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Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork ☆

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ABSTRACT

The effects of dietary supplementation in pigs with plant extract (PE) from Lippia spp., titrated in verbascoside (5 mg/kg feed), from weaning to slaughter (166 days), on carcass characteristics, meat quality, collagen characteristics, oxidative stability and sensory attributes of *Longissimus dorsi* (LD) muscle were examined. Ten pigs per treatment were slaughter at a live weight of 109.5 ± 1.4 kg. No influence on carcass characteristics, LD meat quality parameters and collagen characteristics were observed. Dietary PE increased ($P < 0.001$) α-tocopherol levels in LD muscle. Raw LD of pig fed PE showed lower ($P < 0.001$) lipid oxidation levels than controls. A reduction ($P = 0.05$) of fat odor and rancid flavor intensity in cooked LD muscle stored at 4 °C for 24 h was observed in the treated group. This study shows that PE is an effective antioxidant in pork meat, enhancing oxidative status and sensory attributes, without affecting other meat quality parameters.

1. Introduction

In recent years considerable attention has been given to the improvement of meat quality. Studies have shown that some feed additives (vitamins, mineral and antioxidant) improve pork sensory and nutritional characteristics (Nuernberg et al., 2002; Swigert, McKeith, Carr, Brewer, & Culbertson, 2004). In particular, additives for the control of lipid oxidation in meat and meat products have become increasingly important. Lipid oxidation is a major deteriorative phenomenon that negatively affects flavor, color and nutritional value of meat (Asghar, Gray, Buckley, Pearson, & Boeren, 1988) and is responsible to the potential formation of toxic compounds (Addis & Park, 1989). In addition to microbial spoilage, it causes loss of pork quality and thus determines the shelf life of pork products.

Many synthetic and natural substances have been investigated as potential antioxidants to prevent lipid oxidation. The trend is to decrease the use of synthetic antioxidants due to consumer concerns over safety and toxicity (Coronado, Trout, Dunsea, & Shah, 2002). Therefore, the search for natural additives, especially from plants, has increased in recent years (Meyer, Suhr, Nielsen, & Holm, 2002). Compounds from natural sources such as grains, oilseeds, spices, fruits and vegetables have been investigated (Que, Mao, & Pan, 2006; Sebranek, Sewalt, Robbins, & Houser, 2005).

Some plant extracts contain phenolic compounds that have anti-inflammatory, antimicrobic and antioxidant activities (Pereira, Valentão, Pereira, & Andrade, 2009).

Phenylpropanoid glycosides (PPG), like other phenolic compounds, are powerful antioxidants by direct scavenging of reactive oxygen and nitrogen species, or by acting as chain-breaking peroxyl radical scavengers (Afanasev, 2005). Beside phenolic compounds, verbascoside, shows the highest scavenger activity in the PPG (Wang et al., 1996) and has high antioxidant power in comparison with other phenolic compounds (Aleo, Ricci, Passi, & Cataudella, 2005).

Phenylpropanoids, particularly verbascoside, are the most abundant compounds in Verbenaceae extracts (Pascual, Slowing, Carretero, Sanchez Mata, & Villar, 2001). Previous studies showed that dietary supplementation with plant extracts of Verbenaceae (PE), improved the plasma oxidative status in weaning piglets (Pastorelli, Rossi, & Corino, 2012), in lacaune ewes (Casamassima, Palazzo, Martemucci, Vizzarri, & Corino, 2012) and Italian hares (*Lepus corsicanus*) (Palazzo, Vizzarri, Cinone, Corino, & Casamassima, 2011). Moreover, Rossi, Corino, Pastorelli, Durand, and Prost (2009) found that PE has greater antioxidant power compared to other phenolic compounds and compared to a water soluble analog of vitamin E (trolox).

On this basis the PE containing verbascoside could be used as a dietary supplement in association and/or partial replacement of

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synthetic vitamin E in animal feeding to enhance meat quality and consumer well-being. The literature contains no data on the effects of dietary supplementation of plant extracts of Verbenaceae, titrated in verbascoside, on meat quality.

The objective of the present study was to assess the effectiveness of long term supplementation of porcine diets with PE on carcass characteristics, meat quality parameters, oxidative stability and sensorial properties of *Longissimus dorsi* (LD) muscle. The influence of dietary PE on collagen characteristics and vitamin E content of LD muscle was also determined.

2. Material and methods

2.1. Animals and experimental design

Forty Dalland pigs, half barrows and half females, of average live weight (LW) 7 ± 0.4 kg, were assigned, on the basis of weight and sex, to two dietary treatments: control diet (C) and diet supplemented with plant extract (PE) to obtain 5 mg verbascoside/kg feed. The dose of plant extract in the feed was chosen on the basis of a previous study in piglets (Corino, Rossi, Musella, Cannata & Pastorelli, 2007).

