**Influence of Dietary Fat and Vitamin E on Shelf Life of Ground Broiler Breast and Thigh Meat**

by

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## Abstract

A series of experiments were conducted to determine the effects of dietary fat sources and vitamin E supplementation on shelf life of ground broiler breast and thigh meat. The first experiment evaluated the influence of dietary fat sources and inclusion levels on ground breast and thigh meat shelf life stored under refrigerated conditions (2°C) with either oxygen permeable or impermeable packaging. Corn oil resulted in higher raw ground thigh meat lipid oxidation rate than lard. Microbial counts and lipid oxidation increased with refrigerated storage of raw ground meat and was higher in thigh than in breast muscle. Oxygen impermeable packaging appeared to reduce lipid oxidation.

Experiment two evaluated the influence of dietary fat sources on ground breast and thigh meat shelf life stored under frozen conditions (-18°C) for 3 and 6 months with either permeable or impermeable packaging. Oxidative deterioration was nearly 3-fold higher in ground thigh than in ground breast meat. At 3 months of storage, higher levels of oxidation occurred with corn oil than lard; with higher fat inclusion level and with oxygen permeable packaging. Ground meat in oxygen impermeable packaging showed lower lipid oxidation than oxygen permeable film. An improvement in oxidative stability and a reduction of bacterial growth were seen in ground meat samples stored in impermeable packaging at 3 months of storage.

Two final experiments investigated the effects of vitamin E supplementation on ground breast and thigh meat (either raw or cooked) shelf life under refrigerated and frozen storage. Data from both experiments confirmed that dietary vitamin E supplementation at high levels (>120 IU/kg) significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat. Microbial and oxidative changes that occur during refrigerated and frozen storage of ground and cooked broiler meat were positively influenced by dietary vitamin E supplementation. Significant effects were achieved with thigh meat (especially cooked) but not with breast meat. Lipid oxidation increased during refrigerated storage on both raw and cooked ground meat. Unsaturated lipid content of poultry meat may justify the need for different packaging types or diet supplementation with antioxidants to optimize the quality and shelf life of ground poultry meat.

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# CHAPTER I

## GENERAL INTRODUCTION

Broiler chicken production greatly increased in the USA throughout the 1980s and 1990s due to consumers becoming more health conscious as well as unprecedented increase in exports. Increase in per capita consumption has also been attributed to the introduction of products that are further processed and easier for the consumer to prepare. Currently, most poultry production in the United States is confined to the Southeastern states. Factors such as vertical integration, low cost of production, drive for efficiency in all phases of production, improved flock health programs and mechanized processing have been the primary drivers for the expansion of the broiler industry in the US. Broiler chicken production is expected to expand further in 2011 and USDA predicts broiler production next year to be 37 billion lbs (live), 3% higher than in 2010 and 5.8% higher than in 2009.

Consumption of broiler chicken meat has also been increasing globally over the last 50 years and developments of technology have allowed the poultry industry to meet this increasing market demand. Poultry constitute about 30% of the world's total meat consumption (FAO, 2006).The U.S. poultry industry is the world’s biggest producer and second largest exporter of poultry meat globally. As much as 18% of the total production is being exported to other countries (USDA, 2010). The consumption of poultry meat in

the U.S. (broilers, other chicken, and turkey) is considerably higher (83.6 lb per capita) than beef and pork (46.7 and 58.5 lbs per capita, respectively), but less than total red meat consumption. Poultry dark meat has been considered as a major underused raw material due to the increasing demand for further-processed white meat products (i.e. breast meat). Dark meat contains more fat and higher bone to meat ratio, making it less valuable, as the consumers pay for less meat per unit weight. Boneless skinless dark meat production has been steadily increasing, as heavy (>3kg) market weight reduces the labor cost associated with manual deboning.

Appearance (i.e., color, pigmentation, defects, etc.) is the major criterion for visual evaluation of meat quality and purchase decision by consumers. However, other quality attributes, such as tenderness, drip loss, cook loss, water holding capacity and pH are equally important during the preparation of meat, both by retail customers and consumers. Consumers discriminate against fresh or frozen, as well as whole or ground meat products that have lost their fresh appearance. Hence, processors are extremely concerned about the shelf-life of raw retail products sold fresh (refrigerated) and frozen, as premature spoilage results in significant economic losses. Recent retailing trends, such as marketing ready-to-eat convenience products have resulted in increased production of pre-cooked and restructured meat products. Although these products are more shelf-stable than raw products, they are equally susceptible to spoilage through lipid oxidation and off-flavor development.

# CHAPTER II

# LITERATURE REVIEW

## SHELF LIFE OF FRESH AND FROZEN POUTRY MEAT

Shelf life can be defined as the length of time that food and other perishable items are given before they are considered unacceptable for sale from a sensory, nutritional or safety perspectives (Brown et al., 2007). Extending the shelf life of poultry meat and products are a major goal for the poultry industry. Because fresh poultry meat is highly perishable, it is essential to maintain the shelf life of the product as long as possible. Both microbial and oxidative changes lead to spoilage of fresh and frozen poultry meat. .

### I. Microbial Spoilage:

Foods of animal origin have higher loads of bacteria than most other foods because carcass contamination and cross-contamination can occur at several points during the slaughter process. Microbial load can greatly increase on poultry carcasses due to cross-contamination during scalding, defeathering, evisceration and immersion chilling from other birds or processing equipment. Thus, the shelf-life of chicken products greatly depends on the level of microbial contamination at packaging.

Microbial spoilage occurs primarily due to the formation of metabolites from the utilization of nutrients by the microflora present in the meat. In addition to nutrient availability, factors such as water activity, pH, oxygen, and temperature also influence the growth of microorganisms on meat. Temperature is the most important factor

affecting the growth of bacteria and ultimately shelf life of poultry meat. Poultry products are often subjected to variations in holding temperatures during processing, storage, distribution, and display at retail sale. Broiler carcasses maintained under optimal conditions (< 4°C) should have a shelf life of 12-14 d. The rate of spoilage is 2 to 3 times faster at 10 and 15°C, respectively (Daud et al., 1978). The initial microbial load of meat is mostly mesophilic (microorganism with a growth optimum of 20 to 45$^{o}$C, a minimum of 15 to 20$^{o}$C and a maximum of ≤45$^{o}$C) with a level of $10^2$ to $10^4$ CFU/cm$^2$ (Dainty and Mackey, 1992). When meat is refrigerated, the growth of psychrotrophic bacteria (microorganism with a growth optimum of less than 15$^{o}$C) is favored. Because of a shorter generation time than the other microorganisms under aerobic conditions and low temperatures, *Pseudomonas* spp. usually prevail under conditions of poor refrigeration temperature (10°C), constitute up to 50 to 90% of the overall microbial population and spoil the meat (Dainty and Mackey, 1992). The bacterial load present immediately after processing directly affects product shelf life. If the initial bacterial load can be reduced, the shelf life can be increased dramatically. Also, less time is required for bacterial populations to reach numbers high enough to produce spoilage when large numbers of bacteria are present initially (Brown, 1957). Total number of bacteria on poultry stored at 0°C decreases after a few days of storage, because conditions for the growth of most species of bacteria are no longer optimal (Ayres et al., 1950). An extended shelf life of meat under anaerobic conditions is observed because the spoilage caused by lactic acid bacteria (LAB) that grow on meat occurs later compared to the spoilage caused by the aerobic bacteria under aerobic conditions. However, some LAB, like *Lactobacillus sakei*, can cause spoilage by production of hydrogen sulfide from the degradation of cysteine

even under glucose-limiting (raw meat) or $O_2$-limiting conditions (Egan et al., 1989). Barnes and Impey (1968) characterized bacterial isolates from spoiled poultry as pigmented and non-pigmented pseudomonads, while, *Acinetobacter*, *Pseudomonas putrefaciens*. and *P. fluorescens* have been shown to be a primary source of spoilage of fresh poultry (Russell et al., 1995). *Pseudomonas* spp. reside on the exterior of birds, equipment, walls, floors, and water supply of the processing plant. Psychrotrophic bacteria, such as the pseudomonads, grow well at refrigeration temperatures (<4°C), and can multiply on the surface of poultry meat using glucose and other carbohydrates as energy sources. Once glucose has been depleted, bacteria can grow by utilizing amino acids found in skin and muscle. Pooni and Mead (1984) reported that amino acid metabolism produces odorous end products, making food unacceptable to the consumer. Cox et al. (1975) and Thornley et al. (1960) observed that spoilage defects of poultry, such as putrid and ammonia-like odors, occur when spoilage bacterial populations reach $10^6$ to $10^7$ CFU/$cm^2$. The bacteria associated with white and dark poultry meat have been reviewed extensively (Barnes and Impey, 1968; Barnes, 1976; Lillard and Ang, 1989). It is important to note differences between the breast muscle (i.e., white meat) and leg muscles (i.e., dark meat) due to differences in moisture, protein, and fat (Xiong et al., 1993), amino acid profile (Hamm, 1981), and heme concentration (Saffle, 1973).

## II. Oxidative Rancidity

Meat oxidation is a very general process that can affect lipids, pigments, proteins, DNA, carbohydrates, and vitamins (Kanner, 1994). In muscle and fat tissue, oxidation continues postmortem and affects the shelf-life of meat and meat products. Lipid oxidation is one of the primary mechanisms of quality deterioration in foods, especially

in meat products (Kanner, 1994; Morrissey et al., 1998). This becomes more important because of a trend toward increasing the (long-chain) polyunsaturated fatty acid (PUFA) content in meat products. To maximize the oxidative stability of meat, antioxidants, such as α-tocopheryl acetate, are added to feeds. The beneficial effect of dietary supplementation for the subsequent enhancement of lipid stability in muscle foods has been extensively reported for poultry, beef cattle, veal calves, and pigs (Gray et al., 1996; Jensen et al., 1998). Currently, dietary supplementation with vitamin E may be one of the most effective means of extending oxidative stability of further processed raw and cooked meat products.

In the presence of oxygen, light, heat or metals, unsaturated fatty acids are converted to fatty acid radicals which undergo further reduction to produce more free radicals or peroxides, which in turn act on other fatty acids. Antioxidants inhibit lipid oxidation by blocking the formation of fatty acid radicals and by reacting with free radicals to terminate this chain reaction. Oxidation depends on both intrinsic factors (unsaturated fatty acids concentration, myoglobin, enzymes and iron) and extrinsic factors (light, temperature and oxygen consumption) (Jensen et al., 1998; Underland, 2001).

The relative protective effect of an antioxidant against oxidative rancidity is also dependent on storage time (Chahine, 1978). However, within a specific storage period, increasing the amount of antioxidants reduces the intensity of fat oxidation. Increase in storage temperature also affects the oxidation rate of unsaturated fats (Villwock and Hartfiel, 1982). Lipid oxidation is a major cause of rancidity and undesirable odors and flavors, which in turn affect the functional, sensory, and nutritive values of meat products

(Gray et al., 1996). The development of undesirable flavors is caused by rapid oxidation of the highly unsaturated phospholipid fraction. Hydroperoxides, initial products of oxidation, tend to decompose to form aldehydes, ketones, alcohols, and lactones. These short-chain carbon compounds can impart off-flavors and off-odors to the product (Pearson et al., 1983). Oxidative rancidity leads to formation of malonaldehyde or derivatives of this compound. Malonaldehydes are commonly measured by thiobarbituric acid (TBA) test, using tetraethoxypropane as a standard (Sinnhuber and Yu, 1958).The degree of fat oxidation in meat usually is determined by the TBA method, which is used as an indicator of rancidity (Guille´n-Sans and Guzma´n-Chozas, 1998).

The changes that occur in poultry meat during storage are generally chemical and microbiological in nature and are primarily influenced by temperature and oxygen. Both vacuum and modified atmosphere packaging have been proven to prevent off-flavor development and prolong shelf life in precooked refrigerated and frozen products. Microbial levels, as well as pigment and unsaturated lipid contents of poultry meat, may justify the need for different packaging types than those used with red meat to optimize the appearance and shelf life of ground poultry (Dawson et al., 1995). Ground meat packed with low $O_2$ permeable packaging has shown to decrease microbial growth and lipid oxidation (Dhananjayan et al., 2006; Keokamnerd et al., 2008; Patsias et al., 2006).

## IMPACT OF NUTRITION ON MEAT QUALITY

In poultry meat industry, the production of ready-to-cook (RTC) and ready-to-eat (RTE) products is the fastest growing market segment. This further processing segment includes products that have been subjected to some degree of cooking (partial or full) to

facilitate ease of preparation by retailers and consumers. Poultry meat enjoys popularity with consumers because of its nutritionally favorable fat level and composition (i.e., enhanced amounts of unsaturated fatty acids). However, high amounts of unsaturated fatty acids can increase lipid oxidation rate with subsequent development of off-flavors and off-odors that affect nutritive value, sensory characteristics and meat quality (Barroeta, 2007).

## I. Dietary Fat

Many different fat sources have been used as feed ingredients by the broiler industry. Animal fats and vegetable oils are usually added to broiler diets to increase energy concentration and to improve productivity. Other reasons for fat supplementation include alteration of the fatty acid profile of meat products (value-added products) and development of adipose tissue The addition of fat to diets, also improves the absorption of fat-soluble vitamins, increases the palatability of the rations and increases the efficiency of consumed energy (Nayebpor et al., 2007). Tallow and lard are the most common sources of saturated animal fat sources in broiler diets. These fat sources contain high amount of saturated fatty acids rich in palmitic (C16:0) and steric acids (C18:0). Compared to less saturated fat such as poultry fat and vegetable oils, lard contains lower levels of the polyunsaturated fatty acids, such as linoleic and linolenic acid (Peebles et al., 1997). Previous research indicate that individual polyunsaturated fatty acids (PUFA) are absorbed better than saturated fatty acids (Sklan, 1978). Fat inclusion in broiler diets must take into account the effect on carcass fat quality, because dietary fatty acids are incorporated with little change into body fat (Olomu and Baracos, 1991; Scaife et al., 1994). Previous research also indicate effect of dietary fat on abdominal fat

characteristics in broilers (Yau et al., 1991). Diets containing a relatively high concentration of linoleic acid (C18:2) have been correlated with soft fat tissues and high susceptibility of meat to oxidation (Zollitsch et al., 1997). Smink et al. (2008) reported that digestibility and the proportion of C16:0 in abdominal fat and breast meat was found to be higher in broilers fed tallow when compared with birds fed vegetable oil.

Vegetable oils rich in PUFA are commonly used in broiler diets. The digestibility of corn oil is approximately 90% and contains high proportion of fatty acids compared to poultry fat and commercial animal-vegetable blend (Squires et al., 1991). Oxidation can be prevented by adding antioxidants in the feeds. Antioxidants help protect the oxidative spoilage of fats in feed and in meat, while light, oxygen and high temperatures can accelerate the oxidative process. The efficiency of fat utilization by poultry is dependent on fatty acid composition. Many factors influence fat absorption that will be influenced by free fatty acids (FFA) or triglycerides and the position of fatty acids in triglyceride molecules affect absorption. The digestibility of FFA decreases when the length of the carbon chain and saturation increases. Increasing the content of unsaturated fatty acids in relation to saturated fatty acids helps improve the absorption of saturated fatty acids (Young and Garrett, 1963). Oleic and linoleic acids readily form mixed micelles with bile salts in which saturated acids are then solubilized; monoglycerides of the fatty acids appear to be more effective than the FFA. Fat digestion is facilitated by the combined action of bile acids, lipase, and colipase. Beef tallow has a lower fat digestibility and a lower metabolizable energy (ME) content than vegetable oils, and these have been attributed to higher content of long-chain saturated fatty acids (Blanch et al., 1995). Several studies have shown better utilization of unsaturated fats, leading to higher ME for

unsaturated fats than for saturated fats. Zollitsch et al. (1997) have reported that unsaturated vegetable oils produce lower fecal energy losses and higher ME than animal fats. This higher ME of unsaturated fat could be expected to cause higher fat deposition, because the energy could be stored as triglycerides in fat depots. However, Vila and Esteve-Garcia (1996) found that vegetable oil produced less abdominal fat deposition in broilers than animal fat at different levels of fat inclusion. Abdominal fat deposition increased with increasing fat inclusion level in birds fed animal fats, whereas it remained constant in birds fed vegetable oil. Sanz et al. (1999) found less abdominal fat in broilers fed vegetable oil than in those fed fat from animal sources. Mickelberry et al. (1966) reported that the influence of fat composition in the diet was more affected in abdominal fat than in fat from breast meat, thigh meat and liver. Hrdinka et al. (1996) determined different oils on their ability to influence both adipose tissue and intramuscular fat. They found abdominal fat and subcutaneous fat had similar fatty acid composition with higher content of PUFA, which was more influenced by the dietary fat than the intramuscular fat. Crespo and Esteve-Garcia (2001) reported that sunflower and linseed oils caused lower percentages of abdominal fat at 6 and 10% inclusion levels. In male broilers, abdominal fat increased with increased fat concentration. While in females, abdominal fat deposition showed a significant interaction. Increased fat content of the diets produced higher abdominal fat deposition for tallow and olive oil, whereas it remained constant for sunflower and linseed oils. Supplementation of concentrates with 4% and 8% lard improved body weight gains and feed conversion ratio (Rutkowski et al., 1997). Birds fed diets containing 30% fat showed more rapid growth than lower fat levels. High fat and high energy starter diets have been shown to influence body weight and body

composition of broilers at 11 d of age. Leveille et al. (1975) reported that birds fed 8% supplemental corn oil showed an increase in carcass protein and higher protein retention. Furthermore, in young broilers, the overall dietary energy value of fat has been found to decrease with degree of saturation (Wiseman and Salvador, 1991).

Many studies have shown changes in long chain PUFA metabolism in body systems (Cherian and Sim, 1993). There have been attempts to increase the n-3 fatty acid content of poultry by supplementation of poultry diets with oils rich in n-3 fatty acids. Birds supplemented with linseed oil, rich in linolenic acid oil had significantly higher levels of n-3 fatty acids and higher n-3:n-6 ratios than those supplemented with the same level of menhaden oil (Chanmugan et al., 1992). Levels of eicosapentaenoic acid were increased in the group fed linseed oil or menhaden oil compared to those fed corn oil. Feeding linseed oil, which is rich in α-linolenic acid, to chicks depresses the amount of arachidonic acid but concomitantly raises levels of eicosapentaenoic acid in organ lipids presumably by enhancing (n-3) PUFA formation.

Fats high in polyunsaturated fatty acids are highly sensitive to oxidation reactions during storage and are likely to turn rancid at high temperatures. The presence of fats and oils in poultry rations provides a suitable medium for rancidity as feed nutrients react with oxygen to form free radicals (Bishawi, 1993). Rancidity deteriorates the nutritive value of feeds and thus causes economic losses by adversely affecting performance and health of broilers (Engberg et al., 1996). Award et al. (1983) reported that the consumption of poultry diets containing rancid fat (0.2 - 6.0%) was associated with high mortality (65%), diarrhea, reduced feed intake and reduced body weight gains.

Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life. In practice, meat is stored and cooked for consumption. These processes of cooking and storage of meat promote degradation of its lipid fraction (Jensen et al., 1997; Ruiz et al., 1999, 2001; Grau et al., 2001). The negative consequences of lipid oxidation can be overcome by the use of antioxidants in the diet, such as α-tocopherol, which prevents lipid oxidation and increases the shelf life of meat. Thus, it is of great commercial interest to assess the protective effect of α-tocopherol during storage and cooking processes of poultry meat (Ahn et al., 1995; King et al., 1995; De Winne and Dirinck, 1996; Ruiz et al., 1999; Bou et al., 2001; Grau et al., 2001).

## II. Dietary Vitamin E Supplementation and its role in preventing oxidation

Dietary vitamin E supplementation has been used to maintain overall health in broilers and to enhance the antioxidant activity that inhibits lipid oxidation reaction in the meat. Vitamin E is the major chain reaction interrupting fat-soluble biological antioxidant and has a practical importance in increasing shelf-life of meat (Grau et al., 2001). There are several factors that can affect the intestinal uptake of vitamin E and its subsequent deposition in tissues. The major factors affecting vitamin E absorption are its dietary concentration and the concentration and the fatty acid profile of dietary fat. Absorption of vitamin E occurs mostly via the lymphatic system, the molecules being transported to the liver inside triglyceride-rich chylomicrons. Vitamin E is then secreted by the liver and incorporated into very-low-density lipoproteins (VLDL). It is transported to the interior of cells inside low-density lipoproteins (LDL), which are recognized and removed from the plasma by LDL-specific receptors. Vitamin E acts as an immunomodulator, however

the most important function is the protection of membrane from attack by free radicals. Vitamin E is found in biological membranes in a system stabilized by physicochemical forces that include lipid-lipid interactions between α-tocopherol and polyunsaturated phospholipids. At this site, α-tocopherol protects PUFA, which are extremely susceptible to oxidation, from peroxidation by free radicals produced in many cases by enzymes of the membrane itself (NADPH oxidase). Despite its low molar concentration in the membrane, vitamin E is the principal lipid-soluble antioxidant that acts at the level of cellular membranes to prevent peroxidation and to modulate the metabolism of arachidonic acid via cyclooxygenase and lipoxygenase pathways (Blumberg, 1994). Therefore, the NRC (1994) recommended levels of vitamin E for poultry are intended to prevent deficiency symptoms related to oxidative damage.

