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Natural extracts in animal nutrition: animal well–being and products quality

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NATURAL EXTRACTS IN ANIMAL NUTRITION:
ANIMAL WELL–BEING AND PRODUCTS QUALITY

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"… Non vogliate negar l'esperienza
di retro al sol, del mondo sanza gente.
Considerate la vostra semenza
fatti non foste a viver come bruti
ma per seguir virtute e canoscenza."

(Dante Alighieri, Divina Commedia, Inferno canto XXVI, 116–120)

Abstract

The main objective of this thesis was to have a deeper knowledge about the influence of feeding strategies with several natural plants extracts on pig performance and meat quality in different phases of pig production. To achieve this objective, a set of 4 studies were performed (chapter 2– 6).

The first study evaluated the effect of dietary supplementation with a mixture of algae plus polyphenols (AM) in lactating sow on maternal and piglets' performance and sows' reproductive data until the subsequent farrowing. This study showed that integration of AM in sow diet decreased the fat mobilization during lactation. Moreover, dietary AM inclusion in lactating sows positively affected body weight and average daily gain in piglets at 21 days. Moreover, sows that received AM had more total number of piglets born at the subsequent farrowing.

Regarding the effects of plant extracts on meat and product quality, two different studies were performed. In the second study of the thesis, the impact of dietary integration with vitamin E and verbascoside (AOX) in pigs, on carcass characteristics, meat quality, shelf life of pork under modified atmosphere packaging (MAP) was evaluated. This study demonstrated the positive effects of plant extracts as antioxidant. Particularly, highlighted that dietary AOX positively affected carcass dressing percentage and pork oxidative and colour stability. Moreover, sensory analysis revealed that, at 15 days under MAP, meat from AOX was comparable with fresh meat in appearance and aroma.

In the same way, the third study showed that vitamin E and verbascoside (AOX) also affected the quality of derived product like smoked cured ham. Results relieved infact that the AOX dietary inclusion affected the seasoning losses and influenced the consumers' preference of smoked cured ham, without affecting other quality parameters.

Finally, the fourth experimental trial examined the impact of 3 % hydrolysable tannins (HT) from chestnut extract and two levels of polyunsaturated fatty acid (PUFA) in the diets on growth performance, carcass traits, meat quality and boar taint compounds in entire males. Results showed that dietary HT reduced the feed efficiency but not feed intake and the pigs' final weight. These results show that performance, carcass composition and meat quality traits are not affected by dietary 3% chestnut extract supplementation in entire male. The boar taints compounds like androstenone, skatole and indole tended to be lower in HT group. The cytochrome mRNA expression in the liver and colon mucosa was not affected by the diet. No evident relationship between dietary PUFA level and boar taint compound levels was observed.

Overall, these results contribute to improve the knowledge regarding beneficial effects of plant extracts. The inclusion of bioactive components contained in natural extracts can

be considered an innovative approach to improve pig wellbeing and pork quality without negative effects on animal production.

Key words: Swine; Pig production; Natural extracts; Pig performance; Meat quality.

Riassunto

L'obiettivo principale di questa tesi è stato quello di avere una conoscenza più approfondita circa l'influenza delle strategie alimentari con diversi estratti naturali di piante sulle performance dei suini in diverse fasi della produzione e sulla qualità della carne. Per raggiungere questo obiettivo, sono stati eseguiti una serie di 4 studi (Capitolo 2– 6).

Il primo studio ha valutato l'effetto della supplementazione dietetica con una miscela di alghe più polifenoli (AM) in scrofe in lattazione sulle prestazioni materne e dei suinetti e sui dati riproduttivi delle scrofe fino al parto successivo. Questo studio ha dimostrato che l'integrazione di AM nella dieta della scrofa ha diminuito la mobilizzazione dei grassi durante l'allattamento. Inoltre, l'inclusione nella dieta di AM nelle scrofe in lattazione ha influenzato positivamente il peso corporeo e il guadagno medio giornaliero nei suinetti a 21 giorni di lattazione. Inoltre, le scrofe che hanno ricevuto AM risultano avere un piu' elevato numero di suinetti nati al parto successivo.

Per quanto riguarda gli effetti di estratti di piante sulla qualità della carne e dei prodotti, sono stati eseguiti due studi differenti. Nel secondo studio della tesi, è stato valutato l'effetto dell'integrazione alimentare con vitamina E e verbascoside (AOX) sulle caratteristiche della carcassa, la qualità della carne e shelf life della carne confezionata in atmosfera modificata (ATM). Questo studio ha dimostrato gli effetti positivi degli estratti vegetali come antiossidanti. In particolare, ha evidenziato che la dieta AOX ha influenzato positivamente la percentuale della carcassa, l'ossidazione e la stabilità del colore della carne suina. Inoltre, l'analisi sensoriale ha rivelato che, a 15 giorni in ATM, la carne dal gruppo AOX era paragonabile alla carne fresca per aspetto e l'aroma.

Allo stesso modo, il terzo studio ha dimostrato che la miscela di vitamina E e verbascoside (AOX) ha influenzato la qualità del prodotto derivato come lo speck. I risultati rivelano che l'inclusione nella dieta di AOX ha influenzato le perdite stagionatura dello speck ed ha influenzato la preferenza dei consumatori, senza influire sugli altri parametri di qualità.

Infine, il quarto studio ha esaminato l'effetto della supplementazione dietetica con estratto di castagna (3%) contenente tannini idrolizzabili (HT) e due livelli di acidi grassi polinsaturi (PUFA) nella dieta di suini non castati sulle performance di crescita, caratteristiche della carcassa, la qualità della carne e dei composti che determinano il tipico odore di verro nelle carni. I risultati hanno mostrato che la dieta HT ha ridotto l'efficienza alimentare, senza influenzare il *feed intake* e peso finale dei suini. Questi risultati dimostrano che le performance, la composizione della carcassa e la qualità della carne non sono influenzati dalla supplementazione dietetica con 3% di estratto di castagno nei suini interi. I composti come androstenone, scatolo e indolo tendono ad essere più bassi nel gruppo HT. L'espressione dei citocromi nel fegato e nella mucosa del colon non sono stati

influenzati dalla dieta. Non è stata osservata alcuna relazione evidente tra il livello dietetico PUFA e livelli di composti che determinano il tipico odore di verro nelle carni.

Nel complesso, questi risultati contribuiscono a migliorare le conoscenze riguardanti gli effetti benefici degli estratti vegetali. L'inclusione di componenti bioattivi contenuti negli estratti naturali può essere considerata un approccio innovativo per migliorare il benessere e la qualità della carne di suino, senza effetti negativi sulla produzione animale.

Key words: Suini; Produzione suina; Estratti naturali; Performance dei suini; Qualità della carne.

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CHAPTER 1: LITERATURE REVIEW

RESEARCH CONTEXT

Swine products are economically important, considering that pork is the most produced and consumed meat in the EU (FAOSTAT, 2010). The swine industry needs to increase both efficiency and production in order to meet this high demand for food production. However, the international market requires high standards of quality assurance regarding environmental, ethical and animal welfare problems in the production of meat. The need to safeguard the welfare of animals and, at the same time, produce safe food of the highest quality is the objective of future livestock production systems. Swine nutrition, genetics, and management have been widely studied to improve production and meat quality parameters (Rosenvold and Andersen, 2003; Dugan *et al*., 2004). Several studies have also indicated that consumers specify a higher willingness–to–pay for food from animals whose welfare has been respected (Lusk, 2011; Tonsor *et al*., 2009; Zander and Hamm, 2010). Thus, feeding (Figure 1.1) through dietary supplementation with different additives is considered the key component to improving both animal welfare and meat quality parameters.

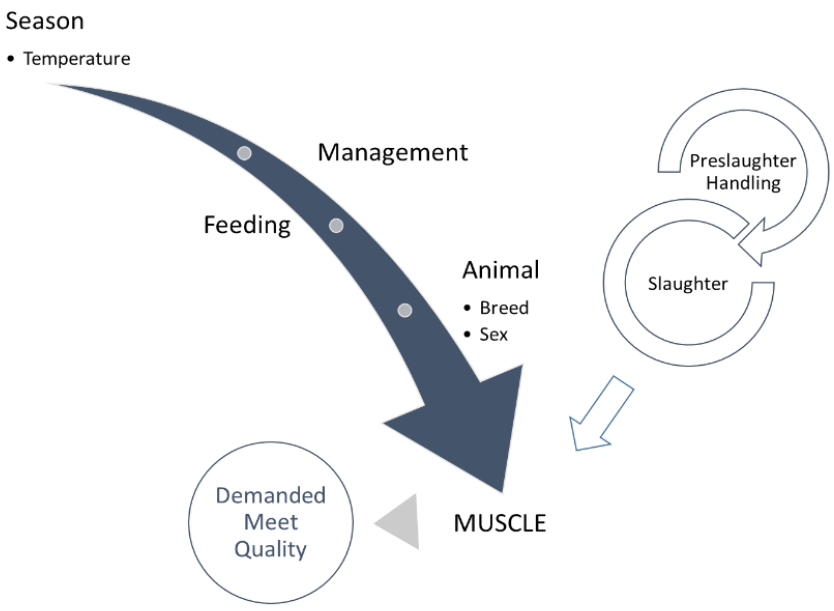

Figure 1.1. Schematic representations of a systems biology approach in meat science with mutual interaction factors of importance, which need to be understood in quantitative terms in order to develop the ideal decision support systems for future meat quality control (adapted from Andersen *et al*., 2005).

PLANT EXTRACTS

Research on natural substances now encompasses a wide variety of multidisciplinary fields including nutrition, health/well–being, and food. One of the most important methods to improve the health of pigs is the modulation of the defence system and also microbial ecology in the digestive tract, which plays an important role in regulating pig performance and health. Public awareness of the potential health risks associated with the use of in–feed antibiotics, growth hormones and various synthetic pharmaceuticals, combined natural approaches to food production, have changed consumer attitudes (Greathead *et al*., 2003). The potential risks to human health from the use of antibiotics led in 2006 to their ban as growth promoters throughout the European Union (Regulation (EC) No. 1831/2003). Risks to human health include the possibility of antibiotic residues in meat and the exchange of plasmids from antibiotic–resistant bacteria from swine to human pathogens, making them resistant to antibiotics (Dewey *et al*., 1997; Silbergeld *et al*., 2008; Maron *et al*., 2013). Changes in the legislation controlling the use of animal additives have stimulated an interest in bioactive natural substances as alternative performance enhancers. Thus, the use of plants and extracts has been replaced by synthetic chemicals in traditional animal healthcare. The search for alternatives to antibiotic growth promoters and the increased demand for natural substances by consumers have stimulated the study of the effects of herbs, spices and their active compounds in animal feed.

Plants produce a wide range of active principles with a low molecular weight, known as secondary metabolites. Through secondary biochemical pathways, plants synthesize several compounds, often in protection against damage (Reymond *et al*., 2000). These secondary metabolites do not play any role in the plants' primary metabolic needs, but increase their overall chances of survival (Harborne, 1993). Some of the roles of secondary metabolites are relatively simple: they play a host of general, protective roles (e.g. antioxidant, free radical–scavenging) and defend the plant against microorganisms such as bacteria, fungi, and viruses. The range of secondary metabolites can be subdivided into a number of distinct groups based on their chemical structure; alkaloids, terpenes, and phenolic compounds. Many papers have focused on the clarification of the biochemical structures and their positive role as phytochemicals. The majority of these papers describe in vitro investigations of the potential antioxidant and antibacterial mechanisms (Al–Mariri and Safi, 2014; Kahkonen *et al*., 1999). Some papers have also explored the in vivo effects in animal studies and the effect on meat or derived products. Plants have assumed great importance in animal nutrition due to their high content in bioactive molecules. Phenolic compounds, extracted from plants, have been reported to have many biological activities including antiallergic, anticancer, anti–inflammatory, anti–microbic and antioxidant activities (Manach *et al*., 2004; Li *et al*., 2014). Novel plant extracts such as seaweeds have

recently attracted interest as functional dietary ingredients due to the health benefits related to the fact that they contain sulfur polysaccharides, florotannins, catechins, carotenoids, tocopherols and diterpenes, which are characterized by strong anti–microbial, anti–oxidant, anti–inflammatory and immunomodulatory activities (Zubia *et al*., 2009; Maghin *et al*., 2014).

Effects of plant extracts in vivo animals

Farm animals in intensive farming systems are exposed to oxidative stress, which is induced by several factors such as nutrition (consumption of high–PUFA or rancid diet; intake of mycotoxins, heavy metals, fungicides and pesticides, and nutritional deficiencies such as selenium or exogenous antioxidants), the environment (stress–related practices such as weaning, the animal's production status such as early lactation; and heat stress) as well as physiological stress (pathogenic infections, vaccination and transportation) (Salobir *et al*., 2012). Oxidative stress is a metabolic disturbance that affects organisms and also the health status of the animals. Oxidative stress is defined as an imbalance when the production rate of Reactive Oxygen Species (ROS) exceeds the capacity of the antioxidant defence and repair mechanisms, leading to oxidative biomolecule damage. When free radical production exceeds the capacity of endogenous antioxidant barriers in the organism and the antioxidant defense activity is low, it potentially causes damage to cellular components, induces harmful autoimmune responses, and causes oxidative stress (Metcalfe and Neil, 2010; Costantini, 2008). Oxidative stress can be measured directly by detecting free radical production, or indirectly by detecting antioxidant molecules or oxidative damage biomarkers. Oxidative stress causes alterations in the physiology and behavior of animals, resulting in poor growth performance, an impaired immune system and increased susceptibility to several diseases (Zhu *et al*., 2012; Lykkesfeldt and Svendsen, 2007). Active principles from plants are normally used by the feed industry as sensory additives, flavouring and appetizing substances. Plants act along the animal digestive tract to improve feed intake, and enhance the nutrient digestion (Frankic *et al*., 2009). The mechanisms of action in the organisms is still not completely understood. However, herbs and their extracts could be have an effect on immune response, morphological and histological modifications in the gastrointestinal tract and changes in the intestinal microbiota.

Antioxidant activity. The growing interest in the bioactivity of natural compounds is due to their efficacy against oxidative stress (Dai and Mumper, 2010).Dietary supplementation of plant extracts has been evaluated in several animal species due to their efficacy as antioxidants to prevent several diseases (Stover and Watson, 2014). Most active natural compounds have been suggested to act as antioxidants (Rhodes, 1996). There is some

evidence that dietary intervention with phenolic compounds changes oxidative stress biomarkers in pig intestine and liver (Di Gincamillo *et al*., 2013; Di Giancamillo *et al*., 2015). Several studies have reported that dietary plant polyphenols in pigs enhance the oxidative stress responses of the organism (Pastorelli *et al*., 2012; Rossi *et al*., 2013a). In particular, brown macroalgae have a higher content of phenol components with higher free–radical scavenging properties (Suhaila *et al*., 2012). One study found that the inclusion of plant extracts in pigs' diet reduced the DNA damage in lymphocytes, which thus highlighted their potentially beneficial properties on the immune system under dietary–induced oxidative stress (Frankič *et al*., 2010).

Antimicrobial activity. Growth performance, immune activities, enteric microbiota populations have been improved by natural feed additives, with a reduction in diarrhea and mortality (Huang *et al*., 2011; Mukhopadhya *et al*., 2012). Several plant extracts reveal a wide spectrum of antibacterial activity against microorganisms, including Escherichia, salmonella, staphylococcus (Hammer *et al*., 1999; Dorman and Deans, 2000). The active compounds of plant extracts act as an inhibitor of enzymatic activity or prevent the development of functional organelle such as flagella (Ankri and Mireman 1999; Burt et al., 2007). Seaweeds also exert a prebiotic activity (Liu et al., 2015) and makes these compounds interesting in livestock production aimed at improving animal health and welfare (Deville, 2007). The prebiotic activity of sulfur polysaccharides is related to a positive effect on gut health (Michell *et al*., 1996). Studies in weaned piglets have shown that dietary supplementation, with laminarin, positively affected the enteric microbiota, promoting lactobacilli and bifidobacteria growth (Reilly *et al*., 2008). Another study highlights how the dietary association of laminarin and fucoidan, reduces the microbial count of Escherichia coli in the intestinal tract of weaned piglets (O'Doherty *et al*., 2010).

Performance. Plant extracts and spices as single compounds or as mixed preparations can enhance both the health status and growth performance of swine. Some studies have highlighted that plant extracts can be used as an alternative growth promoters in livestock production (Franz *et al*., 2010). Plant extracts, therefore present an opportunity to enhance lactating sow and litter performance when used as dietary supplements. The supplementation with oregano in pre–farrowing and lactating sows increases productive performance and piglet health (Allan and Bilkei, 2005). In addition, growing pigs fed a diet with plant extracts, containing polyphenols, improved daily gain and feed use, and reduced mortality (Walter and Bilkei, 2004). Weaner piglets receiving an oregano supplement had a higher weight gain and lower incidence of disease compared with the control (Sads and Bilkei, 2003). In the grower–finisher period, the supplementation of different levels and sources of plant extracts has shown benefits on growth performance. Pigs that had a diet supplemented with garlic had a higher growth performance compared to the control diets (Cullen *et al*. 2005; Janz *et al*., 2007). A higher average daily gain and feed conversion ratio

was observed with the use of a herb mixture (great nettle, garlic, wheat grass) in the diet of pigs from 25 to 105 kg (Grela *et al*., 1998).

Effects of plant extracts on meat quality

The inadequate intake of antioxidants and several environmental stressors could induce oxidative stress, reducing animal health and also the meat quality parameters (Rock, 2009; Archile–Contreras, 2011). In fact, a high production of ROS also leads to degenerative damage of cellular structure (Bekhit, 2013) and oxidative phenomena in muscle (Karakaya 2011; Insani *et al.*, 2008). Oxidative processes lead to modifications of lipids and proteins and organoleptic and nutritional properties of meat and meat products, which can then lead to economic losses (Insani *et al*., 2008).

Lipid oxidation is a complex process and is related to meat fat content, fatty acid composition, antioxidant content, and other factors such as light, oxygen access and storage temperature (Kanner, 1994). The presence or absence of these factors affect consumers' acceptance of meat and determine changes in nutritional meat quality (Karakaya *et al*., 2011; Contini *et al*., 2014). Physical and chemical changes alter meat quality during the conversion of muscle to meat, including discoloration, development of odours and textural changes (McMillin, 1996). The oxidation that develops during storage negatively affects the colour indices, flavour and acceptability of fresh meat (Glitsh, 2000). Furthermore, the fat alteration due to oxidation could also have nutritional implications for human health. In fact, their derived molecules, such as fatty acid peroxides and peroxy radicals, are mutagenic (Bösinger *et al*., 1993; Guardiola, *et al*., 1996).

To improve animal health and product quality, antioxidant molecules such as synthetic vitamin E are usually added in the farm animals' diet. Due to consumer concerns over safety and toxicity, current practice is to decrease the use of synthetic antioxidants. The search for natural additives, especially from plants, has thus increased (Meyer *et al*., 2002) in order to decrease oxidative processes in animals and their products (Coronado *et al*., 2002; Wenk, 2003).

Most natural compounds that exert an antioxidant activity are obtained from plant resources, such as culinary herbs, spices, vegetables, such as fruits, leaves and oilseed products (Shahidi and Zhong, 2010). Natural antioxidants boost the endogenous antioxidant system against oxidative stress in farm animals, and prevent lipid and protein oxidation in meat and meat products (Falowo *et al.*, 2014). Figure 1.2 shows the possible biological effects of diet antioxidant supplementation. The antioxidant molecules accumulated *in vivo* and *post–mortem* can impact on animals and animal food, preventing oxidative stress and oxidative rancidity, respectively (Salami *et al*., 2015). One study showed that antioxidants are incorporated within cell membranes more efficiently when

given *in vivo* to animals than when added post–mortem to preserve meat from oxidative damage (Mitsumoto *et al*., 1993). Dietary antioxidant supplementation is one of the main strategies for preventing tissue oxidation and helping to decrease meat oxidative phenomena (Corino *et al*., 1999; Rossi *et al*., 2013b).

Botanicals rich in polyphenols, such as rosemary extract, grape pomace, grape seed extract, green tea, have been tested as natural antioxidants in feeds. The use of bioactive compounds in plant materials as natural antioxidants preserves meat from oxidative deterioration by direct scavenging of free radicals (Augustyniak *et al*., 2010). Several studies have shown the positive effects of active compounds from oregano, garlic and rosemary on meat colour parameters and sensory properties (Cullen *et al*., 2005; Omojola *et al*., 2009). In addition, feeding pigs with 10 ml *Melissa*, *Origanum* or *Salvia* for 30 days before slaughter improved the red index value of pork after five days of storage (Lahučký *et al*., 2010). Using additives in combination may have more positive effects compared to single antioxidants; two or more antioxidant substances together can act synergically (McCarthy *et al*., 2001).

