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The influence of animal nutrition on meat quality

Sabrina Ratti

Tutor:
Prof. Carlo Corino

Coordinator:
Prof. Giovanni Savoini

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CHAPTER 1

Foreword

1.1 Animal nutrition and meat quality

Meat quality is a difficult term to define. Food and Agriculture organization of the United Nations (FAO) define meat quality as the compositional quality (lean to fat ratio) and the palatability factors such as visual appearance, smell, firmness, juiciness, tenderness, and flavour. The nutritional quality of meat is objective yet "eating" quality, as perceived by the consumer, is highly subjective.

The main sensory factors which influence purchase are color and visible fat, while texture and flavour affect the enjoy of consuming meat. The visual aspect of meat include colour parameter, texture and marbling. The importance of these traits differ and are intrinsic indications highly related with consumers' expectations of meat quality (Verbeke et al., 2005).Color is one of the one of the most important factors that affect consumer choice at the point of purchase (Gracia & de Magistris, 2013). Thus, it is very important to improve color stability because it will increase meat products shelf life by increasing the time that meat will be visually accepted by consumers.

Moreover, consumer prefers "healthy" products with high nutritional value in regard of macronutrients such as proteins, and fats, e.g. the composition of poly-unsaturated vs saturated fat. The quantity and the quality of fat depend on several factors such as specie, sex, diet, age, and genotype (Corino et al.,2002).

Generally, meat quality parameters are influenced by several factors, such as breed, genotype, feeding, pre slaughter handling, stunning, slaughter methods, chilling and storage conditions (Rosenvold and Andersen, 2003).

Animal feeding is a key factor for improve meat quality parameters through dietary supplementation with different additives.

1.2 The effect of dietary supplementation with plant extract on meat quality

In last decades great attention has been given to the improvement of meat quality.

Lipid oxidation is a major cause of chemical spoilage in food systems. Different strategies are used to increase meat antioxidant activity reducing the oxidation products with a positive impact on ageing, cancer and cardiovascular disease (Decker & Xu, 1998). Some intervention are aimed to increase the amount of exogenous antioxidants (vitamin polyphenol, microelement) in animal diet reduce the amount of pro-oxidants and/or to modify the oxidisable substrate (fatty acid composition). Among the antioxidant components of muscle tissues, α-tocopherol plays a central role since it is the major endogenous lipid-soluble

chain breaking antioxidant and is one of defensive barriers of muscle against lipid oxidation (Monahan et al.,1992). To delay this process, synthetic antioxidants such as vitamin E, have been successfully used in animal feeding to improve meat quality (Corino, et al 1999a; Corino, et al 1999b) and processed pork products (Zanardi, et al 2000). This is achieved by preventing deteriorative changes linked to oxidative reactions in lipids and protein and improve color stability (Zhang, et al. 2010).

Lipid oxidation can induce changes in sensorial quality, nutritive value, and product functionality, which are perceived as negative by consumers (Yeates, et al.2010). Physical and chemical changes alter meat quality during the conversion of muscle to meat including discoloration, development of odours and textural changes (McMillin, 1996). In fact, antioxidant dietary supplementation in animal present the advantage that living animals may efficiently distribute the compounds throughout the tissues and the resulting enriched meat ensure a high amounts in humans (Bou et al., 2009).

Antioxidants are used to prevent the oxidative deterioration of foods and thus minimizing oxidative damage in humans, enhancing health. In response to recent claims that synthetic antioxidants have the potential to cause toxicological effects and consumers increased interest in purchasing natural products, the meat industry has been seeking sources of natural antioxidants (Karre et al., 2013).

Shelf-life of meat and meat products can be extended by the presence of natural antioxidants coming from different sources such as plant extracts. Many plants and their extracts have been reported to have a high antioxidant anti-inflammatory, antimicrobic capacities due to their content of polyphenolic compound (Moyo et al., 2012; Pereira, et al. 2009). Beside phenolic compounds, verbascoside, shows an high antioxidant power in comparison with other phenolic compounds (Aleo, et al. 2005). (Trial 2, 3).

Several studies have shown that dietary supplementation with plants extract improve animal's antioxidant status and consequently meat and derived product quality. Dietary supplementation with plant extracts containing verbascoside improved the plasma oxidative status in pig (Pastorelli, et al. 2012; Rossi et al. 2013) and in lacaune ewes (Casamassima, et al. 2012). This antioxidant status effect is related to o the increased serum levels of vitamins A and E (Palazzo et al., 2011). Marcinčák et al, (2011) found that poultry dietary supplementation with *Melissa officinalis, Achillea millefolium and Crataegus oxyacantha* positively affect sensory properties and oxidative stability of chilled and frozen chicken, reducing oxidation in thigh and breast muscles stored under chilling (4°C) or freezing conditions (-18°C). Previous study showed that dietary supplementation with natural antioxidants is required to improve the sensory characteristics and shelf life of omega-3 enriched products (Mairesse et al., 2011).

Other studies showed positive effects of active components from oregano, garlic and rosemary on the colour and sensory properties of meat (Cullen et al., 2005; Fasseas et al., 2008; Simitzis et al., 2008; Stetzer et al., 2008; Omojola et al., 2009). In fact, as previously observed the combined use of different antioxidant subtances may have greater effects when compared to single antioxidants, due to the synergic effects of different molecules (McCarthy et al., 2001). (Trial 1)

Previous study found that a long term dietary supplementation with plant extract from Lippia spp., titrated in verbascoside is an effective antioxidant in pork meat, enhancing oxidative status, Vitamin E content and sensory attributes, without affecting other meat quality parameters (Rossi et al., 2013). It was hypothesized tha tplant extract may improve vitamin E status in vivo by protecting α-tocopherol from oxidative decay, thus increasing muscle concentration. A similar result was observed by Iglesias et al., (2012) who reported that the procyanidins could repair oxidized α-tocopherol in the medium-long term, and could delay ascorbic acid depletion in fish muscle.

In order to respond the demand of consumers for safety and high quality meat production, the present thesis is focused on the use of a dietary antioxidant strategy to enhance the healthy benefits of meat and improve the overall quality.

1.3 Genetic impact on meat product

Improvements of meat quality traits with traditional breeding programs are very difficult, because heritability of them is very low. Animal genetics may have a smaller influence on many quality aspects, but in some cases, genetic variation can make a difference. Indeed, genetic variation may affects the propensity of the animal to convert food into fat vs muscle and hence the texture.

For example beef meat colour depend on the concentration of myoglobin and haemoglobin, that are is affected by factors such as age, exercise, diet of the animal, as well as genetic and environmental factors (Livingston & Brown, 1981).

Duroc is usually used as a sire breed due to its higher feed intake, better feed conversion ratio (Raj et al., 2010), excellent growth rate that is important factor for fattening pig production (Suzuki et al., 2002), and better average daily gain (Latorre et al., 2003). Alonso et al. (2009) stated that carcass traits of Duroc lines (progeny of crossbreeding with Duroc) are better than traits of original pure lines without any loss of meat quality. In addition to its advantages in intramuscular fat deposition and carcass traits, Duroc reportedly has a good feed conversion ratio and adequate daily gains that produce a faster growth than other breeds such as Large White pigs, Pietrain and its crossed breeds (Blanchard et al., 1999; Lattore et al., 2003). Furthermore, Cameron et al. (1990) suggested that the

introduction of Duroc to a pig breeding program may help to improve the meat and eating quality.

Reverter, et al., (2003) found a positive genetic correlation between intramuscular fat in beef and tenderness perceived by consumers. Same results was found by Fernandez et al., (1999) who established a positive correlation between texture sensory parameters in meat pork and breeding depending on the quantity of intramuscular fat.Thus genetic selection can increase the quantity and the quality of meat to meet the needs of consumers (Troy D.J., & J.P. Kerry 2010).

1.4 Sensory evaluation of meat and meat product

Sensory Evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to the characteristics of food and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing. The principal uses of sensory evaluation techniques are in quality control, product development and research in the field of food science. The primary function of sensory testing is to conduct valid and reliable tests to provide data on which sound decisions may be made (Meilgaard et al., 1999).

Sensory quality is one of the main factor that influences consumer choices. Appearance, colour, aroma, taste and texture are important sensory characteristics that influence consumer's preference of food, but in the meantime are the most difficult to identify and quantify.

In the case of meat the most important drivers of overall liking are colour, tenderness, juiciness, chewiness, aroma and flavour (Grunert, et al. 2004).

Sensory analysis of meat is difficult to perform because the high variability of muscles, animals, breeds (Trial 4), feeding conditions and cooking methods (Braghieri et al., 2012).

The relation between meat colour and sensory characteristic has been studied since 1950, when Urbain (Urbain, 1952) described how consumers chosen meat by the colour and Adams and Hoffmans (1972) found that consumers related meat colour with freshness; moreover, the colour of meat was also associated by consumers with the way in which animals were raised or feed (De Marchi et al., 2005; Pelicano et al., 2005).

Meat farmers have a particular interest in the colour of meat as quality parameters for different reasons.
1) Maintain uniformity of colour as indicator of freshness,
2) Prevent any factors that causes lipid oxidation during storage
3) Optimize colour and appearance
4) Respond to consumers expectations (Perez-alvarez, 2006).

Color changes influence the acceptability of meat, and represent an indicator of quality and freshness (Carpenter, et al. 2001). Lipid oxidation produces compounds, such as fatty acid peroxides, cholesterol hydro peroxide, and peroxy radicals that are responsible for the decline in quality of meat and represent health risks (Grün, et al. 2006). (Trial 1, 2 and 3).

In the last decades also meat derived product received more attention than in the past (Nollet &Toldra, 2011) because more difficult to study from a sensory point of view, in fact colour depend on breeding and take place during the different stage of ripening. (Trial 4)

Aroma and the amount of connective tissue in meat are also important sensory characteristics (Hoffman et al., 2007). Of these attributes, consumers consider tenderness to be the most important factor influencing meat quality (Strydom et al., 2000).

Meat texture and flavor are influenced by internal and external factors such as: muscle and connective tissue, lipid composition and cooking method.

Several authors (Hoffman et al., 2007; Calkins & Hodgen, 2007) studied the relations between physical parameters and sensory characteristics of meat, such as muscle fiber and overall tenderness or quantity and composition of intramuscular fat and flavor.

Due to the nature of such product a specific training program is needed to standardize sensory evaluation practices among assessors.

Although several studies has been conducted on the parameters influencing sensory characteristics of meat, the sensory description of this product (sensory profile) often used terms refer to defects (odor and unpleasant flavor, or not conform appearance) or to the product rating like preference, hiding the real nature of the differences between the samples (Risvik, 1994) .

In the case of meat, a trained panel is used on the product raw or after cooking , to assess the quality during purchasing or consuming respectively.

Sensory evaluation is generally performed on cooked meat or derived product and concerns characteristics as such tenderness, chewiness, juiciness, smell and flavor. On fresh meat sensory characteristic investigated are appearance, colour and aroma.

For whole-muscle meat products, the major descriptive attributes are tenderness, juiciness and flavor.

Tenderness is inversely proportional to the force required to compress meat between the molars and is mainly influenced by two factors: the presence of collagen in particular the proportion of collagen degrading during cooking , and the contraction of the muscle fibers during the cooking. Another important factor influencing meat tenderness is the presence of marbling fat (Lawrie ,1998).

Juiciness is the amount of juice perceived during chewing and depend by the amount of water present in the product (immediate juiciness not durable) and

the quantity of fat, stimulating salivation ,gives a feeling of juiciness more persistent. The first element is strictly dependent on the ability the meat to retain water (water holding capacity), and then to have low losses of water during conservation, thawing or cooking (Lawrie, 1998).

Flavour, like smell, is an olfactory sensation but in some conditions, different temperature, humidity and partial decomposition and solubilization of food in the mouth, it can be more intense than smell, perceived externally.

The sensory evaluation of these parameters can be very simple when is related to animal species and their typical aroma, but can be more complex when it's necessary to recognize particular aromas, both pleasant and unpleasant, as presented in table 1. In particular meat aromas developed during cooking, so it's important that the meat is served hot (Mottram, 1992). To avoid the influences of external odor and the flavor, samples of meat are served without salt, oil and any other seasoning. Furthermore, the judges should rinse the mouse with bread or apple pieces and water low salt content between samples in order the flavor of the previous sample not remain in the mouth and influence judgment.

Table 1: Meat descriptive attributes by AMSA (1994)

JUICINESS	MUSCLE FIBER AND OVERALL TENDERNESS	CONNECTIVE TISSUE AMOUNT
8 Extremely Juicy	8 Extremely Tender	8 None
7 Very Juicy	7 Very Tender	7 Practically None
6 Moderately Juicy	6 Moderately Tender	6 Traces
5 Slightly Juicy	5 Slightly Tender	5 Slight
4 Slightly Dry	4 Slightly Tough	4 Moderate
3 Moderately Dry	3 Moderately Tough	3 Slightly Abundant
2 Very Dry	2 Very Tough	2 Moderately Abundant
1 Extremely Dry	1 Extremely Tough	1 Abundant
FLAVOR INTENSITY	OFF-FLAVOR CHARACTERISTIC	OFF-FLAVOR INTENSITY
8 Extremely Intense	A Acid	8 Extermely Intense
7 Very Intense	L Liver	7 Very Intese
6 Moderately Intense	M Metallic	6 Moderately Intense
5 Slightly Intense	F Fish-like	5 Slightly Intense
4 Slightly Bland	O Old(Freezer Burned)	4 Slightly Bland
3 Moderately Bland	U Rancid	3 Moderately Bland
2 Very Bland	B Bitter	2 Very Bland
1 Extremely Bland	SO Sour	1 Extremely Bland
	X Other (describe)	

When it's necessary to analyze meat derived products scientific literature present several protocol that can be used. For example Pérez-Cacho, et al. (2005), developed standards for sensory evaluation of a Spanish dry-cured sausage; Benedini, et al. (2012) studied the sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content or in "Sensory Analysis of Foods of Animal Origin" edited by L.M.L. Nollet and F. Toldra (2011) where the authors provide a detailed description of the sensory aspects of different food of animal origin and, if necessary discussed samples preparation and methods.

1.5 Meta analytical approach to define nutritional meat quality

Over the last 50 years there has been a considerable increase of scientific information. For a given construct, there are numerous studies that analyze the problem on several aspects, but often are of little relevance as were performed on small samples.
In such instances, statistical methods dealing with the analysis of summary (literature) data, known as meta-analyses, must be used.
This type of research has been favored by the development of internet and data-base containing the most relevant scientific articles.

Glass (1976) defines meta- analysis as "the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings".

Meta-analysis is a quantitative statistical techniques that aims to combine information from individual researches with the purpose of obtaining a single data synthesis.

The earliest meta-analysis may have been that of Karl Pearson in 1904, which he applied in an attempt to overcome the problem of reduced statistical power in studies with small sample sizes.

The meta- analysis begins to be part of the scientific literature only by half 900 and only in the last 25 years has expressed considerable interest . For this is still a growing field of research and development.

In particular, in the last decades, research in animal sciences and in nutrition, are increasing, so it's necessary, in some circumstances, summarize the data present in scientific literature.

Several authors (Phillips, 2005; Sauvant, et al. 2008; St-Pierre, 2001) found that meta-analysis is useful statistical tool to extrapolate general response equations from single experiments and to gain statistically reliable, robust response laws for animal science.

Whereas meta-analysis is commonly used in medical and genetic studies, its use in animal science is relatively recent with the main focus on metabolic processes in ruminants (Bermingham et al., 2008; Dragomir, et al. 2008; Schmidely, et al. 2008) or pigs (Schulin-Zeuten et al., 2007; Trefan et al 2013). In contrast, meta-analysis for meat quality traits has been relatively sparse (Dunshea et al., 2005).

The meta- analysis can (Glasser and Duval, 2008): provide uniformity in results dissimilar ; increase the statistical power of comparisons of small samples; improve the accuracy of the estimate ;studying subgroups of units within various studies ;provide information on possible new studies .

In planning the meta-analysis, the same principles apply as planning any other study. That is, one forms a hypothesis, defines eligibility, collects data, tests the hypothesis, and reports the results.

To conduct a meta-analysis according to Glass et al. (1981), one goes through the following steps:

1) Select the independent and dependent variables of interest
2) Locate all relevant and usable studies containing information about the effect of interest.
3) Code the characteristics of each study that might relate to the size of effects obtained in different studies.
4) Calculate effect size estimates for independent-dependent variable pairs of interest.

5) Calculate the mean effect size(s) across studies.
6) Regress effect size estimates on coded study characteristics to assess the relationship between study results and study characteristics.

In the field of animal nutrition, Sauvant et al (2008) published an article "Meta-analyses of experimental data in animal nutrition" that reported the step to conduct a meta analysis with this type of data. The first step concerns the definition of the study objectives and the identification of the criteria to be used in the selection of prior publications to be used in the construction of the database (PICOS criteria: P=population; I=Intervention; C= comparison, O= outcome; S= study design). Publications must be scrupulously evaluated before being entered into the database; it is important to encode each record with pertinent descriptive attributes (experiments, treatments, etc.) to serve as important reference points for the rest of the analysis. This phase is followed by a study of the meta-system made up of the database to be interpreted. These steps condition the definition of the applied statistical model. Variance decomposition must account for inter- and intrastudy sources; dependent and independent variables must be identified either as discrete (qualitative) or continuous (quantitative). Effects must be defined as either fixed or random. Often, observations must be weighed to account for differences in the precision of the reported means. Once model parameters are estimated, extensive analyses of residual variations must be performed. The roles of the different treatments and studies in the results obtained must be identified. Often, this requires returning to an earlier step in the process. Thus, meta-analyses have inherent heuristic qualities.

1.6 Objective

The aim of this study was to investigate the effect of dietary supplementation in animal feeding with plant extract and additives on meat quality, oxidative stability and sensory parameters of fresh meat and derived product.
The obtained results indicate that that dietary supplementation is able to improve oxidative stability and sensory attributes of fresh meat derived from pork, horse and donkey, and cooked ham without affecting other quality parameters.

The effect of dietary vitamin E and verbascoside on meat quality and oxidative stability of Longissimus Dorsi muscle in medium-heavy pigs

2. The effect of dietary vitamin E and verbascoside on meat quality and oxidative stability of *Longissimus Dorsi* muscle in medium-heavy pigs

AUTHORS: Raffaella Rossi, Sabrina Ratti, Grazia Pastorelli, Antonio Crotti, Carlo Corino. *Università degli Studi di Milano, Department of Health, Animal Science and Food Safety, Via Celoria 10, 20133 Milan, Italy*

2.1 Abstract

The aim of the study was to investigate the effect of verbascoside and vitamin E dietary mixture (AOX) on the physical, chemical and sensory parameters of Longissimus Dorsi (LD) muscle in pigs slaughtered at 135 kg of live weight (LW). One hundred and fifty pigs, half castrated males and half females, were assigned to two experimental groups: the first group was fed a commercial diet (CTR) and the second group the same diet with the addition of AOX, containing vitamin E and verbascoside from Verbenaceae extract. Pigs were fed on the basis of 9% of metabolic weight (LW0.75). The antioxidant mixture was administered to pigs 38 days before slaughter. Twenty pigs per treatment (10 castrated males and 10 females) were randomly selected and the left LD muscle was sampled. Physical, chemical and sensory parameters were evaluated. Oxidative stability and color indices were assessed during eight days of refrigerated storage at 4 °C. Sex and dietary treatment did not affect (P b 0.05) the physical and chemical parameters of LD muscle. Dietary AOX improved (P b 0.01) oxidative stability and color indices in LD muscle during refrigerated storage. Sensory characteristics were unaffected by sex and dietary treatment. The results suggest that dietary AOX containing verbascoside is an effective antioxidant in LD muscle, enhancing oxidative status and color indices without affecting other meat quality parameters.

