THE IMPACT OF FEEDING STRATEGIES ON THE QUALITY AND OXIDATIVE STABILITY OF BREAST MEAT FROM BROILERS REARED UNDER HEAT STRESS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
Department of Food Science
at Aarhus University

By

Donavyn Tate Coffey
Lexington, Kentucky
Advisor: Dr. Margrethe Therkildsen, Professor of Food Science
Aarhus, Denmark

ABSTRACT OF THESIS

Heat stress negatively influences meat quality owing to oxidative stress. This study investigated the effect of dietary antioxidants, mannose rich fraction (MRF), and DHA rich microalgae meal (DHA) on oxidative stability and meat quality of *Pectoralis major* from broilers reared under cyclical heat stress. A total of 1200 1-day old Cobb 500 chicks were fed one of five dietary treatments from 1 to 42d: (1) a corn-soy commercial level diet with 100% inorganic minerals; (2) a corn-soy diet with 100% organic minerals (Bioplex + EconomasE); (3) Diet 2 + MRF (Actigen); (4) Diet 2 + DHA (1% Forplus); (5) Diet 2 + MRF + DHA. Starting on day 19 all birds were subject to cyclical heat stress (6 hours at 33.3°C and 18 hours at 21.1°C).

Lipid oxidation was significantly increased ($P < 0.05$) and protein oxidation was numerically increased during 7 days of retail storage in broilers fed DHA, but that oxidative instability was mitigated when DHA was fed in combination with MRF. MRF alone exhibited significantly lower ($P < 0.5$) protein carbonyl content during storage. Antioxidant enzyme, catalase, showed no treatment effect in activity, but MRF and MRF+ DHA broilers showed significantly higher glutathione peroxidase (GPx) activity.

Visual scoring for white striping and wooden breast myopathies, cook loss, and purge loss did not show a treatment effect. DHA and MRF+DHA broilers did show a significant decrease ($P < 0.5$) in tenderness from d0 to d7 of storage, and on d7 MRF+DHA exhibited a significantly higher ($P < 0.5$) puncture force. Redness (a^*) was decreased in DHA broilers on d0, while lightness was improved by DHA on d7. On average all fillets were classified as pale ($L^*>56$), likely as a result of heat stress. These results suggest that feeding strategies, specifically MRF, could provide oxidative stability in birds reared under cyclical heat stress and/or consuming a diet high in polyunsaturated fatty acids.

ACKNOWLEDGEMENTS

I extend my sincerest gratitude to my advisor, Dr. Margrethe Therkildsen, whose encouragement, guidance, and knowledge have been irreplaceable in the completion of this project and the journey thereto. I am honored and humbled by your willingness to advise me in this project, despite the great distance, and your continued patience at every step of the way.

I am also profoundly grateful to my supervisor and mentor, Dr. Rebecca Delles, without whom this project would not have been possible. Her insight and example in both career and daily life served as incredible inspiration over the course of this project. I am forever grateful for her technical knowledge, careful teaching and abundant patience.

My deepest thanks go to Alltech, as it has been an incredible opportunity to do research here. Specifically, I thank Dr. Karl Dawson for funding this project and Janna Norton for years of encouragement and opportunity. Additionally, the assistance and guidance of Dr. Tuoying Ao, Mike Ford, Phyllis Glenney, and Dr. Shelby Roberts have been instrumental. I would also like to thank Dr. Youling Xiong for opening his laboratory to me and for all of his encouragement and advice along the way, as well as Alma D. True for sharing her immense technical knowledge.

