IMPACT OF OXIDIZED CORN OIL AND SYNTHETIC ANTIOXIDANT ON SWINE PERFORMANCE, ANTIOXIDANT STATUS OF TISSUES, PORK QUALITY AND SHELF LIFE EVALUATION

BY

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DISSERTATION

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ABSTRACT

The objective of the study was to evaluate the effect of antioxidant protected (AOX) or unprotected fresh or oxidized corn oil on animal performance, oxidative status of tissues, pork quality, shelf-life, and antioxidant activity of skeletal muscle of finishing pigs. The experimental design was a complete randomized block design (CRBD) in a factorial arrangement (2×2), with 2 levels of corn oil (fresh or oxidized corn oil) and 2 levels of antioxidant (AOX; with or without AOX). A total of 160 barrows were fed for 56 days one of the following experimental diets: fresh oil, fresh oil + AOX, oxidized oil or oxidized oil + AOX. Animal performance was evaluated and oxidative status of blood, liver and jejunum was determined, including thiobarbituric acid reactive substances (TBARS), free carbonyl, glutathione peroxidase (GPx) as oxidative indicators, as well as fatty acid composition, vitamin A and vitamin E concentration. After the live phase, a total of 32 barrows were selected for pork quality and shelf life evaluation. Subjective and objective quality traits were assessed. Loin chops were collected for drip loss, proximate analyses as well as sensory attributes at 0, 7 and 14 d of aging, that include juiciness, tenderness, off-flavor and oxidized odor. Also, loin chops and ground Boston Butt were evaluated for discoloration percentage and TBARS after 0, 7, 14 and 21 d in the retail display case. Fatty acid, and vitamin A and vitamin E concentration were determined in backfat and belly fat samples. Finally, diaphragm and loin samples were collected and AOX enzyme activity determinations including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were performed. Barrows fed diets formulated with fresh oil had an increased cumulative ADG ($P<0.03$), ADFI ($P<0.04$) after 56 d and increased final weight ($P<0.03$) when compared to animals fed

oxidized oil. An increase ($P<0.06$) in TBARS values, a decrease ($P<0.03$) in GPx enzyme activity and a decrease ($P<0.01$) in Vitamin E concentration were observed in plasma from barrows fed diets formulated with oxidized oil. Also, an increase ($P<0.04$) in free carbonyl was detected in plasma from barrows fed diets formulated with AOX unprotected oil. In regards to pork quality and shelf-life, increased hot carcass weight ($P<0.01$) and carcass yield ($P<0.01$) were observed for pigs fed fresh oil and increased ($P<0.05$) carcass yield was observed for pigs fed AOX protected oil. Increased ($P<0.03$) moisture content was observed in loins from animals fed AOX unprotected oil and increased ($P<0.04$) moisture content and decreased ($P<0.05$) fat content were observed for animals fed oxidized oil. After 14 d of retail display, TBARS values were decreased ($P<0.001$) for loins from animals fed diets containing AOX protected fresh oil, and the decrease ($P<0.001$) continued until after 21 d. In backfat, vitamin A concentration was increased ($P<0.02$) in barrows fed fresh oil, and no oil or AOX effects were found for vitamin E concentration. In belly fat, an increase in vitamin A ($P<0.05$) and vitamin E ($P<0.03$) concentrations were observed for barrows fed AOX protected oil. Finally, SOD activity was increased ($P<0.04$) for diets formulated with AOX. Also, CAT and GPx enzyme activity were increased ($P<0.01$) in diaphragm compared to loin. In conclusion, oxidized oil had a negative impact on animal performance affecting ADG and ADFI resulting in a lighter final weight, also, increasing lipid oxidation and affecting antioxidant systems such as GPx and vitamin E in tissues. Pork quality was not impacted; however, shelf-life was positively affected by AOX protected fresh oil decreasing discoloration and lipid oxidation (TBARS). Finally, CAT and GPx activity were

increased in muscles with higher oxidative metabolism, and SOD enzyme activity was increased in animals fed AOX protected oils.

Dedicated to my Wife Arlette and my Family Hugo, Patricia, Adrian and Aline for supporting me with love on each step of the way

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CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Ethanol production is increasing in the U.S. and globally. The increase in ethanol production is expected to expand cereal grain usage to support this demand. From this process many co-products are derived that are suitable to be used in livestock feeds, due to their nutritional value, such as dry distiller's grains with soluble **(DDGS)** and vegetable oils. As corn is one of the most used cereals in ethanol production, DDGS may contain at least 10% fat as corn oil (Stein, 2007) but previous literature has reported a fat percentage range between 9 to 12% for DDGS (Cromwell et al., 1993). Fatty acid profile of corn oil, as in the rest of vegetable oils, contains high levels of polyunsaturated fatty acids (**PUFA**; NRC, 1998). Therefore, with the inclusion of DDGS from corn, oxidized lipids could be present in swine diets particularly, if feedstuffs are stored for extended periods of time under oxidative conditions, such as high temperature and moisture (Gabriel et al., 1978). Limited information is available about DDGS and corn oil oxidation levels, probably due to seasonal and regional crop variation, and ethanol plant differences. Gabriel et al. (1978) found that distillable fraction from corn and olive oil was susceptible to change its fatty acid profile when oils were thermally oxidized.

Oxidative damage in feedstuffs represents economical loss because of the negative impact on feed quality, resulting form oxidizing of the lipid fraction of ingredients and nutrients. Oxidized feed may negatively affect animal performance, resulting in more feeding days to reach market weight. Finally, all these changes in animal performance could be reflected in body composition, pork quality, and shelf-life

of fresh and ground pork leading to a decrease in overall acceptance of pork from animals fed under these dietary conditions.

The following literature review, will address previous research regarding oxidative stress, natural and synthetic antioxidants, as well as the effect of oxidized supplementary energy sources and antioxidant diet supplementation in animal performance, oxidative status of tissues, pork quality and shelf-life.

OXIDATIVE STRESS

Oxidized supplementary energy sources in feed may be an important source of lipid peroxides. Once the lipid peroxides are in the organism, they could be acting as free radicals. Different mechanisms have been hypothesized regarding the metabolic pathways that oxidized lipids follow once they are ingested. Some studies (Aw and Williams, 1992; Aw et al., 1992) suggested that absorption of lipid peroxides depend on the antioxidant **(AOX)** enzymes activity that metabolize lipid peroxides in mucosa of the digestive tract. The same authors also reported an increase in lipid peroxides when Glutathione **(GSH)** was decreased in liver and kidney. Lipid peroxides are absorbed and peroxides metabolic sub-products are released into lymph associated to lipoproteins (Aw, 2005). Suomela et al. (2005) demonstrated that pigs fed oxidized sunflower oil resulted in oxidized triacylglycerols in chylomicrons and very low density lipoproteins **(VLDL)**.

On the other hand, the antioxidant-oxidant balance will depend on the equilibrium between antioxidant systems and free radicals in tissues. A deficiency in the antioxidants systems or/and an excess of free radicals will produce an imbalance in the antioxidant-oxidant status of tissues producing a state known as oxidative stress (Dröge, 2002). There

are two major types of free radicals: reactive oxygen species **(ROS)** and reactive nitrogen species **(RNS)**. From the ROS, superoxide anion **($O_2^{\cdot}$)** is produced during the mitochondrial electron chain (Figure1.1); whereas, the major RNS free radical is the nitric oxide radical **($NO^{\cdot}$)** which is produced during the oxidation of the L-arginine (Dröge, 2002). Superoxide anion is converted into hydrogen peroxide by the enzyme superoxide dismutase **(SOD)**. Resulting hydrogen peroxide will be converted into water by catalase **(CAT)** or glutathione peroxidase **(GSH-Px)**. Both free radical types (ROS and RNS) have physiological functions under balanced circumstances. Reactive oxygen species radicals participate in control of ventilation, smooth muscle relaxation and oxidative stress responses for homeostasis maintenance; whereas RNS participates in control of vascular tone in smooth muscle (Dröge, 2002). A mechanism of the redox homeostasis is depicted in the Figure 1.2. However, excessive production of ROS has pathophysiological implications, basically related with ageing and carcinogenic processes. Some studies have demonstrated that free radicals affect AOX enzyme activity (Melov et al., 2000) and AOX enzymes gene expression (Vina et al., 2006). Increased SOD expression has been related with increased life span (Honda and Honda, 1999; Ishii, 2000). Also mutagenesis, tumor formation and progression of tumor masses are facilitated by free radicals, especially by ROS (Ha et al., 2000). Tissue specific generation of free radicals and adaptation to them has been recognized, such as in skeletal muscle (Jackson, 1999). Hydroxyl radicals are produced in muscle fibers during contractile activity due to the increase of superoxide and hydrogen peroxide during mitochondrial respiration (O'Neill et al., 1996). Increased amount of hydroxyl radicals in skeletal muscle can favor lipid peroxidation of the lipidic fraction associated with muscle

cells. Lipid oxidation of the lipidic fraction of skeletal muscle as a result of an oxidative stress process can be detected in tissues by Thiobarbituric Acid Reactive Substances **(TBARS)** determination, such as malonaldehyde (**MDA**; Tarladgis et al., 1960).

ANTIOXIDANTS

Due to susceptibility of tissues to oxidative stress, some antioxidants systems are present in living organisms. The most important endogenous AOX systems are SOD, CAT and GSH-Px (Dröge, 2002). Exogenous antioxidants, provided by the diet can be classified as natural or synthetic. Natural antioxidants are those elements provided in the diet that act either as structural part of enzymes (such as selenium as cofactor of GSH-Px) or reacting with free radicals (such as Vitamin E that spares GSH-Px activity; Ullrey, 1981); whereas synthetic antioxidants such as ethoxyquin, tertbutylhidroqinone **(TBHQ)**, butylated hydroxytoluene **(BHT),** and butylated hydroxyanisole **(BHA)** are added to ingredients in the diet that are susceptible to oxidation such as cereal grains, animal fats, vegetable oils, fish oil and meal, and vitamin premixes (Thorisson et al., 1992; De Koning, 2002). Ethoxyquin (Figure 1.3) is an aromatic amine scavenger of radicals produced from lipid oxidation ending the oxidation of unsaturated fatty acids (Berdikova-Bohne et al., 2008). Ethoxyquin not only breaks the oxidation chain in fish meal and oil, also two sub-products from ethoxyquin oxidation have been identified with strong antioxidant activity as radicals acceptors (methyquinoline and a non-fluorescent quinolone) in fish meal and oil (Thorisson et al., 1992; De Koning, 2002).

USE OF ANTIOXIDANTS IN ANIMAL NUTRITION

Some information has been generated regarding the effect of natural or synthetic antioxidants, such as ethoxyquin, in feed and feedstuffs; however, limited information is available on its antioxidant effect in animal performance (Table 1). Due to the high amount of unsaturated fatty acids in fish oil, fish meal is susceptible to lipid oxidation. Therefore, use of synthetic antioxidants such as ethoxyquin, propyl gallate, ascorbyl palmitate and monoglyceride citrate (Spark, 1982; Hawrysh et al., 1992; De Koning, 2002), is a common practice with fish meal (often used in livestock diets as a protein source). Berdikova-Bohne et al., (2008) reported no significant effects of ethoxyquin on animal performance when added to Atlantic salmon at different levels. Wang et al., (1997) evaluated ethoxyquin at 0 or 125 ppm in starter and grower broiler diets when non-oxidized or oxidized fat was included at 3.5% of the diet. In this study, authors reported increased weight gain and feed intake in starter and grower diets when no oxidized fat was included (either with or without ethoxyquin).

Conversely to synthetic antioxidant research, a large amount of information has been generated regarding the effect of natural antioxidant compounds in the oxidative status of several tissues. Vitamin E and selenium (Se) are the most frequently studied. Vitamin E is a well known chain-breaking antioxidant with high tissue activity (Pettigrew and Esnaola, 2001) due to its ability to react with lipid peroxide, sparing GSH-Px enzyme activity (Ullrey, 1981; Figure 1.4). Vitamin E level and its antioxidant activity in tissues have been widely studied in poultry. Dietary vitamin E supplementation increases vitamin E concentration and reduces TBARS production in tissues. Sheehy et al. (1994) found a decrease in α-tocopherol and an increase in TBARS in chicken tissues when

thermally oxidized sunflower oil was fed to chickens. Vitamin E concentration in muscle was increased and a TBARS production decreased in stored meat from animals supplemented with vitamin E when diets were formulated with tallow compared to those fed with vegetable oil (Renerre et al., 1999). No effect of vitamin E on antioxidant enzyme activity was found in the same study. Also, Carreras et al. (2004) reported increased levels of vitamin E in skeletal muscle (being higher in oxidative muscle fiber type, such as leg, than in glycolytic fiber types such as breast); whereas lipid oxidation (TBARS) was decreased in skeletal muscle, when chickens were supplemented with vitamin E. Lauridsen et al. (1999a; 1999b) found that rapeseed oil added at 6% rate of the diet of finishing pigs supplemented with vitamin E increased α-tocopherol concentration in plasma and skeletal muscle, but not in liver; whereas TBARS production was decreased in liver and skeletal muscle, but not in plasma. Increased levels of α-tocopherol has been reported in piglet serum and liver (Ching et al., 2002) as well as skeletal muscle in finishing pigs (Mason et al., 2005) when diets were supplemented with α-tocopherol acetate. Authors also reported that skeletal muscle from pigs supplemented with α-tocopherol had increased oxidative stability. Regarding GSH-Px activity some authors have reported either steady activity (Lauridsen et al., 1999a; 1999b; Carreras et al., 2004) or an increased or decreased enzyme activity (Renerre et al., 1999) when vitamin E was supplemented to poultry and pigs.

Selenium is the other dietary component that has been widely studied due to its importance in selenoenzymes, such as GSH-Px. Selenium is incorporated into the catalytic site of the antioxidant enzyme GSH-Px and seven forms of GSH-Px have been described in humans; however only five of them are selenoenzymes (Papp et al., 2007).

Glutathione peroxidase isoform 4 is ubiquitously expressed with antioxidant activity (Papp et al., 2007). Selenium dependant AOX activity has been widely evaluated in swine nutrition. Increased GSH-Px activity in plasma (Mahan et al., 1999), liver and muscle (Zhan et al., 2007) has been reported when growing-finishing pigs were fed either with an organic or inorganic Se source. Zhan et al. (2007) also reported that pigs supplemented with an organic Se source (selenomethionine) in the diet showed decreased MDA levels in skeletal muscle.

USE OF VEGETABLE OILS IN ANIMAL NUTRITION

Some researchers have investigated the effects of a variety of supplementary energy sources, either vegetable/animal sources or fresh / oxidized sources in livestock diets (Table 2). Finishing pigs fed high levels of oil containing n-3 fatty acids (rapeseed oil at 2%) resulted in increased weight gains, probably due to the unsaturation level of fatty acids in these ingredients (Leskanich et al., 1997). However, Lauridsen et al., (1999a) did not find differences in animal performance (during the growing-finishing period) at a higher rapeseed oil inclusion (6% of the diet). Also animal performance has been evaluated when hydrogenated oils have been included (Gläser et al., 2002) with no differences observed in weight gain, feed intake or feed conversion. These results were also corroborated by Averette-Gatlin et al. (2003), when choice white grease (CWG) partially hydrogenated was included at a rate of 5% of the diet and no differences by treatment effect were found in finishing pigs.

Regarding carcass characteristics and meat quality evaluation, effect of vegetables oils has been widely evaluated in swine. Finishing pigs fed diets with high content of n-3

fatty acids (rapeseed oil) did not demonstrate differences when subjective evaluation of the Longissimus dorsi muscle was performed (Leskanich et al., 1997). However, in the same study when shoulder was subjectively evaluated for firmness, shoulders from animals fed with rapeseed oil were less firm than those fed with the control diet (tallow as a supplementary energy source). The decrease in fat firmness is associated with an increase in PUFA (α-linolenic acid) deposition in carcass (Leskanich et al., 1997). A change in fatty acid profile in fat associated with different muscle masses was demonstrated by Rhee et al. (1988) when finishing pigs were fed with canola oil at 0, 10% and 20% of the diet. Authors reported an increase of PUFA in muscle and a decrease in total saturated fatty acids. Also, PUFA deposition was increased when rapeseed oil was included at a higher rate (6%) as it was demonstrated by Lauridsen et al. (1999b). High content of trans fatty acids that are rich in monounsaturated fatty acids (MUFA; such as oleic acid) from partially hydrogenated rapeseed oil fed to finishing pigs resulted in an increased trans fatty acids deposition in backfat (Gläser et al., 2002) and a decreased PUFA in loin fat (Averette-Gatlin et al., 2003). This increase in MUFA deposited in carcass fat resulted in an increased fat firmness (Gläser et al., 2002; Averette-Gatlin et al., 2003).

