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Mate extract as feed additive for improvement of beef quality

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ABSTRACT

Mate (*Ilex paraguariensis* A.St.-Hil.) is generally recognized as safe (GRAS status) and has a high content of alkaloids, saponins, and phenolic acids. Addition of mate extract to broilers feed has been shown to increase the oxidative stability of chicken meat, however, its effect on beef quality from animals supplemented with mate extract has not been investigated so far. Addition of extract of mate to a standard maize/soy feed at a level of 0.5, 1.0 or 1.5% *w*/w to the diet of feedlot for cattle resulted in increased levels of inosine monophosphate, creatine and carnosine in the fresh meat. The content of total conjugated linoleic acid increased in the meat as mate extract concentration was increased in the feed. The tendency to radical formation in meat slurries as quantified by EPR spin-trapping decreased as increasing mate extract addition to feed, especially after storage of the meat, indicating higher oxidative stability. Mate supplementation in the diet did not affect animal performance and carcass characteristics, but meat from these animals was more tender and consequently more accepted by consumers. Mate extract is shown to be a promising additive to feedlot diets for cattle to improve the oxidative stability, nutritive value and sensory quality of beef.

1. Introduction

Oxidation of lipids and proteins is the major non-microbiological factor involved in quality deterioration of meat and meat products (Carlsen, Andersen, & Skibsted, 2001; Carlsen, Møller, & Skibsted, 2005; Lund, Heinonen, Baron, & Estévez, 2011; Morzel, Gatellier, Sayd, Renerre, & Laville, 2006). The resistance to oxidation of muscle tissue and meat depends on the balance between prooxidants and antioxidants present in the product as the most important internal factor together with fatty acid profile of the lipids in the meat. The oxidative stability of meat from non-ruminant animals like poultry and pigs may be improved by adding antioxidants or natural antioxidants from plants to the feed (Kumar, Yadav, Ahmad, & Narsaiah, 2015). The meat fatty acid composition from non-ruminants may likewise be made healthier by changing the lipid composition of the animal feed towards higher degree of unsaturation.

For ruminants, the rumen microbiota will decrease the degree of lipid unsaturation prior to absorption and the effect of the feed composition on the meat will be modulated. An increase in content of plant rich in bioactive compounds like antioxidants for ruminant feed may, however, change the microflora of the rumen subsequently affecting meat composition and quality (Butler, 2014; Kumar et al., 2015; Patra & Saxena, 2011).

The epidemiological evidence of an increased risk of colorectal cancer due to a high intake of red meat seems also to be related to the oxidative stability of meat and especially to the high content of bioavailable iron as a prooxidant (Oostindjer et al., 2014). Red meat includes meat from pigs and cattle, and since the relationship between feed intake and meat stability is better documented for pigs, it seems timely to investigate the effect of addition of plant rich in bioactive components to feed for cattle on performance, carcass characteristics and meat quality. Nellore cattle, the most common cattle breed in Brazil, was fed mate (*Ilex paraguariensis* A. St.-Hil.), which is generally recognized as safe (GRAS status) and is native to the South America region where there are the highest beef production and consumption in the world (OECD data, 2017). Mate has a high content of alkaloids, saponins, and phenolic acids (Heck & de Mejia, 2007) and has been shown to increase the oxidative stability of chicken meat both when

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added to the meat during cooking and when added as an extract to the drinking water of broilers (Racanicci, Danielsen, & Skibsted, 2007; Racanicci, Menten, Alencar, Buissa, & Skibsted, 2011). Herein, the effect of adding extract of mate as a source of bioactive compounds including antioxidants at increasing levels to cattle feed will be followed through animal performance, carcass and meat quality, changes in the metabolic profile of meat and resistance to oxidation, and sensory quality and consumer acceptance.

2. Materials and methods

2.1. Animals and sampling

Forty-eight Nellore steers (*Bosindicus*, minimal genetic variation due to common semen donor), raised at Embrapa Pecuária Sudeste (São Carlos, SP) with an average age of 21 months and an initial weight of 419 kg were individually fed during 94 days with the same basic diet, differing by mate extract levels (0%, 0.5%, 1.0%, and 1.5% *w*/w). Diets were composed of grounded corn grain (51.3% *w*/w), corn silage (43.0% *w*/w), mineral supplement (1.2% *w*/w), soybean meal (1.0% *w*/w), urea (1.0% *w*/w), sodium bicarbonate (1.0% *w*/w), and monensin (0.03% *w*/w). The final diet was composed of 11% crude protein and 72% of total digestible nutrients. Diets were balanced using 0%, 0.5%, 1.0% and 1.5% *w*/w of food grade kaolin as an inert ingredient. Mate extract (particles of 40 mesh, maximum of 6% water content, 6% of organic matter, and a maximum caffeine content of 6%) without addition of excipient was the product of Centroflora Group (Botucatu-SP, Brazil) produced from fresh leaves of mate (*Ilex paraguariensis* A. St.-Hil.) by water:ethanol 75:25 *v*/v extraction at 90 °C.

