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Effects of aging and dietary supplementation with polyphenols from *Pinus taeda* hydrolysed lignin on quality parameters, fatty acid profile and oxidative stability of beef

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Abstract

Context. The inclusion of *Pinus taeda* hydrolysed lignin (PTHL) in beef diets could improve quality and stability of meat, but effects could vary through the aging period (AP).

Aim. The aim was to evaluate the effects of the PTHL inclusion in the diet of finishing beef cattle on meat quality, fatty acid composition and oxidative stability at Days 1, 8, 11 and 15 of aging.

Methods. Forty Limousin bulls (340 ± 42 kg) were fed *ad libitum* on a total mixed ration (TMR). The control group received exclusively TMR for 120 days, while the experimental group received the same TMR as the control group but supplemented with PTHL (Oxifenol, I-Green, Padua, Italy; 35 g/day per head at 1–90 days and 70 g/day per head at 91–120 days).

Key results. Diet did not influence the chemical composition, pH, cooking loss, Warner–Bratzler shear force and hydroperoxide content. The Warner–Bratzler shear force ($P < 0.001$) decreased, while lightness ($P < 0.01$) and hydroperoxides ($P < 0.001$) increased through the aging period. Thiobarbituric acid reactive substances were similar for both diets at 1 and 15 days. However, thiobarbituric acid reactive substances at 8 and 11 days were higher for control than for the PTHL diet ($P = 0.023$ for interaction). Protein carbonyls were higher for control than for the PTHL diet at 8 days ($P = 0.003$ for interaction), but similar for both diets for the other dates. Saturated, monounsaturated and polyunsaturated fatty acids varied through the AP with PTHL diet, while no changes were observed with control diet ($P < 0.01$ for interactions). At 11 days, the n-6 : n-3 ratio passed from being the minimum value with the PTHL diet to be the maximum with control diet ($P < 0.01$ for interaction).

Conclusions. The effects of PTHL inclusion in bull finishing diets depends on the AP but, generally, may result in beef with meat with beneficial effects on human health.

Implications. Including PTHL in the diet of finishing bulls can be useful to improve meat quality, favouring the use of natural waste substances deriving from vegetal production.

Additional keywords: aging time, antioxidants, chemical composition, oxidation, tenderness.

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Introduction

Natural antioxidants obtained from leaves, seeds or flowers of plants contain bioactive substances such as phenolic compounds (e.g. gallic acid, carnosic acid, caffeic acid and rosmarinic acid), flavonoids (e.g. catechin, quercetin, apigenin, kaempferol, naringenin and hesperetin) and volatile oils (e.g. eugenol, carvacrol, thymol, menthol; Lorenzo *et al*. 2018*a*, 2018*b*). These substances have demonstrated several positive effects on human health status (Huang 2018) and are positively perceived by consumers, because they are recognised as natural and not synthetic chemical-additive compounds. Recently, the use of these substances has increased in meat production, using the following two main strategies: (1) as a dietary supplement in meat-producing animals (as in the current study) and (2) by the direct application on meat and meat products. The use of antioxidants as dietary supplements influences animal metabolism, oxidative balance and immune system functionality (Brambilla *et al*. 2008), increasing, finally, overall animal welfare and reducing the use of antimicrobial substances (Baptista *et al*. 2018). Moreover, the use of natural antioxidants as feed additives improves productivity and feed efficiency due to their anti-inflammatory and antimicrobial activities and through a decrease in ruminal bio-hydrogenation (Falowo *et al*. 2018).

Several experiments have been conducted to study effects of diets containing antioxidants on quality (Karami *et al*. 2011; Cimmino *et al*. 2018; Modzelewska-Kapituła *et al*. 2018; Zhao *et al*. 2018), fatty acid (FA) profile (Mele *et al*. 2007; Vasta *et al*. 2007, 2009; Muíño *et al*. 2014) and oxidative stability (O'Grady *et al*. 2006*a*, 2006*b*; Luciano *et al*. 2009; Jerónimo *et al*. 2012; Muíño *et al*. 2014) of meat from ruminants. In general, the supplementation of diets with natural antioxidants is considered a good strategy for modifying the FA profile of meat in response to consumer demands (Cimmino *et al*. 2018). In addition, the inclusion of natural antioxidants delays the lipid oxidation of meat (Lorenzo *et al*. 2018*c*), improving, in consequence, its shelf-life. However, results obtained using diets containing antioxidants are contradictory because some authors have not found benefits on the FA profile (Muíño *et al*. 2014) or on oxidative stability of meat (O'Grady *et al*. 2006*a*, 2006*b*; Luciano *et al*. 2009; Muíño *et al*. 2014). The cause of differences among authors are unknown but might be related to (1) the effects of antioxidants in the diets possibly varying through the aging period (AP; Luciano *et al*. 2009; Guerra-Rivas *et al*. 2016) and (2) the high variability of the vegetal origin of bioactive compounds and, consequently, of their chemical composition. Polyphenolic substances are widely represented in vegetal by-products. The main substance of polyphenolic origin constituting vegetal biomass of agricultural by-products is lignin, a structural biopolymer substance in plant cell walls. The chemical structure of lignin is highly irregular and extremely challenging and its physical and chemical properties are highly dependent on the plant species, botanic region and the isolation processes. Consequently, to study the effects of antioxidant inclusion in the diets of ruminants requires specific research for each particular case. Moreover, the interactions between antioxidants and rumen physiology and microbiome are not well known.

The *Pinus taeda*, commonly known as loblolly pine, is one of several native pines on south-eastern United States and is the second-most common species of tree in United States, after red maple. In the current study, *Pinus taeda* hydrolised lignin (PTHL) was included in the finishing diets for bulls. Its use has been recommended to reduce oxidative stress, thereby improving immune system activity and metabolism in farm animals including cattle. However, its effects on meat quality, FA composition, lipid and protein oxidation and shelf life of meat through the AP have not been studied in detail. Thus, the aim of the present study was to test the hypothesis that the dietary inclusion of PTHL modifies the changes during aging of the technological properties (colour parameters, cooking loss and tenderness and FA profile), the lipid and protein oxidation and the nutritional characteristics of the *Longissimus thoracis* (LT) muscle.