The oxidative stability of poultry meat depends on the levels of α-tocopherol present in cell membrane phospholipids, which is dependent on the level of α-tocopheryl acetate added to the diet (Wen et al., 1997). Dietary supplementation with α-tocopherol has been shown to increase vitamin E in muscle tissues and improve the oxidative stability of meat during storage (Carreras et al., 2004).

Vitamin E requirements are linked to the dietary polyunsaturated fatty acid (PUFA) content, because of the protective effect of vitamin E on lipid peroxidation. On the other hand, dietary PUFA interferes with vitamin E absorption. Vitamin E protects fatty acids and cholesterol from oxidation by trapping free radicals. In this process, vitamin E releases a hydrogen atom, which is captured by a peroxyl radical which is thereby reduced to form a hydroperoxide. Vitamin E radicals are stable and do not react with PUFA. Diets for fast growing broilers are generally rich in polyunsaturated fatty

acids and the degree of unsaturated fatty acids in carcass fat is thereby increased. Furthermore, dietary oils can alter the fatty acid composition of mitochondrial and microsomal lipids (Asghar et al., 1990). However, increasing the degree of unsaturation of the muscle membranes by dietary manipulation increases the peroxidation in tissues and muscle foods (Pikul et al., 1984). Thus, there is a need to increase the antioxidant capacity of the tissues, and this is readily achieved by feeding higher levels of vitamin E.

To ensure optimum end-product quality, it is necessary to consider the entire production chain from farm- to-fork. Many studies have focused on the impact of dietary supplements such as vitamin E or fatty acids (Jensen et al., 1998) on meat quality. Supplementation of vitamin E significantly improved meat stability against oxidative deterioration in beef, pork and turkey meat (Mitsumoto et al., 1993; Phillips et al., 2001; Guo et al., 2001). Vitamin E cannot be synthesized by animals and therefore its presence in animal tissue reflects the dietary level and availability. Due to its lipophilic character, the vitamin E absorption depends on animals' fat digestion and absorption. The most commonly used commercial form of vitamin E for animals dietary supplementation is the acetate ester of α-tocopherol. The esters display no antioxidant activity and they are hydrolysed in the gut, releasing the native α-tocopherol that possesses the antioxidant activity. Dietary vitamin E supplementation significantly increases the α-tocopherol content of muscle membranes in many animals and functions as a lipid antioxidant and free radical scavenger (Lauridsen et al., 1997; Guo et al., 2001). The anitioxidant activity of vitamin E depends on the amount of α-tocopherol content in cell membranes, which inhibits the propagation of the lipid oxidation chain reaction (Asghar et al., 1988). In poultry, the amount of α-tocopherol deposited in the muscle cell membranes is related to

the amount of vitamin E included in the diet and the length of the feeding period (Sheehy et al., 1991). Supplementation of 200 mg/kg of Vitamin E in the diet might be needed to reach the potential of antioxidant properties (Sheehy et al., 1991: Morrissey et al., 1998). This level of α-tocopheryl acetate supplementation in feed significantly improved the oxidative stability of both raw and cooked muscle during storage at 4°C for up to 8 d (Monahan et al., 1990). Jensen et al., 1995 reported that raw chicken meat supplemented with levels as low as levels of 100 mg/kg of vitamin E provided several benefits for the oxidative stability of both white and dark meat during 8 d of storage with no development of lipid oxidation detected. In cooked chicken meat, 200 mg/kg of vitamin E in the diet is recommended to inhibit lipid oxidation rate if the birds are fed polyunsaturated fat sources (Jensen et al., 1995).

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# CHAPTER III

# INFLUENCE OF DIETARY FAT AND PACKAGING ON SHELF LIFE OF GROUND BROILER BREAST AND THIGH MEAT DURING REFRIGERATED STORAGE

## ABSTRACT

This study was conducted to determine the microbial quality and oxidative stability of ground raw chicken meat stored under refrigerated conditions. A 2 x 2 factorial arrangement of two dietary fat sources [corn oil (CO) vs. lard (LD)] and two inclusion levels (low; 2% and high; 6%) was used in this study. Each of the four dietary treatments was fed to 8 replicate pens of 9 birds (288 birds total) to 49 d of age. Upon processing, boneless-skinless breast and thigh meat (6 birds/pen) were ground separately, pooled by pen, formed into patties and sealed in trays with either oxygen permeable or impermeable film (4 replicate pens dietary treatment/packaging type). Meat samples were analyzed for lipid oxidation (TBARS) and microbial spoilage (aerobic plate counts, *pseudomonas,* lactic acid bacteria, and Yeast and Molds) following 1, 3, 6, 12 and 18 d of refrigerated storage at 2°C. TBARS values increased during 18 d of storage and birds fed CO had significantly ($P<0.05$) higher lipid oxidation in thigh meat than those fed LD at 1 and 12 d of storage. TBARS value of breast meat was higher ($P<0.05$) in oxygen permeable packaging (3.27 MDA/kg of meat) as compared to oxygen impermeable packaging (2.77 mg MDA/kg of meat) at 3 d of storage. Interactions between fat sources, inclusion levels

and packaging types were observed for thigh meat on day 1 of the storage period. Thigh meat with 6% CO showed higher ($P<0.05$) lipid oxidation than 2% CO packed in permeable film (2.43 vs. 0.89 mg MDA/kg of meat) which was similar to thigh meat from LD treatment. In both packaging types, meat with higher fat level (6%) had higher TBARS values irrespective of the fat source. Microbial profiles among the treatments were not significantly different ($P>0.05$), however, all samples reached 7 $\log_{10}$ CFU/g after day 6 of refrigerated storage.

**Key words:** broilers, ground meat, storage, shelf-life

## INTRODUCTION

Fresh poultry meat is highly perishable food, and extending the shelf-life of poultry meat and products is a major challenge for the poultry industry. Further processed poultry products are the fastest growing market segment which includes portioned as well as ground boneless and skinless breast and thigh meat to provide ready-to-cook convenience to consumers. Supplementation of poultry diets with unsaturated fats increases the proportion of unsaturated fatty acids in breast and thigh meat and, therefore, affects the nutritional composition of further processed products. Such changes in fatty acid composition increase the susceptibility of meat to oxidation (Kanner, 1994; Ruiz et al., 2001). Lipid oxidation in meat not only reduces the nutritive and sensory value, but may also affect microbial activity during storage and ultimately product shelf-life. Many studies have shown that in raw meat, lipid oxidation could develop during storage, induced by enzymatic and inorganic iron activity (Lin and Hultin 1976; Asghar et al., 1988; Kanner et al., 1988). Because unsaturated fatty acids are susceptible to undergoing

lipid oxidation, their enrichment in the meat may result in higher susceptibility to lipid oxidation, especially when the meat is stored under commercial display. Bartov and Frigg (1992) reported that in raw chicken meat microbial spoilage preceded noticeable oxidative rancidity. However, lipid oxidation can be triggered early during a further processing step such as grinding (Rhee et al., 1996), which disrupts the meat compartmentalization and releases higher amounts of non-heme iron. Free iron has strong catalytic activity that promotes lipid oxidation in meat (Kanner et al., 1988).

Vacuum and modified atmosphere packaging have proven effective in prolonging the shelf life in raw, refrigerated and frozen poultry meat (Dawson et al., 1995). Ground meat packaged with low $O_2$ permeable film has shown lower microbial growth and lipid oxidation that conventional oxygen permeable packaging (Dhananjayan et al., 2001; Keokamnerd et al., 2008; Patsias et al., 2006).

The objective of this study was to evaluate the interrelationship between the microbial spoilage and oxidative rancidity in ground broiler meat. The fat content and composition of broiler chicken breast and thigh meat were altered through dietary means. Raw ground meat samples were then packaged with either oxygen permeable or impermeable film and stored under refrigerated storage conditions for extended periods of time to assess these changes.

## MATERIALS AND METHODS

### Sample Collection and Treatment

A total of 288 male (Ross x Ross 708) broilers were raised in 32 floor pens to 49 d of age in pens prepared with clean pine shavings. The study involved four dietary

treatments in a 2 x 2 factorial arrangement of two dietary fat sources [corn oil (CO) vs. lard (LD)] and two inclusion levels (low; 2% and high; 6%). Each of the four diets was fed to 8 replicate pens of 9 birds (288 birds total) to 49 d of age. The experimental diets were provided on a three stage feeding program in with 0.91 kg starter, 1.81 kg grower, and withdrawal to 49 d of age. Feed and water were available *ad libitum* and lighting was continuous throughout the study. Nutrient composition of experimental diets is presented in Table 1. Body weight, feed efficiency (adjusted for mortality), and mortality were determined at completion of the study.

At 49 d of age, 6 birds were processed at the Auburn University Poultry Science Department Pilot Processing Facilities simulating commercial processing practices as described previously (Saenmahayak et al., 2010). Upon processing, boneless-skinless breast and thigh meat were pooled by replicate pen and meat type, ground (LEM Meat Grinder; LEM Products, OH, with 0.5 cm head size grinder) formed into patties (approximately 100 g, 1.5 cm thickness) and sealed in trays with either oxygen permeable or impermeable film (Cryovac, Inc.; Duncan, SC). This resulted in 4 replicate pens per dietary treatment and packaging type. Permeable film (E2300; 50001$cm^3/m^2$/24 hr atm @ 40°F, 0% RH) had a higher oxygen transmission rate than impermeable film (B4700; 3-650001$cm^3/m^2$/24 hr atm @ 40°F, 0% RH). All meat samples (960 samples) were stored refrigerated (at 2°C) for 0, 3, 6, 12 and 18 d.

**Microbiological Analysis**

Ground meat samples (10 g) were homogenized in 90 ml of peptone water (Oxoid Ltd., Basingstoke, UK) for 2 min using a Stomacher (Model 400 Lab Blender, Seward Ltd., London, UK). A 10-fold dilution of the meat homogenate was prepared in peptone

water and a sample of 100 µl was plated in duplicate. Total aerobic plate counts were performed on Plate Count Agar (PCA; Oxoid Ltd., Basingstoke, UK), and plates were incubated aerobically at 37°C for 24 h. *Pseudomonas* spp. were counted on Cephaloridine Fucidin Cetrimide Agar (CFC; Acumedia Manufactures Inc., Baltimore, MD). Plates were incubated aerobically at 25°C for 48 h. The lactic acid bacteria (LAB) were determined using de Man, Rogosa, and Sharpe Agar (MRS; Acumedia Manufactures Inc., Baltimore, MD) and incubated anaerobically at 25°C for 48 h in anaerobic jars with a disposable Anaerobic Gas Pak (AneroGen; Oxoid Ltd., Basingstoke, UK). Yeasts and molds were counted on potato dextrose agar (PDA; Acumedia Manufactures Inc., Baltimore, MD) at 25°C for 5 d. Bacterial numbers were reported as $\log_{10}$ colony forming units per g of meat.

**Thiobarbituric Acid Reactive Substances (TBARS)**

Samples (10 g of ground meat) were homogenized with 30 ml of DW (Deionized water) for 2 min and 2 ml of homogenate was mixed with 4 ml of TCA/TBA (Trichloroacitic acid/Thiobarbituric acid) reagent [15% TCA (w/v) and 20 mM TBA] and 100 µl BHA (Butylated hydroxyanisole). Solution was heated 15 min in boiling water, cooled for 10 min in cold water, and centrifuged at 3000 rpm for 10 min. Supernatant was used to read the absorbance at 531 nm against blank that contained all reagents minus the sample. The absorbance was calculated from a calibration curve prepared using 1,1,3,3-tetramethoxypropane (TEP) as a standard. The TBARS was expressed as mg of malondialdehyde (MDA) per kg of meat (mg MDA/kg).

**Statistical Analysis**

Live performance data was analyzed as four dietary treatments (CO and LD at 2 and 6%) using proc GLM procedure (SAS Institute, 2002-2003). For the shelf life study, the design involved fat source, inclusion level and packaging type in a factorial arrangement (2 x 2 x 2). Separate analyses were done for each meat type (breast and thigh meat). The data were statistically analyzed using PROC MIXED procedure for the ANOVA (SAS Institute, 2002-2003) to test for significant differences ($P<0.05$). The main factors considered in analysis were dietary treatments, packaging types and meat types.

## RESULTS AND DISCUSSION

The main effect of adding unsaturated fat or oil is to induce the deposition in body lipids of polyunsaturated fatty acids (PUFA) that are not synthesized by chickens (linoleic and linolenic acids) in fatty and muscle tissues, and long-chain PUFA in muscles (Pinchasov and Nir, 1992). The effect of type of fat and inclusion level in the diet on fatty acid composition of ground breast and thigh muscle tissues is summarized in Tables 2 and 3, respectively. As expected, the fatty acid composition of both breast and thigh meat reflected the dietary fat profile. Breast meat had higher protein and lower fat content than thigh meat. Dietary corn oil increased polyunsaturated fatty acid content of both meat types, whereas the use of lard increased saturated fatty acids. However, fat level of these two meat types was not altered by dietary inclusion levels. The results are consistent with other researchers who reported significant changes in composition after feeding difference amounts of certain fatty acids (Scaiffe et al., 1994; Pinchasov and Nir. 1992).

Live and processing performance of broilers varied significantly ($P<0.05$) among the dietary treatments in this study (Table 4). A significant interaction between fat source and inclusion level was detected ($P<0.05$). Birds fed 2% CO had higher BW, CCW and LCW ($P<0.05$) as compared to 2% LD treatment at 49 d of age (Table 4). No differences in feed conversion and abdominal fat were detected among the dietary treatments ($P>0.05$).

Microbial profile of ground breast and thigh meat during refrigerated storage from 1 to 18 d did not differ ($P>0.05$) due to dietary treatments (Figures 1, 3-5). However, all meat samples reached 7 $\log_{10}$ CFU/g after 6 d of storage (Carcasses were not treated with any antimicrobials). This is consistent with results by Sawaya et al., (2007). TBARS values increased during 18 d of storage for both meat types (Figure 1). Birds fed CO exhibited higher ($P<0.05$) lipid oxidation in thigh meat than those fed LD, but this was only significant at day 1 (0.66 vs. 0.03 MDA/kg of meat) and day 12 (24.61 vs. 13.93 MDA/kg of meat) of storage. Fat source and inclusion levels did not affect of ground breast meat TBARS values ($P>0.05$). However, a significant ($P<0.05$) interaction between fat source, inclusion level and packaging type was observed during the first day of storage for thigh meat (Figure 2). This interaction was due to higher TBARS values with CO than LD under oxygen permeable as compared to impermeable film packaging. Raw ground meat packed in oxygen permeable packaging showed higher lipid oxidation than impermeable packaging. Permeable packaging and high fat content (CO>LD) in meat samples accelerated lipid oxidation due to oxygen availability during storage. However, this effect was only significant at day 3 of storage for breast meat.

Based on TBARS values, oxidative deterioration after 7 d of storage was higher in thigh than in breast meat. Impermeable packaging had decreased rate of lipid oxidation in

ground white meat at 1 and 3 d of storage (Figure 6). Meat packed in permeable packaging showed lower lipid oxidation than oxygen impermeable film. Fats with polyunsaturated fatty acids are highly sensitive to oxidation reactions during storage. An increase in the content of unsaturated fatty acids in relation to saturated fatty acids increases the absorption of the saturated fatty acids (Young and Garrett, 1963).

In conclusion, dietary fat supplementation affected live and processing performance of broiler chicken at 49 d of age. Meat composition and fatty acid profile was also altered with dietary fat source and inclusion level. Lipid oxidation increased with refrigerated storage of raw ground meat and a higher rate of lipid oxidation in thigh than breast muscle. Corn oil resulted in a higher raw ground thigh meat lipid oxidation rate than lard. Both meat types reached 7 $\log_{10}$ CFU/g after day 6 of refrigerated storage. Microbial and unsaturated lipid content of poultry meat may justify the need for different packaging types or diet supplementation such as antioxidants to optimize the quality and shelf life of ground poultry meat.

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Table 1. Nutrient composition of experimental diets.

| Compositions | Starter | | | | Grower | | | | Finisher | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Corn | | Lard | | Corn | | Lard | | Corn | | Lard | |
| | 2% | 6% | 2% | 6% | 2% | 6% | 2% | 6% | 2% | 6% | 2% | 6% |
| Moisture (%) | 11.81 | 10.61 | 11.73 | 10.93 | 12.53 | 11.91 | 12.62 | 12.05 | 13.84 | 13.39 | 13.74 | 13.44 |
| Crude Protein (%) | 28.4 | 27.8 | 25.9 | 26.2 | 24.4 | 25.0 | 25.3 | 24.8 | 20.1 | 21.2 | 19.3 | 20.2 |
| Crude Fat (%) | 6.18 | 9.01 | 7.41 | 11.2 | 6.62 | 11.7 | 5.14 | 8.30 | 5.10 | 8.64 | 6.00 | 8.85 |
| Ash (%) | 6.65 | 6.67 | 6.17 | 6.13 | 5.80 | 6.13 | 6.04 | 6.55 | 4.61 | 5.20 | 4.99 | 4.96 |
| Metabolizable Energy (Mcal/lb) | 1.53 | 1.57 | 1.57 | 1.64 | 1.58 | 1.66 | 1.54 | 1.60 | 1.60 | 1.65 | 1.62 | 1.66 |
| Phosphorus (%) | 1.08 | 1.07 | 0.92 | 1.06 | 0.95 | 1.01 | 0.90 | 1.02 | 0.74 | 0.79 | 0.72 | 0.83 |
| Calcium (%) | 1.93 | 1.78 | 1.93 | 1.99 | 1.76 | 1.58 | 1.69 | 1.86 | 1.18 | 1.05 | 1.33 | 1.24 |
| Sodium (%) | 0.22 | 0.26 | 0.22 | 0.21 | 0.26 | 0.20 | 0.24 | 0.24 | 0.22 | 0.20 | 0.20 | 0.21 |

Table 2. Compositions and fatty acid profiles of broiler breast meat.

| Composition | Corn | | Lard | |
|---|---|---|---|---|
| | 2% | 6% | 2% | 6% |
| Moisture (%) | 74.4 | 73.4 | 72.8 | 73.9 |
| Protein (%) | 22.7 | 23.3 | 23.4 | 22.6 |
| Fat (%) | 2.0 | 1.8 | 2.4 | 2.2 |
| Saturated Fatty Acids (% of fat) | 30.8 | 26.8 | 32.8 | 32.4 |
| Myristic (C14:0) | 0.73 | 0.64 | 0.88 | 1.08 |
| Palmitic (C16:0) | 22.2 | 19.5 | 24.4 | 23.3 |
| Stearic (C18:0) | 7.31 | 6.30 | 7.17 | 7.35 |
| Monounsaturated Fatty Acids (% of fat) | 39.4 | 33.2 | 45.3 | 43.2 |
| Oleic (C18:1) | 34.3 | 30.0 | 38.6 | 37.7 |
| Polyunsaturated Fatty Acids (% of fat) | 29.3 | 39.7 | 21.2 | 23.7 |
| Linoleic (C18:2) | 22.5 | 33.7 | 16.0 | 19.1 |
| Arachidonic (C20:4) | 3.53 | 2.80 | 2.64 | 2.08 |
| Trans Fatty Acids (% of fat) | 0.6 | 0.3 | 0.7 | 0.6 |
| Omega 3 Fatty Acids (% of fat) | 1.07 | 1.46 | 0.77 | 0.96 |
| Omega 6 Fatty Acids (% of fat) | 27.34 | 37.61 | 19.76 | 22.20 |
| Omega 9 Fatty Acids (% of fat) | 34.29 | 30.04 | 38.64 | 37.66 |

Table 3. Compositions and fatty acid profiles of broiler thigh meat.

| Compositions | Corn | | Lard | |
|---|---|---|---|---|
| | 2% | 6% | 2% | 6% |
| Moisture (%) | 70.5 | 70.5 | 71.3 | 71.7 |
| Protein (%) | 18.5 | 18.5 | 18.2 | 17.9 |
| Fat (%) | 11.2 | 8.9 | 9.3 | 8.0 |
| Saturated Fatty Acids (% of fat) | 28.3 | 26.5 | 31.4 | 33.0 |
| Myristic (C14:0) | 0.55 | 0.55 | 0.79 | 1.22 |
| Palmitic (C16:0) | 21.2 | 20.0 | 23.8 | 24.9 |
| Stearic (C18:0) | 6.14 | 5.52 | 6.48 | 6.30 |
| Monounsaturated Fatty Acids (% of fat) | 43.0 | 38.1 | 41.4 | 45.3 |
| Oleic (C18:1) | 36.9 | 33.5 | 35.8 | 38.6 |
| Polyunsaturated Fatty Acids (% of fat) | 28.2 | 34.9 | 26.5 | 20.9 |
| Linoleic (C18:2) | 24.3 | 31.6 | 23.1 | 18.1 |
| Arachidonic (C20:4) | 1.50 | 1.19 | 1.39 | 0.96 |
| Trans Fatty Acids (% of fat) | 0.5 | 0.5 | 0.7 | 0.8 |
| Omega 3 Fatty Acids (% of fat) | 0.92 | 1.01 | 0.89 | 0.87 |
| Omega 6 Fatty Acids (% of fat) | 26.64 | 33.51 | 25.24 | 19.65 |
| Omega 9 Fatty Acids (% of fat) | 36.91 | 33.51 | 35.84 | 38.62 |

Table 4. Influence of dietary fat source and inclusion level on live and processing performance at 49 d of age.

| Treatment | BW[1] (g) | FC[2] | CCW[3] (g) | LCW[4] (g) | Abdominal fat (g) |
|---|---|---|---|---|---|
| | * | NS[5] | *** | *** | NS |
| 2% Corn | 3578[a] | 1.880 | 2566[a] | 2517[a] | 49 |
| 6% Corn | 3403[ab] | 1.937 | 2451[ab] | 2401[b] | 50 |
| 2% Lard | 3363[b] | 1.918 | 2379[b] | 2331[c] | 48 |
| 6% Lard | 3443[ab] | 1.916 | 2461[ab] | 2407[b] | 54 |
| SEM[6] | 47.18 | 0.02 | 31.50 | 31.67 | 1.99 |

[1]BW=Body weight
[2]FC=Feed conversion adjusted for mortality
[3]CCW=Chilled carcass weight
[4]LCW=Lean carcass weight
[5]Not significant (P>0.05).
[6]SEM = Pooled Standard Error of the Mean.
[ab]Means within a column with difference superscripts differ significantly.
*(P<0.05); **(P<0.01); ***(P<0.001).

