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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.

2.4.2. Untargeted polar metabolites screening of meat extract by ^{1}HNMR spectroscopy

The experiments were performed on a 9.4 T Bruker Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 5 mm PABBI probe head with gradients, automated tuning and matching accessory (ATMATM), BCU-I for regulation of temperature, and a Sample-Xpress sample changer. The experiments were acquired at 300 K in fully automatic mode without sample rotation. Before the ^{1}H NMR spectra acquisition, a delay of 5 min was ensured for each sample inserted in the magnet for temperature equilibration. Automatic tuning, matching (ATMATM), locking (LOCK), phase, shimming (TOPSHIM), and a saturation pulse (25 Hz) were achieved using standard Bruker routines by ICON-NMR interface. Thirty-two scans and four prior dummy scans of 65,536 data points were acquired with a spectral width of 20.5524 ppm, a receiver gain of 90.5, an acquisition time of 3.98 s, a recycle delay of 4 s, and mixing time was set to 10 ms. The water suppression was achieved using the NOESY-presaturation pulse sequence (Bruker 1D noesygppr1d pulse sequence) with irradiation at the water frequency (O1 = 1881.95 Hz) during the recycle and mixing time delays. FIDs were multiplied by a 0.3 Hz exponential multiplication function prior to Fourier transformation, only a zero order phase correction was allowed, and the TMSP-d4 signal was calibrated at 0.0 ppm. Furthermore, ^{1}D JRES, 2D ^{1}H-^{1}H TOCSY and 2D ^{1}H-^{13}C HSQC experiment were performed on selected samples in order to confirm the assignment of metabolites.

The spectral data were exported to Amix version 3.9.3 for screening of samples. A bucketing was done within 0.90–9.00 ppm, dividing the region into 400 sequential segments or "bins", obtaining an integral for each. The regions of carnosine (8.50–8.30 ppm), residual water (5.00–4.65 ppm), and both the left (9.00–15.00 ppm) and right (0.90– − 5.00 ppm) empty regions have been excluded (those regions are irrelevant for the present investigation or exclude in order to minimize pH variations). Principal components analysis (PCA) was performed by different pre-processing methods (scaling to unit variance and Pareto) as well as no scaling. The best results of PC's analyses were achieved using no scaling method with buckets width of 0.05 ppm.

2.4.3. Quantitative polar metabolite profile of meat extract by ^{1}HNMR spectroscopy

Quantitative^{1}H NMR spectra were acquired on an 11.7 T Agilent DD2 500 MHz NMR Spectrometer. A standard solvent suppression pulse sequence PRESAT was used to saturate the residual water peak, using a 90°pulse width of 7.9 μs, a SW of 20.55 ppm, 256 scans, 32 K total acquired complex points, and a relaxation delay of 4 s. The data were phase corrected, baseline corrected and the TMSP-d4 was calibrated using VnmrJ 4.0 software (Agilent Technologies, USA).

The assignment of peaks in the spectra was based on the database present in the Chenomx NMR suite 7.7 software (Chenomx Inc., Edmonton, AB, Canada) in order to identifying the polar metabolites in beef extract. From the database information, a spectral fingerprinting of metabolites composed by a Lorentzian peak shape model matching the actual spectrum was generated. Individual metabolite concentrations were determined using the 500-MHz library from Chenomx NMR Suite 7.7 by referencing the TMSP-d4 concentration (1 mM). The average metabolite concentration according to the animal diet was calculated using the values of three replicate beef samples. Assignment of the ^{1}H NMR spectra was obtained by literature data (Graham et al., 2010; Tsai, Tsai, Wu, Tseng, & Tsai, 2010) or by adding standard compounds.

2.4.4. Quantitative determination of polar metabolites and CLA in meat extract by high-resolution accurate ESI and APCI mass spectrometry

Mass spectrometry analyses of the methanol/water extract of beef were performed by flow-injection, using an Accela 1250 HPLC system coupled with an autosampler Accela AS and a high resolution mass spectrometer LTQ OrbitrapVelos (Thermo Fisher Scientific, Bremen, Germany), which was equipped with an electrospray source operating in the positive and negative ion modes. The injection volume of 10 μL was injected at a flow rate of 300 μL min^{-1} of an isocratic mobile phase consisting of methanol/water (1:1 *v*/v) with 0.1% formic acid for polar metabolites. Sample cleanup to minimize matrix effect was performed on-line by using a RAM BSA-octyl column (Hypersil phase, 100 μm particle size; 5 cm × 5 mm) to prevent matrix effect. Metabolite identification was carried out by high-resolution MS and MS/MS spectra assignment and by standard addition. Positively identified metabolites were then quantified by external standard calibration curves.

For conjugated linoleic acid (CLA) analysis, the mass spectrometer was equipped with an atmosphere pressure chemical ionization (APCI) source operating in the positive mode. The injection volume of 10 μL of the extract was injected at a flow rate of 300 μL min^{-1} of a mobile phase consisting of acetonitrile and the quantification was carried out by external standard calibration curves. Sample cleanup was performed on-line by using a RAM BSA-octyl column (Hypersil phase, 100 μm particle size; 5 cm × 5 mm) to minimize matrix effect.

2.5. Redox stability of fresh and aged beef by EPR spin-trapping technique

The redox status of fresh and 14-day aged meat was investigated by the spin-trapping EPR technique (Bolumar, Skibsted, & Orlien, 2012; Carlsen et al., 2001) probing PBN radical adduct formation in meat slurries incubated at 65 °C. Samples of 0.5 g of fresh beef or beef aged for 14 days at 4 °C were homogenized in 5.0 mL of 50 mM2-(N-morpholino)-ethane-sulfonic acid (MES, Sigma-Aldrich, Stenheim, Germany) buffer pH 5.7 under ice bath cooling using an Ultra Turrax homogenizer (Janke & Kunkel, Staufen, Germany). Then, 450 μL of 400 mM of α-tert-butyl-phenyl-nitrone (PBN, Sigma-Aldrich, Stenheim, Germany) dissolved in ethanol were added to the homogenate. Samples were vortexed briefly and centrifuged for 10 min at 10,000 rpm and 10 °C. Aliquots of 1.0 mL of the centrifuged solutions were incubated over 4–5 h in water bath at 65 °C before recording the EPR spectra. Every 1 h, one aliquot was collected, cooled at room temperature and transferred to a 50 mL micropipette (Blaubrand Intramark, Wertheim, Germany) located in the EPR cavity through a flow system. The X-band EPR spectra of the slurries were obtained at room temperature in an EPR spectrometer Bruker EMXplus (Bruker BioSpin, Rheinstetten, Germany). The EPR parameters were: microwave power 1 mW, sweep width 50 G, center field 3940 G, modulation frequency 100 kHz, modulation amplitude 2 G, ns = 16 scans.