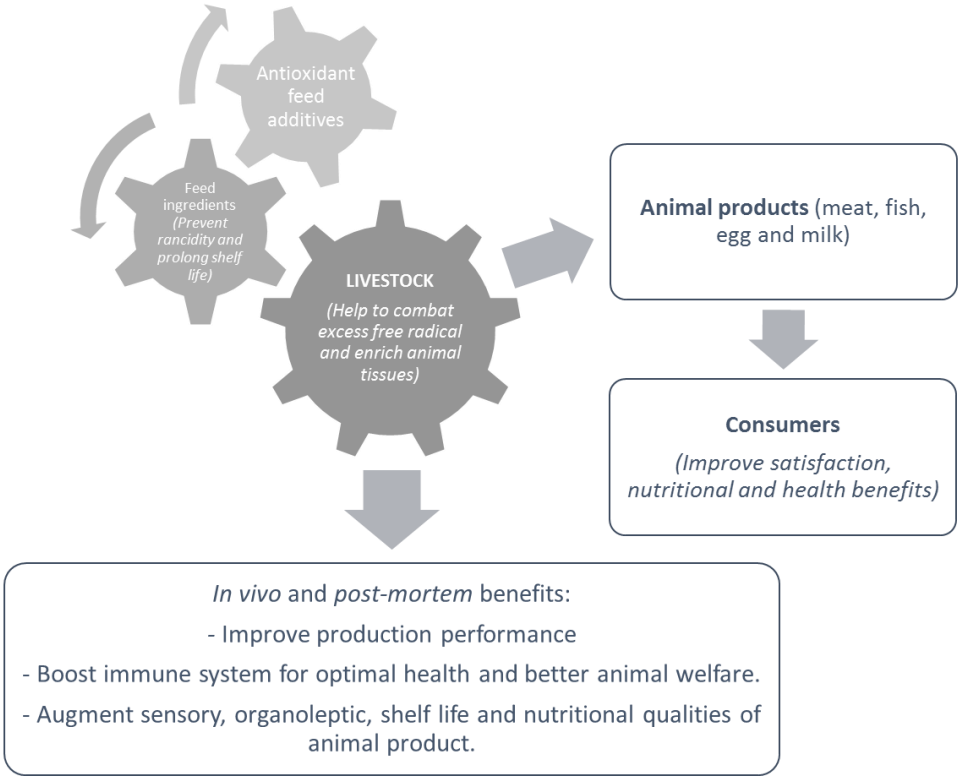

Figure 1.2. Schematic concept of in vivo and postmortem benefits of antioxidants in livestock. PUFA = polyunsaturated fatty acid (adapted from Salami *et al*., 2015).

A plant extract mixture including carvacrol (*oregano*), cinnamaldehyde (*cinnamon*), and capsicum oleoresin (*Mexican pepper*) in pig–fattening periods positively affects drip loss, cooking loss and colour of the *Longissimus Dorsi* (LD) muscle. Furthermore, the inclusion in pigs' diet of a plant extract mixture increased the sensory quality of the fresh and the cooked meat (Kołodziej–Skalska, 2011). Plant extract mixes, composed of oregano essential oil and sweet chestnut wood extract was found to decrease the lipid oxidation of meat more than the control group, and the meat from a mixed group of plant extracts scored more highly in consumer tests (Ranucci *et al*., 2015).

Another study highlighted that a dietary mixture containing polyphenols and vitamin E, administered to pigs 38 days before slaughter, improved oxidative stability and color indices in LD muscle during refrigerated storage (Rossi *et al*., 2013b). A swine diet supplemented with vitamin E and lemon balm (*Melissa officinalis*) for 10 days before slaughter enhanced the oxidative stability of stored pork in comparison with no supplement group. In addition, the mixture had higher effects on meat colour parameters than the control and the vitamin E supplemented diet (Bahelka *et al*., 2011).

Effect of plant extract on boar tainted meat

Given that meat attributes such as food quality or safety are now standard, consumer interest has focused on other dimensions such as animal welfare. Boar taint, which is the distinctive unfavorable odor and flavor of meat from non–castrated males, is one of the main problems in the meat production of non–castrated pigs (Bonneau, 1998). A standard method used to prevent the off–odors is the surgical castration of male piglets, because it reduces sex–specific behavior as the animals mature. Surgical castration is usually conducted without pain relief; however, this practice has been increasingly criticized in recent years. In fact, castration without pain relief, can lead to heterogeneous consumer preferences (Liljenstolpe, 2008).

Castration has become progressively more controversial as social concerns regarding animal welfare in Europe have increased. While regulations concerning piglet castration without pain relief in conventional pig production differ between European countries, there has been an EU–wide ban on piglet castration without pain relief in organic farming since the beginning of 2012. The EU is now considering a ban on surgical pig castration by 2018. Pig welfare has received interest in investigating alternatives to castration in order to control boar taint. Several alternatives methods have been investigated (Zamaratskaia and Squires, 2009). Three alternatives are likely for future pig production. Firstly, castration can be conducted using anaesthesia and/or analgesia, with different options for sedating the piglets such as injections (Prunier *et al*., 2006). Secondly, immunocastration which involves a vaccination against boar taint which temporarily inhibits the sexual development

of male pigs and thereby prevents the occurrence of boar taint (Kim *et al*., 2007). Thirdly, dietary treatment of non–castrated male pigs can be applied in the fattening period, combined with measures to reduce and detect boar taint in meat (Giersing *et al*., 2006).

Boar taint is characterized by the typical odor and flavor of pork which is perceived as unpleasant by several consumers. The three main compounds contributing to boar taint are androstenone, a steroidal pheromone produced in the Leydig cells of the testis, and skatole and indole, microbial degradation products of tryptophan, which have a fecal odor (Jensen *et al*., 1995). The accumulation of skatole and indole, is the main cause for the decreased consumer acceptance of boar meat (Lunde *et al*., 2010; Lundström *et al*., 2009; Xue and Dial, 1997). High levels of sex steroids, including androstenone, decrease the metabolism and subsequent elimination of skatole from the body, resulting in increased levels of skatole in the fat of the animals (Babol *et al*., 1999). Several studies have reported that hepatic metabolism of skatole is reduced by androstenone via its inhibiting effect on CYP450 enzymes (Rasmussen *et al*., 2011; Doran *et al*., 2002; Zamaratskaia *et al*., 2007; Chen *et al*., 2008).

Although dietary treatment solutions have been reviewed (Jensen, 2006; Prunier and Bonneau, 2006), the effect of diet on the hepatic skatole metabolism requires further clarification. Androstenone concentration is only moderately influenced by nutrition (Zamaratskaia and Squires, 2009; Zammerini *et al*., 2012). In addition, several feeding strategies are known to influence the concentrations of skatole in adipose tissue. Effective feeding strategies and feed additives to prevent high skatole concentrations in adipose pig tissue have been reviewed by Wesoly and Wiler, (2012). The rate of the microbial metabolism of tryptophan leads to the production of skatole (Figure 1.3).

Nutritional approaches can influence the microbial ecosystem in the gut of pigs to reduce skatole forming microbes. Several studies have revealed decreased concentrations of skatole in intestinal contents, faeces, blood plasma and fat tissue when swine are fed a supplemented diet with chicory root or chicory inulin (Jensen and Hansen, 2006; Aluwe *et al*., 2013), sugar beet pulp (Jensen *et al*., 1995; Knarreborg *et al*., 2002) or raw potato starch (Claus *et al*., 2003; Zamaratskaia *et al*., 2005). Plant extracts influence the microbial ecosystem due to their antimicrobial properties (Huang *et al*., 2011; Michiels *et al*., 2009) and research has focused on controlling skatole formation and its accumulation in adipose tissue. The antimicrobial activity of particular plant extracts, such as medicinal herbs, essential oils and spices or tannin–rich preparations, could offer a new approach to reduce skatole formation.

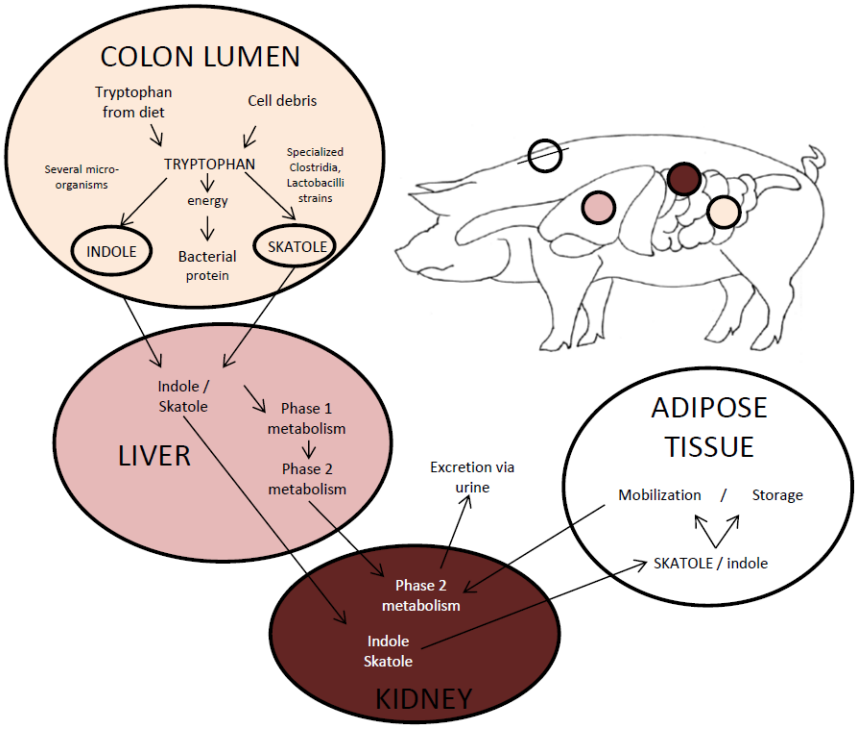

Figure 1.3. Cascade of physiological events leading to skatole formation, further metabolism and accumulation of skatole in adipose tissue (adapted from Wesoly and Wiler, 2012).

The addition of tannin–rich extracts inhibits microbial activity directly or by reducing the availability of proteins for bacterial metabolism. A recent study highlighted that the accumulation of skatole decreased with higher levels (2–3%) of hydrolysable tannin supplementation which was proportional to a high activity of hepatic CYP450, presumably induced by tannin ingestion (Čandek–Potokar *et al.*, 2015).

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CHAPTER 2: BIOLOGICAL FUNCTIONS AND HEALTH PROMOTING EFFECTS OF BROWN SEAWEEDS IN SWINE NUTRITION

Biological functions and health promoting effects of brown seaweeds in swine nutrition

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ABSTRACT

Marine macroalgae could be an important supplement in animal nutrition for their health promoting effects. In recent years, the search of natural substances as substitutes of prophylactic antibiotics increased. Seaweeds, in particular brown algae, possess distinctive compounds such as laminarin and fucoidan, studied for their biologically active functions. Recent studies have shown that these bioactive components can positively affect the health and wellbeing improving intestinal mucosa metabolism, and have anti–microbial, anti–inflammatory and immunomodulatory effects. The present work is focuses on the health promoting activities of seaweeds, in particular brown algae, as swine dietary supplement.

Keywords: Brown seaweed, Health, Swine, Dietary supplementation.

INTRODUCTION

Recently, prophylactic use of antibiotics in livestock have been banned by European Community with the consequence of a growing research towards new, safe and natural ingredients, like plant extract, that would have antimicrobial properties (Pulz and Gross, 2004). In this context, seaweed extracts have assumed great importance in animal nutritionfor the high content in bioactive molecules (Bahar *et al*., 2012). Seaweed or marine macroalgae are divided in three categories: red (*Rhodophyta*), brown (*Phaeophyta*) and green (*Chlorophyta*) (Suhaila *et al*., 2012).

The seaweeds have shown interest as functional dietary ingredient due to its several health benefits related to their content of sulfated polysaccharides, phlorotannins, diterpenes, omega–3 PUFAs, minerals and vitamins (Miyashita, 2006; Zou *et al*., 2008). In particular brown macroalgae have the higher content of water solubles polysaccharides, laminarin and fucoidan, compared to red and green seaweeds (Gupta and Abu–Ghannam, 2011) which contain phenol component with higher free–radical scavenging properties (Suhaila *et al*., 2012).

The following biologically active functions of brown seaweeds are discussed in the present study: prebiotic function such as anti–microbial activity and improvement of digestibility; antioxidant, anti–inflammatory, and immunomodulatory activities.

Prebiotic function

Anti–microbial activity: Seaweeds are rich in sulfated polysaccharides in particular laminarin and fucoidan, that can act as prebiotics with positive effect on gut health (Michell *et al*., 1996; Vidanarachchi *et al*., 2009). These compounds are effective against Gram positive bacteria (Nightingale *et al*., 2007; Pesando and Caram, 1984), and Gram negative such as Escherichia coli (Kamenarska *et al*., 2011).

Laminarin consists mainly of β–glucans that reveal prebiotic function, increasing Bifidobacteria and Lactobacilli species in the large intestine (Jaskari *et al*., 1998). Gut health is indirectly modulated by laminarin, with the microbial production of short–chain fatty acids (SCFA), in particular butyrate (Deville *et al*., 2007; Lynch *et al*., 2010). This SCFA is well known to be the main energy source for intestinal cells, stimulating cell growth (Rossi *et al*., 2010). Moreover, fucoidans are also studied for their antibacterial properties (Shibata *et al*., 2003).

Recent study (O'Doherty *et al*., 2010) showed that dietary supplementation with laminarin (300 mg/kg) and fucoidan (236 mg/kg), independently or in combination, for 21 days increased daily gain in weaned piglets. In particular, authors found that laminarin was able to reduce in Escherichiacoli population. Similarly, Reilly *et al*., (2008) found that dietary

supplementation with laminarin (1.5 g/kg) had a positive effect on the bacteria population *Lactobacilli*, *Enterobacteria* and *Bifidobacteria*, in weaned pigs. Moreover, recently seaweed has been considered as a dietary supplementation in piglets, modulating at weaning the negative changes in gut morphology and microbial populations (Mukhopadhya *et al*., 2012).

Influence on digestibility: McAlpine *et al*., (2012) have highlighted the effect of the seaweed extract on diet digestibility. The authors found that inclusion of laminarin (300 mg/kg) and fucoidan (240 mg/kg) in weaned piglets increased the coefficient of apparent total tract digestibility (CTTAD). The piglets fed with seaweed extract had a higher CTTAD of nitrogen, dry matter, and non–digestible fiber or NDF compared with pigs fed by basal diet. Also Gahan *et al*., (2009) showed that laminarin and fucoidan mixture in piglet diet (4 g/kg feed) could replace lactose dietary supplementation (60 g/kg) without adversely affecting growth rate and feed efficiency in antibiotic free diet. Another study reveals that the dietary supplementation with laminarin (300 mg/kg feed) has a positive effect on diet digestibility, enhancing growth performance in pig (Walsh *et al*., 2013).

Antioxidant function

Brown algae have antioxidant properties due to the presence of phenols, flavonoids, tannins, and phlorotannins (Balboa *et al*., 2013). O'Sullivan *et al*., (2011) studied in vitro antioxidant effects in five different brown algae. They found that two brown algae, Pelvetiacanaliculata and Fucus serratus, prevent H2O2–mediated superoxide dismutase (SOD) depletion and ensurea DNA protective effects. In another study Zhang *et al*., (2004) reported that dietary supplementation with *Porphyra* in mice (200 mg/kg feed) reduce the risk of lipid peroxidation in the aging process.

In contrast, a recent study reported that the dietary inclusion of different levels of seaweed extract (from 2.5 g/kg to 10 g/kg feed) did not enhance plasma oxidative status (Michiels *et al*., 2012).

Anti–inflammatory

Brown macroalgae showed also anti–inflammatory effects (Heo *et al*., 201). In fact, a lower expression of pro–inflammatory cytokines in the colon was observed in piglets after laminarin dietary supplementation (Sweeney *et al*., 2012). Moreover, laminarin dietary supplementation (600 mg/kg) significantly increased gut mucins gene expression (MUC 2 and 4), with a protective effect on epithelial cells (Smith *et al*., 2011). The ex vivo anti–inflammatory response of extracts of brown seaweed on swine colon was also evaluated. Colon samples were homogenized and mixed with 10 µg/ml of bacterial lipopolysaccharides by Escherichia coli to induce a pro–inflammatory responsein relation

to seaweed extract inclusion (1 mg/ml). The results demonstrated that extract of brown algae had anti–inflammatory activity reducing the pro–inflammatory cytokine response (Bahar *et al*., 2012).

Immunomodulatory

Laminarin over that antimicrobial has also immunomodulatory function (Soltanian *et al*., 2012). In a recent trial Leonard *et al*., (2010) studied the effect of seaweed extract (1.8 g/day) in the diet of pregnant sows. They found an increase of immunoglobulin G (IgG) in the colostrum of sows fed with seaweed and consequently an increase of serum IgG in piglets. Also Katayama *et al*. (2011) observed that dietary supplementation with 0.8% of seaweed extract increased immunoglobulin A and G, enhancing the immune system in pigs.

CONCLUSION

The biological activities of brown seaweeds could be used to improve health and welfare of pig. The prebiotic effect and the antimicrobial activity of laminarin and fucoidan may are beneficial for the preventive treatment of gastrointestinal diseases and to enhance diet digestibility in the post–weaning piglet. Moreover, laminarin exerts an anti–inflammatory activity, reducing the pro–inflammatory cytokine response. The brown seaweed dietary supplementation might positively affect immune system, enhancing immunoglobulin production.

In conclusion the bioactive components in seaweed extract, revealed beneficial effects and can be used as dietary supplement in pig to sustain the production, enhancing health.

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CHAPTER 3: DIETARY SUPPLEMENTATION WITH ALGAE AND POLYPHENOLS IN LACTATING SOWS: EFFECTS ON SOWS AND PIGLETS PERFORMANCE

Dietary supplementation with algae and polyphenols in lactating sows: effects on sows and piglet performance

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ABSTRACT

This study evaluated the effects of a diet supplemented with a mixture of algae plus polyphenols on sow and piglet performance and sow reproductive data during the lactation period. In the trial, 84 sows were divided into two groups consisting of sows fed a control (CTR) and Algatan Mater® (AM) diet, respectively. Feed intake, backfat thickness, blood samples at entry in the farrowing room and at day 21 of lactation, and sow reproductive data at subsequent farrowing were collected. In addition, the antioxidant status of sows was analysed. For each litter, weight at birth, after cross–fostering and at day 21 of lactation were recorded. Backfat loss tended to be lower (P = 0.07) in the AM diet sows than the control group. The AM diet improved (P < 0.05) body weight and average daily gain of piglets at day 21 of lactation (P = 0.014). PON–1 and haptoglobin were higher in the AM diet sows (P = 0.02; P = 0.03, respectively) than the CTR group. The total number of piglets born in the AM diet group was higher (P = 0.04) than the control group at the subsequent farrowing. The AM diet also improved lactating sow and piglet performance. Finally, the effects of AM diet on the sow reproductive data at the subsequent farrowing were demonstrated. Further research is needed to explore the mechanism of action of this natural mixture on the gastrointestinal tract and immune system.

Keywords: Swine, Natural extracts, Livestock performance, Lactating sow.

INTRODUCTION

High performance and productivity are the main aspects to consider in relation to high profits in pig farming (Engblom *et al*., 2007). The number of piglets produced per sow and the high number of heavy piglets at weaning represent the most important economic factors. For a good performance in terms of the piglet's growth period, a healthy start is essential (Rydhmer *et al*., 1989). However, to obtain a fast growing litter, the sow needs to have a high milk production. A recent study highlighted that pregnant sows are characterized by elevated oxidative stress during late gestation and lactation (Berchieri–Ronchi *et al*., 2011). In farm animals, oxidative stress is involved in several pathological conditions, negatively affecting animal health, welfare and productive parameters (Lykkesfeldt and Svendsen, 2007). Furthermore, oxidative stress affects milk production, reproductive performance and the longevity of sows (Zhao *et al*., 2013; Zhao *et al*., 2011).

Dietary supplementation with antioxidants in farm animals is an effective strategy for enhancing animal health and welfare (Salami *et al.*, 2015; Franz *et al*., 2010). Most natural active compounds such as polyphenols and algae extract have been suggested to act as antioxidants or as antibiotics (Dagli, 2012; Kamenarska *et al*., 2009; Michiels *et al*., 2012). Plant extracts or mixed preparations can play an important role in supporting both the health status and growth performance of the livestock production (Frankic *et al*., 2009; Maghin *et al*., 2014). Plant extracts act along the animal digestive tract, thus improving the feed intake, enhancing nutrient absorption and the production of endogenous secretions. They also decrease fermentation and the production of toxic metabolites, and exert beneficial effects on the microbial population (Costa *et al*., 2013; Hashemi and Davoodi, 2011). There is some evidence that dietary intervention with polyphenol compounds affects oxidative stress biomarkers in the pig intestine and liver (Di Giancamillo *et al*., 2013; Di Giancamillo *et al*., 2015). Several studies have reported that dietary plant polyphenols in pigs improve the oxidative stress responses of the organism (Pastorelli *et al*., 2012; Rossi *et al*., 2013). Algae extracts have also shown interesting activities due to the presence of several nutraceutical constituents that affect health thanks to sulfated polysaccharides, phlorotannins, diterpenes, omega–3 PUFAs, and the mineral and vitamin content (Miyashita, 2006). Distinctive compounds such as laminarin and fucoidan have been found to exhibit antimicrobial (O'Doherty *et al*., 2010; Reilly *et al*., 2008) activity, which can act as prebiotics with a positive effect on gut health.

The objective of this study was therefore to study the effects of the dietary supplementation of sow diets with a mixture of algae extracts plus polyphenol (Algatan Mater®) during lactation on oxidative stress status, backfat thickness, sow and piglet performance, and sow reproductive data at the subsequent farrowing.