Finally, and most importantly, I thank my parents, Troy and Allison Coffey, for their endless love, continual support and fervent prayers. It is because of the faith which they first exemplified that I find myself able to persevere in all things by and for the glory of God.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Meat quality is the sum of a muscle-based food’s appearance, texture, taste, aroma and functional attributes, and it is ultimately dependent on the consumer perception. Any detriment to these critical attributes in fresh or processed meat will affect the consumer acceptance of a given product. Critical causes of meat quality deterioration are lipid and protein oxidation. Upon the transition from muscle to meat there is a drastic decrease in antioxidant activity, thus the muscle-based foods are highly susceptible to oxidative damage, a major determinant of shelf life in meat (Xiong, 2000)

Reactive oxygen species (ROS), the entity most responsible for oxidative damage, can be generated by a number of mechanisms that involve unsaturated phospholipids, metal catalysts, heme pigments and other oxidizing agents. Radical ROS are highly reactive and can initiate a damaging cascade of reactions oxidizing lipids, proteins, nucleic acid, vitamins and pigments (Smet, 2008). Poultry in particular are vulnerable to lipid oxidation due to their higher content of polyunsaturated fatty acids (PUFAs), compared to other meat products, such as beef and pork (Decker et al., 2010). Lipid oxidation manifests as a number of undesirable and rancid sensory qualities that arise when secondary lipid oxidation products are produced. Moreover, primary products of lipid oxidation, lipid hydroperoxides, when in the presence of a metal catalyst can initiate protein oxidation (Refsgaard et al., 2000). Oxidation of amino acid residues on myofibrillar proteins can significantly affect protein folding and functionality, leading ultimately to deterioration of the texture and water holding capacity of muscle foods (Davies et al., 1987; Liu et al., 2010; Rowe et al., 2004).

Oxidation is ubiquitous in meat products, but the degree to which a muscle food is susceptible to oxidative damage is influenced, in part, by genetic and environmental factors.

In terms of genetics, certain strains of boilers bred with the intention of a higher market weight in a shorter time, exhibit a greater propensity toward idiopathic muscle abnormalities such as white striping or wooden breast syndrome (Petracci et al., 2015). These abnormalities are associated with changes in redox homeostasis and biomarkers involved in increased oxidatives stress (Abasht et al,. 2016). Moreover, myopathies associated with these broiler strains exhibit an upregulation of genes associated with heat shock, oxidative stress and hypoxia (Mutryn et al., 2015). The oxidative damage believed to be at the root of these myopathies manifests in the meat as symptoms visible to the consumer resulting in an economic loss for the industry. Environmental stressors also serve as a causal or compounding factor of meat quality deterioration and oxidative stress (Zahoor et al., 2016). Temperature and associated heat stress is an environmental factor of global concern because of its effect on the end product. Thus, heat stress is damaging to meat quality owing to increased oxidation (Zhang et al., 2012; Mujahid et al., 2005).

Prevalent myopathies such as white striping and wooden breast have been confirmed to be genetically predisposed and environmentally triggered. However, the symptoms of these conditions are comparable to other nutritional myopathies such as Vitamin E/Selenium deficiency which manifest in response to a depleted antioxidant capacity. Thus, targeting the broiler's antioxidant defense system has been among the earliest approaches to overcoming the obstacle of genetic myopathies within the poultry industry (Kuttapan et al., 2012a). Moreover, the study of nutritional interventions as a means of affecting the transcriptome, often referred to as Nutrigenomics, presents itself as a sustainable solution in the modern geopolitical climate. Where poultry are concerned, most nutritional intervention studies have investigated live animal performance characteristics in response to diet. Fewer studies have expounded upon the effects of diet on meat quality.

Microalgae, for instance, is a significant source of fat soluble carotenoids that has the potential to benefit or improve meat quality. Carotenoids have been shown to have a beneficial effect on water holding capacity and some complementary effects on oxidative stability of the meat and animal products (Rajput et al., 2014; Wang et al., 2017). A study on minced pork showed that samples of increased carotenoid content also had lower TBARS values and higher sensory evaluations over time, suggesting a prolonged oxidative stability (Kim et al., 2013). Carotenoids such as β-carotene, are also natural coloring agents that have a positive effect on the redness and yellowness of chicken breast, an important purchase factor in major poultry consuming countries like the US and China (Rajput et al.,2014; Toyomizu et al., 2001). Most studies on algae supplementation in broilers have used *Spirulina* or *Chorella,* while other algae species, such as Schizochytrium, are less explored. Further investigation is needed to determine if algae supplementation in broilers has the potential to result in a functional food of increased stability and shelf life.

