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Longissimus dorsi chemical composition and fatty acid profile in Murrah Buffalo (*Bubalus bubalis*) Heifers Fattened in Drylot with Hormonal Implantation and Lead Spheres in the Uterus

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Abstract: The purpose of the study was to examine the fatty acid profiles in *longissimus dorsi* (LD) of buffalo heifers of breed Murrah (*Bubalus bubalis*) muscle. It has been subdued to three types of treatment: EH – Heifers with ovarian activity (with fertile cycle) but empty; HOR – heifers with implantation of growth promoter (20 mg of stradiol benzoate and 200 mg of Progesterone); LEA – heifers with 100 spheres of lead which have been inserted into the fallopian tubes through an insemination pipette. The contents of moisture, ash, fat, crude protein and cholesterol were around 76.5%, 1.0%, 1.8%, 20.5% and 40 mg/100g, respectively, to the three types of treatment (EH, HOR and LEA). The main fatty acids detected were palmitic (C16:0), stearic (C18:0), oleic (C 18:1ω9) and linoleic (C18:2ω6) with concentrations varying from 22.61% (EH) to 25.85% (HOR), 20.53% (HOR) to 24.43% (EH), 35.10% (EH) to 36.65 (LEA), 3.87% (EH) to 4.96 (LEA), respectively. Palmitic (C16:0), stearic (C18:0) and EPA (C20:5ω3) concentrations presented significant difference ($P<0.05$) among the treatments. The buffalo meat resulting from the LEA treatment showed higher concentrations of polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids and mainly higher concentrations of linoleic (C18:2ω6); EPA (C20:5ω3) and DHA (C22:6ω3).

Key words: Fatty acids, cholesterol, female buffaloes, *Bubalus bubalis*, hormonal implant, lead spheres

Introduction

Buffalo, *Bubalus bubalis*, is an important alternative to cattle in several countries, as it presents advantages in terms of adaptation to certain climates, low nutritional requirements, resistance to several parasites and diseases and higher yield of meat (Soares & Arêas, 1995). The number of buffaloes world-wide totals about 160 millions, the Asian and African countries being the major producers (Carpenter *et al*., 1990). In Brazil, buffalo numbers rose from 500000 in the 1980s to more than 2500000 at present (Mattos, 1992) and numbers continue to increase. Besides the expressive growth, researches accomplished by several groups demonstrated that the buffaloes presents great productivity and adaptation capacity to the Brazilian conditions (Nogueira *et al*., 1989; Baruselli *et al*., 1993; Oliveira *et al*., 1994; Villares, 1994). Buffalo meat has been found to contain less intramuscular lipids (Sharma *et al*., 1986; Rao & kowale, 1993) and more polyunsaturated fatty acids (Sinclair *et al*., 1982; Sharma *et al*., 1986) compared with beef. Consumption of meat is associated with ingestion of fat and protein. Polyunsaturated fatty acids (PUFA) are elements essential for the evaluation of nutritional quality and conservation characteristics of the meat. Although work has been done on the lipids of main muscles of the buffalo, the fatty acid composition of intramuscular lipids has bee litle studied.

In appropriate conditions of nutrition, the utilization of growth-promoting implants, they increase the gain in weight, alimentary efficiency, retention of nitrogen and minerals and deposition of minerals and protein in the carcass (Rumsey, 1982, Cecava, 1994). The use of implant with estradiol benzoate and progesterone (Synovex-S®) for heifers in growth has been increases in the gain in weight from 20 to 35%, improvements in the alimentary efficiency and increase in the dry matter consumption (Rumsey, 1982, Rumsey *et al*., 1992, Cecava, 1994, Gerken *et al*., 1995, Herschler *et al*., 1995, Preston *et al*., 1995, Rumsey *et al*., 1996, Foutz *et al*., 1997, Rumsey *et al*., 1999) for confined animals. Implant it for animals in pasture it also resulted an increase in the gain in weight of 17% (Rumsey *et al*., 1996, Paisley *et al*., 1999). The use of growth-promoting implants in females it has been causing an increment in the protein deposition in the carcass (Perry *et al*., 1991, Herschler *et al*., 1995) and an increase in the value of market of the carcass (Herschler *et al*., 1995). The finished of females is influenced by the oestrous cycle that harms the acting, the alimentary efficiency and the carcass quality. The oestrus can be considered a suppress of the gain in weight in females (Crowe *et al*., 1995; Grassland *et al*., 2000).