Due to its high content in PUFA, vegetable oils are highly susceptible to oxidation (NRC, 1998). Supplementary energy sources such as vegetable oils (Engberg et al., 1996; Nwanguma et al., 1999) and CWG have demonstrated susceptibility to controlled thermal oxidation due to their high content of unsaturated fatty acids (Dibner et al., 1996; De Rouchey et al., 2004). Sheehy et al., (1994) evaluated the effect of oxidized sunflower oil inclusion at 4% of poultry diets and authors did not find any impairment in animal

performance. Engberg et al. (1996) also demonstrated a slight negative effect in average daily weight gain in broilers when oxidized rapeseed (at 9%) and soybean oil (at 2%) was oxidized up to 156 meq/kg of peroxide values. Nwanguma et al. (1999) also reported decreased average daily weight gain and body weight when rats were fed corn oil that was thermally oxidized. Oxidized and rancid CWG fed at 6% of the diet resulted in a negative effect on feed intake, affecting weight gain, when peroxide values reached 40meq/kg of fat after 5 days of thermal oxidation (De Rouchey et al., 2004). Also impaired animal performance was observed when poultry fat was added at 1.9% in broiler diets and 4.2 meq/kg of peroxide value in the final diet (Dibner et al., 1996).

Though animal performance has been widely studied when animals were fed oxidized vegetable oil, scarce information regarding the effects of oxidized animal fat or vegetable oil in livestock feed on meat quality and shelf life has been generated. From previous research it is well known that fatty acid profile of the carcass changes when vegetable oil is added to non-ruminant diets (Wood et al., 2008). Early studies demonstrated that thermal oxidation changes the fatty acid profile of the distillable fraction of oxidized corn and olive oil (Gabriel et al., 1978). Thus, if dietary oxidized fat or synthetic dietary AOX is consumed by animals then it is possible that animal performance and oxidative status of tissues may be affected.

FATTY ACID COMPOSITION AND INFLUENCE ON LIPID AND PROTEIN OXIDATION

Lipid deposition in finishing pigs has been described previously and anatomical fatty acid partitioning evaluated. Kloareg et al. (2006) reported that fat deposition in the

backfat compartment is late when compared to fat deposited in the visceral compartment or carcass when lipid partitioning was evaluated in pigs from 90 to 150 kg of bodyweight. Fatty acid profile differences in adipose tissue and muscle by specie were described by Enser et al., (1996) who observed that the main difference is that linoleic acid (18:2n – 6) is higher in non-ruminant (pigs) when compared to ruminant (sheep and beef) adipose tissue and muscle. Also, Kloareg et al. (2006) observed that fatty acid in backfat was higher in 18:2 and 18:3; whereas fatty acid profile of pig carcass was higher in 18:1, and reduced deposition in 18:2.

Fatty acid profile in non-ruminant tissues is susceptible to manipulation via dietary supplementary energy sources (Nguyen et al., 2003; Wood et al., 2008). Leick (2008) reported an increase of 18:2 cis and total PUFA in jowl and backfat as DDGS diet inclusion rate increased form 0 to 60%. Increased PUFA concentration in diets will increase oxidation susceptibility leading not only to lipid oxidation, but also to protein oxidation in tissues (Faustman and Cassens, 1990; Brewer, 2004). Lipid oxidation reduces shelf-life due to production of undesirable rancid flavors and odors (Jensen et al., 1998). Oxidation can also lead to discoloration in meat. Meat color is driven by the myoglobin state. Oxymyoglobin renders meat an attractive red color. However, oxymyoglobin is oxidized to metmyoglobin producing a brownish color (discoloration) negatively affecting consumer acceptance. Furthermore, both lipid and protein oxidation have been positively correlated, thus the oxidation of lipid fraction could lead to the oxidation of the protein fraction (Faustman and Cassens, 1990). Thus, if dietary oxidized fat or synthetic dietary AOX is deposited in adipose tissue associated with muscle then it is possible that discoloration and lipid oxidation of meat could be affected overtime.

MUSCLE FIBER TYPES

Muscle fiber types can be delineated by myosin heavy chain (MHC) or enzymatic differences (Pearson and Young, 1989; Gondret et al., 2005). These histochemical characteristics are defined by different myosin heavy chain (MyHC) gene isoforms, corresponding to slow twitch I, and fast twitch IIA, IIX (this fiber has been included recently to the classification as fast twitch intermediate) and IIB, which are identified using heterologous monoclonal antibodies against each isoform (immunochemistry; Schiaffino and Reggiani, 1994; Lebret et al. 1999; Chang et al. 2003). Another characteristic that makes distinctive skeletal muscle fibers is the type of energy used as a substrate. Oxidative fibers use fatty acids as the main source of energy (aerobic); whereas glycolytic fibers (anaerobic) use carbohydrates (glucose/glycogen) to produce energy (Gondret et al. 2005). Jackman and Willis (1996) reported that mitochondria content was increased, and citrate cycle and electron transfer chain enzymes were higher in oxidative muscle fibers types when compared glycolytic muscle fibers, resulting in a higher fat oxidation as an energy source for the oxidative muscle fibers. Also, type II fibers have increased glycolytic enzymes such as lactate dehydrogenase indicating the major use of glucose/glycogen as an energy source (Lefaucher et al. 1989).

Lipid oxidation has been evaluated previously in lipidic fraction associated with skeletal muscle. Production of TBARS could depend on the type of energy source fed (saturated or unsaturated fatty acids) that is going to be deposited in fat associated with muscle, lipid percentage of muscle, muscle fiber type metabolism (type I or oxidative, and type II or glycolytic) and oxidative stress imposed to the muscle (Renerre et al.,

1999; Vina et al., 2006). Thus, if dietary oxidized fat or synthetic dietary AOX is deposited in adipose tissue associated with different muscle types then it is possible that AOX enzymes activities varies according to muscle metabolism.

Thus, we hypothesize that oxidized corn oil fed to pigs during the finishing period not only will negatively impact animal performance and tissue oxidative status; but will also accelerate lipid oxidation in the lipid fraction of meat due to the highly unsaturated fatty acid profile of corn oil and the presence of lipid peroxides originated from the oxidized diet. This accelerated lipid oxidation in pork will be detrimental to meat quality. Also, we hypothesize that AOX protected corn oil fed to pigs during the finishing period not only will positively impact animal performance and tissue oxidative status; but will also protect against lipid oxidation in the lipid fraction of meat.

OBJECTIVES OF THE STUDY

I.- Evaluate animal performance and oxidative status of finishing pigs fed oxidized corn oil and a synthetic antioxidant blend.

II.- Evaluate pork quality and shelf-life (lipid oxidation) of finishing pigs fed oxidized corn oil and a synthetic antioxidant blend.

III.- Evaluate antioxidant enzyme activity in oxidative and glycolytic muscle types of finishing pigs fed oxidized corn oil and a synthetic antioxidant blend.

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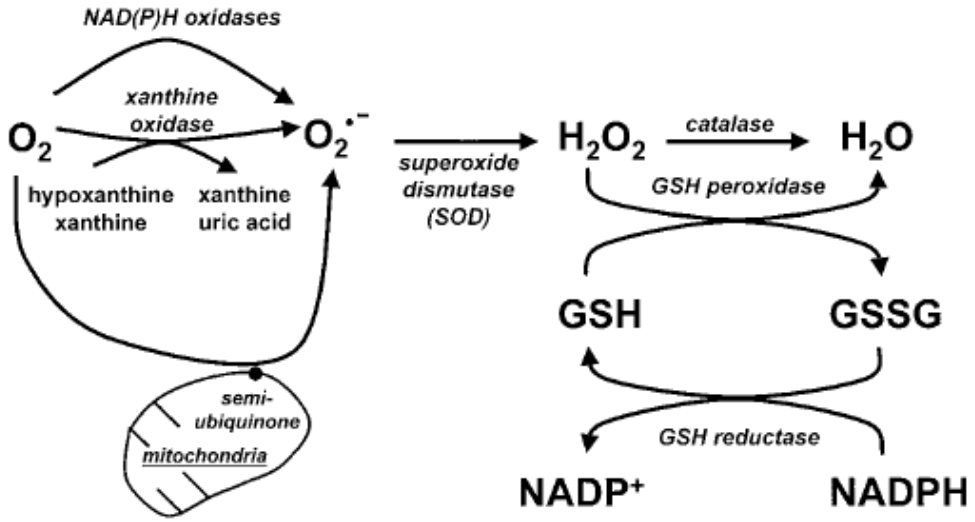
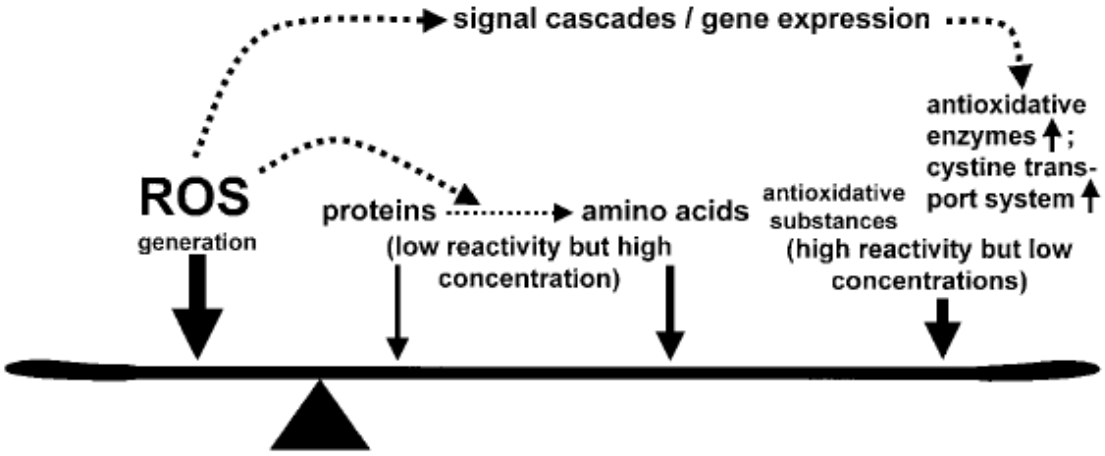
FIGURES AND TABLES

Figure 1.1. Pathways of ROS production and clearance



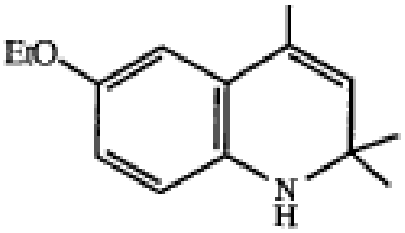
Dröge, 2002

Figure 1.2. Mechanisms of redox homeostasis. Balance between ROS production and various types of scavengers



Dröge, 2002

Figure 1.3. Ethoxyquin structure



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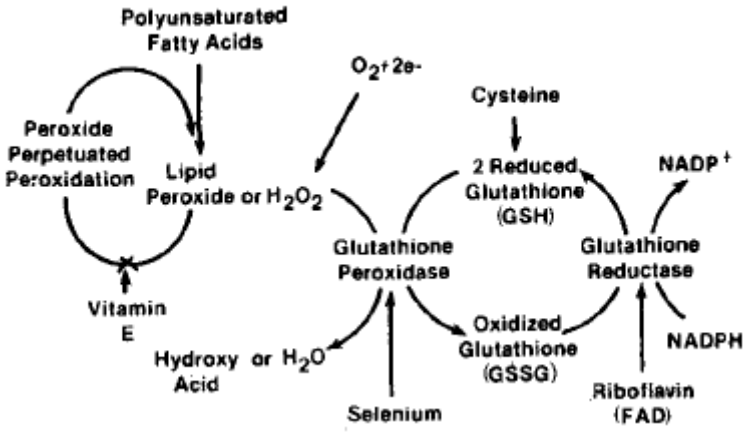
Thorisson et al., 1992

Figure 1.4. Interrelationship between vitamin E and selenium



Ullrey, 1981

Table 1.1. Literature review on the effect of antioxidant to livestock diet supplementation in performance and tissue oxidative status

Citation	Species	Fat source	Antioxidant	Results
Iritani et al., 1980	Rats	Corn oil	α-tocopherol at 80 ppm	Animal performance not evaluated; Increased TBARS[a] in rat metabolism
Sheehy et al., 1994	Chickens	Sunflower oil	α-tocopherol	Increased final weight and decreased TBARS in tissues.
Dibner et al., 1996	Chickens and pigs	Poultry fat	Ethoxyquin at 125 ppm	Decreased feed conversion
Wang et al., 1997	Chickens	Fat	Ethoxyquin at 125 ppm	Increased weight gain and feed efficiency when fat was fresh
Renerre et al., 1999	Turkeys	Tallow, rapeseed or soybean oil	α-tocopherol at 200 ppm	No effect in antioxidant enzymes activity in skeletal muscle
Lauridsen et al., 1999(a)	Pigs	Rapeseed oil	α-tocopherol at 100 or 200 ppm	Increased vitamin E concentration in tissues
Lauridsen et al., 1999(b)	Pigs	Rapeseed oil	α-tocopherol at 100 or 200 ppm	Increased vitamin E concentration and decreased TBARS in skeletal muscle
Carreras et al., 2004	Chickens	Lard	α-tocopherol at 100 ppm Enrofloxacin at 50 mg/L	Increased SOD[b] activity and vitamin E concentration and decreased TBARS in skeletal muscle
Fernández Dueñas et al., 2008	Piglets	Canola oil and tallow	Vitamin C at 150 ppm β-carotene at 350 ppm	No effects in animal performance or oxidative status of plasma

[a] TBARS = Thiobarbituric acid reactive substances; [b] SOD = superoxide dismutase

Table 1.2. Literature review on the effect of oxidized energy sources fed to livestock in performance and tissue oxidative status

Citation	Specie	Fat source	Oxidation level	Results
Carpenter and L'Estrange, 1966	Rats and Chickens	Tallow	Peroxide value 100 µmoles / g tallow	No effect in animal performance
Gabriel and Alexander, 1977	Rats	Corn and olive oil	Carbonyl value 170- 190 meq/kg of oil	No effect in animal performance
Iritani et al., 1980	Rats	Corn oil	Peroxide value Up to 14.5 meq / kg of diet	Animal performance not evaluated; Increased TBARS[a] in rat metabolism
Sheehy et al., 1994	Chickens	Sunflower oil	Peroxide value Up to 66.3 meq / kg of oil	Decreased final weight and increased TBARS in tissues
Dibner et al., 1996	Chickens and pigs	Poultry fat	Peroxide value 212.5 meq / kg of fat	Decreased final weight and increased feed conversion.
Engberg et al., 1996	Chickens	Rapeseed-soybean oil	Peroxide value 156 meq / kg of oil	Decreased weight gain and feed intake
Wang et al., 1997	Chickens	Fat	Peroxide value 39.4 meq / kg of diet	Decreased weight gain and feed intake
Nwanguma et al., 1999	Rats	Corn oil	Oxodiene value of oil 3.95	Decreased final weight, weight gain and feed intake; Increased MDA[b] in tissues
DeRouchey et al., 2004	Piglets	Choice White Grease	Peroxide value Up to 105 meq / kg of fat	Decreased weight gain and feed intake
Suomela et al., 2005	Pigs	Sunflower oil	Peroxide value 190 meq / kg of oil	Increased oxidized chylomicrons and LDL[c] in plasma
Fernández Dueñas et al., 2008	Piglets	Canola oil and tallow	Peroxide value Canola oil, 7.98 meq / kg Tallow, 17.21 meq / kg	No effects in animal performance or oxidative status

[a] TBARS = Thiobarbituric acid reactive substances; [b] MDA = Malonaldehyde; [c] LDL = Low density lipoprotein

CHAPTER II

EFFECTS OF OXIDIZED CORN OIL AND SYNTHETIC ANTIOXIDANT BLEND ON ANIMAL PERFORMANCE AND OXIDATIVE STATUS OF TISSUES OF FINISHING PIGS

ABSTRACT

The objective of the study was to evaluate the effect of protected or unprotected fresh or oxidized corn oil on animal performance and oxidative status of pigs. The experimental design was a complete randomized block design (CRBD) in a factorial arrangement (2×2), with 2 levels of corn oil (fresh or oxidized corn oil) and 2 levels of antioxidant (AOX was provided by NOVUS Inc., St. Charles, MO, USA; with or without AOX). A total of 160 barrows were fed for 56 days one of the following experimental diets: fresh oil, fresh oil + AOX, oxidized oil, or oxidized oil + AOX. Peroxide values of fresh and oxidized oil were determined and proximate analyses and fatty acid profile of the feed was determined. Average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F) were calculated. Blood, liver and jejunum samples were collected to determine the oxidative status of these tissues, including thiobarbituric acid reactive substances (TBARS), free carbonyl, glutathione peroxidase (GPx) and protein, as well as fatty acid composition. Finally, vitamin A and vitamin E concentration in tissues were determined. Peroxide level of the fresh oil was 1 mEq/kg; whereas peroxide value of the oxidized oil was 182.3±5.32 mEq/kg. Oxidative stability was increased in diets formulated with AOX compared with diets formulated with unprotected oil. Barrows fed diets formulated with fresh oil had an increased accumulated ADG ($P<0.03$), ADFI ($P<0.04$) after 56 d and increased final weight ($P<0.03$). Increased ($P<0.07$) G:F

was observed for barrows fed diets with AOX after 28 d of feeding, without any effect on G:F after 56 d. Animals fed AOX protected diets also showed increased ADG ($P<0.001$) at week 3, ADFI ($P<0.05$) at week 3 and 4, and G:F ($P<0.001$) at week 3. An increase ($P<0.06$) in TBARS values, a decrease ($P<0.03$) in GPx enzyme activity and a decrease ($P<0.01$) in Vitamin E concentrations were observed in plasma from barrows fed diets with oxidized oil. An increase ($P<0.04$) in free carbonyl was detected in plasma from barrows fed diets formulated with AOX unprotected oil. In conclusion, oxidized oil had a negative impact on animal performance affecting ADG and ADFI resulting in a lighter final weight. Also, oxidized oil increased lipid oxidation and affected antioxidant systems such as GPx and vitamin E in tissues.