The gastro intestinal tract (GIT) represents a complex ecosystem that is pivotal in broiler nutrient absorption and immune response (Lan et al, 2005). Of the known stressors or challenges in broiler production, heat stress and pathogenic microbes can be detrimental to the efficiency and integrity of the GIT (Pearce et al., 2013). Moreover, Quiao et al. (2012) showed a distinct correlation between the oxidative stress of an organism and the presence of pathogenic microbes in their GIT. Therefore, there is a possibility that improving gut health could inhibit oxidative stress. There are few studies examining the effect of mannose rich fraction (MRF) on meat quality. Performance trials have shown that MRF is effective in restoring gut health and beneficial microbial populations by providing alternative adhesion sites for undesirable enterobacteria with Type 1 fimbriae. Challenge trials using various pathogens have shown that broilers supplemented with MRF have a better immunological response compared to control (Agunos et al., 2007). In porcine MRF has also been shown to be an effective replacement of antibiotic growth promoters (AGPs) (Edwards et al., 2014). A differential expression heat stress trial by Nolin et al. (2013) showed downregulation of inflammatory genes and an upregulation of genes related to nutrient absorption in ileum tissue samples from MRF supplemented broilers. By decreasing competition with pathogens and improving nutrient absorption in the GIT, MRF could potentially increase the deposition of minerals essential to the broiler’s antioxidant defense system and thereby prolong shelf life of the meat. Supplementation with MRF has also been shown to downregulate expression of those genes within the heat shock family in broilers subjected to heat stress (Edens et al., 2014). This same family of genes is often associated with oxidative instability which suggests that MRF may have an indirect antioxidative role in muscle foods.

Increasing concerns with meat quality and the pressures of changing antibiotic growth promoters (AGP) regulations warrant further investigation into alternative supplementation in broiler production. Based on the evidence presented, the aim of this study was to evaluate the effect of nutrient intervention on meat quality in the presence of an environmental stressor. Specifically, mannose rich fraction (MRF) and a DHA rich mircroalgae meal were fed to investigate their effect on oxidative stability and meat quality over time as well as antioxidant capacity in breast meat from broilers reared under cyclical heat stress.

CHAPTER 2

LITERATURE REVIEW

2.1. Poultry Industry

Poultry meat is the second most consumed protein worldwide and the most consumed in America, home of the largest broiler industry in the world. In the U.S. the per capita consumption of broiler chicken climbed to more than 90 pounds in 2015 (National Chicken Council, 2016b). The popularity of poultry meat is likely owed to its relative affordability, mild taste, convenience to cook, religious amicability, and associated health attributes such as low fat and cholesterol (Petracci et al., 2013a). Protein scandals such as BSE along with evidence of the negative health effects of red meat (i.e. cardiovascular disease, diabetes, cancer) have solidified the global preference for poultry as a safe and lean protein (Petracci & Cavani, 2011). Moreover, increased global protein consumption is the product of a rapidly increasing global population and global GDP (FAO, 2016). The aforementioned circumstances have resulted in a building pressure on the poultry industry over the last few decades to increase both the amount and rate of production. There is a demand to produce a larger bird at a younger age (Kuttappan et al., 2016). Immense improvements have been made in strain selection, feed conversion, fat reduction, and breast size (Petracci & Cavani, 2011). Such genetic and production progress have culminated so that a market ready broiler today is twice the size in half the time when compared to production in the 1950s (National Chicken Council, 2016a). Over time it has been evidenced that these hybrids selected for growth rate and breast size meet the needs of a growing debone market but also have some inherent effects on meat quality (Tijare et al., 2016; Kuttappan et al., 2016).

Challenging the bird to grow at such rates has resulted in myopathies such as white striping and wooden breast as well as pale, soft and exudative (PSE) characteristics in the meat

(Petracci and Cavani., 2011; Kuttappan et al., 2013a). These quality issues affect both the appearance and functionality of the poultry meat, particularly the breast which is otherwise the most valuable cut of the bird (USDA ERS, 2017). When poultry meat was primarily retailed in whole-bird portions these abnormalities were less of a concern, as they were not obvious at the point of purchase, due to the skin covering the visual defect. However, as the market preference has shifted to deboned, cut portions quality issues have become more apparent to the consumer. Visual appearance is the singular most important parameter when the consumer is making a purchase decision (Kuttappan et al., 2012b). The quality issues associated with fast growing broilers are known to change the visual appearance of the meat so that the perceived quality and perceived healthiness is altered. Often this results in rejection making these abnormalities a direct cause of economic loss and a chief concern for the poultry industry (Kuttappan et al., 2013b).