2.6. Determination of relative intramuscular fat by low field ^{1}H NMR spectroscopy

One cylindrical sample, 8 mm in diameter and 1 cm in height, was collected from each forty-eight beef steaks (one per animal), as described by Corrêa, Forato, and Colnago (2008) ou (Corrêa et al., 2008). The relaxometric time measurements (T_1 and T_2) were performed in a bench top time-domain NMR Bruker spectrometer Minispec MQ 20 (Bruker BioSpin, Rheinstetten, Germany), equipped with a permanent magnet of 0.47 T (19.9 MHz for ^{1}H), a heater module Bruker N_2 Temperature Controller, and 10 mm probe. Analyses were performed at controlled temperature 25.0 ± 0.1 °C. The longitudinal relaxation time (T_1) values were obtained using inversion-recovery pulse sequence, applied π/2 and π pulses with duration of 2.42 μs and 4.68 μs, respectively, recycle time of 4 s and 8 scans. CP-CWFP sequence has been performed with a train of π/2 pulses with duration of 2.42 μs, separated by an interval time T_p = 300 μs, recycle time of 4 s, 8 scans and number of echoes 500, and the acquisition time was 0.02 ms. The rate M_c/M_0 was obtained after normalizing the values of M_0 using M_c as obtained from Eq. (3). T_1 and T_2 were calculated using Eqs. (1) and (2) combined with the magnitude of NMR signals after the pulse (M_0), in steady state (M_c). The T^* was obtained by mono-exponential fitting of decay after the quasi-stationary state (QSS), Eq. (3).

$$T_1 = \frac{T^*/_2}{M_z M_0} \quad (1)$$

$$T_2 = \frac{T^*/_2}{1 - (M_z/M_0)} \quad (2)$$

$$M_c = \frac{M_0 x T_2}{(T_1 + T_2)} \quad (3)$$

2.7. Meat quality parameters

Fresh and 14-day aged beef were analyzed. For objective color determination, steaks were exposed to atmospheric air for thirty minutes at room temperature prior to the analyses, and CIE tristimulus values coordinates L*, a* and b* were measured at three different locations across the surface of the steaks randomly selected using a Hunter Lab colorimeter model MiniScan XE (Hunter Associates Laboratory, Reston, VA, USA), with illuminant D65 and a 10° observer. Then, the pH was measured at three different locations across the surface using a Testo pH-measuring instrument, model 230 (Testo Limited, Hampshire, UK). The water holding capacity was obtained by the difference between the weights of a meat sample of approximately 2 g, before and after submission to a pressure of 10 kgf cm^{-2} for 5 min as described by Hamm (Hamm, 1986). For cooking loss and shear force measurements, the same steak of 2.5 cm thickness was weighed and cooked in a Tedesco combination oven at 170 °C (Tedesco, Caxias do Sul, RS, Brazil) until the temperature at the center of the sample reached 70 °C, controlled by a thermocouple. Samples were then cooled at room temperature and weighed again. Cooking loss was calculated by difference between the weights before and after cooking, and expressed as percentage. Steaks were transferred to a cooler and held for 24 h, after which, eight cores (1.27 cm in diameter) were removed per steak, parallel to the fiber grain. Peak shear force was determined on each core perpendicular to the fiber grain using a 1.016 mm Warner Bratzler probe in a TA-XT Plus Texture Analyzer (Stable Micro Systems, Surrey, UK) with a calibration weight of 10 kg. Full peak shear force was recorded and maximum shear force calculated as the average of the eight cores. The experimental design was completely randomized with four treatments and twelve replications, with analysis of variance and regression depending on the levels of mate extract added to the animal diet.

2.8. Sensory descriptive analysis and consumer acceptance

A ten-member trained panel was used to perform the descriptive sensory analysis. Frozen samples were placed in a refrigerator at 5 °C overnight prior to the sensory analysis. Steaks were removed from the refrigerator and cooked according to each type of sensory test. For descriptive analysis, five steaks per treatment were evaluated and cooked in a TC06 Tedesco combined oven model at 170 °C, until an internal temperature of 75 °C is reached. Steaks cut into cubes of 1.5 cm of each side and were salted with 1.0 g and pan-fried with soybean oil until reach an internal temperature of 75 °C. Samples were presented for each panelist in a balance design assigned by Fizz Software version 2.41 (Biosystemes, Couternon, France). Eight samples were evaluated per session. Attribute ratings were electronically collected using a nine-point descriptive scale for beef characteristic aroma (1 = extremely bland; 9 = extremely intense), beef characteristic flavor (1 = extremely bland; 9 = extremely intense), strange aroma (1 = extremely intense; 9 = none), strange flavor (1 = extremely intense; 9 = none), tenderness (1 = extremely tough; 9 = extremely tender) and juiciness (1 = extremely dry; 9 = extremely juicy).

Concerning to sensory acceptance, samples were randomly assigned to non-trained panellists (n = 100), who analyzed two treatments (control and 1.5% mate extract). A pool of samples was made combining steaks, which came from 10 animals of each treatment. Attribute ratings were collected using nine-point hedonic scales for flavor, texture and overall acceptance (1 = dislike extremely; 9 = like extremely). Experimental design was completely randomized with four treatments and 12 replicates, with diet as fixed factor. The data were analyzed by ANOVA using XLSTAT software. Trained and consumer panels were conducted at Meat Analysis Laboratory at Embrapa Pecuária Sudeste and Dietetic Techniques Laboratory at Centro Universitário Central Paulista (UNICEP), respectively. Human Research Ethical Committee from Federal University of São Carlos approved the protocol and written consent (CAEE 08551012.3.0000.5504) which participants were required to complete.

2.9. Statistical analyses

The experimental design was completely randomized with four treatments and twelve replications, and data obtained for animal performance, carcass, meat quality and sensory descriptive traits were analyzed by analysis of variance, with diet as fixed effect. For acceptance test, a t-test at the 5% significant level was applied. XLSTAT (Addinsoft, Paris, France) software was employed.

In the untargeted polar metabolites screening of meat, principal components analysis (PCA) was performed by different pre-processing methods (scaling to unit variance and Pareto) as well as no scaling. The best results of PC's analyses were achieved using no scaling method with buckets width of 0.05 ppm. Results from quantitative determination of polar metabolites were analyzed by ANOVA and when there was significant difference ($p < 0.05$), a Tukey's test was applied.

Multivariate data analyses were performed on Statistica software (version 12, StatSoft, Tulsa, OK, USA) using the correlation method on the data taken from the quantitative metabolomics approach as determined by combined NMR spectroscopy and ESI-MS. Variables were pre-selected based on variability of the data values using one-way analysis of variance (ANOVA, $p = 0.05$). Principal Component Analysis (PCA) was carried out by use of the mean-centered data for metabolite concentrations determined in the methanol/water meat extract as x-variables. Three animals representing each treatment were selected as samples to investigate any clustering of the data.

3. Results and discussion

Animal performance and carcass characteristics are shown in Table 1. No significant difference ($p > 0.05$) neither in animal performance nor carcass traits between animals submitted to control diet and those supplemented with different levels of mate extract were found. Dry matter intake, average daily gain, and overall feed conversion are in agreement with literature values reported for Nellore (*Bos indicus*) steers (D'Oliveira, do Prado, dos Santos, & Sakaguti, 1997; Ezequiel, Galati, Mendes, & Faturi, 2006).

The mean values for hot carcass weight (261.9 kg) were similar to reported values for castrated Nellore steers finished in feedlot with the same age (da Silva et al., 2008). The average result found for carcass yield were 54.7% and are in the range of variation considered adequate (55.7 to 58.7%) for Nellore steers (Lorenzoni, Campos, Garcia, & Silva, 1986).