Material and methods

Animals and experimental design

The protocol for animal research was approved by the Ethics Committee for animal testing – CESA (process number 2-X/17) of the Department of Veterinary Medicine of the University of Bari Aldo Moro, Bari, Italy. Handling and sampling were designed to minimise stress and health risks. Animals were examined daily by farm personnel and weekly by an experienced veterinarian.

Forty Limousin bulls with an average age of 6.5 months and an average initial weight of 339.5 ± 41.52 kg were used. At the beginning of the trial, experimental animals were individually identified and weighed. Then, bulls were randomly subdivided into two groups of 20 individuals each, reared in two separate pens with a cement floor, measuring 12 × 18 m and equipped with feeders and automatic waterers. A group was treated as the experimental group (PTHL) and the other one as the control group (CON). Experimental animals were weighed again the day before slaughter. Initial and final weight data obtained were used to calculate the average daily weight gain. Bulls were slaughtered at a final weight of 521.5 ± 33.31 kg and an average age of 10.5 months.

Experimental diets

All bulls were finished intensively, and they were fed *ad libitum* a total mixed ration (TMR) that was administered with a mixer wagon once per day, after removal of the TMR remaining from the previous day. The ingredients and the determined nutrient content (Association of Official Analytical Chemists (AOAC) 2000) of TMR are shown in Table 1.

The CON group received exclusively TMR for 120 days while the experimental group received the same TMR as did the CON group, but supplemented with PTHL (Oxifenol, I-Green, Padua, Italy, www.igreen-srl.com, October 2019). The administering rate was 35 g/day per head on Days 1–90 and 70 g/day per head on Days 91–120, according to the instructions of I-Green, on the basis of several empirical trials (detailed results of these trials were not made available by the company). The supplement was orally administered to each head of the PTHL group in the self-locking head gate in the

Table 1. Ingredients and determined nutrient composition (according to the methods of Association of Official Analytical Chemists (AOAC)) of total mixed ration (TMR)

The control group received exclusively the TMR for 120 days until the end of the experiment at 10 months of age. The experimental group received the same experimental TMR as did the control group, but supplemented with *Pinus taeda* hydrolysed lignin (Oxifenol, I-Green, Padua, Italy). Inclusion rate was 35 g/day per head at 1–90 days and 70 g/day per head at 91–120 days, as per manufacturer instructions. DM, dry matter

Item	Concentration
Ingredients[B] (% of fed diet)	
Wheat straw	15.0
Maize (ground)	44.0
Soybean meal 44% crude protein	14.0
Barley (ground)	12.5
Wheat bran	11.0
Hydrogenated triacylglyceride from palm oil	1.00
Mineral and vitamin premix	2.50
Determined nutrient composition	
DM (% of the as-fed diet)	86.7
Organic matter (% of DM)	85.2
Crude protein (% of DM)	15.9
Crude fibre (% of DM)	9.2
Neutral detergent fibre (% of DM)	25.6
Acid detergent fibre (% of DM)	10.1
Acid detergent lignin (% of DM)	2.63
Ether extract (% of DM)	3.94
Ash (% of DM)	4.01

feeding front when TMR was unloaded, as described by Maggiolino *et al*. (2019). It was mixed with water to obtain a cream, which was then administered directly in the mouth using a large syringe.

The determined composition and the antioxidant activity of PTHL was calculated according methods described by Gerardi *et al*. (2015) and Blando *et al*. (2016; Table 2). Phenolic compounds were analysed by high-performance liquid chromatography and mass spectrometry with an electrospray ion source and photodiode array detection, as described by Blando *et al*. (2016). The peak area of each analyte was recorded at the wavelength of maximum absorbance determined from UV–visible spectra within the wavelength range of 190–600 nm.

The oxygen radical absorbance capacity (ORAC) assay was conducted using a Trolox standard (20 µmol/L), fluorescein (200 nmol/L) and 2,2′-azobis(2-methyl)-propionamidine dihydrochloride (60 mmol/L) in phosphate buffer (75 mmol/L, pH 7.0; Blando *et al.* 2016). All reaction mixtures were prepared in triplicate. Calculated ORAC values per sample (ORAC number = $\text{slope}_{\text{sample}}/\text{slope}_{\text{Trolox}}$) are expressed as µmol Trolox equivalents (TE) per gram of DW or µmol TE/100 g of dry weight.

Antioxidant capacity was assessed based on the TE antioxidant capacity, a decolorisation assay that is primarily used to assess hydrophilic antioxidants. All reaction mixtures were prepared in triplicate, and each sample was independently assayed twice. The % inhibition of absorbance at 734 nm was calculated and plotted as a function of the Trolox reference standard and is expressed for each sample as µmol TE/g of DW.

Table 2. Phenolic composition and antioxidant activity of *Pinus taeda* hydrolysed lignin (according to Gerardi *et al.* (2015) and Blando *et al.* (2016))

TE, trolox equivalents; DM, dry matter; DW, dry weight

Item	Concentration
DM (%)	92.6
Determined composition (g/100 g of DM)	
Vanillin	26.4
Eriodictyol	3.4
Quercetin	2.7
Isorhamnetin	1.6
Rosmarinic acid	1.4
Quercetin ramnoside	13.9
Methyl gallate retunoside	42.3
Epigallocatechin-3-methylgallate	1.5
Ferulic acid derivates	6.7
Antioxidant activity (µmol TE/g DW)	
Trolox equivalent antioxidant capacity	23.9
Oxygen radical absorbance capacity	122.4

Experimental procedures and sampling

Bulls were transported ~10 km to the slaughterhouse and all bulls were slaughtered the same day by using standard commercial procedures in compliance with European Union laws on Animal Welfare in transport (1/2005EC; EC 2005) and the European Community regulation on Animal Welfare for slaughter of commercial animals (1099/2009EC; EC 2009). Animals were stunned (by captive bolt gun), exsanguinated and dressed following commercial dressing-out procedures at the abattoir. Immediately after slaughter, carcasses were chilled at 4°C for 24 h. The pH was recorded at 24 h *post mortem* with a portable pH meter with glass electrode shaped to easily penetrate meat (Carlo Erba pH 710, Carlo Erba Reagenti, Milano, Italy). Before each measurement, the pH meter was automatically calibrated for muscle temperature and using standard solutions with 4 and 7 pH values (Crison, Lainate, Italy). Subsequently, samples of LT muscle (mean weight of 2000 ± 100 g) were removed from the right carcass half between the 11th and 13th thoracic vertebra from each animal and placed in a freezer at 4°C for 24 h until analyses. Samples were stored at 4°C, a 25 mm slice was cut at each storage time (1, 8, 11 and 15 days) and analysed.