MATERIAL AND METHODS

Animals and dietary treatment

This study consisted of 84 mixed parity sows housed on a commercial farrow–to–wean swine operation during two repetitions in May and June 2015 respectively. Pregnant sows were housed in groups in gestation stalls. Sows were moved from the gestation stalls to the farrowing rooms at day 102 ± 2 of gestation and then kept in individual farrowing crates. Sows were assigned to one of two dietary treatments (n = 42 per batch). All procedures involving animals were carried out in accordance with the European Communities Council Directive (86/609/EEC, 1986) and approved by the Italian Ministry of Health (L. n. 116/92). The basal diet was formulated to contain corn, extruded soybean meal, barley, beet pulp, bran wheat, herring, soybean oil and a standard vitamin–mineral premix to meet NRC requirements for nutrient standards (National Research Council, 1998). The treated group (AM) received the same diet supplemented with a mixture containing algae extract plus polyphenols (15 g/d Algatan Mater®; Lombarda Trading). The mixture was administered a few days before farrowing until weaning.

Data collection

Sow backfat thickness was recorded at entry into the farrowing room and at weaning. Backfat thickness at P2 (3 cm respectively from the back midline behind the last rib) was measured with an ultrasonic apparatus (Piglog 105; SFK–Technology, Denmark). At the piglet's birth, several data were collected: date, total number of piglets born, number of piglets born alive, number of stillborn, number of mummies. Cross–fostering of piglets, to standardize litters suckled pigs/sow, occurred within sow dietary treatment few days after farrowing. Pre–starter feed was freely available to the nursing piglets from 10 d of age until weaning. Litter weights were determined within 48 h of birth, before cross–fostering and at day 21 of lactation. The collected data were used to calculate average daily gain (ADG g/d) and percentage pig mortality. The daily feed intake of sows during lactation was recorded weekly. Reproductive performance data of sows were collected, including the weaning– to–service interval (WSI), fertility, weaning–to–estrus interval (WEI), farrowing interval (FI), farrowing rate (FR), total number of piglets born and number born alive.

Collection of blood samples

At entry into the farrowing room and at day 21 of lactation, blood samples were collected from sows (n=21 sows randomly selected) by anterior vena cava puncture. The blood samples were collected in two vacutainer glass tubes – 10 mL – containing or not EDTA (Venoject®, Terumo Europe N.V., Leuven, Belgium) and immediately stored at 4°C.

Samples collected for plasma assays (EDTA tubes) were kept on ice. The samples were then centrifuged for 5min at 8500 ×g at 4°C to obtain plasma and red blood cells (RBCs). Samples for the serum assays (tubes containing no anticoagulant) were left at room temperature for 4 h and then centrifuged for 5min at 5000 ×g at 4°C. Serum and plasma samples were stored at −80°C until assayed.

Hematochemical and Oxidative Stress Parameters

Routine biochemical analyses were run on serum with an automated spectrophotometer (ILAB300 plus, Instrumentation Laboratory S.p.a., Milan, Italy) using reagents provided by the manufacturer of the instrument, except where otherwise specified. The following analytes were measured: alanine aminotransferase (ALT, kinetic IFCC method), aspartate aminotransferase (AST, kinetic IFCC method), total cholesterol (cholesterol oxidase method), high–density lipoproteins (HDL, cholesterol esterase/oxidase reaction after precipitation of LDL and VLDL), urea (urease method), triglycerides (GPO–PAP Trinder's method), non–esterified fatty acids (NEFA, Acetyl CoA synthetase colorimetric method, Randox Laboratories Ltd., Crumlin, Co. Antrium, UK). Low–density lipoproteins (LDL) were calculated by subtracting HDL values from total cholesterol.

Paraoxonase–1 (PON–1) activity was measured in serum using an automated spectrophotometer (Cobas Mira, Roche Diagnostics) and an enzymatic method validated in pigs (Turk et al., 2009). Serum samples (6 µL) were incubated at 37 °C with 89 µL distilled water and 100 µL 0.05 M glycine buffer (pH 10.5) containing 1 mM paraoxon–ethyl (purity > 90%, Sigma–Aldrich) and 1 mM CaCl2. The rate of hydrolysis of paraoxon to p–nitrophenol was measured by recording the increase in absorbance at 504 nm using a molar extinction coefficient of 18,050 L/mol/cm. PON activity, expressed as U/mL, was defined as 1 nmol of p–nitrophenol formed per minute.

Haptoglobin was measured in serum, according to the manufacturer's recommendations, using a commercially available colorimetric assay on an ELISA plate (Tridelta PHASE haptoglobin assay, Tridelta Development Limited, Mynooth, Ireland), previously used in swine species (Chen *et al*., 2003; Salamano *et al*., 2008).

Samples for the plasma assays were transferred to the laboratory of the University of Milan, where the antioxidant status of the sows was evaluated using the Kit Radical Libres (KRL) test (Prost, 1989). This is a biological test that evaluates the antioxidant status of an

organism by testing the antioxidant defense systems. The principle of the KRL test is to submit whole blood and RBC to thermo–controlled free radical aggression in order to mobilize all the families of any free radical scavengers present in the blood in order to neutralize the oxidation processes (Stocker *et al*., 2003). All the chemical and enzymatic antioxidant systems of the sample were triggered to protect cell integrity until lysis occurred.

The total antiradical activity of whole blood and RBC for each sow was evaluated using the KRL biological test (Pastorelli *et al*., 2013, Prost, 1989). In an isotonic saline solution, whole blood and RBC samples were submitted to organic free radicals produced at 37°C from the thermal decomposition of a solution of 2.20–azobis (2–amidinopropane) dihydrochloride (AAPH) (Lara Spiral, Dijon, France). Haemolysis was recorded using a 96–well microplate reader by measuring the optical density decay at 450 nm. For each well, absorbance measurements were performed 75 times, once every 150 s. Results were expressed as the time required to reach 50% of the maximal haemolysis (half–haemolysis time – HT50 – in minutes), which refers to the whole blood resistance to free–radical attack. Intra and inter–assay coefficients of variation of the KRL test were 2.5% and 4%, respectively.

Statistical analyses

Statistical analyses of the data were performed using SPSS (SPSS/PC Statistics 22.0 SPSS Inc., Chicago, IL). In the case of normality and variance homogeneity, two–way ANOVA was performed to evaluate possible significant effects of the dietary treatment and batch on sow and piglet performance. One–way ANOVA was performed to evaluate possible significant effects of the dietary treatment on sow feed intake. For hematochemical parameters and total antioxidant activity of whole blood and RBC were submitted to one–way ANOVA. The values measured at time zero (before treatment) were used as covariates to evaluate possible significant effects of the dietary treatment. Reproductive data at the subsequent farrowing did not have a normal distribution, thus a nonparametric test U Mann–Whitney was applied. Data were presented as means ± SEM, and a value of $P < .05$ was used to indicate statistical significance.

RESULTS AND DISCUSSION

Sow and Piglet performance

Table 3.1 shows the effects of the algae extract plus polyphenol supplementation during sow lactation on the performance of the sows and piglets . No difference in sow parity was observed between the control and AM group. The sow parity was higher ($P < 0.001$) in the Batch 2 than the Batch 1. No interactions between treatment and batch were observed ($P > 0.05$). AM dietary supplementation of sows during lactation did not affect the backfat thickness during lactation. However, the backfat loss tended to be lower ($P = 0.08$) in the AM group than the control group. No interactions between treatment and batch were observed ($P > 0.05$). This suggests that there was a lower fat mobilization when the algae extract plus polyphenol was used during lactation. A similar result was reported by Matysiak *et al*., (2012) who highlighted that losses of backfat during lactation were lower in the plant extract treated sows (capsicum, carvacrol, cinnamaldehyde) than in the control group.

Profitability performance has been evaluated based on piglet performance according to Mahan *et al*., (1991). Lighter pigs at weaning were found to have slower post–weaning growth rates and thus needed a more days to reach a commercial slaughter weight (Wolter *et al*., 2002). In this study, there were no differences in the total numbers of piglets/litter born, stillborn, mummified, born alive, and at cross–fostering, and at day 21 of lactation in relation to dietary treatment. The number of piglets/litter at cross–fostering and at day 21 of lactation was higher ($P > 0.05$; $P > 0.001$; respectively) in the Batch 2 than Batch 1. No interactions between treatment and batch for all piglet/litter parameters were observed ($P > 0.05$). No differences in litter body weight at birth and at cross–fostering and at day 21 of lactation were found in relation to dietary treatment. The litter weight at day 21 of lactation was lower ($P = 0.01$) in the Batch 2 than Batch 1. No interactions between treatment and batch were observed ($P > 0.05$). In addition, no differences in piglet's body weight at birth and at cross–fostering were observed in relation to dietary treatment. Sows fed the AM diet significantly increased the average piglet weight at day 21 of lactation (5.80 versus 5.38 kg, $P < 0.05$).

Table 3.1. Effect of algae extract plus polyphenol supplementation during sow lactation on the performance of sows and piglets.[a]

Sow and piglet performance					SEM	Probability (P)[b]		
	CTR	**AM**	**Batch 1**	**Batch 2**		**Treatment**	**Batch**	**Treatment vs Batch**
No. sow	42	42	42	42				
Parity	3.80	4.50	3.44	4.92	0.23	*0.13*	*< 0.001*	*0.86*
Backfat thickness P2 (mm)								
– at farrowing	21.72	19.95	20.46	21.36	0.60	*0.14*	*0.45*	*0.55*
– at 21 d	16.16	15.73	15.58	16.39	0.47	*0.63*	*0.38*	*0.16*
Delta P2, mm	(–)5.51	(–)4.41	(–)5.02	(–)4.97	0.32	*0.08*	*0.94*	*0.33*
Piglets/litter, n								
– total born	14.82	14.55	14.56	14.85	0.32	*0.70*	*0.67*	*0.09*
– stillborn	0.95	0,73	0.75	0.95	0.14	*0.41*	*0.47*	*0.13*
– mummified	0.55	0.53	0.49	0.59	0.12	*0.93*	*0.68*	*0.11*
– born alive	13.14	13.3	13.1	13.31	0.34	*0.81*	*0.80*	*0.81*
– at cross–fostering	12.66	12.53	12.38	12.85	0.79	*0.38*	*< 0.05*	*0.96*
– at 21 d	11.50	11.15	11.02	11.69	0.11	*0.10*	*< 0.001*	*0.21*
Litter' weight, kg								
– at birth	17.93	18.28	17.88	18.34	0.51	*0.72*	*0.65*	*0.57*
– at cross–fostering	17.61	17,84	17.30	18.19	0.33	*0.71*	*0.16*	*0.18*
– at 21 d	62.00	64.49	60.60	66.17	1.16	*0.26*	*0.01*	*0.49*
Piglets' weight, kg								
– at birth	1.37	1.39	1.36	1.39	0.02	*0.66*	*0.53*	*0.12*
– at cross–fostering	1.39	1.42	1.40	1.42	0.02	*0.44*	*0.69*	*0.12*
– at 21 d	5.38	5.80	5.55	5.66	0.09	*< 0.05*	*0.39*	*0.87*
ADG, g/d	197.88	216.95	195.86	219.77	4.27	*< 0.05*	*< 0.05*	*0.65*
Mortality (%)	9.04	10.86	10.85	8.82	0.84	*0.26*	*0.21*	*0.26*

[a] A total of 84 sows were divided in two groups, the control group (CTR) were fed a commercial diet and sows fed with the same diet supplemented with 15 g/d of mixture of algae plus polyphenols (AM group) at entry to the farrowing room up to 21 d of lactation

[b]Data were analyzed by two–way analysis of variance (ANOVA) with dietary treatment, batch and their interactions as effects.

In the present trial, sows fed the AM diet exhibited a significant increase ($P < 0.05$) in piglet weight at weaning, which usually indicates an improvement in the amount or quality of colostrum and milk, as they are the greatest determinants of litter performance (Kingori, 2012). It has been reported that the inclusion of algae extract in the diet of pregnant sows increased the amount of immunoglobulin G and A in the colostrum of sows, thus improving the quality of colostrum and consequently the immune system in pigs (Leonard *et al*., 2010; Katayama *et al*., 2011).

In our study, the average daily gain (ADG g/d) of piglets from days 1–21 of lactation significantly increased (216.95 versus 197.88 g/d, $P < 0.05$) in sows fed the AM diet. The ADG was higher ($P > 0.05$) in the Batch 2 than Batch 1. No interactions between treatment and batch were observed ($P > 0.05$). No effect of AM diet, batch and their interaction on mortality were observed. Sows in the AM group had the same feed intake in comparison to the CRT group (3.32 versus 3.26 kg/day, $P = 0.565$). Our data are in agreement with the findings of studies on plant extracts such as capsicum, carvacrol, cinnamaldehyde and a mixture of powders containing 5% oregano essential oils of Origanum vulgare subsp. supplemented in sow's diet (Matysiak *et al*., 2012; Tan *et al*., 2015). However, the improvement of piglet ADG observed in this study is in disagreement with previous results (Leonard *et al*., 2011a; Leonard *et al*., 2011b) conducted on sows fed a seaweed supplemented diet (10,0 g) from gestation until weaning, which did not find any effect on piglet ADG in the weaning period.

Hematochemical and Oxidative Stress Parameters

Table 3.2 shows data on the antioxidant activity of whole blood and RBCs, and hematochemical parameters in sows fed the control and AM supplemented diets during lactation (1–21 days of lactation). No dietary treatment differences ($P > 0.05$) were observed for ALT, AST, total cholesterol, HDL, and LDL, NEFA, triglycerides, and urea.

No effect of the AM diet was highlighted on antioxidant activity both in the whole blood and RBCs of sows. The KRL test, used to analyse whole blood, measured the activation rates of the intracellular and extracellular defense mechanisms, taking into account their synergic effects. The mean values for the total antiradical power of whole blood are thus higher than the values observed in RBCs (Pastorelli *et al*., 2013). In this trial, the effect of dietary integration with AM did not affect the whole blood antioxidant defense mechanisms than the control group. Considering that the average lifespan of RBCs is between 60–85 days, the KRL analysis of RBC antioxidant defenses reflects the free radical aggression of the last two–three months. In fact in our trial, the antioxidant defense mechanisms in the red blood cells were not affected by dietary supplementation. Rossi *et al*., (2013) reported a high antioxidant activity of whole blood and red blood cells in post–weaned piglets fed dietary vitamin E supplementation for 60 days. Our data could be

explained by a too short supplementation, from entry in the farrowing room to 21 days of lactation, to detect free radical aggression on the whole blood and RBC values between the control and treated groups.

In sows fed the AM supplemented diet, PON–1 and haptoglobin were greater (P = 0.02; P = 0.03, respectively) than sows fed the CRT diet. Paraoxonase–1 (PON–1) is a serum enzyme synthesized and secreted by the liver (Miyamoto *et al*., 2005). PON–1 plays an important physiological role in lipid metabolism (Feingold *et al*., 1998). It possesses anti–inflammatory properties that limit the production of pro–inflammatory mediators through the hydrolysis of oxidized lipids which are pro–inflammatory compounds. Escribano *et al*., (2015) reported that PON–1 behaves as a negative acute phase protein in pigs since a decrease in its activity was observed after applying an induced experimental inflammation model. Our data showed a higher activity of PON–1 in sows fed AM than the control group considering the PON–1 biomarker against oxidative stress. This is in agreement with studies on rats and rabbits which have shown that vitamin E supplementation increased paraoxonase activity under stress conditions (Sarandöl *et al*., 2005; Jeon *et al*., 2005). Another evaluated index is haptoglobin, which is one of the acute–phase proteins produced during inflammation, infection, malignancy and injury. Serum haptoglobin concentration is considered as an assessment parameter of the health status of pigs (Itoh *et al*., 1993). In the present study, the serum haptoglobin concentration was higher in the AM group than in the control group. According to Regassa and Noakes (1999), higher values of haptoglobin in the AM group may be the consequence of the faster involution of the uterus, as well as the degeneration and regeneration of the endometrium.

Table 3.2. Effect of algae extract plus polyphenol supplementation during sow lactation on the antiradical activity of blood and hematochemical parameters of sows.[a]

	CTR	*AM*	*SEM*	*Probability (P)*[b]
Antiradical activity				
Whole blood, HT50, min	89.83	95.52	2.10	*0.88*
RBC, HT50, min	48.89	49.09	0.52	*0.95*
Hematochemical parameters				
PON–1, UI/L	7.51	8.17	0.16	*0.02*
ALT, UI/L	61.69	59.15	1.95	*0.52*
AST, UI/L	52.58	50.77	3.82	*0.95*
CHOL, mg\dL	66.40	65.64	1.88	*0.85*
HDL, mg\dL	30.64	31.05	0.81	*0.84*
LDL, mg\dL	35.76	34.59	1.64	*0.70*
NEFA, mmol/L	0.22	0.16	0.02	*0.15*
TRYG, mg\dL	18.94	15.08	1.17	*0.12*
Urea, mg\dL	28.19	25.63	0.86	*0.13*
Hapto, mg\dL	1.29	1.52	0.06	*0.03*

[a] A total of 42 sows were randomly selected to collect blood samples at entry to the farrowing room up to 21 d of lactation. The control group (CTR) were fed a commercial diet and the AM group were fed the same diet supplemented with 15 gr/g of a mixture of algae plus polyphenols at entry to the farrowing room up to weaning.

[b]Data were analyzed by one–way ANOVA and data at entry to the farrowing room were used as the covariates in the respective analyses.

Whole blood (HT50, min) = time for half haemolysis of blood samples; RBC (HT50, min) = time for half haemolysis of RBC samples; PON (UI/L) = Paraoxonase–1, UI/L; ALT= Alanine aminotransferase, UI/L; AST= Aspartate aminotransferase, UI/L; CHOL= Cholesterol, mg\dL

HDL= High Density Lipoprotein, mg\Dl; LDL= Low Density Lipoprotein, mg\dL; NEFA= Non Esterified Fatty Acids, mmol/L; TRYG= Triglycerides, mg\dL; Urea= urea, mg\dL; Hapto = Haptoglobin, mg\dL.

Sow Reproductive data

Table 3.3 shows the effects of the algae extract plus polyphenol supplementation during sow lactation on the reproductive data of sows on subsequent farrowing.

Table 3.3. Effect of algae extract plus polyphenol supplementation during sow lactation on the reproduction data at the subsequent farrowing [a]

Reproductive parameters	***CTR***	***AM***	***SEM***	***Probability (P)[b]***
WEI, d*	4.40	4.43	0.06	*> 0.05*
Fertility *	0.90	0.95	0.03	*0.68*
FR *	0.88	0.95	0.03	*0.43*
FI †	148.4	148.1	0.60	*0.47*
Piglets total born, n †	14.43	15.46	0.33	*0.04*
Piglets born alive, n †	12.80	13.60	0.29	*0.18*

*N=40, control CTR; N=39, AM supplemented Algatan Mater®; † N=35, control; N=37, AM supplemented Algatan Mater®;
[a] CTR control group fed a commercial diet and AM group fed the same diet supplemented with 15 g/d of a mixture of algae plus polyphenols at entry to the farrowing room up to weaning;
WEI = weaning–to–estrus interval; FR = farrowing rate; FI = farrowing interval.
[b]Data were analyzed by a non-parametric test.

No effect of the AM supplement diet was observed on weaning–to–estrus interval, fertility, farrowing interval, farrowing rate and number of piglets born alive at subsequent farrowing. The dietary inclusion of AM significantly improved (P = 0.04) the total number of piglets born. The observed higher liveborn rates at subsequent farrowing in the sows that had been fed the AM diet is consistent with the results of other research which reported that sows fed a diet supplemented with plant extract, containing 1000 ppm oregano (dried leaf and flower of *Origanum vulgare*, enriched with 500 g/kg of cold–pressed essential oils of *O. vulgare*) or betaine (natural extract from beetroot) improved liveborn rates in sows. (Allan and Bilkei, 2004; Ramis *et al*., 2011). On the basis of these data, it is reasonable to suggest that ovulation rate and embryotic survival were improved in treated sows. Previous research (Clowes *et al*. 2003) highlighted a decrease in ovarian function when sows mobilized too much body protein. Our data are in agreement with a previous study (Houde *et al*., 2010) which reported that sows with limited back fat loss during lactation had a higher total number of live born piglets. Similarly, as reported by De Rensis *et al*., (2005) a correlation between the highest back fat losses during lactation and the lowest subsequent pregnancy rates has been demonstrated.

CONCLUSION

The data presented demonstrate that the inclusion of an algae extract plus polyphenol in the sow diet improved sow and piglet performance. The mixture also had a positive effect on sow reproductive data. Although there were no apparent deleterious effects of the algae extract plus polyphenol in the present study, further study is needed to determine the optimal concentrations. Basic research is also needed to clarify the effects of the algae extract plus polyphenol on the gastrointestinal and immune systems.

Implications:

- Feed ingredients in sow diets can affect sow health.
- Including an algae extract plus polyphenol in the sow diet enhances the subsequent growth performance of piglets.
- Including an algae extract plus polyphenol in sow diets improves the reproductive data on subsequent farrowing.