2.2. Pale Soft and Exudative (PSE) - like meat

Pale, soft and exudative (PSE) characteristics were first identified in pork and found to be detrimental to the meat as a result of mutations in the HAL or RN genes (Fuji et al., 1991; Milan et al., 2000). Since, the mutations predisposing meat to PSE, RYR1 and PRKAG3, have been selected against in the industrial populations. PSE-like characteristics have also been identified in poultry, and though there has been no genetic marker identified, there have been studies linking PSE to environmental factors (Cavitt et al., 2004; McKee and Sams, 1997). PSE is directly related to the post mortem metabolism within the muscle. After slaughter glycogen is metabolized anaerobically to give lactate. A relatively high rate of post mortem glycolysis can generate unusually acid conditions within 45 minutes of slaughter while the carcass is still hot. This combination of low pH and high temperature optimize enzymatic activity and result in incidences of protein denaturation (Petracci & Cavani, 2011). The denatured proteins are unable to sufficiently bind water, thus decreasing water holding capacity (WHC) and thereby weight, turgor and juiciness. This also lends to further cook loss after purchase. The high temperature and low pH conditions further cause a decrease in redness (a*) and increase in the lightness (L*) of the meat (Zhang et al., 2012). Such changes in color result in meat that is less appealing, visually, to the consumer. Therefore, PSE-like poultry meat is often unsuccessful at retail and must be downgraded to a further processed product (Petracci et al., 2015).

PSE is a well-studied, yet frequent, challenge for the poultry industry, and is common to the breast or flight muscle of the bird. The breast is composed primarily of Type IIB, fast twitch, glycolytic fibers and also has large glycogen stores lending to a high propensity for post mortem glycolysis and lactate production (Petracci & Cavani, 2011). Moreover, if the animal were to undergo a large amount of stress or movement before slaughter the breast muscle would be at a relatively higher temperature as a result of increased flapping or struggle. These conditions further optimize conditions for rapid post mortem glycolysis, and confirm that PSE-like poultry meat is instigated by both genetic and environmental factors (Zhang et al., 2012; Petracci & Cavani, 2011).

Sandercock et al. (2009) showed that broiler strains selected for faster growth rate and breast size had exhibited a different cation regulation and suggested that idiopathic myopathies associated with faster growing broilers could be due to an imbalance in intracellular calcium and other ions. Such an imbalance challenges the integrity of the sarcolemma and may also result in calcium induced activation of cellular proteases that cause membrane dysfunction. Each scenario potentially results in texture and WHC changes. Petracci and Cavani (2011) suggest that these pathologies within hybrid broilers may also bring about PSE-like chicken meat.

2.2.1 PSE and Environment

Environmental stressors including heat stress and transport may also induce PSE, especially when these stressors present themselves in the finishing period before slaughter. Sandercock et al. (2009) suggests that the susceptibility to environmental stressors, chiefly heat stress, is increased in faster growing birds as a result of their decreased thermoregulatory capacity. Thus, existing quality issues associated with fast growing lines would be compounded by muscle damage due to heat. Additionally, acute heat stress, that is no time for adaptation, has been shown to generate ROS and thereby initiate oxidative stress (Akbarian et al., 2016; Mujahid et al., 2005). Mujahid et al. (2005) found a significant increase in superoxide production in heat stressed, skeletal muscle mitochondria. This suggests that the mechanism behind the negative effects of heat stress may very well be interwoven with oxidative damage.