3.1. Mate extract characterization and phenolic analysis in beef

Following the UPLC–ESI-HRMS characterization of the mate extract, over forty phenolic compounds could be identified in the mate extract by their exact mass and fragmentation pattern. From the identified phenolic compounds, 11 compounds of significant peak area were quantified by UPLC–ESI-HRMS and the result of analysis is collected in Table S1. Among the identified phenolic compounds, chlorogenic acid and 1,5-dicaffeoylquinic acids were the most abundant phenolic compounds in the mate extract with a concentration of 12.3 ± 0.01 and 6.0 ± 0.01 mg g^{-1}extract, representing 58.2% and 28.4% of the total

Table 1
Animal performance and carcass traits of animals fed with different levels of mate extract.

	Mate extract supplementation (%)					SEM
	None	0.5	1.0	1.5	*p*-value	
Dry matter intake (kg/day)	10.6 ± 1.3	10.6 ± 1.3	10.3 ± 1.4	9.5 ± 1.2	0.107	0.285
Average daily gain (kg)	1.4 ± 0.4	1.3 ± 0.3	1.3 ± 0.4	1.2 ± 0.4	0.344	0.085
Feed conversion	8.4 ± 2.6	8.9 ± 2.5	8.3 ± 2.2	8.3 ± 2.3	0.599	0.568
HCW (kg)	292 ± 21	289 ± 24	292 ± 25	283 ± 26	0.804	7.5
CY (%)	55.8 ± 1.2	56.1 ± 1.1	56.2 ± 1.5	56.6 ± 1.5	0.624	0.451
REA (cm^2)	56.3 ± 2.8	55.7 ± 2.9	56.2 ± 2.3	56.8 ± 2.6	0.825	0.877
BFT (mm)	10.8 ± 5.6	13.3 ± 5.3	9.0 ± 2.9	10.2 ± 3.4	0.155	1.691

HCW = hot carcass weight; CY = overall carcass yield; REA = rib eye area; BFT = back fat thickness.

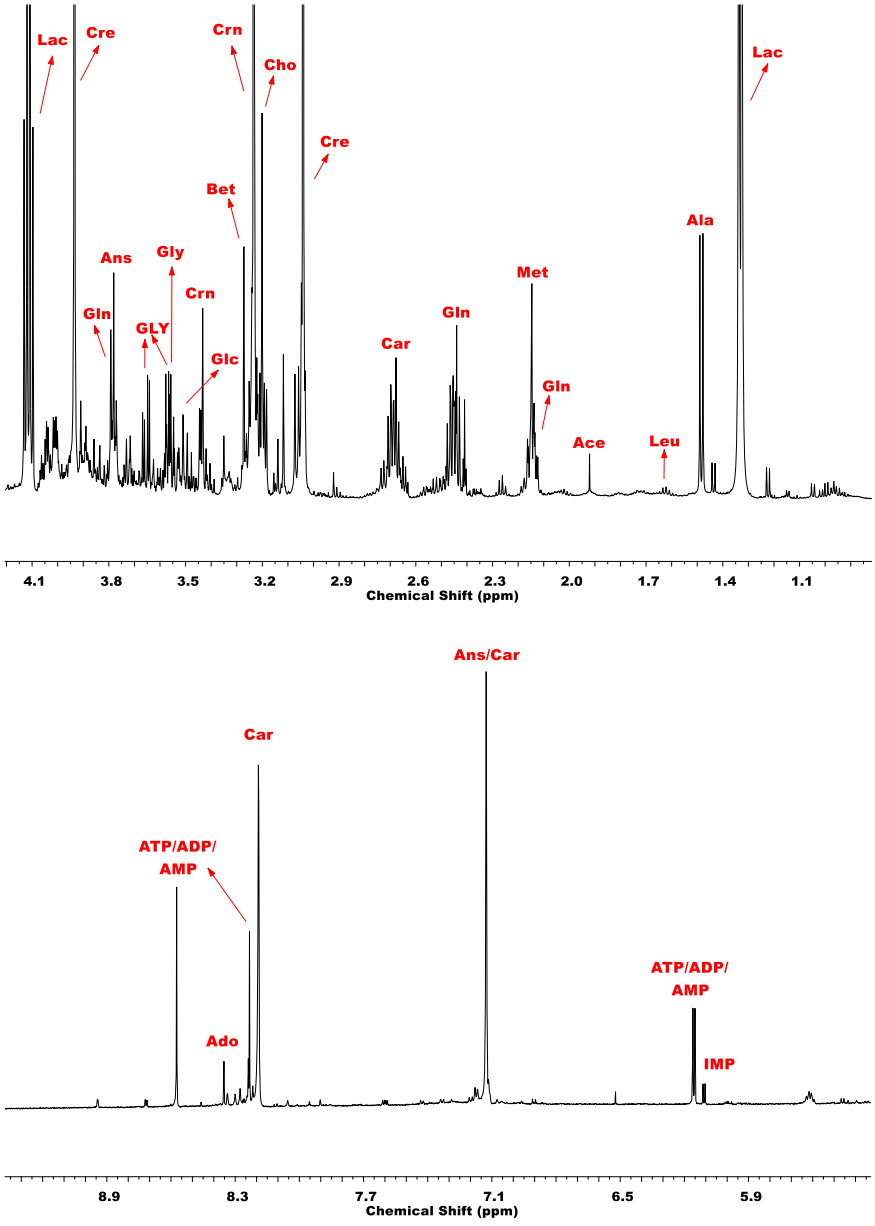

Fig. 1. Typical 500 MHz ^{1}H–RMN spectra recorded for the metanol/water meat extract. Assignments: Ace (acetate); Ado (adenosine); Ala (alanine); Ans (anserine); Bet (betaine); Car (carnosine); Cho (choline); Crn (carnitine); Cre (creatine) Gln (glutamine); Gly (glycine); GLY (glycerol); Glc (glucose); Ile (isoleucine); Lac (lactate); Leu (leucine); Met (methionine); Val (valine).

identified phenolic compounds, respectively. In order to verifying the bioavailability and uptake of these phenolic compounds by the animals, we have performed the analysis of these compounds in meat extracts, however, none of the compounds identified in the mate extract or their known bacterial metabolites (Gonthier, Verny, Besson, Rémésy, & Scalbert, 2003) like ferulic acid, coumaric acid, phenylpropionic and benzoic acid could be detected in meat at a detection limit of 200 nmol L^{-1}, which clearly suggests that the site of action of the phenolic compounds is the rumen.

3.2. Meat metabolomics

In the untargeted polar metabolites screening of meat extract by ^{1}HNMR spectroscopy, approximately 30 compounds were identified in the meat extracts. Seventeen compounds of relevance for meat quality and significantly influenced by the feeding treatment were selected as targeting compounds for a quantitative analysis by a combination of ^{1}H NMR and high-resolution accurate mass spectrometry.