2.2 Introduction

Pig breeding in Italy tends to be focused on the production of heavy pigs, slaughtered at a live weight (LW) of 160 kg±10%, for the production of cuts for processed products. It is aimed at providing thighs for the production of Parma and San Daniele dry-cured hams, which have a protected origin designation. Thus, pork is required to have characteristics corresponding to the qualitative standards described in Council Regulation (EEC)No., 2081/92. These traits result from different farming techniques, including genetics, nutrition, age and weight of animals at slaughter (ECC/2081/92). Medium-heavy swine, slaughtered at 135 kg LW, represents an alternative for the Italian heavy pig farming, with cuts suitable for fresh meat consumption.

Recently, considerable attention has been focused on the improvement of meat quality parameters. The protection of lipids from oxidative phenomena is fundamental to preserve meat color and nutritional quality (Faustman, Sun, Mancini, & Suman, 2010). Color changes influence the acceptability of meat, and represent an indicator of quality and freshness (Carpenter, Cornforth, &Whittier, 2001). Lipid oxidation produces compounds, such as fatty acid peroxides, cholesterol hydroperoxide, and peroxy radicals that are responsible for the decline in quality of meat and represent health risks (Grün, Ahn, Clarke, & Lorenzen, 2006).

Dietary antioxidants are one of the major strategies for preventing lipid oxidation and help to decrease pork oxidative phenomena (Corino, Oriani, Pantaleo, Pastorelli, & Salvatori, 1999; Rossi et al., 2013). In fact, vitamin E is considered as the main synthetic antioxidant molecule against lipid oxidation in cell membranes (Debier & Larondelle, 2005).

The search for safe and effective natural antioxidants has focused on plants, particularly in spices and herbs (Nakatani, 1997; Nkukwana et al., 2014; Que, Mao, & Pan, 2006). Many herbs and their extracts have been reported to have a high antioxidant capacity due to their polyphenolic compound content (Moyo, Oyedemi, Masika, & Muchenje, 2012; Wojdyło, Oszmianski, & Czemerys, 2007). Verbascoside (VB) is the most abundant phenolic compound in Verbenaceae extracts (Quirantes-Pine, Funes, Micol, Segura-Carretero, & Fernandez-Gutierrez, 2009). Our group found that plant extracts containing VB have a greater antioxidant power compared to other phenolic compounds (Rossi, Corino, Pastorelli, Durand, & Prost, 2009). In fact, polyphenol dietary supplementation enhances pig blood antioxidant status (Pastorelli, Rossi, & Corino, 2012; Rossi, Pastorelli, & Corino, 2013a), related to an increase in serum vitamins A and E (Palazzo, Vizzarri, Cinone, Corino, & Casamassima, 2011). We have also found that dietary long term supplementation with plant extract

containing VB improves pork quality parameters and protects α-tocopherol from oxidative decay (Rossi, Pastorelli, Cannata, et al., 2013).

To the best of our knowledge, no studies have reported the effect of short-term dietary supplementation with an antioxidant mixture (AOX), containing vitamin E and VB, on meat quality parameters in medium heavy pigs. In literature, there are no findings on physical, chemical and sensory parameters of Longissimus Dorsi (LD) muscle of medium heavy pigs. The aim of the present work was to characterize the quality of LD muscle in pigs slaughtered at 135 kg LW and to assess the effectiveness of AOX dietary supplementation in decreasing oxidative phenomena.

2.3 Materials and methods

2.3.1 Animal and dietary treatment

The animals used in this experiment were cared for following the European Union guidelines (No. 86/609/EEC) approved by the Italian Ministry of Health (L. 116/92). One hundred and fifty pigs (Duroc × (Landrace × Large White)), half castrated males (surgical castration at 5 days of age) and half females, were assigned to two experimental groups, balanced for body weight and sex. The first group was fed a commercial diet (CTR). The second group had the same diet with the addition of an antioxidant mixture containing vitamin E and verbascoside (AOX) from Verbenaceae extract. The experimental diets were formulated to meet the requirements for all nutrients (NRC, 2012). Pigs were fed a diet with the same amount of all-rac-α-tocopheryl acetate (20 mg/kg in the finishing phase; two-fold the amount reported by NRC, 2012). Two times daily, animals were fed a liquid feed with a water: concentrate ratio of 3:1, with free-choice access to drinking bowls. Pigs were fed a corn-based diet and were rationed on 9% of metabolic weight (LW0.75). The dosage of plant extract in the feed was chosen on the basis of our previous study (Rossi, Pastorelli, Cannata, et al., 2013). The AOX mixture was administered to pigs 38 days before slaughter in daily amounts of 150 mg of vitamin E and 15 mg of verbascoside. The AOX supplement contained a water-soluble extract of Verbenaceae (Lippia spp.) leaves, prepared on an industrial scale by a standardized procedure including ultrasonic extraction with 60% aqueous ethyl alcohol followed by spray-drying with maltodextrins as an excipient. The phenylpropanoid glycosides and benzoic acid content of the feed supplement, according to a certificate of analysis provided by the manufacturer, is: 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) according to Piccinelli, De Simone, Passi, and Rastrelli (2004). To avoid oxidation in the complete feed, the supplement was microencapsulated within a protective matrix of hydrogenated vegetable lipids using spray-cooling technology (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy).

2.3.2 Carcass traits

At 135 kg LW pigs were slaughtered in a commercial slaughterhouse (ProSus, s.c.a., Vescovato, Cremona, Italy). After an on-farm fasting period of 8 h, the pigs were transported to the abattoir, covering a distance of 13 km(travel time: 30 min). The pigs were laired for 4 h with free access to water. Pigs were electrically stunned (220 V, 2.3 A, 8 s head application), and following exsanguination, the carcasses were scalded, dehaired and eviscerated. Hot carcass weight was recorded. Twenty pigs per treatment (10 castrated males and 10 females)were randomly selected and the left Longissimus Dorsi (LD) muscle was removed from the carcass at the last lumbar vertebra and weighted. The LD muscles were vacuum-packed, stored for 4 days at 4 °C, and transported to the laboratory pending analyses. The samples were stored and frozen (−20 °C) until chemical composition, oxidative stability and sensory evaluation were carried out. For the physical analyses, fresh LD samples were used.

2.3.3 Physical parameters

Measurements of pH at 45 min and 24 h post mortem were performed on LD muscles 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 maintenance of calibration was monitored between samples. Color measurements were performed at 45 min on LD samples at the last lumbar vertebra, using a CR-300 ChromaMeter (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 (illuminated C) at a 0° viewing angle. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included. Each data point is the mean of three replications measured at the chop surface. Color indices of LD muscles were also determined at 1, 4 and 8 days of storage (4 °C), to assess color stability. Total losses (drip and cooking losses) were calculated. Drip loss was determined by the method described by Honikel (1998). A slice of fresh LD muscle (40 ±2 g) with the cut surface facing down was placed on a grid, which was placed in a closed plastic container. The weight loss percentage after 24 h of

storage at 4 °C was calculated. For the cooking loss, a fresh slice from each sample was weighed (25 mm thick; average weight: 151 ± 6 g), placed in a plastic bag and cooked to an internal temperature of 70 °C in a 75 °C water bath. The internal temperature was monitored during cooking with a hand-held temperature probe. The cooked samples were cooled for 30 min, blotted dry and weighed. The difference between pre- and post-cookingweights 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. The samples were cut, parallel to the longitudinal orientation of the muscle fibers, into six cylindrical cores (Ø 1.25 cm). Each core was sheared with aWBSF device attached to an Instron Universal TestingMachine (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 (N/cm2) was recorded.

2.3.4 Chemical parameters
Samples of LD were analyzed for dry matter, crude protein, ether extract and ash according to the methods of the Association of Analytical Chemists (AOAC, 2000).

2.3.5 Oxidative stability

Oxidative stability was assessed by the thiobarbituric acid reactive substances (TBARS) method (Monin, Hortos, Diaz, Rock, & Garcia- Regueiro, 2003), during 8 days of refrigerated storage at 4 °C. Briefly, 4 g of minced meat were homogenized in 20 ml of distilled water using an Ultra Turrax T25 homogenizer (IKA, Cincinnati, USA). A total of 5 ml of 25% trichloroacetic acid was added to the homogenate, which was then shaken at 4 °C for 30 min and centrifuged at 5000 ×g at 4 °C for 10 min. The supernatant was filtered with Whatman 52 filter paper. Then 1.5ml of 0.6% thiobarbituric acid was added to 3.5 ml of supernatant and placed at 70 °C for 30 min. Absorbance at 532 nm was measured immediately after cooling and compared with a standard curve of malonaldehyde prepared by hydrolysis of tetraethoxypropane. Determinations were carried out in duplicate. The results were expressed as micrograms of malondialdehyde (MDA) per gram of meat.

2.3.6 Sensory analysis

2.3.6.1 Sample preparation
All LD samples were cooked for 4 min at the maximum power (200 °C) on double-plated grills. A thermocouple (Pentronic AB, 198 Gunnebobruk, Sweden) was inserted in the center of each piece of meat in order to record the

core temperature. The core temperature was not allowed to exceed 68° and therefore the meat was removed from the oven at approximately 60 to 65 °C to avoid post-heating increases and then sliced into 15-mm cubes (Bruce, Beilken, & Leppard, 2005). Four cubes per sample were presented on white plastic plates to each panelist with pairs of cubes identified by 3-digit codes representing their respective strip meat. Cubes were presented to each panelist in an order that ensured that each strip meat was represented an equal number of times in each presentation position within each session (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

2.3.6.2 Sensory evaluation

A selected and trained sensory panel was chosen, consisting of eight members, who were familiar with pork meat and descriptive analysis procedures (ISO International Organization for Standardization, 2010). All assessments were carried out in a sensory laboratory equipped according to ISO International Organization for Standardization (2007) recommendations. Three sessions were conducted to develop a common vocabulary and improve the ability of judges to discriminate between samples, as well as the correct use of the intensity scale. Analysis of variance was performed for each attribute in order to identify those that were not significant in discriminating between samples and to test the discriminant ability of the judges between samples. Attribute references were used during training sessions to calibrate the panel members. The final list of descriptors with the relevant definitions is reported in Table 1. The judges evaluated each sample in triplicate. During training and sampling, panel members had access to unlimited water and unsalted crackers. The judges were presented with a slice of raw LD to evaluate the external appearance and a slice of cooked LD for a tasting test. They were instructed first to score the external appearance and aroma, then to take a bite of the cooked slice (1.5 cm width × 1.5 cm length × 1.5 cm height cubes) and score the texture. They were asked to score texture, flavor and taste during chewing. If necessary, they could taste more than one LD cube. Within each session the design was balanced for order and carry over effects (MacFie et al., 1989). Judges were requested to evaluate the intensity of each attribute by assigning a score between 1 (absence of sensation) and 9 (extremely intense).

2.3.7 Statistical analysis

Statistical analyses of the data were performed using SPSS (SPSS/PC Statistics 18.0 SPSS Inc., Chicago, IL). The data on physical and chemical parameters were analyzed by two-way Analysis of Variance (ANOVA) with dietary treatment, sex and their interactions as effects. The data on TBARS parameters and color indices during storage time were assessed by two-way repeatedmeasures

ANOVA, to evidence the effects of treatment, time, sex and their interactions. The sensory data for each attribute were submitted to ANOVA with samples (CTR-CM, CTR-F, AOX-CM, and AOX-F), judges, replicates and their interactions as effects. Sex was excluded from the model because the difference is not significant (P N 0.05). The significance of these effects was tested with F tests. Means were compared according to the DUNCAN test. The pig was considered the experimental unit for all parameters. Data are presented as means±SEM, and a value of P b 0.05was used to indicate statistical significance.

Table 1. Descriptors and definitions for sensory analysis of LD muscle.

Descriptors	*Definitions*
Appearance	
Pink colour	Characteristic pink color of pork loin
Marbling	Amounts of intramuscular fat running through the muscle of pork loin
Slice omogenity	Slice of pork loin without any defects
Aroma	
Swine	Aroma associated with cooked pork loin
Nut	Aroma associated with roast nuts
Taste	
Salty	Salty taste
Sweet	Sweet taste
Flavour	
Swine	Flavour associated with cooked pork loin
Nut	Flavour associated with roast nuts
Metallic	Flavour associated with blood or rare meat
Texture	
Tender	The force needed to masticate the meat ready for swallowing
Fibrous	Presence of fibers during swallowing
Juicy	The degree of juice released while chewing the meat.
Stingy	Production of a large quantity of saliva for swallowing

2.4 Results and Discussion

Physical parameters - The meat quality parameters, pH values, color indices, total losses and shear force are given in Table 2. No effects of AOX dietary supplementation and sex on pH values and color indices at 45 min postmortem, total losses and shear force were observed. The LD muscle weight was higher (P b 0.05) in the AOX group than the CTR group, which was related to the higher carcassweight of the AOX supplemented pigs (102.6±1.5 kg CTR vs 109.9±2.0 kgAOX). These findings were in disagreement with Paiva-Martins, Barbosa, Pinheiro, and Mourao (2009) who reported a lower carcass weight in pigs fed olive leaves, due to the bitter taste of oleuropein, the main polyphenol found in leaves. In our previous study, the long-term dietary supplementation with plant extract, containing VB, did not affect carcass weight in pigs slaughtered at an average weight of 110 kg (Rossi, Pastorelli, Cannata, et al., 2013). Similar results were obtained in pigs fed different dosages of cranberry pulp, rich in polyphenolic compounds, from25 to 120 kg of LW (Fortier, Saucier, & Guay, 2012). In addition, previous studies in pigs fed vitamin E showed no difference in carcass weight (Cannon et al., 1996; Guo, Richert, Burgess,Webel, Orr, Blair, et al., 2006). The LD muscle pH values reported at 45min and 24 h post-mortem were comparable to previously reported values in pork (Corino, Rossi, Musella, Pastorelli, & Cannata, 2009; Gjerlaug-Enger, Aass, Ødegard, & Vangen, 2010). Dietary supplementation with AOX did not affect the pH values of the LD muscle according to Lahucky, Nuernberg, Kovac, Bucko, and Nuernberg (2010) in pigs fed plant extracts of Melissa, Origanum and Salvia from 30 days before slaughter (105 kg of LW). Rossi, Pastorelli, Cannata, et al. (2013) observed similar results in pigs fed a natural extract containing VB. Mason, Hogan, Lynch, Sullivan, Lawlor and Kerry (2005) also found no difference in pH values of pigs fed vitamin E (200 mg/kg) or green tea catechin (200 mg tea extracts/kg) in the last phase of breeding (80–105 kg LW). Also Guo et al. (2006) reported that different levels of dietary vitamin E (40, 200, and 400 mg/kg) for different periods (3, 6 and 9 weeks) did not affect pH values at 45 and 24 h post-mortem in LD muscles. In the present study, color indices at 45 min post-mortem were not affected by dietary treatment in pigs fed a natural extract containing polyphenols, in accordance with Rossi, Pastorelli, Cannata, et al. (2013) and Paiva-Martins et al. (2009). Janz, Morel, Wilkinson, and Purchas (2007) also reported that dietary supplementation with oregano oil did not affect the color indices of LD muscle. In accordance with our results, Guo et al. (2006) reported no difference in color indices in LD muscle from pigs fed vitamin E. However, in contrast with our findings, Kołodziej-Skalska, Rybarczyk, Matysiak, Jacyno, Pietruszka and Kawęck (2011) reported a more intensive red color a* in LD from pigs fed a plant extract containing carvacrol, cinnamaldehyde and capsicum

oleoresin from 30 to 100 kg LW. The changes of L*, a* and b*values of pork LD muscle in relation to, dietary treatment (CTR vs AOX), sex (CM vs F) and storage time at 4° are shown in Fig. 1(A, B, C). The lightness 'L*' values were significantly affected by storage time (P= 0.010) and dietary treatment (P b 0.001). Sex did not affect (P= 0.067) the lightness 'L*' values in LD muscle. No interactions between time and treatment and sex and treatment were observed (P N 0.005). A significant interaction between sex and treatment was observed (P = 0.011). The 'a*' redness values were significantly affected by storage time (P b 0.001) and an interaction between time and treatment was observed (P = 0.006). No significant effects of dietary treatment and sex were detected (P N 0.05). No interactions between sex and time and sex and treatment were observed (P N 0.05). The yellowness 'b*' values were significantly affected by storage time (P b 0.001) and dietary treatment (P = 0.006) and an interaction between time and treatment was observed (P b 0.041). No interactions between sex and time and sex and treatment were detected (P N 0.05). The increase in lightness 'L*' and yellowness 'b*' values over the 8 day storage period was in agreement with the results reported by Moroney, O'Grady, O'Doherty, and Kerry (2012) in pigs fed seaweed extracts. Jia, Kong, Liu, Diao, and Xia (2012) also reported a decrease in a* value of pork patties during chilled storage, in accordance with the present results. Our data are in agreement with the findings of Trefan, Doeschl- Wilson, Rooke, Terlouw, and Bünger (2013) that reported no effects of sex (CM vs F) on color indices. In the literature, the findings related to dietary antioxidant supplementation and color stability of pork were conflicting. Our results showed that dietary treatment with AOX positively affected lightness 'L*' and yellowness 'b*' values of LD muscle, indicating a high persistent color stability during refrigerated storage. No difference in redness 'a*' value was observed in relation to dietary treatment, although in AOX groups, the a* valuewasmore constant during eight days of refrigerated storage. Our results are in agreement with Jia et al. (2012) who showed that color indices in pork patties are positively affected by the addition of black currant extract. Other experimental studies reported that plant extracts, containing polyphenols, are able to stabilize the color change in pork during chilled storage (Lee et al., 2010; Rodríguez-Carpena, Morcuende, & Estévez, 2011). In contrast, Carpenter, O'Grady, O'Callaghan, O'Brien, and Kerry (2007) reported that color indices in raw and cooked pork patties were unaffected by the addition of grape seed and bearberry extracts. Pork tenderness, measured by shear force, was not affected by dietary treatments, in agreement with previous studies in pigs fed polyphenol- rich extracts (Lahucky et al., 2010; Rossi, Pastorelli, Cannata, et al., 2013). Total losses (drip and cooking losses) did not differ between dietary treatments. Some research has shown no effect of dietary supplement containing polyphenols on cooking and drip loss values of LD muscle from light pigs (110 kg LW) (Rossi, Pastorelli, Cannata, et al., 2013;

Sarker et al., 2010). The findings on muscle drip loss after dietary vitamin E supplementation are conflicting. Guo et al. (2006) reported that dietary supplementation with 200 mg/kg did not affect drip loss values. Other authors have shown that drip loss decreases when vitamin E is added in the diet at the level of 100 mg/kg (Cheah, Cheah, & Krausgrill, 1995).