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CHAPTER 4: DIETARY SUPPLEMENTATION WITH ANTIOXIDANT MIXTURE AFFECTS THE SHELF LIFE OF FRESH PORK PACKAGING UNDER MODIFIED ATMOSPHERE

Dietary supplementation with antioxidant mixture affects the shelf life of fresh pork packaging under modified atmosphere

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ABSTRACT

The effect of dietary integration with vitamin E and verbascoside (AOX) in pigs, on carcass characteristics, meat quality and shelf life of longissimus thoracis et lumborum (LTL) muscle was examined. Physical, chemical, microbiological, sensory parameters and oxidative stability of LTL from 10 pigs per treatment were evaluated for up to 21 days of storage at 4 °C under modified atmosphere packaging. Dietary AOX positively affected ($P < 0.05$) carcass dressing percentage, LTL muscle oxidative and colour stability. A lower ($P < 0.05$) load of Pseudomonas spp. was observed in AOX samples than in controls. Sensory analysis revealed that, at 15 days, meat from AOX was comparable ($P < 0.05$) with fresh meat in appearance and aroma. Overall, the present data show that dietary AOX supplementation in pigs was able to exert antioxidants and antimicrobial effects, enhancing the shelf–life of raw pork under commercial conditions.

***Keywords*:** Antioxidant mixture, Meat quality, Microbiological parameters, Sensory evaluation, Shelf life.

INTRODUCTION

The improvement of meat quality parameters is an important tool for both producers and consumers. In particular, the attention is focused on lipid oxidation and undesired microbial replication that represents the major spoilage phenomena that affect meat quality (Dave and Ghaly, 2011; Fung, 2010) and are responsible for the development of harmful health hazards (Kanner, 2007). In fact, both lipid oxidation and microbiological replication reduce decrease the shelf life of meat, negatively affecting nutritional value, colour and sensory parameters and therefore consumer acceptance (Zhao *et al*., 1994).

A recent study reported that one promising strategy to reduce meat deterioration is the use of antioxidants (Engel *et al*., 2015). Several dietary antioxidants have been employed in order to reduce meat quality decline (Nuernberg *et al*., 2002; Swigert *et al*., 2004). The present trend has moved from synthetic to natural antioxidants and preservative in order to extend the shelf life and enhance food safety (Naveena *et al*., 2008; Sebranek *et al*., 2005). Currently, the functional properties of many plant extracts have been investigated for their potential use as antioxidants and antimicrobials (Škerget *et al*., 2005; Shanmughapriya *et al*., 2008).

To reduce the negative effects of meat lipid deterioration, several antioxidants are placed on meat surface or mixed to minced meat before packaging (Kim *et al*., 2013; Shah *et al*., 2014). Our previous studies reported that pig's dietary supplementation with Verbenaceae extract containing verbascoside was able to protect fresh pork from oxidative phenomena (Rossi *et al*., 2013; Rossi *et al*., 2014).

In addition, several authors have reported that verbascoside possess also antibacterial properties against *Staphylococcus aureus*, *Escherichia coli* and other bacteria (Soler–Rivas *et al*., 2000; Nazemiyeh *et al*., 2008; Rajendran and Basha, 2010). Consequently, it could be also employed as a natural preservative in meat and meat product. In literature, the antimicrobial activities of several natural extracts mixed to minced meat or meat products has been observed (Baldin *et al*., 2016; Georgantelis *et al*., 2007).

To the best of our knowledge, the effects of dietary antioxidant mixture supplementation in pigs on shelf life, microbiological parameters, oxidative stability of pork stored under commercial conditions are still unknown. Therefore, the aim of the study was to evaluate the effect of pigs dietary supplementation with antioxidant mixture containing vitamin E and plant extract (AOX) on physical, chemical, microbiological and sensory shelf life of pork LTL muscle stored in MAP at 4°C up to 21 days.

MATERIAL AND METHODS

Animal and dietary treatment

The animals used in this experiment were cared for following the European Union guidelines (2010/63/EU) and approved by the Italian Ministry of Health (DL 26, 2014 march 4th). The trial was carried out on a commercial farm located in the north of Italy, producing medium–heavy pigs slaughtered at 130 kg of live weight (LW). Seventy pigs (PIC x Max Grow) half males and half females, of an average LW of 95.2 ± 1.2 kg were randomly selected and assigned to two dietary treatments (35 animals in each group). Pigs were kept in 10 pens (5 pens per treatment) balanced for body weight and sex. The CTR group received a commercial diet and the AOX group the commercial diet with antioxidant mixture integration, containing vitamin E and verbascoside from Verbenaceae extract. The diets were formulated to meet the requirements for all nutrients (NRC, 2012). Pigs were fed a corn–based diet two times daily and were rationed on 9% of metabolic weight (LW0.7581). Liquid feed was produced fresh each morning with a water: concentrate ratio of 3:1. The AOX supplement provided a daily amount of 150 mg of vitamin E and 15 mg of verbascoside (Rossi *et al*., 2014). The animals received the antioxidant supplement for 45 days before slaughter.

The AOX supplement was composed by 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 the 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. To define the quantitative analysis of the phenolic compounds the HPLC–UV–DAD methods was employed (Piccinelli *et al*., 2004). Microencapsulation technology with a protective matrix of hydrogenated vegetable lipids (spray–cooling technology) was used in order to protect the supplement from oxidative processes (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy).

Carcass traits and sampling

Pigs were slaughtered in a commercial slaughterhouse (Hauser Carni S.p.a., Mezzocorona, Trento, Italy) at 130.1 ± 1.5 kg LW. Pigs were electrically stunned and following exsanguination, the carcasses were scalded, dehaired and eviscerated. Live weight at slaughter and hot carcass weight were recorded. Carcasses were stored at 2 °C for 24 h. Finally, the left LTL muscle was randomly selected from 10 pigs per treatment (2

pigs per pen), excised from each carcass, weighed, vacuum–packed and then transported at 4 °C to a commercial industry for packaging.

Pork packaging

The LTL were aseptically cut into steaks (~2.5 cm in thickness, ~60 g portion), placed in low oxygen permeable (<1cm3/m2/24 h at STP) polystyrene / ethylvinylalcohol / polyethylene trays and flushed with 70% O2: 20% CO2: 10% N2 using a vacuum–sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt–Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were covered and heat–sealed using a low oxygen permeable (3 cm3/m2/24 h at STP) laminated barrier film with a polyolefin heat sealable layer.

Physical parameters

The pH and colour indices were recorded on different days 0, 6, 12, 15, 18 and 21 of storage in duplicate. The pH measurements were assessed 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.

Colour measurements were defined, using a CR–300 ChromaMeter (Minolta Camera Co., Osaka, Japan), to assess colour stability. The instrument was calibrated on the CIE LAB colour 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. In order to allow blooming, the trays were opened and after 30 minutes the data were collected. Each value was the mean of six replications measured on the chop surface.

The measured colour parameters were used to calculate total colour changes (ΔE) (Boakye & Mittal, 1996) at each sampling, according to the following equation:

$$\Delta E=\sqrt{((L^*-LO^*)2+(a^*-a0^*)2+(b^*-b0^*)2}$$

L*: lightness; L0*: lightness at time zero; a*: redness, where positive and negative values represent red and green colour, respectively; a0*: redness at time zero; b*: yellowness, where positive and negative values represent yellow and blue colour, respectively; b0*: yellowness at time zero.

Water Holding Capacity (WHC) was assessed on different days 0, 6, 12, 15, 18 and 21 of storage. For the determination of WHC, the centrifugation method described by Jauregui,

Regenstein & Backer, (1981) was applied. A filter paper funnel (made by four layers of paper) was prepared and weighed (T, Tare). A piece of 1.5 ± 0.3 g of lean meat was excised from the steak and put into the funnel, that was newly weighed (Pi, initial gross weight). The sample was put in a Falcon 50 ml tube and submitted to centrifugation at 15.000 rpm for 15 minutes at 4°C. Then, once removed the meat piece, the funnel was weighed (Pfin). The WHC of the samples was expressed as water loss percentage, using the following formula:

$$\% \text{ Water loss} = ((Pfin - T) * 100) / (Pi - T)$$

Chemical parameters

Dry matter, crude protein, ether exctract and ash were analysed in duplicate on the samples of LTL according to the Association of Analytical Chemists methods (AOAC, 2000).

Microbiological analyses

For microbial counts, 10 g of each sample were homogenized in 90 ml of diluent solution (0.85% NaCl and 0.1% peptone), and serial 10–fold dilutions were made in sterile saline.

Total Viable Count (TVC) was determined according to ISO 4833: 2003 method. The number of Enterobacteriaceae was determined according to the ISO 21528–2:2004 method; *E. coli* counts were determined according to the ISO 16649–2:2001 method and coagulase positive Staphylococci were determined following the AFNOR 3M 01/9–04/03 method.

Pseudomonas spp. counts were performed on Pseudomonas Agar Base additioned with Pseudomonas CFC Supplement (Oxoid), incubated at 30°C for 48h. Lactobacilli were enumerated on de Man–Rogosa–Sharpe agar (Oxoid). Plates were incubated at 30°C for 48h under anaerobic conditions (AnaeroGen, Oxoid). *Brochothrix thermosphacta* was enumerated according to ISO 13722:1996 method.

Count of spores of sulfite–reducing *Clostridia*, by the ISO 15213:2003 method, after pasteurization of the dilutions.

Salmonella spp. detection was performed by the method ISO 6579:2012. Detection and enumeration of *L. monocytogenes* were performed according to AFNOR methods (BRD 07/4–09/98). Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

Oxidative stability

Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Lorenzo et al., (2014). Briefly, 2 g of meat were dispersed in 5% trichloroacetic acid (10 mL, Sigma–Aldrich, Milan, Italy) and homogenised with an Ultra–Turrax (IKA, Cincinnati, USA) for 40 second. The homogenate was maintained at −10 °C for 10 min and centrifuged (Beckman Coulter Allegra X–22, Fullerton, California, USA) at 2360 ×g for 10 min. The supernatant was filtered through a Whatman No. 1 filter paper (Maidstone, UK). The filtrate (5 mL) was reacted with 0.02 M TBA solution (5 mL, Sigma–Aldrich, Milan, Italy) and incubated in a water bath at 96 °C for 40 min. The absorbance was measured at 532 nm immediately after cooling and compared with a standard curve of malonaldehyde prepared by hydrolysis of 1,1–3,3 tetraethoxypropane (TEP). Thiobarbituric acid reactive substances (TBARS) values were calculated from a standard curve of malonaldehyde with TEP and expressed as mg MDA/kg sample.

Sensory analysis

The sensory panel was composed by 18 people familiar with meat and sensory procedures. The mean age of the assessors was 36 (24–46). All of them were either students or employees of the University of Milan. All assessments were carried out in an equipped sensory laboratory according to ISO 8589 (2007) recommendations.

The sensory shelf life was assessed according to The Sensory Quality System (SQS) developed by Gillette and Beckley (Beckley and Kroll, 1996), using the difference from control method evaluating visual and aromatic attributes (colour and global smell).

Reverse storage design was also utilized to stop the deterioration processes in LTL steaks (Lawless and Haymann 2010). In particular, this method is used to rate each visual and aromatic attributes relative to a gold standard. The gold standard was CTR samples at the beginning of storage (day 0), considering as reference for fresh meat. In order to stop all deterioration processes, the CTR samples were stored at –20°C until sensory evaluation. All steaks (CTR vs AOX) were stored at 4°C for 6, 12 and 15 days and then were frozen at –20°C to be in the same condition of CTR sample. On the evaluation days, the samples were defrosted and removed from trays. The LTL samples were evaluated by the panel in three sessions. Within each session the design was balanced for order and carry over effects (MacFie *et al*., 1989) and the sample were randomized considering sampling storage. The sessions were conducted considering the set of samples defrosted (CTR vs AOX) as stored under normal condition. The panellist evaluated four samples per session (gold standard versus 6, 12 and 15 days of storage). The meat slices (one slice to each pannellist) were individually presented in dishes to each panellist. The judges were not informed about the experimental approach and the samples were blind–coded with 3–digit random numbers. 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 using a 10 cm unstructured line scale with three anchors – 5, 0, + 5. The gold standard is assigned "0" on the scale, and those samples less intense in each attribute compared to the standard are rated less than 5, while those more intense rated greater than 5 (Pecore and Kellen, 2002). The sensory shelf life is represented by the storage time at which the LTL samples differed from gold standard.

Statistical analyses

The SPSS (SPSS/PC Statistics 23 SPSS Inc., Chicago, 207 IL) was used to analyse the physical chemical and microbiological parameters. The analysis was performed with a MIXED model ANOVA that included the fixed effects of dietary treatment and/or storage time and the random effect of each pen. Linear regression was used to evaluate correlation between muscle TBARS and redness values. The sensory data for each attribute were submitted to one–way ANOVA only for the treatment effect. The significance of these effects was tested with T tests. Means were compared according to the DUNCAN test. The pen was considered the experimental unit for all parameters. Data are presented as means ± SEM, and a value of $P \leq 0.05$ was used to indicate statistical significance.

RESULTS AND DISCUSSION

In literature, the prevention and/or reduction of oxidative stress with natural antioxidant supplementation has been already observed (Costa *et al*., 2010; Pastorelli *et al*., 2013). Moreover, dietary supplementation with natural antioxidant in the finishing pigs breeding phase was able not only to reduce oxidative stress markers, but also to improve pork quality (Haak *et al*., 2006; Lahucky *et al*., 2010; Rossi *et al*., 2013).

In addition, the development in food processing and preservation techniques, including meat storage in modified atmosphere packaging, was already stated as useful for colour protection and in the reduction of oxidative phenomena (Cayuela *et al*., 2004; Hayes *et al*., 2010). In fact, most of the shelf life properties of pork meat are extended by use of MAP.

Considering that dietary supplementation with natural substances was able to improve both animal welfare and pork oxidative stability, in the present study we have deepened the possible effects as preservative in pork stored under commercial conditions up to 21 days.

In the following sections, all the results obtained monitoring the parameters considered will be specifically discussed.

Carcass characteristics

The data on carcass and LTL muscle characteristics are reported in Table 4.1. Dietary supplementation with AOX positively affected ($P < 0.01$) dressing percentage. No effects on LTL muscle weight and length were observed. In our previous study, we observed a higher carcass weight and LTL muscle weight in medium–heavy pigs fed the same antioxidant mixture for 38 days before slaughter (Rossi *et al*., 2014). Previously, no effects of long–term dietary supplementation with plant extract, containing verbascoside, on carcass weight in pigs slaughtered at 110 kg was observed (Rossi *et al*., 2013). Recently, Hanczakowska, Świątkiewicza and Grelab (2015) and Ranucci *et al*., (2015) reported no effects of dietary supplementation with natural antioxidants on pigs carcass characteristic.

The dissimilar results should be related to the different pigs genetic or dietary integration employed.

Table 4.1. Carcass and LTL muscle characteristic in pig fed pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX).

Item	*CTR*	*AOX*	*P–value*
Carcass weight, kg *	103.35 ± 1.51	104.6 ± 1.25	*0.359*
Dressing percentage, % *	79.02 ± 0.22a	80.10± 0.11b	*0,006*
LL weight, kg	4.06 ± 0.09	4.22 ± 0.10	*0,281*
LL length, cm	51.7 ± 1.03	52.5 ± 0.94	*0,584*
pH, 24 h	5.61 ± 0.03	5.62 ± 0.02	*0,950*

*n= 10; for other parameters n=5; data are reported as mean ± SEM. a, b for difference P < 0.05. CTR, control diet; AOX, antioxidant mixture supplemented diet.

Physical parameters

The pH values at 24 h are reported in Table 4.1. This parameter was stable for all the period considered (data not shown), the mean values ranging from 5.63 to 5.67, without significant differences ($P > 0.05$) between the dietary treatments.

Water–holding capacity is an important property of meat that influences the quality of pork for fresh consumption. The data showed that WHC ($P > 0.05$) was not affected by dietary treatment (Figure 4.1). The values observed in the present study were in agreement with previous data obtained from similar pork cuts, and fall within the range observed by Brøndum *et al*., (2000). The results of other recent studies are different from those obtained in the present research, but it should be related to the different pigs genetic type and slaughter weight (Park *et al*., 2012; Marinova *et al*., 2015). However, it has to be noted that different methods for WHC determination are currently used, sometimes with variable results.

Otherwise, considering the storage time, a significant effect ($P < 0.001$) was detected on WHC. In particular, a decrease of water loss from T0 (day of packaging, 24 hours from slaughtering) and the other sampling times, followed by a stable trend were observed. In our condition, the significantly higher capability of retaining water by the meat after 6 days of storage could be due to an initial loss of water due to the natural leakage from cut surfaces and the type of packaging, as stated by Moeseke and Smet (1999). No previous studies reported the WHC values of pork steaks for fresh consumption stored in MAP at 4°C for 21 days. A previous study of Hayes *et al*., (2010) reported that in raw minced pork patties stored in MAP at 4°C for 12 the addiction of lutein, sesamol, ellagic acid and olive leaf extract did not affect WHC.

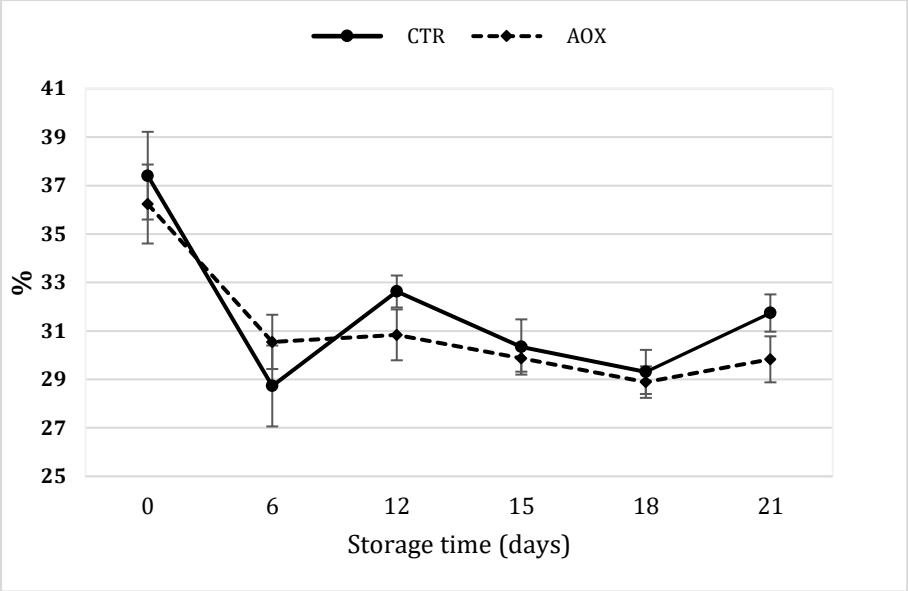

Figure 4.1. Water–holding capacity 641 in LTL muscle from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX) in relation to storage time at 4 °C under modified atmosphere packaging. n = 5; data are reported as mean ± SEM. Effect of time, $P < 0.001$; treatment, $P = 0.694$; time*treatment, $P = 0.474$.

The changes in colour indices measured at the surface of LTL muscle in relation to dietary treatment during 21 days of refrigerated storage in MAP are shown in Figure 4.2 (A, B, C). The LTL muscle colour characteristics presented significant differences ($P < 0.05$) in relation to dietary treatments and storage time.

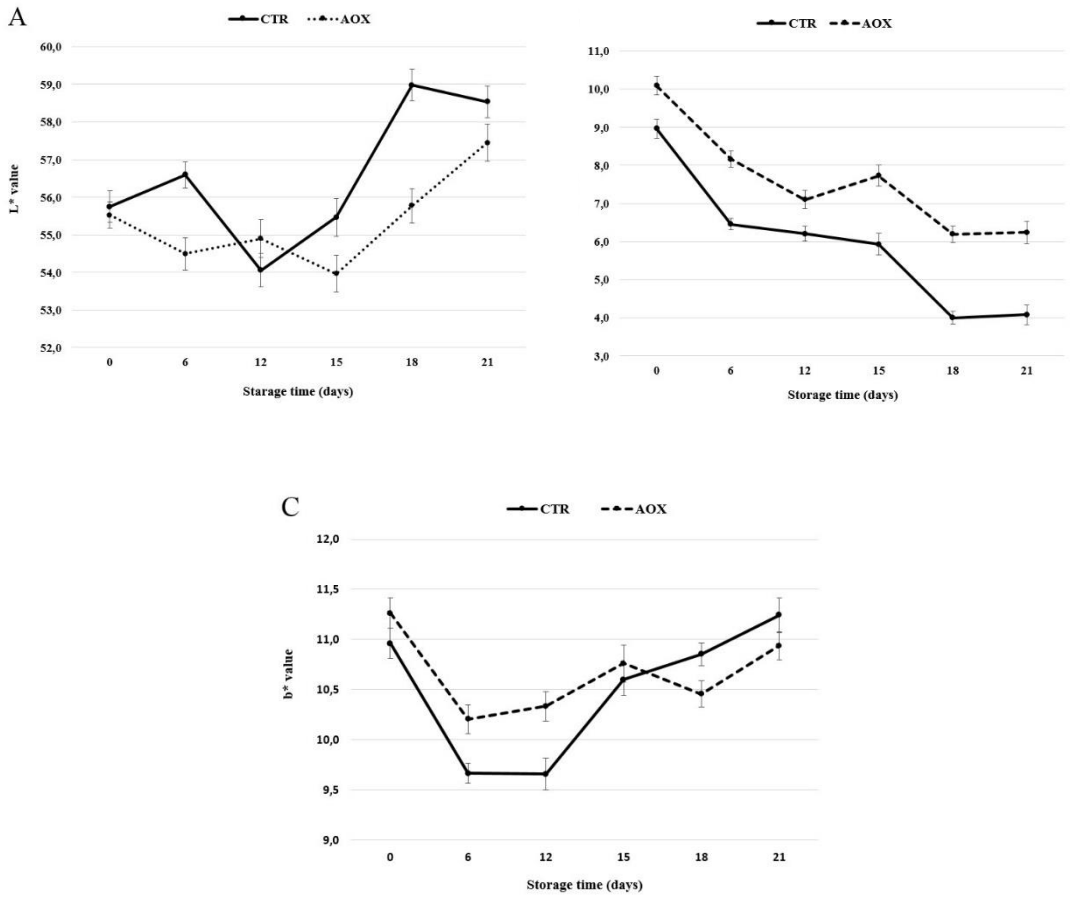

Figure 4.2. Longissimus thoracis et lumborum muscle colour parameters in relation to storage time at 4 °C under modified atmosphere packaging from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX). Lightness L* values (A), redness a* values (B) and yellowness b* values from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX). n = 5 ; data are reported as mean ± SEM. Lightness, L* values: effect of time, $P = 0.003$; treatment, $P = 0.255$; time*treatment, $P < 0.001$. Redness, a* values: effect of time, $P < 0.001$; treatment, $P = 0.017$; time*treatment, $P = 0.154$. Yellowness, b* values: effect of time, $P < 0.001$; treatment, $P = 0.782$; time*treatment, $P = 0.260$.