3.2.1. ^{1}H NMR metabolite untargeted sample screening: Principal components analyses (PCA) of the full ^{1}H NMR spectra

In order to find the most suitable clustering representation of the ^{1}H NMR spectra of beef extracts from animals fed different levels of mate extract, we have performed a standard principal component analysis (PCA) on the complete data set. We have identified three significant PC's in our data set, which together explain 96% of the total variance (PC1 85%, PC2 7%, and PC3 4%). Fig. S1 (A, B and C) at supporting information shows the clustering and trends of beef extracts from animals fed different levels of mate extract. The samples of beef extracts from animal feed without mate extract show a higher variability for PCA results; however, these samples trend to stay in the positive-value region of both PC1 (Figs. S1A and S1B) and PC2 (Fig. S1C). The loadings plot (Fig. S1D and S1E) suggests that the signals of anserine, carnosine, lactate, and carnitine provided a high variability for these samples. The samples of beef extracts from cattle fed with mate extract show trends towards small clusters. The samples with 1.0% of mate extract added to feed formed a small tight cluster in the positive-values region of the PC1 and PC2, and negative-values of the PC3 (almost in the midpoint of the PCA model). The signals of anserine, carnitine, carnosine, lactate, creatine, glycerol, and IMP were identified for loadings plot (Figs. S1D, S1E, and S1F) as responsible for this small tight cluster. Unlike samples from beef from feeding with 1.0% of mate extract, the samples from feeding with 0.5% and 1.5% of mate extract demonstrated a higher variability over the scores plot in Figs. S1A and S1B. However, the samples from meat from feeding with 1.5% of mate extract grouped more loosely in the positive-values region of PC3 almost in the center of PCA model (Fig. S1C). The signals for the metabolites creatine, carnitine, lactate, carnosine, glycerol, anserine, and IMP were responsible for the main information of the samples into the both groups (0.5% and 1.5% of mate extract). Thus, following analysis of the information from^{1}H NMR spectra as an initial data screening, we performed a quantification of the individual metabolites for each beef sample (Fig. S1).

3.2.2. Quantitative metabolite determination by ^{1}H NMR and high-resolution accurate mass spectrometry

Based on ^{1}H NMR spectra for meat extracts as the spectrum shown in Fig. 1 for the 0.5% mate extract-feeding group, 19 metabolites could be quantified using quantitative ^{1}H NMR analysis. The compounds are listed in Table 2 together with the median concentration in all four-treatment groups for cattle feeding. The concentrations were estimated for meat extracts and corrected by the method previously described (Esteve, Martínez-Granados, & Martínez-Bisbal, 2014; Grossi, do Nascimento, Cardoso, & Skibsted, 2014; Want et al., 2013) and are presented in Table 2.

Considering that overlapping peak regions in the NMR spectrum gave high variance values for concentration of some compounds, only nineteen metabolites were quantified by the Chenomx NMR suite 7.7 software library. Moreover, peaks in the aromatic region had variations of chemical shift due to slightly differences in pH and ionic strength complicating quantification (Graham et al., 2010). In this case, assignments were based on signal shape and multiplicity assuming a chemical shift variation allowed by Chenomx library and, also, by comparison with other data base sources and references (Graham et al., 2010; Straadt, Aaslyng, & Bertram, 2014) and by 2D homonuclear and heteronuclear experiments (^{1}H-^{1}H TOCSY and 2D ^{1}H-^{13}C HSQC). For some of these compounds, high-resolution accurate mass spectrometry was used for quantification and the results are shown in Table 3.

Table 3 displays the mean concentration of polar metabolites found in meat extract as determined by ESI-HRMS analysis. > 30 metabolites could be identified using high-resolution accurate mass spectrometry targeted analysis with electrospray ionization (ESI) or with atmospheric pressure chemical ionization (APCI). Table 3 lists only the metabolites not detected by ^{1}H NMR or which had high variance of the concentration values based on replicate ^{1}H NMR analysis. From the 15 polar metabolites identified and quantified by MS, only succinate, glutamine, creatinine, lysine, and arginine show significant variation difference among treatments ($p < 0.05$) for extracts of meat from control animals and animals fed with mate extracts. The metabolites proline, asparte, glutamate, choline, serine, inosine, citrate, glutathione, phenylalanine, and hypoxanthine shows standard deviations for feeding group greater than the variability of the variable values among meat extract from animals fed different diets.

Despite the fact that no significant changes were observed in the ^{1}H NMR qualitative spectral profiling of samples from animals fed different diets, important differences in the metabolite concentrations among the animal treatments are observed. Among the 19 polar metabolites quantified by ^{1}H NMR, Table 2, only the concentrations of isoleucine, alanine, creatine, glycine, carnosine, carnitine, NADH, IMP and ATP/ADP/AMP differ significantly ($p < 0.05$) among treatments and all increased with mate extract supplementation at some level. The highest metabolite levels were in most cases achieved for the supplementation with 1.0% or 1.5% of mate extract in the feed. Clearly supplementation with mate extracts to the feed changes the animal metabolism as can be seen by quantitative MS and ^{1}H NMR data.

Notably, concentration of carnosine and NADH, both endogenous antioxidants, was higher in the samples from animals fed with 1.0 or 1.5% of mate extract compared to other treatments. Increased variations in the concentration of these two metabolites could be related to β-alanine metabolism (Mei, Cromwell, Crum, & Decker, 1998). However, as shown in Table 2, dietary supplementation with mate extract did not to affect significantly the concentration of β-alanine in meat extracts of meat from animals supplement with different levels of mate extract.

The diet with 1.5% of mate extract also enhanced the production of IMP in the muscle. This compound has a wide range of functions in the organism (Arihara, 2006; Djenane, Martínez, Sánchez-Escalante, Beltrán, & Roncalés, 2004; Rabie & Szilágyi, 1998) and has been in focus as attractive meat-based bioactive compounds as also well as anserine, carnosine, creatine, and leucine in muscle tissue manipulated by animal diet (Arihara, 2006).

The metabolites L-carnitine and betaine are involved in the fatty acid metabolism related to energy production in the muscle (Hoppel, 2003; Rabie & Szilágyi, 1998; Zhan, Li, Xu, & Zhao, 2006). High levels of L-carnitine together with a high level of glycerol for animals supplemented with mate extract in comparison with control fed suggest an increasing lipolysis and triglyceride oxidation to provide energy for biosynthetic pathways like protein synthesis (Fritz & McEwen, 1959; Hoppel, 2003; Rasmussen & Wolfe, 1999; Straadt et al., 2014; Trabi, Keller, & Jonsson, 2013). At the same time, a higher concentration of the branched-chain amino acid leucine was found for animals submitted to diets supplemented with mate extract (Table 2). Leucine has a fundamental role for the initiation of anabolic processes in the muscle including myofibrillar protein synthesis (Churchward-Venne et al., 2012). Myofibrillar proteins have been recognized to be involved in protection of muscles against oxidation (Frederiksen, Lund, Andersen, & Skibsted, 2008) and are related to meat tenderness (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002; Lund et al., 2011; Morzel et al., 2006). Mate extract addition to animal feed may accordingly induce a reduction in the intramuscular fat content and an increase in the protein synthesis leading to the production of more lean beef.

Table 2
Average concentration of polar metabolites in beef (n = 3 per feeding treatment, μmol·g^{-1} meat) as obtained by quantitative ^{1}H-NMR of the methanol/water extract of meat from animals fed with different levels of mate extract.