Physicochemical analyses

All physicochemical analyses were performed in duplicate for each muscle sample. Before colour measurements, LT samples were allowed to bloom directly in contact with air for 30 min. Objective measures of meat colour (CIE 1976), including lightness (L*, a greater L* value is indicative of a lighter colour), redness (a*, a greater a* value is indicative of a redder colour) and yellowness (b*, a greater b* value is indicative of a more yellow colour) were determined using a Minolta CR-300 colourimeter (light source D65, Minolta Camera Co., Osaka, Japan). Reflectance measurements were collected from a 0° viewing angle with A-pulsed xenon arc lamp, with a reading surface of 8-mm diameter. For each sample of

meat, three measurements were taken on each sample by rotating the detector system 90° from the previous on three different points. Then, nine readings per sample were taken at each point and averaged for statistical analyses. The colourimeter was calibrated on the Hunter-laboratory colour-space system by using a white title (L* = 99.2, a* = 1.0, b* = 1.9). The a* and b* values were used to determine chroma $(C^*) = (a^2 + b^2)^{1/2}$ and hue (radians, H°) = $\tan^{-1}$ (b/a) according to De Palo *et al*. (2012).

The chemical composition was analysed only at Day 1 of aging. Muscle samples were cleaned, epimysium removed and, then, triturated in a domestic blender until a homogeneous mass was obtained. Then, moisture was determined by the difference between the initial weight of sample and the dried weight of sample after drying at 105°C for 24 h in the oven; protein concentration was calculated according to ISO937:1978 (International Organisation for Standardisation (ISO) 1978), intramuscular fat concentration according to ISO1443:1973 (ISO 1973) and ash following ISO 936:1998 (ISO 1998). Each sample was homogenised with a mixture of chloroform and methanol (1 : 2, vol/vol) solution for the extraction of total lipids from intramuscular fat (De Palo *et al*. 2014).

The cooking losses were measured as described by Wheeler *et al*. (1995). Briefly, two meat pieces (cuboids of 2.5-cm sides and ~8-cm length) were obtained from each slice with the cut being made transverse to the direction of the muscle fibres. Steaks were thawed in a refrigerator for 24 h and weighed using a precision balance (Model TX3202 L, Shimadzu, Milan, Italy). Then, samples were placed together in a griddle and baking sheet and roasted in an electric oven, preheated to 150°C (Star model, Fischer & Paykel Appliances, Milton Keynes, UK), until the internal temperature of the samples reached 70°C. Internal temperature was monitored using K-type thermocouples inserted in the geometric centre of samples and readings were taken with a digital reader (Model TM-361, Tenmars Electronics Co. Ltd, Taipei, Taiwan). Samples were cooled at room temperature until they reached an internal temperature of 25°C, measured using an insertion thermometer (Model 106, Testo Spa, Milan, Italy). Samples were then weighed to determine the weight loss, which was expressed as weight loss percentage.

The Warner–Bratzler shear force (WBSF) was analysed as described by Lorenzo and Carballo (2015). All samples were cut perpendicular to the muscle-fibre direction at a cross-head speed of 3.33 mm/min. A texture analyser (TA-XT2, Stable Micro Systems, Godalming, UK) was used. Seven pieces of meat of 1 × 1 × 2.5 cm (height × width × length) were removed parallel to the muscle-fibre direction. Samples were completely cut using a WB shear blade with a triangular slot cutting edge (1-mm thickness) at a cut speed of 20 cm/min. Maximum shear force, shown by the highest peak of the force–time curve, represents the maximum resistance of the sample to the cut.

Analyses of thiobarbituric acid reactive substances (TBARS), protein carbonyls and hydroperoxide

Minced sample (5 g) was placed in a 50-mL test tube and homogenised with 15 mL of deionised distilled water. An aliquot of homogenate (1 mL) was transferred to a glass tube for the TBARS determination and 0.05 mL of butylated hydroxytoluene (7.2% in ethanol) was added along with 1950 mL of TBA–trichloracetic acid (TCA)–HCl (0.375% TBA, 15% TCA and 0.25 N HCl). The sample solution was shaken and then incubated at 90°C for 15 min in a thermostatic bath. After this period, samples were cooled to room temperature (15–30°C) and then centrifuged at 2000*g* for 15 min, at 15°C. Supernatant absorbance at 531 nm was measured against a blank containing 2 mL of TBA–TCA–HCl solution in 1 mL of distilled water. The TBARS were calculated comparing with a standard curve constructed with 1,1,3,3-tetramethoxypropane, and the concentration of lipid oxidation was expressed as milligrams of malondialdehyde per kilogram of meat (Buege and Aust 1978).

A volume of 2 mL of homogenate (previously prepared for the TBARS determination) was added with 4 mL of CH_3OH and 2 mL of $CHCl_3$. The samples were vortexed for 30 s and were added with 2 mL of $CHCl_3$ and 1.6 mL of 0.9% NaCl. The samples were shaken for 1 min and then centrifuged at 3500*g* for 10 min at 4°C. A sample of 2 mL of lipid extract was collected from the lower chloroform phase and processed with 1 mL of $CH_3COOH/CHCl_3$ and 50 µL of KI (1.2 g/L mL distilled water). Samples were stored for 5 min in a dark room and 3 mL of 0.5% of CH_3COOCd was added and the samples were then vortexed and centrifuged at 4500*g* for 10 min at 40°C. Absorbance at 353 nm was measured against a blank tube in which meat homogenate was replaced by 2 mL of distilled water (De Palo *et al.* 2013). Results are expressed in micromoles per gram, according to Buege and Aust (1978).