Table 2. Physical parameters of *Longissimus Dorsi* muscle from pig fed control (CTR) or antioxidant mixture (AOX) supplemented diet.

| | CTR[a] | | AOX[a] | | P | | |
Item	CM	F	CM	F	*Treat*	*Sex*	*T*S*
L. w., kg	$5.4^B \pm 0.3$	$5.0^B \pm 0.4$	$6.0^A \pm 0.4$	$5.8^A \pm 0.1$	*0.005*	*0.238*	*0.754*
pH, 45 min	6.16 ± 0.07	6.11 ± 0.06	6.12 ± 0.04	6.14 ± 0.06	*0.964*	*0.784*	*0.597*
pH, 24 h	5.49 ± 0.04	5.55 ± 0.05	5.61 ± 0.03	5.60 ± 0.04	*0.122*	*0.419*	*0.445*
L*	44.29 ± 0.82	41.52 ± 0.75	40.63 ± 0.88	42.28 ± 0.76	*0.191*	*0.764*	*0.117*
a*	6.73 ± 0.71	7.60 ± 0.49	7.63 ± 0.44	7.78 ± 0.70	*0.415*	*0.441*	*0.582*
b*	5.68 ± 0.20	5.20 ± 0.14	5.64 ± 0.20	5.58 ± 0.28	*0.463*	*0.248*	*0.361*
T. losses %	24.02 ± 0.48	23.04 ± 0.52	21.68 ± 0.91	22.87 ± 0.75	*0.135*	*0.898*	*0.195*
S. Force, kg/cm²	23.92 ± 0.49	22.84 ± 0.29	23.33 ± 0.39	23.92 ± 0.49	*0.798*	*0.799*	*0.391*

[A,B]Values in the same row are different for $P < 0.01$. [a] n=10; data are reported as mean $\pm$ SEM. AOX antioxidant mixture; CTR, control diet; CM, castrated males; F, females; T*S, Treatment*Sex

Figure 1. Longissimus Dorsi colour parameters in relation to storage time at 4°C: lightness 'L*' values (A), redness "a*" values (B) and yellowness "b*" values (C) from castrated males (CM) and females (F) pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX).
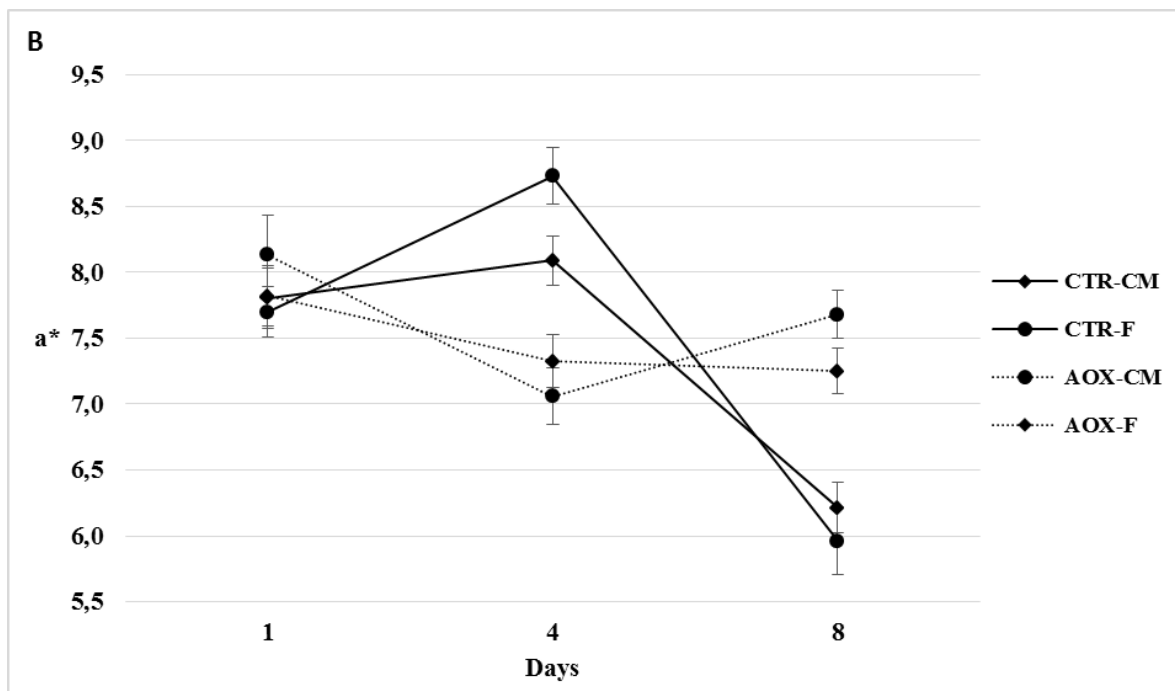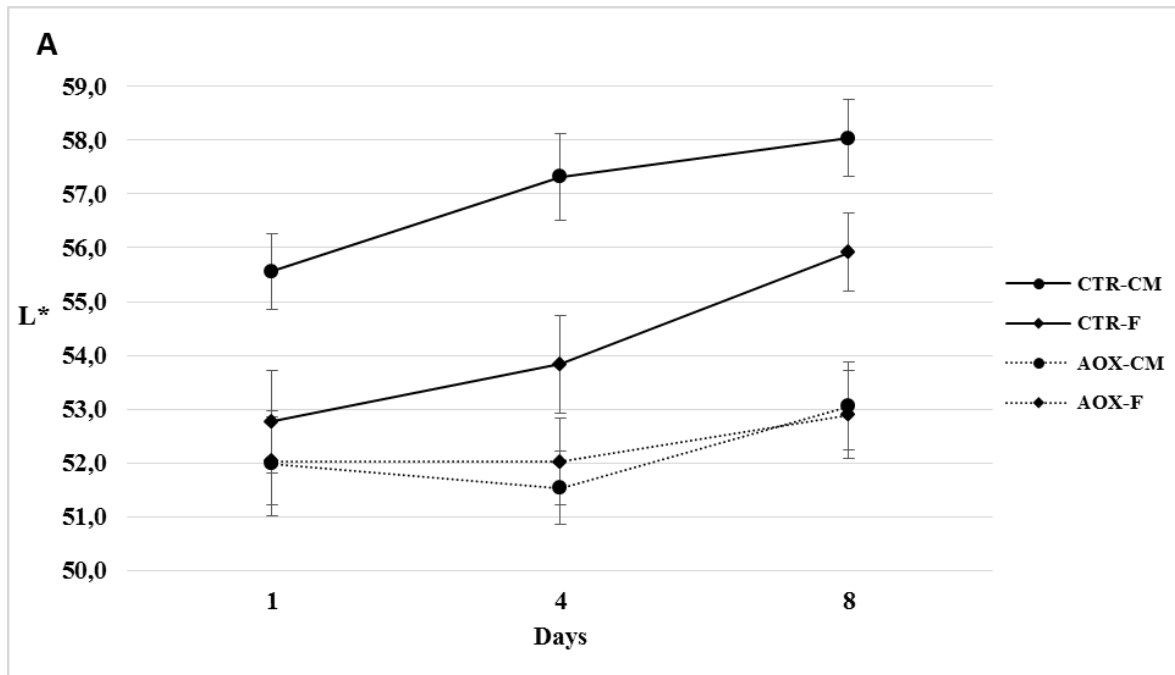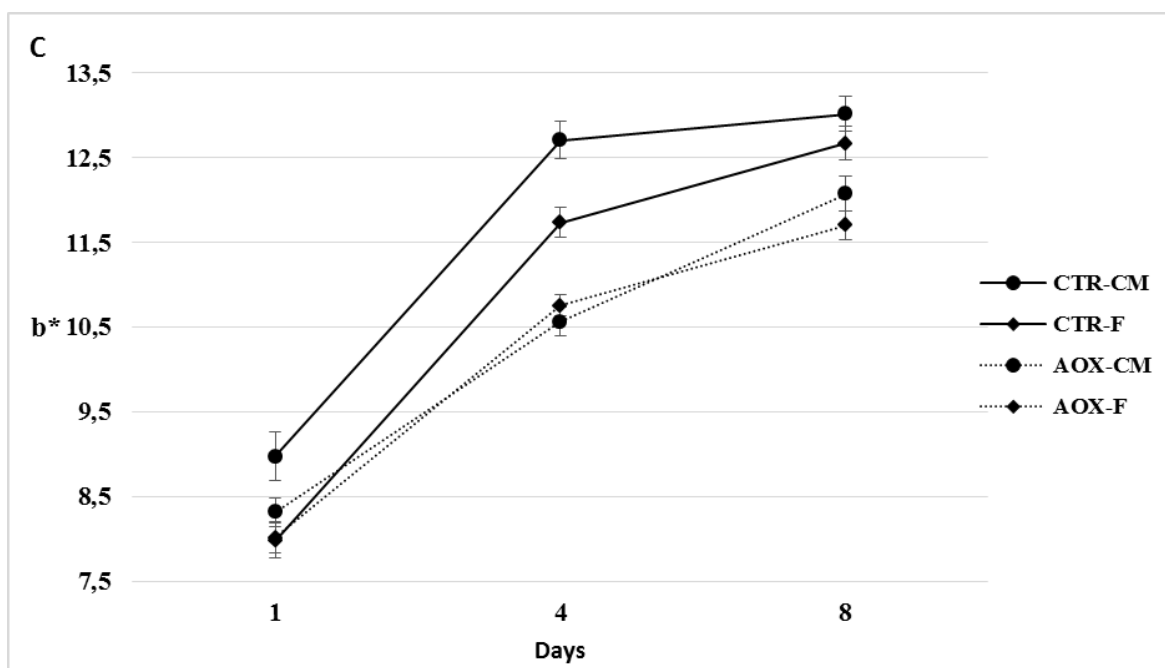
n = 20; data are reported as mean ± SEM. CTR-CM, castrated males fed control diet; CTR-F, female fed control diet; AOX-CM, castrated males fed antioxidant mixture supplemented diet; AOX-F, females fed antioxidant mixture supplemented diet.

lightness 'L*' values: effects of Treatment P<0.001, Time P=0.010, Sex P=0.067, Time*Treatment P=0.335, Time*Sex P= 0.823, Treatment*Sex P=0.011.

redness "a*" values: effects of Treatment P=0.741, Time P<0.001, Sex P=0.833, Time*Treatment P<0.001, Time*Sex P= 0.240, Treatment*Sex P=0.745.

yellowness "b*" values: effects of Treatment P=0.006, Time P<0.001, Sex P=0.158, Time* Treatment P=0.041, Time*Sex P= 0.806, Treatment*Sex P=0.347.

Chemical parameters - Dietary treatments and sex did not affect (P N 0.05) the chemical parameters of LD muscle (Table 3). Chemical composition did not differ in relation to dietary treatment with AOX in agreement with results reported in the literature (Kołodziej-Skalska et al., 2011; Mason et al., 2005; Rossi, Pastorelli, Cannata, et al., 2013). Similar results were observed in pigs fed vitamin E (500 mg/kg feed) and vitamin E plus before slaughter (110 kg LW) (Bahelka, Nürnberg, Küchenmeister, & Lahučký, 2011). The intramuscular fat values of LD from medium-heavy swine were higher than those reported for light pigs (Corino et al., 2009),which was related to the higher slaughter weight. The values were near to those reported in heavy pigs, slaughtered at 160 kg LW (Corino et al., 2002, 2008).

Table 3. Chemical parameters of Longissimus Dorsi (LD) muscle in pigs fed control (CTR) or antioxidant mixture (AOX) supplemented diet

| Item | CTR[a] | | AOX[a] | | *P* | | |
	CM	F	CM	F	*Treat*	*Sex*	*T*S*
Dry Matter, %	27.93 ± 0.39	27.72 ± 0.32	27.31 ± 0.23	28.14 ± 0.50	*0.816*	*0.468*	*0.223*
Crude Protein, % w.w	22.92 ± 0.57	22.75 ± 0.47	22.63 ± 0.94	22.65 ± 0.89	*0.494*	*0.793*	*0.726*
Ether Extract, % w.w	3.42 ± 0.31	3.07 ± 0.38	3.43 ± 0.21	3.47 ± 0.22	*0.496*	*0.605*	*0.502*
Ash, % w.w	1.78 ± 0.18	1.55 ± 0.23	1.58 ± 0.14	1.61 ± 0.12	*0.724*	*0.581*	*0.489*

a n=10; data are reported as mean ± SEM; w. w., wet weight. AOX antioxidant mixture; CTR, control diet; CM, castrated males; F, females; T*S, Treatment*Sex

Oxidative stability - The dietary inclusion of AOX decreased (P = 0.002) oxidative phenomena in LD muscle. Sex did not affect oxidative stability of LD muscle (P = 0.835). Time of storage affected oxidative stability in LD muscle (P b 0.001) (Fig. 2). No interactions between sex and time and sex and treatment were observed (P N 0.05) The TBARS values are in the range reported by Apple et al. (2007) in pigs slaughtered at about 120 kg LW. The present findings indicated that pigs fed AOX had a higher protection fromlipid oxidation than the CTR group, in agreement with the literature reporting a lower TBARS concentration in the muscle of pigs fed plant antioxidants, containing polyphenols (Haak et al., 2006; Lahucky et al., 2010; Mairesse et al., 2010). Dietary supplementation with AOX, containing vitamin E (50 mg/kg feed) and verbascoside (5 mg/kg feed) during the last phase of fattening (38 days) increased the oxidative stability in LD muscle, enhancing the shelf life of meat.

Figure 2. Oxidative stability during refrigerated storage at 4°C of Longissimus Dorsi muscle from castrated males (CM) and females (F) pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX).
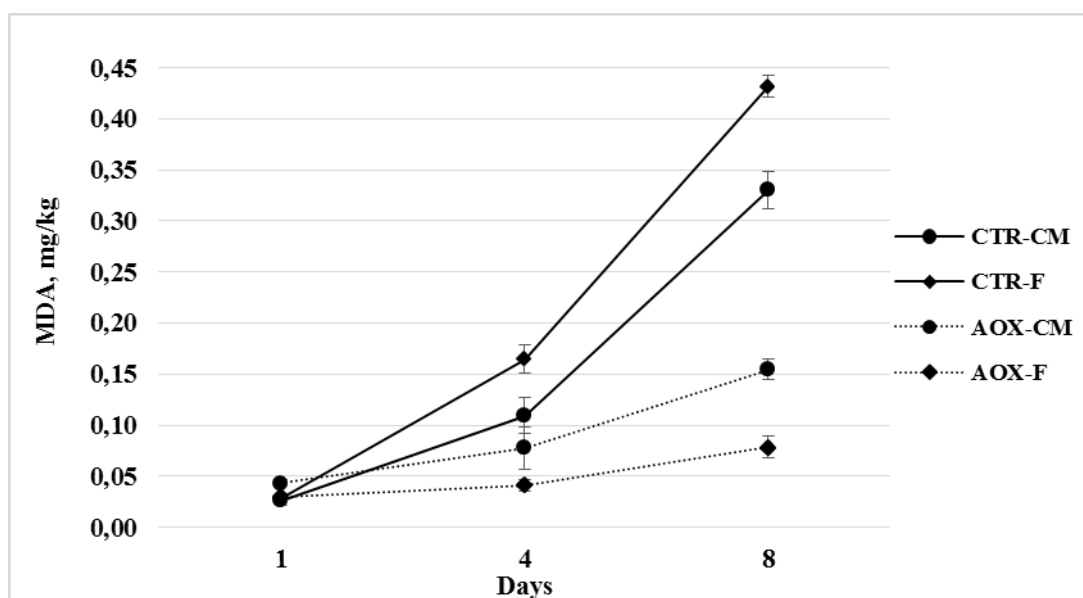
n=20; data are reported as mean ± SEM. CTR-CM, castrated males fed control diet; CTR-F, female fed control diet; AOX-CM, castrated males fed antioxidant mixture supplemented diet; AOX-F, females fed antioxidant mixture supplemented diet. Effects of Time P < 0.001; Treatment P = 0.002; Sex P=0.835; Time*Treatment P < 0.001; Sex* Treatment P= 0.147; Sex*Time P=0.841.

Sensory profile - The F values for appearance, aroma, taste, flavor and texture parameters of LD sensory profile are reported in Table 4. Results indicated that dietary supplementation with AOX did not affect (P N 0.05) LD sensory quality. In particular the judges presented differences (P b 0.001) for all the descriptors. This is common in sensory evaluations due to the different use of the scale (Lea, Naes, & Rodbotten, 1997). There was no significant difference (P N 0.05) between sample, repetition and interactions. The results indicated that the mean scores for each descriptor could be assumed to be satisfactory for the sensory profile of LD muscle. The spider plot of the sensory profile is reported in Fig. 3. All the considered parameters related to appearance, aroma, flavor, taste and texture were comparable in both experimental groups. Our results suggest that dietary treatment with AOX, containing verbascoside, did not affect the sensory quality of LD. This is in agreement with our previous work (Rossi, Pastorelli, Cannata, et al., 2013) which found that the addition of 5 mg verbascoside/kg feed had no effect on the sensory characteristics of cooked LD muscle during storage. Jo, Son, Son, and Byun (2003) reported that the addition of 0.1% irradiated green tea leaf extract had no negative effect on either the physical or

sensory properties of raw and cooked pork patties. Furthermore, Valencia, O'Grady, Ansorena, Astiasarán, and Kerry (2008) found that the addition of 200 mg/kg green tea catechins and green coffee antioxidants in fresh pork sausages had no effect on color, texture and flavour during storage. However, some authors reported that dietary natural extracts, containing polyphenols, improve the texture of meat. Omojola, Fagbuaro, and Ayeni (2009) found that supplementing pig diets with garlic (0.50, 1.00 and 1.50% for 120 days) had a positive effect on the juiciness of cooked meat. In other studies, supplementing pig diets with garlic significantly increased the texture of cooked LD muscle (Cullen, Monahan, Callan, & O'Doherty, 2005). The expert panel indicated that LD muscles from medium-heavy swine showed a good acceptability with average values of approximately 7, with a scale from 1 to 9, for both experimental groups.

Table 4. Sensory evaluation: F value and statistical significance of treatments (CTR and AOX), Judges (n=8), Replicates (n=3) and their interaction for each sensory descriptor.