	Mate extract supplementation (%)				
	None	0.5	1.0	1.5	p-value
Leucine	0.183 ± 0.032	0.197 ± 0.006	0.230 ± 0.010	0.233 ± 0.01	0.109
Isoleucine	0.118 ± 0.003^{b}	0.128 ± 0.002^{b}	0.166 ± 0.011^{a}	0.143 ± 0.011^{ab}	0.002
Valine	0.166 ± 0.030	0.184 ± 0.006	0.201 ± 0.024	0.133 ± 0.01	0.085
Lactate	36.9 ± 3.2	40.2 ± 3.5	52.4 ± 2.3	49.2 ± 1.6	0.071
β-alanine	0.217 ± 0.04	0.231 ± 0.05	0.209 ± 0.14	0.298 ± 0.05	0.251
Alanine	1.52 ± 0.35^{b}	1.93 ± 0.06^{ab}	2.37 ± 0.12^{a}	2.14 ± 0.17^{ab}	0.034
Methionine	0.66 ± 0.16	0.68 ± 0.12	0.91 ± 0.045	0.90 ± 0.20	0.283
Creatine	5.70 ± 1.4^{c}	8.48 ± 0.4^{bc}	20.3 ± 1.98^{a}	10.2 ± 0.3^{b}	< 0.0001
Glycerol	1.74 ± 0.42	2.11 ± 0.22	2.47 ± 0.47	2.36 ± 0.07	0.452
Glycine	0.666 ± 0.037^{b}	0.688 ± 0.076^{b}	0.794 ± 0.085^{ab}	0.941 ± 0.09^{a}	0.047
Carnosine	5.82 ± 0.56^{b}	7.99 ± 0.57^{ab}	9.06 ± 1.00^{a}	9.08 ± 0.83^{a}	0.013
L-carnitine	0.629 ± 0.119^{ab}	0.516 ± 0.071^{bc}	0.383 ± 0.021^{c}	0.736 ± 0.040^{a}	0.007
Betaine	0.51 ± 0.051	0.57 ± 0.089	0.51 ± 0.021	0.70 ± 0.08	0.078
Anserine	0.626 ± 0.061	0.89 ± 0.074	0.772 ± 0.141	0.879 ± 0.04	0.113
Glucose-6-phosphate	1.58 ± 0.97	2.39 ± 0.26	2.76 ± 0.63	2.50 ± 0.46	0.417
NADH	0.25 ± 0.073^{b}	0.32 ± 0.02^{b}	0.52 ± 0.02^{a}	0.49 ± 0.06^{a}	0.002
Adenosine	0.411 ± 0.091	0.288 ± 0.035	0.326 ± 0.018	0.367 ± 0.03	0.236
IMP	1.29 ± 0.13^{b}	1.87 ± 0.30^{ab}	2.03 ± 0.36^{ab}	2.30 ± 0.14^{a}	0.041
ATP/ADP/AMP	0.335 ± 0.067^{c}	1.56 ± 0.11^{ab}	0.921 ± 0.105^{bc}	1.84 ± 0.41^{a}	0.001

^{a,b,} Means in the same row with different superscripts are significantly different (p < 0.05).

Table 3
Mean concentration of the polar metabolites in beef (n = 12 per feed treatment, μmol·g^{-1} meat) as obtained by ESI-HRMS of the methanol/water extract of meat from animals fed with different levels of mate extract.

	Mate extract supplementation (%)				
	None	0.5	1.0	1.5	p-value
Proline	0.033 ± 0.003	0.032 ± 0.002	0.031 ± 0.003	0.030 ± 0.003	0.078
Succinate	0.075 ± 0.005	0.110 ± 0.008	0.152 ± 0.006	0.117 ± 0.005	$3.9\ 10^{-4}$
Aspartate	0.012 ± 0.002	0.010 ± 0.001	0.007 ± 0.002	0.009 ± 0.002	0.559
Glutamine	0.965 ± 0.014	1.125 ± 0.018	0.818 ± 0.019	0.894 ± 0.011	0.012
Glutamate	0.084 ± 0.006	0.096 ± 0.006	0.084 ± 0.003	0.101 ± 0.007	0.844
Creatinine	0.146 ± 0.002	0.144 ± 0.001	0.146 ± 0.002	0.155 ± 0.002	$4.2\ 10^{-4}$
Choline	0.094 ± 0.002	0.084 ± 0.001	0.070 ± 0.001	0.073 ± 0.001	0.457
Serine	0.161 ± 0.009	0.176 ± 0.008	0.159 ± 0.004	0.170 ± 0.003	0.136
Lysine	0.046 ± 0.001	0.045 ± 0.004	0.037 ± 0.002	0.039 ± 0.001	0.016
Inosine	0.973 ± 0.014	0.912 ± 0.018	0.942 ± 0.013	0.982 ± 0.012	0.276
Arginine	0.053 ± 0.001	0.059 ± 0.001	0.045 ± 0.001	0.050 ± 0.001	0.022
Citrate	0.047 ± 0.003	0.050 ± 0.005	0.028 ± 0.003	0.046 ± 0.003	0.507
Glutathione	0.624 ± 0.015	0.660 ± 0.017	0.578 ± 0.011	0.595 ± 0.015	0.939
Phenylalanine	0.036 ± 0.005	0.038 ± 0.005	0.030 ± 0.004	0.036 ± 0.005	0.703
Hypoxantine	0.210 ± 0.003	0.198 ± 0.003	0.189 ± 0.002	0.197 ± 0.002	0.918

Higher levels of IMP as mate extract diet supplementation in the 1.5% concentration suggest a slow *post mortem* metabolism and may infer that these animals were less stressed (Straadt et al., 2014). An improvement of meat quality may be achieved by decreasing the formation of inosine through dephosphorylating of IMP, since this process has been shown to be associated with a high susceptibility to stress (Straadt et al., 2014). Furthermore, IMP is well known as a non-volatile flavor enhancer in meat and it is desirable to keep its concentration high by preventing its degradation during meat processing (Fuke & Konosu, 1991; Tikk et al., 2006).

In order to follow the development of eating quality of meat, ^{1}H NMR was used to determine the metabolites considered most important during meat ageing with special focus on proteolysis. Several chemical, physical and biological changes take place in ageing and influence most of the components of meat. For example, structural proteins of muscle fibers are degraded to smaller peptides or amino acids by enzymatic proteolysis (Feidt, Petit, Bruas-Reignier, & Brun-Bellut, 1996; Graham et al., 2010; Grossi et al., 2014; Paredi, Raboni, Bendixen, de Almeida, & Mozzarelli, 2012). Animal metabolism prior to slaughtering also affects the development of *post mortem* changes. Thus, the metabolic changes processed after 14 days of ageing were investigated in order to determine the effect of diet in the *post mortem* metabolism (Fig. S2 supporting information—metabolite concentration changes after 14 days of storage at 4 °C). As expected an increase in the level of amino acid and amino acids derivatives after 14 days of ageing was observed. This proteolysis is most advanced in the samples representing the group fed 1.5% of mate extract and aged for 14 days as seen by the highest concentration of amino acids. For these group, also important metabolites as carnosine, carnitine, creatine and glycerol had shown to increase in concentrations after ageing of meat.