Meat samples (2 g) were homogenised in 20 mL of 0.15 M KCl for 2 min. Two aliquots of homogenate (50 µL each) were added with 1 mL 10% TCA and then centrifuged at 1200*g* for 3 min at 4°C, to measure protein oxidation. The first aliquot was used as a standard and added with 1 mL of 2 M HCl solution. The second aliquot was added with 1 mL of 2 M HCl containing 10 mM 2,4-dinitrophenyl hydrazine. Samples were incubated for 1 h at room temperature (15–30°C) and shaken every 20 min, and then 1 mL of 10% TCA was added. The samples were vortexed for 30 s and centrifuged three times at 1200*g* for 3 min at 4°C and the supernatant was removed. Care was taken not to disrupt the pellet. The pellet was washed with 1 mL of ethanol : ethyl acetate (1 : 1), shaken, and centrifuged three times at 1200*g* for 3 min at 4°C, and the supernatant was removed. The pellet was then dissolved in 1 mL 20 mM sodium phosphate–6 M guanidine hydrochloride buffer. Samples were then shaken and centrifuged at 1200*g* for 3 min at 4°C. Carbonyl concentration was calculated on the 2,4-dinitrophenyl hydrazine-treated sample at 360 nm, with a Beckman Coulter DU800 (Beckman Instruments Inc., Brea, CA, USA) and is expressed as nanomoles carbonyl per milligram protein. Protein concentration was calculated according to Biuret assay (Tokur and Korkmaz 2007; De Palo *et al.* 2013).

FA methyl ester (FAME) analyses

The FAME were prepared by transesterification of the lipid extract, as described by De Palo *et al*. (2015), using methanol in the presence of 3% hydrochloric acid in methanol (vol/vol). Then, FA were determined with a Trace GC Thermo Quest Gas Chromatograph (Thermo Electron, Rodano, Milan,

Italy) equipped with a flame ionisation detector, after their esterification with methanol in the presence of 3% hydrochloric acid in methanol (vol/vol). The derivatives were separated on a capillary column (Supelco SP-2380 fused-silica column, 60-m length, 0.25-mm internal diameter and 0.20-mm film thickness; Sigma-Aldrich, St Louis, MO, USA). Injector and detector temperatures were held at 260°C. Column oven-program temperatures were as follows: T1 = 80°C, hold 1 min; T2 = 150°C ramp at 15°C/min, hold 2 min; T3 = 220°C ramp at 5°C/min, hold 2 min; and T4 = 250°C ramp at 15°C/min, hold 5 min. The flow rate of the carrier gas (helium) was set at 0.8 mL/min. Identification of FAME was based on the retention times of reference compounds (Sigma-Aldrich, St Louis, MO, USA) and mass spectrometry. The FA composition was expressed as the percentage of total FAME (Supelco^{TM} 37 Component FAME Mix, Catalog Number 47885-U, Sigma-Aldrich). Nutritional implications were assessed by calculating the amount of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 and n-6 FA, as well as the PUFA : SFA and the n-6 : n-3 ratios. Moreover, the atherogenic index (AI) and thrombogenic index were calculated according to Ulbricht and Southgate (1991).

Statistical analyses

The datasets of initial and final liveweights, daily gain and dressing percentage were subjected to ANOVA using the GLM by SAS software (SAS 9.4; SAS Institute Inc, Cary, NC, USA), considering the dietary treatment as a fixed effect.

The other data were subjected to nested ANOVA using the general linear modelling by SAS software (SAS 9.4), according to the following model:

$$y_{ijk} = \mu + \alpha_i + P_j + A_k + (P{\times}A)_{jk} + \varepsilon_{ijkl},$$

where y_{ijk} represents all the meat-qualitative patterns as dependent variables, μ is the overall mean, α_{ij} is the constant, P is the effect of the jth PTHL inclusion in the diet ($j = 1, 2$), A is the effect of the kth aging time ($k = 1, \ldots, 4$), $P \times A$ is the effect of the interaction of the jth PTHL inclusion in the diet and kth aging time, and ε_{ijkl} is the error term. A Tukey test was applied to evaluate the differences among means when the effect of aging time was significant. Chemical composition was assessed exclusively at Day 1 of aging. Consequently, only the dietary supplementation with PTHL effect was analysed. In all cases, the experimental units were 20 per diet and 40 per aging time.

Results and discussion

The PTHL is principally composed of methyl gallate retunoside, vanillin and quercetin ramnoside (Table 2), which are natural phenolic compounds with antioxidant activity. Considering the animals' liveweights and their potential daily intakes of DM (~11 000–13 000 g), the PTHL inclusion ranged from 0.3% to 0.65% (on a DM basis) of the daily intake, a small proportion of the total DM consumed.

Live performance and meat composition

There were no differences in the daily weight gain and final weight between the two experimental groups, with dressing percentage values being similar ($P > 0.05$), as shown in Table 3. Colour plays a major role as a sensory property by which meat quality is readily assessed and seems to be the single most important sensory attribute affecting consumer purchasing decisions (Luciano *et al*. 2009). Current results (Table 4) indicated that L* was not affected by the diet but increased through the AP in the PTHL group ($P < 0.01$). Similar results were reported by Guerra-Rivas *et al*. (2016) who found that L* tended to increase during aging, particularly after the first 7 days. In the current study, although a* and C* did not show any variation ($P > 0.05$), they tended to be slightly higher with PTHL inclusion, but did not vary with AP. Moreover, b* and H° decreased during the AP with both diets ($P < 0.001$), but these decreases were more evident in the CON meat, which had lower values than did the PTHL meat at 8 ($P < 0.05$), 11 and 14 days ($P < 0.01$). Different results have been obtained about the influence of diets containing antioxidants on the colour of beef meat through the AP. Results obtained in the current study for the effects of the antioxidant inclusion in the diet on meat colour partially agree with those obtained by Luciano *et al*. (2009). Those authors, studying the polyphenolic effects on lamb meat, observed that a* values were higher when polyphenolic compounds were used, as was the case in the current trial. However, Luciano *et al*. (2009) also observed that a* tended to decrease and b* increased with time, unlike the current results. In contrast to the current study, other authors (Karami *et al*. 2011; Andrés *et al*. 2013), studying lamb and goat meat, have reported that polyphenols from different natural sources are unable to affect a* compared with the CON groups. It has been shown that, during aging, b* values are positively correlated with sensory appreciation of meat, whereas a* values are negatively correlated with sensory degradation of colour (Insausti *et al.* 2008). In red meat, lipid oxidation and colour deterioration are believed to be linked, with haem pigments serving as catalysts of lipid peroxidation (Baron and Andersen 2002). However, phenolic compounds are known to have antioxidant properties (Tang *et al*. 2001). In this sense, the PTHL inclusion in bull diets seems to delay meat-colour deterioration, resulting in a higher a* stability and a lower b* decreasing trend. Hue angle (radiant) is a good descriptor of browning processes on meat (Lee *et al*. 2005). Contrary to results obtained in the current study, Luciano *et al*. (2009) recorded an increase of H° values during the AP. In addition, these authors observed that H° did not change when polyphenolic compounds were added in the diet. Moreover, Guerra-Rivas *et al*. (2016) did not find effects of the polyphenol inclusion in the diet on meat C* and H°.