Figure 1. Main effects of dietary fat and inclusion levels on Aerobic Pslate Counts (APC) of (A) Breast meat and (B) Thigh meat during refrigerated storage at 2°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

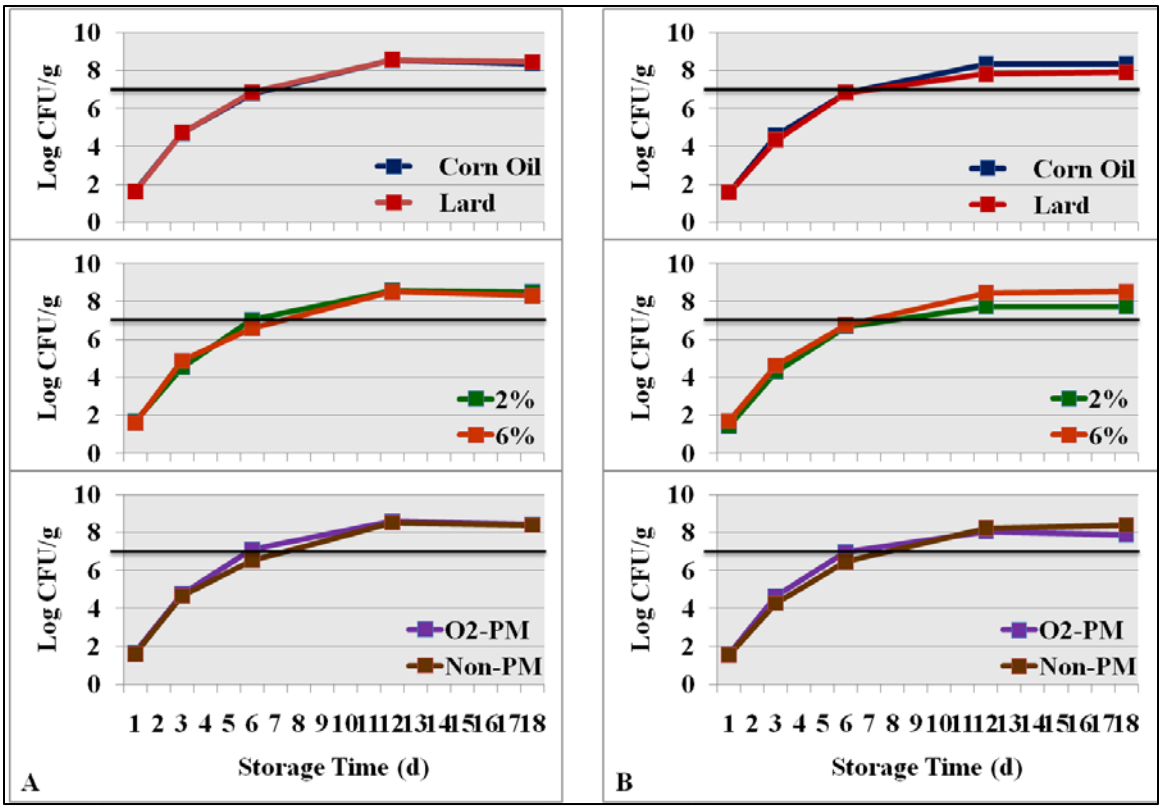

Table 5. Probability (P-value) of Aerobic Plate Counts (APC) of refrigerated storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 18 |
| **Breast Meat** | | | | | |
| Fat Source | 0.86 | 0.94 | 0.91 | 0.85 | 0.62 |
| Inclusion Level | 0.43 | 0.38 | 0.31 | 0.63 | 0.35 |
| Packaging Type | 0.41 | 0.81 | 0.21 | 0.54 | 0.78 |
| SEM[1] | 0.09 | 0.27 | 0.32 | 0.10 | 0.15 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.95 | 0.56 | 0.59 | 0.34 | 0.43 |
| Inclusion Level | 0.26 | 0.43 | 0.84 | 0.20 | 0.18 |
| Packaging Type | 0.89 | 0.36 | 0.14 | 0.85 | 0.40 |
| SEM | 0.15 | 0.29 | 0.25 | 0.39 | 0.41 |

[1]SEM= Pooled Standard Error of the Mean

Figure 2. Interaction of fat source, inclusion level and packaging type for thigh meat at 1 d of storage (SEM = 1.09).

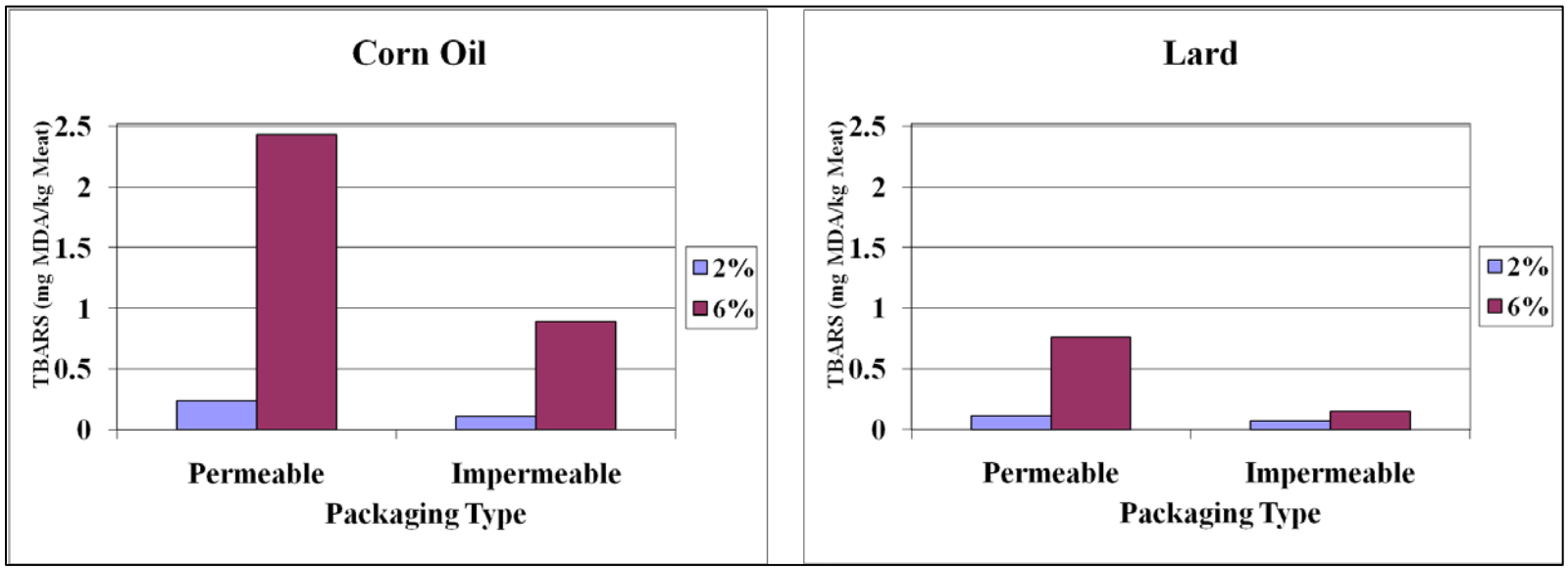

Figure 3. Main effects of dietary fat and inclusion levels on *Pseudomonas* spp. of (A) Breast meat and (B) Thigh meat during refrigerated storage at 2°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

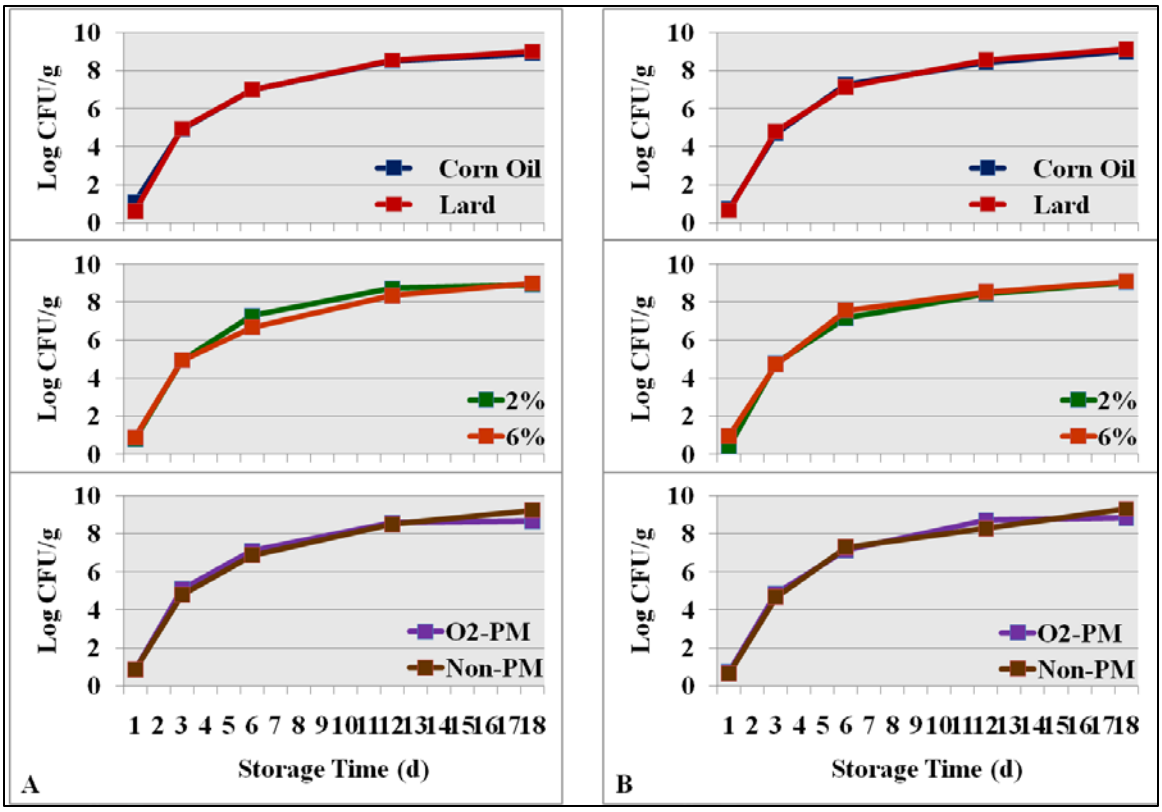

Table 6. Probability (P-value) of *Pseudomonas* spp. of refrigerated storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 18 |
| **Breast Meat** | | | | | |
| Fat Source | **0.04** | 0.93 | 0.98 | 0.86 | 0.42 |
| Inclusion Level | 0.73 | 0.97 | 0.16 | **0.02** | 0.59 |
| Packaging Type | 0.96 | 0.46 | 0.60 | 0.54 | **0.0004** |
| SEM[1] | 0.15 | 0.27 | 0.30 | 0.10 | 0.10 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.74 | 0.76 | 0.65 | 0.44 | 0.53 |
| Inclusion Level | 0.051 | 0.79 | 0.73 | 0.49 | 0.85 |
| Packaging Type | 0.78 | 0.54 | 0.62 | **0.006** | **0.03** |
| SEM | 0.19 | 0.20 | 0.22 | 0.10 | 0.15 |

[1]SEM= Pooled Standard Error of the Mean

Figure 4. Main effects of dietary fat and inclusion levels on Lactic Acid Bacteria (LAB) of (A) Breast meat and (B) Thigh meat during refrigerated storage at 2°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

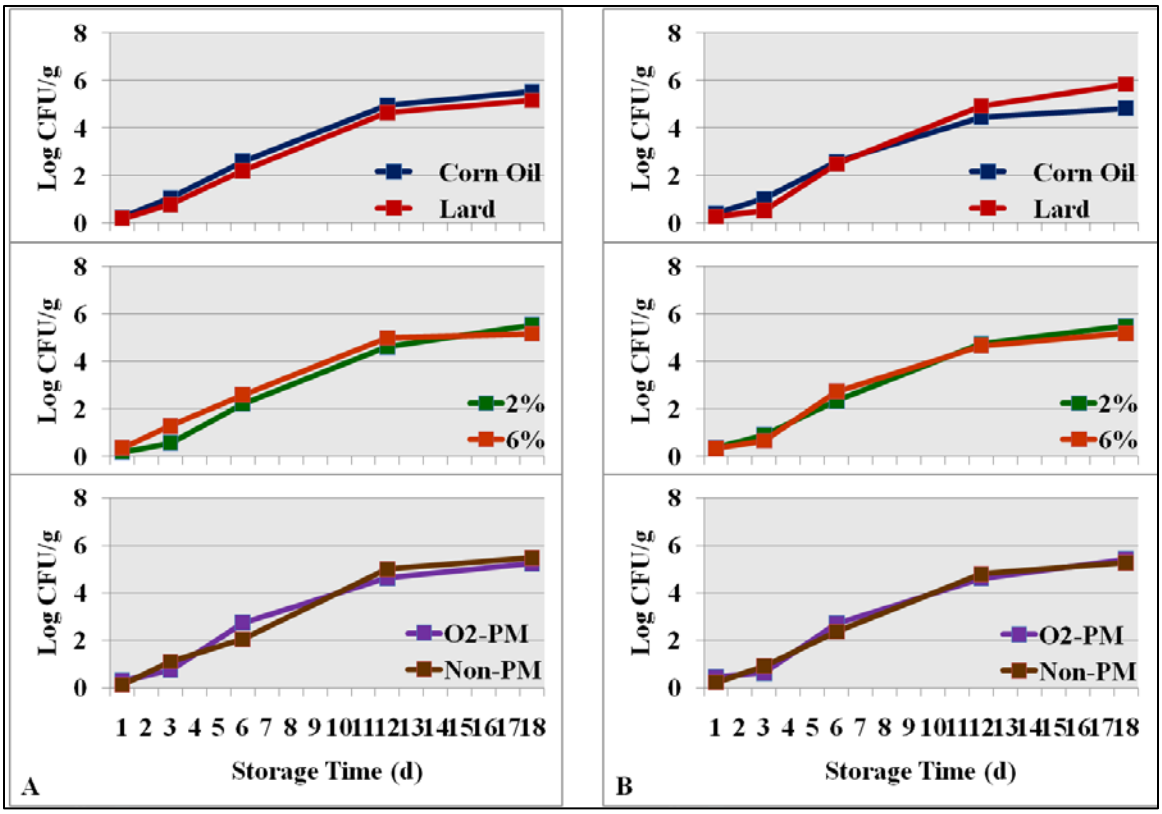

Table 7. Probability (P-value) of Lactic Acid Bacterial (LAB) of refrigerated storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 18 |
| **Breast Meat** | | | | | |
| Fat Source | 0.72 | 0.49 | 0.52 | 0.55 | 0.48 |
| Inclusion Level | 0.60 | 0.08 | 0.55 | 0.46 | 0.47 |
| Packaging Type | 0.25 | 0.37 | 0.26 | 0.47 | 0.65 |
| SEM[1] | 0.11 | 0.28 | 0.42 | 0.36 | 0.37 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.52 | 0.20 | 0.88 | 0.33 | 0.09 |
| Inclusion Level | 0.87 | 0.50 | 0.52 | 0.86 | 0.60 |
| Packaging Type | 0.19 | 0.44 | 0.56 | 0.71 | 0.78 |
| SEM | 0.13 | 0.26 | 0.42 | 0.34 | 0.41 |

[1]SEM= Pooled Standard Error of the Mean

Figure 5. Main effects of dietary fat and inclusion levels on Yeasts and Molds (YAM) of (A) Breast meat and (B) Thigh meat during refrigerated storage at 2°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

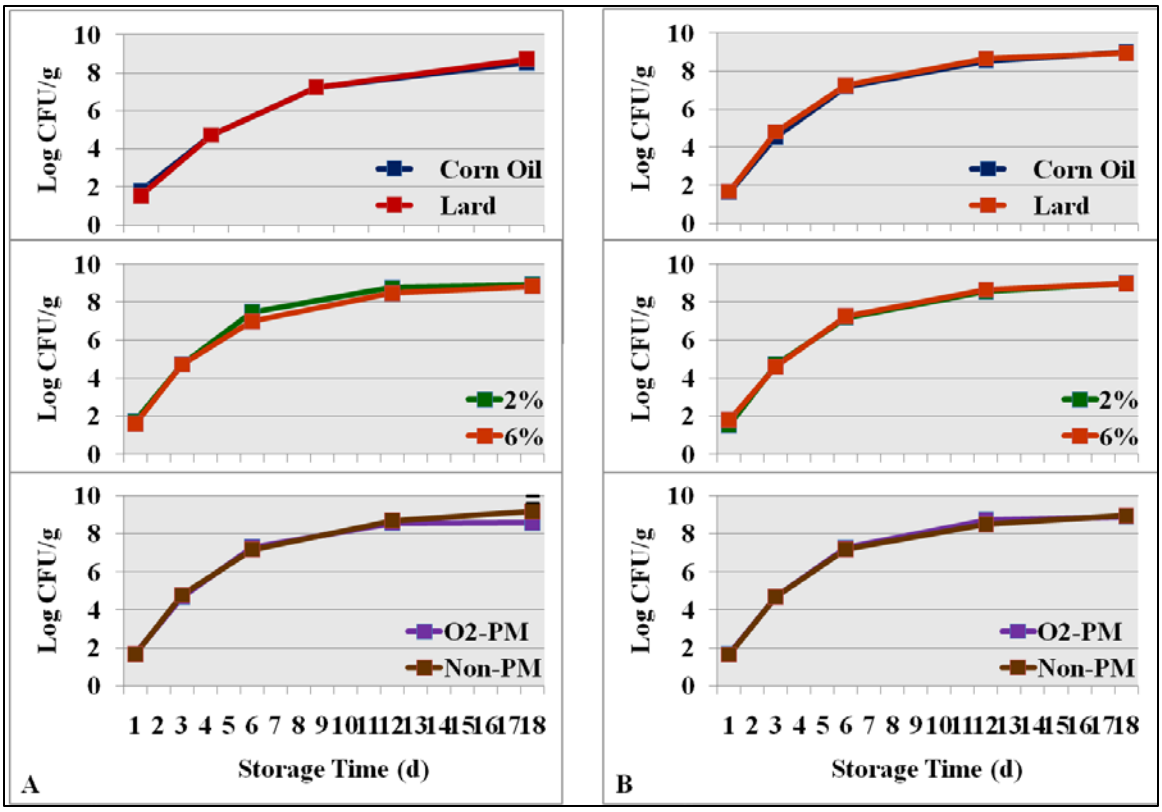

Table 8. Probability (P-value) of Yeasts and Molds (YAM) of refrigerated storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 18 |
| **Breast Meat** | | | | | |
| Fat Source | 0.18 | 0.99 | 0.99 | 0.27 | 0.63 |
| Inclusion Level | 0.47 | 0.91 | 0.24 | 0.10 | 0.62 |
| Packaging Type | 0.95 | 0.58 | 0.76 | 0.48 | **0.002** |
| SEM[1] | 0.14 | 0.15 | 0.29 | 0.12 | 0.12 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.84 | 0.13 | 0.85 | 0.36 | 0.78 |
| Inclusion Level | 0.08 | 0.54 | 0.78 | 0.43 | 0.88 |
| Packaging Type | 0.79 | 0.92 | 0.83 | 0.09 | 0.40 |
| SEM | 0.11 | 0.12 | 0.24 | 0.09 | 0.17 |