For all of the feeding-treatments, the levels of NADH, IMP, and ATP/ADP/AMP seem to deplete during the ageing period. Concerning the nucleotide metabolites, several degradation pathways have been found to be enhanced during *post mortem* changes. IMP could during such ageing be converted to inosine and hypoxantine leading to undesirable development of bitter taste (Dannert & Pearson, 1967; Jones, 1969; Straadt et al., 2014; Terasaki, Kajikawa, Fujita, & Ishii, 1965). Depletion of ATP/ADP/AMP after 14 days of ageing has been shown to occur to maintain the enzymatic functionality of the muscle under anaerobic conditions (Esteve et al., 2014; Graham et al., 2010). The observed

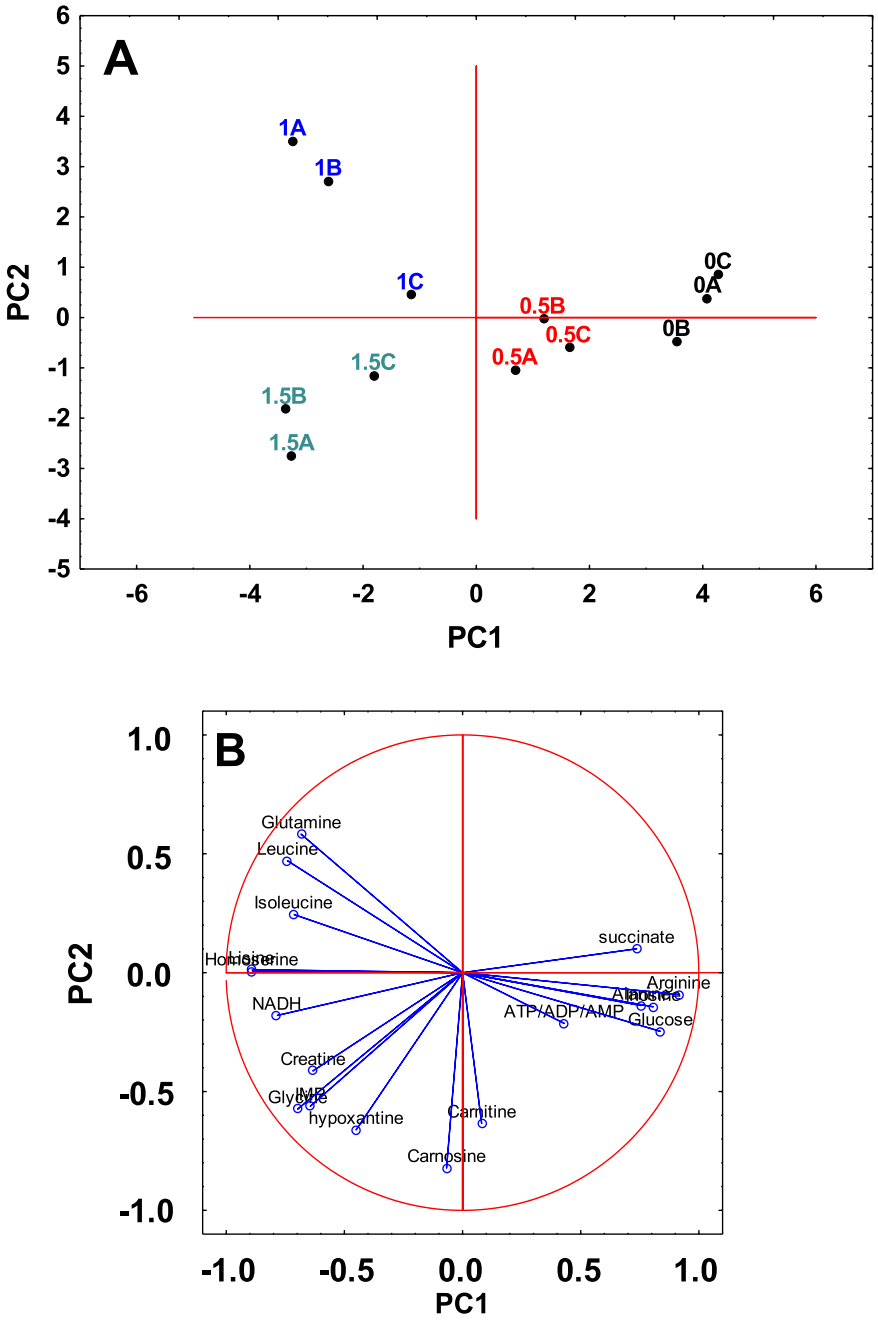

Fig. 2. Principal Component Analysis (PCA) of the quantitative metabolite profile for meat from animals fed with different levels of mate extract. A color-coded number according to mate extract level in the diet represents each meat sample and a letter was used to differentiate animals with the same treatment. Metabolite concentrations as determined by ^{1}H-NMR and ESI-HRMS were selected as variables. A: Two-dimensional plot of samples using the principal components one and two (PC1xPC2) capturing most of the variance in the original dataset (total variance 66.07%). B: Loading plots for PC-variables correlations.

decrease in NADH concentration is expected, since other antioxidants may be protecting the muscle against oxidation instead of participating in regeneration of NADH through redox cycling.

3.3. Principal component analysis of targeted metabolites

In order to observe patterns in the development of metabolites for the four feeding groups, principal component analysis (PCA) was performed for the quantitative ^{1}H NMR and ESI-MS polar metabolite profile of beef from animals fed different levels of mate extract. Variables were pre-selected based on the variability of the metabolites mean values among sample groups (ANOVA, $p = 0.05$). PCA scores plot and loading plots (PC1xPC2) for the beef extracts using targeted profiling are represented in Fig. 2. The variance between sample groups was explained 48.8% by principal component one (PC1) and 17.5% by principal component two (PC2) providing a good discrimination among the different feeding treatments. The polar metabolites accounting for the discrimination among animal feeding treatment in each principal component are described in Fig. 1B. PC1 further differentiates between animals fed higher levels of mate extract (1.0% and 1.5%) from control animals and animals fed with the lowest level of mate extract (0.5%). Meat from animals fed with higher levels of mate extract was associated with high levels of glutamine, leucine, isoleucine, homoserine, lysine, NADH, creatine, glycine, IMP, hypoxanthine, and carnosine. PC2 slightly distinguished the samples from animals fed 1.0% and 1.5% of mate extract. Animals fed with 1.0% of mate extract characterized by higher levels of glutamine, leucine, and isoleucine and animals fed with 1.5% of mate extract by higher levels of NADH, creatine, glycine, IMP, hypoxanthine, carnosine, and carnitine. Samples from animals fed control feed 0.5% and 1.0% of mate extract were closely clustered in the scores plot along the positive part of PC1-axis that is characterized by a high content of succinate, arginine, alanine, inosine, glucose, and ATP/ADP/AMP in comparison to samples from animals fed 1.0 and 1.5% of mate extract.