INTRODUCTION

Traditional finishing pig diets are corn-soybean meal based. However, as corn became the main source for ethanol production, the use of several co-products of ethanol production such as dry distiller's grains with solubles (DDGS) as feedstuff for livestock feed increased (Cromwell et al., 1993; Lumpkins et al., 2004; Stein, 2007). Increased DDGS in finishing diets results in increased inclusion of corn oil in the diet. Therefore, the level of polyunsaturated fatty acid as main component of dietary fat that will be deposited in pig carcass is increased (Leick, 2008).

Animal fats and vegetable oils are used as supplementary energy sources (Carpenter and L'Estrage, 1966). However, vegetables oils, such as corn, canola and sunflower oil, are highly susceptible to lipid oxidation, developing increased peroxide values not only in oil and fats used for feed formulation, but also in the final diet

(Carpenter and L'Estrage, 1966; NRC, 1998). Several authors have reported a negative impact on animal performance when increased levels of oxidized oil or fats were fed to rats, broilers and pigs (Sheehy et al., 1994; Engberg et al., 1996; Dibner et al., 1996; Wang et al., 1997; Nwanguma et al., 1999; DeRouchey et al., 2004)**.** Intake of oxidized lipids from the diet results in lipid hydroperoxides absorption from the intestinal lumen, and therefore the presence of lipids hydroperoxides in plasma and lymph (Aw et al., 1992; Ursini and Sevanian, 2002). Also, low density lipoproteins (LDL) are more susceptible to oxidation when oxidized lipids are present in the diets (Ursini and Sevanian, 2002; Aw, 2005). Oxidation in the lipid fraction of tissues is often detected by thiobarbituric reactive substances (TBARS), whereas protein oxidation is detected by carbonyl formation (Rimbach et al., 1999).

To protect against oxidation, synthetic antioxidants (AOX), such as ethoxyquin, terbutyl-hydroquinone (TBHQ), butylated-hydroxyanisole (BHA) and butylated-hydroxytoluene (BHT) are added in feed formulations. Ethoxyquin is used to protect feed ingredients, such as cereals, animal fats, vegetable oils, fish meal and oil, and lipid soluble vitamins that are susceptible to oxidation (Thorisson et al., 1992; Dibner et al., 1996; Wang et al, 1997; De Koning, 2002). Also, vitamin E is a well known chain-breaking AOX with high tissue activity (Pettigrew and Esnaola, 2001) due to its ability to react with lipid peroxides. Finally, tissues have enzymes with AOX activity, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Antioxidant enzymes metabolize free radicals generated during several metabolic processes (Dröge, 2002). If any AOX system is depleted and oxidized molecules in the diet are increased, the presence of oxidized molecules in tissues could be possible.

As changes in feed formulations have increased the risk of lipid oxidation in finishing diets, the objective of the study was to evaluate the effect of either protected or unprotected fresh or oxidized corn oil on animal performance and oxidative status of finishing pigs.

MATERIAL AND METHODS

Animals and Experimental Design

Animal use for this study was approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC) on April 2008, and the approval number was 08081.

A total of 160 barrows (Sire PIC 337 × Dam PIC C22) were fed a traditional corn-soybean meal diet mixed with corn oil at 5% inclusion, during the last 8 weeks of the finishing period. Experiment was carried-out as a complete randomized block design (CRBD) in a factorial arrangement (2×2), with 2 levels of corn oil (fresh or oxidized corn oil) and 2 levels of antioxidant (AOX; with or without AOX). Antioxidant (SANTOQUIN® Q, to provide 10 ppm Terbutyl-hydroquinone (TBHQ) and 132 ppm of Ethoxyquin (EQ) in the final diets) was provided by NOVUS International Inc. (St. Charles, MO, USA). The four experimental diets (Treatments) are described as follows: Treatment 1) Basal diet (Control) containing fresh corn oil; Treatment 2) Same as 1, but + AOX in the diet; Treatment 3) Basal diet mixed with oxidized corn oil; and Treatment 4) Same as 3, but + AOX in the diet.

Oil Oxidation and Experimental Diets

Oxidation of corn oil was achieved by continuously bubbling air at a rate of 80 L/min (liters per minute) and heating at 95 C° for 48 hours. Peroxide values were determined on a daily basis as described by AOCS (American Oil Chemist Society – Peroxide value Acetic acid- chloroform method, Cd853, 2007). Corn oil was oxidized to a target peroxide value of 300 mEq/kg of oil; thereafter oxidized oil was diluted with fresh oil to reach a target peroxide value of 180 mEq/kg oil or a final feed peroxide value of 9 mEq/kg feed. Further lipid peroxidation level was monitored by oxidative stability of feed. Oxidation of the corn oil was performed at NOVUS Inc. facilities. Control and treatment oils were stored under refrigeration (4C°) to reduce the development of further oxidation. A sample of AOX was retained for oxidative stability analysis, and EQ and TBHQ content were determined.

Experimental diets were mixed on a weekly basis, and composite feed samples were collected and stored at -10C° for further oxidative stability analysis. Prior to mixing, AOX was incorporated in fresh or oxidized corn oil for treatments 2 and 4. Thereafter, 5% of the AOX protected or unprotected corn oil was mixed with the basal diet for the final experimental diets. Basal diet contained 12.4% CP and 3.58 Mcal ME for phase 1; whereas phase 2 contained 16.7% CP and 3.58 Mcal ME. Corn oil was incorporated at 5% rate of the diet; whereas total crude fat of the final diet was 8% in both dietary phases. Basal diet (Table 2.1) was formulated to meet the nutritional requirements for finishing pigs according to the NRC (1998) and lysine was corrected to support the use of Ractopamine (Paylean™) at 4.5g/ton (5 ppm) during the last 28 days of the finishing

period. Vitamin and mineral premixes were added to meet the NRC (1998) requirements for finishing pigs.

Animal Performance

Pigs were housed at the Swine Research Center, University of Illinois at Urbana-Champaign. A total of 160 barrows were penned in groups of 5 to evaluate animal performance. Pens allowed 10.2 ft^2 per head with one feeder and two nipple waterers per pen. Feed and water flow as well as ventilation were checked on a daily basis. Initial body weight for the experiment was 80.2±2.36 kg, and barrows were fed for 28 days with phase 1 experimental diets and the last 28 days with phase 2 experimental diets. Barrows were weighed at the beginning of the trial and on a weekly basis up to the end of the experiment (day 56), and average daily weight gain (ADG) were calculated. Feeders were weighed on a weekly basis (same day as animal weight) and average daily feed intake (ADFI) was calculated as leftover feed subtracted from feed offered. Finally, feed efficiency (G:F) was expressed as ADG divided by ADFI on a weekly basis.

Sample Collection

After the live phase, one pig with the final weight closest to the pen mean was selected from each pen for evaluation (total of 32 barrows) and barrows were transported to the Meat Science Lab at the University of Illinois at Urbana-Champaign for humane harvest after 16 hr of lairage under USDA inspection. Blood was collected after electrical stunning, and jejunum and liver samples were collected after evisceration to perform oxidative status.

Approximately 10 ml of blood were collected in heparinized tubes and placed on ice. Thereafter, blood was centrifuged for 15 minutes at 2500 rcf at 4 C°, and plasma was

separated and frozen at -80 C° for thiobarbituric acid reactive substances (TBARS) determination. At 30 min postmortem, four liver (right lobe) sections of approximately 50g each were collected from each animal, and immediately frozen in liquid nitrogen and stored at -80C° for glutathione peroxidase activity (GPx), vitamin A and vitamin E determination. From each animal, three jejunum sections of approximately 5cm were collected, and immediately frozen in liquid nitrogen and stored at -80C° for TBARS, GPx, free carbonyl, vitamin A and vitamin E determination.

Plasma, jejunum and liver frozen samples were sent to NOVUS Inc. facilities (St. Charles, MO, USA) for laboratory analyses.

Tissue Oxidative Status

Thiobarbituric acid reactive substances were determined using a TBARS assay kit from Cayman Chemical Company (Ann Arbor, MI, USA). Briefly, malonaldehyde (MDA) reacts with thiobarbituric acid (TBA) forming the MDA-TBA product in acidic conditions and high temperatures (90-100C°) and measured colorimetrically at 540 nm. Sample malonaldehyde concentration was compared to a MDA standard curve.

Free carbonyl was determined using an Oxi-Select protein carbonyl ELISA kit from Cell Biolabs Inc. (San Diego, CA, USA). Briefly, carbonyls were measured by an enzyme immunoassay to detect dinitrophenyl (DNP) hydrazone with an anti-DNP antibody. Detection was measured colorimetrically at 450 nm and compared to a standard curve from predetermined reduced and oxidized bovine serum albumin (BSA).

Glutathione peroxidase activity was determined by GPx assay kit from Cayman Chemical Company. Briefly, indirect GPx activity was measured by a coupled reaction with glutathione reductase (GR). Reaction was initiated after cumene hydroperoxide

addition. Oxidation of NADPH to NADP was measured colorimetrically at 340 nm for at least 5 m; GPx activity was expressed as nmol/min/mg of protein and compared to a bovine erythrocyte GPx standard curve over time.

Protein concentration was determined using a Protein Assay kit from Bio-Rad laboratories (Hercules, CA, USA). Briefly, protein determination was measured colorimetrically at 595 nm, when Coomassie blue dye bound to basic and aromatic amino acid residues. Protein concentration was expressed as mg/ml and compared to a predetermined BSA concentration standard curve.

Vitamin A and vitamin E in plasma and liver were determined by high pressure liquid chromatography following the procedures described by Catignani (1985). And fatty acid profile in liver was determined by gas chromatography following the procedures described by Engle et al. (2000).

Statistical Analysis

Animal performance and laboratory analyses were analyzed as a CRBD in a factorial arrangement (2×2) with 2 levels of corn oil (5% of fresh or oxidized corn oil) and 2 levels of antioxidant (with or without AOX). Starting day of the experiment (4 starting days) was used as blocking criteria. Experimental unit for the live phase was the pen with a total of 8 replicates (pens) per treatment. Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included block, main effect of oil, and main effect of AOX, as well as interaction of corn oil × AOX. Least squares differences were separated by probability of difference option (PDIFF) and P values were adjusted by the Bonferroni approach to declare statistical difference at $P<0.05$.

RESULTS AND DISCUSSION

Animal Performance

Peroxide values for fresh corn oil were 1 mEq/kg. Peroxide values for oxidized oil were 316.3±2.22 mEq/kg of corn oil; thereafter the oxidized corn oil was diluted with fresh corn oil to reach a target peroxide value of 182.3±5.32 mEq/kg of corn oil. Several authors have reported a wide range of peroxide values either for supplementary energy sources (from 7.98 to 212.5 meq / kg energy source) or final diet (14.5 to 39.4 meq / kg diet) (Iritani et al., 1980; Dibner et al., 1996; Engberg et al, 1996; Wang et al., 1997; Nwanguma et al., 1999; DeRouchey et al., 2004; Suomela et al., 2005; Fernández-Dueñas et al., 2008). Oxidative stability of four experimental diets was tested by an oxygen consumption test at 0, 2 and 8 weeks. Diets formulated with AOX protected fresh and oxidized oil consumed less O_2 than diets formulated with unprotected fresh and oxidized oil, resulting in increased feed shelf-life (data not shown). Results were likely due to AOX addition to the oil, preventing further oxidation of PUFA and other elements in the diet with high susceptibility to oxidation (De Koning, 2002). Dibner et al. (1996) observed that oil not protected with AOX showed increased deterioration of PUFA and lipid soluble vitamins.

Initial body weight was not different between treatments (80.2±2.36 kg). At the end of the experiment, pigs that were fed oxidized oil (with or without AOX) were lighter ($P<0.03$) than pigs fed fresh oil. Other authors have reported similar differences in final weights when chickens were fed heated sunflower oil (Sheehy et al., 1994), oxidized rapeseed-soybean oil (Engberg et al., 1996) or even oxidized poultry fat (Dibner et al., 1996). Some other authors have reported no differences in final weight of finishing pigs

after feeding oxidized vegetable oils at diet inclusion rate from 2 to 6% (Leskanich et al., 1997; Gläser et al., 2002; Teye et al., 2006; Mitchaothai et al., 2007). Animals fed diets formulated with fresh oil had an increased ($P<0.04$) accumulated ADG after 14d (data not shown), which was maintained until 56 d ($P<0.03$) of feeding. Animals fed fresh oil had increased ($P<0.04$) ADFI after 56d of feeding the experimental diets when compared to animals fed oxidized oil (Table 2.2). Animals fed diets formulated with AOX protected oil had an increased G:F up to 28d ($P<0.07$); however, this G:F was not different between pigs fed diets formulated with or without AOX protected oil throughout the experiment (Table 2.2). Some authors (Dibner et al., 1996; Wang et al., 1997; DeRouchey et al., 2004) have reported a negative impact on ADG, ADFI, and G:F when controlled oxidized fat was fed to chickens and pigs. However, some authors reported no differences in ADG, ADFI and G:F when diets were formulated with oxidized oil or fats in rats, chickens and piglets (Carpenter and L'Estrange., 1966; Sheehy et al., 1994; Nwanguma et al., 1999; Fernández-Dueñas et al., 2008). Lack of negative effects on animal performance might be due to low peroxide values in the oil/fat or final feed. Our results for ADFI are in agreement with DeRouchey et al. (2004) who observed a decrease in ADFI after feeding oxidized choice white grease to nursery pigs for 35 d. This decrease in ADFI may be due to rancid aroma developed by fat or oils oxidized above 40 meq / kg (DeRouchey et al., 2004). No oil × AOX interactions were found for accumulated ADG, ADFI and G:F (Table 2.3). When data was analyzed on a weekly basis, increased ADG ($P<0.001$) at week 3, ADFI ($P<0.05$) at week 3 and 4, and G:F ($P<0.001$) at week 3 for animals fed AOX protected diets was observed (data not shown); however effects were not present for the second dietary phase when weekly intervals response was evaluated.

