Short communication

Fatty acid and cholesterol content, chemical composition and sensory evaluation of horsemeat

I.B. Tonial, A.C. Aguiar, C.C. Oliveira, E.G. Bonnafé, J.V. Visentainer and N.E. de Souza[#]

Department of Chemistry, State University of Maringá, Av. Colombo 5790, CEP 87020-900, Maringá, PR, Brazil

Abstract

This study aimed to determine the fatty acid and chemical composition and cholesterol concentration of horsemeat, and to evaluate its taste acceptability by the Brazilian population. Horsemeat samples (*M. longissimus dorsi*) were obtained from a Paraná State slaughterhouse. The chemical composition revealed a low lipid (2.9%) and high protein content (22.5%). The concentrations of the nutritionally important fatty acids, arachidonic acid (AA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3), were 2.97% and 0.43%, respectively. The polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratio was 0.97, which is within the recommended range. The cholesterol concentration of 40.5 mg/100 g is lower than that of other meat such as chicken, beef, mutton and pork. The sensory evaluation revealed excellent acceptability.

Keywords: *Equus caballus*, *M. longissimus dorsi*, PUFA/SFA, health food, meat quality
[#] Corresponding author. E-mail: nesouza@uem.br

Horses (*Equus caballus*) are usually raised for use in sports and recreation, and as working horses. The use of horses as a meat source has been questioned in terms of food quality despite its nutritional value, low cost and low fat content compared to that of beef (Dufey, 1996). Although horsemeat is considered a delicacy in many cultures, in Brazil most of the horsemeat produced, are exported, with very little being targeted at the internal consumers' market. Slaughtering of horses and commercialization of horsemeat in Brazil are sometimes performed underhandedly and usually with the objective of the adulteration of other meat products (Janssen *et al*., 1990). Although regulations exist for the marketing of horsemeat for human consumption in Brazil, the internal market is negligible, mainly because of the prejudice against this kind of meat (Torres & Jardim, 1985). Furthermore, the commercial preparation of horsemeat is hampered by the absence of guidelines on the processing of the meat (Junqueira *et al*., 2005), which further suppresses its popularity with Brazilian consumers.

Compared to beef, horsemeat has a large percentage of subcutaneous fat and a low percentage of inter- and intramuscular fat (Rossier & Berger, 1998), which makes it a lean meat. The nutritional characteristics of horsemeat currently draw some attention despite the little information on its nutritional value, particularly on fatty acid (Matsuoka *et al*., 1993) and cholesterol concentrations. Polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids have beneficial nutritional and health values. They contribute to a reduction in the occurrence of vascular diseases, carcinoma of the uterine cervix and immunological diseases. Cholesterol is present mainly in animal fats (Bragagnolo & Rodriguez-Amaya, 1995). It is an essential component of cell membranes and lipoproteins, and a precursor of steroid hormones, biliary acids and vitamin D (Maurice *et al*., 1994; Tapiero *et al*., 2003). However, when present in large quantities, cholesterol may cause cardiovascular diseases, which necessitates its determination in foods (Costa *et al*., 2002).

Therefore, the objective of this study was to evaluate the fatty acid and cholesterol composition of horsemeat, and its taste acceptability by the Brazilian population.

The research was based on carcasses of castrated male horses in good health, donated by the Santa Fé Slaughterhouse, Santa Fé Municipality, Paraná State, southern Brazil. Slaughtered animals were nine

to 11 years old, and had a pre-slaughter weight ranging from 460 to 530 kg. The chemical analysis of muscle tissue was carried out on muscle from nine horse carcasses.

In order to determine the chemical composition and characterize the fatty acid properties of the meat, three series of samples of 900 g were taken from the *M. longissimus dorsi* between the 12 - 14th thoracic vertebra. The muscle samples were cleaned of external fat, connective tissue and tendons. The samples were ground in a knife mill, homogenized and frozen at -18 ºC pending analysis in triplicate. Lipid extraction was measured by the Bligh & Dyer technique (1959), protein by using the Kjeldahl method with a conversion factor of 6.25, and moisture and ash according to AOAC (1995).