2.3. White Striping and Wooden Breast

White Striping (WS) and wooden breast (WB) are more recently identified myopathies that are generating a growing global concern. WS has been studied extensively since 2009 and has been visually characterized as white striations running parallel to fibers within the meat (Kuttappan et al., 2016). These white lines of intramuscular deposits occur at different degrees of severity and are commonly classified as normal, moderate or severe based on the grading system established by Kuttappan (2012b). Kuttappan et al. (2012b) evidenced that more than 50% of consumers would probably not or definitely not purchase a fillet affected with moderate or severe WS as they were thought to give the meat a fatty appearance. Thus, this condition is of economical concern for the entirety of the poultry industry. Predominantly in, but not isolated to, the breast muscle, WS also affects tenders and thigh meat (Kuttappan et al., 2013b). Though the exact etiology of WS is unknown, several studies have shown a higher incidence of WS in fast growing broiler lines, heavier birds and thicker fillets (Kuttappan et al., 2013a; Petracci et al., 2013b).

Relative to WS, WB has only been more recently investigated (Kuttappan et al., 2016). WB is characterized by tactile changes in the meat such as pale areas increased in hardness, muscle degeneration due to infiltration of connective tissue and in severe cases, the presence of a bulge on the caudal region of the fillet (Sihvo et al., 2014). WB is also found to be associated with fast growing broiler lines, heavier birds and thicker fillets (Kuttappan et al., 2013a; Petracci et al., 2013b). Overlapping histological features, such as myodegeneration, necrosis, lymphocyte and macrophage infiltration, fibrosis, lipidosis and regenerative changes, suggest that WS and WB may share a common etiology (Kuttapan, 2013b; Kuttapan et al., 2016).

These two myopathies present two primary challenges for the industry i) a decreased visual appeal and ii) a decreased functional capacity (Kuttappan et al., 2016). Visual appearance is the single most important factor in the purchase of raw, packaged meat (Kuttapan et al., 2013b). As a result of consumer rejection of WS and WB affected raw breast, retailers are often forced to discount the product. For this reason the affected meat is often repurposed/downgraded for further processing by the processor resulting in an economical loss on an otherwise valuable part of the bird (Petracci, 2013b). Furthermore, Petracci (2013b) showed a significant change in the proximate composition of WS and WB affected birds, including decreased protein content and increased fat deposition. There is a known impairment of WHC in WS and WB affected fillets as is demonstrated by their increased cook loss and decreased uptake of marinade (Petracci, 2013b; Tijare et al., 2016). This could be a result of a decreased amount of available protein to bind water. Additionally, the perceived value of the chicken breast rests heavily on its role as lean protein source. However, these abnormalities in nutrient composition—increased fat and decreased protein--

could be detrimental to that role and change the nutritional quality of any further processed products made with affected cuts (Petracci et al., 2015).

Again, the origin of either of these abnormalities is unknown, but the association with certain broiler strains suggests that WS and WB are emerging with increased growth rate. It is postulated that extensive hypertrophy may have unsustainable metabolic requirements. The increased fiber size is known to correlate with lower capillarization which has the potential to result in insufficient nutrients and oxygen to the muscle (Kuttappan et al., 2013a; Petracci et al., 2015). Intracellular calcium accumulation has been proposed as a causal factor of increased rate of pH decline, fiber fragmentation and reduced WHC in fast growing broilers (Sandercock et al., 2009). Increased free radical production and subsequent oxidative stress is also of higher incidence in heavier strains, and heat stress exacerbates this quality(Akbarian et al., 2016). RNA sequencing by Mutryn et al. (2015) substantiated these claims by confirming increased expression of genes involved in localized hypoxia, oxidative stress, and higher levels of intracellular calcium in breasts affected with wooden breast. Abasht et al. (2016) showed that WB-affected birds exhibit biomarkers related to oxidative stress, muscle degradation, altered glucose utilization and molecules capable of disrupting redox homeostasis. The elevation of the aforementioned conditions in WS and WB-affected breasts suggests that oxidation may be a causal factor in these conditions or that birds of faster growing strains may be more susceptible to oxidation and thereby WS and WB.

2.4. Heat Stress

In addition to the genetic predisposition of some broiler strains to myopathies, the broiler’s muscle and later meat can be challenged by environmental stressors. Heat stress is a primary concern for poultry production, globally (Lara & Rostagno, 2013). It is further intensified in hot climates or cases of high animal productivity. It is estimated that the losses due to heat stress for the U.S. livestock industry reach up to $2.7 billion per year, and in a

2003 study $128-$165 million of that loss was specific to the poultry industry (St-Pierre, 2013; St-Pierre et al., 2003).

