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Avocado waste for finishing pigs: Impact on muscle composition and oxidative stability during chilled storage

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ABSTRACT

The utilization of agricultural waste materials for pig feeding may be an interesting option for reducing production costs and contributing to sustainability and environmental welfare. In the present study, a mixed diet enriched with avocado waste (TREATED) is used for finishing industrial genotype pigs. The muscle longissimus thoracis et lomborum (LTL) from TREATED pigs was analyzed for composition and oxidative and color stability and compared with muscles obtained from pigs fed a CONTROL diet. Dietary avocado had significant impact on the content and composition of intramuscular fat (IMF), reducing the lipid content in LTL muscles and increasing the degree of unsaturation. This did not increase the oxidative instability of samples. On the contrary, muscles from TREATED pigs had significantly lower lipid and protein oxidation rates during chilled storage. The color of the muscles from TREATED pigs was also preserved from oxidation.

1. Introduction

The feeding background of pigs has a capital influence on the animal production rates, carcass conformation and meat quality (Lebret, 2008). Additionally, feeding consists of about 70% of the total cost in raising pigs (National Research Centre on Pig, 2011). The utilization of waste materials from agricultural production has been proposed as a feasible means to reduce production costs and additionally, guarantee production sustainability and environmental welfare (Kumar, Roy, Lakhani, & Jain, 2014; Westendorf, Zirkel, & Gordon, 1996). Previous studies have tested a variety of food and agricultural by-products for pig feeding including bread waste (Kumar et al., 2014), spent coffee grounds (Sikka & Chawla, 1986), cane molasses (Garg, Pathak, Anjaneyulu, & Lakshmanan, 1986), dairy by-products (Kjos, Øverland, Arnkværn, & Sørheim, 2000) and assorted dehydrated restaurant food waste materials (Myer, Brendemuh, & Johnson, 1999). However, most of these studies focus on the toxicological and nutritional properties of the feeds and overlook the potential impact of such feeding on animal performance and in particular, meat quality.

While recycling food wastes for livestock feeding is an attractive waste disposal alternative, certain US states have banned such practice for health and safety reasons (Myer et al., 1999; Westendorf et al., 1996). In EU, meat wastes are forbidden for animal feeding and hence, this practice is restricted to fruit and fish by-products (Esteban, García, Ramos, & Márquez, 2007). Avocado (*Persea americana* Mill.) is a tropical and subtropical fruit, native to southern Mexico and currently grown in distant countries from four continents (FAOSTAT, 2008). The massive production of avocado in México allows fulfilling the internal demand, exporting to third countries (mostly EU) and still leads to considerable excess of this crop. Avocados may be used for animal feeding and this is particularly applicable for fruits dismissed for human consumption due to lack of adherence to commercial standards. Regarding its potential nutritional value, avocado is known for being an excellent source of unsaturated fatty acids, tocopherols and other phytochemicals with alleged positive biological effects (Wang, Terrell, & Liwei, 2010). In previous studies, avocado oil and phenolic-rich extracts from the peel and the seed have been included in processed porcine patties leading to beneficial effects on their nutritional value and oxidative stability (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estevéz, 2011; Rodríguez-Carpena, Morcuende, & Estévez, 2011; Rodríguez-Carpena, Morcuende, & Estévez, 2012; Utrera, Rodríguez-Carpena, Morcuende, & Estévez, 2012). In particular, the significant increase of monounsaturated fatty acids (MUFA), tocopherols, flavonoids and chlorophylls in porcine patties treated with avocado by-products were identified as responsible for the protection of meat lipids and proteins during storage and processing (Rodríguez-Carpena et al., 2011; Rodríguez-Carpena et al., 2011; Rodríguez-Carpena et al., 2012; Utrera et al., 2012). The application of avocado in animal feeding, however, is scarcely documented

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as just one single article has reported data on the effect of dietary avocado on nitrogen and energy balances in pigs (Grageola et al., 2010). As a result, the impact of this feeding regime on the quality of pork, is ignored. On this line, the composition of meat in terms of intramuscular fat (IMF) and fatty acid composition is paramount as these parameters have a direct influence on particular sensory traits such as juiciness and flavor (Wood et al., 2004). Conversely, the discoloration of meat as well as the onset of lipid and protein oxidation during retail display affects the shelf life and the sensory and nutritional value of pork (Lund, Heinonen, Baron, & Estévez, 2011; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015).

In response to this lack of knowledge, the present study was designed to evaluate the carcass conformation, and quality and oxidative stability of *M. longissimus thoracis et lumborum* (LTL) from pigs fed with avocado.

2. Material and methods

2.1. Animals and sampling

This study was carried out with sixteen male hybrids of commercial genotype pigs (50% York–50% Landrace) with an initial live average weight of 53.77 kg. Pigs were randomly divided into two groups according to the type of feeding during the fattening period: Control group (CONTROL, n = 8) and Treated group (TREATED, n = 8). The composition of the CONTROL diet was as follows (percentage in dry matter): sorghum meal 83.7, soybean meal 12.9, $CaHPO_4H{\cdot}2H_2O$ 1.0, $CaCO_3$ 1.2, NaCl 0.2, vitamins and trace elements 1.0. The TREATED diet contained (percentage in dry matter): sorghum meal 53.7, soybean meal 12.9, avocado paste 30.0, $CaHPO_4H{\cdot}2H_2O$ 1.0, $CaCO_3$ 1.2, NaCl 0.2, vitamins and trace elements 1.0. The share of avocado paste in the TREATED diet (30%) was optimized in a previous trial following digestibility and productivity criteria (unpublished data). The avocado paste was made with avocado waste from packing company. When avocado reached its consumption ripeness, determined objectively by firmness with 23.63 ± 4.0 N of force values (Stable Micro Systems Model TA. XT2, Texture Technologies Corp., Scarsdale, NT), the entire avocado fruit was ground in a screenless hammer mill to obtain a homogeneous paste and then was mixed with the other ingredients. Pigs were in individual pens. Mixed diets and water were supplied to pigs ad libitum. Both mixed diets were isoproteic and provided 14% of crude protein. Additional information about the composition of the mixed diet is provided in Table 1. Digestibility was calculated though the analysis of fecal material according to the procedure described by Paiva-Martinsa, Barbosa, Pinheiro, Mourãoc, and Outor-Monteiro (2009).