Table 3. Effects of including *Pinus taeda* hydrolysed lignin (PTHL) in the diet on *in vivo* and slaughtering performance of finishing beef bulls
PTHL, group supplemented with *Pinus taeda* hydrolysed lignin; and CON, control group; s.e.m., standard error of the means from the feeding group

Parameter	PTHL	Control	s.e.m.	*P*-value
Initial live bodyweight (kg)	339	340	24.9	0.08
Final live bodyweight (kg)	521	522	36.5	0.08
Daily gain (kg)	1.49	1.51	0.11	0.09
Dressing percentage (%)	66.7	67	4.28	0.07

Table 4. Effects of including *Pinus taeda* hydrolysed lignin (PTHL) in the diet and the aging period (AP) on quality traits and chemical composition of beef

WBSF, Warner–Blatzer shear force. s.e.m., standard error of the mean ($n = 20$ and 40 for diet and for AP respectively). Means in the same row followed by no letters or by the same letter are not significantly different (a, b: $P < 0.05$; A, B, C: $P < 0.01$). Means in the same column, for the same parameter, followed by no letters or by the same letter are not significantly different (x, y: $P < 0.05$; X, Y: $P < 0.01$)

Parameter	Treatment group	Aging period (days)					*P*-value		
		1	8	11	15	s.e.m.	Diet	AP period peP[3]	Diet × AP AP
Lightness (L*)	PTHL	36.2A	37.3	38.4	40.0B	0.51	0.25	0.008	0.36
	Control	38.5	37.5	39.4	40.7				
Redness (a*)	PTHL	18.4	18.7	18.6	18.5	0.39	0.081	0.41	0.28
	Control	17.2	18.0	18.2	17.4				
Yellowness (b*)	PTHL	2.60A	1.94ABx	1.55BX	1.85BX	0.131	0.0002	0.0004	0.005
	Control	2.57A	1.58By	0.24C,Y	–0.50CY				
Chroma (C*)	PTHL	18.6	18.8	18.7	18.6	0.39	0.071	0.29	0.37
	Control	17.4	18.1	18.3	17.5				
Hue angle (radians)	PTHL	0.14a	0.11	0.08bX	0.10X	0.008	0.0001	0.0001	0.0001
	Control	0.15A	0.09B	0.01CY	–0.03CY				
Moisture[A]	PTHL	74.6	–	–	–	1.91	0.54	–	–
	Control	74.8	–	–	–				
Protein[A]	PTHL	22.8	–	–	–	0.53	0.82	–	–
	Control	22.2	–	–	–				
Fat[A]	PTHL	1.93	–	–	–	0.070	0.28	–	–
	Control	2.21	–	–	–				
Ash[A]	PTHL	1.15	–	–	–	0.020	0.71	–	–
	Control	1.13	–	–	–				
pH	PTHL	5.72	5.68	5.68	5.70	0.011	0.68	0.49	0.39
	Control	5.72	5.68	5.67	5.71				
Cooking loss (%)	PTHL	28.2	27.5	31.2	29.8	0.71	0.29	0.73	0.70
	Control	26.3	27.4	29.4	27.7				
WBSF (N)	PTHL	76.4A	60.9B	56.6B	59.2B	1.81	0.49	0.0004	0.49
	Control	72.7A	58.9B	58.0B	58.9B				

[A]Measured only at Day 1 of aging.

The proximate composition observed was similar to those reported by other authors for samples from the *longissimus* muscle of bulls (Pateiro *et al*. 2013; De Palo *et al*. 2014; Araujo *et al*. 2016; Modzelewska-Kapituła *et al*. 2018). As expected, diet did not modify the chemical composition of the LT muscle, which is in agreement with the results of other studies conducted on goat meat after polyphenol inclusion in the diet (Karami *et al*. 2011; Cimmino *et al*. 2018). However, chemical composition was analysed only at Day 1 of aging.

The pH values were typical of normal-quality beef (varying between 5.67 and 5.72) and were not affected by the diet or the AP. Results observed in the current trial for pH were similar to those observed by Zhao *et al*. (2018) who did not find differences in the pH of meat from lambs fed diets containing polyphenols and that from the CON. The ultimate pH affects meat quality by altering water-holding capacity and, consequently, cooking loss (Vasta *et al*. 2007). Considering the similar pH values observed between the diet groups, the cooking loss did not show any difference, as expected. However, and despite AP not influencing pH at 24 h *post mortem*, cooking loss tended to be higher at 11 days of aging (not a significant difference; $P > 0.05$) than at the other days considered. The WBSF showed a mean value of 63.3 N, which is considered to be tough meat (WBSF above 62.6 N; Destefanis *et al*. 2008), but there were no differences between the diets.

FA profile

The current results showed significant interactions between the diet and AP for SFA, MUFA and PUFA (Table 5). In fact, the SFA concentration was lower for meat from bulls fed the diet containing PTHL than for meat from bulls fed the CON diet at Days 8 and 11 of aging ($P < 0.0001$). The MUFA concentration was higher for PTHL than for CON diet from Day 1 to Day 11 ($P < 0.0001$), showing similar values for both groups at 15 days of aging. In contrast, the PUFA concentration was higher for PTHL than for CON diet in all AP days considered ($P < 0.001$), and, in the PTHL group, showed lower values from Day 8 of AP ($P < 0.001$). Moreover, the PUFA : SFA ratio (i.e. long chain to n-3, n-3 and n-6) was higher in the PTHL meat across the entire AP ($P > 0.001$). These outcomes are partially in agreement with data reported by Cimmino *et al*. (2018), who noticed that supplementation with polyphenol extract changed the FA composition of goat meat, increasing the MUFA concentration and decreasing the concentration of SFA, without having any effect on the PUFA concentration. Also, Vasta *et al*. (2009) reported that PUFA concentration

