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Cholesterol and fatty acid composition of *longissimus thoracis* from water buffalo (*Bubalus bubalis*) and Brahman-influenced cattle raised under savannah conditions

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ABSTRACT

Male (n = 66) water buffalo (Buffalo) and Brahman-influenced cattle (Brahman) were born, raised, weaned, fattened on grazing savannah and harvested at two different ages (19 and 24 months) to compare lipid composition of the *longissimus thoracis* muscle. Half of the animals were castrated at seven months of age (MOA) to examine the castration effects. At 24 MOA Brahman steers showed the highest content of total lipids ($P < 0.05$). No significant variation was detected in cholesterol content for either the main or interaction effects in the age groups. Some individual fatty acids varied with the species ($P < 0.05$), however, interspecific similarities were found in fatty acid ratios. For health-related indices, only atherogenic index (AI) showed lower values in favor of Buffalo meat ($P < 0.05$) at both harvesting ages. Although, meat derived from both bovid groups was leaner and showed lower cholesterol level, AI indicates that Buffalo meat might be beneficial from a human health standpoint.

1. Introduction

There is an increasing awareness of the role of red meat in the human diet and the onset of degenerative diseases. As a consequence, recommendations on the dietary levels of total lipids, cholesterol, saturated fatty acids and *trans* fatty acids have been indicated by several sources (FAO, 1994; Uauy et al., 2009; USDA/HSS, 2010). Despite the vast amount of research that has been undertaken on fatty acid (FA) compositional patterns of beef, the most urgent and long overdue requirement is a reappraisal of the FA gravimetric profile (in g or mg/100 g raw fresh tissue or total lipids) in terms of its functional effects, through health-related indices such as polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA) and ω6/ω3 ratios (HMSO, 1994), hypercholesterolemic:hypocholesterolemic (Dietschy, 1998; Monteiro, Santos-Silva, Bessa, Navas, & Lemos, 2006) or atherogenic and thrombogenic indices (Ulbricht & Southgate, 1991).

The FA composition and cholesterol content in beef is affected by multiple factors (Giuffrida-Mendoza, 2008; Huerta-Montauti et al., 2007; Martínez, 2007). This suggests that differences could be found in the FA composition and cholesterol content of meat from water buffalo (Buffalo) as compared to beef cattle. The worldwide production of Buffalo as a source of meat for human consumption has turned into an interesting economic activity, particularly in tropical regions, such as the Venezuelan savannahs that experience periodic flooding (Reggeti, Rodriguez, & Silva, 1993; Rodas-González et al., 2014; Vale, 1994). Few studies have been undertaken in tropical and sub-tropical regions to compare the chemical composition of lean meat derived from Buffalo versus that of Brahman-influenced cattle (Giuffrida de Mendoza et al., 2005). Results from studies of this type would allow producers, health professionals and consumers to increase their knowledge about the lipid composition and nutritive properties of these red meat sources. Hence, the primary objective of the present work was to compare gravimetric FA profiles and cholesterol content of lean meats from Buffalo (Murrah × Mediterranean crossbreds) and Brahman-influenced (pooled crossbred and purebred) cattle (Brahman) stocked and fattened under similar savannah grass-feeding conditions, and harvested

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at 19 and 24 months of age (MOA). Additionally, post-weaning castration effects were evaluated.

2. Materials and methods

2.1. Animals' characteristics and management

This trial was conducted using an experimental group of 66 bovids (32 Buffalo and 34 Brahman) which represented two postweaning harvesting age groups (19 and 24 MOA) from a serial slaughter experiment described by Giuffrida de Mendoza et al. (2005) and Rodas-González et al. (2014). The post-weaning animals were chosen from a larger group of 132 bovids (64 Buffalo and 68 Brahman) and were randomly selected from a cow–calf operation located at the western Venezuelan llanos (Hato Los Cocos, Apure State). Further details on animal genetics, grass management and supplementation, animal handling during the experiment, growth and carcass performance have been previously reported by Giuffrida de Mendoza et al. (2005) and Rodas-González et al. (2014). Briefly, all newborns were allowed to stay with their mothers to be fed only with dam's milk. Weaning occurred at approximately seven MOA (231 days for Buffalo and 215 days for Brahman). A random sample of bull calves (16 Buffalo and 17 Brahman) was sent to the slaughtering house the following day after weaning. The remaining group of weaned calves was sent to another ranch (Hato El Charcote, Cojedes State), where half of the group were castrated. Non-castrated (bulls) and castrated (steers) males of both species were kept in the same grazing area until reaching the appropriate ages to be serially harvested at 19 MOA (average, 602 days for Buffalo; 583 days for Brahman) and 24 MOA (average, 736 days for Buffalo; 718 days for Brahman). When reaching pre-planned age endpoints animals from both species were weighed and transported approximately 127 km from the ranch to a abattoir facility at Valencia City, Carabobo State, where they fasted for 12 h with access to water and harvested following conventional procedures (COVENIN, 1983). Given that average difference in chronological ages of the species was less than three weeks (Buffalo were 16 to 19 days older), and carcass physiological maturity at the slaughter ages for these two bovid species did not significantly differ (Rodas-González et al., 2014), the age endpoints were comparable for the purpose of meat science studies.