At the end of the fattening period at a live weight of 100 kg, pigs were electrically stunned and subsequently slaughtered. *Muscles longissimus thoracis et lomborum* (LTL) were removed hot from the carcasses, immediately freed from visible fat, and kept at 4 °C for its analysis within the hour following slaughter. Five slices of each LTL muscle were cut, dispensed in polystyrene trays, wrapped in oxygen-permeable film (14 μm thickness and 10,445 mL/m^2/24 h of oxygen permeability) and stored in the dark at 4 °C for 12 days. Samplings were made at days 0, 3, 6, 9 and 12 days of storage.

Table 1
Analysis of CONTROL and TREATED experimental diets.

	CONTROL		TREATED	
	Mean	SD	Mean	SD
Moisture (g/100 g feed)	12.64	0.36	30.54	1.44
Crude Protein (g/100 g feed)	13.40	1.64	12.24	1.74
Ether extract (g/100 g feed)	0.31	0.09	3.22	0.13
Ash (g/100 g feed)	3.91	0.10	3.84	0.16
Tocopherol (mg/kg feed)	164.62	18.77	273.03	16.24
Digestibility (% dry matter)	87.33	1.81	86.25	1.85
Gross energy (kJ/g feed dry matter)	17.84	–	25.58	–

SD: standard deviation.

2.2. Analytical methods

2.2.1. Chemical composition of feeds and muscles, intramuscular fat isolation and fatty acid profiles

Moisture, protein, ether extractives, nitrogen free extractives (NFE) and ash were determined in feeds and muscles using official methods (AOAC, 2000). Intramuscular total lipids were extracted from muscles using the Folch method (Folch, Lees, & Sloane-Stanley, 1957). For the analysis of the fatty acid profiles of the LTL muscle, fatty acid methyl esters (FAMEs) were prepared by methylation with cold methanolic solution of potassium hydroxide (Cert, Moreda, & Pérez-Camino, 2000). FAMEs were analyzed by gas chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethyleneglycol capillary column (Supelcowax-10, Supelco, Bellefonte, Pa., U.S.A.) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). Oven temperature ramp was 180 °C to 250 °C. Injector and detector temperatures were 250 °C. Carrier gas was Helio with a constant pressure flow of 22 psi. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, Mo., U.S.A.). Results were expressed as percentage of the total fatty acids analyzed.

2.2.2. Tocopherol quantification

Prior to analysis, IMF and ether extractives from feeds were dissolved in isopropanol (1:10, v/v). Tocopherol determination was performed on a Shimadzu "Prominence" HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a quaternary solvent delivery system (LC-20AD), DGU-20AS on-line degasser, SIL-20A auto-sampler, RF-10A XL fluorescence detector, and CBM-20A system controller. Separation was made on a reversed-phase C18 column (150 mm length × 4.6 mm i.d., 5 μm particle diameter) manufactured by Phenomenex (USA) with the mobile phase being methanol:water (97:3 v/v) at a flow rate of 1.5 mL min^{-1}, and peaks were registered at 285 and 335 nm as excitation and emission wavelength, respectively. The mobile phases were filtered by a Millipore vacuum filtration system equipped with a 0.45 μm pore size filter. Samples were injected (2 μL) by the aid of the auto-sampler. Identification and quantification of the peaks were done by comparison with α-tocopherol and γ-tocopherol standards (0.2–14 μg/mL). Results were expressed as μg of α- or γ-tocopherol/g fresh matter.

2.2.3. Objective color measurement

Surface color measurements of loin slices at days 0, 3, 6, 9 and 12 days of storage were performed using a Minolta Chromameter CR-400 (Minolta Camera Corp., Meter Division, Ramsey, N.J., U.S.A.), which consisted of a measuring head (CR-400), with an 10 mm diameter measuring area and a data processor. Before each measuring session the chromameter was calibrated on the CIE color space (CIE, 1978) system using a white tile. One measurement consisted of three consecutive flashes of illumination to obtain a mean value. Color measurements were made at room temperature (approximately 22 °C) with illuminant D65 and a 0° angle observer. The L*a*b* values were recorded from the average across each loin slice surface. The L*, a* and b* values (CIE L*a*b* color system) were assessed as a measure of respectively lightness, redness and yellowness; through them Chroma and Hue values were calculated. Numerical total color differences (E) were calculated to assess the total color change undergone by slice loin as a result of feeding type of pigs (ΔE_{C-T}) and the days of storage of slice loins (ΔE_{0-3}), (ΔE_{3-6}), (ΔE_{6-9}), and (ΔE_{9-12}). Therefore, ΔE_{C-T} was calculated between samples from the C group (C) and T group (T), ΔE_{0-3} was calculated between 0 (0) and 3 (3) days of storage, ΔE_{3-6} was calculated between 3 (3) and 6 (6) days of storage, ΔE_{6-9} was calculated between 6 (6) and 9 (9) days of storage and ΔE_{9-12} was calculated between 9 (9) and 12 (12) days of storage as follows:

$$\Delta E_{C-T} = [(L^*_T - L^*_C)^2 + (a^*_T - a^*_C)^2 + (b^*_T - b^*_C)^2]^{1/2}$$
$$\Delta E_{0-3} = [(L^*_3 - L^*_0)^2 + (a^*_3 - a^*_0)^2 + (b^*_3 - b^*_0)^2]^{1/2}$$
$$\Delta E_{3-6} = [(L^*_6 - L^*_3)^2 + (a^*_6 - a^*_3)^2 + (b^*_6 - b^*_3)^2]^{1/2}$$
$$\Delta E_{6-9} = [(L^*_9 - L^*_6)^2 + (a^*_9 - a^*_6)^2 + (b^*_9 - b^*_6)^2]^{1/2}$$
$$\Delta E_{9-12} = [(L^*_{12} - L^*_9)^2 + (a^*_{12} - a^*_9)^2 + (b^*_{12} - b^*_9)^2]^{1/2}.$$