Table 5. Effects of including *Pinus taeda* hydrolysed lignin (PTHL) in the diet and aging period (AP) on total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, PUFA : SFA ratio, long chain n-3, n-3, n-6, n-6 : n-3 ratio, atherogenic index (AI) and thrombogenic index (TI) of beef (% on total fatty acid methyl ester)

SFA, (C8:0 + C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0). MUFA, 3(C12:1 + C14:1 + C16:1 + C17:1 + C18:1 cis n-9 + C18:1 trans n-9 + C20:1+C22:1). PUFA, (C18:2n-6 + C18:3n-3 + C18:3n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3). Long-chain n-3, (C20:5n-3 + C22:5n-3 + C22:6n-3). *n*-3, (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3). n-6, (C18:3n-6 + C20:3n-6 + C20:4n-6). AI, [C12:0 + (4 × C14:0) + C16:0) / [(ΣMUFA) + (ΣPUFA)]. TI, (C14:0 + C16:0+C:18:0) / [(0.5 × ΣMUFA) + (0.5 × n-6) + (3 × n-3) + (n-3 : n-6)]. s.e.m., standard error of the mean (n = 20 and 10 for diet and for AP respectively). Means in the same row followed by no letters or by the same letter are not significantly different (a, b: $P < 0.05$; A, B, C: $P < 0.01$). Means in the same column, for the same parameter, followed by no letters or by the same letter are not significantly different (x, y: $P < 0.05$; X, Y: $P < 0.01$)

Parameter	Treatment group	Aging period (days)					*P*-value		
		1	8	11	15	s.e.m.	Diet	AP	Diet × AP APaAP
SFA	PTHL	48.3A	45.0BX	40.9CX	48.1A	0.68	0.0001	0.0001	0.0001
	Control	50.5	50.6Y	49.6Y	48.9				
MUFA	PTHL	40.3AX	43.6BX	48.3CX	41.5AB	0.64	0.0001	0.0001	0.0001
	Control	37.7Y	38.2Y	39.6Y	40.2				
PUFA	PTHL	13.3AX	13.6AX	10.7BX	12.1AB	0.33	0.0002	0.35	0.007
	Control	7.99Y	8.03Y	8.58Y	7.93Y				
PUFA : SFA	PTHL	0.28X	0.30X	0.27X	0.25X	0.009	0.0001	0.41	0.57
	Control	0.16Y	0.16Y	0.17Y	0.16Y				
Long-chain n-3	PTHL	2.04aX	2.12bX	2.03aX	2.18bX	0.052	0.0001	0.03	0.02
	Control	1.18Y	1.16Y	1.17Y	1.23Y				
n-3	PTHL	2.69X	2.63X	2.51X	2.65X	0.029	0.0001	0.48	0.51
	Control	1.58Y	1.48Y	1.32Y	1.49Y				
n-6	PTHL	10.7X	10.9X	8.19X	9.43X	0.329	0.0001	0.71	0.007
	Control	6.41Y	6.54Y	7.25Y	6.45Y				
n-6 : n-3	PTHL	4.00	4.19	3.27X	3.56X	0.131	0.0001	0.29	0.004
	Control	4.08	4.44	5.48Y	4.33Y				
AI	PTHL	0.76X	0.61X	0.78X	0.87X	0.034	0.0001	0.43	0.03
	Control	1.11Y	1.14Y	1.10Y	1.07Y				
TI	PTHL	1.20AX	1.06X	0.99BX	1.19X	0.026	0.0001	0.004	0.06
	Control	1.54Y	1.51Y	1.43Y	1.43Y				

Table 6. Effects of including *Pinus taeda* hydrolysed lignin (PTHL) in the diet and aging period (AP) on saturated fatty acid (SFA) profile (% of total fatty acid methyl esters) of beef

s.e.m., standard error of the mean (n = 20 and 10 for diet and for AP respectively). Means in the same row followed by no letters or by the same letter are not significantly different (a, b: $P < 0.05$; A, B, C: $P < 0.01$). Means in the same column, for the same parameter, followed by no letters or by the same letter are not significantly different (x, y: $P < 0.05$; X, Y: $P < 0.01$)

SFA	Treatment group	Aging period (days)					*P*-value		
		1	8	11	15	s.e.m.	Diet	AP	Diet × AP
C8:0	PTHL	0.06	0.06	0.06	0.05	0.003	0.35	0.42	0.61
	Control	0.06	0.07	0.06	0.05				
C10:0	PTHL	0.33	0.26	0.27	0.27	0.011	0.48	0.27	0.39
	Control	0.29	0.28	0.28	0.30				
C12:0	PTHL	1.48	1.78	1.00a	2.48b	0.159	0.07	0.03	0.08
	Control	1.37	1.61	1.08	1.22				
C14:0	PTHL	3.55AX	2.88AX	5.09B	4.69B	0.286	0.0005	0.0024	0.0081
	Control	5.11Y	5.49Y	5.30	5.51				
C15:0	PTHL	0.52	0.52	0.48	0.49	0.018	0.48	0.52	0.54
	Control	0.50	0.56	0.54	0.43				
C16:0	PTHL	24.6X	21.2X	23.3X	25.1x	0.52	0.0001	0.48	0.02
	Control	29.0Y	28.9Y	30.3Y	28.1y				
C17:0	PTHL	0.67	0.58	0.67	0.54	0.027	0.61	0.51	0.29
	Control	0.61	0.66	0.57	0.61				
C18:0	PTHL	16.8AX	17.4AX	9.70BX	14.2AX	0.006	0.0001	0.0001	0.0001
	Control	13.2Y	12.7Y	11.0Y	12.2Y				
C20:0	PTHL	0.12	0.17	0.12	0.12	0.007	0.41	0.23	0.37
	Control	0.13	0.16	0.17	0.16				
C22:0	PTHL	0.16	0.22	0.19	0.21	0.007	0.19	0.42	0.52
	Control	0.21	0.17	0.18	0.15				

increased in muscle after polyphenol (proantocyanidins) inclusion in the diet of sheep. In contrast, Muíño *et al*. (2014) did not observe any effect of supplying polyphenols on the FA profile of lamb meat, with similar concentrations of SFA, MUFA and PUFA with and without polyphenols. Kafantaris *et al*. (2018) found that the inclusion of grape pomace polyphenol extract in the diet was able to reduce the n-6 : n-3 ratio in lamb meat, by increasing the n-3 concentration, similar to what was observed in the current study for Day 11 of aging.