Tissue Oxidative Status

Barrows fed oxidized oil showed increased ($P<0.06$) plasma TBARS values, decreased ($P<0.03$) plasma GPx enzyme activity and decreased ($P<0.01$) plasma vitamin E concentration. Some authors (Sheehy et al., 1994; Engberg et al. 1996) observed increased plasma TBARS from chickens fed oxidized oil when compared to chickens fed fresh oil. However, no changes in plasma TBARS values were reported either when oxidized canola oil (7.98 mEq/kg of canola oil) was fed (Fernández-Dueñas et al., 2008) or vitamin E and copper were supplemented (Lauridsen et al., 1999) in pig diets. Probably, this lack of altered TBARS, as reported in earlier studies, may be due to insufficient dietary oxidative challenge. Regarding GPx activity in chickens, some authors reported no effect of oxidized oil or fat in plasma (Engberg et al., 1996) or liver GPx (Wang et al., 1997). Similarly, no change in GPx activity in plasma was reported either when oxidized canola oil was fed (Fernández-Dueñas et al., 2008) or in liver when vitamin E and copper were supplemented in pig diets (Lauridsen et al., 1999). It is possible that luminal and mucosal GPx activity was sufficient to eliminate lipid peroxides from dietary sources in the earlier studies, but not in our experiment. In regards of plasma vitamin E concentration, some other authors reported similar concentrations to those observed in our experiment when chickens were fed oxidized rapeseed-soybean (Engberg et al., 1996) or sunflower oil (Sheehy et al., 1994). This decreased plasma vitamin E concentration in animals may be due to the susceptibility of the natural AOX to be oxidized when interacting with oxidized dietary ingredients. Also, increased ($P<0.04$) free carbonyl was detected in plasma from barrows fed without AOX oil (Table 2.4). Some other studies (Şahin and Gümüşlü., 2004; Larraín et al., 2007) have reported

increased values of protein carbonyl, as the main oxidation marker for proteins modifications, under different oxidative stress conditions. Presence of AOX in the diets could help to reduce oxidation of proteins (carbonyls) in plasma. Finally, an oil × AOX interaction was observed where plasma TBARS from animals fed fresh oil without AOX was increased ($P<0.08$) when compared with animals fed fresh oil with AOX; whereas TBARS in plasma from animals fed oxidized oil with or without AOX were not different (Table 2.5). Wang et al. (1997) reported decreased plasma TBARS of chickens fed fresh oil with synthetic AOX compared to chickens fed fresh oil without AOX, but these TBARS values were not statistically different. Likewise, some other authors (Sheehy et al., 1994; Lauridsen et al., 1999) reported no differences in plasma TBARS for chickens and pigs fed fresh or oxidized oil with or without natural AOX supplementation. Our results suggest that synthetic AOX may be more effective at preventing TBARS development in plasma than natural AOX which are more susceptible to oxidation.

Regarding fatty acid profile, an increase ($P<0.002$) in 14:0 percentage in liver from barrows fed oil without AOX was observed when compared to liver from barrows fed oil with AOX (Table 2.6); however, the magnitude of this change was too small. Enser et al. (2000) reported no differences in 14:0 in liver when increased PUFA diets were fed to finishing pigs; whereas, Soler-Velasquez et al. (1998) reported no differences in liver fatty acid profile when pigs were fed increasing canola oil (up to 10 % of inclusion) and diets supplemented with vitamin E in diets. Finally, an oil × AOX interaction was observed where 20:1 percentage in liver from animals fed fresh oil without AOX was increased ($P<0.03$) when compared with animals fed fresh oil with AOX; whereas 20:1 percentage in liver from animals fed oxidized oil with or without

AOX were not different. Also, 20:5 percentage in liver from animals fed oxidized oil without AOX was decreased ($P<0.05$) when compared with animals fed oxidized oil with AOX; whereas 20:5 percentage in liver from animals fed fresh oil with or without AOX were not different (Table 2.7). Soler-Velasquez et al. (1998) reported a linear increase of 20:1 and a linear decrease of 20:4 in liver when pigs were fed increasing canola oil in diets (up to 10 % of inclusion) similar to what we observed in our experiment. However, Enser et al. (2000) reported no differences for 20:1 in liver when increased PUFA diets were fed to finishing pigs. In regards to 20:5 in livers, Enser et al. (2000) reported this in finishing animals fed diets with increased levels of PUFA. However, other authors reported no differences in liver 20:5 when animals were fed oxidized sunflower oil (Sheehy et al., 1994) or with diets supplemented with natural AOX (Lauridsen et al., 1999).Our results suggest that some PUFA such as 20:5 were preserved in livers with AOX, while in livers without AOX, 20:5 were modified.

CONCLUSIONS

In conclusion, oxidized corn oil fed to finishing barrows affected animal performance, decreased ADFI, thus ADG was also negatively impacted after 56 days. This resulted in lighter final weight when compared to animals fed diets formulated with high quality oil (fresh corn oil). No AOX effects were detected for animal performance of finishing pigs. Poor quality diets containing oxidized oils, also favored the presence of lipid oxidation in tissues, affecting oxidant-antioxidant balance in the tissues. Antioxidant systems such as GPx, and natural AOX compounds such as vitamin E were decreased in some tissues in response to the dietary oxidative challenge.

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TABLES

Table 2.1. Experimental diet composition and calculated analysis

Ingredient, %	Day 0-28	Day 29-56
Yellow Corn, grain	81.550	70.955
Soybean meal, CP 48%	10.920	21.450
Corn oil	5.000	5.000
Dical phosphate	0.910	0.930
Limestone	0.620	0.540
Salt	0.250	0.250
L-Lysine, HCl	0.250	0.270
MHA [a]	0.000	0.060
L-Threonine	0.050	0.070
Vitamin Premix [b]	0.100	0.100
Mineral Premix [c]	0.300	0.300
Paylean™ [d]	0.000	0.025
Mintrex® [e]	0.050	0.050
Calculated Analysis		
Dry matter, %	87.02	86.9
Metabolizable Energy, Mcal/kg	3.58	3.58
Crude protein, %	12.4	16.7
Crude fat, %	8.3	8.1
Total lysine, %	0.75	1.06

[a] Methionine Hydroxyanalogue

[b] Each kg of vitamin premix provided: Vitamin A, 3,300,000 IU; Vitamin D_3, 330,000 IU; Vitamin E, 44,000; Vitamin K, 2,200mg; Vitamin B_{12}, 17.6mg; Riboflavin, 4,400g; d-Pantothenic acid, 12,100mg; Niacin, 16,500mg; Choline, 143,000mg.

[c] Each kg of mineral premix provided: Salt, 2,574g; Iron, 82g; Zinc, 91g; Manganese, 5,710ppm; Copper, 2,290ppm; Iodine, 100ppm; Selenium, 85.7ppm.
[d] Equivalent to 5ppm of Ractopamine
[e] Each kg of organic trace minerals premix provided: Zinc, 250g; Copper, 130g; Manganese, 310g.

Table 2.2. Main effects of oxidized oil and AOX on accumulated animal performance

	Oil		Antioxidant			P< Value	
Parameter [a]	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
Initial wt, kg	80.2	80.3	80.2	80.2	1.32	0.70	1.00
Final wt, kg	139.9	136.0	137.2	138.7	1.86	0.03	0.40
Performance 0 – 28d							
ADG, kg	0.917	0.838	0.845	0.909	0.034	0.12	0.20
ADFI, kg	2.76	2.63	2.60	2.79	0.099	0.26	0.10
G:F, kg	0.323	0.316	0.311	0.328	0.006	0.46	0.07
Performance 28 – 56d							
ADG, kg	1.216	1.153	1.190	1.179	0.027	0.12	0.78
ADFI, kg	3.25	3.08	3.16	3.17	0.054	0.03	0.85
G:F, kg	0.382	0.375	0.386	0.370	0.009	0.58	0.21
Performance 0 – 56d							
ADG, kg	1.066	0.995	1.018	1.044	0.022	0.03	0.37
ADFI, kg	3.00	2.85	2.88	2.97	0.067	0.04	0.14
G:F, kg	0.356	0.349	0.354	0.351	0.004	0.21	0.67

[a] ADG = Average daily gain; ADFI = Average daily feed intake and G:F = Feed efficiency.

Table 2.3. Effect of oxidized oil and AOX interaction on accumulated animal performance

	Oil × Antioxidant					
Parameter [a]	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
Initial wt, kg	80.2	80.3	80.3	80.2	1.33	0.60
Final wt, kg	139.6	140.2	134.8	137.2	2.19	0.61
Performance 0 – 28d						
ADG, kg	0.895	0.938	0.796	0.880	0.048	0.67
ADFI, kg	2.66	2.86	2.55	2.72	0.125	0.89
G:F, kg	0.319	0.327	0.303	0.330	0.009	0.30
Performance 28 – 56d						
ADG, kg	1.228	1.204	1.152	1.153	0.039	0.75
ADFI, kg	3.27	3.22	3.16	3.17	0.054	0.38
G:F, kg	0.388	0.375	0.385	0.365	0.012	0.78
Performance 0 – 56d						
ADG, kg	1.061	1.071	0.974	1.018	0.030	0.57
ADFI, kg	2.96	3.04	2.79	2.91	0.082	0.77
G:F, kg	0.359	0.353	0.349	0.349	0.005	0.60

[a] ADG = Average daily gain; ADFI = Average feed intake and G:F = Feed efficiency.

Table 2.4. Main effects of oxidized oil and antioxidant on jejunum, plasma and liver oxidative status

	Oil		Antioxidant			P< value	
Item	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
Jejunum							
TBARS, nmol/ml	7.18	7.11	7.05	7.23	1.11	0.90	0.76
Plasma							
TBARS, nmol/ml	5.03	5.69	5.50	5.22	0.26	0.06	0.37
GPx, nmol/mg[a]	16.21	13.88	14.78	15.31	0.75	0.03	0.60
Protein, mg/ml	73.96	74.46	74.54	73.88	2.26	0.85	0.80
Free Carbonyl, nmol /mg[a]	20496	26994	28933	18557	3346.6	0.18	0.04
Vitamin A, µg/dL	7.38	7.17	7.13	7.42	0.070	0.77	0.70
Vitamin E, µg/dL	125.27	56.75	83.59	98.44	17.40	0.01	0.20
Liver							
GPx, nmol/mg[a]	137.04	127.03	133.44	130.63	8.41	0.26	0.75
Protein mg/g	19.15	20.20	19.15	20.20	1.31	0.42	0.42
Vitamin A, µg/g	37.01	27.89	32.38	32.52	3.13	0.06	0.97
Vitamin E, µg/100g	290.63	171.51	214.63	247.50	22.97	0.01	0.32

[a] nmol/mg of protein

Table 2.5. Effect of oxidized oil and antioxidant interaction on jejunum, plasma and liver oxidative status

	Oil × Antioxidant					
Item	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
Jejunum						
TBARS, nmol/ml	7.22	7.14	6.89	7.32	1.18	0.65
Plasma						
TBARS, nmol/ml	5.47[b]	4.60[c]	5.54[b]	5.84[b]	0.34	0.08
GPx, nmol/mg[a]	15.25	17.17	14.31	13.45	1.03	0.17
Protein, mg/ml	75.34	72.60	73.75	75.17	2.89	0.42
Free Carbonyl, nmol /mg[a]	26417	14575	31450	22539	4732.7	0.76
Vitamin A, µg/dL	7.44	7.32	6.83	7.51	0.86	0.58
Vitamin E, µg/dL	116.64	133.91	50.54	62.96	19.07	0.83
Liver						
GPx, nmol/mg[a]	141.58	132.50	125.30	128.76	10.42	0.48
Protein mg/g	18.20	20.10	20.11	20.29	1.58	0.51
Vitamin A, µg/g	37.54	36.48	27.21	28.56	4.42	0.79
Vitamin E, µg/100g	291.63	289.63	137.64	205.38	32.49	0.30

[a] nmol/mg of protein

[b,c] Different superscripts in the same row means statistical difference

Table 2.6. Main effects of oxidized oil and antioxidant on liver fatty acid profile

	Oil		Antioxidant			P<value	
Fatty acid, %	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
C14:0	0.56	0.68	0.81	0.44	0.075	0.26	0.002
C16:0	14.86	14.42	14.90	14.38	0.700	0.66	0.61
C16:1	1.45	1.32	1.38	1.39	0.074	0.25	0.89
C18:0	29.55	28.78	29.38	28.95	0.646	0.42	0.64
C18:1cis9	14.75	15.15	14.71	15.18	0.708	0.66	0.61
C18:1cis11	2.57	2.92	2.74	2.75	0.268	0.38	0.97
C18:2Total	12.95	13.95	13.69	13.20	0.590	0.17	0.50
C20:1	0.16	0.15	0.16	0.14	0.007	0.23	0.07
C20:4	7.93	7.34	7.74	7.53	0.634	0.15	0.59
C20:5	12.40	12.54	11.67	13.27	0.655	0.86	0.06
C24:0	2.84	2.75	2.83	2.76	0.140	0.69	0.77

Table 2.7. Effect of oxidized oil and antioxidant interaction on Liver Fatty Acid Profile

	Oil × Antioxidant					
Fatty acid, %	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
C14:0	0.79	0.33	0.82	0.54	0.106	0.42
C16:0	14.71	15.01	15.08	13.75	0.989	0.42
C16:1	1.43	1.46	1.32	1.32	0.104	0.93
C18:0	29.33	29.76	29.43	28.13	0.914	0.36
C18:1cis9	14.85	14.65	14.58	15.72	0.951	0.47
C18:1cis11	2.39	2.75	3.08	2.75	0.379	0.38
C18:2Total	13.02	12.88	14.36	13.53	0.771	0.63
C20:1	0.18^{a}	0.14^{b}	0.14^{b}	0.15^{b}	0.010	0.03
C20:4	7.93	7.92	7.55	7.14	0.690	0.61
C20:5	12.45^{ab}	12.34^{ab}	10.88^{b}	14.20^{a}	0.864	0.05
C24:0	2.91	2.76	2.74	2.77	0.198	0.65

[a,b] Different superscripts in the same row means statistical difference

CHAPTER III

EFFECTS OF OXIDIZED CORN OIL AND SYNTHETIC ANTIOXIDANT BLEND ON PORK QUALITY AND SHELF-LIFE

ABSTRACT

The objective of the study was to evaluate the effect of protected or unprotected fresh or oxidized corn oil on pork quality traits, and shelf-life. A total of 32 barrows were selected (pig with the final weight closest to the pen mean) for pork quality and shelf life evaluation, after being fed for 56 days with one of the following experimental diets: fresh oil, fresh oil + antioxidant (AOX), oxidized oil or oxidized oil + AOX. Backfat depth, subjective color, marbling and firmness as well as objective color and pH were measured on the loin surface 24 h post-mortem. Loin chops were collected for drip loss, proximate analyses as well as sensory attributes at 0, 7 and 14 d of aging, Also, discoloration percentage and thiobarbituric acid reactive substances (TBARS) after 0, 7, 14 and 21 d in the retail display case were evaluated in modified atmosphere packaged loin chops and ground Boston Butt. Finally, fatty acid, and vitamin A and vitamin E concentrations were determined in backfat and belly fat samples. Increased hot carcass weight ($P<0.01$) and carcass yield ($P<0.01$) were observed for pigs fed fresh oil and increased ($P<0.05$) carcass yield was observed for pigs fed AOX protected oil. No main effects of oil or AOX were observed for subjective or objective pork quality evaluations. Increased ($P<0.03$) moisture content was observed in loins from animals fed AOX unprotected oil and increased ($P<0.04$) moisture content and decreased ($P<0.05$) fat content were observed for animals fed oxidized oil. After 14 d of retail display, TBARS values were decreased ($P<0.001$) for loins from animals fed diets containing AOX protected fresh oil,

and the decrease ($P<0.001$) continued until after 21 d. In backfat, vitamin A concentration was increased ($P<0.02$) in barrows fed fresh oil; whereas no oil or AOX effects were found for vitamin E concentration in backfat. In belly fat, an increase in vitamin A ($P<0.05$) and vitamin E ($P<0.03$) concentrations were observed for barrows fed AOX protected oil. No main effects were found for fatty acid profile in backfat and belly fat. In conclusion, neither oxidized oil nor AOX protection had any impact on pork quality; however, AOX protected fresh oil was shown to improve shelf life, decreasing discoloration and TBARS production over time.

INTRODUCTION

Fatty acid pattern deposited in pigs is very closely related to the dietary fatty acid profile. This relationship has been demonstrated in previous research when different energy sources were fed to finishing pigs (Nguyen et al., 2003; Teye et al., 2006; Mitchaothai et al;, 2007; Wood et al, 2008). Vegetable oils, such as corn, canola and sunflower oil are often used as supplementary energy sources in pig diets. Because vegetable oils are high in polyunsaturated fatty acid (PUFA), adipose tissue in pigs will have a high content of PUFA, especially linoleic and linolenic acids (Nguyen et al., 2003) when fed these oil sources. This high content of PUFA in feed ingredients is also highly susceptible to lipid oxidation during storage and is detectable as having elevated peroxides values (Carpenter and L'Estrage, 1966; NRC, 1998).

Increased levels of PUFA fed to pigs can affect pork quality. Deposition of PUFA in pig adipose tissue can result in increased likelihood of lipid oxidation detectable as thiobarbituric reactive substances (TBARS) resulting in rancidity (Tarladgis et al., 1960;

Wood et al., 2003). Furthermore, lipid oxidation can occur during storage when pork is packaged under high oxygen atmosphere (80% O_2 /20% CO_2), such as modified atmospheres packaging (MAP; Jensen et al., 1998). In addition to oxygen reacting with the lipid fraction of muscle tissues, oxygen can also react with proteins, developing protein oxidation products favoring meat color changes resulting in consumer disapproval (Mercier et al., 1998; Brewer, 2004). Therefore, meat package systems such as MAP can favor lipid and protein oxidation development when meat is stored for long periods of time under retail display conditions.

