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Calcium and potassium content in beef: Influences on tenderness and associations with molecular markers in Nellore cattle

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ABSTRACT

Calcium (Ca) and potassium (K) are essential nutrients in animal nutrition. Furthermore, the Ca content can influence meat tenderness because it is needed by the proteolytic system of calpains and calpastatins, major factors in postmortem tenderization of skeletal muscles. K content, which is needed for muscle contraction, can also affect meat tenderness. This study showed that K positively affects the Warner–Bratzler shear force (WBSF), measured at 14 days of meat aging, which means that higher levels of K are related to lower meat tenderness. Additionally, a significant effect ($P \leq 0.015$) of a SNP in the calcium-activated neutral protease 1 (*CAPN1*) gene on Ca content was observed. Metal content in beef can affect not only nutritional values but also meat quality traits. Part of this effect may be related to variation in specific genes.

1. Introduction

Beef is an important part of the diet in most countries. Besides the relevance of metal content in meat for human nutrition, the investigation of the effects of metal levels on meat quality is also important. Brazil is the main global producer of beef and Nellore is the main cattle breed in Brazil. Variation in tenderness is a major concern for the meat industry (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2004) and Zebu cattle produces less tender meat compared to European breeds.

Metals such as calcium (Ca) and potassium (K) can affect meat tenderness through their function in the cells. Research has shown that neutral proteases dependent on calcium ions, calpains are associated with postmortem protein degradation of skeletal muscle (Geesink & Koohmaraie, 1999). The calcium-activated neutral protease 1 gene (*CAPN1*; Gene ID: 281661) codifies the μ-calpain enzyme. Calpastatin (*CAST*; Gene ID: 281039), an enzyme that inhibits the action of *CAPN* and is primarily responsible for the regulation of postmortem proteolytic activity (Koohmaraie, 1996). The increase in postmortem activity of *CAST* is associated with reduced beef tenderness (Pringle, Williams, Lamb, Johnson, & West, 1997). Studies have shown an association between polymorphisms in the *CAPN* and *CAST* genes and meat tenderness in different cattle populations (Barendse, 2002; Casas et al., 2006; Corva et al., 2007; Page et al., 2002; Schenkel et al., 2006).

The potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*, Gene Bank ID: 532060) is involved in the insulin secretion pathway (Alekseev et al., 2010). It is located on bovine chromosome 15, close to a quantitative trait locus (QTL) for meat tenderness (Rexroad Iii et al., 2001). This gene encodes a protein which increases the flow of K into cells where it takes part in the establishment of electrical potential in the cell membrane. K is necessary for muscle contraction and nerve impulses, and along with sodium, it helps maintain the proper balance of fluids in the cells (Knochel & Schlein, 1972). A recent study (Tizioto et al., submitted for publication) found that a single nucleotide polymorphism (SNP) in the *KCNJ11* gene is related to meat tenderness in the same population used in this study. Recent results indicate the importance of genomic variation in the mineral content of different tissues (Morris et al., 2013).

In this study, the association between Ca and K content and meat tenderness was analyzed as well as the effects of SNPs in the *CAPN1*,

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CAST and *KCNJ11* genes on Ca and K contents in a population of Nellore cattle.

2. Materials and methods

2.1. Animals and phenotypic data

Nellore steers (n = 286), offspring of 32 sires chosen to represent the main breeding lineages in Brazil, were used to obtain genotypic and phenotypic data. The half-sib families were produced by artificial insemination in commercial and purebred Nellore dams. The animals were raised and finished in feedlots at the experimental unit of the Embrapa Cattle — Southeast research unit at São Carlos, São Paulo, Brazil. The diet of the animals was previously described (Tizioto et al., 2012). The animals were slaughtered when they reached 5 mm of back fat thickness determined by ultrasound imaging.

After slaughter, 2.5 cm thick steaks from a cross section of the *longissimus dorsi* muscle, between the 12th and 13th ribs, were collected. These were identified, vacuum-packed and analyzed for meat tenderness by Warner–Bratzler shear force (WBSF) measurements. The WBSF was measured in a TA XT2i texture analyzer coupled to a 1.016 mm Warner–Bratzler probe. The analyses of WBSF were done at different aging times: about 24 h after slaughter (WBSF0), after seven days (WBSF7) and 14 days (WBSF14). Samples were aged at 2 °C in a cold chamber manufactured by McQuay–Heatcraft do Brasil Ltda. (São José dos Campos, São Paulo, Brazil).

2.2. Sample preparation for chemical elements determination

Analytical grade reagents and Milli-Q water (Millipore, Billerica, MA, USA) were employed. All stock standard solutions used to prepare the multi-element standard solution were certified with plasma grade and high purity materials from SpecSol, (Jacareí, SP, Brazil). Working standard solutions were prepared daily by diluting appropriate aliquots of the stock solution in ultrapure water.