[1]SEM= Pooled Standard Error of the Mean

Figure 6. Main effects of dietary fat and inclusion levels on lipid oxidation (TBARS) of (A) Breast meat and (B) Thigh meat during refrigerated storage at 2°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

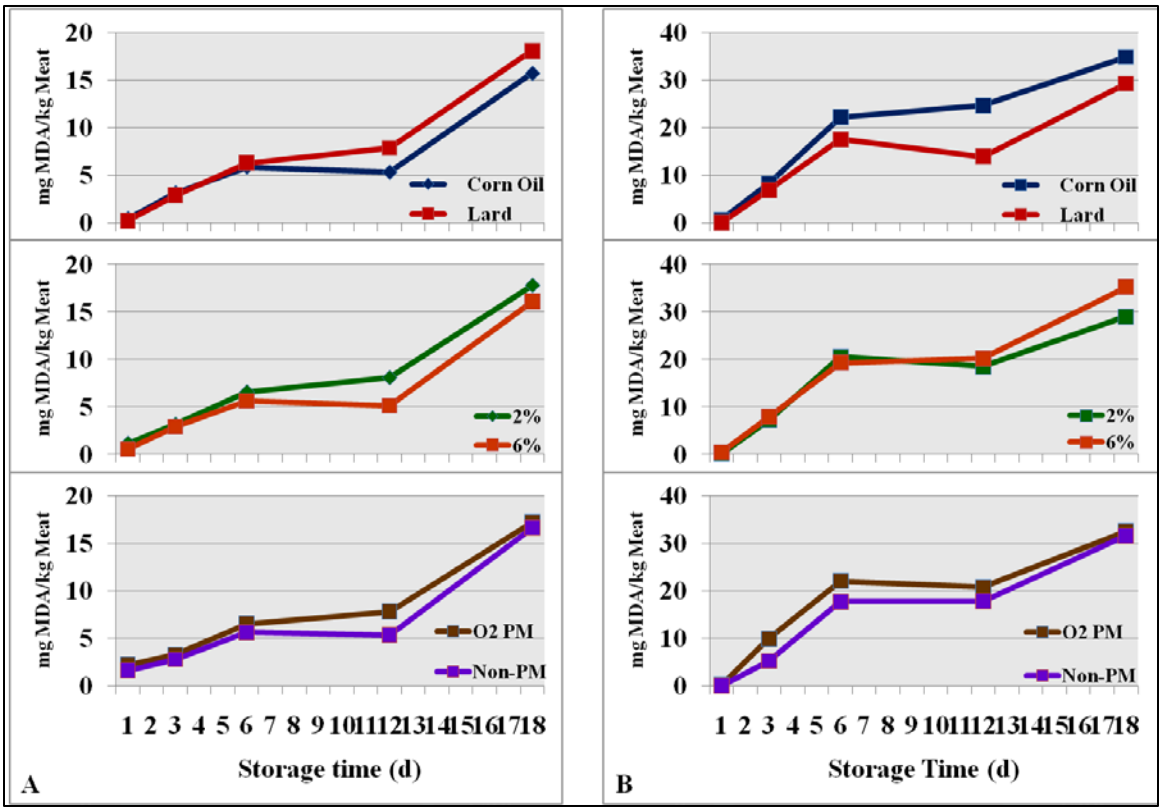

Table 9. Probability (P-value) of lipid oxidation (TBARS) of refrigerated storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 18 |
| **Breast Meat** | | | | | |
| Fat Source | 0.39 | 0.89 | 0.81 | 0.18 | 0.38 |
| Inclusion Level | 0.51 | 0.23 | 0.59 | 0.12 | 0.52 |
| Packaging Type | 0.13 | **0.009** | 0.60 | 0.19 | 0.83 |
| SEM[1] | 0.95 | 0.22 | 1.20 | 1.29 | 1.86 |
| **Thigh Meat** | | | | | |
| Fat Source | **0.04** | 0.59 | 0.20 | **0.009** | 0.24 |
| Inclusion Level | 0.52 | 0.77 | 0.74 | 0.66 | 0.19 |
| Packaging Type | 0.40 | 0.06 | 0.23 | 0.43 | 0.83 |
| SEM | 0.54 | 1.71 | 2.53 | 2.67 | 3.27 |

[1]SEM= Pooled Standard Error of the Mean

# CHAPTER IV

# INFLUENCE OF DIETARY FAT AND PACKAGING ON SHELF LIFE OF GROUND BROILER BREAST AND THIGH MEAT DURING FROZEN STORAGE

## ABSTRACT

A study was conducted to determine the microbial quality and oxidative stability of ground raw chicken meat stored under frozen conditions. The study involved 2 x 2 factorial arrangement of two dietary fat sources [corn oil (CO) vs. lard (LD)] and two inclusion levels (low; 2% and high; 6%). Each of four dietary treatments was fed to 8 replicate pens of 9 birds (288 birds total) to 49 d of age. Upon processing, boneless-skinless breast and thigh meat (6 birds/pen) were ground separately, pooled by pen, formed into patties and sealed in trays with either oxygen permeable or impermeable films (4 replicate pens/dietary treatment/packaging type). Meat samples were stored frozen (-18°C) for 6 months and analyzed for lipid oxidation (TBARS) and microbial spoilage (aerobic plate counts, *pseudomonas* spp., lactic acid bacteria, Yeast and Molds) following 3 and 6 months of frozen storage (-18°C). Samples were removed from frozen storage, thawed and stored for 1, 3, 6, 12 and 15 d at 2°C. After 3 months of frozen storage, thigh meat packed in impermeable packaging had lower ($P<0.05$) APC and decreased rate of lipid oxidation than those packed in permeable packaging at 0 d of storage and breast meat at 0 and 3 d of storage. Following 6 months of storage, CO had

lower (P<0.05) microbial growth than LD in thigh meat with 3 and 6 d of storage, while breast meat had lower (P<0.05) microbial growth in LD than CO. TBARS values increased during 15 d of storage and birds fed 2% fat had significantly (P<0.05) lower lipid oxidation in breast meat than those fed 6% at 0 d of storage. TBARS values of thigh meat were higher in oxygen permeable packaging as compared to oxygen impermeable packaging at 12 and 15 d of storage. Interactions between inclusion levels and packaging types were observed for breast meat on day 1. Breast meat with 6% fat packed in oxygen permeable film showed higher (P<0.05) lipid oxidation than those packed in impermeable film. Oxidative and microbial changes dominate spoilage of frozen ground meat. These changes can be minimized by alterations in fat sources and level, as well as oxygen permeability of packaging.

**Key words:** broilers, ground meat, frozen storage, shelf-life

## INTRODUCTION

Extending the shelf life of poultry meat and products are a major concern for the poultry industry. Because fresh poultry meat is highly perishable, it is essential to maintain shelf life of the product as long as possible. Further processed products are the largest market segment, which includes products that have gone through some degree of cooking or preparation that provides convenience to consumers. In recent years, there has been an increased interest in manipulating fatty acid composition of meat to promote the substitution of animal fat source by unsaturated vegetable oils, a trend that is currently influencing the production of poultry meat and processed meat products. Supplementation of poultry diets with unsaturated fats increases the degree of unsaturated

fatty acids in meat and meat products, which increases the susceptibility of meat to oxidation (Kanner, 1994; Ruiz et al., 2001). Lipid oxidation causes nutritional and sensory value loss as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelf life.

In raw chicken meat, lipid oxidation development is known to be minimal (Bartov and Frigg, 1992) and considered to be little practical relevance because microbial spoilage appears earlier than noticeable oxidative rancidity. However, many studies have indicated that in raw meat, lipid oxidation could develop during storage induced by enzymatic and inorganic iron activity (Lin and Hultin 1976; Asghar et al., 1988; Kanner et al., 1988). Because unsaturated fatty acids are susceptible to lipid oxidation, their enrichment in meat lipid composition may result in higher susceptibility to lipid oxidation, especially when the meat is stored and commercially display. However, lipid oxidation development has been found to occur rapidly in thermally processed chicken meat, influenced by further processing steps such as grinding and cooking (Rhee et al., 1996), which disrupt the meat compartmentalization and release higher amounts of non-heme iron. Free iron has strong catalytic activity that promotes lipid oxidation in meat (Kanner et al., 1988).

The changes that occur in poultry meat during storage are generally chemical and microbiological in nature and are primarily influenced by factors such as temperature, oxygen and light. In order to prevent oxidation and delay microbial spoilage, supplementation of antioxidants in the diets (such as α-tocopherol), packaging types and storage temperature have been focused in meat and meat products. Vacuum packaging and modified atmosphere packaging have been proven to be effective against off-flavors

and to prolong shelf life in precooked refrigerated and frozen products. Microbial, as well as pigment and unsaturated lipid contents of poultry meat, may justify the need for different packaging types than those used with red meat to optimize the appearance and shelf life of ground poultry (Dawson et al., 1995). Several studies have reported that ground meat packed with low $O_2$ permeable packaging has shown decreasing microbial growth and lipid oxidation (Dhananjayan et al., 2001; Keokamnerd et al., 2008; Patsias et al., 2006; Saenmahayak et al., 2009).

The objective of this study was to evaluate the relationship between microbial spoilage and oxidative rancidity in ground broiler meat. The fat content and composition of broiler chicken breast and thigh meat were altered through dietary means. Raw ground meat samples were then packaged with either oxygen permeable or impermeable film and stored frozen for up to 6 months.

## MATERIALS AND METHODS

### Sample Collection and Treatment

A total of 288 male (Ross x Ross 708) broilers were raised in 32 floor pens to 49 d of age in floor pens prepared with clean pine shavings. The study involved four dietary treatments in a 2 x 2 factorial arrangement of two dietary fat sources [corn oil (CO) vs. lard (LD)] and two inclusion levels (low; 2% and high; 6%). Each of the four diets was fed to 8 replicate pens of 9 birds (288 birds total) to 49 d of age. The experimental diets were provided on a three stage feeding program (0.91 kg starter, 1.81 kg grower, and withdrawal) to 49 d of age. Feed and water was available *ad libitum* and lighting was continuous throughout the study.

At 49 d of age, 6 birds per pen were processed at the Auburn University Poultry Science Department Pilot Processing Facilities simulating commercial processing practices as described previously (Saenmahayak et al., 2009). Upon processing, boneless-skinless breast and thigh meat were pooled by replicate pen and meat type, ground (LEM Meat Grinder; LEM Products, OH, with 0.5 cm head size grinder) formed into patties (approximately 100 g, 1.5 cm thickness) and sealed in trays with either oxygen permeable or impermeable film (Cryovac, Inc.; Duncan, SC). This resulted in 4 replicate pens per dietary treatment and packaging type. Permeable film (E2300; 50001$cm^3/m^2$/24 hr atm @ 40°F, 0% RH) had higher oxygen transmission rate than impermeable film (B4700; 3-650001$cm^3/m^2$/24 hr atm @ 40°F, 0% RH). All meat samples (960 total) were then immediately stored frozen (-18°C) for 3 and 6 months. Frozen samples were thawed overnight at 2°C and sampled subsequently at 0, 3, 6, 12 and 15 d of refrigerated storage.

**Microbiological Analysis**

Ground meat samples (10 g) were homogenized in 90 ml of peptone water (PW; Oxoid Ltd., Basingstoke, UK) for 2 min using a Stomacher (Model 400 Lab Blender, Seward Ltd., London, UK). A 10-fold dilution of the meat homogenate was prepared in PW and a sample of 100 µl plated in duplicate. Total aerobic plate counts were performed on Plate Count Agar (PCA; Oxoid Ltd., Basingstoke, UK), and plates were incubated aerobically at 37°C for 24 h. *Pseudomonas* spp. were counted on Cephaloridine Fucidin Cetrimide Agar (CFC; Acumedia Manufactures Inc., Baltimore, MD). Plates were incubated aerobically at 25°C for 48 h. The lactic acid bacteria (LAB) were determined using de Man, Rogosa, and Sharpe Agar (MRS; Acumedia Manufactures Inc., Baltimore, MD) and incubated anaerobically at 25°C for 48 h in

anaerobic jars with a disposable Anaerobic Gas Pak (AneroGen; Oxoid Ltd., Basingstoke, UK). Yeasts and molds were counted on potato dextrose agar (PDA; Acumedia Manufactures Inc., Baltimore, MD) following incubation at 25°C for 5 d. Bacterial numbers were reported as $\log_{10}$ colony forming units per g of meat.

**Thiobarbituric Acid Reactive Substances (TBARS)**

Samples (10 g of ground meat) were homogenized with 30 ml of DW (Deionized water) for 2 min and 2 ml of homogenate was mixed with 4 ml of TCA/TBA (Trichloroacitic acid/Thiobarbituric acid) reagent [15% TCA (w/v) and 20 mM TBA] and 100 µl BHA (Butylated hydroxyanisole). Solution was heated 15 min in boiling water, cooled for 10 min in cold water, and centrifuged at 3000 rpm for 10 min. Supernatant was used to read the absorbance at 531 nm against blank that contained all reagents without the sample. The absorbance were calculated from a calibration curve prepared using 1,1,3,3-tetramethoxypropane (TEP) as a standard. The TBARS was expressed as mg of malondialdehyde (MDA) per kg of meat (mg MDA/kg).

**Statistical Analysis**

For the shelf life study, the design involved fat source, inclusion level and packaging type in a factorial arrangement (2 x 2 x 2). Separate analysis was done for each meat type (breast and thigh meat). The data were statistically analyzed using PROC MIXED procedure for the ANOVA (SAS Institute, 2002-2003) to test for significant differences ($P<0.05$). The main factors considered in analysis were dietary treatments, packaging types and meat types.

## RESULTS AND DISCUSSION

After 3 months of frozen storage (-18°C), all thawed samples tended to show lower microbial growth rate compared to fresh meat samples kept under refrigerated storage (Saenmahayak, 2009) (Figures 1-4). Microbial counts were not affected by any of the dietary treatments ($P<0.05$). However, all counts rapidly increased over time. Microbial spoilage occurred in both meat types in about 9-10 days (based on 7 log cfu). *Pseudomonas* counts showed the same trend with no significant treatment effects ($P<0.05$) for either breast or thigh meat, but a significant ($P<0.05$) increase with storage time. Lactic acid bacteria (LAB) growth rate showed the same trend on both breast and thigh meat and increased as storage time increased. Yeast and molds showed lower counts on day 6 and 12 of storage with non-permeable packaging.

After 6 months of frozen storage, APC were reduced ($P<0.05$) with LD treatment at day 3 and 6 of storage, but no other treatment effects were detected (Figures 5-8). Microbial spoilage occurred approximately at 9-10 days of refrigerated storage. Lower *Pseudomonas* counts were observed in both breast (3 d) and thigh meat (3 and 6 d) with CO treatment. Also with 2% inclusion level, lower pseudomonas counts were detected after 6 days of storage. No differences were detected due to packaging type ($P>0.05$). LAB growth rate was higher with CO in breast meat and LD in thigh meat. However, these differences were not statistically significant. Again, LAB increased with storage time. Yeast and mold counts were higher with corn oil treatment in breast meat but only at 3 and 6 d of storage, whereas the opposite occurred with lard in thigh meat. Also higher yeast and molds counts were seen with 2% inclusion levels. No effect of packaging was detected.

Based on TBARS values after 3 and 6 months of frozen storage, oxidative deterioration was nearly 3-fold higher in ground thigh than in ground breast meat (Figures 9-10). At 3 months, higher levels of oxidation occurred with corn oil than lard; with higher fat inclusion level and oxygen permeable packaging. Meat packed in permeable packaging showed lower lipid oxidation than oxygen impermeable film. Fats with polyunsaturated fatty acids are highly sensitive to oxidation reactions during storage. The improvement of oxidative stability and reduction of bacterial growth were seen in meat samples packed in impermeable packaging at 3 months of storage. Microbial and oxidative changes in poultry meat may justify the need for different packaging types or supplementation with antioxidants to extend the quality and shelf life of ground poultry meat.

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Figure 1. Main effects of dietary fat and inclusion levels on Aerobic Plate Counts (APC) of (A) Breast meat and (B) Thigh meat after 3 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

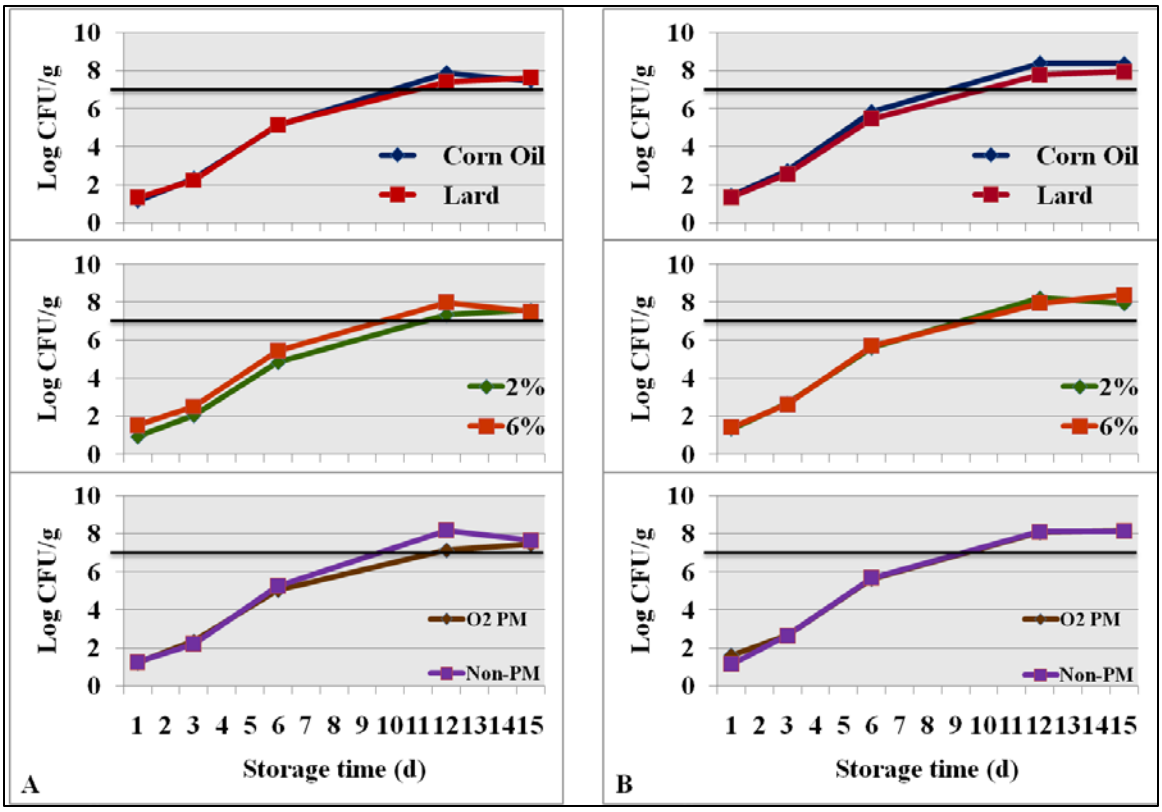

Table 1. Probability (P-value) of Aerobic Plate Counts (APC) after 3 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.40 | 0.80 | 0.94 | 0.46 | 0.59 |
| Inclusion Level | **0.01** | 0.18 | 0.08 | 0.30 | 0.80 |
| Packaging Type | 0.81 | 0.68 | 0.50 | 0.10 | 0.54 |
| SEM[1] | 0.15 | 0.23 | 0.24 | 0.43 | 0.23 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.63 | 0.68 | 0.23 | 0.08 | 0.28 |
| Inclusion Level | 0.60 | 0.93 | 0.70 | 0.43 | 0.24 |
| Packaging Type | **0.02** | 0.98 | 0.85 | 0.91 | 0.96 |
| SEM | 0.13 | 0.29 | 0.20 | 0.24 | 0.26 |