Vitamin E, supplemented in diets, can be deposited in fat associated with muscle tissue as α-tocopherol. This is the most important natural dietary antioxidant to protect against lipid oxidation and increase shelf-life (Jensen et al., 1998). Some synthetic antioxidants such as ethoxyquin (EQ), terbutylhydroquionone (TBHQ) are often used to protect oils and fats from lipid oxidation (Thosrisson et al., 1992; De Koning, 2002). However, the effect of synthetic antioxidants such as EQ and TBHQ on pork quality and shelf-life is not well documented in the literature.

Thus, the objective of the study was to evaluate the effect of either AOX protected or unprotected fresh or oxidized corn oil on pork quality traits, meat discoloration and lipid oxidation as indicators of shelf-life.

MATERIAL AND METHODS

Animal Background

Animal use for this study was approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC) on April 2008, and the approval number was 08081.

Animal handling and experimental design was described in the Chapter II. Briefly, a total of 160 barrows were used for the live phase of the experiment. The experiment was carried out as a CRBD in a factorial arrangement (2×2), with 2 types of corn oil (5% of fresh or oxidized corn oil) and 2 levels of antioxidant (with or without AOX). Antioxidant (SANTOQUIN® Q, to provide 10 ppm Terbutyl-hydroquinone (TBHQ) and 132 ppm of Ethoxyquin (EQ) in the final diets) was provided by NOVUS Inc. (St. Charles, MO, USA). The four experimental diets are the same as described in Chapter II.

After the live phase, the pig with final weight closest to the pen mean was selected from each pen for meat quality and shelf-life evaluation. After 18hr feed withdrawal, 32 barrows were transported to the Meat Science Laboratory, University of Illinois at Urbana-Champaign. Barrows were weighed prior to harvest; thereafter animals were humanely slaughtered under USDA inspection. Hot carcass weight (HCW) was recorded to calculate dressing percentage. Carcasses were chilled at 4 C° for 24 hr.

Pork Quality Evaluation

Meat quality evaluation was conducted 24 hr post-mortem on the left side of the carcass. Backfat was measured at four different anatomical points: 1st, 10th, last rib and last lumbar vertebrae. Carcasses were ribbed between the 10th and 11th rib, and loin

surface was allowed to bloom for 15 minutes. Subjective color, marbling (NPPC, 1999) and firmness (NPPC, 1991) were appraised on the loin surface. At the same anatomical point, instrumental color (L*, a* and b*) values were obtained using a Minolta Chromameter (CR-300; Minolta Camera Co., Osaka, Japan) with illuminant D65 and a 0° observer. Finally, pH was measured with a pH-STAR (SFK Technologies Inc., Cedar Rapids, IA).

After quality evaluations, the loin from the left side of the carcass was removed, and chops were collected for further analysis (Figure 3.1). One 1.3 cm chop was collected, weighed, hung in a Whirlpak™ bag, and weighed again after 24 hr to determine drip loss. A 2.54 cm loin chop was collected and stored at -10 C° for further proximate analysis; moisture was determined as described by method 950.46 of the AOAC (1995) and extractable lipid percentage determined as described by Novakofski et al. (1989).

Sensory Analysis

Three 2.54 cm chops were collected and randomly assigned to 3 different aging days for further sensorial analysis by a trained panel. Sensory analysis was evaluated in chops after 0, 7 and 14 d of aging and included juiciness, tenderness, off-flavors (rancid) and oxidized odor recorded on a 15-cm anchored unstructured line scale, where 0 = extremely dry, tough, no off-flavor and no oxidized odor, and 15 = extremely juicy, tender and off-flavor and oxidized odor. In order to train panelists for off-flavors and oxidized odor detection, loin chops and ground pork patties from untreated pigs were fried either in fresh corn oil, oxidized corn oil, ½ fresh corn oil + ½ oxidized corn oil and refried oxidized corn oil to favor development of oxidized flavor and rancid odor in

chops and ground pork patties. Finally, a backup loin section was vacuum packaged and stored at -20 C°.

Discoloration and Lipid Oxidation

In order to evaluate shelf-life, subjective discoloration percentage and thiobarbituric Acid Reactive Substances (TBARS) were evaluated in fresh and ground pork. Four 2.54 cm chops as representative of fresh pork were collected as described in Figure 3.1 and randomly assigned for TBARS evaluation. Chops were packaged in modified atmosphere (MAP; 80 % oxygen / 20 % carbon dioxide); thereafter, chops were stored at 4ºC for 0, 7, 14 and 21 d. For 7, 14 and 21 d chops were placed under simulated retail display conditions 7 d previous TBARS evaluation. Boneless Boston butt was fabricated according to the National Association of Meat Purveyors (NAMP, 2007). Boneless Boston Butt (NAMP 406A) was ground twice through a Hobart grinder (Hobart Corp, Troy, OH) with a 3/16 in plate and then 0.5 kg sample was placed in four trays, one for each TBARS period (0, 7, 14 and 21 d), packaged (MAP) and stored as described for loin chops. A sample of ground Boston Butt was collected and stored at -10 C° for further proximate analysis as previously described.

After each period (0, 7, 14 and 21d), discoloration percentage was evaluated in chops and ground Boston Butt surface as described by Holmer et al. (2009). Briefly, discoloration percentage was evaluated using a 10 cm scale in which each 1 cm represents 10 % discoloration on the surface of the loin or ground Boston Butt sample.

After subjective discoloration evaluation, loin chops and ground Boston Butt were finely minced (Cuisinart Food Processor, East Windsor, NJ, USA) and TBARS were determined as an indicator of lipid oxidation. Briefly, duplicate samples (~5 g) were

weighed and homogenized for 30 seconds with 45.5 ml of trichloroacetic acid / Phosphoric acid solution and 1 ml of butylhydrotolouene as antioxidant to prevent further oxidation due to sample processing. The homogenate was poured into a beaker through Whatman™ filter paper No. 1 (Maidstone, UK). Five ml of filtered homogenate was reacted with 5 ml of thiobarbituric acid. Test tubes were inverted and incubated at room temperature for 16 to 20 h. Malonaldehyde (MDA) concentration was measured colorimetrically for each meat sample in triplicate using a spectrophotometer (Beckman DU640, Fullerton, CA, USA) at 530nm.

Fatty Acid Profile and Vitamin A and E Concentration in Adipose Tissue

One sample of approximately 50cm^2 of backfat (at 10th rib), and a 50cm^2 belly fat section were collected, placed in a 4oz. Whirlpak™ bag and stored at -20C° for further fatty analysis as well as vitamin A and vitamin E determination. Vitamin A and vitamin E, in back and belly fat were determined by high pressure liquid chromatography following the procedures described by Catignani (1985); while fatty acid profile in back and belly fat was determined by gas chromatography following the procedures described by Engle et al., (2000).

Statistical Analysis

Pork quality data and shelf-life were analyzed as a complete randomized block design (CRBD) in a factorial arrangement (2×2) with 2 levels of corn oil (5% of fresh or oxidized corn oil) and 2 levels of antioxidant (with or without AOX). Harvest day of the experiment (4 starting days) was used as blocking criteria. Experimental unit for the pork quality and shelf-life evaluation was the carcass with a total of 8 replicates per treatment. Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary,

NC). The model included block, main effect of oil, and main effect of AOX as well as interaction of corn oil × AOX. Least squares differences were separated by probability of difference option (PDIFF) and P values were adjusted by the Bonferroni approach to declare statistical difference at $P<0.05$. Finally, shelf-life data (lipid peroxidation and discoloration percentage) were evaluated over time using the PROC MIXED procedure of SAS with the REPEATED command by day (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Live, HCW and Dressing Percentage

Fresh oil increased live ($P<0.04$) and slaughter weight ($P<0.02$) of barrows selected for pork quality evaluation when compared to barrows fed oxidized oil (Table 3.1). This result is in agreement with the live weight observed for the experimental units (pens) at the end of the live phase (Chapter II). Also, carcasses from barrows fed fresh oil were heavier ($P<0.01$) than carcasses from barrows fed oxidized oil. Carcass yield was also increased in barrows fed fresh oil ($P<0.01$) and AOX protected oil ($P<0.05$). Other authors have reported no differences in final weight of finishing pigs fed fresh vegetable oils at inclusion rates from 2 to 6% (Leskanich et al., 1997; Gläser et al., 2002; Teye et al., 2006; Mitchaothai et al., 2007). Similar to final weight, some authors also found no differences in HCW (Leskanich et al., 1997; Teye et al., 2006; Mitchaothai et al., 2007). Decreased live and HCW, as well as carcass yield, in our experiment could be the result of the poor quality of the oxidized oil fed to barrows which was reflected in decreased animal performance, resulting from decreased feed intake and weight gain (Chapter II).

Estimates of Carcass Fat

In general, pigs fed oxidized oil deposited less fat than those fed fresh oil. Barrows fed oxidized oil had decreased backfat depth at the 10th ($P<0.02$) and last rib ($P<0.03$). Also, ground Boston Butt from barrows fed oxidized oil had increased ($P<0.04$) moisture content and decreased ($P<0.05$) extractable lipid content. However, loins from barrows fed unprotected oil (no AOX) had increased ($P<0.03$) moisture content and tended towards decreased ($P<0.07$) extractable lipid content than loins from barrows fed AOX protected oil (Table 3.1). An oil × AOX interaction ($P<0.08$) was found for marbling score in which barrows fed AOX protected fresh oil had a higher marbling score than barrows fed unprotected fresh oil; whereas, marbling score was not different when barrows were fed either AOX protected or unprotected oxidized oil. Also, an oil × AOX interaction ($P<0.03$) was observed for backfat at 1st rib, where barrows fed AOX protected fresh oil were not different than the barrows fed unprotected fresh oil; whereas, barrows fed AOX protected oxidized oil had greater backfat at 1st rib than barrows fed unprotected oxidized oil (Table 3.2). However, no oil × AOX interactions were observed at the other anatomical sites where backfat depth was measured, or for extractable lipid in the loin or Boston Butt. Backfat depth at P_2 of barrows fed fresh corn oil were higher than those reported for barrows fed high PUFA level diets (Kouba et al., 2003; Teye et al., 2006; Mitchaothai et al., 2007). Our results may be due to heavier final weight in our experiment. Similar extractable lipid content in loins has been reported when animals were fed high PUFA level diets (Mitchaothai et al., 2007). However, loin extractable lipid content was higher than those observed by other authors (Lauridsen et al., 1999; Teye et al., 2006; Leick, 2008), but lower than content in loins observed by

Rhee et al. (1988), when animals were fed high levels of DDGS, or high level of PUFA in the diet. Finally, contrary to our results, Phillips et al. (2001) reported no differences in extractable lipid content in ground Boston Butt from barrows supplemented with vitamin E when compared to animals with no supplementation. Thus, decreased fat deposition in loins of pigs fed oxidized oil may be due to decreased feed intake during the live phase (Chapter II). Leick (2008) reported that marbling scores decreased as DDGS inclusion levels increased from 0 to 60% in finishing diets. Marbling scores observed during our experiment are comparable to scores observed at 45% DDGS inclusion rates.

Pork Quality

Corn oil type or AOX did not affect subjective color, firmness, pH or Minolta L*, a*, and b* values. In agreement with our findings, some authors have reported no differences in Minolta a* value (Averette-Gatlin et al., 2003; Teye et al., 2006) and L* and b* value (Leskanich et al., 1997; Leick, 2008) when different sources of PUFA were fed to finishing pigs. However, other authors (Van Oeckle et al., 1996; Averette Gatlin et al., 2003; Teye et al., 2006) reported increased L* and b* values compared to values observed in our experiment when finishing pigs were fed high PUFA level diets (palm oil, soybean oil, linseed or hydrogenated choice white grease). Previous literature and our results suggest that diets containing high PUFA levels and poor quality of fat sources did not affect lightness, redness and yellowness of the loin.

Sensory Analysis

In general, corn oil type or AOX did not affect sensory analyses. Only tenderness scores were higher for loin chops from barrows fed AOX protected oil ($P<0.03$); however the magnitude of changes in tenderness scores was not considerable (Table 3.3) and may

be of minimal importance. Also, an interaction (P<0.08) was observed for oxidized odor score at Day 1.Oxidized odor score from barrows fed unprotected fresh oil was higher than scores for barrows fed AOX protected fresh oil; whereas scores were not different for barrows fed neither AOX protected or unprotected oxidized oil (Table 3.4). However, the magnitude of change in odor scale was not substantial. While some authors (Teye et al., 2006) reported decreased tenderness from animals fed high PUFA diets, others (Ahn et al., 1996; Van Oeckel et al., 1996; Leskanich et al., 1997) observed no differences in palatability of loins from animals fed diets containing high PUFA level when compared to loins from animals fed diets containing traditional energy sources (animal fats). Also, in agreement to our results, Kouba et al. (2003) reported increased abnormal odor of fat, but not abnormal odor or flavor of lean, for animals fed diets high in PUFA when compared to animals fed tallow/soybean oil diets. Oxidized odor observed in our experiment suggests that loins from animals fed fresh oil with no AOX protection were the most susceptible to oxidation at day 1 of evaluation (Lebret et al., 1999).

Discoloration

In general, fresh oil protected with AOX helped to prevent discoloration after 21 days under retail display case conditions. A three way (oil × AOX × day) interaction (P<0.001) was observed where discoloration scores for loins chops were not different among treatments at 1, 7 and 14d. However, after 21d, loins from barrows fed AOX protected fresh oil had decreased (P<0.0001) discoloration percentages scores when compared all other treatments (Figure 3.2)**.** Fresh meat color depends on myoglobin state; oxymyoglobin is more stable than deoxymyoglobin which oxidizes faster than oxymyoglobin to form metmyoglobin. Metmyoglobin is the state responsible for the

undesirable brownish color of fresh meat and this could be due partially to lipid oxidation (Faustman and Cassens, 1990; Brewer, 2004). Previous research showed that metmyoglobin formation can be delayed using MAP systems (Jensen et al., 1998; Behrends et al., 2003); however, it has been also proposed that pigment oxidation is highly related to lipid oxidation, so high content of PUFA might favor meat discoloration (Faustman and Cassens, 1990). In our experiment, loin discoloration percentage scores after 21 d may be due to susceptibility of PUFA to oxidation in meat under high O_2 conditions, such as MAP (O_2 = 80%). Holmer et al. (2009) reported less than 10% discoloration after 28 d under vacuum conditions meaning no O_2 is present to favor lipid and deoxymyoglobin oxidation. Finally, our results also suggest that the AOX blend used in fresh corn oil delayed the formation of metmyoglobin, increasing shelf-life in loins. No interactions for oil × AOX × day were found for ground Boston Butt discoloration percentage scores (Figure 3.3). This lack of differences may be due to the high fat content in shoulder (Boston Butt and Picnic), suggesting that lipid oxidation was developed regardless corn oil type, and overwhelming of any AOX activity, resulting in metmyoglobin formation.

Lipid Oxidation

In general, fresh oil protected with AOX helped to prevent lipid oxidation after 21 days under retail display case conditions. In regard to TBARS production, an oil × AOX × day interaction ($P<0.001$) was observed, where TBARS values for loins chops were not different among treatments at 1 and 7d. However, after 14d, loins from barrows fed AOX protected fresh oil had decreased ($P<0.001$) TBARS values when compared to loins from barrows fed unprotected oxidized oil. Values were similar among AOX protected and

unprotected fresh oil and AOX protected oxidized oil. After 21d, loins from barrows fed AOX protected fresh oil had decreased ($P<0.001$) TBARS values when compared to loins from barrows fed unprotected oxidized or fresh oil, and AOX protected oxidized oil (Figure 3.4). In agreement to other authors (Ahn et al., 1996; Leskanich et al., 1997; Mercier et al., 1998; Lauridsen et al., 1999) vegetable oils fed as supplementary energy sources increased TBARS values of meat when compared to diets containing animal fats. In our experiment, TBARS values were higher since the first day when compared to TBARS values at 1 d as reported by Leick (2008) when animals were fed high DDGS inclusion rates (0 to 60%). Also, in agreement to our results, Leick et al., (2008) reported a significant TBARS increase after 14 and 21 d of retail display case conditions for loins from animals fed DDGS at rates of 30, 45 and 60%. Other authors have reported decreased TBARS values and increased shelf life in loins when natural antioxidants have been supplemented (Mercier et al., 1998; Lauridsen et al., 1999; Boler, 2008). Our results also suggest that the AOX blend used in fresh corn oil decreased lipid oxidation after 14 d maintaining a TBARS value under 1 mg/ kg after 21 d. Finally, no oil × AOX × day interactions were found for TBARS values in ground Boston Butt (Figure 3.5)**.** Our results are in disagreement with other authors that reported decreased TBARS values in ground meat over time when natural antioxidants were supplemented to diets (Phillips et al., 2001; Boler, 2008). This lack of difference may be due to the high fat content in shoulder (Boston Butt and Picnic), and large amount of tissue exposed to O_2 (due to grinding), suggesting that lipid oxidation was developed regardless of corn oil type, and ultimately overwhelming the AOX activity.