Some SFA (C14:0 and C16:0; Table 6) were lower in the PTHL group than in the CON group ($P < 0.001$), but C18:0, on the contrary, was higher in the CON group ($P < 0.01$). For the unsaturated FA, excluding the C16:1, which was lower in the PTHL group ($P < 0.01$) (Table 7), most of the FA were higher in the PTHL group ($P < 0.001$). According to Nanon *et al*. (2014), the inclusion of antioxidants in the diet could modify the ruminal bio-hydrogenation and change the C18:0 concentration. In this regard, several Gram-positive bacteria are related to ruminal bio-hydrogenation of unsaturated FA of the diet. Therefore, feeding PTHL could decrease the bio-hydrogenation of FA by reducing the number and activity of bacteria involved in the bio-hydrogenation of unsaturated FA. In addition, meat from bulls fed the diet containing PTHL had a higher concentration of C18:3 n-6 than did meat from bulls fed the CON diet. This agrees with data of Jafari *et al*. (2016) who noted an increase of C18 PUFA concentration after 24-h *in vitro* incubation of an experimental diet containing leaf fractions of papaya, extracted with hexane or chloroform.

The AI increased through the AP with the PTHL diet, but remained stable with the CON diet. However, in all cases, the AI was lower with the PTHL diet than with the CON diet, in agreement with the results reported by Yakan *et al*. (2016) who found a lower AI in kids after diet supplementation with 450 mg/kg of vitamin E than in the CON group. The PTHL inclusion in bull diets is able to affect the meat FA composition. Since PUFA content is important for human

Table 7. Effects of including *Pinus taeda* hydrolysed lignin (PTHL) in the diet and aging period (AP) on unsaturated fatty acid (UFA) profile (% on total fatty acid methyl esters) of beef

s.e.m., standard error of the mean ($n = 20$ and 10 for diet and for AP respectively). Means in the same row followed by no letters or by the same letter are not significantly different (a, b: $P < 0.05$; A, B, C: $P < 0.01$). Means in the same column, for the same parameter, followed by no letters or by the same letter are not significantly different (x, y: $P < 0.05$; X, Y: $P < 0.01$)

SFA	Treatment group	Aging period (days)				s.e.m.	*P*-value		
		1	8	11	15		Diet	AP	Diet × AP
C12:1	PTHL	0.07ax	0.14b	0.09ax	0.17b	0.003	0.03	0.04	0.03
	Control	0.15y	0.12	0.15y	0.13				
C14:1	PTHL	0.33	0.33	0.31	0.40	0.011	0.48	0.31	0.45
	Control	0.58	0.60	0.59	0.51				
C16:1	PTHL	3.11AX	4.13AX	2.18BX	5.67A	0.159	0.0057	0.0001	0.0008
	Control	6.75Y	7.20Y	7.72Y	7.54				
C17:1	PTHL	0.18	0.24	0.25	0.2a	0.286	0.45	0.38	0.24
	Control	0.25	0.23	0.18	0.21				
C18:1 n-9	PTHL	34.4X	36.3X	32.8	33.0	0.018	0.0001	0.52	0.0047
	Control	27.9Y	27.9Y	29.1	30.0				
C18:1 trans-11	PTHL	1.68AX	1.74AX	0.97B	1.42	0.52	0.0001	0.0001	0.0001
	Control	1.32Y	1.27Y	1.10	1.22				
C20:1	PTHL	0.23	0.18	0.19	0.18	0.027	0.24	0.41	0.35
	Control	0.23	0.23	0.19	0.19				
C22:1	PTHL	0.39	0.56	0.46	0.49	0.006	0.41	0.23	0.39
	Control	0.51	0.56	0.47	0.39				
C18:2 n-6	PTHL	5.91AX	6.25AX	3.35B	5.37AX	0.258	0.0051	0.0037	0.0001
	Control	4.32Y	4.53Y	4.60b	4.34Y				
C18:3 n-3	PTHL	2.64X	2.64X	2.66X	2.03X	0.167	0.0001	0.28	0.16
	Control	1.07Y	1.11Y	1.68Y	1.33Y				
C18:3 n-6	PTHL	0.65Ax	0.51BX	0.49BX	0.47BX	0.024	0.0001	0.0001	0.48
	Control	0.40AY	0.33Y	0.15BY	0.25BY				
C20:3 n-6	PTHL	0.80X	0.76X	0.77X	0.77X	0.006	0.0001	0.72	0.40
	Control	0.35Y	0.35Y	0.31Y	0.31Y				
C20:4 n-6	PTHL	1.31X	1.28X	1.40X	1.25X	0.056	0.0001	0.29	0.71
	Control	0.67Y	0.55Y	0.66Y	0.47Y				
C20:5 n-3	PTHL	0.25	0.25	0.27	0.33	0.006	0.23	0.31	0.42
	Control	0.26	0.25	0.25	0.30				
C22:5 n-3	PTHL	1.00X	1.04X	0.98X	1.03X	0.006	0.0001	0.09	0.63
	Control	0.67Y	0.66Y	0.66Y	0.67Y				
C22:6 n-3	PTHL	0.80X	0.84X	0.78X	0.83X	0.006	0.0001	0.33	0.04
	Control	0.25Y	0.25Y	0.26Y	0.26Y				

nutrition and health (Simopoulos 2009; Kafantaris *et al*. 2018), the PTHL was able to increase both n-3 and n-6 PUFA and their increase in bull meat showed a beneficial effect of the inclusion of this hydrolysed lignin in the bull diets on meat quality.

The reasons for differences observed among studies for the influence of natural antioxidants on traits studied in the current trial are not known, but might be related to the high variability in the chemical composition of the different antioxidants compared in the different studies. As a consequence, it is necessary to conduct studies about the effects of the quality and stability of FA and meat through the AP for each antioxidant.