The lightness (L*) values were not affected by dietary treatment ($P > 0.05$). This finding was in agreement with the results reported by O'Grady *et al*., (2008) in raw meat stored in modified atmosphere pack at 4°C from pigs fed grape seed extract and bearberry. The L* values were significantly affected ($P < 0.01$) by storage time, with a significant interaction ($P < 0.001$) between storage time and dietary treatment. Our data are in agreement with Muhlisin *et al*., (2014) that reported an increase in L* values of LTL samples packed in modified atmosphere.

The redness (a*) values were higher ($P < 0.001$) in LTL muscle from pigs fed AOX than controls. The present data are in agreement with the results of Lorenzo *et al*., (2014) in porcine patties with the addition of grape extract and packed in modified atmosphere. The colour stabilizing effect of dietary antioxidant has been also observed in previous study in pork LD during chilled storage (Jia *et al*., 2012). In our previous study, the long–term dietary supplementation with plant extract, containing verbascoside, did not affect redness values (Rossi *et al*., 2014). Therefore, in the present study the efficacy of dietary antioxidants on the colour stability of pork meat may also be attributed to packaging method and perhaps to the protracted time of storage. The results showed that muscle red colour stability was positively affected by dietary AOX supplementation, indicating a high persistent colour stability during refrigerated storage. The greater persistence of red colour in meat for fresh consumption has a positive influence on the consumers purchase decision, because it represents an indicator of meat freshness (Brewer *et al*., 2002).

A decrease ($P < 0.001$) in redness values were observed in relation to storage time. No interactions between storage time and treatment were observed ($P = 0.084$). These data are in agreement with previous studies that show that meat discoloration is mostly due to the marked decrease of redness. In addiction a negative correlation (r= –0,634; $P < 0.001$) was observed between redness and TBARS values, in agreement with the data of Schmidt et al., (2016) in pork burgers. In fact, Mancini and Hunt (2005) reported a decrease of redness in meat due to myoglobin oxidation and metmyoglobin accumulation.

The b* values were significantly affected only by storage time ($P < 0.001$). Dietary treatment ($P > 0.05$) did not affect yellowness. No interactions between time and treatment were detected ($P = 0.548$). The yellowness values showed an initial decrease and then increase from 12 days of storage not only in controls but also in AOX samples. However, AOX samples showed a lower variation and greater yellowness stability than controls.

The total colour change (ΔE) in LTL muscle from pigs fed CTR or AOX diet are reported in Figure 4.3. The overall trends of total colour change were comparable between experimental groups, but in CTR samples the changes took place more rapidly. Only at 18 days of refrigerated storage under MAP, ΔE values resulted higher in CTR muscle than in AOX samples ($P < 0.05$). Moreover, LTL muscle samples from pig fed AOX supplemented diet exhibited a slight ΔE variation.

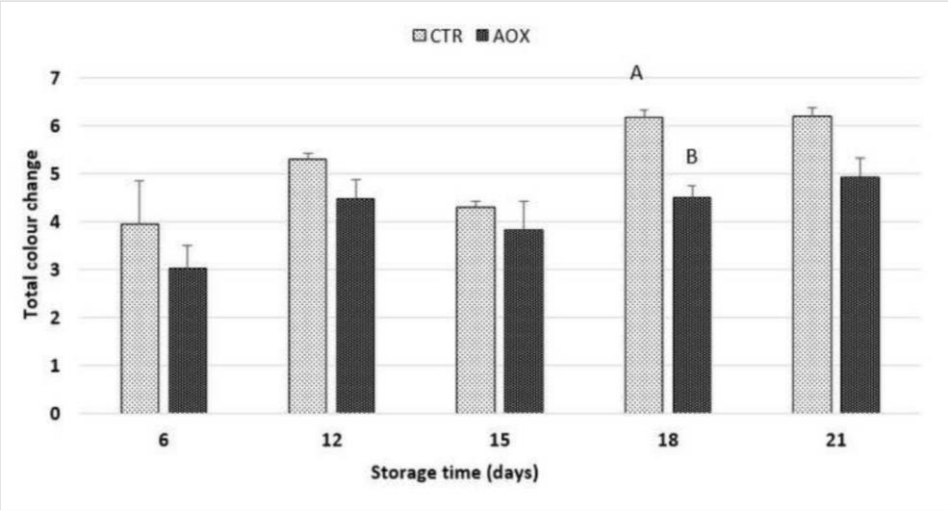

Figure 4.3. Total colour change (ΔE) of Longissimus thoracis et lumborum muscle during 21 days of refrigerated storage at 4 °C under modified atmosphere packaging (MAP) from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX). n= 5; data are reported as mean ± SEM. A, B difference for *P* = 0.001.

Chemical parameters

The chemical composition of the LTL muscle from pigs fed CTR and AOX supplemented diet are reported in Table 4.2. No differences ($P > 0.05$) were observed between experimental groups, in agreement with previous data (Ranucci *et al*., 2015; Kołodziej–Skalska *et al*., 2011; Rossi *et al*., 2013; Lahucky *et al*., 2010). The mean values of intramuscular fat was lower than those reported by Rossi *et al*., (2014) in a medium–heavy pigs and this should be related to the different pig genetic type.

Table 4.2. Nutritional parameters of Longissimus thoracis et lumborum muscle from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX).

Item	***CON***	***AOX***	***P–value***
Moisture, %	72.3 ± 0.21	72.5 ± 0.22	*0,402*
Crude protein, % w.w.	23.1 ± 0.19	22.9 ± 0.26	*0,465*
Ether extract, % w.w.	1.80 ± 0.12	1.88 ± 0.13	*0,794*
Ash % w.w.	1.17 ±0.01	1.16 ±0.02	*0,975*

n= 5; data are reported as mean ± SEM; w.w., wet weight. CTR, control diet; AOX, antioxidant mixture supplemented diet.

Microbiological parameters

The microorganisms usually involved in meat spoilage are lactic acid bacteria (LAB) *Brochothrix thermosphacta*, *Pseudomonas spp.* and Enterobacteriaceae (Pennacchia *et al*., 2011). The microbial growths are shown in Figure.4.4. Total Viable Count (TVC) of LTL muscle stored in MAP exhibited a constant and gradual increase ($P < 0.001$) in loads from 0 to 21 days of refrigerated storage at 4°C. The mean TVC values increase from 3 Log CFU/g at 0 days to 7 Log CFU/g at 21 days, in agreement with Lorenzo *et al*., (2014) that observed the same trend in pork patties stored under MAP for 20 days. As expected, during refrigerated storage in MAP, the microbial populations in raw meat increase following the characteristic microbial growth pattern (Garcıa–Lopez *et al*., 1998).

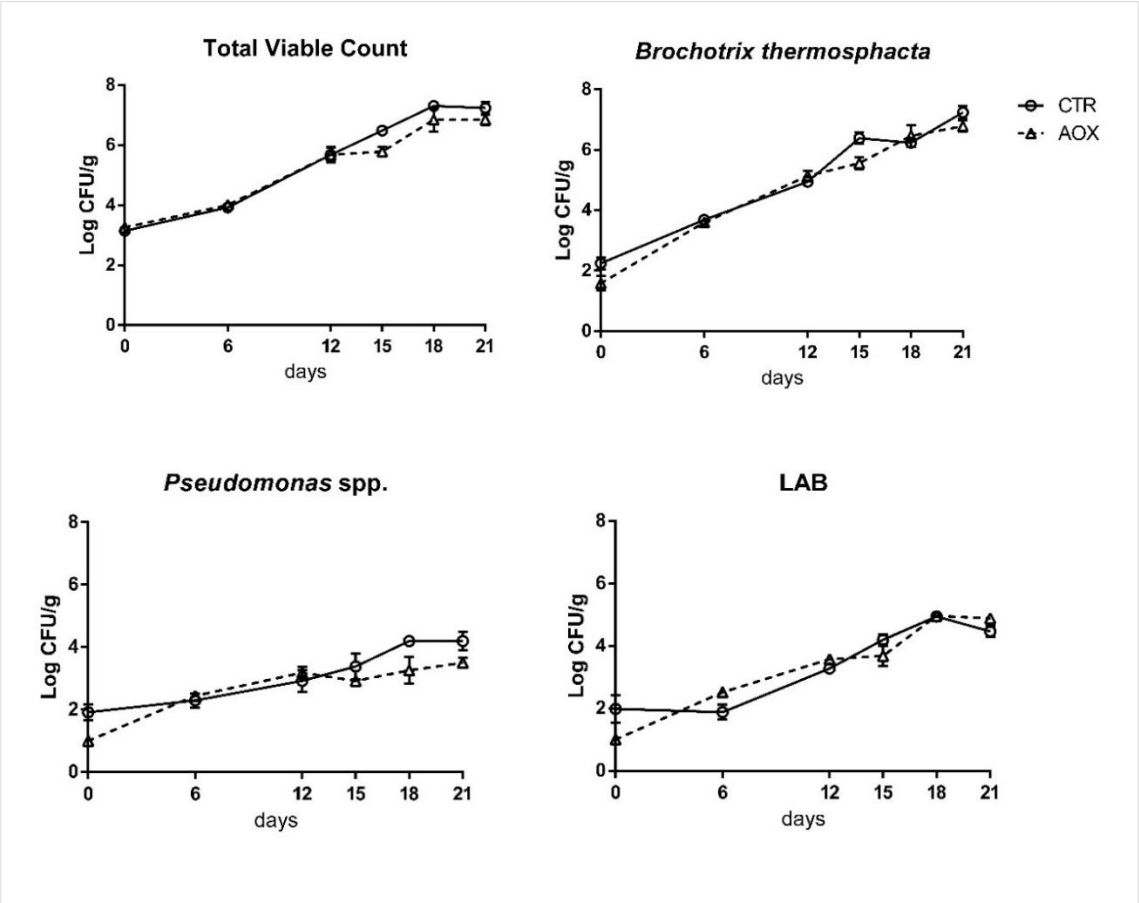

Figure 4.4. Changes in microbial counts of LDL muscle packaged in modified atmosphere and stored at 4°C for 21 days °C as affected by natural antioxidants. A: total viable counts (TVC), B: lactic acid bacteria (LAB), C: Pseudomonas spp. and D: Brochothrix thermosphacta bacteria counts.

No significant difference (P = 0.149) was observed in microbial counts between CTR and AOX groups. Moreover, considering as a threshold limit for the determination of end of the shelf–life the value of TVC of 7.0 log CFU/g (Ercolini *et al*., 2011; Tang *et al*., 2013), in the CTR group this limit was overcome after 15 days, while in AOX muscle it was reached after 21 days.

Lactic acid bacteria (LAB) loads slightly increased (P < 0.001) during the refrigerated storage at 4°C (from 1.98 to 4.46 Log CFU/g in CTR samples and from 1.00 to 4.88 Log CFU/g in AOX samples), without significant differences (P = 0.770) between treatments. A previous study in beef sirloin stored in MAP reported that population of lactic acid bacteria reached a level of 4.3 – 6.6 log CFU/g in the acceptable samples from a sensorial point of view (Pennacchia *et al*., 2011).

Brochothrix thermosphacta, a psychotropic spoilage microorganism usually isolated from raw meat products packaged in different conditions, was characterized by a rapid increase (P < 0.001) from T0 till the end of the trial (7.24 and 6.78 Log CFU/g at T21 in CTR and AOX samples respectively). After 15 days, the samples from both experimental groups exceeded the level of 5 Log CFU/g, recognized as the limit above which it could be likely produced a smell alteration, described as cheese–dairy or sewage–like odour. The *Brochothrix thermosphacta* loads tended to be lower in AOX samples (P = 0.071) compared to CTR.

Pseudomonas spp. are mainly involved in the deterioration of refrigerated meat (Ercolini *et al*., 2007). A gradual increase of this parameter (P < 0.001) was observed from the first sampling time, without overcoming the threshold limit indicating the spoilage according to GMP guide criterion. The same parameter was significantly lower (P = 0.011) in AOX if compared to CTR. The present data seem to indicate that dietary supplementation with AOX mixture containing vitamin E and verbascoside can have inhibitory properties on Pseudomonas spp, a specific spoilage organism in raw pork. This result suggests that dietary verbascoside did exert antimicrobial activities in LTL muscle. In fact, an antimicrobial activity of several natural extracts was reported when they are mixed to minced meat or meat products (Baldin *et al*., 2016; Georgantelis *et al*., 2014; Lorenzo *et al*., 2014).

E. coli, *Enterobacteriaceae* and *Clostridia* resulted below the detection limit (2 Log CFU/g) for all the period considered. These microorganisms could be considered as specific indicators of faecal contamination during slaughter or during processing. Finally, *Salmonella spp.* and *L. monocytogenes* were always absent in all the samples examined.

This is the first experimental study that reported an antimicrobial activity of verbascoside against spoilage and foodborne pathogenic bacteria. The mechanism of action of verbascoside on microbial populations in meat is still unknown. However, it is possible that secondary metabolites of verbascoside possess antimicrobial activity against the growth of microorganisms during meat storage.

In fact, as previously observed in in vitro studies, a natural extract from Verbenaceae, containing verbascoside, inhibited Gram–positive and Gram–negative bacteria growth (Oliveira *et al*., 2007; Pereira *et al*., 2008).

Oxidative stability

The oxidative stability of LTL muscle in relation to dietary treatment and storage time is reported in Figure 4.5. The mean values are in agreement with the data reported by Apple *et al.*, (2007) in pigs muscle. Statistical analysis indicated that TBARS values were significantly ($P < 0.001$) affected by storage time and dietary treatment. The oxidative stability resulted higher in LTL muscle from AOX group than in the other ones. As expected, storage time negatively affected oxidative stability in LTL muscle. A significant interaction between storage time and treatment was also observed ($P < 0.001$).

The TBARS value in AOX group was lower until 15 days of refrigerated storage than the threshold level of fresh meat: 0.5 mg MDA/kg muscle (Lanari *et al*., 1995). However, in CTR groups the MDA reached these values at 6 days. Moreover, the LTL muscle from AOX pigs displayed slight changes during 21 days of refrigerate storage in agreement with data reported by Lorenzo *et al*., (2014).

The TBARS values of LTL muscle from CTR pigs were below 2 mg MDA/kg sample till 18 days and in AOX samples it remained under this limit, that was considered the limiting threshold for meat acceptability (Campo *et al*., 2006).

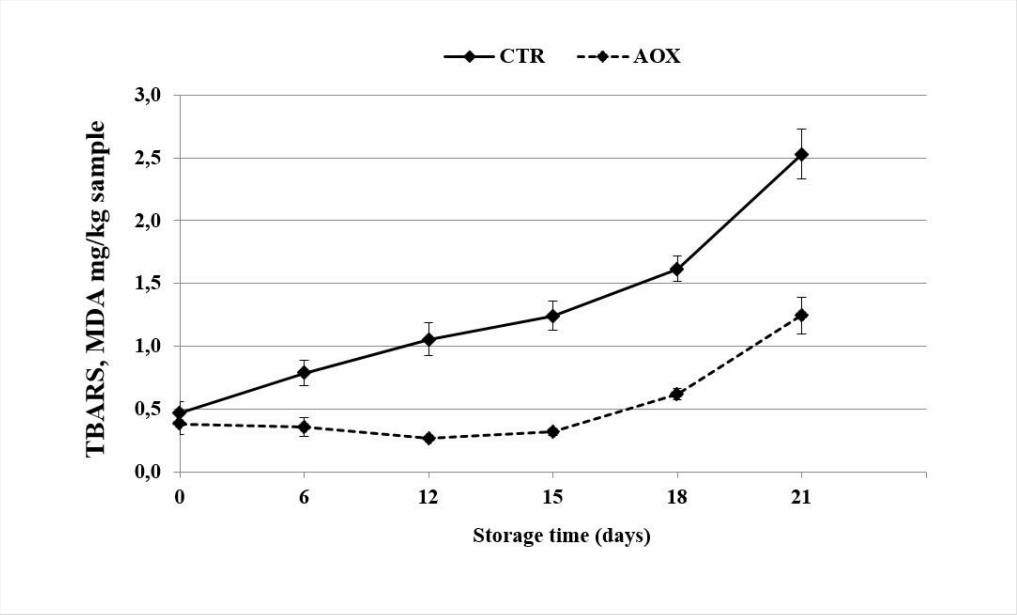

Figure 4.5. Longissimus thoracis et lumborum muscle oxidative stability during 21 days of refrigerated storage at 4 °C under modified atmosphere packaging (MAP) from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX). n= 5; data are reported as mean ± SEM. Effects of time, $P < 0.001$; treatment, $P < 0.001$; time ∗ treatment, $P < 0.001$.

The TBARS values in AOX group showed a high lipid protection from oxidative phenomena, due to the antioxidant properties of Vitamin E and verbascoside. This finding is in agreement with 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; Rossi *et al*., 2014). In contrast, O'Grady *et al*. (2008) did not find any improvement in oxidative stability in raw LTL muscle under MAP on 12 and 16 days of storage by supplementing grape seed extract and bearberry to porcine diets. Dietary supplementation with AOX, containing vitamin E and verbascoside during the last phase of pig fattening, is able to protect muscle from oxidative decay that causes loss of in both sensory and nutritional qualities.

Sensory analysis

The most important characteristics of overall consumer liking are colour, aroma and flavour (Grunert *et al*., 2004). All sensory traits, in particular, those related to aroma and appearance showed a progressive deterioration during refrigerated storage and contribute to the shelf life decline. This is known to cause a decrease in consumer attractiveness and was mainly related with colour parameters deterioration and the development of oxidative phenomena. A consumer preferences test in beef stored in different packaging condition showed that acceptance was related to the preservation of a redness colour (Carpenter *et al*., 2001). The panellist compared samples at different storage times with CTR samples at the beginning of storage (day 0), considering as reference for fresh meat, to detect differences in colour and aroma score, as reported in Figure 4.6. Colour and global smell were enhanced ($P < 0.05$) in LTL muscle from AOX pigs.

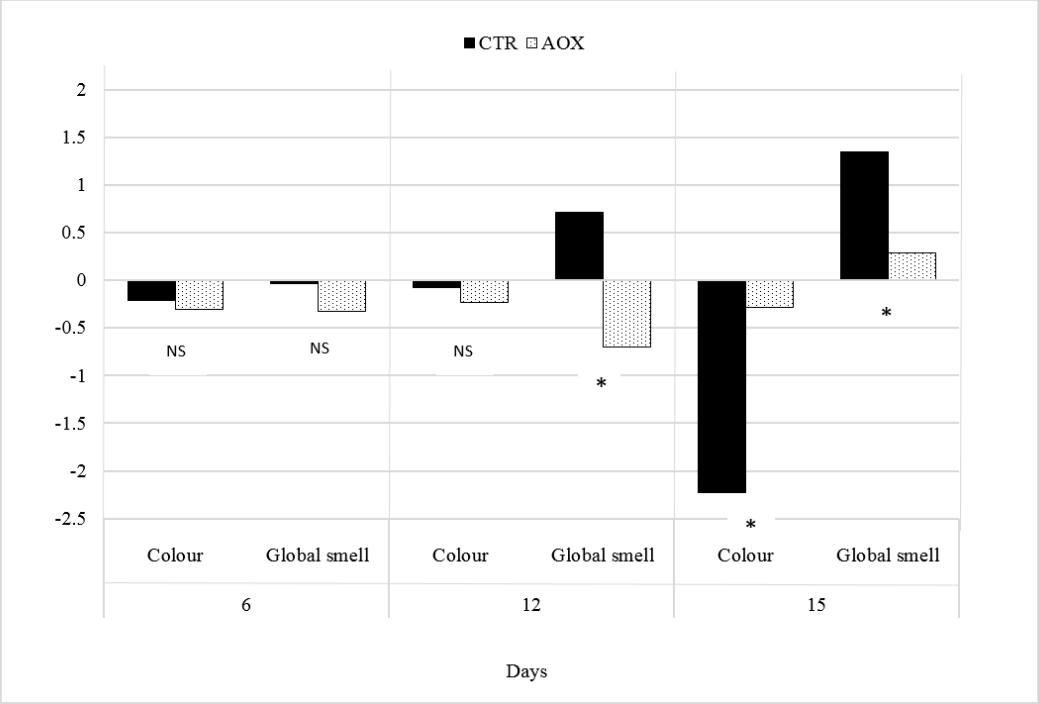

Figure 4.6. Sensory evaluation by the trained judges (N=18) on raw LTL muscle from pigs fed control (CTR) or antioxidant mixture supplemented diet (AOX), conserving at 4°C under modified atmosphere packaging. NS = $P > 0.05$; * $P < 0,05$.