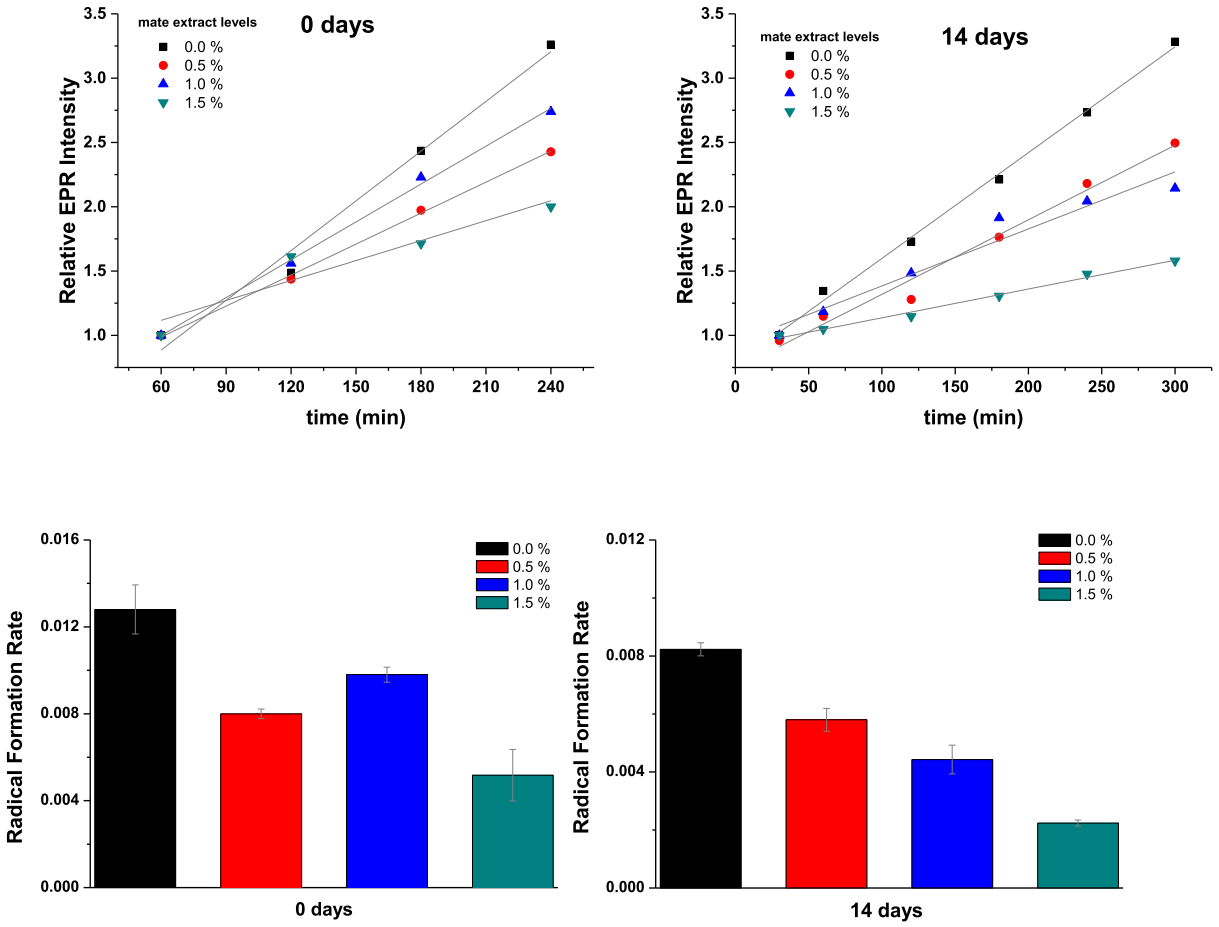

Fig. 3. Radical formation as measured by the relative intensity of PBN radical adduct by electron paramagnetic resonance (EPR) in slurry incubated at 65 °C made from fresh meat and in meat stored at 5 °C for 14 days, PBN concentration of 400 mM. A: EPR intensity was calculated by double integration of EPR signal and expressed as the ratio $Area_{T = xmin}/Area_{T = 0}$. Rates for radical formation were determined by linear regression of relative EPR intensity plots and shown in B for each set of condition.

3.4. EPR spin-trapping and redox stability of beef

Radical formation is an early event in oxidative degradation of proteins and lipids in muscle and in meat, and the tendency of this phenomenon may be used to predict oxidative stability of meat products. The oxidative stability of beef was accordingly determined using the spin trapping technique by monitoring the development of radicals in meat slurries subjected to incubation at 65 °C in the presence of PBN as spin trap. PBN adduct radicals are formed, when short-lived reactive radicals from the components of beef like lipid radicals or protein radicals produced during heating or by metal catalysis are adding to a spin trap double bonds like in PBN to produce a more stable adduct radical (Polovka, 2006). The X-band EPR spectra of meat slurries displayed the characteristic six-line pattern of PBN-adduct radicals (Fig. S3 – supporting information) and the signal was double integrated in order to obtain the area referred to as EPR intensity, which is proportional to radical concentration. The kinetics of the formation of radicals in beef from different animal feeding treatment was accordingly based on the EPR intensity.

Fig. 3 shows the development of PBN radical adducts in meat slurries during 4–5 h of incubation at 65 °C. For fresh meat and for 14-days aged meat, the radical formation could be described as a zero order reaction. Linear regression of relative EPR intensity as function of incubation time was used to determine the rate of radical formation. For fresh meat samples, the formation of radicals in meat slurries was faster than for meat aged for 14 days. For fresh meat an induction period or so-called lag phase of 60 min was seen prior to the development of radicals at a constant rate during the thermal incubation. For 14 days aged meat, the lag phase was shorter and approximately of 40 min. After approximately 4 h for aged meat and after approximately 5 h for fresh meat of incubation, the level of radicals became constant, which may be described as a steady state, where radical consumption reactions exactly balance the reactions forming radicals. The period with constant rate of radical formation between the end of the induction period and the steady state period was used to characterized the oxidative stability of meat (Carlsen et al., 2005; Churchward-Venne et al., 2012; Frederiksen et al., 2008) (Fig. 3). During the incubation at 65 °C, iron is released from the heme porphyrin, thus enhancing the oxidation and initiating the formation of radicals (Kingston, Monahan, Buckley, & Lynch, 1998; Lee, Hendricks, & Cornforth, 1998). However, some metabolites like peptides and proteins may slow down or prevent the radical formation since they as antioxidants promptly scavenge radicals (Decker & Crum, 1993; Saiga, Tanabe, & Nishimura, 2003). The high concentration detected for several metabolites capable of radicals scavenging in meat when using 1.5% of mate extract supplementation in the feed may explain the improved redox stability as compared to the other treatments. The histidine dipeptides, anserine and carnosine, which were found to increase in the meat from animals supplemented with mate extract have thus previously been found effective as radical scavengers and inhibitor of oxidation processes (Decker & Crum, 1993; Djenane et al., 2004; Wu, Shiau, Chen, & Chiou, 2003). Besides the reducing power and radical scavenging ability of these two metabolites (Kohen, Yamamoto, Cundy, & Ames, 1988), their chelating ability towards metal ions may also contribute to the protection against oxidation (Wu et al., 2003). Carnosine may thus act as antioxidant both through scavenging radicals and by metal binding, and we suggest that

Table 4
Sensory descriptive analysis of fresh meat from animals fed different levels of mate extract.

Attributes[1]	Mate extract supplementation (%)				SEM	*p*-value
	none	0.5	1.0	1.5		
Characteristic beef aroma	6.5[b]	6.0[ab]	6.0[ab]	5.9[a]	0.26	0.049
Strange aroma	8.0	7.8	7.9	7.8	0.29	0.913
Characteristic beef flavor	6.1	5.9	5.8	5.5	0.26	0.146
Strange flavor (off-flavor)	7.6	7.6	7.7	7.6	0.31	0.959
Tenderness	4.0[a]	5.0[b]	4.4[ab]	4.6[ab]	0.31	0.020
Juiciness	5.0	5.2	5.4	5.1	0.27	0.365

[a,b] Mean values in the same row with different superscripts are significantly different ($p < 0.05$; SEM, standard error for the mean value).

[1] Beef characteristic aroma (1 = extremely bland; 9 = extremely intense); beef characteristic flavor (1 = extremely bland; 9 = extremely intense); strange aroma (1 = extremely intense; 9 = none);/strange aroma (1 = extremely intense; 9 = none); tenderness (1 = extremely tough; 9 = extremely tender) and juiciness (1 = extremely dry; 9 = extremely juicy)

this metabolite gave a major contribution to the oxidative stability of beef from animals supplemented with mate extract (Wu et al., 2003).