2.2.4. Thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were assessed using the method described by Ganhão, Estévez, and Morcuende (2011) with some modifications. 5 g of fresh loin was dispensed in cone plastic tubes and homogenized with 15 mL perchloric acid (3.86%) and 0.5 mL BHT (4.2% in ethanol). During homogenization, the plastic tubes were immersed in an ice bath to minimize oxidative reactions during extraction of TBARS. The mix was filtered and centrifuged (3000 rpm for 4 min) and 2 mL aliquots were mixed with 2 mL TBA (0.02 M) in test tubes. The tubes were placed in a boiling water bath (100 °C) for 45 min together with the tubes from the standard curve. After cooling, the absorbance was measured at 532 nm. The standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP) solutions in 3.86% perchloric acid. Lipid oxidation of LTL samples was measured at 0, 3, 6, 9 and 12 days of refrigerated storage.

2.2.5. Protein carbonyl quantification

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with dinitrophenylhydrazine (DNPH) according to the procedure described by Ganhão, Morcuende, and Estévez (2010) with slight modifications. Muscle samples were cut into 0.5 cm^3 cubes and lyophilized for 24 h in a Telstar Lyoquest equipment (T[a] condenser: −50 °C; pressure: 0.001 mBar) to improve homogenization and subsequent sampling. Lyophilized loin (1 g) was minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturrax homogenizer for 30 s. A total of 2 equal aliquots of 0.15 mL were taken from the homogenates and dispensed in 2 mL Eppendorf tubes. Proteins were precipitated by cold 10% trichloroacetic acid (TCA) (1 mL) and subsequent centrifugation for 5 min at 2236 *g*. One pellet was TREATED with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed twice with 1 mL ethanol:ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were carefully drained and dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 358 *g* to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using bovine serum albumin as standard. The amount of carbonyls was expressed as nanomol of carbonyl per milligram of protein using an absorption coefficient of 21 $nM^{-1}\ cm^{-1}$ at 370 nm for protein hydrazones. The same samples for the TBARS measurement were used.

2.3. Statistical analysis

All analyses were performed in quintuplicate in each LTL muscle from each animal (n = 8). To assess significant differences between the CONTROL group and the TREATED group a Student "t" test for independent samples was used (SPSS, 1999). The General Linear Model with repeated measures was used to analyze the data of meat color stability, lipid oxidation (TBARS) and protein oxidation (protein carbonyls concentration) over time of refrigerated storage. In the mixed statistical model, the individual pig was considered as a random effect, while the dietary treatment (CONTROL vs TREATED), storage time (days 0, 3, 6, 9 or 12), and the Diet × Time interaction were considered as fixed effects. The Tukey's test was used for multiple comparisons of the means. The significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Impact of dietary avocado on LTL muscle composition

Table 2 displays the proximate composition of muscle LTL from pigs fattened with a CONTROL diet and a diet TREATED with avocado waste. The protein, IMF and moisture contents in LTL muscles were significantly affected by the finishing diet. In particular, pigs TREATED with the avocado waste had significantly smaller amount of IMF and as a result, significantly higher moisture contents. The lower tendency of animals fed on the avocado waste to deposit fat was unexpected given the higher fat, energy and daily intake (1.49 ± 0.23 kg/day vs 2.04 ± 0.24 kg/day for CONTROL and TREATED, respectively) in the TREATED group compared to the CONTROL counterparts. It is generally known that IMF can be promoted by dietary means typically involving a reduction in protein/energy intake (Hocquette et al., 2010). On the other hand, increasing the gross feed energy by increasing dietary fat and keeping an iso-protein level, has a negligible effect on IMF as reported by D'Souza, Pethick, Dunshea, Pluske, and Mullan (2007). According to the present results, including avocado waste in an iso-protein diet leads to a significant reduction in IMF content of LTL muscles. These results have no precedent in the literature since avocado waste has not been studied before in pig feeding and its impact on pork quality was unknown. The relative roles of direct deposition of dietary fat and endogenous synthesis and the regulation of such synthesis by diet components are key factors in pig lipid metabolism. The underlying mechanisms of these complex physiological processes are not well understood. This is particularly applicable to IMF as this depot is not just

Table 2
Chemical composition and fatty acid profile of muscle *M. longissimus thoracis et lumborum* of pigs fed a control diet (CONTROL) and pigs fed an experimental avocado diet (TREATED).

	CONTROL		TREATED		
	Mean	SD	Mean	SD	p[a]
Moisture (g/100 g fresh loin)	71.16	1.40	73.92	1.45	<**0.001**
Protein (g/100 g fresh loin)	24.55	1.58	25.80	0.92	**0.043**
IMF (g/100 g fresh loin)	4.08	1.02	2.07	0.66	**0.004**
Ash (g/100 g fresh loin)	0.99	0.05	1.19	0.03	0.150
γ-Tocopherol (μg/g fresh loin)	3.34	0.39	3.11	0.42	0.464
α-Tocopherol (μg/g fresh loin)	3.70	0.83	5.62	0.92	**0.021**
Fatty acids (% of total fatty acids)					
C12	0.10	0.02	0.07	0.01	**0.019**
C14	1.83	0.27	1.36	0.13	**0.010**
C16	29.32	0.91	26.48	0.84	**0.002**
C17	0.24	0.06	0.19	0.03	0.102
C18	9.83	1.01	9.74	0.75	0.885
C20	0.13	0.03	0.10	0.01	0.072
Σ SFA	41.45	1.15	37.94	0.75	<**0.001**
C16:1 (n−7)	5.20	0.46	5.09	0.76	0.806
C17:1 (n−7)	0.28	0.18	0.24	0.03	0.683
C18:1 (n−9)	46.33	0.98	46.48	1.48	0.864
C20:1 (n−9)	0.70	0.11	0.55	0.07	**0.040**
Σ MUFA	52.50	1.36	52.36	1.61	0.894
C18:2 (n−6)	4.67	0.97	7.61	0.71	**0.001**
C18:3 (n−6)	0.06	0.01	0.07	0.02	0.346
C18:3 (n−3)	0.17	0.05	0.29	0.08	**0.029**
C20:2 (n−6)	0.13	0.03	0.18	0.05	0.105
C20:4 (n−6)	0.78	0.21	1.11	0.53	0.289
C20:3 (n−3)	0.04	0.02	0.04	0.01	0.658
C20:5 (n−3)	0.09	0.01	0.13	0.04	0.122
C22:2 (n−6)	0.14	0.02	0.19	0.03	**0.050**
C22:6 (n−3)	0.06	0.02	0.15	0.03	**0.002**
Σ PUFA	6.14	1.00	9.78	1.04	**0.001**
Σ LC-PUFA	1.24	0.16	1.88	0.27	**0.004**
SFA/UFA	0.71	0.03	0.61	0.02	<**0.001**
n−6/n−3	17.15	2.45	12.26	1.53	0.066