The optimal temperature for broiler growth and performance is 8-22 °C (Lin et al., 2006). Heat stress occurs when the amount of heat produced by the bird exceeds the heat or energy given off by the bird into its environment. This is usually due to irregularly high environmental temperature (Zhang et al., 2012). Heat stressed birds have been shown to spend less time feeding, more time drinking and panting, less time moving and more time with their wings elevated (Lara & Rostagno, 2013). Consequently, heat stress is a known cause of higher mortality, decreased feed consumption, decreased bodyweight and increased feed conversion ratio in broilers (Quinteiro-Filho et al., 2012). Moreover, high rearing temperatures, whether they be acute, chronic, or cyclical, have been shown to have adverse affects on cellular structure and function. This results in changes of both metabolic and transcriptional processes including oxidative metabolism, transcription and translation (Mager and De Kruijff, 1995). Elevated levels of creatine kinase also suggest that there is damage to the muscle cell membrane and therefore its functionality (Sandercock et al., 2006). Altan et al. (2003) showed that antioxidant enzymes GPx, CAT and SOD were also increased in heat stress birds as a kind of protective mechanism against oxidative stress known to arise in heat stressed birds. It is important to note that response to heat stress varies distinctly between breed and strain. Broilers have a greater sensitivity to heat stress than do layers, and the already heat sensitive, high production broiler strains are most sensitive to heat stress (Sandercock et al, 2006; Altan et al., 2003; Soleimani et al., 2011; Geraert et al., 1993).

Heat stress also has significant effects on the broiler meat quality. The decreased feed consumption and increased FCR induced by chronic heat stress have been shown to result in decreased body weight and breast size (Zhang et al., 2012). Thus, profitability is hindered. Moreover, heat stress conditions can impair the bird’s protein metabolism by limiting protein deposition and increasing catabolic rate (Geraert et al., 1996). Acute stress affected meat color in breast meat with increased lightness and decreased red and yellow likely due to denaturation of sarcoplasmic proteins and subsequent increased scattering of light (Owens et al., 2000). Supra-optimal temperatures pre-slaughter can also increase energy demands and therefore glucose mobilization. This results in increased activity of glycolytic enzymes LDH, PK and HK and therefore increased conversion of pyruvate to lactate. This increase in rate of lactate production will result in rapid pH decline during the conversion of muscle to meat and PSE-like conditions especially within the breast (Zhang et al., 2012; McKee and Sams, 1997). Oxidation and heat stress are believed to be closely linked, as increased MDA is considered a marker of heat stress in broilers (Altan et al., 2000). In a follow up study, Altan et al. (2003) compared Ross and Cobb fast growing strains for their susceptibility to heat stress induced lipid oxidation and subsequent damage to cell membrane. Heat stress resulted in higher lipid oxidation for both strains, with Ross showing the greater sensitivity. While strain does play a role in sensitivity to heat stress, Zahoor et al. (2016) evidenced that heat stress is not a primary cause of muscle damage, but rather genetics is the causal factor and high temperatures an exacerbating stressor.

2.5. Oxidation

Oxidation is inherent to metabolism as a necessary component to ATP synthesis (Halliwell & Gutteridge, 1995). However, in excess or when insufficiently balanced with the antioxidant defense system, free radicals or reactive oxygen species (ROS) are destructive to biological systems. ROS exist as both radical and non radical molecules. The radicals are molecules able to exist with an unpaired electron making them very reactive with a number of biological macromolecules and systems including proteins, lipids, vitamins, pigments and nucleic acid (Smet, 2008). Once initiated, ROS can propagate a damaging chain reaction, and continued, unregulated oxidation causes damage and apoptosis. There are existing preventative antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase that counteract oxidative stress. However, these enzymes begin to diminish postmortem, thus oxidative damage is a common hurdle for the meat industry (Xiong, 2000). Oxidation of lipids and proteins, in particular, are a known parameter of deterioration in meat affecting nutritional value, functional properties, flavor, texture and perceived quality (Bekhit et al., 2013; Zhang et al., 2013).