At 15 days of refrigerated storage AOX showed the lower difference ($P < 0.05$) in appearance and aroma from fresh meat, if compared to CTR groups. In fact, the MDA values in CTR group at 15 days were higher than 1 mg /kg muscle, matching with the sensory detection of rancidity (Gray and Pearson, 1987). Global smell tended to increase with storage times, and off–flavor intensity ratings followed the same pattern, as observed in CTR groups that present a high value if compared to fresh LTL muscle.

The LTL muscle from AOX pork exhibited the lower difference scores in all the studied sensory attributes, identifying the meat as fresh, even after 15 days of storage at 4 °C under MAP. This agrees with previous studies, reported that off–flavours were reduced by the addition of natural extract in beef and pork patties and in cooked and ground chicken (Brannan *et al*., 2007; Lorenzo *et al*., 2014).

CONCLUSION

Overall, the results suggest that dietary supplementation with antioxidant mixture containing Vitamin E and verbascoside in the last phase of pig fattening exerts an antioxidant and antimicrobial effects in LTL muscle during refrigerated storage under MAP. In fact, a high colour and lipid stability and a reduction of Pseudomonas spp. growth were observed in LTL from pigs fed AOX. Moreover, AOX has the potential to improve LTL muscle sensory characteristic during refrigerated storage under MAP. Therefore, dietary AOX supplementation results as promising antioxidants and antimicrobial to enhance the shelf life of raw pork under commercial conditions.

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CHAPTER 5: FEEDING STRATEGIES WITH NATURAL EXTRACTS IN ORDER TO EVALUATE THE EFFECT ON SMOKED CURED HAM

Feeding strategies with natural extracts in order to evaluate the effect on smoked cured ham

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ABSTRACT

The effect of dietary supplementation with antioxidant mixture (AOX), containing vitamin E and verbascoside, on nutritional and sensory characteristics of smoked cured ham was examined. Physical and chemical parameters of smoked cured ham from 10 pigs per treatment were evaluated after 5 months of seasoning. Moreover, sensory analysis and consumer's preference were performed. Results showed that the seasoning losses of smoked cured ham tended to be lower in treated group (P = 0.06) than control. Colour parameters, redness and yellowness index, were not affected (P > 0.05) by dietary treatment, while muscle lightness resulted lower in AOX group (P < 0.001) than CT. Nutritional parameters were not affected by dietary treatment. Sensory analysis revealed a significant difference between control and treated group (P < 0.05) in salty and sweety taste. Antioxidant mixture positively affected the consumer preference of smoked cured ham, without affecting other quality parameters.

***Keywords*:** Antioxidant mixture, Smoked cured ham, Sensory evaluation.

INTRODUCTION

The Italian meat products arise from ancient cultural traditions that led over time to produce a wide variety of processed foods, with local and regional origin. Time and experience gave rise to a great variety of local meat products, all characterized by a clear geographical identity with consolidated preparation techniques, and specific sensory properties (Lucarini *et al*., 2013). Among the traditional Italian hams, speck has gained more and more importance in the international market, especially after obtaining the Protected Geographic Indication (PGI) by the European Union, according to EU rules (Council Regulation Nº 1107/96). Speck is prepared from deboned pork legs; the preparation combines two preservation methods: salting with a special mixture of aromas (salt, pepper, juniper, rosemary and laurel) and a mild smoking phase according to Disciplinary of Production of "Speck Alto Adige PGI". This quality certification requires compliance with stringent requirements at all stages of the production chain, from breeding to meat processing, in order to ensure an excellent final quality of smoked cured ham. Changes are mainly related to water loss, stabilization through salt diffusion, intensive proteolysis and lipolysis with the subsequent development of the typical flavor of the product (Toldrà, 1998). A dry–maturation stage allows to enhance and increase flavour and sensory characteristics. Although smoked cured ham is a common food of the Italian consumption pattern, few data are available on the qualitative aspects of this important production. The study of the food composition other than a basic need for studies in nutrition, is also strongly felt by consumers who are increasingly interested in the issue of food quality and safety.

Moreover, in response to recent claims regarding the potential of synthetic antioxidants to cause toxic effects and consumers increased interest in purchasing natural products, the meat industry is searching for natural antioxidants sources (Karre *et al*., 2013). Some studies reported that natural antioxidants added in the preslaughter stages are able to improve the meat quality if added in animal diets. Specifically, the positive effects of natural antioxidants on meat parameters, are characterized by a decrease of lipid oxidation, color loss, and microbial growth (Velasco and Williams 2011; Rossi *et al*., 2013). As previously observed, the combined use of different antioxidant subtances may exert a greater effect when compared to single antioxidants, due to the synergic effects of different molecules (Rossi *et al*., 2014). Furthermore, the use of natural extracts let to a greater colour stability of derived products like salami over three months of ripening (Pastorelli *et al*., 2012).

No previous study reported the effect of dietary supplementation with plant extract, on smoked cured ham quality parameters and sensory characteristic. The aim of this study was to evaluate the effects of dietary supplementation with plant extract, containing verbascoside, on nutritional and sensory characteristics of smoked cured ham.

MATERIAL AND METHODS

Animal and dietary treatment

The animals used in this experiment were cared for following the European Union guidelines (2010/63/EU) and approved by the Italian Ministry of Health (DL 26, 2014 march 4th). The trial was carried out on a commercial farm located in the north of Italy, producing medium–heavy pigs slaughtered at 130 kg of live weight (LW). Seventy pigs (PIC x Max Grow) half males and half females, of an average LW of 95.2 ± 1.2 kg were randomly selected and assigned to two dietary treatments (35 animals in each group). Pigs were kept in 10 pens (5 pens per treatment) balanced for body weight and sex. The CTR group received a commercial diet and the AOX group the commercial diet with antioxidant mixture integration, containing vitamin E and verbascoside from Verbenaceae extract. The diets were formulated to meet the requirements for all nutrients (NRC, 2012). Pigs were fed a corn–based diet two times daily and were rationed on 9% of metabolic weight (LW0.7581). Liquid feed was produced fresh each morning with a water: concentrate ratio of 3:1. The AOX supplement provided a daily amount of 150 mg of vitamin E and 15 mg of verbascoside (Rossi *et al*., 2014). The animals received the antioxidant supplement for 45 days before slaughter.

The AOX supplement is composed by 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 the 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. To define the quantitative analysis of the phenolic compounds the HPLC–UV–DAD methods was employed (Piccinelli *et al*., 2004). Microencapsulation technology with a protective matrix of hydrogenated vegetable lipids (spray–cooling technology) was used in order to protect the supplement from oxidative processes (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy).

Sample preparation

Pigs were slaughtered in a commercial slaughterhouse (Hauser Carni S.p.a., Mezzocorona, Trento, Italy) at 130.1 ± 1.5 kg LW. Pigs were electrically stunned and following exsanguination, the carcasses were scalded, dehaired and eviscerated. Carcasses were stored at 2 °C for 24 h. Finally, the left thighs were randomly selected from 10 pigs per treatment (2 pigs per pen), and then transformed in smoked cured ham, following Disciplinary of Production). The length of the seasoning was 5 months.

Chemical and physical parameters

The smoked cured ham samples were weighted and seasoning loss were calculated. The samples of smoked cured ham were analyzed for dry matter, crude protein, ether extract and ash according to the methods of the Association of Analytical Chemists (AOAC, 2000). Color measurements fat and muscle on smoked cured ham were performed, 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). Each data point is the mean of three replications measured at the chop surface both for fat and muscle area.

Sensory evaluation

A selected and trained sensory panel was chosen, consisting of eight members, who were familiar with meat derived product and descriptive analysis procedures (ISO, 2010). All assessments were carried out in a sensory laboratory equipped according to ISO International Organization for Standardization (ISO, 2010) 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 5.1. The judges evaluated each sample in triplicate. During training and sampling, panel members had access to unlimited water and unsalted crackers. Samples were offered in a randomized order and codified by a number with three casual number in order to minimize possible error. The judges were presented with a slice of smoked cured ham to evaluate the external appearance and for a tasting test. They were instructed first to score the external appearance and aroma, then to take a bite of the smoked cured ham slice and score the texture. They were asked to score texture, flavor and taste during chewing. 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).

Table 5.1. Descriptors and definitions for sensory analysis of smoked cured ham.

Descriptor	Definition
Appearance	
Red colour	Intensity of red colour (meat)
White colour	Intensity of white colour (fat)
Marbling	Amounts of intramuscular fat
Aroma	
Seasoned	characteristic odors of seasoned ham perceived through smell
Smoky	Aroma associated with smoke perceived through smell
Pepper	Aroma associated with pepper perceived through smell
Taste	
Sweet	Salty taste
Salty	Sweet taste
Flavour	
Seasoned	Flavor associated with seasoned ham
Smoky	Flavor associated with smoke
Pepper	Flavor associated with pepper
Juniper	Flavor associated with juniper
Texture	
Tender	The force needed to masticate the meat ready for swallowing
Juicy	The degree of juice released while chewing the meat
Fibrous	Presence of fibers during swallowing

The consumers' preference analysis was done by eighty common consumers, which expressed a preference between the samples. The descriptors evaluated by the common consumers were: global preference, appearance, fat colour, muscle red colour, small, flavor, salty and slice texture. Common consumers were requested to evaluate the preference of each attribute by assigning a score between 1 (undesirable) and 9 (extremely appreciated).

Statistical analyses

Statistical analyses of the data were performed using SPSS (SPSS/PC Statistics 21.0 SPSS Inc., Chicago, IL). The data on physical and chemical parameters were analyzed by one–way Analysis of Variance (ANOVA) to evidence the effect of treatment. The sensory data were submitted to ANOVA with samples, judges, replicates and their interactions as effects. The significance of these effects was tested with F tests. Means were compared according to the DUNCAN test. Data are presented as means±SEM, and a value of $P<0.05$ was used to indicate statistical significance.

RESULTS AND DISCUSSION

The physical parameters of smoked cured ham are given in Table 5.2. Weight of thighs before and after seasoning were not affected by dietary treatment. The seasoning losses of smoked cured ham tended to be lower (P = 0.06) in AOX group than CT one. The seasoning losses are in agreement with Candek–Potokar and Škrlep (2012) that highlighted correlation between this parameter and with pig feeding. Colour is one of the most important characteristic of the dry–cured ham appearance that could influence consumers' choice (Ruiz *et al*., 2002). Colour parameters such as redness and yellowness index, were not affected (P > 0.05) by dietary treatment, while lower muscle lightness has been observed in AOX group than CT (P < 0.001). Similarly, Isabel *et al*., (2009) reported a colour parameters variation in Iberian dry–cured ham from pig fed α–tocopheryl–acetate.

Table 5.2. Characteristic of thighs and color indices of smoked cured ham from pig fed control (CT) or antioxidant mixture (AOX) supplemented diet.

	CT	***AOX***	***SEM***	***P–value***
Characteristics of thighs				
Weight of thighs, kg	8.03	8.03	0.127	*0.989*
Weight after seasoning, kg	5.51	5.64	0.098	*0.242*
Seasoning losses, %	31.35	29.71	0.450	*0.067*
Fat Colour				
L*	76.5	76.9	0.285	*0.467*
a*	1.12	1.17	0.243	*0.925*
b*	8.6	8.3	0.183	*0.370*
Muscle colour				
L*	43.3	40.6	0.392	*<0.001*
a*	9.3	9.8	0.352	*0.351*
b*	8.5	8.7	0.142	*0.651*

n = 10; data are reported as mean ± SEM. CTR, control diet; AOX, antioxidant mixture.

Dietary treatments did not affect (P > 0.05) the chemical parameters of smoked cured ham (Table 5.3). No chemical composition changes were observed in relation to dietary treatment in agreement with Lucarini *et al*., (2013) which reported the same values of moisture, protein and ash. In our study, the amount of fat resulted higher than the value reported to Lucarini *et al*., (2013) (25.1 g/100 g vs 19.1 g/100 g, respectively). Moreover, results showed that smoked cured ham chemical parameters are in agreement with the

Disciplinary of Production. Specifically, the value of crude protein was higher than 20%, mean water:protein ratio was 1.51 and fat:protein ratio was 0.92, both within threshold limits established by Disciplinary of Production of 2 and 1.5, respectively.

Table 5.3. Chemical parameters of smoked cured ham in pigs fed control (CT) or antioxidant mixture (AOX) supplemented diet.

	CT	***AOX***	***SEM***	***P–value***
	Chemical analysis of total slice			
Moisture, (g/100 g)	42.09	41.55	0.955	*0.602*
Protein, (g/100 g)	27.89	26.19	0.742	*0.262*
Fat, (g/100 g)	23.98	26.21	1.515	*0.479*
Ash, (g/100 g)	5.60	5.07	0.205	*0.203*

n = 10; data are reported as mean ± SEM. CTR, control diet; AOX, antioxidant mixture.

The F values for appearance, aroma, taste, flavor and texture parameters of smoked cured ham sensory profile are reported in Table 5.4. Sensory analysis revealed a difference between CT and AOX ($P < 0.05$) in salty and sweet taste. In particular, the judges presented differences ($P < 0.001$) for all the descriptors. This is common in sensory evaluations due to the different use of the scale (Lea *et al*., 1997). There was no significant difference ($P > 0.05$) 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 smoked cured ham.

Table 5.4. Sensory evaluation: F value and statistical significance of treatments (CT and AOX), judges (n = 8), replicates (n = 3) and their interaction for each sensory descriptor.

Descriptor	F value					
	Treatments	Judges	Replicates	T * J	T * R	J * R
Red colour	1.18	7.10**	3.06	0.76	0.94	1.83
White colour	0.44	10.26***	0.12	1.03	5.28 *	0.87
Marbling	0.02	6.56 **	1.64	0.67	2.74	0.56
Seasoned aroma	1.20	3.92 ***	1.28	3.44 *	3.59	1.15
Smoky aroma	0.25	5.31**	0.03	1.35	2.34	0.81
Pepper aroma	1.34	7.85 ***	0.46	2.05	1.36	1.84
Sweet	5.3*	11.48 ***	0.16	1.13	1.75	0.93
Salty	8.35 *	10.27 ***	0.70	1.42	2.14	1.23
Seasoned flavor	2.26	11.16 ***	0.25	1.07	0.11	0.71
Smoky flavor	0.01	10.49 ***	3.03	0.32	1.24	1.76
Pepper flavor	0.56	11.56 ***	0.30	0.44	1.06	1.02
Juniper flavor	0.45	34.50 ***	0.08	0.89	0.63	0.76
Tender	0.01	10.65 ***	3.08	1.27	0.10	1.06
Juicy	0.10	3.62 *	1.69	0.83	0.06	0.35
Fibrous	0.91	3.42 *	0.41	0.66	3.74*	0.37

CT, control diet; AOX, antioxidant mixture; T ∗ J, treatment ∗ judges; T ∗ R, treatment ∗ replicates; J ∗ R, judges ∗ replicates.
*** Significant at P < 0.001.
** Significant at P < 0.01.
* Significant at P < 0.05.

The spider plot of the sensory profile is reported in Fig. 5.1. The considered parameters related to appearance, aroma, flavor and texture were comparable in both experimental groups, while the parameters related to taste were not equal. The spider plot of the consumer's preference is reported in Fig. 5.2. The consumer test revealed that smoked cured ham from AOX were preferred ($P < 0.05$) than CT. Consumers perceived a difference for global preference, fat colour, smell, flavor and slice texture. Antioxidant mixture positively affected the consumer preference of smoked cured ham, without affecting other quality parameters. There is no literature about smoked cured ham preferences, although previous studies highlighted that texture, smell and appearance are the main characteristics that affect the consumer's decision to purchase seasoning products (Morales *et al.*, 2013; Font–i–Furnols *et al.*, 2014).

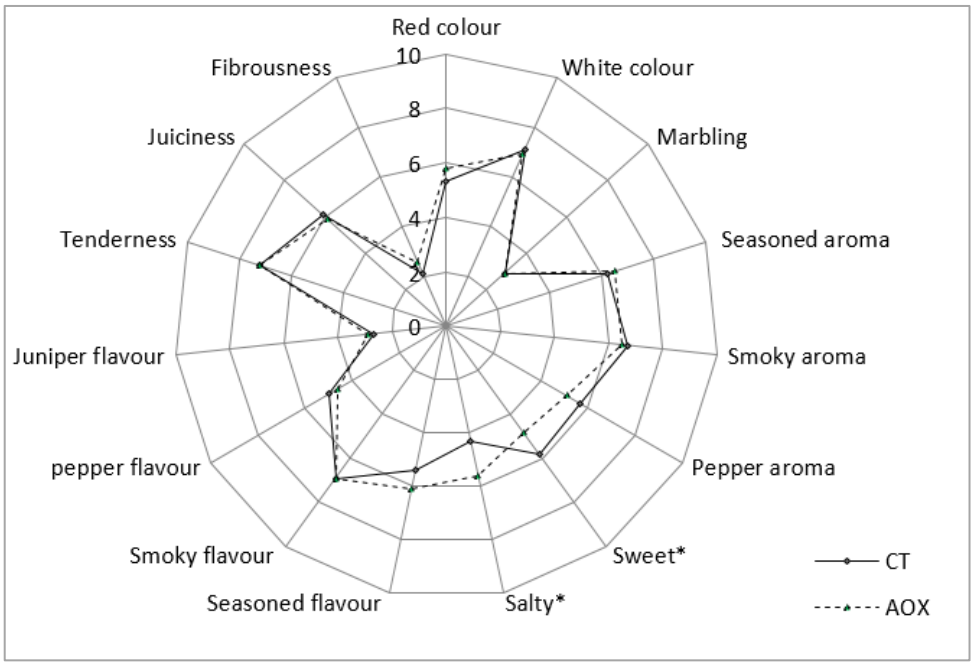

Fig. 5.1. Spider plot of the sensory profile of smoked cured ham fed control diet (CT) or diet supplemented with antioxidant mixture (AOX). N=8; * Significant at P < 0.05.

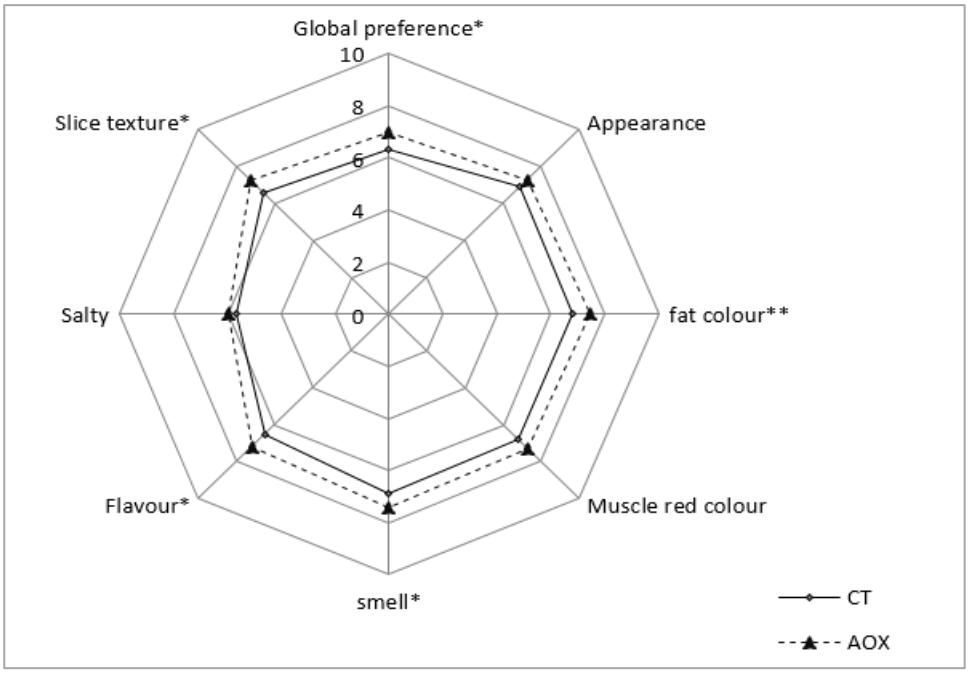

Fig. 5.2. Spider plot of the consumer's preference of smoked cured ham fed control diet (CT) or diet supplemented with antioxidant mixture (AOX). N=80; * Significant at *P* < 0.05; ** Significant at *P* < 0.01.

CONCLUSION

The present data showed that dietary plant extract supplementation, containing different active principles in swine improved the seasoning losses of smoked cured ham, without affecting physical and chemical parameters. In addition, short–term supplementation with plant extracts positively affected the consumer's preference of smoked cured ham, without affecting the nutritional characteristics. Considering the limited literature about smoked cured ham, further studies are needed to determine the effects of plant extracts on this derived product.