2.2. Sample collection

At 48 h postmortem, *longissimus thoracis* (LT; COVENIN, 1982) were removed and one 2.54-cm thick steak was obtained from the subprimal posterior end of each animal and individually vacuum packaged. All vacuum-packaged LT steaks were immediately frozen at −30 °C and stored at −20 °C until further chemical analyses were performed.

Samples were partially thawed at 4 °C (to avoid fluids losses), trimmed of visible adipose and connective tissue, homogenized in a Black & Decker™ food processor (Model HC306 1-1/2-Cup One-Touch Electric Chopper, New Britain, CT, USA), packaged (Whirl-pak bags, Nasco, Fort Atkinson, WI) and stored at −20 °C until final preparation for the chemical analyses.

2.3. Lipid extraction and determination of cholesterol

Total lipids (g/100 g fresh muscle) from LT samples were extracted according to the method described by Folch, Lees, & Stanley (1957). Total lipids were determined gravimetrically using duplicates of a 5 ml-aliquot of the lipid extract.

The cholesterol content was determined in duplicate for each sample, according the method described by Rhee, Dutson, & Smith (1982). A 4 ml-aliquot of the extract was evaporated under a stream of nitrogen, saponified with alcoholic potassium hydroxide, separated with pyrogallol, acidified and prepared for a colorimetric assay (Searcy & Berquist, 1960), using a Shimadzu UV-2101® PC spectrophotometer (Nakagyo-ku, Kyoto, Japan) at a wavelength of 490 nm. A standard curve was constructed using various cholesterol solutions made of purified cholesterol (Cholesterol Nutritional Biochemical Corporation ® SCW, Cleveland, Ohio, USA) of different contents. Values were expressed in mg/100 g fresh muscle tissue.

2.4. Fatty acid composition and calculations of health-related indices

A duplicate of an aliquot of the lipid extract, equivalent to 20 mg of total lipids free of solvent was used to prepare the FA methyl esters (FAME) according to the method described by Giuffrida de Mendoza et al. (2005), to determine the FA composition. The FAME were extracted with *iso*-octane and stored at −20 °C, after flushing with a nitrogen stream. For each sample, the *iso*-octane layer was transferred to a vial containing 5.0 mg of C21:0 (methyl heneicosanoate) used as internal standard. Samples were stored at −20 °C until performing the chromatographic analysis.

The extracts containing FAME were evaporated under a nitrogen stream at room temperature, and redissolved in 0.5 ml of *iso*-octane. The quantitative determination of SFA, PUFA and monounsaturated fatty acids (MUFA) was performed in a gas chromatograph (GC) (Perkin Elmer Auto System™, Waltham, Massachusetts, USA) equipped with a flame ionization detector, using 1 μl of sample as injection volume. The chromatographic separation was carried out in a capillary column (Supelco™, SP 2560, Sigma-Aldrich, St. Louis, Missouri, USA) of 100 m × 0.25 mm ID × 0.20 μm of film thickness of *bis*-cyanopropyl–polysiloxane. A temperature program was used, with the initial oven temperature of 140 °C maintained by 2.0 min at a rate of 2.7 °C/min. The temperature column increased to 220 °C at a rate of 0.8 °C/min until reaching a final temperature of 240 °C; this temperature was maintained by 9.4 min. The injection port and detector were maintained at 150 °C. Helium served as the carrier gas at a inlet pressure of 2.21 kg/cm^2. TURBOCHROM software (PE-NELSON™, Cupertino, CA, USA) was used for GC data analysis.

The SFA, MUFA and PUFA content was identified by comparing the retention times with those of pure reference standards obtained from Nu-Chek Prep, Inc.™ (Elysian, MN, USA), which contains the following compounds: C12:0, C14:0; C14:1, C15:0, C16:0, C16:1 *cis*, C16:1 *trans*, C17:0, C17:1, C18:0, C18:1, C18:1 *trans* (metil elaidate), C18:1 *trans* (metil vaccenate), C18:2, C18:2 *trans–trans*, C18:3, C18:3 (γ metil linoleate), C20:0, C20:1, C20:1 *trans*, C20:2, C20:3, C20:3 (metil homo γ linoleate), C20:4, C20:5, C21:0, C22:0, C22:1, C22:2, C22:4, C22:6, C24:0 and C24:1. The individual FA content, in relation to the total of intramuscular lipids, was calculated and expressed in g/100 g total lipids.

Atherogenic index (AI), and thrombogenic index (TI) were calculated according to Ulbricht & Southgate (1991) as follows:

$$AI = (C12:0 + 4 \times C14:0 + C16:0)/[\Sigma MUFA + \Sigma(n\text{-}6) + (\Sigma(n\text{-}3)]$$

$$TI = (C14:0 + C16:0 + C18:0)/[0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n\text{-}6) + 3 \times \Sigma(n\text{-}3) + \Sigma(n\text{-}3)/\Sigma(n\text{-}6)].$$

Hypercholesterolemic/hypocholesterolemic ratio (H/h), was calculated according to Monteiro et al. (2006), as follows:

$$H/h = (C14:0 + C16:0)/(C18:1 + C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:4 + C22:5 + C22:6).$$

2.5. Data analysis

Data were analyzed using the MIXED model procedures of SAS (SAS Inst. Inc., Cary, NC) version 9.2 (SAS, 2003).

Total lipids, cholesterol and FAs were analyzed separately because those analyses were conducted in different times for each age endpoint (19 and 24 MOA). Therefore, the variation associated with species, male class and their interactions within each post-weaning harvesting age