2.5.1. Lipid Oxidation

Of the macronutrients, lipids are the most vulnerable to oxidative changes that alter meat quality (Kanner, 1994). Polyunsaturated fatty acids (PUFA) and phospholipids are particularly susceptible oxidative targets. Though lower in fat relative to other meats, poultry are particularly vulnerable to lipid oxidation because they have a higher degree of unsaturated fat within the cell membrane. (Decker et al., 2010). The carbon-carbon double bonds in PUFAs weaken the carbon-hydrogen bond, making the hydrogen more readily abstracted by a ROS generated in the mitochondria or an existing lipid radical. The abstraction of such vulnerable hydrogen within the methylene group of a PUFA is often the initiation of lipid oxidation.

Initially lipid oxidation involves a ROS removing a hydrogen adjacent to a double bond within the PUFA. This generates a lipid radical that reacts with oxygen to form a lipoperoxyl radical able to abstract hydrogen from neighboring lipids. This is an autopropogative chain reaction that results in a hydroperoxide (LOOH) primary end-product (Decker et al., 2010). Presence or absence of hydroperoxides is an indicator of the meat's oxidative damage but they are not detrimental to the acceptability of the meat. It is when these primary products interact with metal ions and undergo subsequent reactions that volatile, secondary end-products such as alcohols, aldehydes and ketones are formed. Secondary end products significantly impact the flavor of the meat and are responsible for the rancidity that is characteristic of unacceptable meat products. Hexanal, known to give a grassy flavor, is the most common secondary product found in chicken thigh and breast meat (Ajuyah et al 1993). Propanal (alcoholic), pentanal (pungent) and nonanol (soapy) are also common secondary products that contribute to rancidity. Not all secondary oxidation products give a bad flavor (Jayasena et al., 2013; Decker et al., 2010); some bring a sweet or fatty taste to the meat.

Extrinsic factors associated with processing such as freezing and thawing, deboning, grinding, cooking and restructuring are often accelerants of lipid oxidation and the associated off flavors as they damage the integrity of the cell and bring unsaturated lipids into direct contact with cellular oxidative constituents such as heme (Xiao et al., 2011). Oxidation of phospholipids can also be thermally induced; thus, oxidative damage occurs more rapidly after cooking. Warmed-over-flavor is the term used to describe the specific rancid flavors that can be identified in refrigerated cooked meat as a result of thermally induced lipid oxidation. In order to mitigate such oxidative deterioration packaging, temperature control and antioxidant supplementation strategies can be employed.

The degree of susceptibility of a piece of meat to lipid oxidation is affected by a myriad of conditions including species, anatomical location, age and sex, environmental temperature, diet and phospholipid composition (Min et al., 2008; Gray & Pearson, 1987). Chief among these causal factors is the nutrient composition of the animal’s diet. Intake, type and amount of dietary fats is a major determinant of the meat’s susceptibility to lipid oxidation. Increased supplementation of dietary fat in the feed stuff will lead to changes in the fatty acid composition of the animal. Specifically, an increase in readily oxidizable fats in the form of PUFA supplementation will increase both the unsaturation in the membranes and the susceptibility to oxidation (Kanner, 1994). Leaner meats, such as the chicken breast, do tend to exhibit greater oxidative stability and slower increase in TBARs in comparison to more energy dense protein sources such as beef. This is likely related to their lower concentration of heme, and therefore reactive iron cations, which limits the development of secondary end-products (Min et al., 2008).

2.5.2. Protein Oxidation

While lipid oxidation has been reviewed extensively and is accepted to be well understood, protein oxidation is still being studied. ROS, both radical and non-radical, that arise from lipid peroxidation are able to oxidize and modify protein side chains (Reefsgaard et al., 2000). Therefore, factors affecting lipid oxidation may also contribute to protein oxidation (Xiao et al., 2011). In fact, Coetzee and Hoffman (2001) found that the degree of protein oxidation measured by carbonyl content is coupled to lipid oxidation in chicken. Similar results have been found in other species (Mercier et al., 1995; Estevez et al., 2008). Additionally, mechanical actions that harm the integrity of the cell membrane bring pro-oxidants into contact with cellular proteins in the presence of oxygen, thereby making the proteins vulnerable to oxidation (Zhang et al., 2013; Soladoye et al., 2015).