Another mechanism studied to avoid the manifestation of the oestrus, went to introduction of small lead spheres (sterilized) inside the uterine horn, being used a pipette of artificial insemination (Marques *et al*., 2001). The heifers with it implants of lead they presented higher average daily gain in weight (1.0 kg) than the empty finished heifers (0.8 kg). However, it was not observed alterations in the carcass yield and thickness of covering fat. The higest gain in weight was attributed to the hormonal alterations caused in the heifers implanted with lead spheres, once the same ones provoked several macroscopic alterations in the wall of the uterus, in function of the presence of strange body in its interior. In bubalos heifers, Albuquerque (2002) it observed, through histological cuts, that the lead spheres implanted inside the horn uterus were recognized as strange body, they unchained an inflammatory reaction and they were expelled of its interior through the peristaltic activity.

Thus the purpose of the study was to investigate the influence of three types of treatment: EH – Heifers with ovarian activity (with fertile cycle) but empty; HOR – heifers with implantation of growth promoter (20 mg of stradiol benzoate and 200 mg of Progesterone); LEA – heifers with 100 spheres of lead which have been inserted into the fallopian tubes through an insemination pipette, on the Longissimus dorsi muscle composition and fatty acid profiles of heifers Murrah buffaloes (Bubalus bubalis)

Materials and Methods

Animals were finished in the the beef catle Section of Iguatemi Experimental Farm, wich belongs to the State University of Maringá,

Maringá, PR, Brazil. Twenty female buffalo heifers of breed Murrah were used, with alive weight of 350 kg and 16 months of age, distributed in three treatments: empty heifers (EH) with 6 animals ; heifers wth hormonal implant (HOR) with 7 animals, heifers with lead sphere (LEA) with 7 animals, introduced in the womb. Hormonal implant (Synovex-hR – 20 mg de stradiol benzoate of and 200 mg of testosterone) and 100 lead spheres of 4mm e. d. in the womb on the ovary activity. Feeds for these animals contained 55% of sugarcane silage, 33% of soybean meal, 11% of corn and 1% of common salt. Slaughtering started after 84 days of drylot. Slaughtering started after 84 days of drylot. After the establishement of the *rigor mortis* state, a 20-cm-thick section of the *Longissimus dorsi* muscle(LD), corresponding to the 11th tp the 12th rib section, was taken from the left side of each carcass. All samples were stored in freezer (-18°C), after removal of all external fat. Chemical analysis were carried out in triplicate using homogenized thawed muscle samples.

For the moisture, ash and protein contents were determined as described by Cunniff (1998). Lipids were extracted from the muscle tissues using Folch *et al.* (1957) method. Meat samples (10.00±0.01g) were homogenized with 90 mL of chloroform-methanol (2:1 v/v) solution for 2 minutes. After blending, 30 mL of chloroform and 30 mL of deionized water were added and the mixture was once more homogenized. A 0.58% aqueous NaCl solution was added to the homogenate, causing the chloroform layer (containing lipid) to separate from the methanol-water phase. The lipid extract was transferred to a 250 mL flask and the solvent evaporated under a nitrogen flux. The lipid content was gravimetrically determined.

The extraction and quantification of the cholesterol were carried out by the method of Al-Hasani *et al.* (1993), with modifications (Rowe *et al.*, 1999). Samples of the LD muscle (5-10 g) were placed in a 250 mL flat-bottom flask. Around 3 µL was injected into a gas chromatograph. A Shimadzu 14A (Japan) fitted with 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 (300 °C) in a fused silica capillary column (25 m, 0.25 mm i.d.), coated with SE-30 (0.25 µm phase thickness). The carrier gas was hydrogen (1.5 mL-min-1) and the makeup gas was nitrogen (25 mL min-1). Cholesterol identifications were made by comparing the relative retention time peaks from samples with standards from Sigma (USA). For peak integration a CG-300 Computing integrater (CG Instruments, Brazil) was used. Transesterification and fatty acid composition

Methyl esters were prepared by transmethylation according to the procedure of the method 5509 of ISO (1978), using KOH 2 mol L-1 in methanol and n-heptane. Fatty acid methyl esters (FAME) were analyzed using a Shimadzu 14A (Japan) gas chromatograph equipped with flame ionization detector and fused silica capillary column (50 m x 0.25 mm and 0.20 µm of Carbowax 20M). The column temperature was programmed at 10 K min-1 from 150 to 240 300 °C. The injection port and detector were maintained at 220 °C and 245 °C, respectively. The carrier gas was hydrogen (1.2 mL min-1) and the makeup gas was nitrogen (30 mL min-1). The split used was 1:100. The identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from Sigma (USA).