Fatty Acid Profile and Vitamin A and Vitamin E Concentration

In general, corn oil type or AOX impacted vitamin A and E concentration in adipose tissue, while fatty acid profile was not greatly affected. Vitamin A concentration was decreased ($P<0.02$) in backfat of barrows fed oxidized oil (Table 3.5). An oil × AOX interaction ($P<0.07$) was found for vitamin E concentration in backfat, where vitamin E concentration from barrows fed unprotected fresh oil was lower than barrows fed AOX protected fresh oil. However, vitamin E concentration in backfat from barrows fed unprotected oxidized oil was higher than barrows fed AOX protected oxidized oil**.** Also, an oil × AOX interaction was found for 20:4 fatty acid percentage, where 20:4 percentage in backfat from barrows fed AOX protected fresh oil was lower ($P<0.02$) than percentage from barrows fed unprotected fresh oil; whereas backfat from barrows fed either AOX protected or unprotected oxidized oil were not different (Table 3.6)**.** In belly fat, a decrease in vitamin A ($P<0.05$) and vitamin E ($P<0.03$) concentration were observed for barrows fed unprotected oil (Table 3.7). No oil × AOX interactions were found for vitamin A, vitamin E and fatty acid profile of belly fat (Table 3.8). Boler (2008) reported increased levels of vitamin E in adipose tissue as vitamin E levels in diet increased when finishing pigs were fed DDGS at 10 % inclusion rate. Leskanich et al., (1997) reported increased backfat vitamin E concentration for animals fed tallow/soybean diets when compared to rapeseed/fish oil diets; however, when vitamin E was supplemented to the rapeseed/fish oil diets, concentration of vitamin E in tissues was the highest. Some other authors (Lauridsen et al., 1999; Eichenberger et al., 2004) observed decreased α-tocopherol levels in muscle and backfat of animals fed diets with rapeseed or sunflower oil (no AOX supplemented) compared to animals fed diets supplemented with vitamin E.

However, all these studies provided diets specifically enriched in vitamin E, thus it is not surprising that vitamin E concentration increases. Our study, which used a synthetic AOX source and not vitamin E, would indicate that vitamin E concentration in backfat decreases with increased oxidative status of the diet, being higher in fresh versus oxidized oil fed pigs. The synthetic AOX, however, was unable to protect against vitamin E depletion in backfat in pigs fed oxidized oil. Our results suggest that lipid soluble vitamins are susceptible to depletion and with no dietary AOX protection, vitamin concentrations in tissues may be inadequate to protect against further oxidative stress. Also, fresh energy sources (such as corn or rapeseed oil) protected with natural or synthetic AOX spared vitamin E as an antioxidant for oxidative stress.

Regarding fatty acid profile in back and belly fat, our results are in agreement with those results reported by several authors (Van Oeckel et al., 1996; Leskanich et al., 1997; Teye et al., 2006) where no changes in muscle and adipose tissue fatty acid profile were observed for animals fed different supplementary energy sources to provide enriched PUFA ingredients to the diet. However, some other authors have reported important fatty acid profile changes affecting primarily linoleic (18:1 cis) and linolenic (18:2) acid in muscle (Rhee et al., 1988; Ahn et al., 1995; Glässer et al., 2002; Averette-Gatlin et al., 2003; Mitchaothai et al., 2007) and adipose (Lauridsen et al., 1999; Glässer et al., 2002; Averette-Gatlin et al., 2003; Kouba et al., 2003; Mitchaothai et al., 2007; Leick, 2008) tissue when animals were fed high levels of vegetable oils. Our results suggest that no important changes in belly or backfat fatty acid profile took place when diets with oxidized corn oil were fed. It has been shown that vitamin E at different levels increases protection against lipid oxidation, thus protecting PUFA (Monahan et al., 1992; Jensen et

al., 1998; Lauridsen et al., 1999; Boler, 2008). However, it is also important to note that presence of pro-oxidant molecules either deposited or generated in tissues could be contributing to lipid oxidation (Jensen et al., 1998; Wood et al., 2003); therefore increased shelf-life of loins from barrows fed AOX protected fresh oil in our experiment, may be due to AOX activity of the EQ + TBHQ blend added to fresh energy source before diet mixing.

CONCLUSIONS

In conclusion, oxidized corn oil inclusion in finishing diets of barrows did not adversely affect subjective and objective quality traits in pork. Also, AOX protection did not seem to improve pork quality. However, shelf-life of fresh and ground pork was greatly affected by oxidized oil, resulting in rapid color discoloration, increased lipid oxidation and low quality visual appraisal. Also, our results suggest a strong antioxidant activity of the AOX blend used in the experiment, resulted in a delay in oxidation development and an increased shelf-life of products coming from animals fed protected diets.

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FIGURES AND TABLES

Table 3.1. Main effect of oxidized oil and antioxidant on meat quality evaluation

	Oil		Antioxidant			P< value	
Item	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
Number of animals	16	16	16	16	-	-	-
Farm wt, kg	140.6	136.5	137.2	139.9	2.07	0.04	0.18
Slaughter wt, kg	136.7	132.1	132.9	135.9	2.00	0.02	0.12
Hot carcass wt, kg	106.6	102.1	102.9	105.8	1.91	0.01	0.07
Carcass yield, %	78.0	77.3	77.4	77.9	0.34	0.01	0.05
Subjective [a]							
Color score	2.44	2.56	2.50	2.50	0.13	0.50	1.00
Marbling score	1.63	1.50	1.38	1.75	0.15	0.55	0.08
Firmness score	2.50	2.50	2.38	2.63	0.13	1.00	0.18
Loin pH	5.56	5.59	5.55	5.59	0.18	0.18	0.16
Minolta values							
L*	49.16	48.45	49.39	48.21	0.71	0.47	0.23
a*	7.09	7.60	7.10	7.59	0.27	0.15	0.16
b*	3.39	3.80	3.62	3.56	0.25	0.20	0.85
Back fat, in							
First rib	1.77	1.73	1.69	1.80	0.06	0.60	0.21
Tenth rib	1.30	1.15	1.18	1.27	0.05	0.03	0.18
Last rib	1.21	1.02	1.06	1.17	0.05	0.02	0.13
Last lumbar	0.96	0.92	0.91	0.96	0.04	0.52	0.39
Drip loss, %	2.61	2.81	2.43	2.99	0.41	0.70	0.28
Loin Moisture, %	74.58	73.98	74.71	73.84	0.26	0.12	0.03
Boston Butt Moisture, %	61.44	63.54	63.15	61.82	0.70	0.04	0.17
Loin Fat, %	2.49	2.97	2.28	3.18	0.33	0.32	0.07
Boston Butt Fat, %	21.98	19.44	19.87	21.56	0.84	0.05	0.17

[a] NPPC color and marbling standards (NPPC, 1999); NPPC firmness standards scale 1-5 (NPPC, 1991).

Table 3.2. Effect of oxidized oil and antioxidant interaction on meat quality evaluation

	Oil × Antioxidant					
Item	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
Number of animals	8	8	8	8	-	-
Farm wt, kg	139.3	142.0	135.2	137.8	2.07	0.97
Slaughter wt, kg	135.3	138.2	130.6	133.5	2.38	0.98
Hot carcass wt, kg	105.3	108.0	100.6	103.6	2.20	0.90
Carcass yield, %	77.8	78.1	77.0	77.6	0.38	0.66
Subjective [a]						
Color score	2.50	2.38	2.50	2.63	0.19	0.51
Marbling score	1.25[b]	2.00[c]	1.50[bc]	1.50[bc]	0.21	0.08
Firmness score	2.38	2.63	2.38	2.63	0.18	1.00
Loin pH	5.53	5.58	5.58	5.60	0.04	0.55
Minolta values						
L*	49.73	48.59	49.06	47.84	0.98	0.97
a*	7.03	7.15	7.17	8.04	0.36	0.29
b*	3.44	3.33	3.81	3.80	0.33	0.87
Back fat, in						
First rib	1.81[b]	1.73[bc]	1.58[c]	1.88[b]	0.08	0.03
Tenth rib	1.25	1.35	1.11	1.19	0.07	0.85
Last rib	1.15	1.26	0.96	1.08	0.07	1.00
Last lumbar	0.93	0.99	0.90	0.94	0.06	0.83
Drip loss, %	2.63	2.60	2.24	3.39	0.54	0.26
Loin Moisture, %	74.93	74.23	74.50	73.46	0.37	0.65
Boston Butt Moisture, %	62.13	60.75	64.18	62.90	1.92	0.96
Loin Fat, %	2.05	2.93	2.51	3.44	0.46	0.97
Boston Butt Fat, %	21.11	22.86	18.63	20.26	1.18	0.97

[a] NPPC color and marbling standards (NPPC, 1999); NPPC firmness standards scale 1-5 (NPPC, 1991).
[b, c] Different Superscripts in the same row means statistical difference.

Table 3.3 Main effect of oxidized oil and antioxidant on sensory evaluation

	Oil		Antioxidant			P< value	
Item, [a]	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
Juiciness Score							
Day 1	8.14	8.34	8.21	8.27	0.230	0.48	0.84
Day 7	8.49	8.51	8.57	8.43	0.202	0.90	0.47
Day 14	8.71	8.40	8.31	8.80	0.192	0.27	0.08
Tenderness Score							
Day 1	7.31	7.51	7.19	7.63	0.312	0.56	0.22
Day 7	8.73	8.48	8.53	8.68	0.172	0.32	0.56
Day 14	8.86	8.33	8.27	8.93	0.216	0.06	0.03
Off-Flavor Score							
Day 1	0.36	0.41	0.51	0.27	0.114	0.76	0.16
Day 7	0.91	0.75	1.01	0.65	0.228	0.43	0.08
Day 14	0.57	0.63	0.52	0.68	0.203	0.71	0.34
Oxidized Odor Score							
Day 1	0.84	0.54	0.81	0.59	0.149	0.18	0.30
Day 7	0.73	0.68	0.79	0.62	0.196	0.80	0.38
Day 14	0.38	0.53	0.37	0.54	0.112	0.33	0.25

[a] 15-cm anchored unstructured line scale, where 0 = extremely dry, tough, no off-flavor and no oxidized odor, and 15 = extremely juicy, tender and off-flavor and oxidized odor

Table 3.4. Effect of oxidized oil and antioxidant interaction on sensory evaluation

	Oil × Antioxidant					
Item, [a]	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
Juiciness score						
Day 1	8.16	8.13	8.26	8.41	0.299	0.74
Day 7	8.60	8.38	8.54	8.49	0.241	0.65
Day 14	8.33	9.09	8.29	8.51	0.271	0.33
Tenderness score						
Day 1	7.19	7.43	7.20	7.83	0.397	0.59
Day 7	8.81	8.64	8.25	8.71	0.243	0.21
Day 14	8.53	9.20	8.01	8.65	0.288	0.94
Off-Flavor score						
Day 1	0.51	0.21	0.50	0.33	0.161	0.71
Day 7	1.15	0.66	0.86	0.64	0.265	0.50
Day 14	0.53	0.61	0.51	0.75	0.235	0.65
Oxidized Odor score						
Day 1	1.14[b]	0.54[c]	0.48[c]	0.64[bc]	0.208	0.08
Day 7	0.95	0.51	0.64	0.73	0.240	0.20
Day 14	0.26	0.50	0.48	0.59	0.154	0.67

[a] 15-cm anchored unstructured line scale, where 0 = extremely dry, tough, no off-flavor and no oxidized odor, and 15 = extremely juicy, tender and off-flavor and oxidized odor

[b, c] Different Superscripts in the same row means statistical difference.

Table 3.5. Main effects of oxidized oil and antioxidant on Vitamin A, Vitamin E and fatty acid profile of backfat

	Oil		Antioxidant			P<value	
Fatty acid, %	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
Vitamin A, µg/100g	16.63	8.45	11.86	13.22	3.097	0.02	0.68
Vitamin E, µg/100g	504.4	378.2	442.1	440.5	55.49	0.11	0.98
C14:0	1.18	1.19	1.10	1.27	0.093	0.99	0.21
C16:0	21.71	21.84	21.92	21.63	1.733	0.96	0.91
C16:1	3.50	3.97	3.63	3.84	0.222	0.15	0.52
C18:0	15.81	14.90	13.54	17.18	1.481	0.67	0.10
C18:1cis9	35.71	36.81	37.99	34.53	1.351	0.56	0.08
C18:1cis11	3.55	3.72	3.51	3.76	0.342	0.73	0.60
C18:2Total	16.07	15.53	15.88	15.71	0.901	0.68	0.90
C20:1	0.40	0.37	0.35	0.42	0.032	0.52	0.18
C20:4	0.64	0.62	0.71	0.55	0.081	0.84	0.18
C20:5	0.67	0.48	0.64	0.51	0.088	0.13	0.30
C24:0	0.74	0.57	0.72	0.59	0.102	0.25	0.39

Table 3.6. Effect of oxidized oil and antioxidant interaction on Vitamin A, Vitamin E and fatty acid profile of backfat

	Oil × Antioxidant					
Fatty acid, %	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
Vitamin A, µg/100g	14.41	18.85	9.32	7.59	3.849	0.35
Vitamin E, µg/100g	432.3[ab]	576.5[a]	452.0[ab]	304.5[b]	77.20	0.07
C14:0	1.13	1.24	1.08	1.30	0.131	0.71
C16:0	23.21	20.20	20.63	23.05	2.450	0.28
C16:1	3.18	3.83	4.09	3.85	0.314	0.17
C18:0	12.70	18.93	14.37	15.44	2.095	0.23
C18:1cis9	37.54	33.88	38.44	35.18	1.879	0.92
C18:1cis11	3.59	3.51	3.42	4.02	0.483	0.49
C18:2Total	15.88	16.27	15.89	15.16	1.282	0.67
C20:1	0.40	0.40	0.31	0.43	0.045	0.17
C20:4	0.87[a]	0.42[b]	0.55[b]	0.69[ab]	0.113	0.02
C20:5	0.67	0.67	0.61	0.35	0.125	0.32
C24:0	0.83	0.65	0.61	0.53	0.145	0.73

[a, b] Different Superscripts in the same row means statistical difference.