IMF: intramuscular fat; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LC-PUFA: long-chain PUFA.
[a] Significance level in student "T"-test. Bold p-values denote significant differences ($p \leq 0.05$).

an ectopic location of subcutaneous lipids, but is characterized by specific metabolic features governed by particular gene expression pathways (Hocquette et al., 2010). In fact, profuse literature indicates that IMF is less affected than back-fat thickness by differences in diet (Hocquette et al., 2010). This observation, however, may not be applicable to the present results. In contrast to previous studies in which feeding low protein/energy ratios to fattening pigs led to an increase in IMF in diverse muscles (Goerl, Eilert, Mandigo, Chen, & Miller, 1995; Ventanas, Ventanas, Tovar, García, & Estévez, 2007) the present results may not be explained by such ratio. The main source of crude energy in the diet TREATED with avocado was the avocado oil instead of the carbohydrates from the diet in the study carried out by Ventanas et al. (2007). It is hence likely that the avocado oil and in particular, its fatty acid profile, had an influence on lipid metabolism and IMF depot. Previous studies carried out by Isabel, Cordero, Olivares, Daza, and Lopez-Bote (2014) in pigs and Sanz, Flores, Perez De Ayala, and Lopez-Bote (1999) in chickens provide support to the aforementioned hypothesis. According to these studies, feeding on diets enriched in unsaturated fatty acids (i.e. linoleic acid) leads to lower fat deposition including lower IMF contents. Recently, the hypolipidemic effect of avocado oil has been proven in rats fed a high-fat diet (Padmanabhan & Arumugam, 2014). The IMF content has relevant consequences for meat quality in terms of eating quality traits, nutritional value and caloric delivery to humans. It is generally known that IMF has a positive influence on pork juiciness and overall flavor while IMF reduction compromises consumer acceptability of pig meat (Lawrie, 1998). The IMF content in the LTL muscles from pigs fed avocado may be in the limit to guarantee pork eating quality (Lawrie, 1998). On the other hand, a significant reduction of the total lipid content in loins from pigs fed on avocado may be seen as an advantage as current consumer's trends indicate preferences to leaner pork (Ngapo, Martin, & Dransfield, 2007).

The feeding background affected the level of α-tocopherol in LTL muscle. TREATED pigs had significantly higher levels of α-tocopherol than those from CONTROL pigs. This result could be explained because avocado tissues including the peel, the seed and the pulp are rich in diverse natural antioxidants such as α-tocopherol (Rodríguez-Carpena et al., 2011; Wang et al., 2010). Vitamin E cannot be synthetized by animals and has to be provided by diet, hence dietary Vitamin E is commonly supplemented as acetate ester of all-rac-α-tocopherol (Wood & Enser, 1997). The higher α-tocopherol level in TREATED pigs was expected as a reflection of the tocopherol concentration in the diet in agreement with other reports (Ventanas et al., 2007; Wood & Enser, 1997). This finding is particularly important because α-tocopherol has been proven to extend the shelf life of pork acting post-mortem as an inhibitor of lipid oxidation and an enhancer of color stability during chilled display of fresh pork (Haak et al., 2006; Wood & Enser, 1997). On the same line, dietary tocopherols have other benefits such as controlling protein and cholesterol oxidation (Haak et al., 2006), and improving sensory and technological features such as water holding, juiciness and pork flavor (Ventanas et al., 2007).

3.2. Impact of dietary avocado on LTL lipid composition

Significant differences were found for some major fatty acids since pigs fed CONTROL diet had higher levels of saturated (lauric, myristic and palmitic acids) fatty acids and significantly lower of polyunsaturated (linoleic acid, alpha-linolenic, docosadienoic and docosahexaenoic acids) fatty acids compared to samples from pigs fed on the avocado paste (Table 2). IMF from TREATED pigs showed higher long chain polyunsaturated fatty acid than that from CONTROL pigs. As expected, the saturated/unsaturated ratio was higher in pigs fed on the CONTROL diet. As reported by Wood and Enser (1997), dietary fatty acids are absorbed unchanged into the blood stream in monogastric animals and incorporated from there into tissues. Hence, meat fatty acid composition can be changed through dietary means. Avocado pulp and oil have high content in unsaturated fatty acids (Rodríguez-Carpena et al., 2011) and hence the differences found in muscles from both treatments were expected and consistent with the fatty acid composition of the diets. The linoleic acid, in particular, cannot be synthesized and tissue concentrations respond rapidly to dietary changes (Wood & Enser, 1997). To this regard, pigs fed the TREATED diet had the higher levels of linoleic acid in IMF because linoleic acid is one of the most abundant in the avocado oil (Rodríguez-Carpena et al., 2011). According to Wood and Enser (1997) increased deposition of α-linolenic acid could lead to increased synthesis of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) through multiple steps of desaturation and elongation. Consistently, in this study, DHA was significantly higher in TREATED pigs than in the CONTROL counterparts. In agreement with our results, Morel, Leong, Nuijten, Purchas, and Wilkinson (2013) reported increased levels of EPA and DHA levels in back-fat and loins of pigs fed fish oil. Conversely, Durand-Montgé, Realini, Barroeta, Lizardo, and Esteve-Garcia (2010) reported EPA increases but no DHA in pigs with a diet high in linolenic acid (>9% linseed oil).