Statistical analysis: The experimental data are showed as mean±standard deviations and were statistically confronted for one-way ANOVA. Data were processed in the Statistica 5.1 Software (StatSoft, USA, 1996).

Results and Discussion

The chemical composition and fatty acid profiles of the experimental treatments are shown in Tables 1 and 2, respectively. Moisture, ash, crude protein and lipids there was no significant difference (P<0.05) among the three treatments EH, LEA and HOR. The contents of moisture, ash, protein and lipids were around 76%, 1%, 20% and 1.8%, respectively, for all treatments. There is few literature data for the centesimal composition in buffalo heifers´

Table 1: Effect of treatments on *Longissimus dorsi* muscle composition of the Murrah buffalo (*Bubalus bubalis)*

Treatment	Moisture (%)	Ash (%)	Protein (%)	Lipids (%)	Cholesterol (mg/100g)
EH	76.40±0.12	1.00± 0.06	20.52±1.72	1.84±0.51	38.46^{a}±0.83
LEA	76.50±1.80	1.02± 0.08	20.72±0.95	1.67±0.58	37.27^{a}±0.59
HOR	76.63±0.22	1.04± 0.08	20.27±1.62	1.75±0.87	41.85^{b}±1.55

Each value is the average of samples in triplicates, with standard deviations. The means followed by different letters in the same columns, are different among themselves by the Tukey test at 5%. EH – seven heifers with ovarian activity (displaying estral cycle), however, empty; HOR – six heifers with growing promoting implant (20 mg of estradiol benzoate and 200 mg of progesterone); LEA – seven heifers with 100 lead spheres introduced in uterine horn, using a insemination pipette.

Longissimus dorsi muscles, for us to make comparisons. Verma & Mehra (1998) studying Murrah Buffalos, without any treatment found values of 23.42% for crude protein, when compared to bovine meat, with 18.30%, affirming that the buffalo meat´s quality showed higher levels of protein. In one of the rare works,

Rao *et al.* (1996) obtained 17.73% for lipids in buffalo meat submitted to refrigeration. But, these animals had approximately 10 years of age and the analyses were made with meat from the thigh region of the female buffalos. Sharma *et al.* (1986) found for total lipids in the meat of Murrah buffalos slaughetered with the weight of 250 kg, 1.03%, 0.99%, 0.66% and 0.55% for the *Longissimus dorsi*, *Psoas major*, *Biceps femoris* and *Semitendinosus*, respectively. The values found by Sharma *et al.* (1986) for buffalo meat intramuscular lipids were lower than this work´s values. Even so, it may be inferred that the buffalo meat intramuscular lipids present lower values when compared to other types of meat. Silva *et al.* (2001) and Silva *et al.* (2002) accomplished studies with bovines and obtained 1.0% and 1.8% for lipids in *Longissimus dorsi* muscles of ½ Nelore vesus ½ Red Angus and Limousin versus Nelore and Simental versus Nelore heifers, respectively. All those muscles, as well as the buffalo heifer used in that study, underwent the removal of the external fat. In relation to the cholesterol content, there was significant difference (P<0.05) among the treatments. The buffalo heifer´s muscle that received the HOR treatment, showed a higher value, 41.85 mg/100g. The obtained values for the EH and LEA treatments were 38.46 and 37.27 mg/100g, respectively. Rao *et al.* (1996) obtained 80 mg/100g for the cholesterol in the thigh meat of the Murrah female buffaloes with approximately 10 years of age. Rowe *et al.* (1997) analyzing the cholesterol content in the bovine meat, of several regions

Table 2:Treatment effects on the fatty acid profile *Longissimus dorsi* muscle of the Murrah buffalo (*Bubalus bubalis)*