				F value		
Descriptors	Treatments	Judges	Replicates	T*J	T*R	J*R
Pink colour	1.27	14.33***	0.37	0.56	0.92	0.42
Marbling	2.13	7.50***	2.15	0.48	3.63*	0.26
Slice omogenity	0.08	14.32***	1.62	2.25	1.33	0.95
Swine aroma	0.42	61.56***	0.83	1.98	0.90	0.54
Nut aroma	0.01	76.67***	0.20	0.51	0.06	0.98
Salty	0.01	43.66***	0.08	0.94	0.94	0.50
Sweet	0.55	15.36***	0.92	0.44	0.29	0.50
Swine flavour	3.25	46.76***	1.02	0.92	0.17	1.51
Nut flavour	3.56	24.63***	0.46	1.30	0.37	1.51
Metallic flavour	0.76	20.70***	0.51	0.88	1.79	1.57
Tender	1.57	17.52***	2.22	0.38	0.99	1.07
Fibrous	1.83	9.19***	0.42	0.23	0.77	1.18
Juicy	1.11	13.38***	0.92	1.22	0.44	0.98
Stingy	1.48	7.80***	0.06	0.48	0.24	0.94

CTR control diet; AOX antioxidant mixture; T*J, Treatment*Judges; S*R, Treatment*Replicates; J*R, Judges* Replicates; Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Figure 3. Spider plot of the sensory profile of LD muscle from pig fed control diet (CTR) or diet supplemented with antioxidant mixture (AOX)
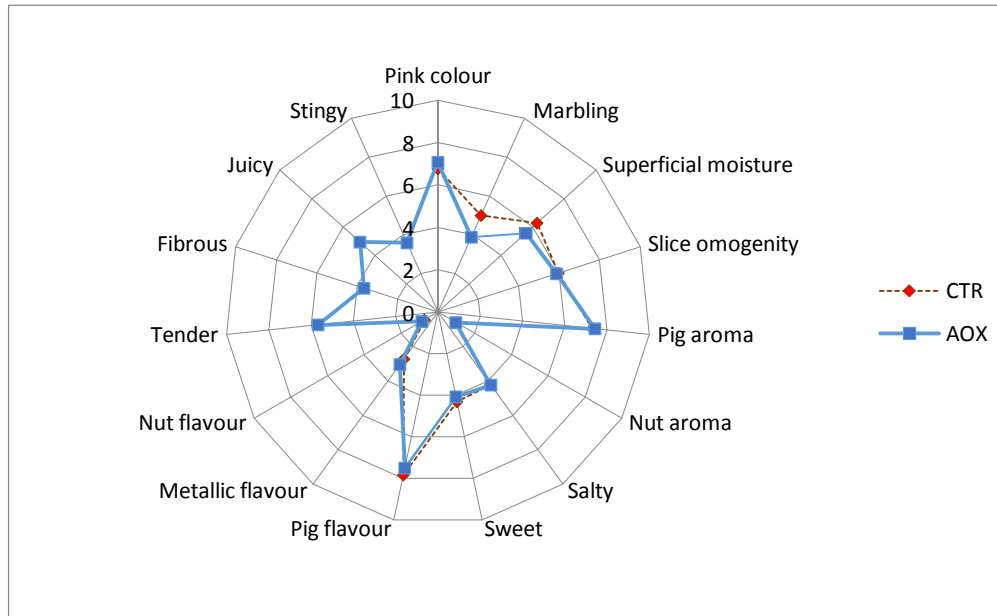
2.5 Conclusions

We found that LDmuscle from medium-heavy swine, slaughtered at 135 kg LW, has a high level of quality for fresh meat consumption. The physical and chemical parameter values are within the range of those expected for light and heavy pigs. The sensory acceptability was good. Dietary supplementation in the last phase of fattening with an AOX mixture, containing verbascoside, exerts an antioxidant effect on LD muscle, enhancing color parameters and oxidative stability. No detrimental effects on physical, chemical and sensory parameters were observed. We believe that our findings reveal the potential of polyphenols in enhancing quality parameters in pork. Further research is needed to study the effectiveness of dietary polyphenols in pork-derived products.

2.5.1 Acknoledgements

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CHAPTER 3

Effect of long term dietary supplementation with plant extract on meat quality in heavy pigs

3. Effect of long term dietary supplementation with plant extract on meat quality in heavy pigs.

AUTHORS: C. Corino[1], G. Pastorelli[1], R. Rossi[1], S. Ratti[1] and G. Maiorano[2], *1 University of Milan, Department of Health, Animal Science and Food Safety, Milan Italy, 2 University of Molise, Department of Agricultural, Environmental and Food Sciences, Via De Sanctis snc, 86100 Campobasso, Italy.*

3.1 Background and Objective

In Italy pork production is oriented to 'heavy pigs" slaughtered at an higher live weight (160 kg ± 10%) than in other European countries. For this purpose the pork must present chemical and physical characteristics conform to qualitative standards described in the regulations for Parma dry cured ham (Council Regulation (EEC) No. 2081/92). These traits result from different farming techniques, including genetics, nutrition, age and weight of animals at slaughter. In the recent years considerable attention has been given to the improvement of meat quality parameters. Some plant extracts contain phenylpropanoid glycosides that have many biological activities such as anti-inflammatory, antimicrobial and antioxidant. Verbascoside, are the most abundant compounds in Verbenaceae extracts (Pereira et al., 2009). In literature no previous study reported the effect of long term supplementation of dietary PE containing verbascoside on meat quality parameters in heavy pigs. The objective of the present study was to assess the effectiveness of long term supplementation of porcine diets with PE on meat quality parameters of Longissimus dorsi (LD) muscle in heavy pigs.

3.2 Materials and Methods

Twenty Dalland female pigs of an average live weight (LW) of 7 kg, were assigned to two dietary treatments: control diet (C) and diet supplemented with plant extract (PE) to obtain 5 mg verbascoside/kg feed. The animals fed a corn-based diet that contained the same amount of vitamin E (33 mg/kg in the finishing phase; threefold the amount reported by NRC 2012). The animals were slaughtered at 170 kg LW. At slaughter LD muscle of all animals was sampled for determination of physical and chemical meat quality parameters (Figure 1). Drip and cooking losses were determined by the method of Honikel, (1998).

Moisture, protein, lipid and ash content were determined in accordance with AOAC (2000) methods. Cholesterol was extracted using the method of Maraschiello et al. (1996) and then quantified by HPLC. Data were analyzed by one-way analysis of variance (ANOVA) where diet was the main factor (SPSS/PC Statistics 18.0 SPSS Inc., Chicago, IL).

3.3 Results and Discussion

The pH values did not differ between treatments (Table 1). The value of pH and colour indices are in line with those reported in literature for heavy pigs (Corino et al., 2002; Corino et al., 2009). Total losses (drip and cooking loss) resulted lower in LD muscle of PE group than control. This data are in agreement with Kołodziej-Skalskaet al., (2012) that reported a lower drip and cooking losses values in LD muscle of pig fed plant extract compared with the control group. No effect on pork LD muscle chemical composition due to dietary supplementation of plant extract was observed (Table 2). Cholesterol content was affected by dietary treatment, resulting lower in LD muscle from PE group than control. No other chemical parameters were affected.

TABLE 1. Physical parameters of Longissimus Dorsi muscle in pigs fed control or plant extract (PE) supplemented diet

	Control	PE	SEM	*P*
pH ,45min	6.35	6.28	0.06	*0.614*
pH, 24 h	5.56	5.55	0.02	*0.834*
Color parameters:				
L*	46.92	46.13	0.53	*0.749*
a*	10.60	9.22	0.35	*0.051*
b*	5.91	5.54	0.21	*0.389*
Drip Loss, %	3.42	2.93	0.13	*0.068*
Cooking Loss, %	16.15	15.34	0.27	*0.146*
Total Losses %	19.02	17.82	0.29	*0.038*
Shear Force, kg/cm^2	2.54	2.30	0.07	*0.130*

TABLE 2. Chemical parameters of Longissimus Dorsi muscle in pigs fed control or plant extract (PE) supplemented diet

	Control	PE	SEM	*P*
Moisture, %	71.97	71.27	0.22	*0.121*
Crude Protein, % tq	22.83	23.29	0.19	*0.252*
Ether extract , %	2.80	2.87	0.18	*0.876*
Ash, %	1.54	1.77	0.07	*0.133*
Cholesterol, mg/100 g	57.8	51.0	1.78	*0.047*

3.4 Conclusion

The results of the present study suggested that long term supplementation with PE contained verbascoside positively affect LD meat quality parameters. The reduction of total losses results interesting from a technological point of view. Moreover dietary PE are able to reduce cholesterol content, improving the nutritional quality of meat. Further studies are needed to clarify the optimal length of PE dietary supplementation and to investigate its effect on sensory parameters and on cooked and ripening products.

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The effect of dietary supplementation with plant extract on meat quality in Equidae

4. The effect of dietary supplementation with plant extract on meat quality in Equidae

AUTHORS: C. Corino [a], S. Ratti[a], R. Rossi [a], G. Pastorelli [a], F. Maghin[a], G Martemucci.[b], A D'Alessandro.[b], D.[c] Casamassima. *[a] Università degli Studi di Milano, Department of Health, Animal Science and Food Safety, Via Celoria 10, 20133 Milan, Italy; [b] Università degli Studi di Bari, Department of Scienze Agro-Ambientali e Territoriali, Bari, Italy; [c] Università degli Studi del Molise, Dipartimento di Scienze Animali, Vegetali e dell'Ambiente,Campobasso, Italy*

4.1 ABSTRACT

The effects of dietary supplementation with plant extract (PE), containing verbascoside (0.5 mg/kg metabolic weight) on meat quality, oxidative stability and sensory parameters of *Longissimus Lumborum* (LL) muscle in Equidae were investigated. Twelve donkeys and horses, were assigned to two experimental groups: the first group was fed a commercial diet (C) and the second one, the same diet with dietary PE. The trial lasted 6 months and at slaughter, the LL muscle were sampled. Dietary treatment did not affect (P>0.05) physical and chemical parameters of muscle in both donkey and horse. Dietary PE improved (P<0.01) oxidative stability in donkey muscle during refrigerated storage. Sensory characteristics of LL muscle were positively affected (P<0.05) by dietary PE in both donkey and horse. The results suggest that dietary PE containing verbascoside is able to improve oxidative stability and sensory attributes of Equidae meat, without affecting other quality parameters.

4.2 Introduction

In some European countries, like Spain, Italy and France, Equidae meat is traditional and regularly consumed (Sarriés, Murray, Troy, & Beriain, 2006). In particular, horse meat production in Italy has grown in the recent years, being the twelfth largest producer in the EU in 2012 with 18,125 tons (FAOSTAT, 2012). Even if donkey breeding is less popular than horse, its meat represented an interesting source of protein that can increase the income of local farmers (Polidori, Beghelli, Cavallucci, &Vincenzetti, 2011).

Meat derived from these species has excellent nutritional properties, rich in bioavailable iron, and low in fat and cholesterol (Badiani, Nanni, Gatta, Tolomelli, & Manfredini, 1997; Polidori, Vincenzetti, Cavallucci, Beghelli, 2008; Tateo, De Palo, Ceci, & Centoducati, 2008). The fatty acid profile, with a higher omega 3 fatty acids content compared to beef and pork, make this product more suitable for human health (Lee, Seong, Oh, Ko, Kim, & Jeong, 2007). Moreover, this meat is appreciated for its slightly sweet taste, due to the high content of glycogen (Rødbotten, Kubberød, Lea, & Ueland, 2004).

Meat is subjected to a quality deterioration and one of the main causes is related to oxidative phenomena. It is a complex process associated to meat fat content, fatty acids composition, antioxidants content, and other factors such as light, oxygen and storage temperature (Kanner, 1994). Moreover, the accumulation of compounds derived from cholesterol oxidation, such as fatty acid peroxides, cholesterol hydroperoxide, and peroxy radicals, represents a health risks for consumers (Bösinger, Luf, & Brand, 1993; Guardiola, Codony, Addis, Rafecas, Boatella, 1996).

Several compounds exert antioxidant activities, improving colour, flavour and oxidative stability in meat (Ladikos, & Lougovois, 1990). Among the most known antioxidant, the interest in natural extract, containing polyphenols, has been growing in recent years. Polyphenols are extracted from many plants and spices and a high antioxidant capacity has been confirmed (Wojdyło, Oszmianski, & Czemerys, 2007; Moyo, Oyedemi, Masika, & Muchenje, 2012). Verbascoside is the most abundant phenolic compound in *Verbenaceae* extracts and exhibits a higher antioxidant activity compared with other phenolic compounds (Pascual, Slowing, Carretero, Sanchez Mata, & Villar, 2001; Rossi, Corino, Pastorelli, Durand, & Prost, 2009).

An effective strategy for preventing lipid oxidation is dietary supplementation with antioxidant in farm animals (Lahucky, Nuernberg, Kovac, Bucko, & Nuernberg, 2010; Rossi, Pastorelli, Cannata, Tavaniello, Maiorano, & Corino, 2013). Several studies reported that oregano essential oil supplementation, rich in polyphenols, positively influences lamb meat quality, retarding lipid oxidation (Simitzis, Deligeorgis, Bizelis, Dardamni, Theodosiou, & Fegeros, 2008). Also, Kolodziej-Skalska, et al. (2011) have shown that the dietary supplementation with plant extracts mixture (carvacrol, cinnamaldehyde and capsicum oleoresin) enhanced pork quality. Furthermore, recent studies showed that dietary supplementation with natural extract, containing verbascoside, improved pork quality parameters (Rossi, et al., 2013; Rossi, Ratti, Pastorelli, Crotti, & Corino 2014).

No previous study reported the effect of dietary supplementation with plant extract, containing verbascoside, on meat quality parameters and sensory characteristic in Equidae. The aim of the present study is to evaluate the effects

of dietary supplementation with plant extract, containing verbascoside, on meat quality parameters, oxidative stability and sensory characteristics in Equidae.

4.3 Materials and methods

4.3.1. Animals and diets.

Twelve weaned males donkeys of the Martina Franca breed and twelve weaned males Avelignese horses were selected and reared in two different farms. Donkeys and horses were divided in two experimental group fed a control diet (CH for horse and CD for donkey) or a diet supplemented with plant extract supplement (PE), containing 0.5 mg verbascoside/kg metabolic weight (PEH for horse and PED for donkey). The PE supplement contained a water-soluble extract of Verbenaceae (Lippia spp.) leaves (Rossi, et al., 2013). The horses were fed *ad libitum* oat hay and wheat straw (2:1 ratio) with concentrate feed (4 kg/d). Composition of commercial feed was: crude protein (18.3%), crude fibre (3.8%) and fat (3.5%). Commercial feed was composed by corn, wheat, soybean meal, wheat by-products, field beans (Vicia faba minor) and mineral/vitamin premix. The donkeys were fed *ad libitum* grass hay and oat straw (1:1 ratio), oat grain (2 kg/d) and concentrate feed (0.5 kg/d). Composition of commercial feed was: crude protein (16.1 %), crude fibre (3.8%) and fat (3.6%). Concentrates feed was composed by barley, oat, wheat by-products, alfalfa meal, field beans (Vicia faba minor) and mineral/vitamin premix. The experimental diets were administered for 6 months.

The animals used in this experiment were cared for in following with European Union guidelines (No. 86/609/EEC) approved by the Italian Ministry of Health (L. 116/92).

4.3.2 Slaughter and sampling procedures

The animals were slaughtered at 12 months of age. After an on-farm fasting period of 8 hours, the animals were transported to the abattoir. The animals were laired for 4 hours with free access to water. The animals were stunned with a captive bolt, slaughtered, skinned and eviscerated according to current European Union regulations (Council Directive 95/221EC). Samples of *Longissimus Lomborum* (LL) muscle of donkey and horse (N=12 and N=12 respectively) were collected from each right half carcass after chilling for 24 h at 1°C. The samples were cut crosswise to the fibres in slices of 15 mm and each slice was vacuum packed in coded plastic bags free from flavour transmission and immediately stored at -20 C° until laboratory analyses. The day before assessment all samples were transferred to a cooling chamber with a temperature of 4° C, pending analyses.

4.3.3 Physical and chemical parameters

Measurement of pH were performed using a pH meter (HI 9023 microcomputer, Hanna Instruments, Vila do Conde, Portugal). Colour measurements were determined, using a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan). The instrument was calibrated on the CIE LAB colour space system using 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. The measurement values were given in the color spectrum Commission Internationale d'Eclairage (CIE), where L* is lightness; a* is redness; and b* is yellowness. Each data point is the mean of three replications measured at the chop surface.

Samples of LL were analysed for dry matter, crude protein, ether extract and ash according to Association of Analytical Chemists methods (AOAC, 2000).

4.3.4. Measurement of Oxidative Stability

Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Bidlack, Okita, &Hochstein (1973). The LL muscle samples were analyzed as raw meat stored at 4° C for 0 and 96 hours. Briefly, 5 g of minced meat was homogenized in 25 ml of distilled water using an Ultra Turrax T25 homogenizer (IKA, Cincinnati, USA). To the homogenate was added 25 ml of 20% trichloroacetic acid and centrifuged at 5000 g and 4°C for 10 min. The supernatant was filtered with Whatman 52 filter paper. To 1.5 ml of supernatant 1,5 ml of 0.02 M thiobarbituric acid was added and placed at 100°C for 20 min. The absorbance at 532 nm was measured immediately after cooling and compared with a standard curve of malonaldehyde prepared by hydrolysis of tetraethoxypropane. Determinations were made in duplicate. The results were expressed as microgram of malondialdehyde (MDA) per gram of meat.

4.3.5. Sensory analysis

4.3.5.1. Sample preparation

The slices of 15 mmwere cooked for 4 min at greatest power (200 °C) on double-plated grills. A thermocouple (Pentronic AB, 198 Gunnebobruk, Sweden) was inserted in the centre of each piece of meat to register the core temperature. The core temperature was not allowed to exceed 68° and therefore the horse and donkey meat was removed from the oven at approximately 60 to 65°C to avoid post-heating rise; then sliced into 15-mm cubes for presentation

to panelists (Heather, Shane, Beilken, &Leppard, 2005). Two cubes per samples were presented on white plastic plates to each panelist.

4.3.5.2. Sensory evaluation

A selected and trained sensory panel, consisting of 8 members, familiar with meat and sensory procedures was chosen. All assessments were carried out in an equipped sensory laboratory according to ISO 8598 (2007) recommendations. The training period of the judges lasted for 2 months. The aim of this training session was to develop a common vocabulary to describe the Equidae meat samples. The final list of descriptors with the relevant definitions is reported in Table 1 for both donkey and horse. The sensory profile was evaluated according to EN ISO 13299 (2010) and the panel evaluated the samples in triplicate. Four samples (CD vs PED and CH vs PEH) were evaluated in each session. They were asked to score texture, flavor and taste during chewing. If necessary, they could taste more than one cubes, which was then swallowed. Judges were requested to evaluate the intensity of each attribute using a 10 cm unstructured line scale with two anchors (from weak to strong) according to ISO 4121 (2003). Within each session the design was balanced for order and carry over effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

4.3.6. Statistical analysis

Data on carcass characteristics and LL muscle parameters were analyzed by one-way analysis of variance (ANOVA) where diet was the main factor. Data related to oxidative stability (TBARS value) during storage time were assessed by two-way repeated measures ANOVA, to evidence the effects of treatment, time, and their interactions. The sensory data for each attribute were submitted to Analysis of Variance (ANOVA) with dietary treatment (CD/CH and PED/PEH), judges, replicates and their interactions as effects. The significance of these effects was tested with F test. Means were compared according to DUNCAN test with a level of significance at $p < 0.05$. The means of the dietary treatment averaged across judges and replicates were submitted to Principal Component Analysis (PCA) in order to interpret sensory differences among LL muscles. All statistical procedures were computed using SPSS 21.0 (SPSS/PC Statistics SPSS Inc., Chicago, IL, 2009).

Table 1. Attributes and definitions of sensory profile for both donkey and horse *Longissimus Lumborum* muscle

Attibutes		Definition
Appearance		
	Colour	Intensity of red colour
Aroma		
	Typical	Typical aroma associated with cooked meat
	Metallic	Aroma associated with blood or rare meat
Taste		
	Sweet	One of the four basic tastes caused by water solutions of various substances perceived on the tip of the tongue
	Salty	One of the four basic tastes caused by water solutions of various substances perceived on the tip of the tongue
Flavour		
	Typical	Typical flavour, resulting from placing cooked meat in the mouth, involving taste in water solution and smell at the moment of swallowing
	Metallic	Flavour associated with blood or rare meat
Texture		
	Tender	The force needed to masticate the meat ready for swallowing(chewing 5 times)
	Juicy	Wet sensation in the mouth caused by a product after compression between the teeth
	Fibrous	Presence of fibers during chewing

4.4. Results and Discussion

Recently, considerable attention has been focused on the improvement of meat quality parameters (Rossi, et al., 2014; Carpenter, O'Grady, O'Callaghan, O'Brien, & Kerry, 2007). Several studies have showed that dietary supplementation with plant extract, rich in polyphenols, improve shelf-life and quality of animal products, reducing oxidative phenomena (Falowo, et al 2014; Franz, Baserb, & Windischc, 2010). No data have been reported on the effects of dietary supplementation with plant extracts on meat quality parameters in donkey and horse.