[1]SEM= Pooled Standard Error of the Mean

Figure 2. Main effects of dietary fat and inclusion levels on *Pseudomonas* spp. of (A) Breast meat and (B) Thigh meat after 3 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

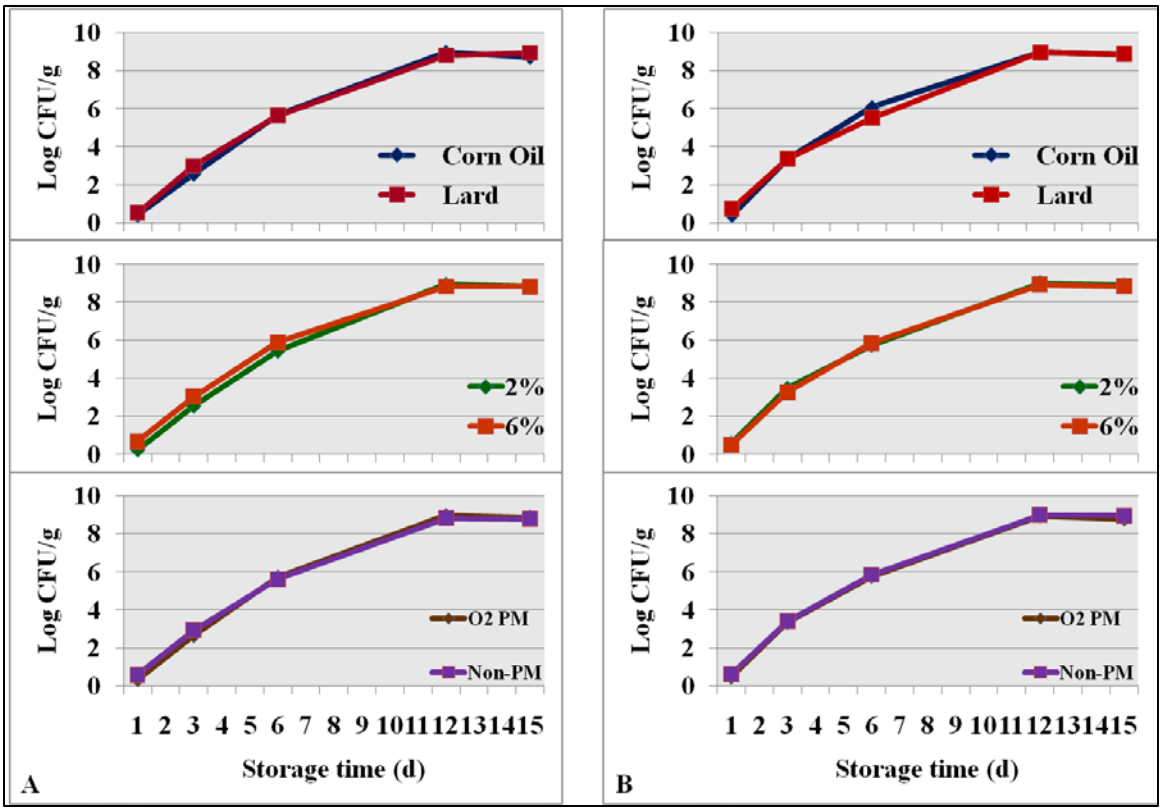

Table 2. Probability (P-value) of *Pseudomonas* spp. after 3 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.67 | 0.16 | 0.92 | 0.09 | **0.03** |
| Inclusion Level | 0.12 | 0.12 | 0.10 | 0.23 | 0.85 |
| Packaging Type | 0.41 | 0.36 | 0.72 | 0.14 | 0.36 |
| SEM[1] | 0.19 | 0.21 | 0.19 | 0.07 | 0.07 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.11 | 0.98 | **0.04** | 0.86 | 0.94 |
| Inclusion Level | 0.60 | 0.53 | 0.69 | 0.44 | 0.48 |
| Packaging Type | 0.55 | 0.87 | 0.79 | 0.69 | 0.06 |
| SEM | 0.15 | 0.25 | 0.18 | 0.07 | 0.07 |

[1]SEM= Pooled Standard Error of the Mean

Figure 3. Main effects of dietary fat and inclusion levels on Lactic Acid Bacteria (LAB) of (A) Breast meat and (B) Thigh meat after 3 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

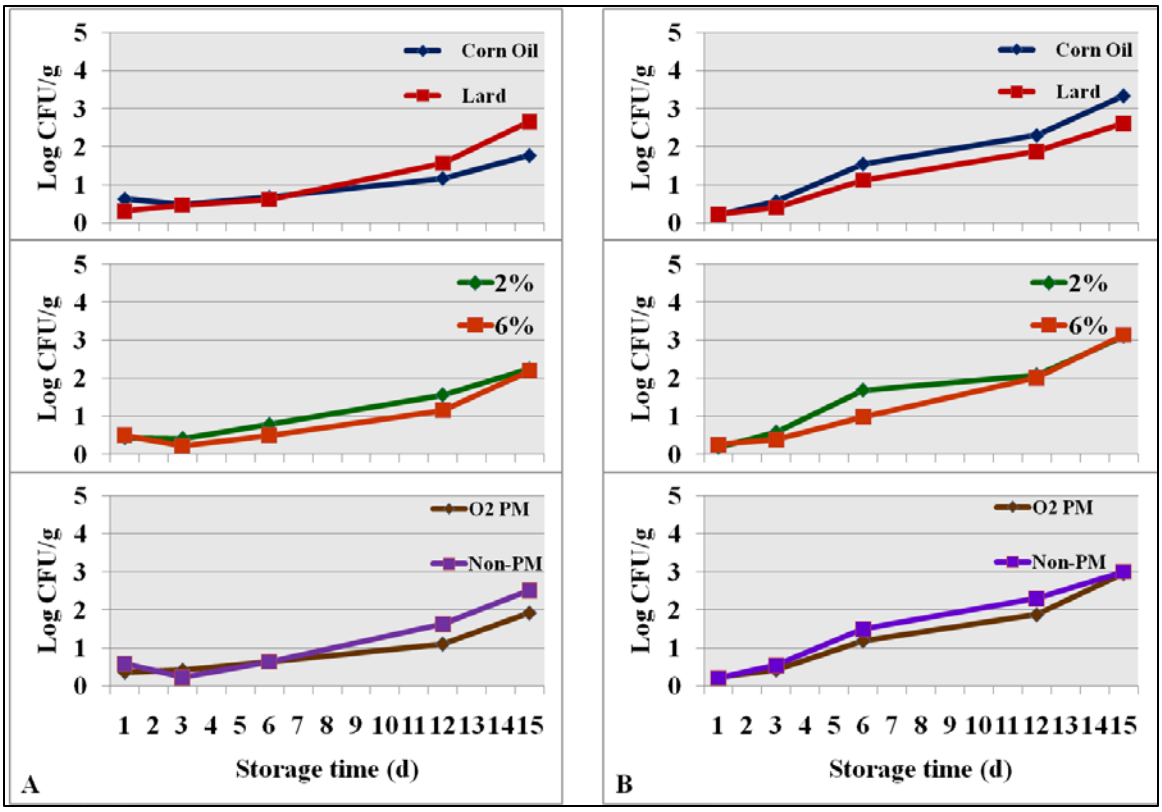

Table 3. Probability (P-value) of Lactic Acid Bacterial (LAB) after 3 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.06 | **0.048** | 0.86 | 0.48 | 0.14 |
| Inclusion Level | 0.60 | 0.16 | 0.42 | 0.47 | 0.93 |
| Packaging Type | 0.20 | 0.16 | 0.97 | 0.35 | 0.32 |
| SEM[1] | 0.12 | 0.10 | 0.24 | 0.38 | 0.41 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.92 | 0.43 | 0.44 | 0.43 | 0.17 |
| Inclusion Level | 0.61 | 0.34 | 0.22 | 0.98 | 0.63 |
| Packaging Type | 0.92 | 0.59 | 0.58 | 0.43 | 0.93 |
| SEM | 0.10 | 0.15 | 0.39 | 0.38 | 0.37 |

[1]SEM= Pooled Standard Error of the Mean

Figure 4. Main effects of dietary fat and inclusion levels on Yeasts and Molds (YAM) of (A) Breast meat and (B) Thigh meat after 3 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

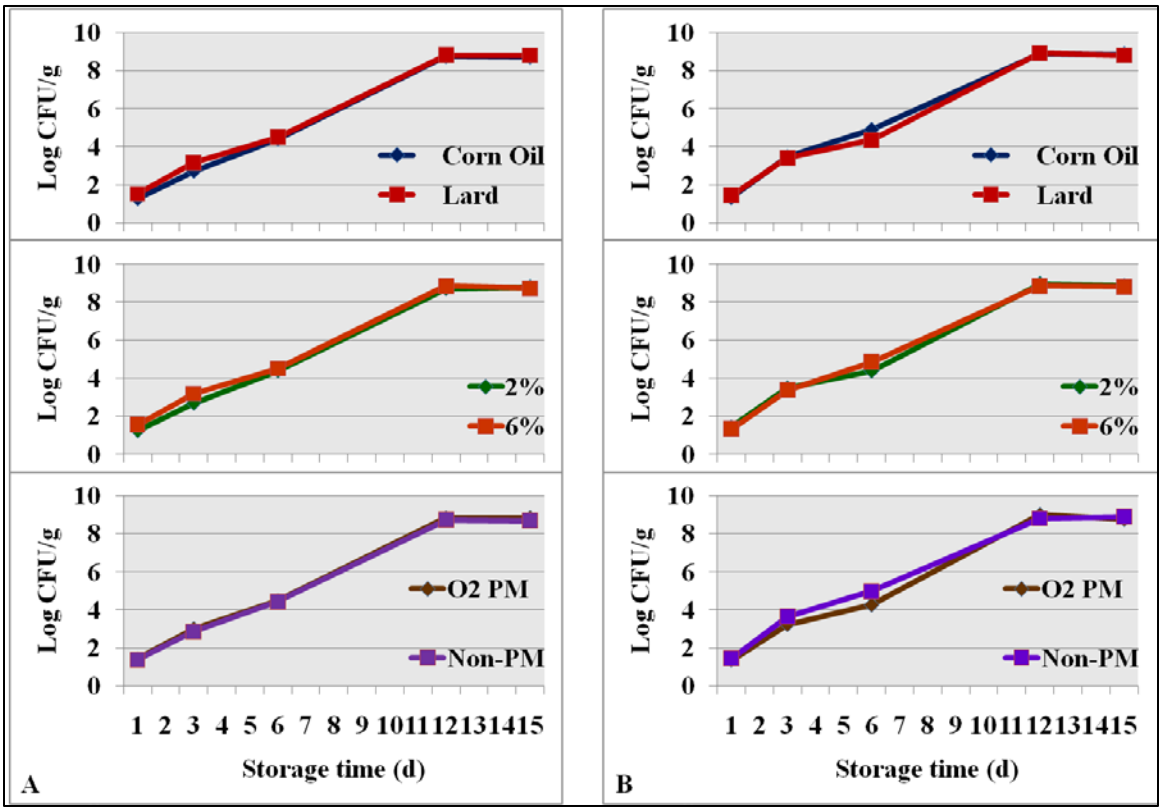

Table 4. Probability (P-value) of Yeasts and Molds (YAM) after 3 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.34 | 0.14 | 0.84 | 0.63 | 0.44 |
| Inclusion Level | 0.15 | 0.09 | 0.82 | 0.34 | 0.50 |
| Packaging Type | 0.88 | 0.59 | 0.90 | 0.53 | 0.19 |
| SEM[1] | 0.15 | 0.21 | 0.31 | 0.11 | 0.08 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.54 | 0.87 | 0.09 | 0.80 | 0.48 |
| Inclusion Level | 0.63 | 0.71 | 0.17 | 0.09 | 0.56 |
| Packaging Type | 0.64 | 0.24 | **0.04** | **0.004** | 0.11 |
| SEM | 0.14 | 0.24 | 0.23 | 0.05 | 0.05 |

[1]SEM= Pooled Standard Error of the Mean

Figure 5. Main effects of dietary fat and inclusion levels on Aerobic Plate Counts (APC) of (A) Breast meat and (B) Thigh meat after 6 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

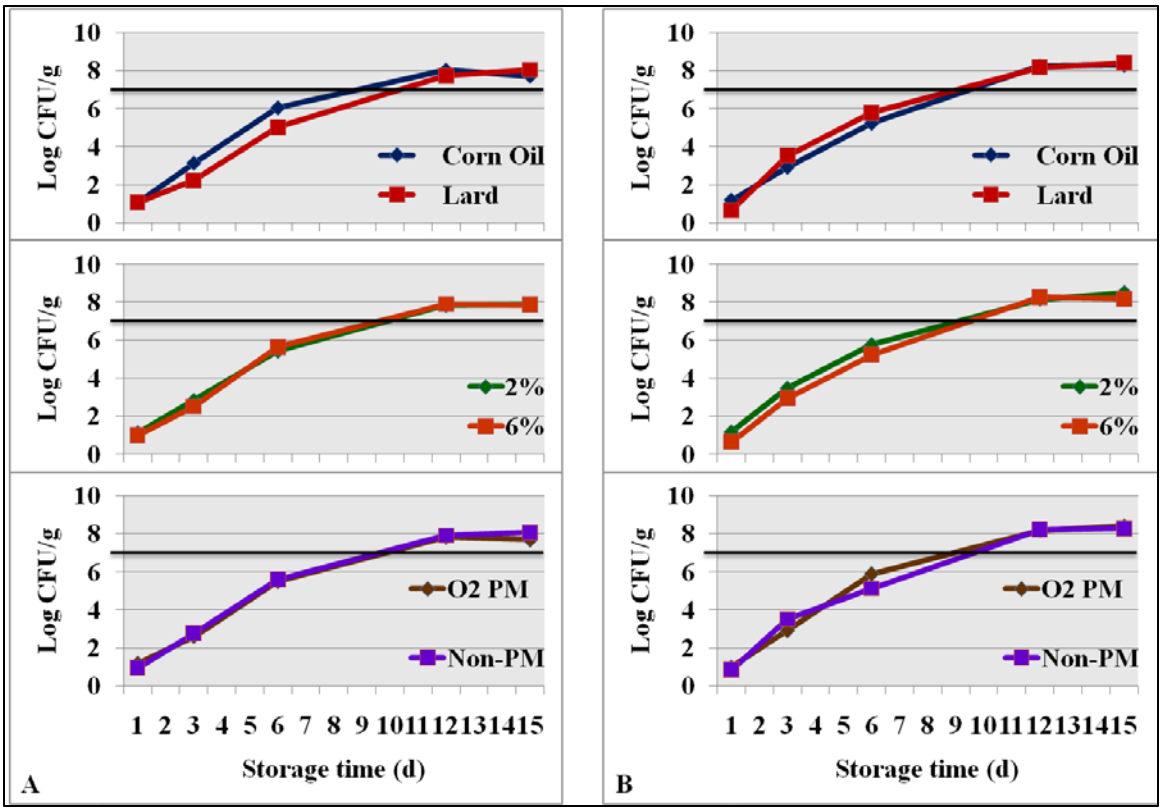

Table 5. Probability (P-value) of Aerobic Plate Counts (APC) after 6 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.95 | **0.01** | **0.03** | 0.48 | 0.35 |
| Inclusion Level | 0.61 | 0.36 | 0.68 | 0.91 | 0.72 |
| Packaging Type | 0.33 | 0.64 | 0.81 | 0.84 | 0.26 |
| SEM[1] | 0.18 | 0.24 | 0.32 | 0.30 | 0.24 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.063 | 0.13 | 0.27 | 0.77 | 0.74 |
| Inclusion Level | 0.059 | 0.18 | 0.30 | 0.67 | 0.29 |
| Packaging Type | 0.58 | 0.14 | 0.12 | 0.12 | 0.66 |
| SEM | 0.19 | 0.27 | 0.35 | 0.14 | 0.22 |

[1]SEM= Pooled Standard Error of the Mean

Figure 6. Main effects of dietary fat and inclusion levels on *Pseudomonas* spp. of (A) Breast meat and (B) Thigh meat after 6 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

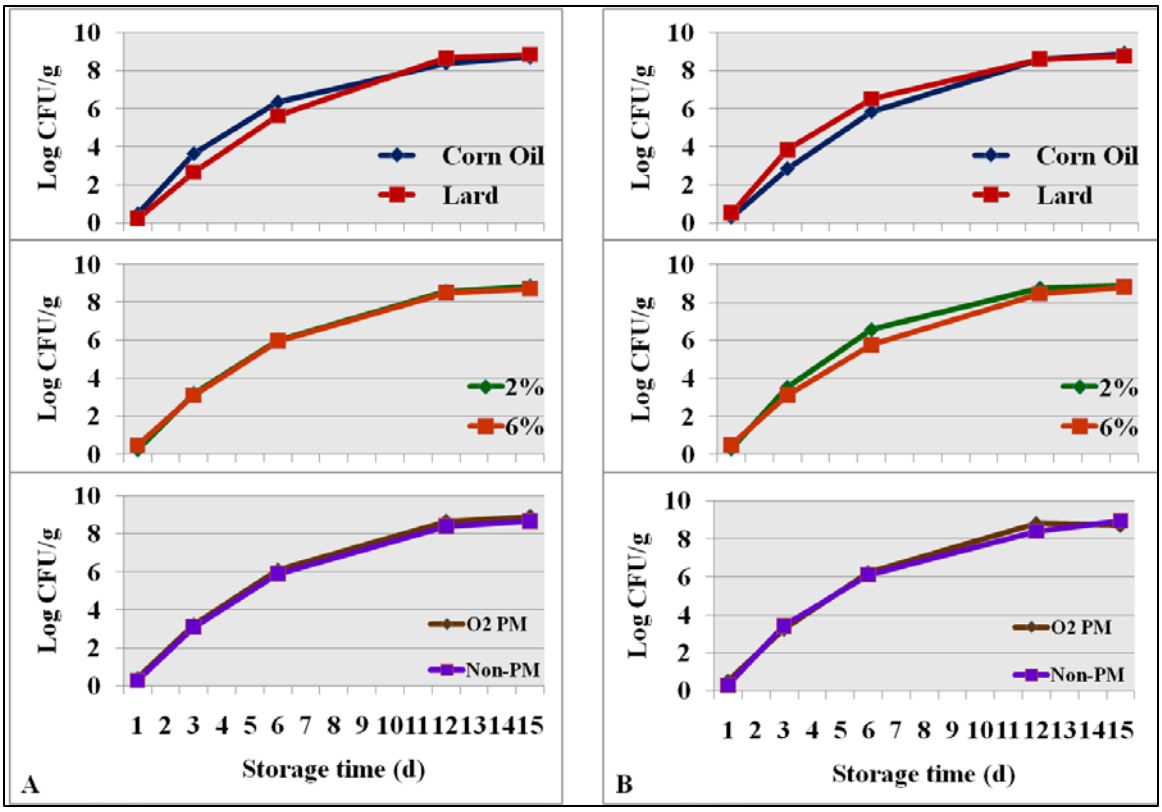

Table 6. Probability (P-value) of *Pseudomonas* spp. after 6 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.24 | **0.01** | 0.052 | 0.15 | 0.51 |
| Inclusion Level | 0.23 | 0.80 | 0.89 | 0.73 | 0.58 |
| Packaging Type | 0.61 | 0.79 | 0.60 | 0.17 | 0.27 |
| SEM[1] | 0.15 | 0.26 | 0.25 | 0.12 | 0.15 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.40 | **0.005** | **0.03** | 0.99 | 0.41 |
| Inclusion Level | 0.42 | 0.19 | **0.01** | 0.16 | 0.70 |
| Packaging Type | 0.40 | 0.60 | 0.65 | 0.06 | 0.25 |
| SEM | 0.18 | 0.23 | 0.21 | 0.15 | 0.13 |

[1]SEM= Pooled Standard Error of the Mean

Figure 7. Main effects of dietary fat and inclusion levels on Lactic Acid Bacteria (LAB) of (A) Breast meat and (B) Thigh meat after 6 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