Total lipid fatty acids were transesterified with 12% (wt/vol) boron trifluoride in methanol (Joseph & Ackman, 1992). The fatty acid esters were separated by gas chromatography in a CP 3380 (Varian, USA) equipped with a flame ionization detector and fused silica capillary column CP Select CB (FAME) cyanopropyl (100 m long, 0.25 mm i.d., and 0.25 µm stationary phase). Gas flows were 0.6 mL/min for the carrier gas (H_2), 30 mL/min for the auxiliary gas (N_2), 30 and 300 mL/min for the flame (H_2) gas and synthetic air, respectively. The sample injection split ratio was 1/100. The column temperature was 197 ºC for 23 min and was raised to 255 ºC at 20 ºC/min, and kept at this temperature for 15 min. Both injector and detector temperatures were 240 ºC. The injection of 1.5 mL samples was carried out in triplicate. The peak areas were determined with the software, Workstation version 5.0 (Varian). For fatty acid identification, retention times were compared with those of standard methyl esters (Sigma, St. Louis, M.O., USA) and values were also in line with the certified reference material (RM 8415) distributed by the National Institute of Standards and Technology (NIST). Repeatability tests were performed by injecting a pattern and a sample consecutively six times a day. Reproducibility tests were also carried out; injecting the pattern and the sample twice a day for three days under the same experimental conditions. No significant differences ($P < 0.05$) were found between the results obtained in either of the tests.

Cholesterol was extracted and quantified by the direct saponification method described by Al-Hasani *et al.* (1993). The cholesterol concentration was quantified in a gas chromatographer 14 A (Shimadzu, Japan) equipped with a flame ionization detector and fused silica capillary column 25 m long with a 0.25 mm i.d. and a 0.2 µm SE-30 (Quadrex, USA). The injector, column and detector temperatures were 260, 300, and 300 °C, respectively. The gas flows used, were: 1.5 mL/min for the carrier gas (H_2), 25 mL/min for the make-up gas (N_2), 300 mL/min for the synthetic air and 30 L/min for the H_2 flame gas. The sample injection split rate was 1/150. The peaks were integrated with an Integrator-Processor CG-300 (CG Instrumentos Analíticos). Cholesterol was identified by comparison with Sigma standards (USA). Sample cholesterol was quantified after the method linearity was verified by analysis of different concentrations of standard cholesterol samples with 0.20 mg/mL of 1 5 alpha-cholestane.

Horsemeat samples of approximately 500 g were cut into small pieces (2 x 4 cm), salted (1.3% wt/wt), homogenized and grilled in an electric grill at 160 °C with an internal temperature of the grilled samples of 105 °C. The samples were then served in ceramic dishes immediately after preparation and under normal light. Acceptability was evaluated by 30 (voluntary) meat consumer testers, 20 – 50 years

Table 1 Chemical composition and cholesterol concentration of the *M. longissimus dorsi* of horses

Chemical composition	g/100 g meat ± s.d.
Moisture	73.3 ± 1.7
Ash	1.1 ± 0.1
Protein	22.5 ± 1.1
Total lipids	2.9 ± 0.3
Cholesterol (mg/100 g meat)	40.5 ± 2.6

Each value is an average of nine samples (fresh) analysed in triplicate, with standard deviations (s.d.).

Table 2 Fatty acid composition, and sum and ratio of fatty acids of the fat in the *M. longissimus dorsi* of horses compared with fat from the same muscle from other species

Fatty acids (%)	Means ± s.d.	Goat*	Beef**	Chicken***
14:0	2.30 ± 0.24	8.27	20.4	0.03
14:1n-5	0.23 ± 0.01	0.10	nd	0.09
16:0	25.94 ± 0.73	24.00	24.0	22.19
16:1n-9	0.35 ± 0.04	nd	nd	0.38
16:1n-7	2.88 ± 0.26	0.88	nd	3.79
17:0	0.46 ± 0.01	0.37	nd	0.16
17:1n-9	0.25 ± 0.02	0.49	38.4	0.05
18:0	7.47 ± 0.23	13.14	17.9	7.51
18:1n-9	22.15 ± 0.61	27.58	nd	35.59
18:1n-7	1.58 ± 0.03	2.62	nd	1.42
18:2n-6	22.88 ± 0.33	3.43	29.8	23.44
18:3n-6	7.80 ± 1.06	0.34	nd	0.18
18:3n-3	0.43 ± 0.02	1.01	7.4	1.34
21:0	0.42 ± 0.01	0.42	nd	nd
20:3n-6	0.34 ± 0.01	nd		0.22
20:4n-6	2.97 ± 0.22	nd	3.3	1.37
23:0	0.19 ± 0.01	nd	nd	nd
20:5n-3	0.43 ± 0.02	nd	nd	nd
22:4n-6	0.92 ± 0.05	nd	nd	nd
PUFA	35.8 ± 1.13	4.9	73.0	27.3
MUFA	27.4 ± 0.66	32.3	45.6	41.8
SFA	36.8 ± 0.80	62.9	47.0	31.0
n-6	34.9 ± 1.13	3.9	46.6	24.2
n-3	0.86 ± 0.02	1.01	20.9	2.00
n-6/n-3	40.60 ± 1.16	3.90	2.23	12.62
PUFA/SFA	0.97 ± 0.21	0.08	0.15	0.88