Physical and Chemical parameters - The available literature on physical and chemical parameters of Equidae meat is lacking, in particular no studies on the effect of natural extract dietary supplementation are reported. The pH, color indices and chemical parameters of LL muscle from donkeys and horses are reported in Table 2 and Table 3 respectively. No effect of PE dietary supplementation on meat physical and chemical parameters were observed.

Muscle pH values were within the range expected for horse and donkey meat and the values are comparable with the data reported in literature (Tateo, et al., 2008; Polidori, Cavallucci, Beghelli, & Vincenzetti, 2009). Dietary supplementation with plant extract did not affect the pH values of red meat according to O'Grady, Maher, Troy, Moloney, & Kerry (2006) in beef cattle fed with plant tea catechins and rosemary extract for 103 days before slaughter.

In the present study, color indices of muscle were not affected by dietary treatment with natural extract containing polyphenols. This results are in agreement with Rossi, et al., (2013) and Janz, Morel, Wilkinson, & Purchas (2007) who reported that dietary supplementation with plant extract did not affect the color indices of LD muscle in pig. In literature no data on the effect of dietary plant extract on LL muscle colour indices in donkey and horses are reported.

The chemical parameters of LL muscle from Martina Franca donkey are in line with those reported by Polidori, et al., (2011), excepting for intramuscular fat values that resulted lower than those reported in the present study.

Chemical parameters of LL muscle from horses are in line with data reported by Pomianowski, Rotkiewicz, & Borowski, 1994. The intramuscular fat of LL muscle were within the range of 3.1% and 5.4%. Also Sarriés, & Beriain (2005) reported a similar content of intramuscular fat in meat foals from Burguete breed.

Table 2. Influence of dietary plant entract on chemical and physical characteristics of *Longissumus Lumborum* muscle in donkey[1]

Item	CD	PED	P
pH	5.7 ± 0.01	5.6 ± 0.01	0.121
Color indexes:			
L*	58.3 ± 2.81	56.7 ± 1.98	0.652
a*	14.5 ± 1.63	12.6 ± 0.91	0.326
b*	-3.56 ± 0.85	-3.53 ± 0.59	0.978
Chemical composition:			
Dry matter	27.9 ± 0.57	28.3 ± 0.67	0.636
Protein, % [2]	20.6 ± 0.49	20.7 ± 0.39	0.891
Fat, % [2]	4.8 ± 1.0	5.1 ± 0.87	0.843
Ash, % [2]	1.0 ± 0.01	1.0 ± 0.02	0.936

[1]Data are reported as mean values ± SEM; n= 6; CD, Control donkey; PED, Plant extract donkey.

[2] Data expressed as percentage of wet weight

Table 3. Influence of plant extract on chemical and physical characteristics of *Longissumus Lumborum* muscle in horse[1].

| Item	CH	PEH	P
pH	5.7 ± 0.10	5.6 ± 0.02	0.453
Color indexes:			
L*	58.87 ± 0.84	57.76 ± 1.95	0.584
a*	14.1 ± 1.13	12.7 ± 0.17	0.365
b*	1.82 ± 0.53	1.07 ± 0.34	0.355
Chemical composition:			
Dry matter	27.7 ± 0.79	27.1 ± 0.77	0.630
Protein, % [2]	20.7 ± 0.57	21.1 ± 0.24	0.496
Fat, %[2]	3.6 ± 1.0	3.9 ± 0.71	0.816
Ash, %[2]	1.0 ± 0.01	0.9 ± 0.01	0.621

[1]Data are reported as mean values ± SEM; n= 6; CH, Control horse; PEH, Plant extract horse.

[2] Data expressed as percentage of wet weight

Oxidative stability of LL muscle - In Figure 1 and 2 are reported the data on oxidative stability of LL muscle during refrigerated storage from donkey and horse, respectively. In donkey, dietary inclusion of PE decreased oxidative phenomena (P<0.001) during refrigerate storage. As expected, storage time negatively affect oxidative stability (P<0.001) in LL muscle. Interactions between dietary treatment and time were observed (P<0.001). There are no previous studies regarding TBARS values in donkey meat. The present findings indicated that donkeys fed PE had a higher protection from lipid oxidation than the CD group. The data are in agreement with the literature reporting a lower TBARS concentration in muscle of animals fed plant antioxidants, containing polyphenols (Simitzis, et al., 2008, Rossi, et al 2013, 2014).

The TBARS values in horse meat showed no difference between PE and control group (P>0.05). Storage time negatively affected oxidative stability in LL muscle (P<0.001). No interactions between dietary treatment and time were observed (P>0.05). The TBARS values recorded after 0 h of refrigerated storage are in agreement with Peiretti, Medana, Visentin, Giancotti, Zunino, & Meineri (2011). In horse, PE dietary supplementation is not able to contrast meat oxidation. Our findings could be related to the different polyunsaturated fatty acids content of meat in horse than in donkey (21-41,5 % *vs* 13-25,16 % respectively) (Lorenzo, Sarriés, Tateo, Polidori, Franco, & Lanza, 2014; Karatosidi, Marsico, & Tarricone, 2013; Polidori, et al., 2009).

Fig. 1. Oxidative stability of *Longissimus Lomborum* muscle from donkey fed control (CD) or plant extract supplemented diet (PED) during refrigerated storage at 4 °C.
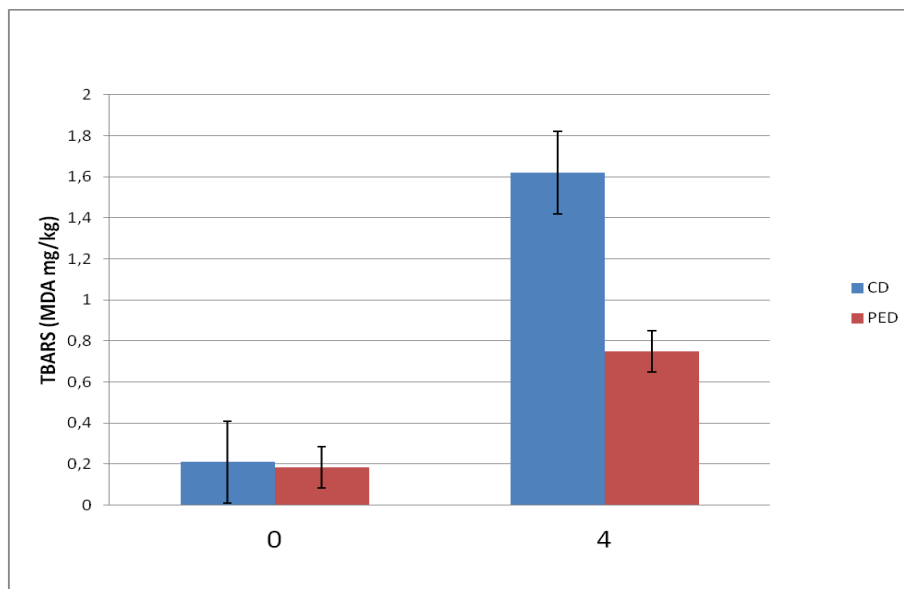
n = 6; data are reported as mean ± SEM. Effects of treatment, P <0.001; time, P < 0.001; treatment∗time, P < 0.001.

Fig. 2. Oxidative stability of Longissimus Lomborum muscle from horse fed control (CH) or plant extract supplemented diet (PEH) during refrigerated storage at 4 °C.
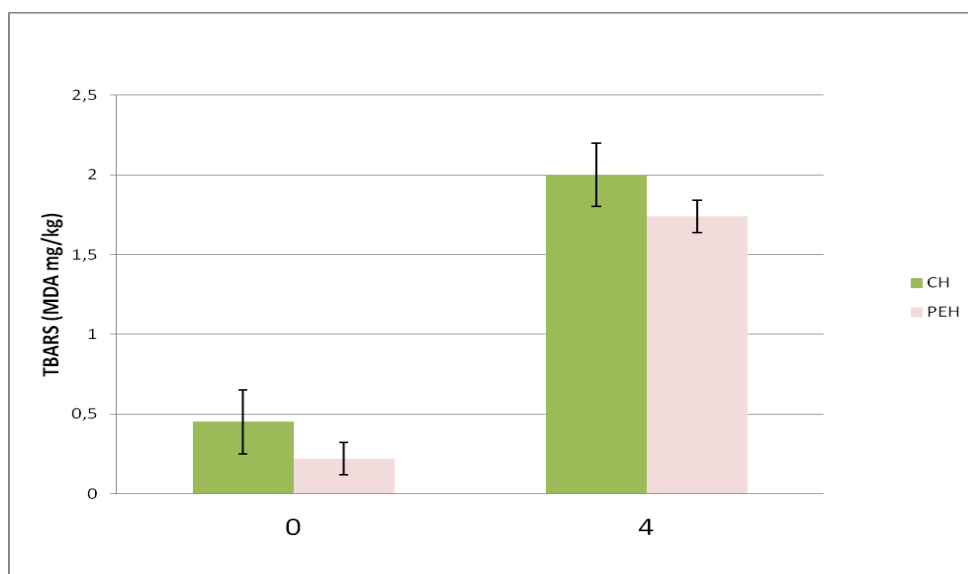
n = 6: data are reported as mean ± SEM. Effects of treatment, P > 0.05; time, P < 0.001; treatment∗time, P >0.05.

Sensory evaluation - In Figures 3 and 4 are reported the spider plot for donkey (CD and PED group) and horse meat (CH and PEH group) for all sensory attributes. The F values for replicates and interactions were not significant for all the attributes, and in donkey (Table 4) and horse meat (Table 5). These results indicate that the mean scores for donkey and horse meat given by the panelist for each attributes could be assumed satisfactory for the sensory profile.

Sensory evaluation showed that PE dietary supplementation affect *LL* aroma and texture in both donkey and horse. In particular, in donkey LL muscle colour, overall aroma, sweetness, tenderness and fibrousness are improved (P<0.05) by PE dietary supplementation (Figure 3). The same result was obtained in LL muscle from horse fed PE: the sensory descriptors related to overall and metallic aroma, saltiness and tenderness resulted higher (P<0.05) in PEH group than CH group. Moreover a less fibrousness was observed in PEH group than CH group (P<0.05).

These results are in agreement with previous studies that found that dietary supplementation with natural antioxidant, improve texture attribute of meat. In particular, Ghazalah & Ali (2008) highlight that feeding broiler with natural extract (0.5 and 1.0% rosemary) for 42 days had a positive effect on taste, texture, aroma and overall acceptability of meat. The same results were found by Cullen, Monahan, Callan, & O'Doherty, 2005 in pork *Longismus Dorsi* muscle for texture parameters. However, some authors reported that natural antioxidant, have no effect on meat sensory characteristics in different species. O'Grady, et al., (2006) found that dietary supplementation with natural extract in beef did not affect meat sensory parameters. The same result was obtained by Rossi, Ratti, Pastorelli, Crotti, & Corino (2014) in pig fed antioxidant mixture for 38 days.

Doth donkey and horse sensory descriptors were averaged across assessors and submitted to Principal Component Analysis (PCA) in order to evaluate the results from a multidimensional point of view. A multidimensional space based on significant sensory data is reported in the Bi-Plot (Figure 5). The variance explained by the first two principal components (PC) was 88% (PC1= 61% and PC2 = 27%).

From left to right along the first component (PC1) donkey and horse meat samples were well distinguished as specie, while along PC2 it can be seen that the samples were separated according to dietary treatments.

Bi-plot also shows the relationship between the sensory attributes and their influence on the space. In fact texture attributes, sweet taste and metallic (aroma and flavour) are located along PC1 in the right hand panes and negatively correlated to salty taste, colour appearance (red) and typical (aroma and flavour). The PC2 that accounts for less variation, is positively related to tenderness and typical aroma, which are located at positive values and negatively related to fibrousness. As expected sweet is negatively correlated to salty taste.

Comparison of samples and attributes shows that meat derived from dietary integration with PE are better characterized by more descriptors in both specie. The PED meat is more red, salty and "typical" than control group (CD). Also PEH meat resulted more tender, juicy and aromatic than CH group.

The PCA model well described the Equidae samples in relation to dietary treatment with PE. Moreover, the samples assumed a well-defined position in the space for both donkey and horse meat. This data indicate that the judges had the same perception of the sensory aspects of the Equidae samples and are able to categorize them.

The integration of these results with those obtained by ANOVA allows us to state that there are differences in describing and perceiving meat samples from a qualitative point of view. Our findings are in agreement with data observed by Webb & O'Neill (2008) in contemporary consumers. The main sensory criteria associated with meat quality are: juiciness, taste, and tenderness.

Table 4. Sensory evaluation in donkey meat: F value and statistical significance of samples (n = CD and PED), judges (n = 8), replicates (n = 3) and their interaction for each sensory attributes.

Attributes	Fvalue					
	Samples	Judge	Replicates	S_*J	S_*R	J_*R
Red	8.89***	2.46 n.s.	3.74 n.s.	2.26 n.s.	3.49 n.s.	1.41 n.s.
Typical aroma	11.49**	45.32***	3.46 n.s.	2.23 n.s.	0.17 n.s.	5.55**
Metallic aroma	0.10 n.s.	33.49***	3.01 n.s.	0.18 n.s.	2.10 n.s.	2.53*
Sweet	8.50*	88.42***	2.76 n.s.	7.14**	15.28***	4.62**
Salty	1.76 n.s.	52.34***	2.23 n.s.	1.89 n.s.	2.12 n.s.	1.83 n.s.
Typicalflavour	0.00 n.s.	40.97***	1.15 n.s.	1.11 n.s.	2.60 n.s.	1.59 n.s.
Metallicflavour	0.50 n.s.	82.76***	6.24*	0.49 n.s.	0.21 n.s.	0.68 n.s.
Tender	4.89 *	6.90**	3.45n.s.	1.04 n.s.	1.54 n.s.	1.03 n.s.
Juicy	2.17 n.s.	28.90***	3.32 n.s.	0.89 n.s.	4.25 n.s.	2.67 n.s.
Fibrous	6.28*	16.01***	3.01 n.s.	1.36 n.s.	0.09 n.s.	1.80 n.s.

S_*J, Sample*Judge; C_*R, Sample * Replicates; G_*R= Judge *Replicates
*** Significant at $P < 0.001$; ** Significant at $P < 0.01$; * Significant at $P < 0.05$; n.s. = no significant.

Table 5. Sensory evaluation in horse meat: F value and statistical significance of samples (n = CH and PEH), judges (n = 8), replicates (n = 3) and their interaction for each sensory attributes.

Attributes	F value					
	Samples	Judges	Replicates	S*J	S*R	J*R
Red	0.48 n.s.	16.68***	2.08 n.s.	0.68 n.s.	2.37 n.s.	2.00 n.s.
Typical aroma	6.66*	28.76***	5.73*	3.79*	3.03 n.s.	2.27 n.s.
Metallic aroma	5.06*	21.00***	0.38 n.s.	2.08n.s.	2.29 n.s.	0.49 n.s.
Sweet	15.72**	46.24***	2.54 n.s.	2.72 n.s.	1.35 n.s.	3.04*
Salty	5.61*	29.94***	0.50 n.s.	3.79*	0.51 n.s.	2.37n.s.
Typical flavour	0.86 n.s.	31.56***	3.36 n.s.	1.05 n.s.	0.49 n.s.	2.05 n.s.
Metallic flavour	0.12 n.s.	24.30***	0.79 n.s.	0.64 n.s.	1.89 n.s.	1.75 n.s.
Tender	11.34**	8.86***	1.19 n.s.	1.24 n.s.	2.94 n.s.	1.16 n.s.
Juicy	0.15 n.s.	8.07***	1.47 n.s.	1.27 n.s.	3.49 n.s.	0.77 n.s.
Fibrous	5.19*	27.27***	1.82 n.s.	1.88 n.s.	1.74 n.s.	1.73 n.s.

S*J, Sample * Judge; C*R, Sample * Replicates; G*R= Judge * Replicates
*** Significant at $P < 0.001$; ** Significant at $P < 0.01$; * Significant at $P < 0.05$; n.s. = no significant.

Figure 3: Spider plot of the sensory profile of LL muscle from donkey fed control diet (CD) or diet supplemented with plant extract (PED).
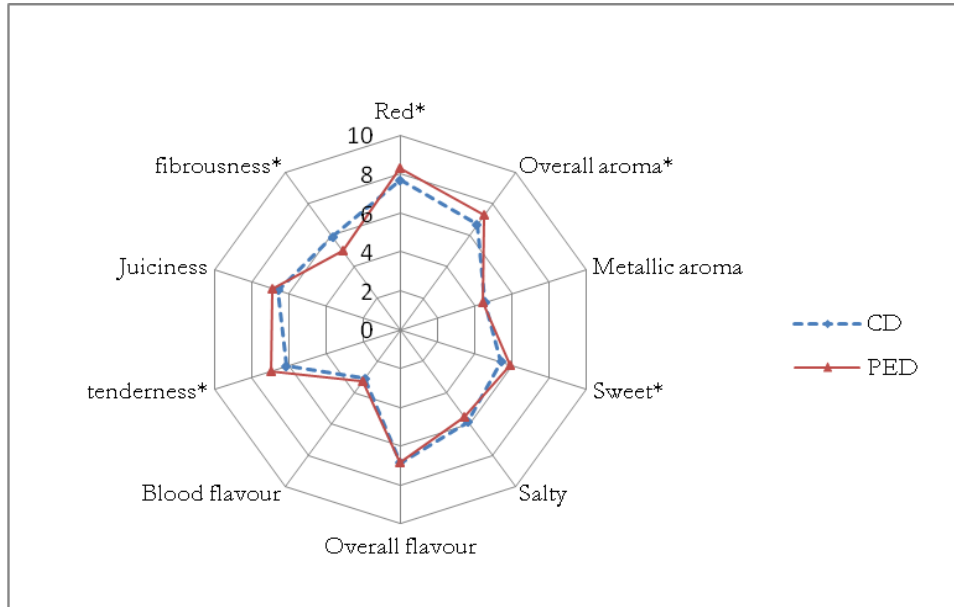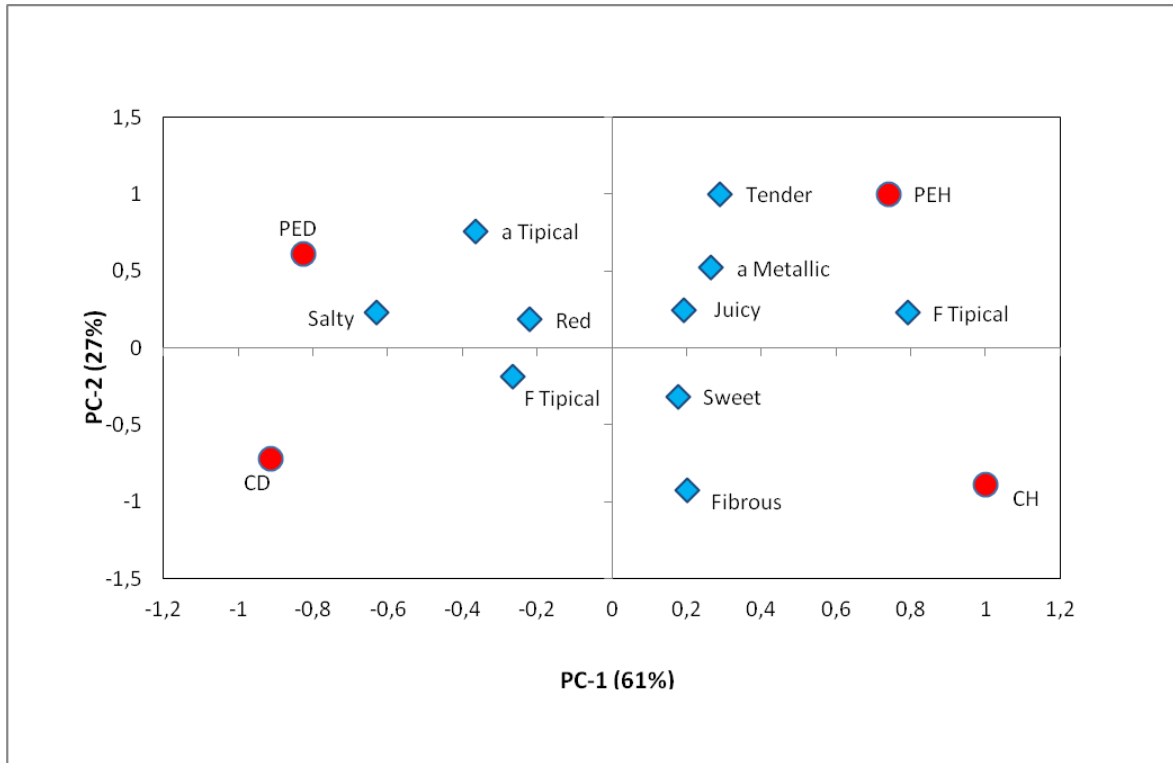