Increasing the degree of unsaturation is linked to fat softness, which is generally regarded as a drawback from a meat processing perspective (Lawrie, 1998). On the other hand, the long-chain PUFA, EPA, DPA and DHA offer a wide range of potential health benefits and are commonly recognized as essential nutrients in the human diet. Taking this into consideration, the fatty acid composition of IMF from TREATED pigs is associated with better nutritional value compared with pigs fed CONTROL diet. As demonstrated in this and other studies (Wood & Enser, 1997.; Ventanas et al., 2007; Durand-Montgé et al., 2010) manipulation of pig feeds provides an effective method for modifying the fatty acid composition on pig fat depots, and thereby, modify the human dietary fat intake from pork toward healthier profiles. It is however, worth mentioning that further processing and culinary treatment can largely modify the fatty acid composition of fresh meat (Bonoli, Cabani, Rodríguez-Estrada, & Lercker, 2008).

3.3. Impact of dietary avocado on lipid oxidation during chilled storage of LTL muscle

In our study, the occurrence of lipid oxidation during chilled storage of fresh loins led to an ongoing accumulation of TBARS as shown in Fig. 1. TBARS concentration increased in LTL along with chilled storage in both groups of samples. In LTL muscles from CONTROL pigs TBARS increased from 0.18 to 0.38 mg MDA/Kg of fresh loin. The amount of TBARS in LTL muscle from pigs fed the TREATED diet was significantly lower than in muscles from CONTROL pigs during the entire chilled storage. An increase of TBARS during chilled storage of fresh pork was similarly reported by Estévez, Morcuende, and Cava (2003); Lund, Hviid, Claudi-Magnussen, and Skibsted (2008), and Rodríguez-Carpena et al. (2011). Oxidative processes lead to the degradation of lipids and proteins and are one of the primary causes of quality deterioration in meat, including loss of flavor, color, texture deterioration and

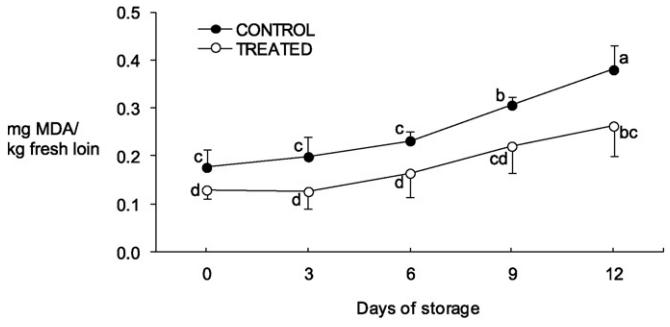

Fig. 1. Evolution of TBARS in muscle LTL of pigs fed a control diet (CONTROL) and pigs fed an experimental avocado diet (TREATED) during refrigerated storage (+4 °C/12 days). Footnote: a–d: Different letters denote statistical significance between samples.

nutritional value, besides generation of toxic compounds (Kanner, 1994). Since the onset of oxidative reactions is the outcome of the imbalance between pro-oxidant and antioxidant factors, high concentrations of unsaturated fatty acids may increase the susceptibility of meat to undergo oxidation (Wood & Enser, 1997) while increases in tocopherol depots protect muscle tissue against oxidative reactions (Wood & Enser, 1997). Whereas the highly unsaturated fat in LTL from TREATED pigs predicted larger oxidation levels, the present results indicate that these samples had effective antioxidant compounds that kept TBARS values at low levels. To this regard, the dietary enrichment of α-tocopherol probably enhanced the oxidative stability of LTL muscle from TREATED pigs. Many other authors reported that providing α-tocopherol-enriched feeds to pigs led to a decrease of TBARS during chilled storage of fresh pork (Haak et al., 2006; Lund et al., 2008). The direct addition of avocado oil and avocado extracts (from peel and seed) to porcine patties has been proven to be an effective strategy to inhibit lipid oxidation during storage and processing of these products (Rodríguez-Carpena et al., 2011; Rodríguez-Carpena et al., 2012). The authors attributed the antioxidant properties to diverse bioactive compounds including tocopherols, chlorophylls and polyphenols. It is, however, unknown to which extent the other natural antioxidants found in great quantities in the avocado fruit (i.e. chlorophylls and polyphenols) are bioavailable, potentially accumulated and still bioactive as such or after metabolization in animal tissues. Recent studies have described the beneficial effect of feeding phenolic-rich diets on the oxidative stability of pork (Zhang et al., 2015).

Avoiding lipid oxidation is a relevant goal because their products are linked with negative effects to human health such as carcinogenesis, cytotoxic, hepatotoxic and mutagenic process, among others (Kanner, 1994). The results from this study show that feeding pigs with avocado waste protected LTL muscle against lipid oxidation and that may improve the quality of fresh pork in terms of safety, and sensory and nutritional quality.

3.4. Impact of dietary avocado on color stability during chilled storage of LTL muscle

Fig. 2 shows the evolution of instrumental lightness (A), redness (B) and yellowness (C) during chilled storage of fresh LTL muscles. The L* value (Fig. 2A) of LTL muscles from pigs showed a significant decrease upon completion of storage (day 0 vs. day 12) while this parameter did not change over time in samples from pigs fed the TREATED diet. At the end of the chilled storage (day 12), the LTL muscles from TREATED pigs had higher L* values than muscles from pigs fed the CONTROL diet. Redness (Fig. 2B) of LTL muscles varied throughout the chilled storage and no significant differences between groups were observed. Evolution of yellowness (Fig. 2C) was also variable during storage but in this case, significant differences were found between groups. At day 12 of storage, yellowness was higher in LTL muscle from pigs fed the CONTROL diet than in muscles from pigs fed the TREATED diet.

