolution of beef cattle males intact or castrated at different ages. Pesq. Agropec. Bras. 29, 1631-1635.

Restle, J., Grassi, C. & Feijo, G.L.D., 1996. Carcass characteristics of beef cattle bulls or steers castrated with burdizzo or knife, under pasture conditions. J. Braz. Soc. Anim. Sci. 25, 334-344.

Ribeiro, E.L.A., Hernandez, J.A., Zanella, E.I., Shimokomaki, M., Prudencio-Ferreira, S.H., Youssef, E., Ribeiro, H.J.S.S., Bogden, R. & Reeves, J.J., 2004. Growth and carcass characteristic of pasture fed LHRH immunocastrated, castrated and intact Bos indicus bulls. Meat Sci. 68, 285-290.

Rowe, A., Macedo, F.A.F., Visentainer, J.V., Souza, N.E. & Matsushita, M., 1999. Muscle composition and fatty acid profile in lambs fattened in drylot or pasture. Meat Sci. 51, 283-288.

Rule, D.C., MacNeiL, M.D. & Short, R.E., 1997. Influence of sire growth potential, time on feed, and growing finishing strategy on cholesterol and fatty acids of the ground carcass and longissimus muscle of beef steers. J. Anim. Sci. 75, 1525-1533.

StatSoft, 1996. Statistica 5.1 software. Tucksa, USA.

VanKoevering, M.T., Gill, D.R., Owens, F.N., Dolezal, H.G. & Strasia, C.A., 1995. Effect of time on feed on performance of feedlot steers, carcass characteristics, and tenderness and composition of longissimus muscles. J. Anim. Sci. 73, 21-28.

Zweiacher, E.R., Durham, R.M., Boren, B.D. & Gaskins, C.T., 1979. Effects of method and time of castration of feeder calves. J. Anim. Sci. 49, 5-9.