*Values are different for P<0.05

Figure 4. Spider plot of the sensory profile of LL muscle from horsefed control diet (CH) or diet supplemented with plant extract (PEH).
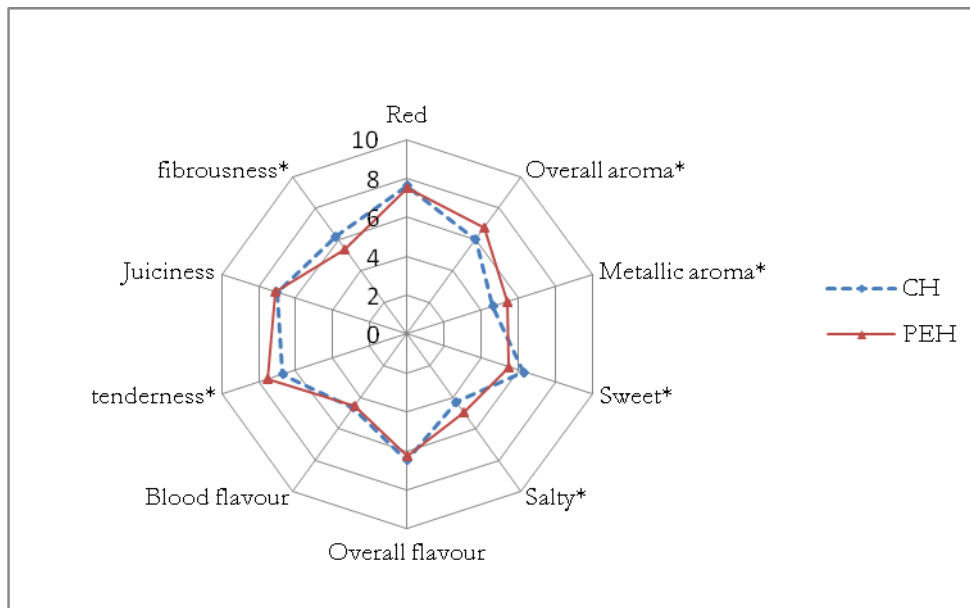
*Values are different for P<0.05

Figure 5. Bi-plot obtain by PCA model of donkey and horse meat sensory data.

- CD, donkey receive a control diet; • CH, horse receive a control diet; • PED, donkey receive supplemented diet with plant extract; • PEH, horse receive supplemented diet with plant extract
- Sensory attributes (F, Flavour; A, Aroma).

4.5. Conclusion

The present data show that dietary PE supplementation in Equidae improve oxidative stability and sensory parameter in LL muscle without affect phisycal and chemical parameters. These results suggest the opportunity to enhance the eating quality of donkey and horse LL muscle. In addition, supplementation with PE is active as an antioxidant in muscle due to the reduced levels of lipid oxidation biomarkers. These are interesting results since no previous studies reported an improvement of meat quality in Equidae in relation to dietary PE. Further studies are needed to determine the optimal length and dosage of dietary supplementation in both donkeys and horse meat and meat product.

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CHAPTER 5

Nutritional and sensory quality of cooked ham from 135 kg lw pigs

5. Nutritional and sensory quality of cooked ham from 135kg lw pigs

AUTHORS: S. Ratti, R. Rossi, G. Pastorelli and C. Corino, *University of Milan, Department of Health, Animal Science and Food Safety, Milan Italy.*

5.1 Introduction

Cooked ham is one of the most commonly consumed pork product in several European countries including Italy. Besides the technological guidelines for the production of cooked ham, genetic and breeding conditions of pigs have an important role on the quality of the final product (De Winne and Dirink 1997; Lindahl et al. 2006). Pigs in Europe are reared to different slaughter weights. The Italian heavy pig production, with an average slaughter weight of 160-170 kg, is designed to products of Protected Designation of Origin (PDO), such as Parma and San Daniele dry cured hams (Lo Fiego et al. 2005). The cooked product has not a PDO due to the great amount of raw thighs imported from other European countries. In these countries, pigs are slaughtered at a live weight of 90–110 kg and are mainly destined to produce fresh meat, whereas thighs are exported to be transformed in cooked ham. Italian regulations concerning cured products obtained from pork was aimed to characterize and classify Italian hams according to parameters that mainly include the weight of the thighs, the technological process and the proximate composition of processed products (Ministerial Decree, G.U. n 231, 04.10.2005). Medium-heavy swine represents an alternative for the Italian heavy pig farming. Medium-weight pigs are suitable for fresh meat consumption and the production of cooked ham. In literature no data are available on cooked ham quality from medium-heavy swine. This research was carried out to define the physicochemical and sensory parameters of cooked hams from medium-heavy swine thighs and processed under commercial guidelines. The quality of cooked ham from two Italian heavy pig genetic types slaughtered at a live weight of 135 kg was investigated.

5.2 Materials and Methods

All data were recorded in a commercial scale in a meat processing plant located in Northern Italy. Three hundred pigs balanced for body weight and sex, half PIC 1050 x TOP D (PT) and half ANAS F1 (Italian Large White x Italian

Landrace) x Duroc (AD), of initial body weight of 60 kg were selected from two different farms. Pigs were fed commercial diets on the basis of 9% of metabolic weight (LW0.75). At an average weight of 135 kg, pigs were slaughtered in a commercial slaughterhouse (Pro Sus, s.c.a., Vescovato, Cremona, Italy). Twenty pigs per treatments (10 castrated males and 10 females) were randomly selected and the left thighs were sampled (figure 1). All the thights were weighed and processed under commercial guidelines for production of cooked hams (Citterio, Rho, Milan, Italy). After processing, cooked hams quality parameters were evaluated. For the chemical analysis, a slice of about 1,5 cm thickness was taken from each sample. After removing the external fat layer, the slice was finely minced and moisture, protein, lipid and ash content in accordance with AOAC (2000) methods were determined. Selected and trained sensory panel, consisting of 12 members, familiar with pork products (EN ISO 13299, 2010) was chosen. Data were analyzed by one-way analysis of variance (ANOVA) where genetic type was the main factor (SPSS/PC Statistics 18.0 SPSS Inc., Chicago, IL).

5.3 Results and Discussion

Genetic type significantly affects chemical parameters of cooked ham (Table 1). The dry matter resulted significantly higher (P<0.01) in ham from AD than PT. Also the fat content was affected by genetic type and resulted tendentially higher (P=0.09) in cooked ham from AD group than PT. Sensory evaluation showed no significant difference in texture, flavor, aroma and visual descriptors as reported in Figure 2.

TABLE 1. Chemical properties (expressed as % of wet weight) of cooked ham from PIC 1050 x TOP D (PT) and ANAS F1 (Italian Large White x Italian Landrace) x Duroc (AD) pigs slaughtered at 135 kg.

Trait	Genetic Type		
	PT	AD	*P*
Dry matter, %	30.2 ± 0.47	32.1 ± 0.36	*0.005*
Crude Protein, %	20.19 ± 0.51	20.26± 0.36	*0.904*
Ether extract, %	6.52 ± 0.38	7.52 ± 0.43	*0.090*
Ash,%	2.54 ± 0.05	2.54 ± 0.04	*0.982*

Figure 2. Visual representation of cooked ham sensory profile
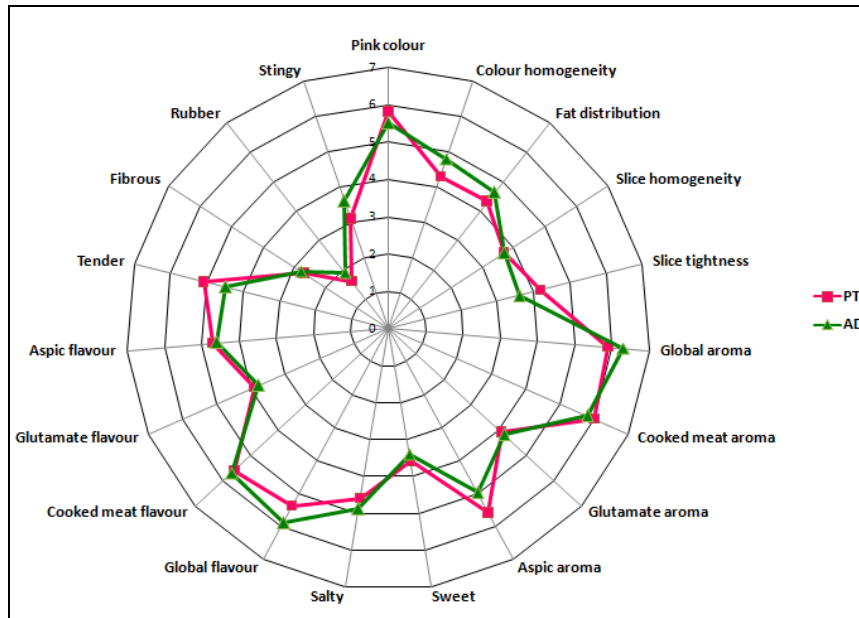
5.4 Conclusion

The evaluation of chemical detectable parameters of ham represents an important tool to define and characterize this product. Genetic type significantly affects some chemical parameters of cooked ham from medium-weight pigs, without affecting sensory parameters.

Further studies are required to better characterize this product and to identify the genetyc type more suitable for the production of cooked ham from medium-weight pigs.

5.4.1 Acknoledgements

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CHAPTER 6

Effect of dietary linseed on the nutritional value and quality of pork and pork products: Systematic review and meta-analysis

6 Effect of dietary linseed on the nutritional value and quality of pork and pork products: Systematic review and meta-analysis

AUTHORS: Carlo Corino, Raffaella Rossi, Susanna Cannata, Sabrina Ratti.
Università degli Studi di Milano, Department of Health, Animal Science and Food Safety, Via Celoria 10, 20133 Milan, Italy

6.1 Abstract

Nutritional quality of pork is a significant factor for consumers' health. Feeding n-3 PUFA to pigs, using linseed, improves pork nutritional quality. A meta-analysis involving 1006 pigs reported in 24 publications was carried
out to assess the effects of dietary linseed on alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) content in muscle and adipose tissue. Data showed positive effects of n-3 PUFA on muscle fatty acid composition: ALA + 137%, EPA + 188%, DPA + 51% and DHA + 12%. Same results were observed in adipose tissue: ALA + 297%, EPA + 149%, DPA + 88% and DHA + 18%. A positive correlation between dietary treatment and ALA and EPA content in muscle (P b 0.001) and adipose tissue (P = 0.036) was observed. A significant association between DPA (P= 0.04) and DHA (P=0.011) and live weight in muscle was observed. Feeding linseed to pig improves the nutritional pork quality, raising the n-3 PUFA content in muscle and adipose tissue.

6.2 Introduction

In recent years, consumer interest in the relationship between diet and health has increased the demand for functional foods. Omega-3 fatty acids are recognised to be functional components that may reduce the incidence of cardiovascular disease (Beilin & Mori, 2003; Connor, 2000; Kris-Etherton, Harris, & Appel, 2002).
It has been assumed that the physiological requirement for long chain (LC) n-3 PUFA can be satisfied by the consumption of plant foods (such as linseeds, walnuts, soybean and canola oils), containing their precursor alpha-linolenic acid (ALA). Conversion of ALA into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans is low (Portolesi, Powell, & Gibson,

2007), and the conversion to DHA is better in infants than in adults, as also recently reported in pigs (Brenna, Salem, Sinclair, & Cunnane, 2009; De Quelen, Boudry, & Mourot, 2010). In pigs, it has been observed (Kloareg, Noblet, & Van Milgen, 2007) that approximately one-third of the supplied n-3 that was deposited resulted from the conversion of ALA to EPA and DHA.

Currently, authorities (EFSA, 2012; FAO/WHO 2008) recommend that the macronutrient distribution range in adults include the consumption of 20% to 35% of total energy (E) from fat although one authority suggest that it is possible to increase the amount till 40% (AFSSA, 2010), with a maximum of 10% of E from SFA and up to 6–11% of E from PUFA. The intake of n-3 fatty acids should be 0.5–2% of E and the intake of n-6 fatty acids should be 2.5–9% of E. Recommendations concerning the n-6/n-3 ratio suggest a value between 5:1 and 10:1. Nutritionists have expressed concern that the typical Western diet provides too much n-6 and not enough n-3 PUFA (Givens & Gibbs, 2008). One way to increase the intake of n-3 PUFA, without changing the consumers' nutritional behaviour, would be to fortify traditional foods such as meat and meat products with n-3 PUFA. Indeed meat is one of the major sources of fat in the diet with an average European per capita consumption of around 98 kg (Llorens Abando & Martinez Palou, 2006).Meat fat contains a high amount of SFA associated with some modern diseases (Wood et al., 2003).

Feeding n-3 PUFA to pigs using linseed could improve the nutritional quality of pork, but may adversely affect its sensory qualities due to the susceptibility of n-3 PUFA to oxidation (Lyberg, Fasoli, & Adlercreutz, 2005).

The aim of this work was to identify and summarise the main effects or the effect orientation of dietary enrichment with n-3 PUFA using linseed and to review works showing the effect of increasing PUFA n-3 content in pig tissues on meat quality. Considering the great amount of literature that investigates the effects of dietary linseed on pork quality traits and the high results variability, due to the different experimental conditions, there is a need to obtain a statistical synthesis. A meta-analysis was conducted in order to establish the real effect of the orientation of linseed dietary exposure on nutritional meat quality parameters from a set of comparable studies. Further, because few data are available on physical and sensory characteristics of meat quality of pork fed linseed, a critical review was performed.

6.3 Materials and methods

Animal Care and Use Committee approval was not required for this study because the data were obtained from an existing database.

6.3.1 Literature search

A systemic literature search was carried out by search of journals, book articles and abstracts from CAB Abstracts (ISI) to identify articles published between January 1975 and April 2013. The structured strategy included the following keywords applied as follows: "pork" OR "pig" AND "n-3 PUFA" OR "Omega 3" OR "linseed" OR "flaxseed" AND "fatty acids". A manual review of the reference list of the selected articles was conducted to identify additional articles for possible inclusion. Additional studies were identified from the reference lists of retrieved articles. The literature search focused exclusively on articles published in peer-reviewed journals for the methodological accuracy of the studies. Two independent reviewers, evaluated the eligibility of each article. The reviewers were blinded to author, institution, and journal of publication. Articles were excluded based on abstract review only if both reviewers independently believed the inclusion criteria were not met. Otherwise, all the remaining studies were assessed using the complete papers. Any disagreements between the two reviewers were resolved by a third reviewer.

6.3.2 Study selection

To be included in the reviewthe studies needed to satisfy the following criteria: (1) data collected from January 1975 up to April 2013; (2) English, French or Italian language; (3) study carried out in crossbred pigs from about 25 to 160 kg of live weight (LW); (4) linseed supplemented diet; (5) study assessed both control and linseed supplemented diets using isoenergetic and isoproteic diets; (6) study reported fatty acid composition of Longissimus thoracis et lumborum (LTL) muscle and/or adipose tissue. Our principal aim was to evaluate the effect of linseed dietary treatment on meat quality parameters, referring in particular to fatty acid composition of muscle and adipose tissue. For this reason experimental trials, involving different genetic types, body weight and length of linseed supplementation, in which meat quality parameters were evaluated, were selected. Considering the restricted amount of trials we include in the meta analysis animals with average initial weight of 49.4 kg (25 to 85 kg LW) and average final weight of 98.4 kg (from 50 kg to 160 kg LW). Dietary linseed supplementation of included studies ranged from 30 to 103 days. Growth performances (average daily gain, average daily feed intake) were not evaluated due to the inadequate number of results. Not one of the examined studies explicitly reported blinded analyses of the results and nine studies were classified as randomised because they reported that the trial involved random assignment of animals to treatment groups. Some studies reported dietary comparisons were not relevant to this article, or if there were more than one comparison group, only the results addressing the objectives of this article were extracted. The outcomes evaluated were the fatty acid composition of LTL

muscle and subcutaneous adipose tissue. The data on ALA, EPA DPA and DHA content in intramuscular fat (IMF) and adipose tissue were subjected to a meta-analysis. The lipid extraction methods were not taken into account for the restricted results available. Moreover, considering the limited number of data we decided to include linseed oil and extruded linseed, with awareness of the different fat digestibility. In fact, fat digestibility of ground linseed was considerably lower than after extrusion (51% vs. 81% and 90% for two different extrusion procedures) (Noblet, Jaguelin-Peyraud, Quemeneur, & Chesneau, 2008). In addition, linseed oil had a higher digestibility in pigs (92.6% apparent ileal digestibility) than ground linseed but comparable to extruded linseed (Duran-Montgé, Lizardo, Torrallardona, & Esteve-Garcia, 2007).

6.3.3 Data extraction

A database was created, including detailed description of each reference: author's name, publication year, animals used (gender, breed, weight), housing condition (group size), design details (randomization and blinding), control and experimental diets (including description of n-3 fatty acids), source and dose of linseed, duration of feeding, tissues sampling, statistical analyses (mean value, standard deviation/standard error and P value) and fatty acid composition of Longissimus thoracis et lumborum (LTL) muscle and/or adipose tissue. Lipid extraction method was not taken in account.

6.3.4 Statistical analysis

The inputs for meta-analysis were statistical analysis results reported in literature: means or difference in means, standard error/standard deviation and P-value. The effect sizes were calculated using Hedges' g approach. The random-effect model was used to determine the overall weighted mean difference. The random-effects model, assumes that the treatment effect is not the same across studies. The goal is to estimate the average effect in the studies that are weighted equally (Sauvant, Schmidely, Daudin, & St-Pierre, 2008). The heterogeneity of effect size across trials was tested by I2 statistic (Higgins & Thompson, 2002). Categorical characteristic (sex)was treated as moderator, while continuous characteristics (live weight, length of dietary treatment, dietary ALA content and linseed % feed)were examined as covariates using random effects (method of moments) of meta-regression. The subgroup analysis refers to the effect in a group with the same categorical characteristics. Publication bias was investigated using "trim and fill" procedure (Duval & Tweedie, 2000). Based on conventional standards, effect sizes of g equal to 0.20, 0.50, and 0.80 were considered small, medium, and large respectively (Cohen, 1988). The data were analysed with the program Comprehensive Meta Analysis v2.2 (Borenstein, Hedges, Higgins, & Rothstein, 2010).