Fresh meat discoloration occurs during chilled storage and it is generally associated with metmyoglobin accumulation in the meat surface (Faustman, Mancini, & Suman, 2010). Discoloration process is typically defined by decreases of lightness and redness and increase of yellowness. The discoloration of chilled pork is usually linked to the onset of lipid oxidative reactions (Estévez et al., 2003; Faustman et al., 2010; Haak et al., 2006). The increase of yellowness in particular may be due to the accumulation of Schiff pigments from lipid to protein complexes as a result of oxidative stress according to Chelh, Gatellier, and Sante-Lhoutellier (2007). The present data leads to similar conclusions since LTL muscle from pigs fed the CONTROL diet had higher lipid oxidation rates and a more intense discoloration during chilled storage than the TREATED counterparts. The numerical total color difference (ΔE) between pigs fed the CONTROL and TREATED diets at the days 0, 3, 6, 9 and 12 were 0.95, 0.79, 0.94, 1.98 and 5.92, respectively. The ΔE values calculated between samples at each day of the refrigerated storage

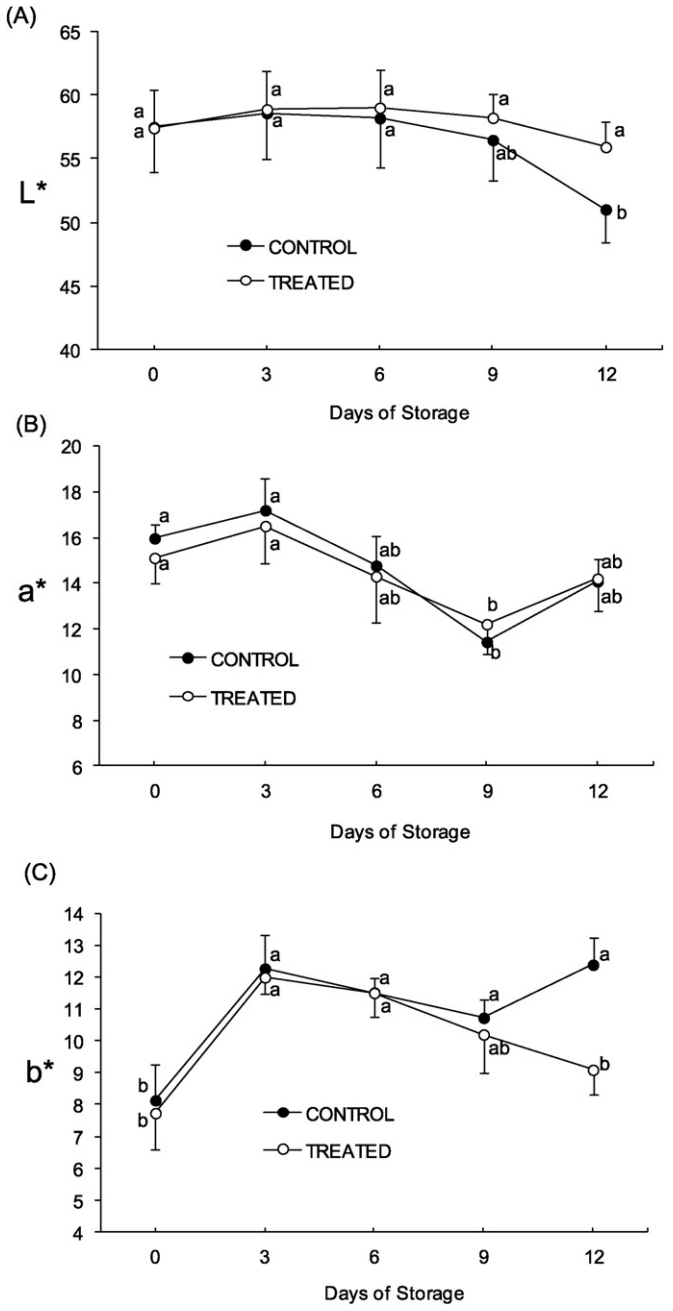

Fig. 2. Evolution of instrumental lightness (A), redness (B) and yellowness (C) in muscle LTL of pigs fed a control diet (CONTROL) and pigs fed an experimental avocado diet (TREATED) during refrigerated storage (+4 °C/12 days). Footnote: a–b: Different letters denote statistical significance between samples.

confirmed the significant impact of the dietary background on the color displayed by LTL muscles during chilled storage. The antioxidant defense provided by the avocado-enriched diet could have also protected heme pigments against oxidation. Other authors have previously reported the effectiveness of dietary α-tocopherol in minimizing the color changes occurred during chill storage of pork (Estévez et al., 2003). It is worth mentioning that according to Francis and Clydesdale (1975), ΔE values higher than 2 can be considered as a noticeable visual difference between two given meat samples. Hence, consumers may be able to appreciate color differences between groups of samples at day 12 of chilled storage. The stability of color in pork during chill storage is especially important because consumers regard discoloration as an indicator of freshness and wholesomeness (Faustman et al., 2010).

3.5. Impact of dietary avocado on protein oxidation during chilled storage of LTL muscle

The evolution of protein oxidation during the chilled storage of LTL muscles is displayed in Fig. 3. The oxidative damage to proteins occurred during chilled storage of LTL muscles as assessed by the accumulation protein carbonyls. Significant differences between treatments were found at days 9 and 12 of chilled storage. LTL muscle slices from pigs fed the CONTROL diet had significantly higher levels of protein carbonyls than pigs fed the TREATED diet. The concentration of protein carbonyls in pork subjected to chilled storage is highly variable as reviewed from literature by Estevéz (2011) and ranges from 1 to 3 nmol carbonyls/mg protein. The formation of protein carbonyls in meat respond to the oxidative δ-deamination of alkaline amino acids induced by diverse reactive oxygen species in the presence of transition metals such as iron (Estevéz, 2011). While the mechanisms of protein carbonylation may differ from that of lipid oxidation (Soladoye et al., 2015) the occurrence of both phenomena is sometimes described to occur simultaneously. In fact, some authors reported strong links and significant correlations between lipid and protein oxidation in pork (Lund et al., 2008, 2011; Ventanas et al., 2007). Consistently, results from this study show that the occurrence of protein oxidation followed a similar pattern to the oxidative degradation of lipids. In this case, it is plausible to hypothesize that lipids and proteins were likely affected by similar pro- and antioxidant factors. On this line, protein and lipid oxidation was found to be inhibited in chilled meat from pigs fed tocopherol-enriched diets (Haak et al., 2006). Regarding avocado fruit, in particular, Rodríguez-Carpena et al. (2011) and Utrera et al. (2012) reported that the addition of phenolic-rich extracts from avocado seed and peel alleviated both lipid and protein oxidation during storage and processing of porcine patties. According to the present study, dietary avocado may also have similar antioxidant benefits in porcine muscles. Unlike the direct addition of bioactive compounds to food systems, the supply of avocado to livestock is profitable from a productive point of view, innocuous for consumers and beneficial for meat quality.