Table 3.7. Main effects of oxidized oil and antioxidant on Vitamin A, Vitamin E and fatty acid profile belly fat

	Oil		Antioxidant			P<value	
Fatty acid, %	Fresh	Oxidized	No AOX	AOX	SEM	Oil	Antioxidant
Vitamin A, µg/100g	7.60	10.50	5.28	12.81	2.53	0.43	0.05
Vitamin E, µg/100g	307.3	440.7	262.1	485.9	63.97	0.16	0.03
C14:0	1.18	1.26	1.20	1.25	0.098	0.52	0.68
C16:0	22.51	24.29	24.25	22.55	1.140	0.28	0.30
C16:1	4.39	4.37	4.53	4.23	0.310	0.98	0.49
C18:0	14.37	12.70	12.94	14.13	1.740	0.51	0.64
C18:1cis9	38.22	37.38	37.58	38.02	1.194	0.63	0.80
C18:1cis11	2.27	2.84	2.29	2.82	0.363	0.28	0.32
C18:2Total	14.77	14.71	14.83	14.64	0.802	0.96	0.87
C20:1	0.32	0.36	0.33	0.36	0.036	0.40	0.54
C20:4	0.78	0.83	0.83	0.78	0.085	0.73	0.72
C20:5	0.50	0.77	0.64	0.62	0.105	0.07	0.91
C24:0	0.71	0.49	0.58	0.60	0.103	0.13	0.90

Table 3.8. Effect of oxidized oil and antioxidant interaction on Vitamin A, Vitamin E and fatty acid profile belly fat

	Oil × Antioxidant					
Fatty acid, %	Fresh	Fresh + AOX	Oxidized	Oxidized + AOX	SEM	P<value
Vitamin A, µg/100g	3.74	11.46	6.83	14.16	3.580	0.96
Vitamin E, µg/100g	169.9	444.8	354.3	527.0	90.44	0.58
C14:0	1.14	1.22	1.25	1.28	0.132	0.82
C16:0	24.19	20.84	24.30	24.27	1.591	0.31
C16:1	4.62	4.15	4.44	4.30	0.438	0.72
C18:0	12.85	15.89	13.03	12.37	2.461	0.47
C18:1cis9	38.09	38.35	37.06	37.70	1.688	0.92
C18:1cis11	1.96	2.57	2.62	3.05	0.514	0.87
C18:2Total	14.89	14.64	14.77	14.64	1.133	0.97
C20:1	0.28	0.36	0.38	0.35	0.051	0.30
C20:4	0.74	0.83	0.92	0.74	0.120	0.28
C20:5	0.52	0.48	0.77	0.77	0.144	0.91
C24:0	0.74	0.66	0.43	0.54	0.141	0.50

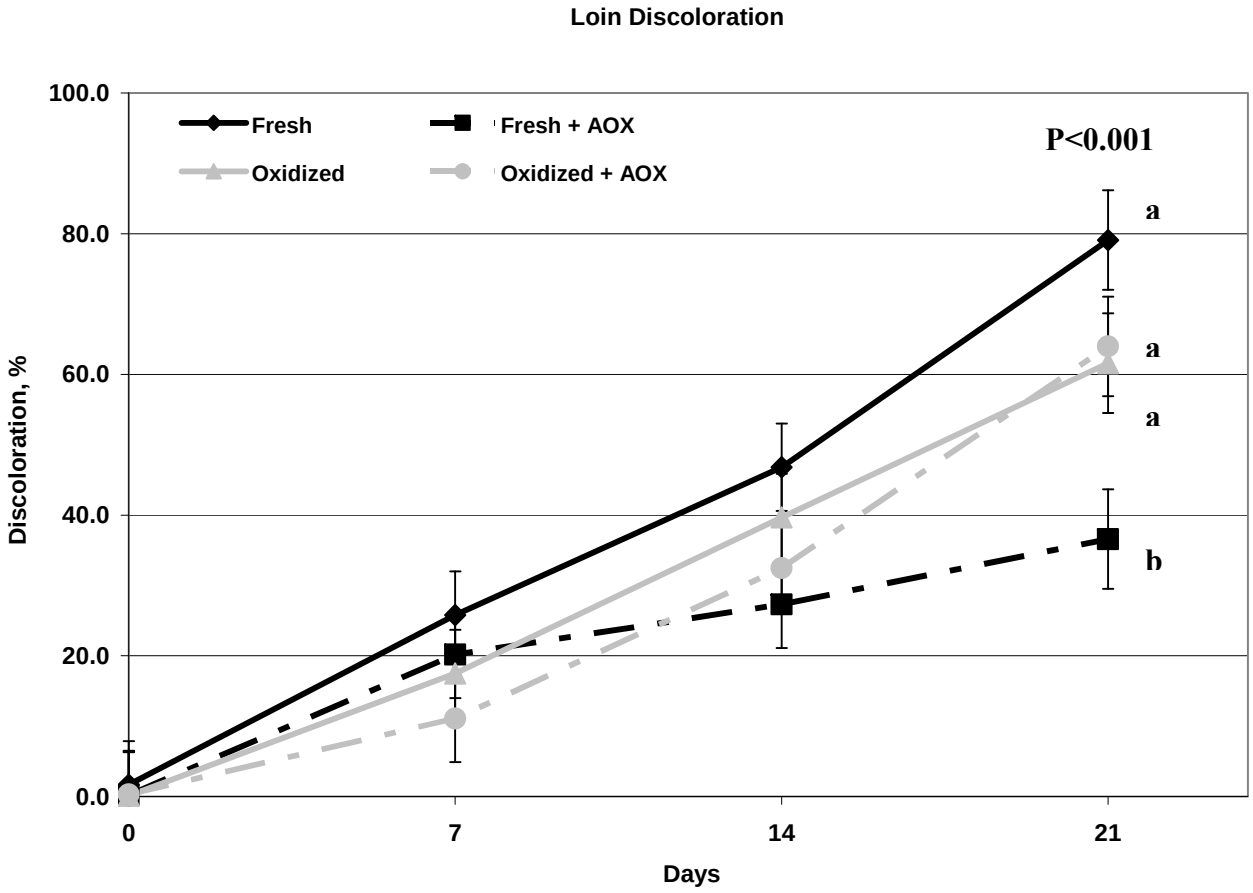
Figure 3.1. Loin sections for sensory attributes evaluation and TBARS determination

Cranial 10^{th} rib Caudal

Backup Loin Section	Drip	PA	Sensory	Sensory	Sensory	TBARS	TBARS	TBARS	TBARS

Chops (2.54cm) sections were randomly assigned for 1, 7 and 14d for sensory analysis and 1, 7, 14 and 21d for TBARS determination.

Figure 3.2. Effect of oil × antioxidant interaction over time (21 days) on loin discoloration (%)



[a, b] Different Superscripts in the same evaluation day means statistical difference.

Figure 3.3. Effect of oil × antioxidant interaction over time (21 days) on ground Boston Butt discoloration (%)

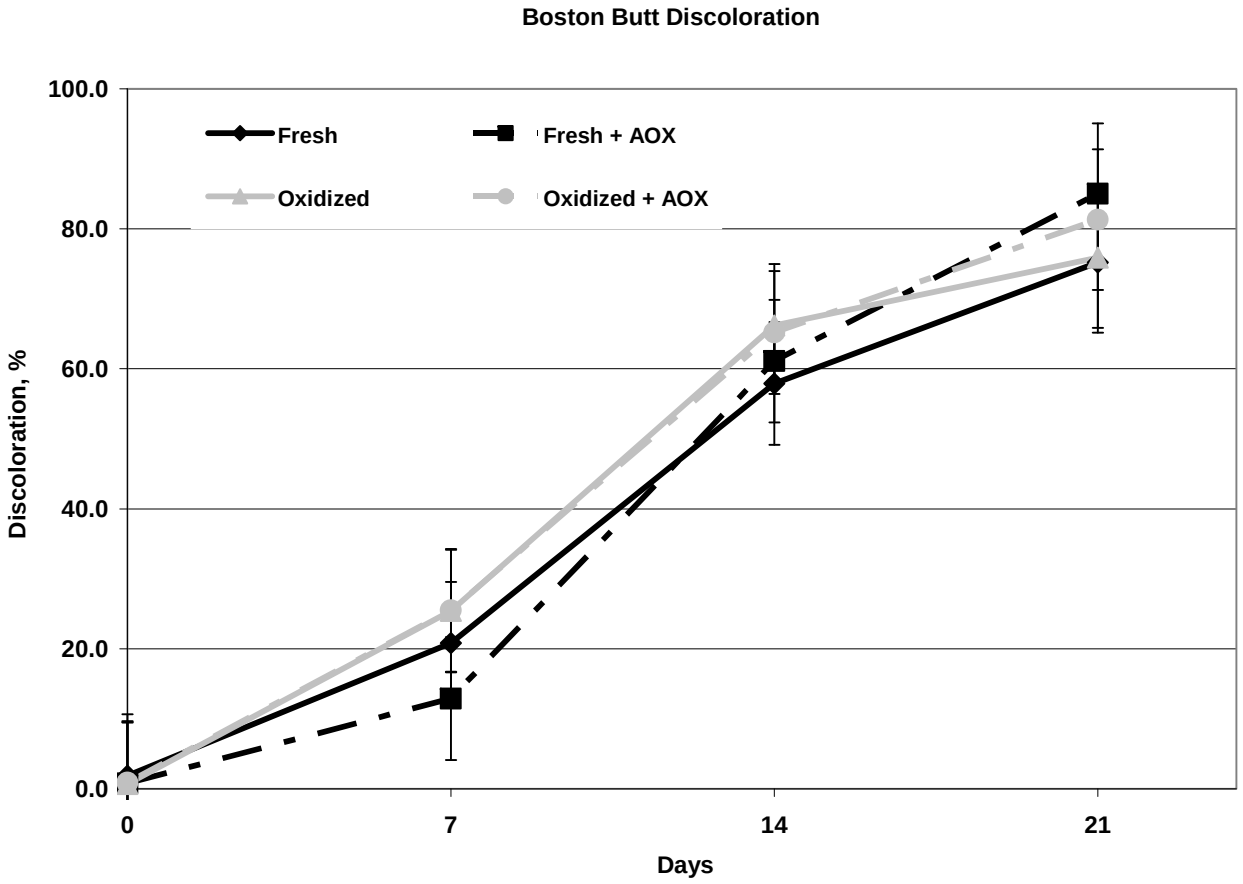
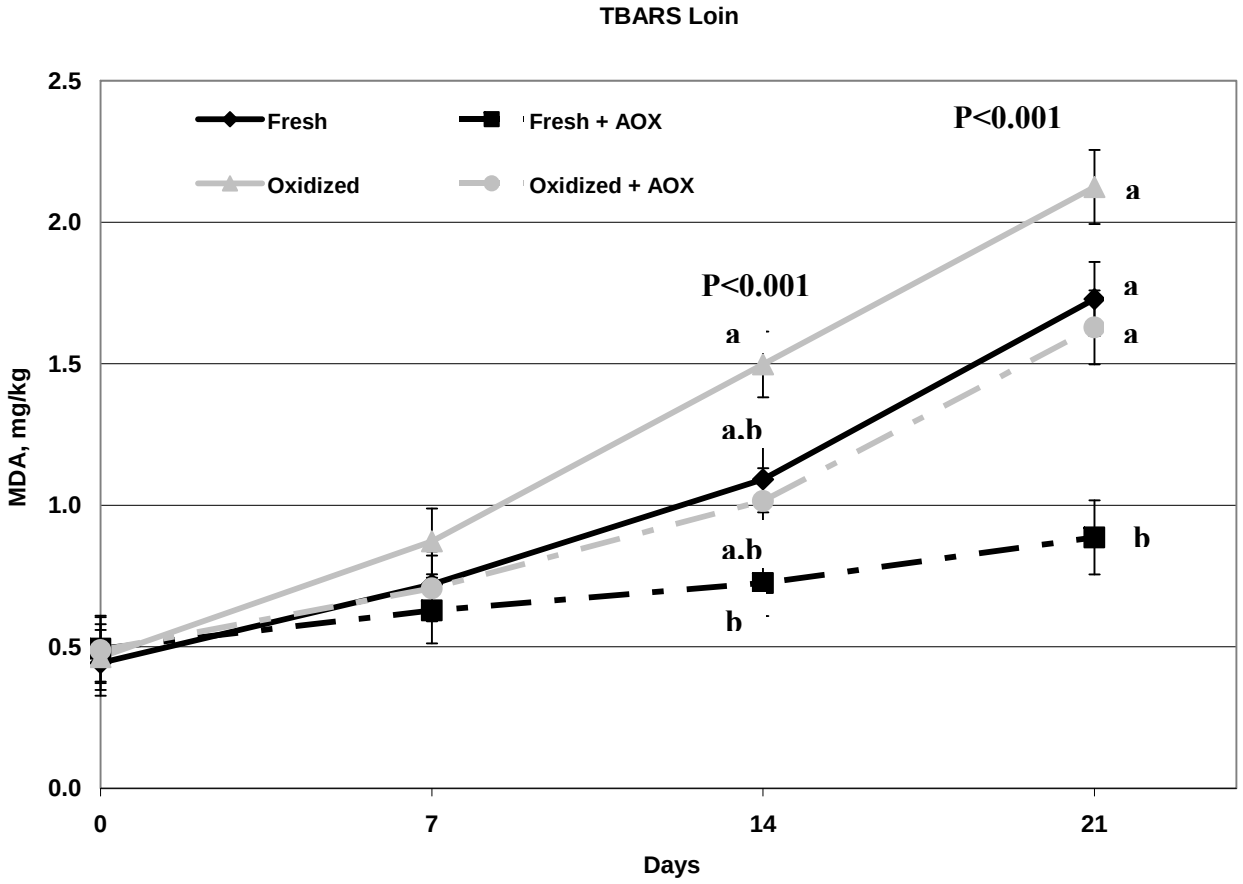
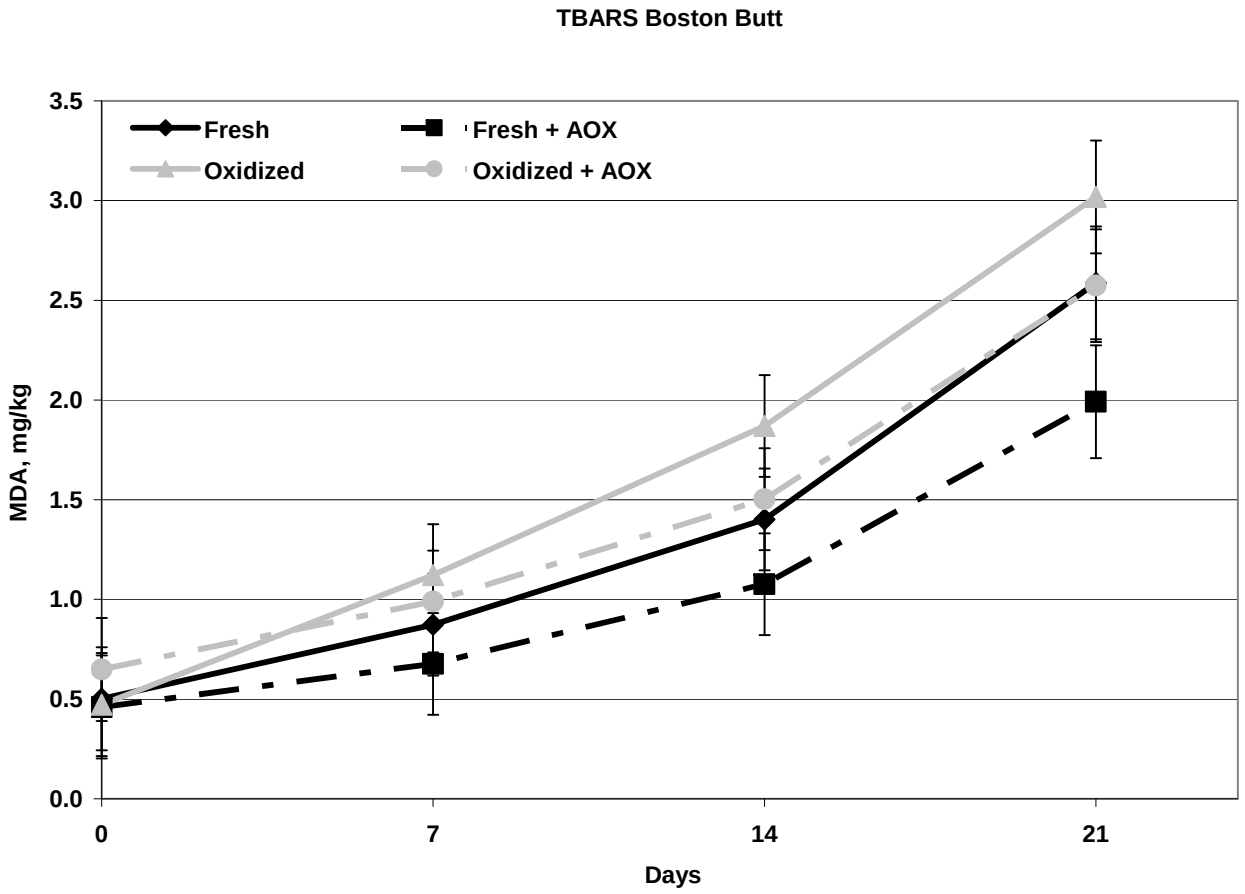


Figure 3.4. Effect of oil × antioxidant interaction over time (21 days) on shelf-life (TBARS) of loin.



[a, b] Different Superscripts in the same evaluation day means statistical difference.

Figure 3.5. Effect of oil × antioxidant interaction over time (21 days) on shelf-life (TBARS) of ground Boston Butt



CHAPTER IV

EFFECTS OF OXIDIZED CORN OIL AND SYNTHETIC ANTIOXIDANT BLEND ON ANTIOXIDANT ENZYME ACTIVITY OF OXIDATIVE AND GLYCOLYTIC MUSCLE FIBER TYPES

ABSTRACT

The objective of the study was to evaluate the effect of protected or unprotected fresh or oxidized corn oil on antioxidant (AOX) enzyme activity of oxidative (diaphragm) and glycolytic (loin) muscles of pigs. A total of 32 barrows were selected for AOX enzyme activity evaluation, after being fed for 56 days with one of the following experimental diets: fresh oil, fresh oil + AOX (blend of ethoxyquin and TBHQ), oxidized oil or oxidized oil + AOX. Diaphragm and loin samples were collected and AOX enzyme activity determinations including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were performed. All activities were related to protein concentration for each muscle. Superoxide dismutase activity was increased ($P<0.04$) for diets formulated with AOX. Also, CAT and GPx enzyme activity were increased ($P<0.01$) in diaphragm compared to loin. In conclusion, CAT and GPx activity were increased in muscles with higher oxidative metabolism, and SOD enzyme activity was increased in animals fed AOX protected oils.