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CHAPTER 6: EFFECT OF ADDED HYDROLYSABLE TANNINS COMBINED WITH HIGH DIETARY PUFA SUPPLY IN THE GROWER–FINISHER DIET OF ENTIRE MALES ON THE GROWTH PERFORMANCE, CARCASS CHARACTERISTICS MEAT QUALITY AND HEPATIC SKATOLE AND INDOLE METABOLISM

Effect of added hydrolysable tannins combined with high dietary pufa supply in the grower–finisher diet of entire males on the growth performance, carcass characteristics meat quality and hepatic skatole and indole metabolism

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ABSTRACT

The aim of this study was to determine the impact of 3 % hydrolysable tannins (HT) and two levels of polyunsaturated fatty acid (PUFA) in the diets on growth performance, carcass traits, meat quality and boar taint compounds in entire males. Forty–four entire males (BW 25 kg) were randomly assigned to four dietary treatments. Two basal grower (25–60 kg BW) and finisher (60–105 kg BW) diets, which were supplemented either with 2% soy oil (H = high PUFA) or 2% tallow (L = low PUFA), were formulated. The L and H diets were with either 3 (+) or 0% (–) chestnut extract rich in gallotannins. Moreover, to study in depth the effect of hydrolysable tannins (HT) and PUFA levels, the cytochrome (CYP) isoenzymes mRNA expression in the liver and colon mucosa was determined. Compared to HT– diet, feed efficiency but not feed intake, was lower (P = 0.01) in the HT+ group. Except for the liver weight, which was lower (P < 0.05) in the HT+ than the HT– group, carcass characteristics were not affected by diet. With respect to meat quality, HT+ treatment resulted in lighter (P = 0.04, greater L* value) and less red (P = 0.08; lower a* value) loins compared to HT–. Water holding capacity (%) of loins from HT+ pigs was greater (P > 0.04) than from HT– pigs. Indole adipose tissue levels tended to be lower (P = 0.08) in HT+ compared to HT–. Skatole concentration in the adipose tissue of pigs fed the H– diet was greater compared to pigs fed the L– diet, with intermediate levels in the H+ and L+ groups (TAN × PUFA interaction; P = 0.05). Androstenone levels were in general low and did not differ between dietary treatment groups. Nevertheless, androstenone levels were positively correlated (P < 0.05) with the weight of testes, bulbo–urethral and salivary glands. Hepatic and colon mucosa gene expression of CYP isoenzymes were unaffected by the dietary treatments, except for the lower (P = 0.04) hepatic CYP1A2 gene expression in HT+. These results show that performance, carcass composition and meat quality traits are not affected by dietary 3% chestnut extract supplementation in entire male. No evident relationship between dietary PUFA level and boar taint compound levels was observed.

Keywords: Pigs, Hydrolysable tannins, PUFA, Meat quality, Boar taint.

INTRODUCTION

Surgical castration of male pigs without anaesthesia and analgesia is a major welfare concern especially in Europe and the search for viable alternatives is an ongoing process. Raising entire males (EM) is one of the envisioned alternatives but despite positive aspects regarding performance and carcass quality, major challenges arise from managing the pigs in the grower finisher period, carcass and meat quality and especially controlling boar taint (Zamaratskaia and Squires, 2009; Bee *et al*., 2015). Boar taint, depicted as a urinary and fecal odour and flavour, originates primarily from the testicular steroid androstenone (Patterson, 1968) and skatole, a metabolite derived from bacterial catabolism of tryptophan in the hindgut (Yokoyama and Carlson, 1979), respectively. These compounds are lipophilic, accumulate in the lipid fraction, and consequently affect odour and flavour of pork from EM and ultimately consumer acceptance (Font–i–Furnols, 2012).

Wesoly and Weiler (2012) recently presented in a review, mechanisms by which feed ingredients like chicory, raw potato starch and sugar beet pulp can switch microbial metabolism from primarily proteolytic to primarily saccharolytic. Consequently, less skatole and indole is produced and ultimately incorporated in pork from EM. Results of recent studies suggest that also bioactive compounds from plants in the diet like hydrolysable tannins (HT) have also the potential to reduce bacteria–mediated skatole and indole production in the colon, resulting in lower tissue levels of the two boar taint compounds in the adipose tissue (Wealleans *et al*., 2013; Čandek–Potokar *et al*., 2015, Bee *at al*., 2016). A recent study revealed that dietary inclusion of 3% HT was related to lower apoptosis of intestinal epithelial cells in EM, limiting the availability of L–tryptophan from cell debris and consequently microbial mediated skatole production (Bilić–Šobot *et al*., 2016).

Up to recent, there was no evidence that androstenone tissue concentration could be influenced by nutrition (Zamaratskaia and Squires 2009; Zammerini *et al*., 2012). However, Jen and Squires (2011) found that EM fed with activated carbon for 28 d in the finishing period had lower plasma as well as tissue androstenone levels compared to a control group. The authors hypothesized that androstenone, like oestadriol, undergoes an enterohepatic circulation (Ruoff and Dziuk, 1994) and activated carbon could act as an absorbent thereby diminishing androstenone level. Recently, Wealleans *et al*. (2013) and Bee *at al*., (2016) observed that androstenone concentration linearly decreased with increasing dietary HT intake. However, in these studies the HT supplemented diet was offered only in the finisher and not in the grower period. The authors hypothesized that if HT interferes with the development of androgen secretion and is offered to the pig at early life, it may be possible to decrease androstenone levels before pigs reach puberty. Furthermore, Moerlein and Tholen (2015) reviewed data from published studies and observed that EM with low

androstenone, skatole, and indole levels had significantly greater polyunsaturated (PUFA) levels and lower saturated fatty acid (SFA) levels in the adipose tissue. As fatty acid composition of the lipids can be easily manipulated by nutrition, the aforementioned findings give rise to the interesting possibility that androstenone and/or skatole levels are related to the lipid metabolism in pigs.

Consequently, we hypothesized that the inclusion of HT and high dietary supply of PUFA in the grower and finisher diet could diminish boar taint compounds in the adipose tissue as well as in the intramuscular fat. Thus, the aims of this study were to investigate the effects of dietary HT and high and low PUFA supplementation on androstenone, skatole and indole tissue levels in EM and possible regulation of hepatic clearance of androstenone and skatole.

MATERIAL AND METHODS

Animal trial

The Swiss cantonal Committee for Animal Care and Use approved all procedures involving animals (27428). Forty–four Swiss Large White EM weighing 26.0 ± 4.95 kg were randomly selected and assigned to four dietary treatments: H–, H+, L–, L+. The grower (25–60 kg BW) and finisher (60–105 kg BW) diets H– and H+ had a high PUFA content originating from a 2% inclusion of soy oil whereas diets L– and L+ contained 2% tallow resulting in 1.38% lower PUFA level (Table 6.1). Furthermore, in two of the grower and finisher H+ and L+ diets, 3% HT extract obtained from chestnut (Silvateam, San Michele Mondovì Italy) was added. All diets were formulated to meet nutrient requirements according to the Swiss feeding recommendations for swine (Agroscope 2015) and were isocaloric and isonitrogenous. The experimental diets were offered *ad libitum* in a pelleted form. All pigs were reared in group pens, equipped with automatic feeders and individual pig recognition system (SchauerMaschinenfabrik GmbH. & Co KG, Prambachkirchen, Austria) as described previously by Bee *et al*., (2008). Determination of individual feed intake as well as feeding behaviour in group–housed pigs were assessed. The pigs were switched from the grower to the finisher diet when the average BW of all 44 pigs was 60 kg.

Table 6.1. Feed ingredients (%), nutrient and tannin composition of the experimental diets.

	Grower diet[1]				*Finisher diet*[1]			
	H–	***L–***	***H+***	***L+***	***H–***	***L–***	***H+***	***L+***
Wheat	49.75	49.75	49.75	49.75	42.59	42.59	42.59	42.59
Barley	10.56	10.56	10.56	10.56	27.70	27.70	27.70	27.70
Corn	2.43	2.43	2.43	2.43				
Wheat starch	7.00	7.00	7.00	7.00	9.09	9.09	9.09	9.09
Soy extraction meal	12.71	12.71	12.71	12.71	9.70	9.70	9.70	9.70
Potato protein	2.47	2.47	2.47	2.47	0.59	0.59	0.59	0.59
Wheat bran	5.00	5.00	5.00	5.00	1.14	1.14	1.14	1.14
Soy oil	2.00		2.00		2.00		2.00	
Tallow		2.00		2.00		2.00		2.00
Arbocel	3.00	3.00			3.00	3.00		
Hydrolysable tannins[2]			3.00	3.00			3.00	3.00
Dicalcium phosphate	1.45	1.45	1.45	1.45	1.05	1.05	1.05	1.05
Mono–sodium phosphate	0.40	0.40	0.40	0.40				
Calcium carbonate	1.38	1.38	1.38	1.38	0.88	0.88	0.88	0.88
NaCl	0.08	0.08	0.08	0.08	0.46	0.46	0.46	0.46
Natuphos 5000 G	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L–lysine–HCl	0.34	0.34	0.34	0.34	0.36	0.36	0.36	0.36
DL–methionine	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L–threonine	0.07	0.07	0.07	0.07	0.10	0.10	0.10	0.10
Mikrogrit	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Pellan[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral–vitamin premix[4]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Analysed nutrient and tannin composition, g/100 kg DM								
Total ash, g/kg	59.4	59.4	59.4	59.4	48.3	48.3	48.3	48.3
Crude fibre, g/kg	47.8	47.8	47.8	47.8	47.6	47.6	47.6	47.6
Crude protein, g/kg	161.4	161.4	161.4	161.4	161.4	161.4	161.4	161.4
Crude fat, g/kg	41.6	41.6	41.6	41.6	40.05	40.05	40.05	40.05
SFA	0.80	1.24	0.80	1.24	0.75	1.18	0.75	1.18
MUFA	0.68	1.30	0.68	1.30	0.65	1.26	0.65	1.26
PUFA	2.51	1.44	2.51	1.44	2.45	1.38	2.45	1.38
Total hydrolysable tannin, g/kg[5]	Nd	Nd	14.81	14.81	Nd	Nd	14.81	14.81
Ellagitannin, g/kg	Nd	Nd	1.51	1.51	Nd	Nd	1.51	1.51
Gallotannin, g/kg	Nd	Nd	11.38	11.38	Nd	Nd	11.38	11.38
Gallic acid, g/kg	Nd	Nd	1.915	1.92	Nd	Nd	1.915	1.92
Calculated DE content, MJ/kg DM[6]	13.54	13.54	13.54	13.54	13.54	13.54	13.54	13.54

[1]Grower diet formulated for pigs in the BW range of 25 to 60 kg; finisher diet formulated for pigs in the BW range of 60 to 110 kg; H = diets had a high PUFA content and were supplemented without (H–) or with (H+) a chestnut powder containing hydrolysable tannin. L = diets had a low PUFA content and were supplemented without (H–) or with (H+) a chestnut powder containing hydrolysable tannin.

[2]Chestnut powder.

[3]Binder that aids in pellet formation.

[4]Supplied the following nutrients per kg of diet: 20000 IU vitamin A, 200 IU vitamin D3, 39 IU vitamin E, 2.9 mg riboflavin, 2.4 mg vitamin B6, 0.010 mg vitamin B12, 0.2 mg vitamin K3, 10 mg pantothenic acid, 1.4 mg niacin, 0.48 mg folic acid, 199 g choline, 0.052 mg biotin, 52 mg Fe as FeSO4, 0.16 mg I as Ca(IO)3, 0.15 mg Se as Na2Se, 5.5 mg Cu as CuSO4, 81 mg Zn as ZnO2, 15 mg Mn as MnO2.

[5] Total hydrolysable tannins and gallic acid was estimated on the basis of previous study (Bee *at al*., 2016).

[6]The digestible energy coefficients from each feed ingredient were obtained from the Swiss Feed Database (https://www.feedbase.ch) and taking into account the relative amount of each feed ingredient in the diet, digestible energy content was calculated.

Slaughter procedure, carcass measurements, tissue sampling and meat quality assessment

Following the procedure described in detail by Bee *et al*., (2016), pigs were slaughtered at 170 d of age at the research abattoir of Agroscope Posieux (Switzerland) after feed was withdrawn for 16 h. At exsanguination, blood samples were collected into 10 mL vacutainer glass tubes, kept on ice until being processed for analysis. After splitting the dehaired carcass, liver, kidney, testicles, salivary (mandibular) and bulbo–urethral glands were weighed. Liver samples were excised within 2 min after evisceration, rinsed with PBS and stored at −20°C in RNA–Later solution (1018087 Qiagen, Basel, Switzerland) until RNA extraction. Subsequently, the hot carcasses were weighed and the pH and temperature of the longissimus dorsi muscle (LD) was measured with a pH meter (WTW pH197–S, WTW, Weilheim, Germany) equipped with a WTW Eb4 electrode (WTW) at the 10th rib location before chilling at 3°C for 1 d.

The following day, the left carcass sides were weighed, scanned by dual–energy X–ray absorptiometry (GE Healthcare i–DXA, Nova–Logic, St–Légier, Switzerland) in human thick mode. Afterwards, carcasses were fabricated into the major primal cuts (shoulder, loin, ham, and belly) and the lean and backfat percentage were calculated (Bee *et al*., 2004).

Meat quality traits

After carcass dissection was completed and following the protocol described by Pardo *et al*., (2013), LD was removed for the determination of ultimate pH, meat colour, water holding capacity as percentage drip, cooking and thaw loss as well as shear force.

Feed and meat analysis

Feed and LD samples, after being milled with a 1 mm sieve and freeze–dried, respectively, were analysed for dry matter content at 105°C for 3 h and ash content after incineration at 550°C. Using the Kjeldahl procedure (Leco FP–2000 analyser, Leco, Mönchengladbach, Germany) N content of the feed was analysed and CP expressed as 6.25 × N. The content of crude fibre was analysed after digestion with successively H_2SO_4 and KOH, washed with acetone, dried at 130°C and then ashed (EN 71/393, ISO 6865:2000, VDLUFA 6.1.4). The fatty acid profile of the diets, LD and adipose tissue was obtained by gas chromatography with in situ transesterification as previously described in detail (Ampuero *et al*., 2014). The content of gallic acid and HT in the diets were determined as outlined by Johnson *et al*., (2014).

Analysis of boar taint in fat

Androstenone, skatole and indole concentrations in the adipose tissue and LD were analysed according to Ampuero *et al*., (2011). Briefly, the adipose tissue samples of subcutaneous fat were liquefied in a microwave oven for 2 × 2 min at 250 W. The liquefied lipids were centrifugated for 2 min at room temperature. The water was then removed and 0.5 mL of water–free liquid fat, kept at approximately 47°C, was placed in 2.0 mL Eppendorf tubes in duplicates and an internal standard was added (1 mL methanol containing 0.469 mg/L androstanone and 0.05 mg/L 2–methylindole). After vortexing for 30 s, the tubes were incubated for 5 min at 30°C in an ultrasonic–water bath, kept at 0°C in an ice–water bath for 20 min and then centrifuged at 11'000 g for 20 min. Finally, the liquid fraction was filtered (0.2 µm filter) and transferred into a vial for androstenone, skatole and indole analysis with a high–performance liquid chromatography system. Concentrations were expressed per g of LD and adipose tissue. The quantification limits were 0.3 µg/g tissue for androstenone and 0.03 µg/g tissue for skatole and indole.

RNA isolation, primer design and quantitative Real–Time PCR

Total RNA extraction from the liver and colon was performed using Nucleospin® RNA XS kit (740902, Macherey–Nagel, Oensinger, Switzerland) as previously described (Bee et al. 2016). Primers for cytrochrome CYP1A1, CYP1A2, CYP2A19, CYP2E1 and CYP3A29 in both liver and colon mucosa were designed using Primer–Blast service (Ye *et al*., 2012) offered by the National Institute of Health and verified for specificity using the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov). Primers for the CYP1A2 gene were the same as used by Rasmussen *et al*., (2011). The target and housekeeping gene primers and National Center for Biotechnology Information accession numbers are shown in annex Table 1. For each primers pairs, the efficiency of amplification was determined in three independent experiments. These genes were evaluated for their expression in pig livers and colon via quantitative real–time PCR as outlined in detail by Bee *et al*., (2016). The expression of each targeted gene was evaluated using the ΔΔ–Ct method (with efficiency corrections) and normalized using GAPDH as housekeeping genes. All the calculations were performed using the Eco–Illumina Study software (Labgene, Chatel–St–Denis, Switzerland).

Statistical analyses

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included dietary PUFA level, HT level and the PUFA × HT interaction as fixed effects and litter as random effect. Least squares means were calculated and considered statistically significant at $P \leq 0.05$ and tendencies were denoted at $P \leq 0.10$ and > 0.05. Pearson correlations between boar taint compounds and weight of testes, accessory sex glands and CYP gene isoforms expression were determined using CORR procedure.

RESULTS AND DISCUSSION

Dietary effects on growth performance, carcass characteristic and organ weight

The effect of dietary HT and PUFA level on growth performance of grower–finisher pigs are reported in Table 6.2. Despite similar growth rate in the grower period, feed intake tended (P = 0.10) to be greater in the HT group resulting in impaired (P = 0.01) feed efficiency. In the finisher period, dietary HT inclusion impaired (P = 0.01) feed efficiency as well but this was caused primarily by a reduced (P = 0.01) average daily gain despite a similar feed intake. Dietary PUFA level had no (P > 0.05) effects on these traits in the grower and finisher period.

Table 6.2. Effect of dietary hydrolysable tannin and PUFA level on growth performance of grower–finisher pigs[1].

	H	L	H	L		P–values[2]		
Item	–	–	+	+	SEM	Tan	PUFA	TanxPUFA
Body weight, kg								
At birth	1.68	1.64	1.75	1.71	0.109	*0.36*	*0.64*	*0.98*
At start of grower period	25.60	25.42	26.51	26.64	1.553	*0.42*	*0.98*	*0.91*
At start of finisher period	66.45	62.60	65.86	66.83	2.377	*0.44*	*0.54*	*0.31*
At slaughter	112.76	109.00	108.49	110.40	3.791	*0.63*	*0.76*	*0.34*
Average daily gain, kg/d								
Grower period	0.83	0.76	0.80	0.82	0.029	*0.58*	*0.46*	*0.14*
Finisher period	0.94	0.94	0.87	0.88	0.037	*0.01*	*0.94*	*0.71*
Grower and finisher period	0.89	0.85	0.83	0.85	0.029	*0.27*	*0.69*	*0.27*
Total feed intake, kg								
Grower period	83.8	77.9	84.6	86.5	2.831	*0.10*	*0.48*	*0.17*
Finisher period	124.1	119.4	118.2	118.4	6.571	*0.34*	*0.53*	*0.49*
Grower and finisher period	207.7	197.5	202.9	205.3	8.535	*0.80*	*0.50*	*0.28*
Average daily feed intake, kg/d								
Grower period	1.71	1.59	1.73	1.77	0.058	*0.10*	*0.48*	*0.17*
Finisher period	2.51	2.43	2.40	2.40	0.093	*0.33*	*0.56*	*0.56*
Growing and finishing period	2.11	2.01	2.06	2.09	0.071	*0.82*	*0.51*	*0.31*
Gain–to–feed, kg/kg								
Grower period	0.49	0.48	0.47	0.47	0.008	*0.01*	*0.34*	*0.34*
Finisher period	0.38	0.39	0.36	0.37	0.008	*<0.05*	*0.19*	*0.63*
Grower and finisher period	0.42	0.42	0.41	0.41	0.006	*≤0.001*	*0.56*	*0.78*

[1] H = high dietary PUFA level; L = low dietary PUFA level; – = without hydrolysable tannin supplementation; + = with hydrolysable tannin (3%) supplementation

[2] Probability values for hydrolysable tannin supplementation (TAN), dietary PUFA level (PUFA) and TAN × PUFA interaction.

It was expected that diets being isocaloric, dietary fat source would have no impair growth performance (Kim *et al*., 2014). By contrast, the current study demonstrated that dietary HT supply impaired feed efficiency in both the starter and finisher period. This impairment was caused by a similar growth rate despite greater intake in the starter period and a slower growth rate despite a similar feed intake in the finisher period. One possible explanation for these findings is that HT or their microbial degradation products negatively affected nutrient digestibility and consequently, their absorption. The current findings on feed efficiency are in agreement with those of Bee *et al*., (2016) whereas they contradict the findings of Čandek–Potokar *et al*., (2015). The latter found a significant reduction in feed intake and a concomitant decrease in growth and thus similar feed efficiency with increasing HT level. As in the present study, in the experiments of Bee *et al*., (2016) and Čandek–Potokar *et al*., (2015), chestnut HT extract was used at the same inclusion level but the composition of the basal diet differed markedly between the studies. Thus, it cannot be excluded that interactions between dietary HT and feed ingredients of the basal diets may have affected nutrient digestibility. The decrease of feed intake revealed by Čandek–Potokar *et al*., (2015) could be due not only to the 3% chestnut extract, but also due to the inclusion of 7.0% rapeseed meal in the basal diet, that contributes to an increase the amount of condensed tannin (Naczk *et al*., 1998), which ultimately might have negatively affected palatability of the diet (Bell, 1993).

Data on feeding behaviour (Table 6.3) suggest that dietary HT inclusion had no ($P > 0.05$) impact on the palatability of the diet. By contrast, pigs offered the diets supplemented with soy oil had fewer ($P \leq 0.001$) visits at the feeding station but concomitantly ingested more ($P < 0.05$) feed per visit compared to pigs fed the diets supplemented with tallow. This finding is in agreement with Solà–Oriol *et al*., (2011) that highlighted a preference of pigs for vegetable over animal fat.

Table 6.3. Effect of dietary hydrolysable tannin and PUFA level on feeding behavior of grower–finisher pigs[1].