Each value is the mean of nine samples analysed in triplicate.
PUFA - polyunsaturated fatty acids; MUFA - monounsaturated fatty acids; SFA - saturated fatty acids; n-6 - omega-6 fatty acids, n-3 - omega-3 fatty acids; PUFA/SFA - polyunsaturated fatty acids/saturated fatty acid ratio. nd – not detected.
* Matsushita *et al*. (2007); ** Padre *et al.* (2007); *** Boroski *et al*. (2008).

of age. The testers evaluated the samples using the 9-score hedonic scale: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely, according to the methodology described by Meilgaard *et al*. (1987).

Analysis was processed using Statsoft Statistic for Windows 5.0 (1995). The results were analyzed by ANOVA and the Tukey test, using 5% as level of significance.

The values of the chemical composition and cholesterol concentration in the present study (Table 1) are similar to those obtained by Badiani *et al*. (1997) who found that samples contained 0.9%, 0.98% and 19.8% moisture, ash and protein, respectively; while the total lipid content (2.93%) is in agreement with results (3.10%) reported by Devic & Stamenkovic (1989).

Table 2 depicts the fatty acid composition, sums of fatty acid groups and their ratios in horsemeat. In relation to other meat species such as the goat (Matsushita *et al*. 2007), cattle (Padre *et al*. 2007) and chicken (Boroski *et al*. 2008), the qualitative and quantitative fatty acid composition were different, except for six fatty acids.

Hambleton *et al*. (1980) found that the main fatty acids in horse carcasses in descending order of concentration were oleic, palmitic and linoleic acids. In the present study the major fatty acids were palmitic (16:0, 25.9%), linoleic (18:2n-6, 22.9%), and oleic (18:1n-9, 22.2%) acid. Therefore, although the concentrations of the major fatty acids were within a similar range, their relative concentrations differed from those reported by Hambleton *et al*. (1980). The horsemeat contains nutritionally important fatty acids such as arachidonic acid (AA - 20:4n-6: 2.97%) and eicosapentaenoic acid (EPA - 20:5n-3: 0.43%); and 36.8% saturated fatty acids (SFA) and 36.8% PUFA with a PUFA/SFA ratio of 0.97, which characterize it as a healthy meat according to the reference values the Department of Health and Social Security (1984). It considers values lower than 0.45 as inappropriate for human health due to their association with cardiac diseases. However, these latter values obtained in the present experiment differ from those of Badiani *et al*. (1997) who reported 34.8% SFA, 46.5% MUFA and 18.6% PUFA.

The cholesterol concentration was 40.5 mg/100 g meat, which is within the range of 33.2 - 57.3 mg/100 g meat found by Junqueira *et al*. (2005) in male and female horses at different body weights. Research indicates that this characteristic is directly influenced by sex and herd handling (Sinclair *et al*. 1990). In general, horsemeat has a low cholesterol content compared to other meat such as chicken, mutton, beef and pork (Bragagnolo & Rodriguez-Amaya, 1995; Souza *et al*. 2004).

Among the 30 meat consumer testers, the following acceptability ratings were obtained: 15 rated the meat as liking it extremely, 10 liked it very much, four moderately and one slightly, giving a mean test score of 8.3, which means that the product's sensorial acceptability index was approximately 100% as all testers accepted the meat as excellent.

Despite its low consumption by Brazilians, horsemeat was viewed as excellent in the sensory evaluation. The cholesterol concentration of the horsemeat was lower than that report for meat from other species. From the nutritional point of view it is a good source of proteins and has a low total lipid content. The PUFA/SFA ratio is also within the recommended range for a healthy diet.

References

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.

AOAC, 1995. Official methods of analysis (15th ed.). Association of Official Analytical Chemists, Inc., Arkington, Virginia, USA.

Badiani, A., Nanni, N., Gatta, P.P., Tolomelli, B. & Manfredini, M., 1997. Nutrient profile of horsemeat. J. Food Comp. Anal. 10, 254-269.

Bligh, E.G. & Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Boroski, M., Perini, J.A.L., Rota, E.M., Matsushita, M., Souza, N.E. & Visentainer, J.V., 2008. Effects of condiments and fatty acids composition in meat chicken. Rev. Nac. Carne. 382, 72-77.