The antioxidant protection of proteins would also contribute to these benefits since oxidized proteins have been reported to display impaired functionality and digestibility and pork with high protein oxidation rates is tougher and less juicy than pork in which these oxidative reactions are controlled (Lund et al., 2008; Lund et al., 2011; Soladoye et al., 2015).

4. Conclusions

The usage of avocado waste for finishing pigs seems to have benefits in terms of meat quality and shelf life. The modifications in the composition of LTL muscles in terms of lower IMF, more unsaturated fat and larger tocopherols concentrations may be regarded as positive effects of dietary avocado. The protection of muscle lipids and proteins against oxidative reactions by the dietary avocado paste has also benefits in terms of meat quality and safety. The contribution of dietary avocado with other bioactive phytochemicals different from tocopherols may be investigated as they may also account for some of the benefits observed in terms of oxidative stability.

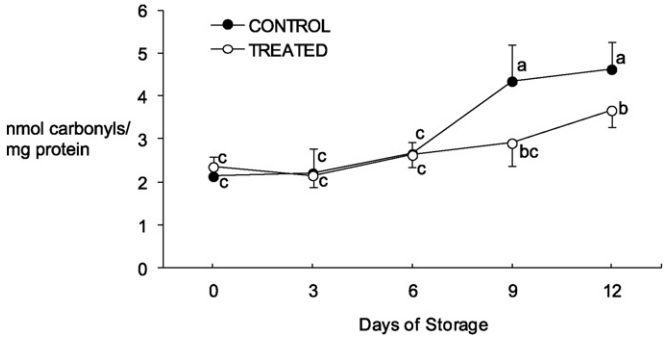

Fig. 3. Evolution of protein carbonyls in muscle LTL of pigs fed a control diet (CONTROL) and pigs fed an experimental avocado diet (TREATED) during refrigerated storage (+4 °C/12 days). Footnote: a–c: Different letters denote statistical significance between samples.

References

AOAC (2000). *Official Methods of Analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Bonoli, M., Cabani, M.F., Rodríguez-Estrada, M.T., & Lercker, G. (2008). Effect of processing technology on the quality and composition of lipids of precooked chicken patties. *International Journal of Food Science and Technology*, *43*, 296–308.

Cert, A., Moreda, W., & Pérez-Camino, M.C. (2000). Methods of preparation of fatty acid methyl esters (FAME). Statistical assessment of the precision characteristics from a collaborative trial. *Grasas y Aceites*, *51*(6), 447–456.

Chelh, I., Gatellier, P., & Sante-Lhoutellier, V. (2007). Characterisation of fluorescent Schiff bases formed during oxidation of pig myofibrils. *Meat Science*, *76*, 210–215.

CIE (1978). Recommendations on uniform color spaces–color equations, psychometric color terms. *Supp nr 2 to CIE Publ nr 15 (E-1.3.L) 1971 (9TC-1-3)*. Paris, France: CIE.

D'Souza, D.N., Pethick, D.W., Dunshea, F.R., Pluske, J.R., & Mullan, B.P. (2007). Nutritional manipulation increases intramuscular fat levels in the longissimus muscle of female finisher pigs. *Australian Journal of Agricultural Research*, *54*, 745–749.

Durand-Montgé, P., Realini, C.E., Barroeta, A.C., Lizardo, R.G., & Esteve-Garcia, E. (2010). *De novo* fatty acid synthesis and balance of fatty acids of pigs fed different fat sources. *Livestock Science*, *132*, 157–164.

Esteban, M.B., García, A.J., Ramos, P., & Márquez, M.C. (2007). Evaluation of fruit–vegetable and fish wastes as alternative feedstuffs in pig diets. *Waste Management*, *27*, 193–200.

Estevéz, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science*, *89*, 259–279.

Estévez, M., Morcuende, D., & Cava, R. (2003). Oxidative and colour changes in meat from three lines of free-range reared Iberian pigs slaughtered at 90 kg live weight and from industrial pig during refrigerated storage. *Meat Science*, *65*, 1139–1146.

FAOSTAT (2008). *Statistical Databases, Agricultural Datahttp://faostat.fao.org/*

Faustman, C., Mancini, R., & Suman, S.P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science*, *86*, 86–94.

Folch, L., Lees, M., & Sloane-Stanley, C.H. (1957). A simple method for the isolation and purification of total lipid from animal tissues. *The Journal of Biological Chemistry*, *226*, 497–509.

Francis, F.J., & Clydesdale, F.M. (1975). *Food Colorimetry: Theory and Applications*. Westport, CT: AVI Publishing.

Ganhão, R., Estévez, M., & Morcuende, D. (2011). Suitability of the TBA method for assessing lipid oxidation in meat system with added phenolic-rich material. *Food Chemistry*, *126*, 772–778.

Ganhão, R., Morcuende, D., & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on color and texture deterioration during chill storage. *Meat Science*, *85*, 402–409.

Garg, A.K., Pathak, N.N., Anjaneyulu, A.S.R., & Lakshmanan, V. (1986). Utilization of cane molasses as a source of energy in the diet of young pigs. *Agricultural Wastes*, *17*, 225–228.

Goerl, K.F., Eilert, S.J., Mandigo, R.W., Chen, H.Y., & Miller, P.S. (1995). Pork characteristics as affected by two populations of swine and six crude protein levels. *Journal of Animal Science*, *73*(12), 3621–3626.