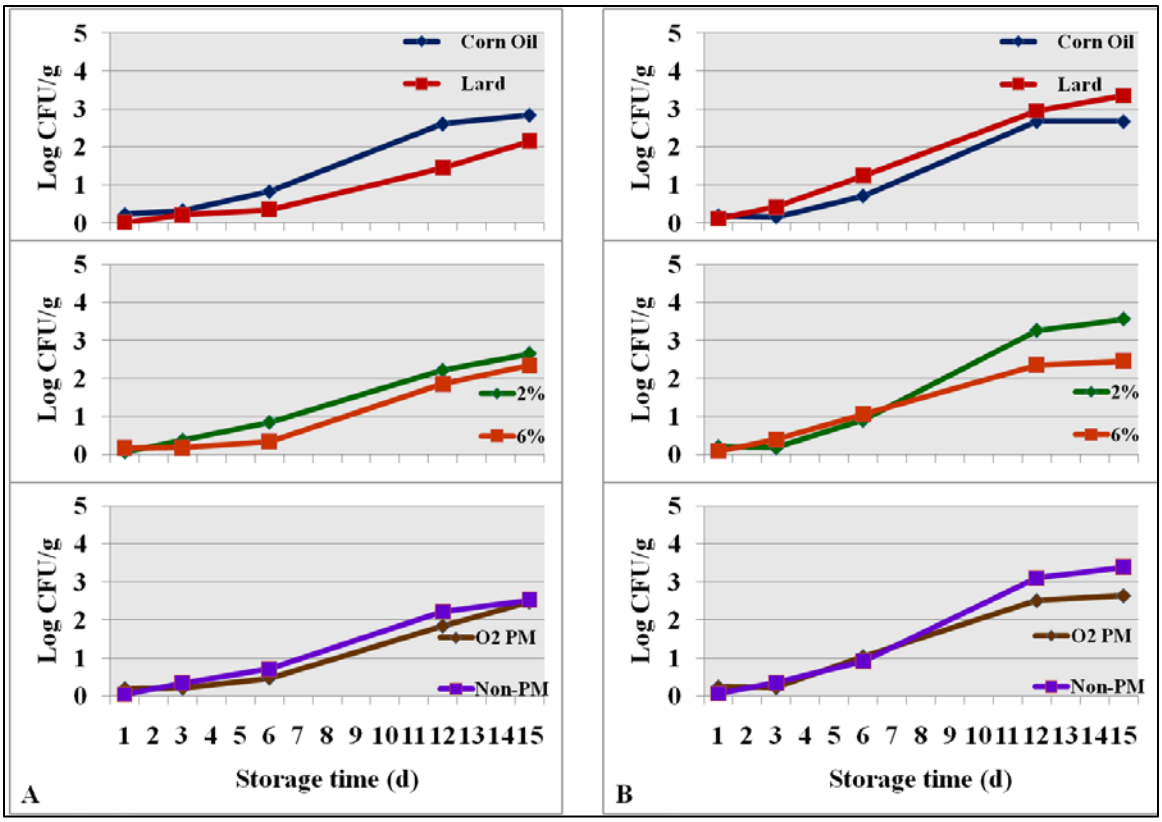

Table 7. Probability (P-value) of Lactic Acid Bacteria (LAB) after 6 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | **0.045** | 0.48 | 0.25 | 0.06 | 0.23 |
| Inclusion Level | 0.34 | 0.25 | 0.24 | 0.54 | 0.57 |
| Packaging Type | 0.20 | 0.39 | 0.56 | 0.53 | 0.93 |
| SEM[1] | 0.08 | 0.12 | 0.29 | 0.42 | 0.39 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.44 | 0.21 | 0.25 | 0.71 | 0.35 |
| Inclusion Level | 0.27 | 0.31 | 0.73 | 0.22 | 0.14 |
| Packaging Type | 0.13 | 0.52 | 0.78 | 0.42 | 0.31 |
| SEM | 0.08 | 0.14 | 0.32 | 0.51 | 0.51 |

[1]SEM= Pooled Standard Error of the Mean

Figure 8. Main effects of dietary fat and inclusion levels on Yeasts and Molds (YAM) of (A) Breast meat and (B) Thigh meat after 6 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

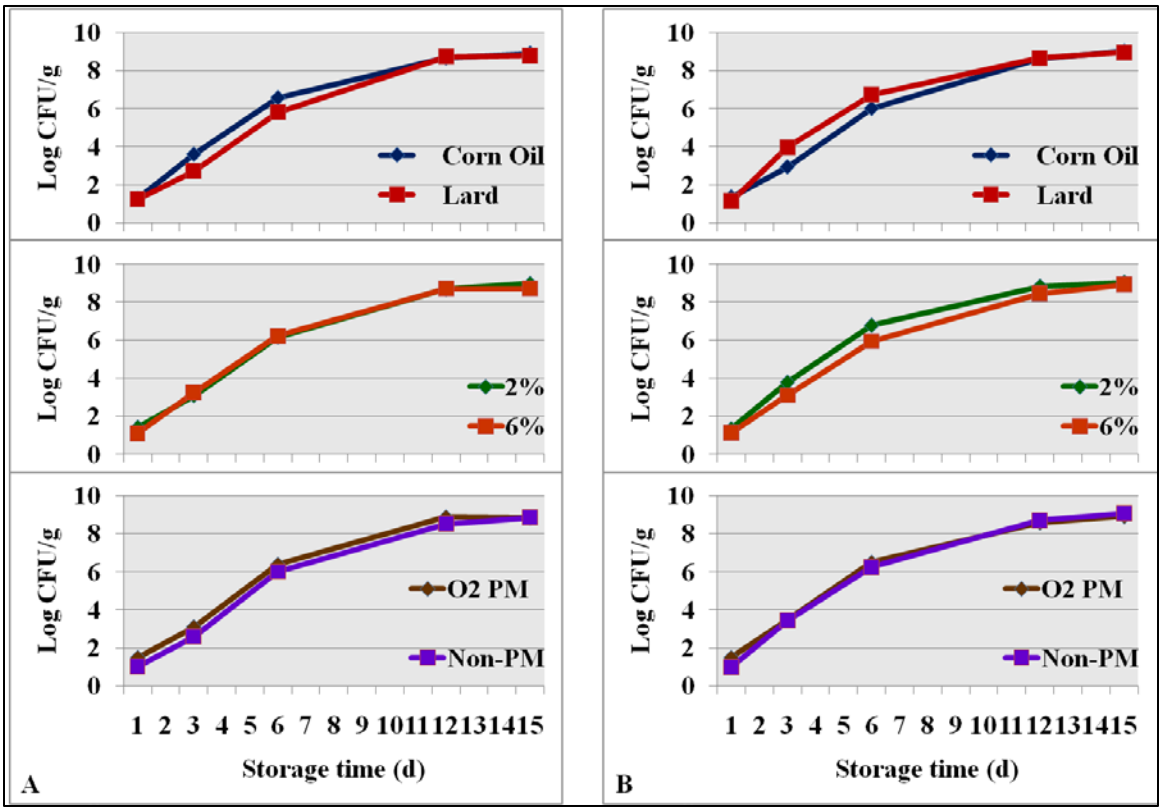

Table 8. Probability (P-value) of Yeasts and Molds (YAM) after 6 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.92 | **0.007** | **0.04** | 0.71 | 0.50 |
| Inclusion Level | 0.38 | 0.59 | 0.84 | 0.87 | 0.08 |
| Packaging Type | 0.19 | 0.71 | 0.29 | 0.09 | 0.86 |
| SEM[1] | 0.24 | 0.21 | 0.25 | 0.15 | 0.11 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.44 | **0.005** | **0.02** | 0.74 | 0.79 |
| Inclusion Level | 0.45 | 0.051 | **0.01** | **0.005** | 0.55 |
| Packaging Type | 0.06 | 0.93 | 0.41 | 0.30 | 0.46 |
| SEM | 0.18 | 0.24 | 0.22 | 0.08 | 0.15 |

[1]SEM= Pooled Standard Error of the Mean

Figure 9. Main effects of dietary fat and inclusion levels on lipid oxidation (TBARS) of (A) Breast meat and (B) Thigh meat after 3 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

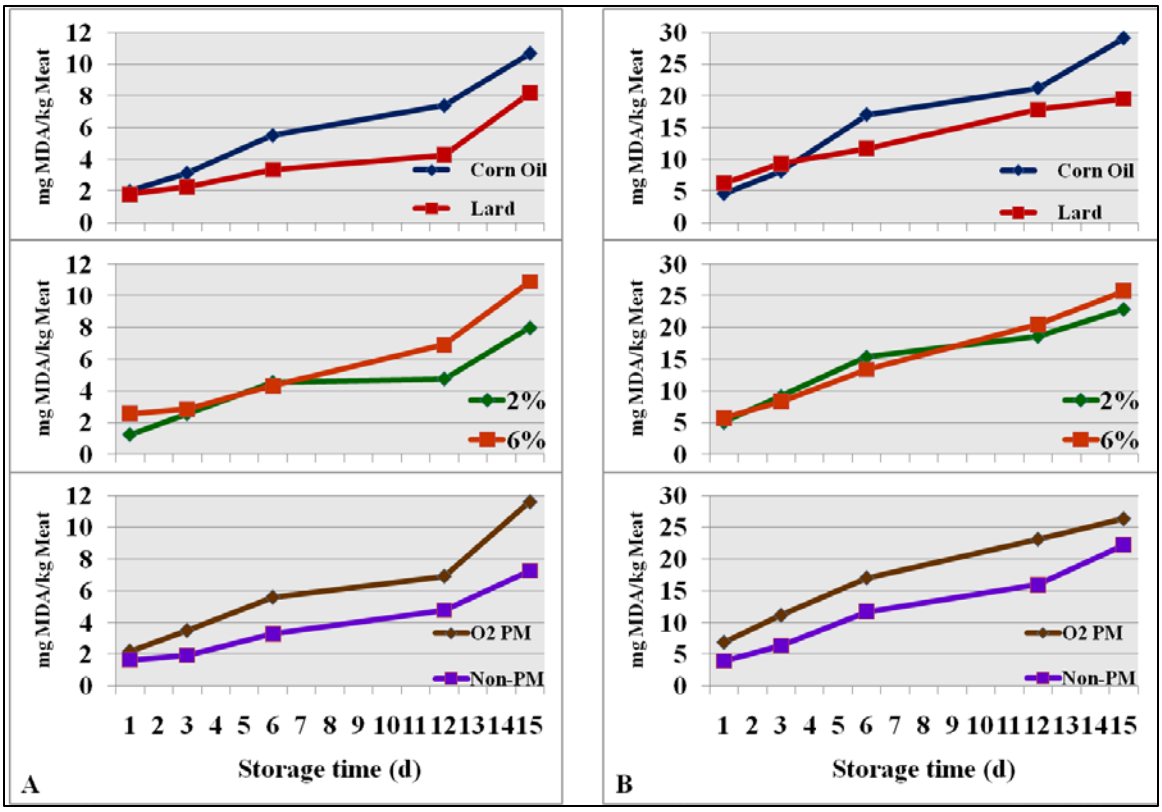

Table 9. Probability (P-value) of lipid oxidation (TBARS) after 3 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.16 | 0.46 | 0.14 | 0.30 | 0.35 |
| Inclusion Level | **0.04** | 0.20 | 0.37 | 0.54 | 0.66 |
| Packaging Type | **0.0006** | **0.02** | 0.21 | 0.45 | 0.60 |
| SEM[1] | 1.07 | 1.76 | 1.83 | 3.18 | 3.10 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.25 | 0.68 | 0.14 | 0.54 | **0.0002** |
| Inclusion Level | 0.65 | 0.78 | 0.57 | 0.74 | 0.20 |
| Packaging Type | **0.048** | 0.11 | 0.14 | 0.19 | 0.07 |
| SEM | 1.00 | 2.05 | 2.49 | 3.81 | 1.54 |

[1]SEM= Pooled Standard Error of the Mean

Figure 10. Main effects of dietary fat and inclusion levels on lipid oxidation (TBARS) of (A) Breast meat and (B) Thigh meat after 6 m of frozen storage at -18°C. Each graph represents a factor (type of fat (Corn Oil vs. Lard), inclusion level (2% vs. 6%), and package type ($O_2$-PM vs. Non-PM)).

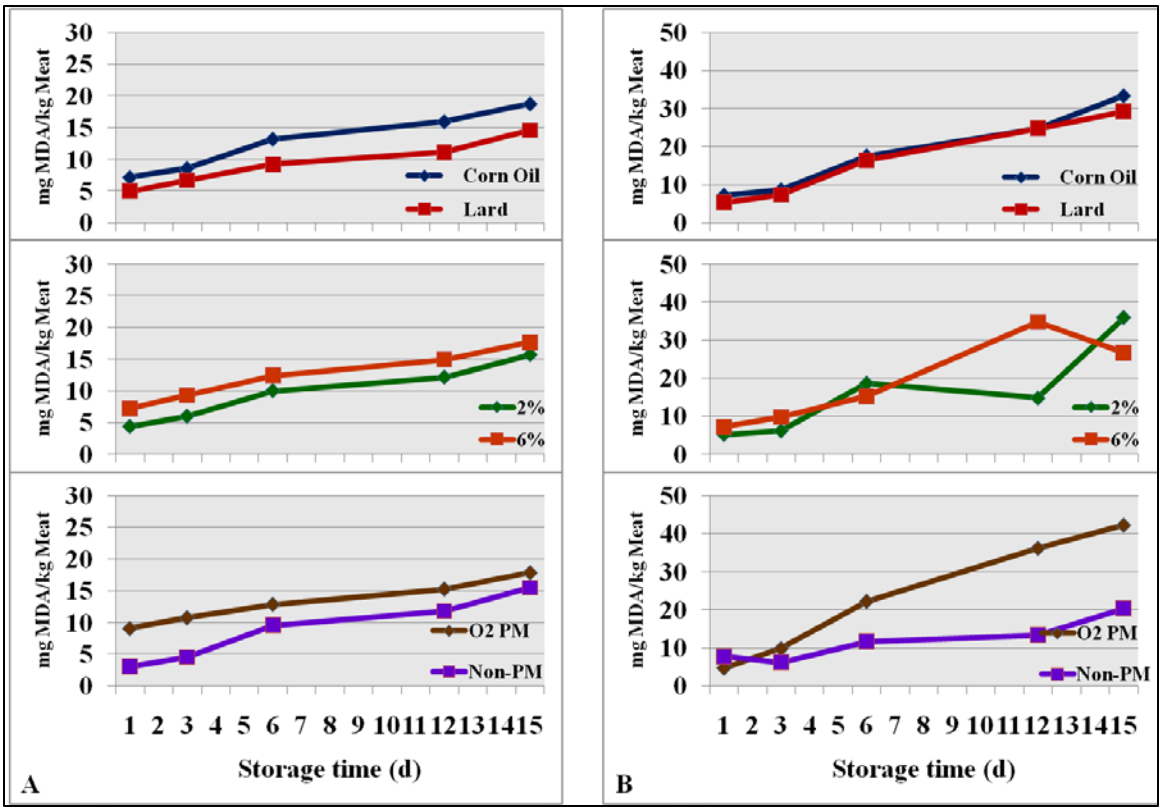

Table 10. Probability (P-value) of lipid oxidation (TBARS) after 6 m of frozen storage.

| Factors | Storage Time (d) | | | | |
|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 15 |
| **Breast Meat** | | | | | |
| Fat Source | 0.71 | 0.38 | 0.09 | 0.13 | 0.34 |
| Inclusion Level | 0.03 | 0.78 | 0.86 | 0.29 | 0.26 |
| Packaging Type | 0.36 | 0.13 | 0.08 | 0.29 | 0.10 |
| SEM[1] | 0.41 | 0.69 | 0.89 | 1.41 | 1.79 |
| **Thigh Meat** | | | | | |
| Fat Source | 0.24 | 0.63 | 0.82 | 0.99 | 0.57 |
| Inclusion Level | 0.22 | 0.15 | 0.52 | **0.02** | 0.21 |
| Packaging Type | 0.06 | 0.15 | 0.051 | **0.007** | **0.006** |
| SEM | 1.10 | 1.17 | 2.49 | 3.81 | 1.54 |

[1]SEM= Pooled Standard Error of the Mean

# CHAPTER V

# DIETARY VITAMIN E SUPPLEMENTATION AND SHELF LIFE OF GROUND BROILER CHICKEN MEAT DURING REFRIGERATED STORAGE

## ABSTRACT

This study was conducted to determine the microbial spoilage and oxidative stability of ground raw and cooked breast and thigh meat (during refrigerated storage) from broiler chickens fed graded levels of vitamin E. A total of 480 female broilers were assigned to 4 dietary vitamin E treatments (IU/kg of feed): 30 (basal level), 60, 120 and 240. Each of the 4 dietary treatments was fed in a three-stage feeding program to 12 replicate pens of 10 birds and reared to 49 d of age. Upon processing, one-half of the replicate pens from each dietary treatment were used for raw and the other half for cooked meat treatments. Boneless-skinless breast and thigh meat (6 birds per pen) was ground, pooled by pen, formed into patties, vacuum packaged (oxygen impermeable) either as raw or cooked meat (internal temperature of 80°C), and held at 2°C. Samples were analyzed for microbial spoilage (aerobic plate counts; APC, lactic acid bacteria; LAB, and yeast and molds; YAM) and lipid oxidation (TBARS) following 1, 3, 6, 12 d of refrigerated storage (2°C). Microbial numbers increased with storage time in both raw breast and thigh meat ($P < 0.05$), with APC counts on thigh meat reaching 7 log10 CFU/g after 12 d of storage. Cooking reduced microbial counts and slowed the rate of microbial growth in breast and thigh meat during refrigerated storage. Vitamin E supplementation affected microbial

counts only for raw (APC on 6 d) and cooked (APC and LAB on 6 and 12 d, and YAM on 12 d) thigh meat, with levels of 240 IU/kg significantly impeding microbial growth as compared with basal levels. Lipid oxidation increased during refrigerated storage on both raw and cooked ground meat (breast < thigh meat). Dietary vitamin E supplementation at levels >120 IU/kg significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared with the basal level. In this study, microbial and oxidative changes that occur during refrigerated storage of ground cooked broiler meat appeared to correlate and were positively influenced by dietary vitamin E supplementation.

**Key Words:** vitamin E, shelf-life, lipid oxidation

## INTRODUCTION

Vitamin E (α-tocopheryl acetate) is the major chain reaction interrupting fat-soluble biological antioxidant (Villaverde et al., 2004) and has practical importance in increasing the shelf life of meat. There are several factors that can affect vitamin E intestinal uptake and, therefore, its deposition. Two of the major factors affecting vitamin E absorption are its dietary concentration and, as it is a fat-soluble substance, the concentration and the fatty acid profile of dietary fat. The inclusion of polyunsaturated fatty acids (PUFA) in poultry diets improves general lipid digestion and absorption (Young and Garret, 1963). Oxidative damage occurs in the living animal due to an imbalance between the production of reactive oxygen and defense mechanism of the animal against oxidative stress. Oxidation is inherent to metabolism, but an excessive formation of reactive species in oxidation processes can cause damage to vital

components in biological systems (Halliwell et al., 1995). Oxidation increases as a result of high intake of oxidized lipids, oxidation of sensitive polyunsaturated fatty acids (PUFA), or a low intake of nutrients involved in the antioxidant defense system (Morrissey et al., 1998). Consequently, poultry meat is enriched with polyunsaturated fatty acids and readily susceptible to oxidative deterioration (Kanner, 1994). Oxidation is a very general process, which affects lipids, pigments, proteins, DNA, carbohydrates, and vitamins in tissues (Kanner, 1994). In muscle and fat tissue, oxidation continues postmortem and affects the shelf-life of meat and meat products. To maximize the oxidative stability of meat, antioxidants (mostly α-tocopheryl acetate) are added to feeds. The beneficial effect of dietary supplementation of vitamin E to enhance the stability of lipids in muscle foods has been reported for poultry, beef cattle, veal calves, and pigs (Gray et al., 1996; Jensen et al., 1998). In addition, several studies also demonstrated the beneficial effects of dietary natural antioxidants on the oxidative stability of meat or meat products (Tang et al., 2000; Mason et al., 2005; Haak et al., 2006; O'Grady et al., 2006; Goni et al., 2007). The oxidative stability of poultry meat depends largely on the α-tocopherol present in cell membrane phospholipids, which in turn is dependent on the level of vitamin E added to the diet (Wen et al., 1997). Dietary supplementation with vitamin E has been shown to improve the oxidative stability of meat during storage (Carreras et al., 2004). The major objective of this study was to evaluate the influence of vitamin E supplementation level effects on microbial spoilage and lipid oxidative stability of either raw or cooked ground chicken breast and thigh meat when stored under refrigerated storage.

## MATERIALS AND METHODS

### Sample Collection and Treatment

A total of 480 female (Ross x Ross 708) broilers were raised in 48 floor pens to 49 d of age in floor pens with new pine-shavings. Four dietary treatments were fed to 12 replicate pens of 10 birds (480 birds total) to 49 d of age. The experimental diets were provided on a three stage feeding program of 0.91 kg/ bird starter, 1.81 kg/bird grower, and withdrawal feeds to 49 d of age. There were four dietary treatments (IU of vitamin E/kg of feed): (1) 30 [Basal level], (2) 60, (3)120, and (4) 240, and to each treatment 2% corn oil was added. Previous studies by Saenmahayak et al. (2009, 2010), showed that birds performed better on 2% corn oil supplemented diets. Feed and water were provided *ad libitum*.