A closed-vessel microwave digestion system (Ethos-1600, Milestone-MLS, Sorisole, Italy) equipped with optic fiber temperature and pressure sensors was used for sample digestion. Sample masses of 100 mg of beef samples were digested with microwave assistance using 2 mL of sub-boiled concentrated HNO_3, 2 mL of H_2O_2 (30% w/w) and 6.0 mL of ultrapure water in closed vessels made of perfluoroalkoxy copolymer resin (PFA). The heating program was in three steps: (1) heating ramp of 10 min with maximum temperature of 120 °C (1300 W); (2) second heating ramp of 15 min with maximum temperature of 170 °C (1500 W); (3) temperature at 170 °C during 35 min.

After digestion, the samples and blank solutions were transferred to 10.0 mL volumetric flasks and made to volume with deionized water. The metal concentrations were determined by a Vista Pro-CCD ICP-OES spectrometer (Varian, Mulgrave, Australia). The wavelengths were chosen according to the least spectral interference and the highest intensity emission for each element. The optimized parameters used in the ICP-OES are described in Table S1. A linear calibration was calculated with up to five points, which was prepared with standard analytical solutions.

The accuracy of the proposed method was evaluated by analyzing the certified reference material Bovine Liver 1557b and Bovine Muscle 8414 from the National Institute of Standards and Technology (NIST Gaithersburg, MD, USA). To assess possible contamination during sample preparation, blank samples of ultrapure water were prepared using the same procedure as for the beef samples.

2.3. DNA extraction

Straws of frozen semen obtained from Brazilian artificial insemination centers were used to extract DNA from bulls using the standard phenol-chloroform method (Sambrook, Fritsch, & Maniatis, 1990). For the steers, 5 mL blood samples were used and DNA extractions were performed by a salting-out method as described in Tizioto et al. (2012). DNA concentration was measured by spectrophotometry, and the quality was verified by the 260/280 ratio, followed by inspection of integrity through agarose gel electrophoresis.

2.4. SNPs genotyping

For the *CAPN1* gene, the synonymous SNP c.3379G > A (rs17872099), located in exon 5, was genotyped in 133 animals. For the *CAST* gene, genotypes for 178 animals were determined for SNP c.2959A > G (AF159246), located in the 3′UTR region (Barendse, 2002).

Two SNPs in *KCNJ11* were genotyped in 286 animals, one (c.1526C > T; NCBI_ss#537718973) a synonymous mutation located in the coding region and the other (c.2342T > C; NCBI_ss#537718995) located in the 3′UTR region. The genotypes for all SNPs were determined by real-time PCR, using TaqMan™ assays (Applied Biosystems, Foster City, CA, USA) (Table S2).

2.5. Statistical analysis

The content of Ca and K given in mg g^{-1} were transformed into logCa and logK to achieve a normal distribution.

A mixed model was used with fixed effects of contemporary groups (CGs) and age of the animal at measurement (linear effect), pH and metal content as covariates, and sire as the random effect. The CG included the effects of birthplace, breeding season, and slaughter date.

Analyses were performed by restricted maximum likelihood using the PROC MIXED procedure in the Statistical Analysis System (SAS) (SAS Institute Inc., 2000) using model 1:

$$Y_{ijklm} = \mu + CG_i + S_j + b_1\left(A_{ijk} - a\right) + b_2\left(P_{ijkl} - p\right) + b_3\left(M_{ijklm} - m\right) + e_{ijklm} \quad (1)$$

where:

- Y_{ijklm} observation of individual *l*, of age A, offspring of sire *k* with genotype *j* for the marker, belonging to contemporary group *i*;
- μ overall mean;
- CG_i fixed effect of the contemporary group *i*;
- S_j random effect associated with the father of animal *k*, $N \sim (0, \sigma_t^2)$;
- b_1 linear regression coefficient associated with animal's age;
- b_2 linear regression coefficient associated with pH of the samples;
- b_3 regression linear coefficient associated with Ca or K content of the samples;
- A_{ijk} animal age on the date of measurement, a is the mean age of measurement;
- P_{ijkl} value for each observation of pH, p is the mean pH of the samples;
- M_{ijklm} value for each observation of metal content (Ca or K), m is the mean Ca or K of the samples;
- e_{ijklm} random error associated with each observation, assumed normally distributed with mean zero and variance σ^2.

To evaluate the effects of markers in *CAPN1*, *CAST* and *KCNJ11* on the Ca and K content in the meat, the model described in Eq. (2) was used.

$$Y_{ijkl} = \mu + CG_i + M_j + S_k + b_1\left(A_{ijkl} - a\right) + e_{ijkl} \quad (2)$$

where:

- Y_{ijklm} observation of individual *l*, of age A, son sire *k* with genotype *j* for the marker, belonging to contemporary group *i*;
- μ overall mean;