FATTY ACIDS	EH	LEA	HOR
C14:0	1.41±0.39	1.58±0.40	0.86±0.53
C15:1ω5	0.41±0.04	0.28±0.05	0.46±0.04
C16:0	22.61^{a}±1.00	23.60^{a}±0.61	25.85^{b}±0.36
C16:1ω9	0.46±0.04	0.39±0.05	0.48±0.04
C16:1ω7	1.60±0.23	1.78±0.33	1.93±0.33
C16:1ω5	0.54±0.17	0.37±0.02	0.43±0.02
C16:2ω6	0.57±0.10	0.47±0.03	0.54±0.06
C17:0	1.18±0.17	0.89±0.08	1.08±0.11
C17:1ω9	0.72±0.12	0.59±0.06	0.70±0.17
C18:0	24.43^{b}±1.33	20.60^{a}±0.83	20.53^{a}±0.82
C18:1ω9	35.10±0.94	36.65±0.98	36.28±0.45
C18:1ω7	3.58±0.33	3.38±0.86	3.59±0.63
C18:2ω6	3.87±0.72	4.96±1.16	4.00±0.09
C18:3ω3	0.62±0.09	0.57±0.13	0.67±0.18
C18:4ω6	0.47±0.04	0.75±0.09	0.51 ±0.05
C20:4ω6	0.45±0.06	0.46±0.13	0.45±0.25
C20:5ω3	1.22^{a}±0.27	1.55^{a}±0.26	0.77^{b}±0.32
C21:0	0.36±0.05	0.55±0.08	0.36 ±0.09
C22:6ω3	0.40±0.16	0.58±0.10	0.51±0.08
PUFA	7.60±0.88	8.76±0.80	6.94±0.64
MUFA	42.41±1.36	43.44±0.48	43.87±0.07
SFA	49.99±0.66	47.22±1.48	48.68±0.68
ω6	5.36±0.85	6.65±0.81	5.50±0.64
ω3	2.24±0.22	2.07±0.62	1.95±0.10
PUFA/SFA	0.15±0.10	0.18±0.05	0.14±0.07
ω6/ω3	2.39±2.65	3.21±0.41	3.82±0.63

Each value is the average of samples in triplicates, with standard deviations. The means followed by different letters in the same lines, are different among themselves by the Tukey test at 5%. PUFA, MUFA, SFA, ω6 and ω3: polyunsaturated, monounsaturated, Saturated, omega-6 and omega-3 fatty acids fatty acids. EH: seven heifers with ovarian activity (displaying estral cycle), however, empty. HOR: six heifers with growing promoting implant (20 mg of stradiol benzoate and 200 mg of progesterone); LEA: seven heifers with 100 lead spheres introduced in uterine horn, using a insemination pipette.

of the animals carcass such as rump, topside, knuckle, liver and ground meat fried in vegetable oil found 57, 40, 38, 265 and 32 mg/100g, respectively. Silva *et al.* (2001) and Silva *et al.* (2002) in their studies in raw bovine meat, obtained 30, 40 and 42 mg/100g of cholesterol, for *Longissimus dorsi* muscles of ½ Nelore versus ½ Red Angus and Limousin versus Nelore and Simental versus Nelore heifers, respectively. All those muscles, as well as the buffalo heifer´s one utilized in that study, underwent the removal of the external fat. Rowe *et al.* (1999) analyzing the cholesterol content in the lamb meat finished in pastures and drylot, obtained 62 and 58 mg/100g, respectively. In table 2, The fatty acids are ordered according to their chromatographic retention time and the values are the percentual average in relation to the total fatty acids. The data shows that the palmitic (C16:0) and stearic (C18:0) acids were the predominant saturated fatty acids (SFA) with values from 22.6% (EH) to 25.9% (HOR) and 20.5% (HOR) to 24.4% (EH), respectively. There was significant difference (P>0.05), for these two acids among the three treatments. Sharma *et al.* (1986) found 26% for the palmitic acid and 25% for the esteric acid in Longissimus dorsi muscle of Murrah buffalos, values equal to the ones obtained in this work. Rao & Kowale (1993) achieved for these two acids 16.6% and 18.8%, values lower than the ones reported by Sharma *et al.* (1986) and the ones shown in this study. Values lower for the esteric acid for Australian buffalos´ intramuscular lipids were described by Sinclair *et al.* (1982).