At 49 d of age, 10 birds per pen (480 total) were processed at the Auburn University Poultry Science Department Processing Plant simulating commercial processing practices. Upon processing, boneless-skinless breast and thigh meat (6 birds per pen) were ground separately, pooled, formed into patties (approximately 100 g, 1.5 cm thickness) and sealed in vacuum packaging either raw or cooked (at 177°C to an internal temperature of 80°C in a commercial radiant oven). A total of 96 samples, 48 from ground breast meat (24 raw samples and 24 cooked samples) and 48 from ground thigh meat (24 raw samples and 24 cooked samples), were analyzed for lipid oxidation (TBARS) and microbial spoilage (aerobic plate counts, lactic acid bacteria; LAB, and Yeasts and Molds; YAM). Meat samples were measured fresh (refrigerated at 2°C) at 1, 3, 6, and 12 d of storage.

**Microbiological Analysis**

Ground samples of 10 g were homogenized in 90 ml of peptone water for 2 min using a Stomacher (model 400 Lab Blender, Seward Ltd., London, UK). A 10-fold dilution series of the meat homogenate was made in peptone water (Oxoid Ltd., Basingstoke, UK) and plated duplicate sample of 100 µl each. Total aerobic plate counts were performed on Plate Count Agar (PCA; Oxoid Ltd., Basingstoke, UK), and plates were incubated aerobically at 37°C for 24 h. The LAB were determined using deMan, Rogosa, and Sharpe Agar (MRS; Acumedia Manufactures Inc., Baltimore, MD) and incubated anaerobically at 25°C for 48 h in anaerobic jars with a disposable Anaerobic Gas Pak (AneroGen; Oxoid Ltd., Basingstoke, UK). Yeasts and molds were counted on potato dextrose agar (PDA; Acumedia Manufactures Inc., Baltimore, MD) following incubation at 25°C for 5 d. Bacterial numbers were reported as $\log_{10}$ colony forming units per g of meat.

**Thiobarbituric Acid Reactive Substances (TBARS)**

Samples (10 g of ground meat) were homogenized with 30 ml of DW for 2 min and 2 ml of homogenate was added with 4 ml of TCA/TBA reagent [15% TCA (w/v) and 20 mM TBA] and 100 µl BHA. Solution was heated 15 min in boiling water, cool for 10 min in cold water, and centrifuged at 3000 rpm for 10 min. The supernatant was read against a blank (contains all reagents minus sample) at 531 nm. The absorbance was calculated from a calibration prepared using 1,1,3,3-tetramethoxypropane (TEP) as a standard. The TBARS number was expressed as mg of malondialdehyde (MDA) per kg of meat (mg MDA/kg).

**Statistical Analysis**

A completely randomized design was used to assign pens to the various treatments. The data were statistically analyzed using the General Linear Model procedures of SAS 9.1.2 software (SAS Institute, 2002-2003), with replicate pens as the error term. The Tukey's test was used to compare and separate means when main effects were significant ($P<0.05$).

## RESULTS AND DISCUSSION

Microbial populations in ground meat were assessed after 1, 3, 6 and 12 d of storage at 2°C. Microbial numbers increased with storage time in both raw ground breast and thigh meat. In Raw and cooked breast ground meat, vitamin E supplementation had no significant effect ($P>0.05$) on the microbial profile (APC, LAB and YAM) during the 12 d refrigerated storage. However, vitamin E supplementation affected microbial counts (APC) only for raw meat on 6 d and cooked meat on 6 and 12 d for thigh meat (Figure 1). APC counts on ground raw thigh meat reached 7 $\log_{10}$ CFU/g after 12 d of storage. Lactic acid bacteria (LAB) growth rate showed the same trend on both breast and thigh meat and increased as storage time increased (Figure 2). As expected, cooked meat had lower microbial counts and slowed microbial growth in both breast and thigh meat. YAM counts were lower on d 12 of storage for cooked thigh meat with vitamin E levels of 240 IU/kg significantly impeding microbial growth as compared to basal levels (Figure 3). The results of this study are consistent with those reported by Govaris et al. (2010) in which the dietary supplementation of 150-300 mg vitamin E/kg of feed had no inhibitory effect on LAB and psychrotrophic bacterial counts in breast samples stored for up to 12

days. Other studies have also reported limited influence of dietary vitamin E on the APC in raw pork and beef meat (Cannon et al., 1995; Chan et al., 1995). However, Asghar et al. (1991) reported that bacterial growth was greater in chops from pigs fed diets with supplemental vitamin E, which had lower drip losses.

Lipid oxidation increased during refrigerated storage on both raw and cooked ground meat with thigh meat exhibiting a higher rate of oxidation than breast meat (Figure 4). Dietary vitamin E supplementation at levels higher than 120 IU/kg significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared to the basal level. There was more development of lipid oxidation in cooked than in ground raw breast and thigh meat. This indicates that cooking triggers lipid oxidation (Rhee et al., 1996. These results are in agreement with those reported by Cortinas et al. (2005), who observed that as the amount of polyunsaturated fatty acids and vitamin E level increased in the diet, higher and lower lipid oxidation development, respectively, was detected in cooked than raw chicken meat. Previous studies have found that poultry thigh meat had higher amounts of vitamin E than in breast meat, however, it tended to oxidize faster (Lin et al., 1989; Higgins et al., 1999). In another study by Narciso-Gaytan et al. (2010), broiler chickens fed soybean oil and low dietary vitamin E concentration (33 mg/kg) showed the highest rate of lipid oxidation. The level of vitamin E reduced lipid oxidation rate over storage time, in this study showing a higher antioxidant activity. Raw thigh meat was susceptible to lipid oxidation over storage time in birds reared on feeds containing lower vitamin E level in the diet. These results indicate that raw fresh thigh meat are more likely to develop lipid oxidation during

storage and that, to prevent lipid spoilage, a higher concentration of vitamin E supplementation than the currently used commercial levels should be considered.

This study suggests that dietary vitamin E supplementation at higher levels (>120 IU/kg) significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared to the basal level. Thigh meat reached 7 $\log_{10}$ CFU/g after day 12 of refrigerated storage. Microbial and oxidative changes that occur during refrigerated storage of ground and cooked broiler meat were positively correlated with dietary vitamin E supplementation. Significant effects were achieved in thigh meat (especially in cooked) but not in breast meat. Cooking reduced microbial counts and slowed the rate of microbial growth in breast and thigh meat during refrigerated storage. Lipid oxidation increased during refrigerated storage on both raw and cooked ground meat (breast < thigh meat).

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Figure 1. Main effects of vitamin E supplementation on Aerobic Plate Counts (APC) of (A) Raw breast meat; (B) Cooked breast meat; (C) Raw thigh meat and (D) Cooked thigh meat during refrigerated storage at 2°C.

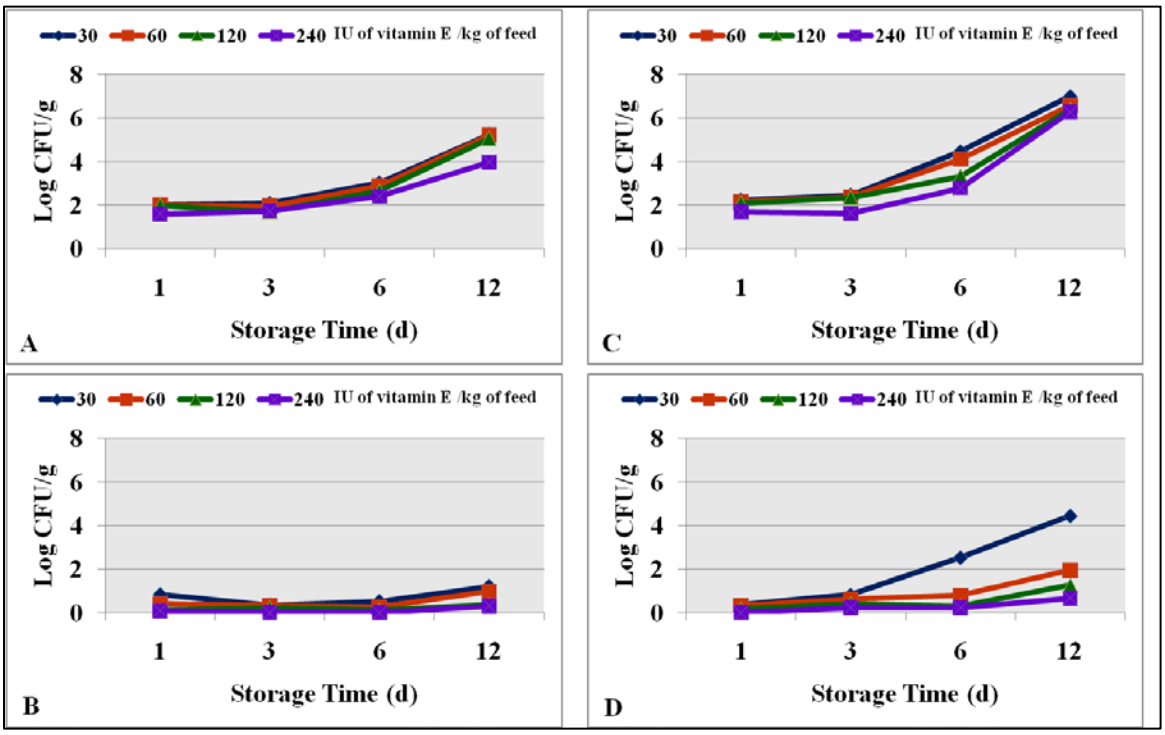

Table 1. Probability (P-value) of Aerobic Plate Counts (APC) of refrigerated storage.

| Treatments | Storage Time (d) | | | |
|---|---|---|---|---|
| | 1 | 3 | 6 | 12 |
| **Breast Meat** | | | | |
| Raw | 0.24 | 0.20 | 0.78 | 0.40 |
| SEM[1] | 0.16 | 0.13 | 0.42 | 0.52 |
| Cooked | 0.10 | 0.77 | 0.46 | 0.21 |
| SEM | 0.20 | 0.23 | 0.22 | 0.34 |
| **Thigh Meat** | | | | |
| Raw | 0.13 | 0.21 | **0.003** | 0.64 |
| SEM | 0.22 | 0.22 | 0.26 | 0.37 |
| Cooked | 0.44 | 0.46 | **0.006** | **0.006** |
| SEM | 0.18 | 0.21 | 0.45 | 0.62 |

[1]SEM= Pooled Standard Error of the Mean

Figure 2. Main effects of vitamin E supplementation on Lactic Acid Bacteria (LAB) of (A) Raw breast meat; (B) Cooked breast meat; (C) Raw thigh meat and (D) Cooked thigh meat during refrigerated storage at 2°C.

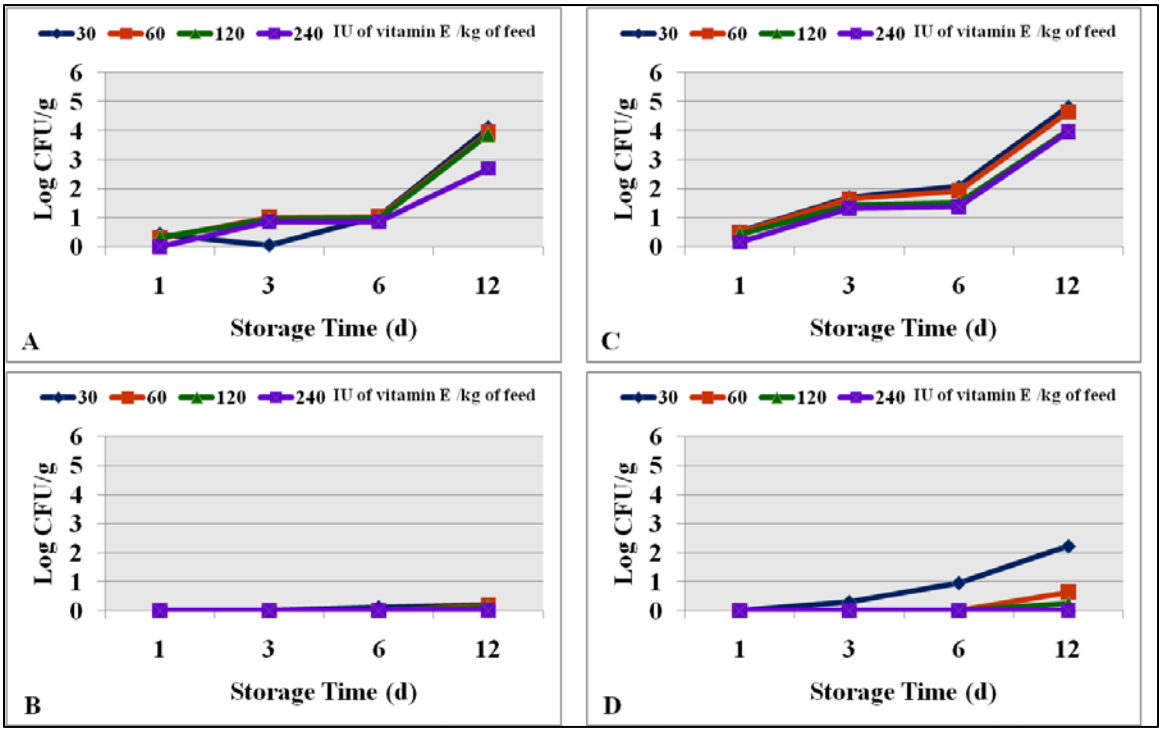

Table 2. Probability (P-value) of Lactic Acid Bacteria (LAB) of refrigerated storage.

| Treatments | Storage Time (d) | | | |
|---|---|---|---|---|
| | 1 | 3 | 6 | 12 |
| **Breast Meat** | | | | |
| Raw | 0.30 | 0.98 | 0.98 | 0.28 |
| SEM[1] | 0.18 | 0.31 | 0.31 | 0.53 |
| Cooked | BDL[2] | BDL | BDL | BDL |
| SEM | 0 | 0 | 0.12 | 0.14 |
| **Thigh Meat** | | | | |
| Raw | 0.051 | 0.84 | 0.44 | 0.41 |
| SEM | 0.22 | 0.34 | 0.33 | 0.44 |
| Cooked | BDL | 0.17 | **0.01** | **0.049** |
| SEM | 0 | 0.09 | 0.21 | 0.45 |

[1]SEM= Pooled Standard Error of the Mean

[2]BDL=Below Detection Limit (< 2 log CFU/g)

Figure 3. Main effects of vitamin E supplementation on Yeast and Mold (YAM) of (A) Raw breast meat; (B) Cooked breast meat; (C) Raw thigh meat and (D) Cooked thigh meat during refrigerated storage at 2°C.

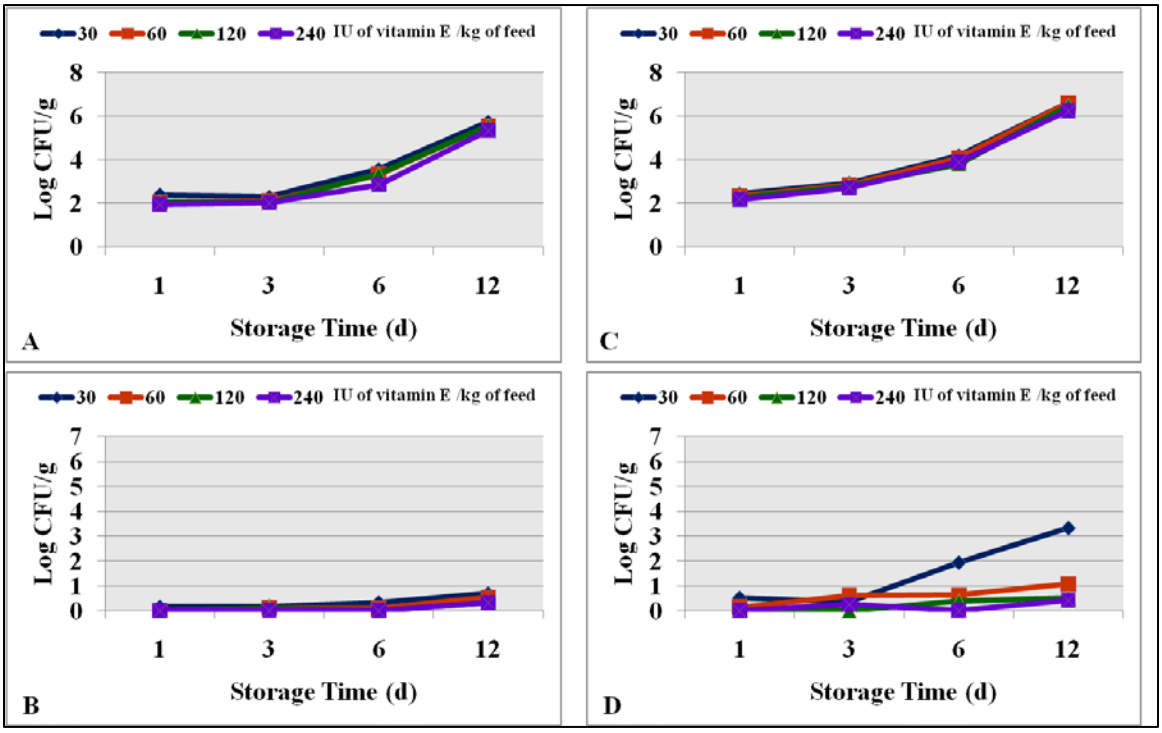

Table 3. Probability (P-value) of Yeast and Mold (YAM) of refrigerated storage.

| Treatments | Storage Time (d) | | | |
|---|---|---|---|---|
| | 1 | 3 | 6 | 12 |
| **Breast Meat** | | | | |
| Raw | 0.30 | 0.50 | 0.72 | 0.86 |
| SEM[1] | 0.16 | 0.13 | 0.38 | 0.36 |
| Cooked | 0.17 | 0.64 | 0.32 | 0.75 |
| SEM | 0.24 | 0.09 | 0.12 | 0.22 |
| **Thigh Meat** | | | | |
| Raw | 0.17 | 0.98 | 0.88 | 0.68 |
| SEM | 0.10 | 0.34 | 0.40 | 0.26 |
| Cooked | 0.33 | 0.64 | 0.07 | **0.02** |
| SEM | 0.19 | 0.36 | 0.50 | 0.72 |

[1]SEM= Pooled Standard Error of the Mean

Figure 4. Main effects of vitamin E supplementation on lipid oxidation (TBARS) of (A) Raw breast meat; (B) Cooked breast meat; (C) Raw thigh meat and (D) Cooked thigh meat during refrigerated storage at 2°C.