6.4 Results

Study characteristics - The CAB abstracts (ISI) search on dietary linseed for the fatty acid composition of muscle and adipose tissue yielded 460 publications. Fig. 1 shows the flow chart of identifying and including trials. After the exclusion criteria (n = 415) only 45 papers were selected. Among these references there were some studies in which no control diet was reported (n = 5). In some experiments additional dietary ingredient, which could interact with linseed were supplied (n = 12). Other researchers reported fatty acid composition of different muscles than LTL or subcutaneous adipose tissue (n = 4). After applying exclusion criteria, 21 articles were selected. Examination of the reference lists of these articles yielded three additional articles. In total, 24 articles were included (18 for adipose tissue) in the meta-analysis. Tables 1 and 2 show selected characteristics of the studies that met the criteria for analysis in muscle and adipose tissue respectively. Of the selected articles, 22 reported that feed was supplied for ad libitum consumption. Only 10 papers reported the average daily gain (ADG) and 7 reported the average daily feed intake (ADFI). Of the selected studies, 15 reported that carcasses were chilled for 24 h at 4 °C (Beckova & Vaclavkova, 2010; Duran-Montgé, Realini, Barroeta, Lizardoa, & Esteve-Garcia, 2008; Enser, Richardson, Wood, Gill, & Sheard, 2000; Kouba, Enser, Whittington, Nute, & Wood, 2003; Lu, Zhang, & Yin, 2008; Nuernberg et al., 2005; Pieszka, 2007; Romans, Johnson, Wulf, Libal, & Costello, 1995; Wiecek, Rekiel, & Skomial, 2010), 2 studies reported that were chilled for 48 h at 1 °C (Riley, Enser, Nute, & Wood, 2000; Romans et al., 1995) and the others reported no information. All the studies reported that samples were packaged and stored at −20 ° C pending analyses, except for four studies that did not report any information (Eastwood, Kish, Beaulieu, & Leterme, 2009; Kralik, Margeta, Suchy, & Straková, 2010; Matthews, Homer, Thies, & Calder, 2000; Wilfart, Ferreira, Mounie, Robin, & Mourot, 2004). The lipid extraction method from muscle and adipose tissues was also evaluated. Of the 24 experimental studies only three experimental studies used Soxhlet lipid extraction (one study in adipose tissue and three in LTL muscle) (Eastwood et al., 2009; Flachowsky, Schulz, Kratz, & Glodek, 2008; Kralik et al., 2010). One study (Karolyi, Rimac, Salajpal, Kljak, & Štoković, 2012) reported transesterification using the method of Park and Goins (1994). The other 20 studies used lipid cold extraction with chloroform and methanol according to the methods of Folch, Lees, and Stanley (1957) or Bligh and Djer (1959).

Intramuscular fat and fatty acid composition of muscle and adipose tissue - Several studies reported the effect of dietary linseed on pork quality (reviewed by Wood et al., 2008). A linear increase of more than 40% in the IMF content was observed in

pigs fed 10% linseed diet for 90 days (Huang, Zhan, Luo, Liu, & Peng, 2008). No differences in total lipid content in both muscle and adipose tissue have been reported in many other studies (Guillevic, Kouba, & Mourot, 2009a; Matthews et al., 2000). Regarding fatty acid, an increase in muscle ALA, EPA and DPA content, proportional to the their dietary content has been reported, but contrasting data have been reported for DHA (Matthews et al., 2000; Nuernberg et al., 2005). Other authors (Wilfart et al., 2004) have confirmed an increase in muscle DHA in pig fed linseed but have shown that it is not related to the ALA content in the diet. In adipose tissue an increase in ALA, EPA, DPA and DHA content proportional to their dietary amount and to the length of supplementation has been reported (Matthews et al., 2000; Nuernberg et al., 2005; Wilfart et al., 2004).

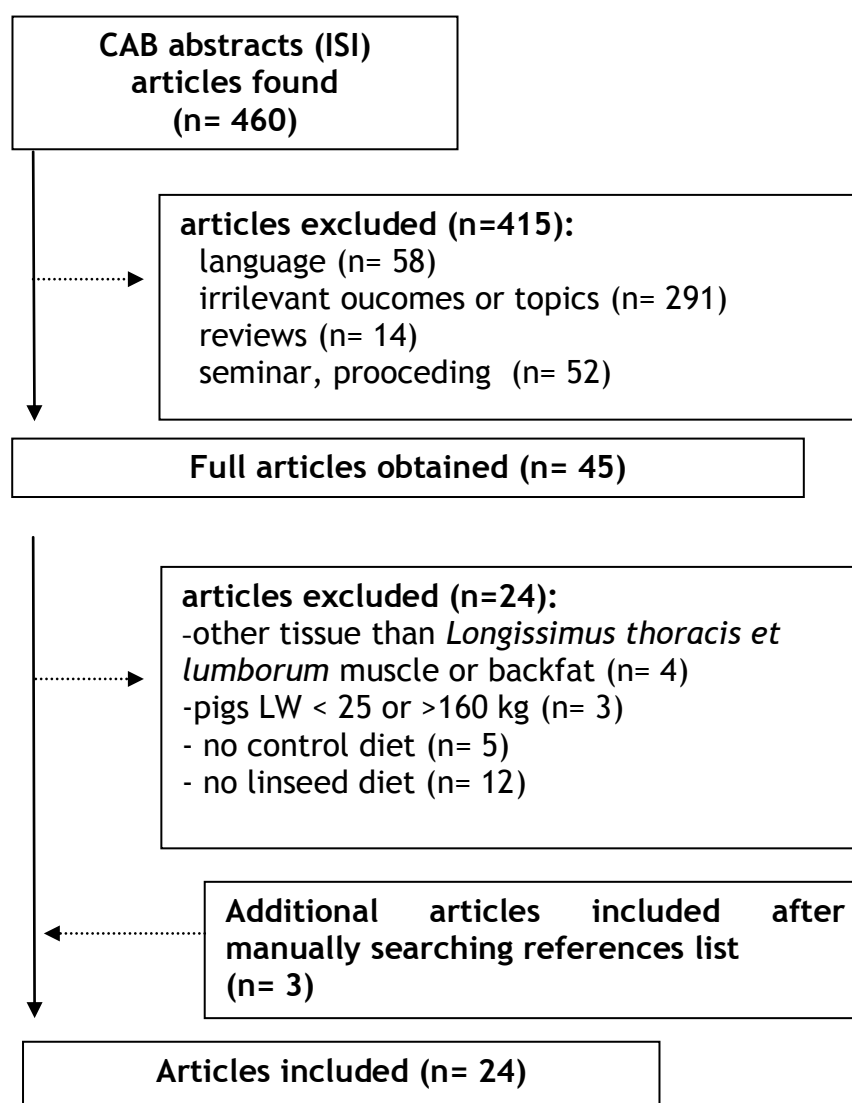**Figure 1.** Selection process of trials.

Table 1
Characteristics of included trials: effect of dietary linseed on fatty acids composition of LD muscle.

References	Year	Gender	Genetic	Weight (kg)	Feeding	Performance	Linseed (% feed)	ALA (g/kg feed)	Vit E (mg/kg feed)	Duration (days)
Beckova et al.	2010	F	(LWxL)x(HxP)	40–90	Ad libitum	ADG	13.4	6.7	31.4	63
Corino et al.	2008	CM	LW × (LxP)	80–110 80–160	Ad libitum	ADG/ADFI	5 extr.	6.7	170	30 80
Duran Montge et al.	2008	F	D × L	60–100	Ad libitum	n.r.	9.68 oil	47.1	15	50
Eastwood et al.	2009	CM, F	n.r.	85–115	Ad libitum	ADFI	0.5 1.0 1.5	2.3 3.1 5.5	40	30
Enser et al.	2000	F,M	n. r.	25–95	Ad libitum	n.r.	1	4.5	100	85
Flachowsky et al.	2008	CM, F	D;HxD;PxH	30–120	Individual feeding	n.r.	2.5	1.3	36	103
Fontanilla et al.	1997	CM	L × D	26–96	Ad libitum	ADG/ADFI	4 oil	12.0	n.r.	82
Guillevic et al.	2009a	CM	(LWxL) × P	50–105	Ad libitum	ADG/ADFI	4.2 extr.	5.6	40	60
Haak et al.	2008	F, CM	(HN) × P	40–100	Ad libitum	n.r.	3	4.7	100	120
Hoz et al.	2003	F	LW × GY	50–100	Ad libitum	n.r.	3 oil	12.1 12.5	200	56
Huang et al.	2008	CM	L × LW	35–115	Ad libitum	n.r.	10	10.2 12.4	200	60 90
Karolyi et al.	2012	CM, F	PIC	27–101	Ad libitum	ADG	3	1.5	155	90
Kouba et al.	2003	F	D × L × LW	40–50 40–93 40–127	Ad libitum	n.r.	6	8.8	150	20 60 100
Kralik et al.	2010	n.r.	LW; L; P	70–100	n.r.	n.r.	2	6.2	–	n.r.
Lu et al.	2008	CM	D × L × LW	45–100	Ad libitum	n.r.	3 oil	10.1	100	60
Matthews et al.	2000	F, CM	n.r.	30–85	Ad libitum	ADG/ADFI	0.5 1.0	2.1 3.3	100	65
Pieszka	2007	F, CM	L	50–105	Ad libitum	n.r.	3	1.1	n.r.	n.r.
Rey et al.	2001	CM	L × LW	50–	Ad libitum	n.r.	0.5 oil	3.1	10	42
Riley et al.	2000	F	LW × L	46–96	Ad libitum	n.r.	1 2 3	3.4 6.0 8.1	200	65
Romans et al.	1995	CM, F	n.r.	86–104	n.r.	ADG	0.5 1.0 1.5	1.8 3.7 5.5	n.r.	25
Wilfart et al.	2004	F, CM	(LWxL) × P	50–105	Ad libitum	ADG/ADFI	5 extr.	5.1	40	65

F, female; M, entire male; CM, castrated male; LW, Large White; L, Landrace; H, Hampshire ; HN, Helvoirt Netherland ; PIC, Pig Improvement Company ; P, Piétrain; D, Duroc; GY, Great York; n. r., not reported; ADG, average daily weight gain; ADFI, average daily feed intake; extr., extruded linseed; LA, linoleic acid (18:2n-6); ALA, alpha-linolenic acid (18:3n-3).

Table 2
Characteristics of included trials: effect of dietary linseed on fatty acids composition of adipose tissue.

References	Year	Gender	Genetic	Weight (kg)	Feeding	Performance	Linseed (% feed)	ALA (g/kg feed)	Vit E (mg/kg feed)	Duration (days)
Beckova et al.	2010	F	(LW × L) × (H × P)	40–90	Ad libitum	ADG	13.4	6.7	31.4	63
Corino et al.	2008	CM	LW × (L × P)	80–110 80–160	Ad libitum	ADG/ADFI	5 extr.	6.7	170	30 80
D'Arrigo et al.	2002	F	LW × GY	48–100	Ad libitum	n.r.	3	13.7	24 vs 31	56
Duran Montge et al.	2008	F	D × L	60–100	Ad libitum	n.r.	9.68 oil	47.1	15	50
Eastwood et al.	2009	CM, F	n.r.	85–115	Ad libitum	ADFI	0.5 1.0 1.5	2.3 3.1 5.5	40	30
Enser et al.	2000	F, M	n.r.	25–95	Ad libitum	n.r.	8	4.5	100	85
Flachowsky et al.	2008	CM, F	D, H × D; P × H	30–120	Individual feeding condition	n.r.	2.5	1.3	36	103
Fontanilla et al.	1997	CM	L × D	26–96	Ad libitum	ADG/ADFI	4 oil	12	n.r.	82
Guillevic et al.	2009a	CM	(LW × L) × P	50–105	Ad libitum	ADG/ADFI	4.2 extr.	5.6	40	60
Huang et al.	2008	CM	L × LW	35–115	Ad libitum	n.r.	10	10.2 12.4	200	60 90
Karolyi et al.	2012	CM, F	PIC	27–101	Ad libitum	ADG	3	1.5	155	90
Kouba et al.	2003	F	D × L × LW	40–50 40–93 40–127	Ad libitum	n.r.	6	8.8	150	20 60 100
Matthews et al.	2000	F, CM	n.r.	30–85	Ad libitum	ADG/ADFI	5 10	2.1 3.3	100	65
Nernberg et al.	2005	F, CM	P × GL	40–105	Feed twice daily	n.r.	5 oil	38.9	12.5	75
Riley et al.	2000	F	LW × L	46–96	Ad libitum	n.r.	1 2 3	3.5 8.1	200	65
Romans et al.	1995	CM, F	n.r.	86–104	No info	ADG	0.5 1.0 1.5	1.8 3.7 5.5	n.r.	25
Wiecek et al.	2010	F, CM	(LW × L) × D	61–104	Ad libitum	ADG/ADFI	4	14.3	30	50
Wilfart et al.	2004	F, CM	(LW × L) × P	50–105	Ad libitum	ADG/ADFI	5 extr.	5.1	40	65

F, female; M, entire male; CM, castrated male; LW, Large White; L, Landrace; H, Hampshire; PIC, Pig Improvement Company; P, Piétrain; D, Duroc; GL, German Landrace; GY, Great York; n.r., not reported; ADG, average daily weight gain; ADFI, average daily feed intake; extr., extruded linseed; LA, linoleic acid (18:2n-6); ALA, alpha-linolenic acid (18:3n-3).

Meta analysis—muscle and adipose tissue - A significant effect of dietary linseed on muscle and adipose tissue fatty acid composition was confirmed by results of meta analysis. As reported in Table 3, feeding dietary linseed (1–13.4% seed or 0.5–9.68% oil) to crossbred pigs during the growing phase increases ($P < 0.05$) ALA, EPA, DPA and DHA content in both tissues. The results showed positive effects of dietary n-3 PUFA from linseed on muscle fatty acid composition: ALA + 137%, EPA + 188%, DPA + 51% and DHA + 12%. The same results were observed in adipose tissue: ALA + 297%, EPA + 149%, DPA + 88% and DHA + 18%. As shown in Figs. 2 and 3 all the studies reported a large significant effect of dietary linseed compared with control in both tissues. The linseed effect for DHA content is small in muscle tissue ($0.20 < g < 0.50$) and resulted medium ($0.50 < g < 0.80$) in adipose tissue. Heterogeneity test, revealing the variation among data, indicated that the treatment effect was significant ($I^2 > 50$) across studies for ALA ($P < 0.001$) DPA ($P < 0.001$) and DHA ($P < 0.001$) in muscle tissue and for DHA ($P = 0.002$) in adipose tissue as shown in Table 3.

Meta-regression - Dietary treatment: a meta-regression was conducted to determine a dose–response relationship between dietary ALA content (ALA g/kg feed) and the outcomes measured in muscle and adipose tissue. The result showed a significant association between dietary ALA and ALA and EPA content in muscle tissue (slope = 0.13; intercept= 0.41, $P < 0.001$; Slope = 0.14; intercept = 0.42, $P < 0.001$ respectively).In adipose tissue a significant association between dietary ALA and EPA content was observed (slope = 0.07; intercept = 0.78, P = 0.036). Dietary ALA content did not affect ($P > 0.05$) the other outcomes analyzed (data not shown). Live weight: a meta-regression was conducted to determine a dose–response relationship between final live weight (kg) and the outcomes measured in muscle and adipose tissue. A significant association was observed for DPA (slope = 0.02; intercept = −0.74, P = 0.04) and DHA (slope= 0.017; intercept= −1.48, P = 0.011) in muscle tissue.

Subgroup analysis - Subgroup analysis was performed by sex, considering CM(castrated males), F (females) and CMF (both genders). The ALA and EPA content in muscle tissue was significantly modified by sex (P b 0.001). The effect size of CM and F group has a major impact and no heterogeneity in muscle tissue: ALA content (g = 1.443; 1.212–1.673 and I2 = 0.00) and EPA content (g= 1.402; 1.199–1.606 and I2= 0.00). The DHA content in adipose tissue was significantly modified by sex. The effect size without F group has a major impact and no heterogeneity (g= 0.894; 0.652–1.137 and $I^2 = 0.00$). The DPA content in muscle and adipose tissue was not modified by sex.

Pubblication bias—muscle and adipose tissue - The Trim and Fill method evidenced moderate bias for ALA EPA and DPA in muscle tissue, as reported in Table 4. The adjusted values did not affect the results.

Meat physical parameters, oxidative stability and sensory feedback - There were no differences for any of the literature search results about pH in muscle. Two studies reported differences for instrumental colour. They found that loin from linseed-fed animals had a darker appearance, as measured by the colour lightness (L*) (Juarez et al., 2011). Their results are in contrast with several reports in literature in which no differences for colour parameters L* (lightness), a*(redness) and b* (yellowness) were found (Corino, Musella, & Mourot, 2008; Haak, De Smet, Fremaut, Van Walleghem, & Raes, 2008; Sheard et al., 2000). No differences have been observed for drip and cooking losses in meat from pigs fed a linseed diet (Haak et al., 2008; Huang et al., 2008; Nuernberg et al., 2005; Riley et al., 2000). The oxidative stability of meat was influenced by its fatty acid composition. A study reported that oxidative stability was lower in LM muscle from heavy pigs fed linseed diet than controls (Corino et al., 2008). A recent research showed that lipid peroxidation was higher in chops of pigs fed linseed diet than control (Guillevic et al., 2009b). Several studies reported an increase in the TBARS (thiobarbituric acid reactive substances) value, expressed as mg malondialdehyde/kg tissue, in fresh pork (Hoz et al., 2003; Kouba et al., 2003; Nuernberg et al., 2005; Rey et al., 2001). Other authors reported an increase of the TBARS value after 6 or 8 days of storage at 4 °C in meat from pigs fed a 3% linseed diet (Rey et al., 2001; Riley et al., 2000). These results are in contrast with that of others that reported no differences between dietary treatments (Enser et al., 2000,Matthews et al., 2000). Another study reported that meat from pigs fed a linseed diet had a lower TBARS content than meat from pigs fed a control diet (Sheard et al., 2000). In all these studies control and linseed supplemented diet contained the same amount of antioxidant molecules. In only one study vitamin E plus selenium was supplemented (170 mg vitamin E and 170 μg as selenite and 80 μg as Se-methionine/kg feed) (Guillevic et al., 2009b). In the other experimental studies the amount of dietary vitamin E in both experimental diet varied from 40 to 200 mg/kg feed (Corino et al., 2008; Hoz et al., 2003; Kouba et al., 2003; Matthews et al., 2000, Nuernberg et al., 2005; Rey et al., 2001; Riley et al., 2000). Dietary linseed affects sensory parameters in pork. A previous study reported that dietary crushed linseed decreased pork odour and flavour and increased fat off-flavour in LTL muscle (Kouba et al., 2003). Feeding 3% linseed oil did not affect the flavour attributes of cooked LM, but cooked biceps branchii muscles had a lower pork flavour and a higher off flavour than control (Lu et al., 2008). A short-term trial reported that loin steak from pig fed linseed resulted more tender and juicy than controls, probably due to the high fat content of linseed diet (Riley et al., 2000). A recent study (Cannata et al., 2010) reported a sensory comparison between n-3 enriched dry-cured hams using linseed with those that represented the control group, Italian typical Parma ham and Italian non-branded ham (16 months of ripening

each product). A negative sensory feedback has been observed for the linseed dry-cured ham from both Italian and French consumer panels. Le Minous, De Broucker, Blochet, Guillevic, and Mourot (2008) reported a lower grade of appreciation, obtained from a 60-consumer test, for paté de champagne from pigs fed linseed diet. Others (Romans et al., 1995) observed differences in sensory attributes reported by both a trained panellist and consumer, in particular more off flavour and dislikes for bacon flavour when pigs were fed linseed, but no differences were observed for pork loin.