Ageing of beef seems to increase the peptide and amino acid content through protein hydrolysis. The lowest radical formation rate for 14-days ageing was found for the meat samples from animals fed with the highest content of mate extract in agreement with higher concentration of radical scavenging metabolites in these samples including the histidine derived peptides and redox active amino acids.

3.5. Relative intramuscular fat and CLA content

The relative intramuscular fat content (IMF) for fresh meat from animals fed with different levels of mate extract in the diet has been determined by low-field NMR using a continuous wave-free precession pulse sequence (CWFP). Obtained IMF values (given by the M_c/M_o ratio) were 0.04 ± 0.02, 0.04 ± 0.01, 0.05 ± 0.02, and 0.02 ± 0.01 for the feed supplementation with 0, 0.5, 1.0, and 1.5% (*w*/w) of mate extract, respectively. The M_c/M_o values were reported to show a positive correlation with the intramuscular fat content and capable of estimate IMF with a precision of 0.5% (Corrêa et al., 2008). From the M_c/M_o values found for beef samples from animals fed with different levels of mate extract in the finishing diet, a large variance of the data is found, as expected, for the IMF of beef from animals in the same feeding regime ($n = 12$, analysis in triplicate). Interestingly, beef samples from animals supplemented with the highest content of mate extract in the diet show a significant decrease in the IMF compared to the other treatments, which is in good agreement with the elevated levels of betaine, L-carnitine, and leucine in beef from animals supplemented with 1.5% of mate extract supporting an increase in the fatty acid metabolism to generate energy for protein synthesis leading to lean meat.

Conjugated linoleic acid (CLA), a bio converted fatty acid found in ruminant products, has received considerable attention from nutritionists due to its health benefits associated with a decrease in animal body fatty and its anticarcinogenic, antioxidant, and anticholesteremic properties (Hayek et al., 1999; Heuvel & Vanden, 1999; Nicolosi, Rogers, Kritchevsky, Scimeca, & Huth, 1996). CLA is known to be formed during ruminal bio hydrogenation especially by *Butyvibrio* spp. bacteria and deposited in the animal tissues (Patra & Saxena, 2011). Tannins and phenolic compounds are suggested to modulate rumen bio hydrogenation by selective inhibition of some bacteria such as *Fusocillus* spp. and *Clostridium proteoclasticum* that transforms vaccenic acid into stearic acid, thus providing more vaccenic acid to be converted to CLA in the animal tissue (Patra & Saxena, 2011). In this study, the increase in the mate extract concentration in the animal feed led to an increase in the CLA concentration. For animals that did not receive the supplementation (control), CLA concentration in meat was of 0.07 ± 0.01 $\mu mol \cdot g^{-1}$ and in the range of values found in the literature. For animals fed with 0.5, 1.0, and 1.5% mate extract, obtained CLA concentration were 0.08 ± 0.01, 0.09 ± 0.01, and 0.12 ± 0.01 $\mu mol \cdot g^{-1}$, respectively (CLA concentration obtained by the analysis of meat from 12 animals of each feed treatment with triplicate injection into the LC-MS system). From the quantitative analysis for CLA in fresh meat, it is clear that addition of mate extract as a supplement to the animal feed modulates the production of CLA and vaccenic acid in the rumen and lead to a CLA enriched beef production for animals fed with mate extract in comparison to control feeding. For beef from animals supplemented with 1.5% of mate extract an increase of about 60% in the intramuscular CLA content is observed, despite a reduced IMF compared to animals fed without mate extract.

3.6. Meat quality parameters

Significant differences among diets as isolated effects were found for meat quality parameters ($p < 0.05$) and as 14-day aged beef was analyzed, there were no interactions between these two factors. Different diets affected luminosity (L*), and shear force as shown in Table S2.

Animals fed with 1% *w*/w of mate extract had the higher luminosity (L*) compared to the other samples. Values for this parameter ranged from 36.54 to 45.11, which are in agreement with average values found for beef (Muchenje et al., 2009). Animals used were castrated and the meat is expected to show higher brightness compared to non-castrated animals due to a higher amount of intramuscular fat (Rodrigues & de Andrade, 2004). Meat from animals fed with the control diet showed higher values for shear force as a measurement of tenderness. Animals fed with 1.0% extract of mate showed the lowest shear force values compared to animals which were fed with the control diet and can be related to the higher content of carnosine, shown by metabolomics study, in this treatment. Carnosine is a natural antioxidant, which can prevent protein oxidation and consequently result in tender meat.

3.7. Sensory descriptive analysis and acceptance

Results from sensory descriptive analysis of fresh beef from animals fed different mate concentrations are shown in Table 4. Characteristic beef aroma and tenderness attributes were affected by the animal diet. The control sample showed the highest value ($p < 0.05$) of characteristic beef aroma, but was only different ($p < 0.05$) from 1.5% treatment. Although 1.5% beef was considered the one with the least characteristic beef aroma, it not seem so relevant, as strange aroma, strange flavor and characteristic beef flavor did not show significant difference ($p > 0.05$), indicating that supplementation with mate in the diet did not affect these attributes. The addition of mate extract to the animal diet is thus concluded to affect the beef tenderness positively, with the control sample being less tender, especially compared to the beef from animals fed with mate extract at 0.5% in the diet.

Table S3 contains the sensory acceptance results. Flavor acceptance was not affected by animal feeding with mate extract. However, regarding to texture (tenderness) and overall acceptance, the beef from animals supplemented with mate extract was found more accepted probably due to its better tenderness, which was observed in the descriptive analysis and also in shear force values. The use of mate extract as an antioxidant in meat has been shown to limit lipid and protein oxidation. Protein oxidation as the cross-linking of myofibrillar proteins may affect tenderness (Huff-Lonergan & Lonergan, 2005; Lund et al., 2011) and bioactive compounds from mate extract or their ruminal metabolites may change the metabolic profile of the muscle tissue improving its redox status and preventing protein oxidation, in effect increasing the meat tenderness. These results are in line with the presence

of metabolites which are related to prevent oxidation such as carnosine, an endogenous antioxidant, which had its concentration increased in beef in animals fed mate supplementation, as stated before.

4. Conclusion

The addition of mate extract, at varying levels from 0.5 to 1.5% *w/w*, in the diet of feedlot cattle did not affect animal performance and carcass characteristics, but these animals presented more tender beef, which was well-received by consumers. Beyond this, beef from animals fed mate supplemented diet was shown to be healthy, with good CLA, creatine and histidine levels and had increased oxidative stability, also showing higher endogenous antioxidants such as carnitine and carnosine. It may be further speculated that this beef, with increased oxidative stability, will also have a lower tendency to induce radical formation in the gastrointestinal tract during digestion following meat consumption, thus in effect lowering the risk of colorectal cancer.

For practical application in beef production these conclusions should, however, be considered together with production costs related to the change in feed by adding mate extract to increase oxidative stability and to improve beef quality.

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Appendix A. Supporting information

Untargeted principal component analysis for full ^{1}H NMR spectra for meat extracts from animals fed different levels of mate extract. Metabolite concentration changes during meat ageing, EPR spectra of meat slurry added of PBN recorded for different period of incubation at 65 °C, concentration of the major phenolic compounds in the mate extract, meat quality parameters for meat from animals fed with different levels of mate extract, and sensory acceptance of meat from animals fed with mate extract and control animals. Supplementary data associated with this article can be found in the online version, at doi: http://dx.doi.org/10.1016/j.foodres.2017.05.033.

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