Item	H –	L –	H +	L +	SEM	P–values2		
						Tan	PUFA	Tan × PUFA
Total of visit during the experiment (d)	703.6	922.3	590.8	821.6	59.81	*0.09*	*<0.001*	*0.92*
Total time spent eating (min)	5283.1	5536.7	5395.6	5391.0	174.1	*0.91*	*0.42*	*0.40*
Total feed intake (kg)	207.5	197.4	202.6	205.0	8.546	*0.82*	*0.50*	*0.29*
Number of visit per day (n/d)	7.25	9.49	6.03	8.41	0.639	*0.08*	*≤0.001*	*0.91*
Time spent eating per day (min/d)	54.28	56.93	55.31	55.19	1.953	*0.82*	*0.43*	*0.38*
Feed intake per visit (g/visit)	378.33	289.79	416.92	323.80	29.35	*0.21*	*<0.05*	*0.94*
Feed intake per min (g/min)	39.19	35.37	37.89	38.84	1.716	*0.35*	*0.22*	*0.05*

[1] H = high dietary PUFA level; L = low dietary PUFA level; – = without hydrolysable tannin supplementation; + = with hydrolysable tannin (3%) supplementation
[2] Probability values for hydrolysable tannin supplementation (TAN), dietary PUFA level (PUFA) and TAN × PUFA interaction

Carcass composition of half–left carcass determined by DXA scan and carcass characteristics and organ weight of grower–finisher pigs are reported in Table 6.4 and Tables 6.5 respectively. Except for lighter (P = 0.001) livers, neither organ weights, testis, bulbourethral and salivary glands nor carcass characteristics were affected by the HT intake or the dietary PUFA level. The HT related differences in feed efficiency were not sufficient to detrimentally affect carcass characteristics either determined by dissection or DXA measurement.

Table 6.4. Effect of hydrolysable tannin and PUFA on carcass composition of half–left carcass determined by DXA scan in finisher pigs[1].

Item	H –	L –	H +	L +	SEM	P–values[2]		
						Tan	PUFA	TanxPUFA
Total mass, kg	42.29	41.30	40.68	41.73	1.514	*0.63*	*0.98*	*0.40*
Bone mass, kg	1.09	1.05	1.05	1.08	0.035	*0.74*	*0.96*	*0.52*
Fat mass, kg	7.48	7.09	7.16	7.56	0.578	*0.88*	*1.00*	*0.45*
Lean mass, kg	33.70	33.16	33.47	33.12	1.060	*0.44*	*0.95*	*0.47*
Tissue mass, kg	41.20	40.25	39.63	40.65	1.484	*0.62*	*0.98*	*0.40*
Fat free mass, kg	34.79	34.22	33.52	34.20	1.092	*0.44*	*0.95*	*0.46*
BMD[3], g/cm2	0.86	0.84	0.82	0.85	0.021	*0.36*	*0.97*	*0.16*

[1]H = high dietary PUFA level; L = low dietary PUFA level; – = without hydrolysable tannin supplementation; + = with hydrolysable tannin (3%) supplementation; DXA = Dual–Energy X–ray Absorptiometry measurements performed in human thick mode.
[2] Probability values for hydrolysable tannin supplementation (TAN), dietary PUFA level (PUFA) and TAN × PUFA interaction
[3]BMD = bone mass density

The lack of effect and concurs with results of previous studies (Bee *et al*., 2016). Moreover, Bee *et al*., (2016) reported that weights of the mandibular glands, bulbo–urethral glands and testes were lower in pigs fed the HT diet. It was hypothesized that this might have been triggered by lower levels of gonadotrophins as observed in male albino rats (Dias *et al*., 2014) resulting in lower androgenic activity of the testes and causing regressive changes of the accessory sex glands. Although not significant, also in the present study bulbo–urethral glands and testes were numerically lighter in the HT compared with the control group. It is noteworthy to mention that compared to the unsupplemented group, bulbo–urethral glands and testes were approximately 10% lighter whereas in the previous study testes weight differed only by 5% (Bee *et al*., 2016). The greater impact might result from the longer feeding period (98 vs. 60 d). In addition, the weights of testes, bulbo–urethral and salivary glands were positively correlated (r = 0.49; $P < 0.001$; r = 59; $P < 0.001$; r = 0.39; $P = 0.008$; respectively) with androstenone adipose tissue levels, in agreement with previous data of Bee *et al*., (2016). Moreover, a positive correlation (r = 0.45; $P = 0.002$) was observed between weight of bulbo–urethral gland and skatole adipose tissue levels. The effects of polyphenolic compounds are in agreement with Mennen *et al*., (2005) who reported a reduction of fertility in animals, antiandrogenic effects, and testicular atrophy in rats as a result of excessive consumption of polyphenols.

In the present study, dietary HT inclusion decreased ($P < 0.001$) markedly the weight of liver. The liver represents the main organ of lipid metabolism and it is very susceptible to lipid peroxidation. In pigs no differences have been found by Bilić–Šobot *et al.*, (2016) regarding the supplementation level of HT on mitotic and apoptotic cell count of liver. Frankic and Salobir (2011) recently reported that dietary HT supplementation inhibited the elevation of plasma alanine aminotransferase, an indicator of hepatic oxidative damage, in young growing pigs. The authors concluded that HT derivatives had a protective rather than a toxic effect on the liver and in line with the current observation hepatotoxic effects are usually linked to an increase in liver weight. Specifically, Maronpot *et al*. (2010) reported that a 10 to 50% increase in liver weight is a typical response to xenobiotic exposure, probably due to a hepatic enzyme induction and liver enlargement rather than hepatocellular necrosis followed by hyperplastic response. In this scenario one could hypothesize that HT derivatives exert protective effects on porcine liver but further studies are necessary to clarify the exact mechanisms involved.

Table 6.5. Effect of dietary hydrolysable tannin and PUFA level on carcass characteristics and organ weight of grower–finisher pigs[1].

Item	H –	L –	H +	L +	SEM	P–values[2] Tan	PUFA	TanxPUFA
Hot carcass weight, kg	89.94	87.56	86.86	88.90	3.110	*0.73*	*0.95*	*0.38*
Carcass yield, %	79.71	80.33	80.06	80.54	0.305	*0.32*	*0.05*	*0.80*
Cold Loss, [3]%	1.92	1.72	2.24	1.66	0.251	*0.59*	*0.12*	*0.44*
Lean meat, [4] %	58.13	58.87	58.14	56.93	0.707	*0.45*	*0.23*	*0.44*
Loin	27.01	26.70	26.86	26.89	0.265	*0.91*	*0.52*	*0.44*
Ham	18.40	18.54	18.33	17.70	0.318	*0.16*	*0.44*	*0.23*
Shoulder	12.75	12.69	12.92	12.31	0.287	*0.69*	*0.19*	*0.28*
Belly	16.56	16.62	16.41	16.23	0.249	*0.19*	*0.75*	*0.55*
Back Fat %	7.35	7.09	7.14	7.42	0.374	*0.84*	*0.98*	*0.43*
10th rib backfat thickness, mm	20.49	17.04	17.65	18.01	1.346	*0.40*	*0.17*	*0.09*
Subcutaneous Fat, [5] %	12.68	12.46	12.35	12.68	0.503	*0.91*	*0.90*	*0.54*
Omental fat, [6] %	1.04	1.02	1.02	1.13	0.084	*0.54*	*0.60*	*0.38*
Organ weight, g								
Liver	1663	1643	1482	1506	0.051	*<0.001*	*0.95*	*0.60*
Kidney	320	299	310	306	0.011	*0.88*	*0.24*	*0.39*
Testis	522	538	490	472	0.040	*0.14*	*0.97*	*0.62*
Bulbourethral gland	153	149	134	138	0.013	*0.16*	*0.98*	*0.67*
Salivary gland	69	75	71	72	0.005	*0.87*	*0.39*	*0.53*

[1] H = high dietary PUFA level by including 2% soy oil; L = low dietary PUFA supplementation by including 2% tallow; – = without hydrolysable tannin supplementation; + = with hydrolysable tannin (3%) supplementation.
[2] TAN = effect of hydrolysable tannin supplementation; PUFA = PUFA level in the diet; TAN x PUFA
[3] Weight loss of the hot carcass during cooling at 2°C for 24 h
[4] Sum of denuded shoulder, back, and ham weight as a percentage of cold carcass weight.
[5] Sum of external fat from the shoulder, back, and ham expressed as a percentage of cold carcass weight.
[6] Omental fat weight expressed as a percentage of cold carcass weight.

Dietary effects on meat quality, skatole, indole and androstenone levels in adipose and cytochrome isoenzyme gene expression

Meat quality traits of the loin are reported in Table 6.5. The loins of pigs fed the HT supplemented diets were lighter (P = 0.04) and tend to be less red (P = 0.08) compared to those fed the unsupplemented diets. Within the HT group, loins of L+ pigs tended to be tougher than those of the H+ pigs, with intermediate values for the H– and L– group (TAN × PUFA interaction; P = 0.07). Previous studies did not find any differences in shear force values in relation to dietary fat sources (Alonso *et al*., 2012, Mitchaothai *et al*. 2007).

The main objective of this study was to highlight the effect of HT and high dietary supply of PUFA in the grower and finisher diet to reduce the deposition of boar taint compounds in the adipose tissue. This objective was based on the results of *in vivo* experiment of Bee *et al*., 2016 suggesting that dietary 3% HT inclusion decrease the boar taint compounds as androstenone, skatole and indole. Moreover, Moerlein and Tholen (2015) observed that EM with low androstenone, skatole, and indole levels had significantly greater PUFA levels in the adipose tissue. Results of this study showed that indole adipose concentration tended to be lower (P < 0.10) in HT compared to unsupplemented HT diets and confirms our previous results (Bee *et al*., 2016). Pigs of the H– group had greater skatole concentration in adipose tissue compared to those of the L– group, with intermediate levels of the H+ and L+ group (TAN x PUFA interaction; P = 0.05). Although not reaching significance, androstenone levels were numerically lower in the HT than the unsupplemented diets. Overall, the androstenone and skatole concentrations were below boar taint thresholds (androstenone > 1.0 ppm; skatole > 0.16 ppm) considered problematic for consumer acceptance (Ampuero and Bee, 2008).

Table 6.6. Effect of dietary hydrolysable tannin and PUFA level on meat quality traits of the loin and androstenone, skatole and indole level in adipose tissue of grower–finisher pigs[1].

	H	L	H	L		P–values[2]		
Item	–	–	+	+	SEM	Tan	PUFA	TanxPUFA
pH								
45 min	6.50	6.18	6.50	6.24	0.208	*0.91*	*0.17*	*0.90*
24 h	5.54	5.56	5.56	5.50	0.028	*0.42*	*0.37*	*0.13*
Temperature								
45 min	6.50	6.18	6.50	6.24	0.208	*0.91*	*0.17*	*0.90*
24 h	4.75	4.88	4.39	4.95	0.356	*0.58*	*0.19*	*0.41*
Color[3]								
L*	47.17	46.67	47.64	49.21	0.755	*0.04*	*0.45*	*0.15*
a*	5.36	5.09	4.78	4.81	0.287	*0.08*	*0.62*	*0.55*
b*	2.58	2.35	2.55	2.76	0.250	*0.29*	*0.96*	*0.23*
Chroma value	5.96	5.63	5.45	5.57	0.348	*0.30*	*0.71*	*0.41*
Water–holding capacity, %								
Drip loss	2.01	2.18	2.06	2.38	0.161	*0.36*	*0.10*	*0.60*
Thaw loss	5.70	6.81	5.85	5.52	0.755	*0.46*	*0.61*	*0.35*
Cook loss	24.73	24.79	25.67	25.32	0.549	*0.09*	*0.73*	*0.63*
Total loss	31.80	32.67	33.65	33.30	0.687	*0.04*	*0.65*	*0.30*
Shear force, kg	8.00^{xy}	8.17^{xy}	8.85^{y}	7.41^{x}	0.432	*0.91*	*0.15*	*0.07*
Boar taint compounds, µg/g adipose tissue								
Androstenone	0.51	0.41	0.37	0.39	0.085	*0.32*	*0.62*	*0.40*
Skatole	0.13^{y}	0.05^{x}	0.09^{xy}	0.12^{xy}	0.026	*0.48*	*0.25*	*<0.05*
Indole	0.05	0.03	0.02	0.02	0.007	*0.081*	*0.16*	*0.21*

^{x,y} Values within a row with different superscripts tend to differ significantly at P ≤ 0.10.

[1] H = high dietary PUFA level by including 2% soy oil; L = low dietary PUFA supplementation by including 2% tallow; – = without hydrolysable tannin supplementation; + = with hydrolysable tannin (3%) supplementation

[2] Probability values for hydrolysable tannin supplementation (TAN), dietary PUFA level (PUFA) and TAN × PUFA interaction

[3] L* = lightness (greater values equal lighter color); r* = Redness (greater values equal redder color); b* = yellowness (greater values equal more yellow color); chroma value (color saturation) = $\sqrt{a*^2 + b^{*2}}$

Effect of hydrolysable tannin and PUFA level on hepatic mRNA cytochrome P450 isoenzyme expression are shown in Table 6.6. Except for the CYP1A2 mRNA liver expression, which was lower (P = 0.04) in pigs fed the HT diets, feeding 3% chestnut extract had no effect on the CYP isoform gene expression in the liver and colon. Similarly, Zamaratskaia *et al*., (2015) did not find any effect of dietary HT supplementation on CYP3A mRNA expression in the liver and colon when pigs received 3% chestnut extract for 78 d. However, they found an increase in CYP3A protein expression and activity. Dietary PUFA level had no (P > 0.05) effects on the CYP isoenzyme mRNA expression in the liver and colon mucosa. In contrast to Bee *et al*. (2016) who found a negative relationship between skatole and indole concentration in the adipose tissue and hepatic of CYP2E1 and CYP2A19 gene expression, in the current study no correlation between boar taint compounds and CYP gene expression level was found. A possible explanation for the discrepancy between the two studies is the fact that androstenone and skatole level in adipose tissue were up to 50% and about three times rispectivelly greater in the study of Bee *et al*. (2016), which might have had an influence on the overall expression level.

Table 6.7. Effect of hydrolysable tannin and PUFA level on hepatic mRNA cytochrome P450 isoenzyme expression in grower–finisher pigs [1].

	H	L	H	L		P–values[2]		
Item	–	–	+	+	SEM	Tan	PUFA	TanxPUFA
Liver								
CYP1A1	1.11	1.19	0.93	1.22	0.285	*0.83*	*0.69*	*0.53*
CYP1A2	1.59	1.24	0.93	1.03	0.200	*0.04*	*0.52*	*0.28*
CYP2A19	2.08	1.62	2.26	1.08	0.812	*0.21*	*0.13*	*0.23*
CYP2E1	1.15	1.45	1.13	1.12	0.166	*0.07*	*0.22*	*0.11*
CYP3A29	1.43	1.24	1.25	1.02	0.109	*0.40*	*0.36*	*1.00*
Colon								
CYP1A1	0.10	0.79	1.25	0.18	0.566	*0.59*	*0.71*	*0.09*
CYP3A39	1.14	1.55	0.10	1.17	0.075	*0.14*	*0.06*	*0.42*

[1] H = high dietary PUFA level by including 2% soy oil; L = low dietary PUFA supplementation by including 2% tallow; – = without hydrolysable tannin supplementation; + = with hydrolysable tannin (3%) supplementation

[2] Probability values for hydrolysable tannin supplementation (TAN), dietary PUFA level (PUFA) and TAN × PUFA interaction.

CONCLUSION

The topic of castration in pig production raises consumer's attention but the presence of unfavorable compounds in meat from EM like androstenone, skatole and indole affect their acceptance. Therefore, efficient and feasible alternatives are urgently needed. One possible way is to supplement diets of EM with components which might modulate development of boar taint. Considering the hypothesis that HT and PUFA level inclusion in the EM diet could be affect the boar taint compounds, this study demonstrates that only administering 3% chestnut extract to EM affects the overall level of boar taint compounds in pork, without detrimental effects on performance, carcass composition and meat quality. HT in chestnut extract numerically reduce androstenone and skatole level. However, overall the levels were surprisingly low, therefore the effects of HT on skatole levels need to be further investigated. PUFA tissue level seems not be related to androstenone and skatole level, which then would contradict the hypothesis. The obtained results will be submitted to a scientific journal after the complete data analysis.

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CHAPTER 7: GENERAL CONCLUSION

This thesis focused on the study of plant extracts as potential health–promoting compounds in animal nutrition. Specifically, it highlighted their influence in the swine wellbeing and nutritional quality improvement of pork.

Overall, efficacy of the plant extracts has been demonstrated by the use of *in vivo* models in all studies performed. Results showed that dietary supplementation with plant extracts can improve the performance in different phases of pig production. Moreover, meat and derived products quality and its sensory parameters resulted in enhanced physical, chemical, oxidative stability.

This thesis leads to specific conclusions that are listed below:

Dietary supplementation with algae extract plus polyphenol in lactating sows improved sows and piglets' performance. The mixture has shown a positive effect on sow's reproduction, increasing the number of total born at the subsequent farrow. Even though no apparent deleterious effects of supplemented diet have been observed in the present study, further studies are needed to determine the optimal algae extracts and polyphenols supplementation level in the diet. In conclusion, the bioactive components in seaweed extract represent an innovative dietary supplement in pig diet with beneficial effects on health and able to sustain the production performance.

Dietary antioxidants are one of the major strategies in preventing lipid oxidation and meat oxidative phenomena. Verbascoside is the most abundant phenolic compound in Verbenaceae extracts. In previous studies, our group found that plant extracts containing Verbascoside have a greater antioxidant activity compared to other phenolic compounds. According to these results, study performed in my PhD course suggests that dietary supplementation with Vitamin E and Verbascoside mixture, in the last phase of pig fattening, exerts an antioxidant and antimicrobial effects and improved pork sensory characteristics in refrigerated storage and modified atmosphere packaging conditions. Moreover, supplemented diet improved the seasoning losses of smoked cured ham, without affecting physical and chemical parameters. As a result, this dietary mixture supplementation can be considered antioxidants and antimicrobial compound able to enhance not only the shelf life of raw pork under commercial conditions but also the consumer's preference of smoked cured ham, without affecting the nutritional characteristics.

The presence of unfavorable compounds in meat from entire male like androstenone, skatole and indole affect their consumer's acceptance. Hydrolysable tannin dietary supplementation in entire male pigs decreases the presence of boar taint in pork. This study demonstrated that the 3 % chestnut extract supplementation in entire male diet reduce

the overall levels of boar taint compounds in pork, without detrimental effects on performance, carcass composition and meat quality.

Concluding, this thesis improved knowledge regarding beneficial effects of plant extracts. The dietary inclusion of bioactive components contained in natural extracts can be considered an innovative approach to improve pig wellbeing and pork quality without negative effects on animal production.

Recommendation for future studies:

In retrospect with the discussed points in this thesis, following topics can be of interest for future research:

- More nutritional studies are required to evaluate the efficacy of algae and polyphenols at different inclusion levels in sows during lactating period in enhancing their healh and productivity. Further studies are required to confirm our findings of the improvement of total number of piglets at the subsequent farrowing, in order to explore the possible effect on reproduction.
- 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.
- The results obtained from the smoked cured ham could be considered as a good starting point for study this type of product. In our knowledge, this topic has never been investigated in scientific literature.
- More nutritional studies are required to evaluate the efficacy of the inclusion of hydrolysable tannin supplementation in entire male pigs in order to overcome the topic of castration and to give to the market a quality product as meat.

LIST OF SCIENTIFIC PAPERS

Paper

Maghin F, Ratti S, Corino C. Biological Functions and Health Promoting Effects of Brown Seaweeds in Swine Nutrition. J Dairy Vet Anim Res 1(1): 00005.
Submitted to Journal of Dairy, Veterinary & Animal Research July 23, 2014.
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Corino C, Maghin F, Rossi R. Nutrizione animale per la sicurezza nutrizionale delle carni suine. Tomo II degli Atti 2016 dell'Accademia dei Georgofili.

Rossi R, Stella S, Ratti S, Maghin F, Tirloni E, Corino C. Dietary supplementation with antioxidant mixture affects the shelf life of fresh pork packaging under modified atmosphere.
Submitted to Food Research International.

Poster

Rossi R., Maghin F., Tucci T., Corino C. Miscela di estratti naturali (Algatan Mater®) nella dieta di scrofe in lattazione: effetti su performance di scrofe e suinetti.
XLII Meeting Annuale SIPAS, Montichiari (BS), Italy, 10–11 Marzo 2016. pp. 183.

Maghin F., Rossi R., Chiapparini S., Corino C. Feeding with natural antioxidant as strategy to improve meat quality.
Workshop NutriOx 2016, Kaiserslautern (DE), Germany, 21 – 23 September 2016.

Conference

Maghin, F., Rossi, R., Ratti, S., Pastorelli, G., Stella, S., Tirloni, E., Corino, C. Antioxidant mixture supplementation in the medium–heavy pigs: effects on performances and shelf life of Longissimus Dorsi muscle (Oral presentations). ASPA 21st Congress–Milano, June 9–12, 2015. Italian Journal of Animal Science 2015; volume 14: supplement 1 pp 20.

Maghin F., Rossi R., Prost M., Corino C. Biological system to assess the antioxidant capability of plant extracts. Workshop NutriOx 2016, Kaiserslautern (DE), Germany, 21 – 23 September 2016.

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"What makes the life cheerful is not to do things that we like, but finding pleasure in the things we have to do" (Goethe).