Animals were shipped one day before the slaughter to a commercial abattoir and held overnight with access to water. Animals were slaughtered and carcasses were chilled overnight at 2° C. All dietary treatments and experimental procedures were approved by the Embrapa Pecuária Sudeste Animal Care Committee (CEUA protocol 06/2014). Animals were stunned by captive bolt and exsanguinated in accordance with guidelines established by the Brazilian Ministry of Agriculture. After twenty-four hours *post-mortem* the left half-carcass between the 12 and 13th rib was removed and 2.5 cm steaks (*Longissimus thoracis et lumborum* muscle) were collected for chemical investigations and sensory analysis. Steaks were placed in plastic bags, vacuum-packed, part of them were aged during 14 days at 0 to 2 °C in a refrigerated chamber and after stored at − 20 °C and − 80 °C for sensory evaluation and for chemical profiling, respectively.

2.2. Animal performance and carcass traits evaluation

Dry matter intake was calculated by weighting offer and leftover daily. Samples were collected for analysis of dry matter, performed in a ventilated oven at 60 °C for 72 h. Animal live weights were obtained every 28 days to calculate daily weight gain.

Carcasses were weighed immediately after slaughter to obtain hot carcass weight (HCW) and then were split into sides and were chilled overnight at 2 °C. Carcass yield (CY) was obtained by multiplying the hot carcass weight by 100, divided by final live weight.

The loin eye area (LEA) was evaluated in *longissimus* muscle between the 12th and 13th ribs. The outer perimeter of *longissimus* muscle was directly traced on tracing paper, and the loin eye area was measured using a transparent scale. Back fat thickness (BFT) was measured in the *longissimus* muscle, in millimeters, using a ruler. The experimental design was completely randomized with four treatments and twelve replications.

2.3. Mate extract characterization and analysis of phenolic compounds in beef

Ten mg of mate extract were homogenized for 1 min using a commercial cell disruptor (Fast Prep®, MP Biomedicals, Solon, OH, USA) with 1.5 mL of cold methanol/water solution (1:1 *v*/v) in homogenization tubes containing ceramic beads at a speed of 5.0 ms^{-1} during 120 s, after that samples were filtered using nylon membranes of 0.45 μm and stored at − 20 °C until the ultra-performance liquid-chromatography electrospray ionization mass spectrometry analysis (UPLC-HRESI-MS). Ten μL of the sample extract were injected into an Accela 1250 HPLC system coupled with an autosampler Accela AS and a high-resolution accurate mass spectrometer LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany), which was equipped with an electrospray source (HESI-II) operating in the negative ion detection mode. The mobile phase at a flow rate of 0.5 mL min^{-1} consisted of a linear gradient from 5% of mobile phase B (methanol containing 0.1% formic acid) and 95% of mobile phase A (0.1% aqueous formic acid) to 80% of mobile phase B over 30 min and then from 80% to 95% of mobile phase B in 5 min which was held 5 min in isocratic mode and then from 95% of the mobile phase B to 5% of B in 7 min.

The analysis of phenolic compounds in beef were carried out by taking a sample of 500 mg of frozen beef which was homogenized for 1 min at 5 m s^{-1} using a commercial cell disruptor (Fast Prep®, MP Biomedicals, Solon, OH, USA) with 1.0 mL of cold isopropanol containing 2% *v*/v of trichloroacetic acid in homogenization tubes containing ceramic beads. Samples were kept on ice throughout the procedure. The supernatant was collected using a micropipette and transferred to 2 mL micro-tube. Tubes were placed into a *speed-vac* for 12 h until dryness. After complete dryness, 500 μL of a solution of 50:50 (*v*/v) water-methanol was added and each tube was vortexed for 2 min. Tubes were again centrifuged and the supernatant was passed through a 0.22 μm hydrophilic filter and 10 μL of the resulting extract injected in the ultra-performance liquid-chromatography (UPLC) system for phenolic analyses.

2.4. Meat metabolomics profile

After feeding of 48 Nellore steers during their finishing for the last 94 days prior to slaughter with a feed supplemented with mate extract, fresh and 14-day aged beef from *Longissimus thoracis et lumborum* muscle were collected and extract of meat samples from different feeding treatment were subjected to ^{1}H NMR screening for untargeted metabolomics, followed by quantitative polar metabolic profile by ^{1}H NMR and high-resolution accurate mass spectrometry as described in the next experimental sections.

2.4.1. Extraction of polar metabolites and CLA from beef

Approximately 0.20 g of frozen beef was homogenized for 1 min using a commercial cell disruptor (Fast Prep®, MP Biomedicals, Solon, OH, USA) with 1.0 mL of cold methanol/water solution (1:1 *v*/v) for polar metabolites extraction or cold chloroform for CLA (Prema et al., 2015) in homogenization tubes containing ceramic beads. Samples were kept on ice throughout the procedure. Then, the homogenates were centrifuged for 10 min at 10,000 × *g* at 10 °C to remove precipitated protein, fat and connective tissue. Supernatants were carefully collected, transferred to Eppendorf tubes and dried in a centrifugal concentrator (*Speed-Vac*, Thermo Savant, Holbrook, NY, USA). The dried extract containing the polar metabolites of beef was re-suspended with deuterium oxide phosphate buffer (0.10 M, pD = 7.3) containing 0.050% *w*/w of the internal chemical shift standard sodium 3-trimethylsilyl-2,2,3,3-d4-propionate (TMSP-d4, from Cambridge Isotopes, Leicestershire, UK), and transferred to a 5 mm NMR precision tube (Vineland, NJ, USA). The same extraction was performed in beef samples after 14 days of storage at 4 °C to investigate changes in the metabolites profile during ageing. For CLA, the dried extract was re-suspended in acetonitrile for further mass spectrometry analysis.