Table 3. Summary of meta-analysis findings in in muscle and adipose tissue.

|	N° of trials	g^1	95% CI	P	I^2	P^2
Muscle						
ALA	29	1.23	0.99; 1.47	*<0.001*	56.16	*<0.001*
EPA	28	1.15	0.97; 1.34	*<0.001*	32.57	*0.051*
DPA	15	1.07	0.68;1.46	*<0.001*	68.46	*<0.001*
DHA	25	0.33	0.07; 0.59	*0.013*	63.86	*<0.001*
Adipose tissue						
ALA	29	1.32	1.15; 1.49	*<0.001*	7.35	*0.353*
EPA	21	1.24	1.02; 1.45	*<0.001*	16.52	*0.244*
DPA	12	1.34	0.99; 1.70	*<0.001*	40.59	*0.07*
DHA	22	0.59	0.34; 0.85	*<0.001*	52.80	*0.002*

[1]Pooled effects size was estimated using a random effects model (g di Hedge's). ALA, alpha-linolenic acid (18:3n-3); EPA, eicosapentaenoic acid (20:5n-3); DPA Docosapentaenoic acid (22:5n-3); DHA, docosaesaenoic acid (22:6n-3); [2] heterogeneity test

	g^1	95% CI
Muscle		
ALA	0.93	0.70; 1.17
EPA	0.90	0.69; 1.10
DPA	0.85	0.48; 1.22
DHA	0.19	-0.09; 0.47
Adipose tissue		
ALA	1.17	0.89; 1.36
EPA	1.05	0.82; 1.28
DPA	1.14	0.79; 1.49
DHA	0.66	0.38; 0.94

Table 4. Adjusted results from "Trim and Fill" method.

1Pooled effects size was estimated using a random effects model (g di Hedge's). ALA, alpha-linolenic acid (18:3n-3); EPA, eicosapentaenoic acid (20:5n-3); DPA Docosapentaenoic acid (22:5n-3); DHA, docosaesaenoic acid (22:6n-3)

Figure 2. The dietary treatment effects on ALA, alpha-linolenic acid (18:3n-3) (A); EPA, eicosapentaenoic acid (20:5n-3) (B); DPA, docosapentaenoic acid (22:5n-3) (C); and DHA, docosaesaenoic acid (22:6n-3) (D) content in muscle tissue. The diamond represents the overall effect size.

Figure 3. The dietary treatment effects on ALA, alpha-linolenic acid (18:3n-3) (A); EPA, eicosapentaenoic acid (20:5n-3) (B); DPA, docosapentaenoic acid (22:5n-3) (C); and DHA, docosaesaenoic acid (22:6n-3) (D) content in adipose tissue. The diamond represents the overall effect size
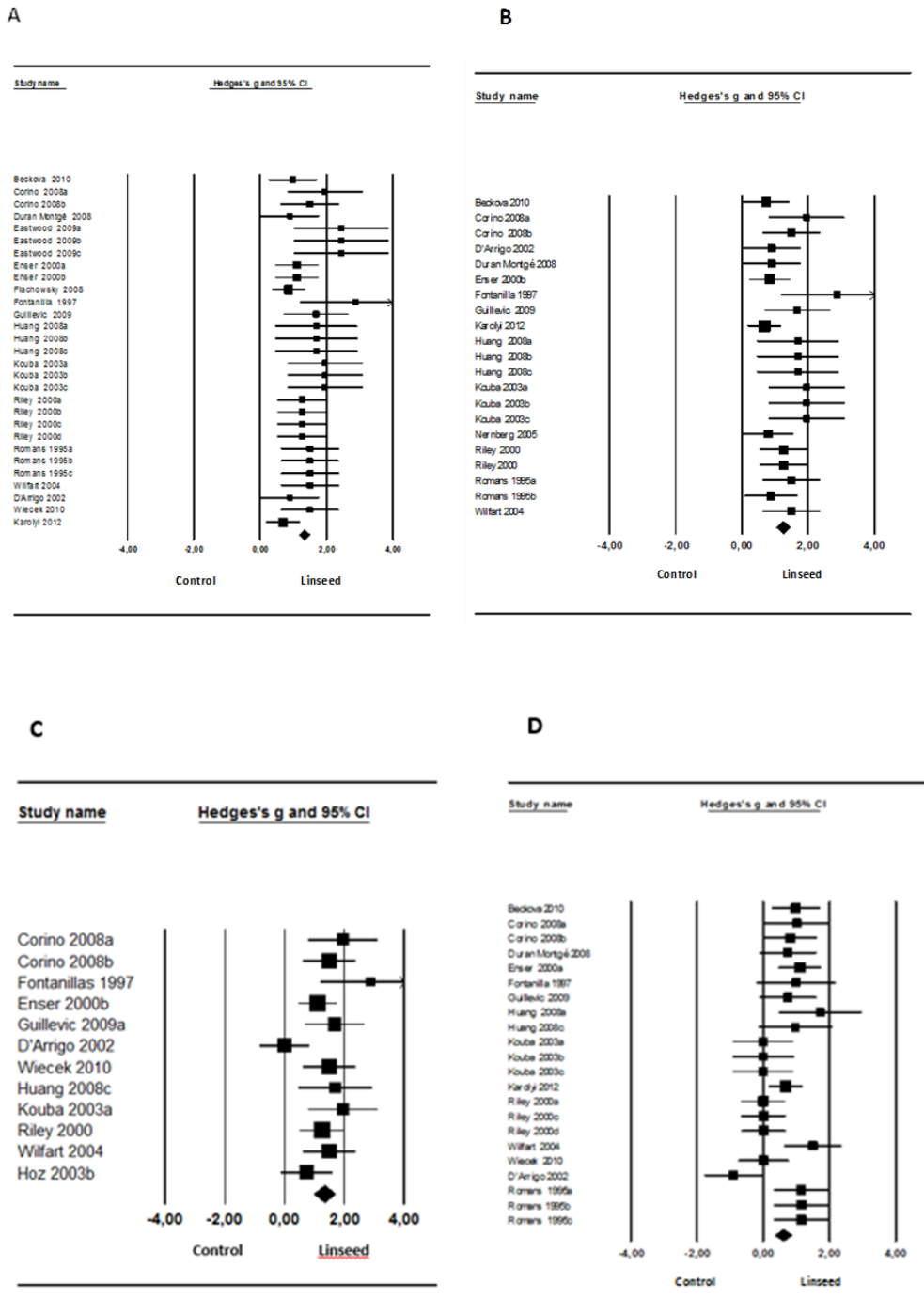
6.5 Discussion

Intramuscular fat and fatty acid composition of muscle and adipose tissue -
The use of linseed in pig diet seems to be a good source of n-3 PUFA, due to its economic and technical sustainability and ALA content (50% of fatty acids). The use of the entire seed could be more practical than the oil, and the whole seed has natural antioxidants, which could slow down the oxidative processes of PUFA (Cunnane, Stitt, Ganguli, & Armstrong, 1990). The available literature showed that dietary fatty acids composition did not affect the IMF percentage. Acetyl-CoA-carboxylase and fatty acid synthase activities were enhanced with the linseed diet in subcutaneous and intermuscular adipose tissue, while malic enzyme activity was decreased in liver and subcutaneous adipose tissue of pigs fed the linseed diet. The result was the lack of diet effect on lipid content of the different tissues (Guillevic et al., 2009a). Instead, some data suggested that the linseed enrichment in muscle would be an important determinant of the extent of improvement in IMF deposition (Huang et al., 2008). This result is supported by recent research that reported how enhancing n-3 PUFA enrichment in muscle leads to a significant increase in IMF content due to alterations in the expression of genes involved in adipogenesis (Luo et al., 2009). Pigs fed linseed diet are amenable to changes in the fatty acid composition of adipose tissue and muscle because these tissues are influenced by the fatty acid composition of the diet (Kouba &Mourot, 1999). Dietary fatty acids are promptly deposited in pig adipose tissue, and there is a linear correlation between the dietary PUFA content and the intramuscular PUFA contents. It is reported that there is a different efficiency in fatty acid deposition: lower amount in the intramuscular than in the subcutaneous adipose tissue (Fontanillas, Barroeta, Baucells, & Codony, 1997; Warnants, Van Oeckel, & Boucque´, 1996). According to the present results, this may be due to a weak deposition of absorbed fat in muscle tissue or to the greater amount of membrane lipids in IMF containing high quantities of PUFA, which are less sensitive to dietary variation. Results from the meta-analysis confirmed that it is possible to improve the nutritional quality of pork by linseed supplementation in pig, enhancing ALA, EPA, DPA and DHA content in both muscle and adipose tissue. A recent study reported that docosapentaenoic acid (DPA) appears principally derived from endogenous elongation from EPA (Mozaffarian & Wu, 2012). DPA can also be retro converted to EPA; in contrast, retro conversion of DHA to DPA is limited. Some lines of evidence suggest that EPA and DHA provide cardiovascular benefits. DPA may also have some positive effects, but its levels appear to depend on endogenous conversion from EPA, and effects of direct DPA consumption are unknown (Mozaffarian &Wu, 2012). Dietary linseed allows ALA in muscle to compete more effectively with LA for the pathways

responsible for producing LC PUFA (Raes, De Smet, & Demeyer, 2004). The results by meta-analysis confirmed what has been reported by a recent research in which DHA is more efficiently incorporated into adipose tissue than muscle (+18% vs. +12% respectively) (Haak et al., 2008). The meta-regression analysis was used to examine whether there was a dose–response relation between linseed dietary treatment (ALA g/kg feed) and the change in n-3 PUFA content in tissues. As expected ALA and EPA appear to be dose dependent: ALA and EPA in muscle tissue increase linearly as the total dietary ALA increases. This effect is also evident when the live weight increases. The lack of effect on DHA may be explained by competition for $\Delta 6$ desaturase activity between ALA and the precursor for DHA (i.e. 24:5 n-3), when the dietary concentration of ALA is high (Cameron, Wood, Wihittington, Penman, & Robinson, 2000). The results from our meta-regression analyses are consistent with these observations. The high intakes of n-3 fatty acids result in their incorporation into membrane phospholipids, where they replace ARA (Crawford et al., 2000). Decreasing the availability of ARA results in a suppression of the biosynthesis of ARA-derived eicosanoids in favour of EPA-derived 3-series prostanoids and 5-series leukotrienes. This suppressing mechanism explains how the n-3 fatty acids may lower the risk of cancer
(Natalie, Rgia, Miyoung, & Mohamed, 2009).

Meat physical parameters, TBARS and sensory feedback - No effect of n-3 PUFA on pH and drip and cooking losses was observed. Overall, no consistent effect is reported on instrumental colour parameters using linseed diet. The n-3 PUFA are particularly susceptible to oxidation that negatively affects sensory parameters and represents a risk to human health. In fact, some molecules produced by lipid oxidation, such as the oxides of cholesterol, could be atherogenic, mutagenic, and carcinogenic agents (Vicente, Sampaio, Ferrari, & Torres, 2012). Quality problems, concerning oxidation, appear to have been controlled by dietary supplementation with vitamin E and selenium in pigs slaughtered at 110 kg of LW and in fresh meat for consumption or fermented pork products that require a short time for ripening (Ansorena & Astiasaràn, 2004). It seems to be different for pork products that require a longer ripening process and that have a longer
shelf-life. Dietary linseed improved nutritional quality of raw material for end products, increasing the n-3 PUFA content and decreasing the n-6/n-3 ratio from 12 to 3 in dry-cured ham (Musella et al., 2009). However, long term n-3 PUFA dietary supplementation in heavy pigs for Parma Ham production, increased the ALA content in green ham. This caused problems with the iodine value, exceeding the level accepted by the Consortium of Parma Ham (Council Regulation (EEC) N_2081/92, 2081/92) and with the sensory characteristics. A consumer test, conducted by two different nationality panels (French and

Italian), evaluated four products and reported a negative discrimination for n-3 enriched dry-cured ham (Cannata et al., 2010).

The use of dietary antioxidant in pig protected meat from oxidation and showed positive effects on colour, nutritive value (Faustman, Sun, Mancini, & Suman, 2010) and flavour (Cardenia et al., 2011). A meta analysis showed a positive effect of dietary vitamin E supplementation on a* value in pork (Trefan et al., 2010). Moreover, the length and dosage of vitamin E supplementation affected α-tocopherol content in muscle and consequently lipid oxidation (Sales & Koukolová, 2011).

The oxidative and colour stability of pork depend mostly on the balance of antioxidant and oxidant substances (Serpen, Gökmen, & Fogliano, 2012). Some studies reported lower α-tocopherol levels in pork tissues containing high n-3 PUFA levels (Jensen et al., 1997; Wang, Leibholz, Bryden, & Fraser, 1996), probably due to increased metabolism of the vitamin E as a result of the n-3 PUFA greater susceptibility to oxidation. A recent study, in pigs fed linseed, reported that 200 mg/kg feed of dietary vitamin E is sufficient to protect LTL muscle from lipid oxidation, increasing α-tocopherol tissue levels (Botsoglou, Govaris, Ambrosiadi, & Fletouris, 2012; Sales & Koukolová, 2011; Trefan et al., 2010). Integration of vitamin E and selenium (170 mg of vitamin E and 250 μg of selenium) was not sufficient to preserve the sensory quality of dry-cured ham from heavy pigs fed a linseed diet (Cannata et al., 2010).

Plant antioxidants can be employed in association and/or partial replacement with synthetic substances (vitamin E) due to their high inhibitory effect on lipid oxidation. A recent study in pig reported that long term supplementation with dietary plant extract, containing verbascoside, is able to increase vitamin E content in LTL muscle (+34% than control), enhancing oxidative stability (Rossi et al., 2013).

Green tea catechin supplementation in pig diet (200 mg/kg feed) improved oxidative stability in LTL muscles (Mason et al., 2005). Plant antioxidant mixture supplementation in pigs fed n-3 PUFA enriched diet is able to reduce MDA content in dry-cured ham, without affecting other quality parameters (Mairesse et al., 2011).

Therefore, considering the high antioxidant power of phenylpropanoid glycoside it could be interesting to investigate its possible influence on meat quality and to evaluate its use in animal feed instead of synthetic vitamin E, which is normally present in feedstuff (Corino, Pastorelli, Pantaleo, Oriani, & Salvatori, 1999; Corino et al 2007).

There are several implications from this meta-analysis for nutritionists working in the field of functional food design. The present data show a clear evidence that dietary linseed can improve nutritional quality of pork and pork products enhancing n-3 PUFA content in both tissue. Despite these strengths, several

doubts remain. First, there is a significant heterogeneity between selected studies and although the source for heterogeneity with sub-group analyses and meta regression was investigated, there persists the possibility that other sources of heterogeneity exist.

The present work provides information to find the optimal dosage, source and length of linseed dietary supplementation in relation to slaughter weight and genetic type. Moreover, nutritional studies are required to evaluate the efficacy of linseed treatment in pork products in enhancing PUFA Omega-3 intake in humans. In conclusion, further studies are required to confirm findings of this meta-analysis and to extend the results to other pork products.

6.5.1 Acknowledgements

6.6 References

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CHAPTER 7

General Discussion

7. General discussion

7.1 General conclusions

The study of meat quality during this three years pointed out the strictly connection between genetic impact, animal nutrition, and quality parameters of meat and meat product.

Recently, considerable attention has been focused on the improvement of meat quality parameters. The protection of lipids from oxidative phenomena is fundamental to preserve meat color and nutritional quality and increase shelf life. Indeed, color changes influence the acceptability of meat, and represent an indicator of quality and freshness. Moreover, lipid oxidation produces compounds, such as fatty acid peroxides, cholesterol hydro peroxide, and peroxy radicals that are responsible for the decline in quality of meat and represent health risks.

Dietary antioxidants are one of the major strategies for preventing lipid oxidation and help to decrease meat oxidative phenomena. The search for safe and effective natural antioxidants has focused on plants, particularly in spices and herbs.

Verbascoside (VB) is the most abundant phenolic compound in *Verbenaceae* extracts.

Our results point out the importance of dietary supplementation with plant extract, containing verbascoside, on oxidative stability, physical, chemical and sensory parameters of different type of meat and derived product.

This thesis leads to some specific conclusions which are listed below;
• Dietary supplementation with an antioxidant mixture containing verbascoside in medium-heavy/heavy swine, exerts an antioxidant effect on *Longissimus Dorsi* muscle, enhancing color parameters and oxidative stability.
• Dietary supplementation with an antioxidant mixture, containing verbascoside, in Equidae, improved oxidative stability and sensory parameter in *Longissimus Lomborum* muscle without affecting physical and chemical parameters. These results suggest the opportunity to enhance the eating quality of donkey and horse meat reducing levels of lipid oxidation biomarkers. These are interesting results since no previous studies reported an improvement of meat quality in Equidae in relation to dietary supplementation with natural antioxidant.
• The evaluation of chemical detectable parameters of cooked ham represents an important tool to define and characterize this product. Genetic

type significantly affects some chemical parameters of cooked ham from medium-weight pigs, without affecting other quality parameters.

• Nutritional quality of pork and pork products can be improved through linseed dietary supplementation, enhancing n-3 PUFA content both in muscle and adipose tissue. Meta-analysis results provides information to find the optimal dosage, source and length of linseed dietary supplementation in relation to slaughter weight and genetic type.

7.2 Recommendation for future studies

On the basis of the discussed points in this thesis, following topics can be of interest for future research:

• More studies focused on polyphenols content are required to clarify the optimal length of plant extract dietary supplementation to enhance quality parameters in fresh meat and meat product.

• The results on cooked ham could be considered as a good starting point for further studies related to study the genetic type more suitable for the production of cooked ham from medium-heavy swine.

• Further studies are required to evaluate the efficacy of linseed treatment in meat and meat products in enhancing PUFA Omega-3 content.

• The data related to meat quality in Equidae could be considered as a good starting point for future studies on this product type.

CHAPTER 8

Summary

The influence of animal nutrition on meat quality

Sabrina Ratti

Abstract:

Food and Agriculture organization of the United Nations (FAO) define meat quality as compositional quality and palatability factors such as visual appearance, smell, firmness, juiciness, tenderness, and flavour. Recently, the attention has been focused on the improvement of meat quality parameters. The protection of lipids from oxidative phenomena is fundamental to preserve meat color, nutritional quality and increase shelf life, enhancing consumer acceptability. Otherwise, it is possible to enhance nutritional quality of meat and meat products, modifying fat content and fatty acid profile. Animal feeding is a key factor for improve meat quality parameters through dietary supplementation with different additives.

Antioxidants are one of the major strategies for preventing lipid oxidation in meat products, decreasing oxidative phenomena and increasing shelf life. The search for safe and effective natural antioxidants has focused on plants extract. Dietary supplementation with antioxidant mixture containing polyphenols in swine, enhance color parameters and oxidative stability in *Longissimus Dorsi* muscle (trial 1 and 2). Moreover, the same plant extract improved oxidative stability and sensory parameter in donkey and horse *Longissimus Lomborum* muscle (trial 3).

Also genetic type significantly affects cooked ham chemical profile, without affecting other quality parameters (trial 4).

Nutritional quality of pork products are improved by dietary linseed supplementation, enhancing muscle and adipose tissue n-3 PUFA content. Meta-analysis data provides information to find the optimal dosage, source and length of linseed dietary supplementation in relation to slaughter weight and genetic type (trial 5).

Our results point out the importance of animal dietary supplementation with plant extract and linseed, on oxidative stability, physical, chemical and sensory parameters of different type of meat and meat product.

CHAPTER 9

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CHAPTER 10

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