Lipid and protein oxidation

The lipid oxidation of bull meat through the AP, measured by peroxide and TBAR values (Figs 1, 2), was shown to be influenced by the diet. In fact, the TBARS concentration was similar for both diets at 1 and 15 days of aging ($P > 0.05$). However, meat from bulls fed diet containing PTHL presented a lower concentration of TBARS than did meat from bulls fed the CON diet at 8 and 11 days of aging ($P < 0.01$), and the concentration increased during the AP in both diets ($P < 0.05$). In contrast, hydroperoxides increased through the AP regardless of the diet. Dietary polyphenol inclusion in ruminants can strongly interfere with FA metabolism (Mele *et al*. 2007; Vasta *et al*. 2007) and, so, with lipid-metabolism pathways, thereby influencing the meat oxidative stability. Generally, increasing the degree of unsaturation of muscle reduces its oxidative stability (Campo *et al*. 2006), but it also has been shown that some FAs (as conjugated linoleic acid) can exert antioxidant activity in meat, thereby reducing lipid oxidation and improving the colour stability (Joo *et al*. 2002). However, considering the protective effect of polyphenols on colour stability, a similar result in TBARS accumulation was expected over the APs considered. Jerónimo *et al*. (2012) showed that the use of dietary polyphenols (i.e. grape-seed extracts) have positive results on lamb meat stability. However, when studying the effects of inclusion of polyphenol diet on lamb meat, other authors (Luciano *et al*. 2009; Muíño *et al*. 2014) have not found any effect on lipid oxidation. Also, some authors (O'Grady *et al*. 2006*a*, 2006*b*), when studying the effects of the diet supplementation with polyphenols from tea (catechins), have not observed an improvement in lipid stability in beef meat. The lack of effects observed in those studies might be due to the susceptibility of lipid oxidation not being detected by TBARS, demonstrating, therefore, a protective effect of dietary polyphenol supplementation against lipid oxidation (Rivas-Cañedo *et al*. 2013).

It is not clear whether lipid oxidation initiate protein oxidation or *vice versa*, or even if the two oxidation processes are coupled (Lund *et al*. 2011). Current results showed that protein carbonyls had similar values at 1, 11 and 15 days of aging. However, protein carbonyl concentration was higher in meat from bulls fed the CON diet than in meat from bulls fed the PTHL diet at 8 days of aging. Previously, Estévez and Heinonen (2010) had observed a protective *in vitro* effect of some polyphenolic compounds on the formation of protein carbonyls. As meat from bulls fed the diet containing PTHL also had a lower concentration of TBARS than did meat from bulls fed the CON diet at 8 days of aging, a lower lipid oxidation could induce a lower protein carbonyl production, as was recorded in the current study.

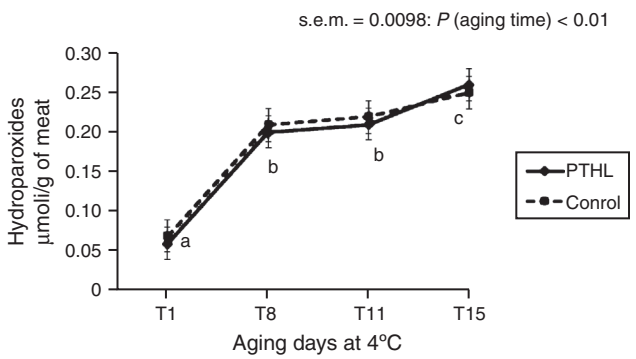

Fig. 1. Effects of aging time on hydroperoxide concentration of *longissimus thoracis*. Diet effect and the interaction between diet and aging period were not significant ($P > 0.05$). s.e.m., standard error of the mean. Means with the same letter are not significantly different (at $P = 0.05$).

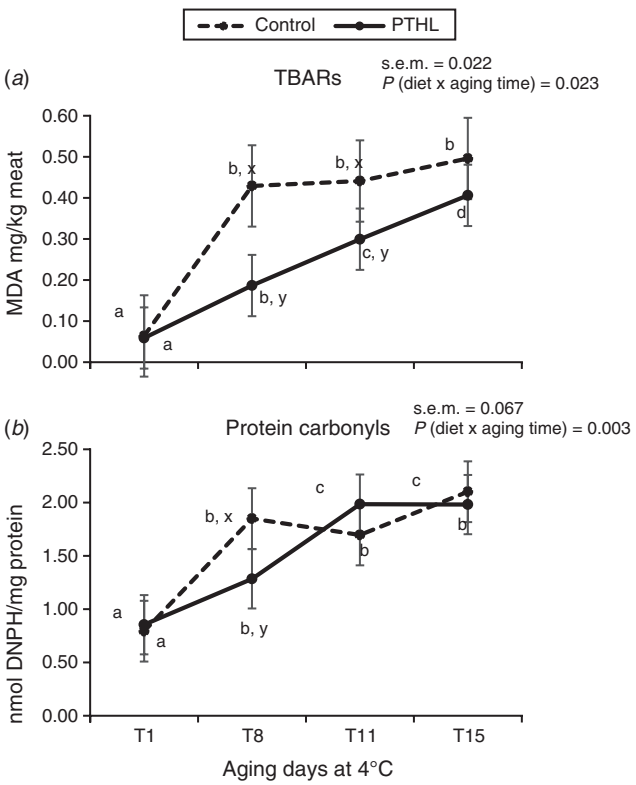

Fig. 2. Effects of including *Pinus taeda* hydrolysed lignin (PTHL) in the diet on (*a*) thiobarbituric acid reactive substances (TBARs; malondialdehyde, MDA) and (*b*) protein carbonyl production (nmol 2,4-dinitrophenyl hydrazine/mg protein) of samples from the *longissimus* muscle of bulls through the aging period. Means in the same line with no letters or the same letter are not significantly different (a, b, c; at $P = 0.05$). Means at the same time with no letters or with the same letter are not significantly different (x, y; at $P = 0.05$). Error bars represent the standard error of the mean (s.e.m.).

Conclusions

(1) The inclusion of PTHL in bull finishing diets at less than 0.65% of DM had no effect on live performance, daily gain, dressing percentage and chemical composition of meat during 15 days of aging at 4°C. However, the inclusion of this natural hydrolysed lignin decreased the lipid oxidation and kept the meat colour stable during its shelf-life, relative to the CON diet.

(2) The modification of FA composition due to the inclusion of PTHL in the diet may result in meat with beneficial effects on human health, because of the improved presence of PUFA compared with SFA, doubling the PUFA : SFA ratio.

(3) Thus, it is recommended to include PTHL in the finishing diet, ranging from 0.3% to 0.65% of DM, to improve the meat and the FA quality of meat from bulls.

Conflicts of interest

The authors declare no conflicts of interest.

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