Grageola, F., Sangines, L., Díaz, C., Gómez, A., Cervantes, M., Lemus, C., & Ly, J. (2010). The effect of breed and dietary level of avocado fat on the N and energy balance in young pigs. *Journal of Animal and Feed Sciences*, *19*, 37–49.

Haak, L., Raes, K., Smet, K., Claeys, E., Paelinck, H., & De Smet, S. (2006). Effect of dietary antioxidant and fatty acid supply on the oxidative stability of fresh and cooked pork. *Meat Science*, *74*, 476–486.

Hocquette, J.F., Gondret, F., Baza, E., Mdale, F., Jurie, C., & Pethick, D.W. (2010). Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal*, *4*, 303–319.

Isabel, B., Cordero, G., Olivares, A., Daza, A., & Lopez-Bote, C. (2014). Differential response of Iberian and lean pig crossbreeds to dietary linoleic acid administration. *Spanish Journal of Agricultural Research*, *12*(2), 419–426.

Kanner, J. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Science*, *36*, 169–189.

Kjos, N.P., Øverland, M., Arnkværn, B.E., & Sørheim, O. (2000). Food waste products in diets for growing–finishing pigs: Effect on growth performance, carcass characteristics and meat quality. *Acta Agriculturae Scandinavica Section A Animal Science*, *50*, 193–204.

Kumar, A., Roy, B., Lakhani, G.P., & Jain, A. (2014). Evaluation of dried bread waste as feedstuff for growing crossbred pigs. *Veterinary World*, *7*(9), 698–701.

Lawrie, R.A. (1998). The eating quality of meat. In R.A. Lawrie (Ed.), *Meat Science* (6th ed.). Cambridge: Woodhead Publishing.

Lebret, B. (2008). Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal*, *2*(10), 1548–1558.

Lund, M.N., Heinonen, M., Baron, C.P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research*, *55*, 83–95.

Lund, M.N., Hviid, M.S., Claudi-Magnussen, C., & Skibsted, L.H. (2008). Effects of dietary soybean oil on lipid and protein oxidation in pork patties during chill storage. *Meat Science*, *79*, 727–733.

Morel, P.C.H., Leong, J., Nuijten, W.G.M., Purchas, R.W., & Wilkinson, B.H.P. (2013). Effect of lipid type on growth performance, meat quality and the content of long chain n − 3 fatty acids in pork meat. *Meat Science*, *95*, 151–159.

Myer, R.O., Brendemuh, J.H., & Johnson, D.D. (1999). Evaluation of dehydrated restaurant food waste products as feedstuffs for finishing pigs. *Journal of Animal Science*, *77*, 685–692.

National Research Centre on Pig (NRC) (2011). Annual progress report. *National Research Centre on Pigs, ICAR, Rani, Guwahati, Assam* (pp. 1–4).

Ngapo, T.M., Martin, J.F., & Dransfield, E. (2007). International preferences for pork appearance: I. Consumer choices. *Food Quality and Preference*, *18*, 26–36.

Padmanabhan, M., & Arumugam, G. (2014). Effect of *Persea americana* (avocado) fruit extract on the level of expression of adiponectin and PPAR-γ in rats subjected to experimental hyperlipidemia and obesity. *Journal of Complementary and Integrative Medicine*, *11*(2), 107–119.

Paiva-Martinsa, F., Barbosa, S., Pinheiro, V., Mourãoc, J.L., & Outor-Monteiro, D. (2009). The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. *Meat Science*, *82*, 438–443.

Rodríguez-Carpena, J.G., Morcuende, D., & Estévez, M. (2011). Avocado by-products as inhibitors of color deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage. *Meat Science*, *89*, 166–173.

Rodríguez-Carpena, J.G., Morcuende, D., Andrade, M.J., Kylli, P., & Estevéz, M. (2011). Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, *59*, 5625–5635.

Rodríguez-Carpena, J.G., Morcuende, D., & Estévez, M. (2012). Avocado, sunflower and olive oils as replacers of pork back-fat in burger patties: Effect on lipid composition, oxidative stability and quality traits. *Meat Science*, *90*, 106–115.

Sanz, M., Flores, A., Perez De Ayala, P., & Lopez-Bote, C.J. (1999). Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *British Poultry Science*, *40*(1), 95–101.

Sikka, S.S., & Chawla, J.S. (1986). Effect of feeding spent coffee grounds on the feedlot performance and carcass quality of fattening pigs. *Agricultural Wastes*, *18*, 305–308.

Soladoye, O.P., Juárez, M.L., Aalhus, J.L., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, *14*(2), 106–122.

SPSS (1999). *SPSS Base 10.0. User Manual: Application Guide*. Republic of Ireland: SPSS.

Utrera, M., Rodríguez-Carpena, J.G., Morcuende, D., & Estévez, M. (2012). Formation of lysine-derived oxidation products and loss of tryptophan during processing of porcine patties with added avocado byproducts. *Journal of Agricultural and Food Chemistry*, *60*, 3917–3926.

Ventanas, S., Ventanas, J., Tovar, J., García, C., & Estévez, M. (2007). Extensive feeding versus oleic acid and tocopherol enriched mixed diets for the production of Iberian dry-cured hams: Effect on chemical composition, oxidative status and sensory traits. *Meat Science*, *77*, 246–256.

Wang, W., Terrell, R.B., & Liwei, G. (2010). Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food Chemistry*, *122*, 1193–1198.

Westendorf, M.L., Zirkel, E.W., & Gordon, R. (1996). Feeding food or table waste to livestock. *Professional Animal Science*, *12*, 129–137.

Wood, J.D., & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, *78*, 49–60.

Wood, J.D., Nute, G.R., Richardson, R.I., Whittington, F.M., Southwood, O., Plastow, G., ... Chang, K.C. (2004). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, *67*, 651–667.

Zhang, C., Luo, J., Yu, B., Zheng, P., Huang, Z., Mao, X., ... Chen, D. (2015). Dietary resveratrol supplementation improves meat quality of finishing pigs through changing muscle fiber characteristics and antioxidative status. *Meat Science*, *102*, 15–21.