Bragagnolo, N. & Rodriguez-Amaya, D.B., 1995. Teores de Colesterol em Carne Suína e bovina e Efeito do Cozimento. Ciênc. Tecnol. Aliment. 15, 11-17. (In Portuguese).

Costa, E.C., Restle, J., Brondani, I.L., Peottono, J., Faturi, C. & Menezes, F.G., 2002. Carcass composition, meat quality and cholesterol content in the Longissimus dorsi muscle of young Red Angus steers confined and slaughtered at different weights. Rev. Bras. Zootec. 31, 417-428.

Department of Health and Social Security, 1984. Report on health and social subjects No 28: Diet and cardiovascular disease, London. Meat Sci. 42, 443-56.

Devic, B. & Stamenkovic, T., 1989. Basic characteristics of the horse meat and the possibilities for its processing. Tehnolo Mesa, 30, 232-237.

Dufey, P.A., 1996. Sensory and physicochemical properties of meat from horses of different age groups. Proc. 42th Int. Conf. Meat Sci. Technol., Lillehamer, Norway, 556-557.

Hambleton, P.L., Slade, L.M., Hamar, D.W., Kienholz, E.W. & Lewis, L.D., 1980. Dietary fat and exercise conditioning effect on metabolic parameters in the horse. J. Anim. Sci. 51, 1330-1339.

Janssen, F.W., Hagele, G.H., Voorpostel, A.M.B. & De Baaij, J.A., 1990. Myoglobin analysis for determination of beef, pork, horse, sheep, and kangaroo meat in blended cooked products. J. Food Sci. 55, 1528-1530.

Joseph, J.D. & Ackman, R.G., 1992. Capillary column gas chromatography method for analysis of encapsulated fish oil and fish oil ethyl esters: collaborative study. J. AOAC Int. 75, 488-506.

Junqueira, A.C.A., Bressan, M.C., Rebello, F.F.P., Faria, P.B., Vieira, J.O. & Savain, S.V., 2005. Proximate composition and cholesterol contents in meat horse (*Equus caballus*, Linneaus) male and female contained by carcass. Ci. Agrotec. 29, 362-368.

Matsuoka, A., Takahashi, T. & Yamanaka, Y., 1993. Lipid and fatty acid composition of M. longissimus thoracis of horse. J. Dairy Food Sci. 42, 89-96.

Matsushita, M., Tazinafo, N.M., Padre, R.G., Oliveira, C.C., Souza, N.E., Visentainer, J.V., Macedo, F.A.F. & Ribas, N.P., 2007. Fatty acid profile of milk from Saanen goats fed a diet enriched with three vegetable oils. Small Rumin. Res. 72, 127-132.

Maurice, D.V., Lightsey, S.F., Hsu, K.T. & Gaylord, R.V.R., 1994. Cholesterol in eggs from different species of poultry determined by capillary GLC. Food Chem. 50, 367-372.

Meilgaard, M., Civille, G.V. & Carr, B.T., 1987. Sensory Evaluation Techniques. London: CRC Press, Inc. p. 281.

Padre, R.G., Aricetti, J.A., Gomes, S.T.M., Goes, R.H.T.B., Moreira, F.B.M., Prado, I.N., Visentainer, J.V., Souza, N.E. & Matsushita, M., 2007. Analysis of fatty acids in Longissimus muscle of steers of different genetic breeds finished in pasture systems. Livest. Sci. 110, 57-63.

Rossier, E. & Berger, C., 1998. La Viande de Cheval: Des Qualite´s Indiscutables et Pourtant Meconnues. CEREOPA–ITEB, Paris, França. (In French).

SAS, 1989. Statistical Analysis System, user's guide for personal computers. 5th ed. Cary, N.C., USA.

Sinclair, A.J., Slattery, W.J. & O´Dea, K., 1990. The analysis of polyunsaturated fatty acids in meat by capillary gas-liquid chromatography. J. Sci. Food Agric. 33, 771-776.

Souza, X.R., Bressan, M.C., Pérez, J.R.O., Faria, P.B., Vieira, J.O. & Kabeya, D.M., 2004. Effects of the genetic group, sex and weight on physico-chemical and properties in lamb meat. Ci. Technol. Alim. 24, 543-549.

Tapiero, H., Townsend, D.M. & Tew, K.D., 2003. Phytosterols in the prevention of human pathologies. Biomed. Pharmaco. 57, 321-325.

Torres, A.P. & Jardim, W.R., 1985. Horse and Other Equine Production. 3rd ed. São Paulo: Nobel, Brazil. 654 p.