Pigs were fed a corn-based diet with same amount of all-rac-α-tocopheryl acetate (30 mg/kg in the finishing phase; threefold the amount reported by NRC, 2012). The PE supplement contained a water-soluble extract of Verbenaceae (*Lippia* spp.) leaves, prepared on an industrial scale by a standardized procedure that included ultrasonic extraction with 60% aqueous ethyl alcohol followed by spray-drying with maltodextrins. The PE supplement did not contain vitamin E. The phenylpropanoid glycosides and benzoic acid content of the feed supplement, according to a certificate of analysis provided by the manufacturer, was: gallic acid, 1.75 ± 0.07; 3.4-dihydroxybenzoic acid, 0.45 ± 0.04; methyl gallate, 1.91 ± 0.09; isoverbascoside, 0.43 ± 0.04 and verbascoside, 4.47 ± 0.08 g/kg. The quantitative analysis of the phenolic compounds was performed by HPLC–UV–DAD (Rastrelli, personal communication). To avoid oxidation in the feed, the supplement was microencapsulated within a protective matrix of hydrogenated vegetable lipids using spray-cooling (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy). The experimental diets were formulated to meet the requirements for all nutrients for all the breeding phases (NRC, 2012), and were presented for ad libitum consumption. According to the gender, the animals were divided into 10 pens (4 pigs/pen) and reared in an environmentally controlled room.

The animals were cared for following the European Union guidelines (No. 86/609/EEC, 1986) approved by the Italian Ministry of Health (L. 116/92).

2.2. Carcass traits

Ten barrows per treatments were slaughtered at 109.5 ± 1.4 kg LW. After an on-farm fasting period of 8 h, the pigs were transported to the abattoir. The pigs were laired for 4 h with free access to water. Pigs were electrically stunned; following exsanguination, the carcasses were scalded, dehaired and eviscerated. Live weight at slaughter and hot carcass weight were recorded.

Dressing percentage was calculated, and midline backfat depth opposite the first rib, last rib, and last lumbar vertebrae was recorded. pH at 24 h postmortem was determined on LD muscle using a pH meter (HI 9023 microcomputer, Hanna Instruments, Vila do Conde, Portugal). The pH probe was calibrated using two buffers (pH 4.0 and 7.0), and calibration was monitored between samples. Color measurements were performed at 24 h postmortem on LD samples at the last lumbar vertebra, using a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan). The instrument was calibrated on the CIE LAB color space system using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras). The colorimeter had an 8-mm measuring area and was illuminated with a pulsed Xenon arc lamp (illuminat C) at 0° viewing angle. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included.

Longissimus dorsi muscle of all animals was removed from the carcass (after 24 h at 2–4 °C) at the last lumbar vertebra, vacuum-packed, and stored frozen (−20 °C) for chemical composition, vitamin E content, oxidative stability and intramuscular collagen (IMC) analyses. For the physical analyses fresh LD samples were employed.

2.3. Physical analyses

Drip and cooking losses were determined as described by Honikel (1998). A slice of fresh LD muscle (45 ± 2 g), was placed with a cut surface facing down on a grid in a closed plastic container. Drip loss was determined as percentage weight loss after 24 h of storage at 4 °C. For cooking loss determination, a fresh 25 mm thick slice from each sample was weighed (130 ± 5 g), placed in a plastic bag and cooked to an internal temperature of 70 °C in a 75 °C water bath. Internal temperature was monitored during cooking with a hand-held temperature probe. Cooked samples were allowed to cool for 30 min, blotted dry and weighed. The difference between pre- and post-cooking weights was used to calculate the percentage loss during cooking.

The Warner–Bratzler shear force (WBSF) was determined in samples cooled at 4 °C for 24 h after heat-treatment. For each sample, 6 cylindrical cores (Ø 1.25 cm), parallel to the longitudinal orientation of the muscle fibers, were taken. Each core was sheared with a WBSF device attached to an Instron Universal Testing Machine (model 4466; Instron Corp., Canton, MA) with a 50 kg tension/compression load cell using a crosshead speed of 50 mm/min. The maximum force (kg/cm^2) was recorded.

2.4. Chemical composition and cholesterol

Samples of LD of all animals were analyzed for dry matter, nitrogen, fat and ash according to Association of Analytical Chemists methods (AOAC, 2000).

Cholesterol was extracted using the method of Maraschiello, Diaz, and Garcia Regueiro (1996) and then quantified by HPLC. A Kontron HPLC (model 535, Kontron Instruments, Milan, Italy) equipped with a C18 reverse-phase column (250 mm × 4.6 mm × 5 μm; Phenomenex, Torrance, CA) was used. The mobile phase was acetonitrile 2 propanol (55:45 vol/vol) at a flow rate of 1.2 mL/min. The detection wavelength was 210 nm and retention time was 13.89 min.

2.5. Alpha tocopherol content

The levels of vitamin E in the LD muscle were determined and quantified as described by Zapel and Csallany (1983) and then quantified by HPLC (Kontron Instruments, Milan, Italy) model 535 equipped with a C18 reverse-phase column (250 cm × 4.6 mm × 5 μm) (Phenomenex, Torrance, CA). The mobile phase was 100% methanol at a flow rate of 1.5 mL/min. The detection wavelength was 292 nm and retention time was 4.1 min.

2.6. Collagen analyses

The IMC characteristics are responsible for the background toughness of meat (Maiorano, Kapelański, Bocian, Pizzuto, & Kapelańska, 2013). To study IMC characteristics, samples of LD from all pigs were thawed, trimmed of fat and epimysium, lyophilized for 48 h, weighed, and hydrolyzed in Duran tubes in 6 N HCl at 110 °C for 18–20 h (Etherington & Sims, 1981) for determination of hydroxyproline (Woessner, 1961) and crosslinking. IMC concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe & Leach, 1958) and expressed as micrograms of hydroxyproline per milligram of lyophilized tissue. Hydroxylysylpyridinoline (HLP) concentration, the principal nonreducible crosslink of muscle collagen (McCormick,