INTRODUCTION

Oxidative stress is defined as an imbalance between free radicals, or pro-oxidants, and antioxidants systems (Droge, 2002). Skeletal muscle is highly susceptible to oxidative stress caused by several endogenous and exogenous pro-oxidants. Endogenous

oxidative stress in muscle is caused by free radicals produced during exercise, especially during aerobic contraction (Jackson, 1999). Exogenous oxidative stress could be influenced by exposure of tissues to several physical factors, such as irradiation and ultraviolet light, and dietary factors, such as polyunsaturated fatty acids (PUFA) making tissues susceptible to oxidative stress (Jensen et al., 1998; Jackson, 1999). Skeletal muscle maintains oxidative-antioxidant balance through antioxidant systems. Antioxidants (AOX) systems can be exogenous such as antioxidants supplemented by diet or endogenous as part of homeostasis systems in tissues, such as antioxidant enzymes (Droge, 2002).

Among the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are the most studied (Droge, 2002; Ji, 2008). Several studies have evaluated AOX activity of pig tissues in response to oxidative dietary challenges and dietary supplementation of antioxidants (Lauridsen et al., 1999; Carreras et al., 2004; Sárraga et al., 2006; Fernández-Dueñas et al., 2008). In pig skeletal muscle, AOX enzyme activity increased as animals reach market age, approximately between 126 to 168 days of age (Xu et al., 2007).

Muscle fiber type also can play an important role in free radical generation and AOX enzyme activity during oxidative stress. Reactive oxygen species (ROS), such as superoxide anion are greatly generated during high O_2 consumption rates, such as exhaustive physical activity in skeletal muscle (Dröge, 2002). Oxidative muscle fiber types are more aerobic and contain more mitochondria compared to glycolytic muscle fiber types (Jackman and Willis, 1996). Diaphragm and soleus are examples of muscles that contain a higher percentage of oxidative or red fibers; whereas longissimus and the

deep and superficial sections of vastus lateralis contain higher percentages of glycolytic or white fibers (Eddinger et al., 1985; Ji et al. 1992; Toniolo et al., 2004). Also, AOX enzyme activity varies by the muscle fiber type and is higher in oxidative fiber than in glycolytic fibers (Ji et al. 1992; Ji, 2008).

Given the inherent differences in free radical generation and AOX enzyme activity between muscles of different fiber types, the objective of the study was to evaluate the effect of either AOX protected or unprotected fresh or oxidized corn oil on AOX enzyme activity of oxidative (diaphragm) and glycolytic (loin) muscle fiber types of finishing pigs.

MATERIAL AND METHODS

Animal Background

Animal use protocol designed for this study was approved by the Institutional Animal Care and Use Committee (IACUC) on April 2008, and the approval number was 08081.

Animal handling and experimental design was described in the Chapter II. Briefly, a total of 160 barrows were used for the live phase of the experiment. Experiment was carried out as a CRBD in a factorial arrangement (2×2), with 2 types of corn oil (5% fresh or oxidized corn oil) and 2 levels of antioxidant (with or without AOX). Antioxidant (SANTOQUIN® Q, to provide 10 ppm Terbutyl-hydroquinone (TBHQ) and 132 ppm of Ethoxyquin (EQ) in the final diets) was provided by NOVUS Inc. (St. Charles, MO, USA).

After the live phase, the pig with final weight closest to the pen mean was selected from each pen for AOX enzyme activity. After 18hr feed withdrawal period, a total of 32 barrows were transported to the Meat Science Laboratory, University of Illinois at Urbana-Champaign. Barrows were humanely slaughtered under USDA inspection.

Muscle Sample Collection and Preparation

Prior to carcass chilling, approximately 50 g of diaphragm as a representative oxidative muscle and 5 g samples (cores) of loin muscle as a representative glycolytic muscle were collected. Samples were immediately wrapped with tin foil and frozen in liquid nitrogen and stored at -80 C° for AOX enzyme activity analyses, including SOD, GPx and CAT.

Frozen samples were homogenized in a buffer solution as described by Young et al. (2003). Briefly, 100 mg of muscle sample was disrupted with 1 ml of the following buffer solution: 50 mM TRIS HCl, 1 mM EDTA, 0.25 M sucrose and a pH of 7.4. Tissue disruption was performed with the Tissue Lyser II (Qiagen, Valencia, CA, USA) at 30Hz shaking speed for 3 min and using stainless steel beads (5 mm diameter). After tissue disruption, samples were centrifuged at 10,000 ×g at 4 C° for 15 minutes, and approximately 300 µl of supernatant were collected and immediately frozen at -80°C until the enzyme activity assays were performed. All three AOX enzyme activities were measured colorimetrically on a Bio-Tek plate reader (Bio-Tek Instruments INC., Winooski, VT, USA).

Antioxidant Enzyme Activity

Copper/zinc (Cu/Zn) dependent SOD activity was determined using a SOD assay kit from Cayman Chemical Company (Ann Arbor, MI, USA). Briefly, superoxide radicals are detected by tetrazolium salt generated from xanthine oxidase addition and incubation for 20m at room temperature. Enzyme activity of Cu/Zn SOD was measured colorimetrically at 440nm; Cu/Zn SOD activity was expressed as nmol/min/mg of protein and compared to bovine erythrocyte Cu/Zn SOD standard curve.

Glutathione peroxidase activity was determined using a GPx assay kit from Cayman Chemical Company (Ann Arbor, MI, USA). Briefly, indirect GPx activity was measured by a coupled reaction with glutathione reductase (GR). Reaction was initiated after cumene hydroperoxide addition. Oxidation of NADPH to NADP was measured colorimetrically at 340 nm for at least 5 m; GPx activity was expressed as nmol/min/mg of protein and compared to bovine erythrocyte GPx standard curve over time.

Catalase activity was determined using a CAT assay kit from Cayman Chemical Company (Ann Arbor, MI, USA). Briefly, CAT enzyme activity was based on methanol reaction in the presence of hydrogen peroxide (H_2O_2) for 20 minutes at room temperature, to produce formaldehyde; the reaction was terminated by potassium hydroxide and colored by a chromogenic compound (Purplad) incubated for 10 minutes at room temperature. Activity was measured colorimetrically at 540nm; CAT activity was expressed as nmol/min/mg of protein and compared to a predetermined formaldehyde standard curve.

Protein concentration was determined as described by Pierce BCA Protein Assay kit from Pierce Biotechnology laboratories (Rockford, IL, USA). Briefly, protein

determination was measured colorimetrically at 558nm, expressed as mg/ml and compared to a predetermined bovine serum albumin (BSA) standard curve.

Statistical Analysis

Antioxidant enzyme activity were analyzed as a complete randomized block design (CRBD) in a factorial arrangement (2 × 2 × 2) with 2 types of corn oil (5% of fresh or oxidized corn oil), 2 levels of antioxidant (with or without AOX) and 2 different muscle fibers (diaphragm as oxidative fiber and loin as glycolytic fiber). Harvest day of the experiment (4 starting days) was considered as blocking criteria. Experimental unit for the antioxidant enzyme activity was the carcass and we had a total of 8 replicates (carcasses) per treatment. Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included block, main effect of oil, main effect of AOX and main effect of muscle fiber type as well as the interaction oil × AOX × muscle fiber type. Least squares differences were separated by probability of difference option (PDIFF) and P values were adjusted by the Bonferroni approach to declare statistical difference at $P<0.05$.

RESULTS AND DISCUSSION

The main effect of oil type on AOX activity was not significant; however, SOD enzyme activity was increased ($P<0.04$) for diets formulated with AOX protected oil (Table 4.1). Superoxide dismutase activity observed in our experiment is in agreement with activity reported by Carreras et al. (2004) for broilers diets supplemented with α-tocopherol, but in disagreement with SOD activity reported by other authors (Renerre et al., 1999; Lauridsen et al., 1999; Young et al., 2005) for muscles from animals fed diets

supplemented with natural AOX (α-tocopherol). On the other hand, CAT and GPx activities reported by same authors were similar to enzyme activities observed in our experiment with no response to AOX supplementation. Catalase activity in our experiment is in disagreement to CAT activity reported by Sárraga et al., (2006) who observed an increase of CAT activity in turkey breast for animals supplemented with natural AOX (α-tocopherol and β-carotene).

A significant main effect of muscle on AOX enzyme activity was observed in our experiment. Superoxide dismutase was not different between diaphragm and loin; however, CAT and GPx enzyme activities were increased ($P<0.01$) in diaphragm when compared to loin, regardless of oil type and AOX protection (Figure 4.1). Superoxide dismutase activity in our experiment is in disagreement to the SOD activity reported by Renerre et al. (1996) in beef diaphragm, turkey sartorius muscle (Renerre et al., 1999), and pig psoas major (Lauridsen et al., 1999) when compared to glycolytic muscle types by same authors. However, the increased CAT and GPx activities in oxidative muscle observed in our experiment compared to loin are in agreement to enzymes activities reported in the literature (Renerre et al., 1996; Renerre et al., 1999; Lauridsen et al., 1999; Hernández et al., 2002; Sárraga et al., 2006). Our results suggest that increased CAT and GPx enzyme activity in diaphragm is probably due to the increased oxidative metabolism of red muscle fibers in comparison to white muscle fibers. Furthermore, Hernández et al., (2002) have proposed that increased CAT and GPx activity can help to prevent lipid oxidation during meat storage. If this is true, then CAT and GPx activity observed in our experiment could be playing an important role for the decreased loin

TBARS values over time in pork from animals fed AOX protected oil reported in Chapter III.

A two-way oil × muscle interaction was found (data not shown) for CAT activity with increased ($P<0.02$) activity in diaphragms from barrows fed diets formulated with fresh oil when compared to diaphragms from barrows fed diets formulated with oxidized oil; whereas loin from barrows fed diets formulated with fresh or oxidized oil were not statistically different. Our results suggest that CAT activity was decreased in oxidative muscles when animals were fed an oxidized source of PUFA. Previous research (Renerre et al., 1999; Young et al., 2003; Young et al., 2005) showed increased CAT activity in muscles with higher proportion of glycolytic muscle fiber types, such as loin of pigs and pectoralis major of turkeys, both fed diets containing high levels of PUFA. This suggests that high content of PUFA in muscle can act as pro-oxidants favoring CAT activity. Finally, no three-way oil × AOX × muscle interaction was observed in this study.

CONCLUSION

Muscle fiber type is a fundamental factor for AOX enzyme activity in skeletal muscle. Muscle with higher proportions of oxidative fibers contains greater CAT and GPx activity. On the other hand, only SOD activity in skeletal muscle seems to be increased by synthetic AOX protection of supplementary energy sources in the diet. Finally, CAT activity is ameliorated if the supplementary energy sources provided in the diet are of a good quality (fresh vs. oxidized).

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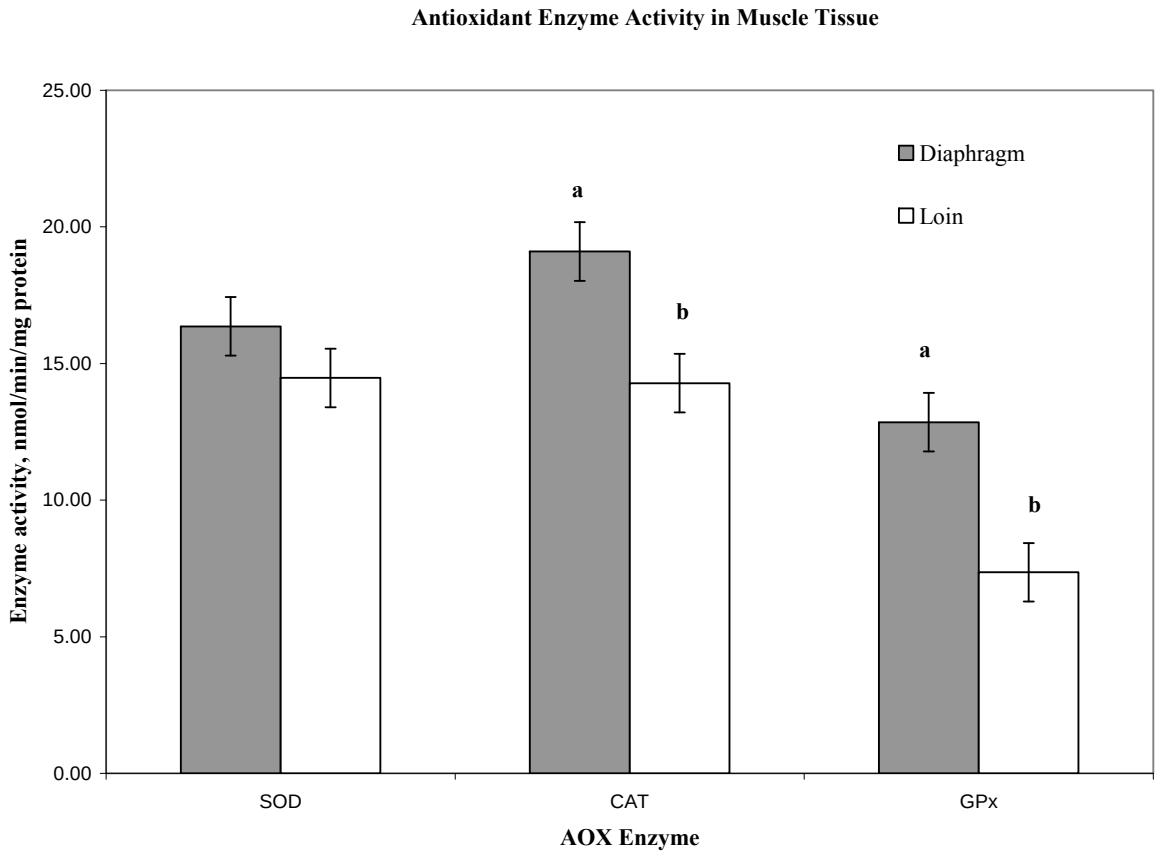
FIGURES AND TABLES

Table 4.1. Main effect of oil and antioxidant enzyme activity in muscle

	Oil		Antioxidant			P-value	
Activity [a]	Fresh	Oxidized	No AOX	AOX	SEM	Oil	AOX
SOD [b]	15.87	14.93	13.83	16.99	1.023	0.53	0.04
CAT [c]	18.08	15.30	15.26	18.12	1.364	0.12	0.11
GPx [d]	10.85	9.37	9.31	10.90	0.828	0.19	0.16

[a] Activity expressed as nmol/min/mg of protein.
[b] SOD = Superoxide dismutase.
[c] CAT = Catalase.
[d] GPx = Glutathione peroxidase.

Figure 4.1. Antioxidant enzyme activity in diaphragm and loin muscle



Diaphragm vs. Loin (D vs. L) were not statistically different for superoxide dismutase (SOD)

Catalase (CAT) and Glutathione peroxidase (GPx) were different between diaphragm and loin ($P<0.01$).

AUTHOR'S BIOGRAPHY

Demián Fernández-Dueñas was born in Ciudad de México, México on June the 5th, 1975 to Hugo Fernández and Patricia Dueñas. He has an older brother Adrian, and a younger sister Aline. He attended the Instituto Juventud del Estado de México high school, after which he attended the Veterinary College of the Facultad de Estudios Superiores Cuautitlan (FES-C) at the Universidad Nacional Autónoma de México (UNAM). During the Veterinary College he earned the Scholarship "UNAM Foundation Scholarship Programs in recognition of distinguished UNAM students to develop studies at foreign Universities" at the University of Montreal, in Montreal, Canada from January 1997 to May 1997. After obtaining his Veterinary Medicine and Animal Science degree he started to work in the feed industry working for two different companies specialized in animal nutrition as a technical consultant. After two years developing his experience in the industry, he decided to start the Master program "Master in Health and Livestock Production Sciences" at the UNAM where he obtained his degree under the direction of Dr. José Cuarón. In 2005, he joined the Meat Science and Muscle Biology Laboratory to pursue his PhD in Animal Science degree at the University of Illinois at Urbana-Champaign under the direction of Dr. John Killefer and Dr. Floyd McKeith. His research focused on the impact of feeding oxidized oil and synthetic antioxidant on animal performance, oxidative status of tissues, pork quality, and shelf-life and antioxidant enzyme activity of skeletal muscle of pigs.

On August the 5th, Demián was married to Arlette Soria from Coatzacolacos, Veracruz, México.