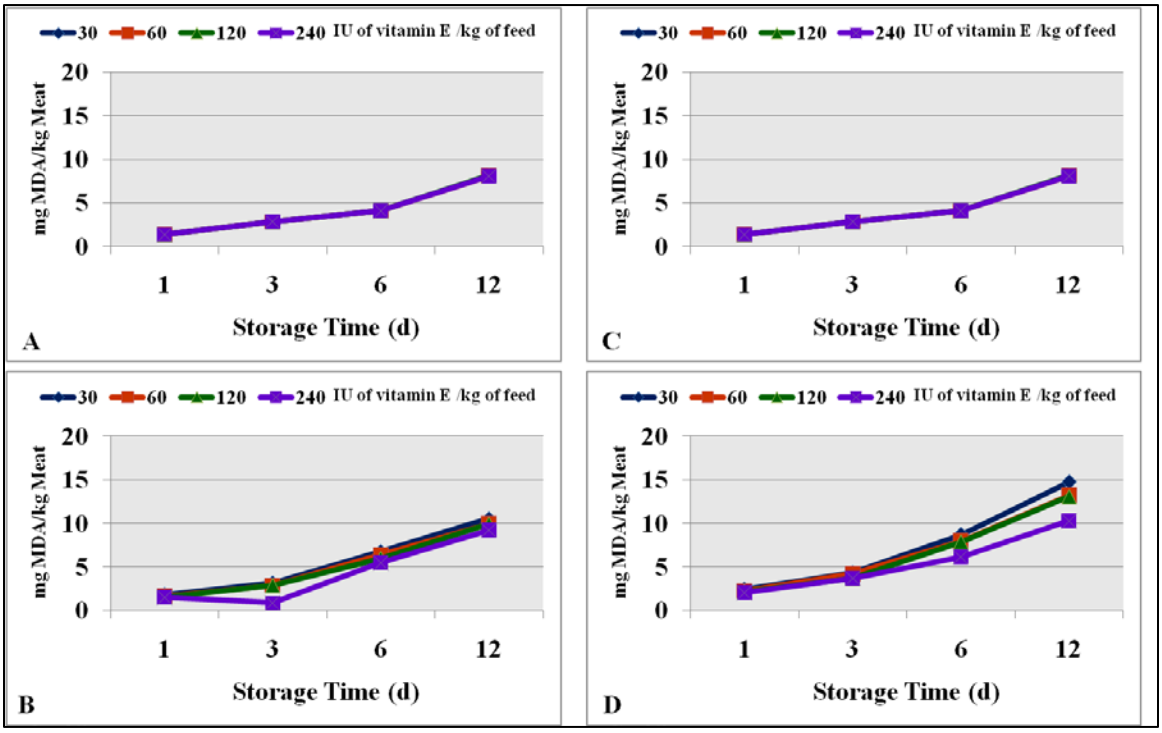

Table 4. Probability (P-value) of lipid oxidation (TBARS) of refrigerated storage.

| Treatments | Storage Time (d) | | | |
|---|---|---|---|---|
| | 1 | 3 | 6 | 12 |
| **Breast Meat** | | | | |
| Raw | 0.85 | 0.81 | 0.34 | 0.75 |
| SEM[1] | 0 | 0.02 | 0.09 | 0.05 |
| Cooked | **0.0002** | **0.04** | 0.17 | **0.009** |
| SEM | 0.04 | 0.28 | 0.10 | 0.23 |
| **Thigh Meat** | | | | |
| Raw | 0.39 | 0.51 | 0.20 | 0.91 |
| SEM | 0 | 0.02 | 0.02 | 0.05 |
| Cooked | **0.01** | **0.0013** | 0.39 | **0.04** |
| SEM | 0.07 | 0.12 | 0.55 | 0.62 |

[1]SEM= Pooled Standard Error of the Mean

# CHAPTER VI

# DIETARY VITAMIN E SUPPLEMENTATION AND SHELF LIFE OF GROUND BROILER CHICKEN MEAT DURING FROZEN STORAGE

## ABSTRACT

This study was conducted to determine the microbial spoilage and oxidative stability of ground raw and cooked breast and thigh meat from broiler chickens fed graded levels of vitamin E during frozen storage. A total of 480 female broilers were assigned to 4 dietary vitamin E treatments (IU/kg of feed): 30 (basal level), 60, 120 and 240. Each of the 4 dietary treatments was fed in a 3 stage feeding program to 12 replicate pens of 10 birds and reared to 49 d of age. Upon processing, one-half of the replicate pens from each dietary treatment were used for raw and the other half for cooked meat treatments. Boneless-skinless breast and thigh meat (5 birds per pen) was ground, pooled by pen, formed into patties, vacuum packaged (oxygen impermeable) either raw or cooked (internal temperature of 80°C), and held under -18°C. Samples were analyzed for microbial spoilage (aerobic plate counts; APC, lactic acid bacteria; LAB, and yeast and molds; YAM) and lipid oxidation (TBARS) following 6, 12, 24, 48 d of frozen storage. Microbial numbers increased with storage time in both raw breast and thigh meat, with APC counts remaining below 3 $\log_{10}$ CFU/g after 48 d of storage. Cooking reduced microbial counts and slowed the rate of microbial growth in breast and thigh meat during frozen storage. Vitamin E supplementation affected microbial counts only for raw breast

(APC on 12 d) and raw thigh (LAB on 12 d), with levels of 240 IU/kg significantly impeding microbial growth as compared with basal levels. Lipid oxidation increased during frozen storage on both raw and cooked ground meat (breast < thigh meat). Dietary vitamin E supplementation at levels more than 120 IU/kg significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared with the basal level. In this study, oxidative changes that occur during frozen storage of ground cooked broiler meat appeared to correlate and were positively influenced by dietary vitamin E supplementation.

**Key Words:** vitamin E, shelf-life, lipid oxidation

## INTRODUCTION

Vitamin E is the major chain reaction interrupting fat-soluble biological antioxidant (Villaverde et al., 2004) and has a practical importance in increasing the shelf life of meat. There are several factors that can affect vitamin E intestinal uptake and its deposition. Two of the major factors affecting vitamin E absorption are its dietary concentration and, as it is a fat-soluble substance, the concentration and the fatty acid profile of dietary fat. Regarding the effect of dietary fat composition, polyunsaturated fatty acids (PUFA) require vitamin E for protection against oxidation. The inclusion of PUFA in poultry diets improves general lipid digestion and absorption (Young and Garret, 1963). Oxidative damage occurs in the living animal due to an imbalance between the production of reactive oxygen and the defense mechanism of the animal against oxidative stress. Oxidation is inherent to metabolism, but an excessive formation of reactive species in oxidation processes can cause damage to vital components in

biological systems (Halliwell et al., 1995). Oxidation increases as a result of high intake of oxidized lipids, oxidation of sensitive polyunsaturated fatty acids (PUFA), or a low intake of nutrients involved in the antioxidant defense system (Morrissey et al., 1998). Oxidation is a very general process, which affects lipids, pigments, proteins, DNA, carbohydrates, and vitamins (Kanner, 1994). In muscle and fat tissue, oxidation continues postmortem and affects the shelf-life of meat and meat products. To maximize the oxidative stability of meat, antioxidants, mostly α-tocopheryl acetate, are added to feeds. The beneficial effect of dietary supplementation with vitamin E to enhance the stability of lipids in muscle foods has been reported for poultry, beef cattle, veal calves, and pigs (Gray et al., 1996; Jensen et al., 1998).

Poultry meat is relatively rich in polyunsaturated fatty acids and readily susceptible to oxidative deterioration (Kanner, 1994). Increasing the degree of unsaturation in the muscle membrane by dietary manipulation increases the susceptibility of chicken meat to oxidative deterioration during storage (Enberg et al., 1996), and decrease in flavor and nutritional value. The oxidative stability of poultry meat depends largely on the contained α-tocopherol present in cell membrane phospholipids, which in turn is dependent on the level of α-tocopheryl acetate added to the diet (Wen et al., 1997). Dietary supplementation with this antioxidant has been shown to increase vitamin E in muscle tissues, improving the oxidative stability of meat during storage (Carreras et al., 2004). The objective of this study was to evaluate the influence of vitamin E supplementation level effects on microbial spoilage and lipid oxidation stability of either raw or cooked ground chicken white and dark chicken meat when stored under frozen storage for different periods of time.

## MATERIALS AND METHODS

### Sample Collection and Treatment

A total of 480 female (Ross x Ross 708) broilers were raised in 48 floor pens to 49 d of age in pens prepared with new pine-shaving bedding. Four dietary treatments were fed to 12 replicate pens of 10 birds (480 birds total) to 49 d of age. The experimental diets were provided in a three stage feeding program of 0.91 kg starter, 1.81 kg grower, and withdrawal to 49 d of age. The four dietary treatments (IU/kg of feed) were: (1) 30 [Basal level], (2) 60, (3)120, and (4) 240, with an added 2% corn oil. Body weight, feed efficiency (adjusted for mortality), and mortality on a pen basis were determined at 49 d of age. Feed and water were provided *ad libitum*.

At 49 d of age, 10 birds per pen (480 total) were processed at the Auburn University Poultry Science Department Processing Plant simulating commercial processing practices. Upon processing, boneless-skinless breast and thigh meat (6 birds per pen) were ground separately, pooled, formed into patties (approximately 100 g, 1.5 cm thickness) and sealed in vacuum packaging either raw or cooked (at 177°C to an internal temperature of 80°C in a commercial radiant oven). Meat samples were then stored frozen (-18°C) for 48 d. A total of 96 samples, 48 from ground breast meat (24 raw samples and 24 cooked samples) and 48 from ground thigh meat (24 raw samples and 24 cooked samples), were analyzed for lipid oxidation (TBARS) and microbial spoilage (aerobic plate counts, lactic acid bacteria; LAB, and Yeasts and Molds; YAM). Meat samples were analyzed after thawing overnight at 2°C following 6, 12, 24, and 48 d of frozen storage.

**Microbiological Analysis**

Ground samples of 10 g were homogenized in 90 ml of peptone water for 2 min using a Stomacher (model 400 Lab Blender, Seward Ltd., London, UK). A 10-fold dilution series of the meat homogenate was made in peptone water (Oxoid Ltd., Basingstoke, UK) and duplicate sample of 100 µl each were plated. Total aerobic plate counts were performed on Plate Count Agar (PCA; Oxoid Ltd., Basingstoke, UK), and plates were incubated aerobically at 37°C for 24 h. The LAB were determined using deMan, Rogosa, and Sharpe Agar (MRS; Acumedia Manufactures Inc., Baltimore, MD) and incubated anaerobically at 25°C for 48 h in anaerobic jars with a disposable Anaerobic Gas Pak (AneroGen; Oxoid Ltd., Basingstoke, UK). Yeasts and molds were counted on potato dextrose agar (PDA; Acumedia Manufactures Inc., Baltimore, MD) following incubation at 25°C for 5 d. Bacterial numbers were reported as $\log_{10}$ colony forming units per g of meat.

**Thiobarbituric Acid Reactive Substances (TBARS)**

Samples (10 g of ground meat) were homogenized with 30 ml of DW for 2 min and 2 ml of homogenate was added with 4 ml of TCA/TBA reagent [15% TCA (w/v) and 20 mM TBA] and 100 µl BHA. Solution was heated 15 min in boiling water, cooled for 10 min in cold water, and centrifuged at 3000 rpm for 10 min. absorbance of the supernatant was measured at 531 nm against a blank that contained all reagents minus sample. The absorbance was calculated from a calibration prepared using 1,1,3,3-tetramethoxypropane (TEP) as a standard. The TBARS number was expressed as mg of malondialdehyde (MDA) per kg of meat (mg MDA/kg).

**Statistical Analysis**

A completely randomized design was used to assign pens to the various treatments. The data were statistically analyzed using the General Linear Model procedures of SAS 9.1.2 software (SAS Institute, 2002-2003), with replicate pens as the error term. The Tukey's test was used to compare and separate means when main effects were significant ($P<0.05$).

## RESULTS AND DISCUSSION

Microbial populations in ground meat were assessed after 6, 12, 24 and 48 d of frozen storage at -18°C. Vitamin E supplementation had no significant effect ($P>0.05$) on microbial profiles (APC, LAB and YAM) of ground raw and cooked breast and thigh meat (Figures 1). Microbial numbers remained below 3 $\log_{10}$ CFU/g on ground raw breast and thigh meat after 48 d of storage. As expected, cooked meat had lower microbial counts and slower microbial growth in both breast and thigh meat. The results of this study concur with those by Govaris et al. (2010), where the dietary supplementation of 150-300 mg vitamin E/kg of feed had no inhibitory effect on LAB and psychrotrophic bacterial counts in breast samples stored for up to 12 days. Lipid oxidation increased during frozen storage of both raw and cooked ground meat with thigh meat exhibiting higher rate of oxidation than breast meat (Figure 2). Dietary vitamin E supplementation at levels higher than 120 IU/kg reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared to the basal level. Cooking resulted in rapid development of lipid oxidation in both raw breast and thigh meat. This indicates that grinding and cooking accelerate lipid oxidation (Rhee et al., 1996). In cooked meat,

the deposition of a higher proportion of unsaturated fatty acids reduces the lipid oxidation stability, particularly when relatively low levels of vitamin E are supplemented in the diet. These results are in agreement with those reported by Cortinas et al. (2005), who observed that as the amount of polyunsaturated fatty acids and vitamin E level increased in the diet, higher and lower lipid oxidation development, respectively, was detected in cooked rather than raw chicken meat. Previous studies have found that poultry thigh meat had higher amounts of α-tocopherol than in breast meat, however, tended to oxidize faster (Lin et al., 1989; Higgins et al., 1999). Broiler chickens fed soybean oil and low dietary vitamin E concentration (33 mg/kg) showed the highest rate of lipid oxidation (Narciso-Gaytan et al., 2010).

The result in this study suggest that dietary vitamin E supplementation at higher levels (>120 IU/kg) significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared to the basal level. Cooking reduced microbial counts and slowed the rate of microbial growth in breast and thigh meat during frozen storage. Microbial counts in both meat types remained below 3 $\log_{10}$ CFU/g through day 48 of frozen storage. Significant effects were achieved in breast and thigh meat, when cooked but not raw. Lipid oxidation increased during frozen storage on both raw and cooked ground meat (breast < thigh meat).

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Figure 1. Main effects of vitamin E supplementation on Aerobic Plate Counts (APC) of (A) Raw breast meat; (B) Cooked breast meat; (C) Raw thigh meat and (D) Cooked thigh meat during frozen storage at -18°C.

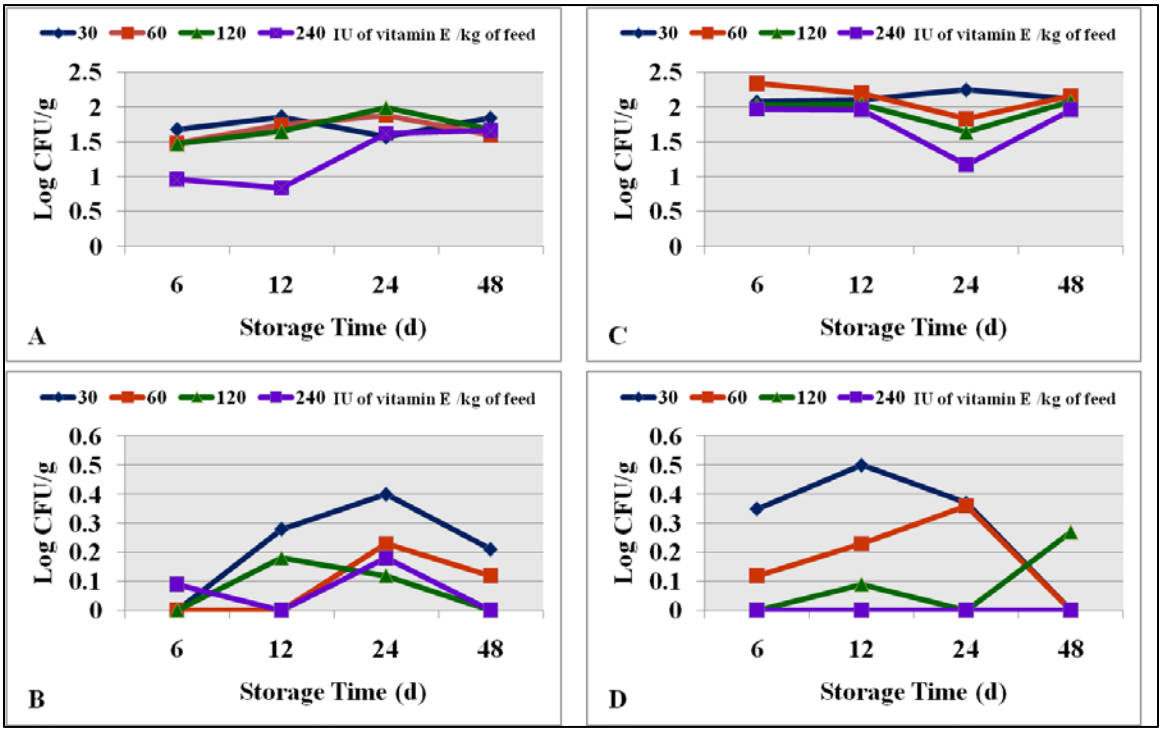

Table 1. Probability (P-value) of Aerobic Plate Counts (APC) of frozen storage.

| Treatments | Storage Time (d) | | | |
|---|---|---|---|---|
| | 6 | 12 | 24 | 48 |
| **Breast Meat** | | | | |
| Raw | 0.86 | **0.02** | 0.16 | 0.55 |
| SEM[1] | 0.20 | 0.18 | 0.23 | 0.20 |
| Cooked | 0.60 | 0.17 | 0.79 | 0.71 |
| SEM | 0.60 | 0.12 | 0.25 | 0.16 |
| **Thigh Meat** | | | | |
| Raw | 0.74 | 0.90 | 0.37 | 0.92 |
| SEM | 0.22 | 0.17 | 0.25 | 0.23 |
| Cooked | 0.06 | 0.26 | 0.46 | 0.25 |
| SEM | 0.08 | 0.16 | 0.22 | 0.12 |

[1]SEM= Pooled Standard Error of the Mean

Figure 2. Main effects of vitamin E supplementation on lipid oxidation (TBARS) of (A) Raw breast meat; (B) Cooked breast meat; (C) Raw thigh meat and (D) Cooked thigh meat during frozen storage at -18°C.

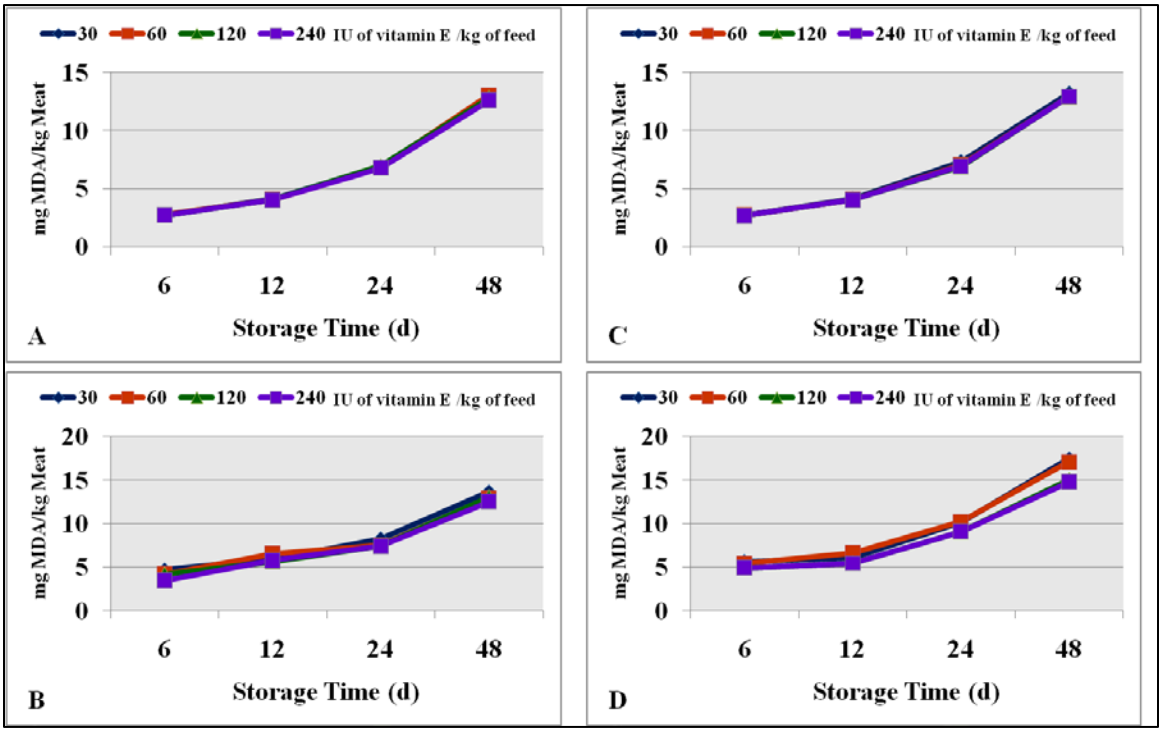

Table 2. Probability (P-value) of lipid oxidation (TBARS) of frozen storage.

| Treatments | Storage Time (d) | | | |
|---|---|---|---|---|
| | 6 | 12 | 24 | 48 |
| **Breast Meat** | | | | |
| Raw | 0.40 | 0.40 | 0.41 | 0.79 |
| SEM[1] | 0.01 | 0.05 | 0.06 | 0.32 |
| Cooked | **0.0002** | 0.20 | **0.02** | 0.26 |
| SEM | 0.17 | 0.30 | 0.23 | 0.34 |
| **Thigh Meat** | | | | |
| Raw | 0.25 | 0.70 | **0.003** | 0.78 |
| SEM | 0.02 | 0.07 | 0.07 | 0.23 |
| Cooked | **0.006** | 0.43 | 0.08 | 0.21 |
| SEM | 0.15 | 0.52 | 0.40 | 1.05 |

[1]SEM= Pooled Standard Error of the Mean

# CHAPTER VII

## SUMMARY

- Dietary fat source influenced the fatty acid composition of broiler chickens. Changes in the fatty acid composition in muscles affected the lipid oxidation stability of meat over storage time in both raw and cooked products. Higher levels of dietary supplementation with vitamin E was necessary to maintain lipid oxidation stability of raw and cooked meat during storage.
- Lipid oxidation was affected by: dietary fat (corn oil>lard), level of dietary fat (6%>2%), packaging film (oxygen permeable>non-permeable), type of meat (thigh>breast) and duration of frozen storage (6 months>3 months).
- Oxidative changes dominate spoilage of fresh and frozen ground meat. Dietary alterations in fat source and level, in addition to type of packaging used may be used to extend the stability of frozen ground meat.

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