The total of saturated fatty acids (SFA) found in this work ranged from 47.22% (LEA) to 49.99% (EH), while SHARMA *et al.* (1986) obtained 54.2% and Sinclair *et al.* (1982) achieved 44.11%. Meaning that the values obtained in this study were intermediate in relation to the already mentioned researchers. In relation to the monounsaturated fatty acids (MUFA), the ones that showed higher concentrations were the oleic acid (C18:1ω9) with values around 36% and the vacenic acid (C18:1ω7) with 3.5%, not showing significant difference among the three treatments. Sinclair *et al.* (1982) achieved 38.83% for the oleic acid and didn't detect the presence of the vacenic acid, surely due to the material used for determining the fatty acids (steel packed column). Sharma *et al.* (1986) obtained lower values 27.8% and 1.4% for the oleic and vacenic acids, respectively. The polyunsaturated fatty acids (PUFA) contents were lower, varying from 6.9% to 8.8% in the present study, with significant difference (P>0,05), only for the eicosapentanoic acid (C20:5ω3) among the three treatments. Sharma *et al.* (1986) and Sinclair *et al.* (1982) found a total of 11.4% and 9.2% for the Longissimus dorsi muscle, respectively. Among the polyunsaturated acids, we emphasize the presence of the eicosapentaenoic (C20:5ω3) and the docosahexaenoic (C22:6ω3) acids not detected by Sinclair *et al.* (1982) and detected by Sharma *et al.* (1986) only in the liver of the Murrah buffalo. Among the polyunsaturated fatty acids, the main ones in our diet are the fatty acids related to ω6 and ω3. Mainly in relation to the linoleic (C18:2ω6) and α-linoleic (C18:3ω3) essential fatty acids for acting as precursors to the synthesis of other fatty acids. The linoleic acid presents itself with a higher percentual for 4.96% of the LEA treatment. For the α-linoleic the highest percentual was the LEA treatment with 0.67. From the nutritional point of view, on general meats, the ratios must be verified between saturated fatty acids and polyunsaturated ones, as well as the ω6 and ω3 ones, of the existing acids.

Enser *et al.* (1997) describes that the reason of total PUFA by total SFA is low due to bio-hydrogenation of the unsaturated fatty acids occurred in the rumen, derived from the diet. The values obtained for the ω6/ω3 ratios can also be small, due to the small quantity of unsaturated fatty acids, that are not hydrogenated in the rumen and that are later absorved by the organism. In a study on sheep and

bovine meat muscles from the United Kingdom, the ratios of total PUFA by total SFA are highlighted, recommended by England´s Department of Health (1994), are the best, when equal to the higher than 0.45. And for the ω6/ω3 ratios, the values would be better when equal to 4.0 or lower than these.

In this work it is verified that the ω6/ω3 ratios are according to the recommended by England's Department of Health, for all the treatments, with the smalles value for the EH treatment with 2.39 and the biggest for the HOR treatment with 3.82. These ω6/ω3 reasons, are better showed when compared to Silva et al's work (2000), where Aberdeen Angus versus Nelore and Simental versus Nelore using soy bran as protein source, cassava husk and corn, sweeping powder and cassava scraping as energy source, obtained reasons of 8.14 and 7.25, respectively, for the analyzed races.

Analyzing the PUFA/SFA ratios achieved for the muscles, we verify that the rations are under the recommendations of England's Department of Health. The highest ratio achieved was for the CHU treatment with 0.18. In Silva *et al.* (2001) work with heifers in confinement with a gradual replacement of corn by the pelletized citrus pulp obtained values of 0.07 and 0.09, for the ω6/ω3 ratios.

Conclusions

Based on the results found in this work and of some others in the literature, we verify that the content of total intramuscular lipids of the Murrah buffalo's Longissimus dorsi muscle, are lower in comparison to other types of meat. In relation to the composition in fatty acids, buffalo meat shows lower concentrations of SFA and higher PUFA concentrations in relation to the bovine, ovine and chicken meats. In relation to the treatments applied to the animals in this work, it is concluded that the female buffalos that received the lead sphere implant on the uterus horn (LEA treatment), resulted in a higher quality meat for showing less contents of cholesterol, of total lipids and of saturated fatty acids and higher concentrations of polyunsaturated fatty acids.

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