Oxidative modifications to the amino acids or polypeptide backbone may result in the unfolding of or conformational changes of the original protein. These changes can give rise to polymerization and decreased solubility, loss of enzyme function, changes in protein digestibility and generation of amino acid derivatives (Martinaud et al., 1997; Lund et al., 2011; Davies et al, 1987). Such damage to the protein could thereby alter functionality including gel-formation, meat binding, solubility, stability, nutritional quality, viscosity, meat tenderness and water holding capacity (WHC) of meat (Xiong, 2000; Lametsch, 2007). The amino acids exposed on the surface of the protein are most readily oxidized; however, the most significant changes in functionality occur when buried amino acid side chains are oxidized. Changes to these inner side chains can result in changes to hydrophobicity and therefore function (Liu & Xiong, 1996; Gao et al., 1998). Dramatic changes in surface hydrophobicity via protein oxidation may result in aggregation and polymerization of proteins causing changes in the physiochemical quality parameters of the meat, namely texture (Lund et al., 2011).

Not all amino acids are equally disposed to oxidation; there are amino acids that are more readily oxidized than others with cysteine and methionine as the most susceptible due to their sulfur atoms (Shacter, 2000; Garrison, 1987; Zhang et al., 2013). It is important to note that calpain and calpastatin, the enzyme and inhibitor most responsible for meat tenderness, are both cysteine proteases. Therefore, under oxidative stress these two enzymes are readily oxidized, and their activity is altered (Zhang et al., 2013) and thus detrimentally affecting tenderness (Rowe et al., 2004).

Direct oxidation of amino acid side chains, fragmentation of the peptide backbone, reaction of proteins with reducing sugars and binding of proteins to non-protein carbonyl compounds are all means to generate protein carbonyls (Headlam & Davies, 2004). Because they arise by various mechanisms, carbonyls are a valuable and measurable by-product of protein oxidation. Their detection using 2,4-dinitrophenylhydrazine (DNPH) is relatively affordable and is considered a relevant methodology for testing protein oxidation within food systems as reviewed by Estévez, M. (2011). DNPH binds the carbonyl to generate hydrazones which can be detected via spectrophotometry at 370nm and then recorded as nmol of DNPH per mg of protein which is detected at 280 nm (Castegna et al., 2000). Though this methodology is widely accepted, it is limited by a lack of specificity which begets an overestimation of carbonyl content. DNPH readily reacts with any form of carbonyl, including those generated from other macromolecules like lipids or nucleic acids. As these cannot be fully removed, carbonyls not generated by protein oxidation are able to contaminate the sample (Fedorova et al., 2013).

2.6. Nutritional Interventions

Nutrition is an underlying factor of the broiler's performance, subsequent meat quality as well as susceptibility to any of the aforementioned myopathies and stressors. Thus, feeding to improve performance, meat quality or consumer acceptance is common practice. Delles and colleagues (2014) successfully improved oxidative stability of chicken breasts by feeding organic minerals as well as algae-based antioxidant containing Se yeast as a replacement for vitamin E (Xiao et al., 2011). The present study is a continuation of those findings and intended to investigate the degree to which these same nutrients can influence meat quality in the presence of environmental stressors. Additionally, this study investigated the effect of DHA rich microalgae meal and mannose rich fraction (MRF) on meat quality based on their antioxidative potential and gut health effects, respectively.

2.6.1. Organic Minerals

Trace minerals such as Zn, Mn, Cu, Fe and Se are essential in the broiler diet in smaller amounts. Zn, for instance, is a necessary cofactor to more than 300 enzymes, Mn is critical for enzymes involved in protein metabolism and antioxidant defense, and Se is essential for proper antioxidant enzyme activity (Ao et al., 2013; Keen et al., 2000; Rotruck et al., 1973). The aforementioned examples are only a small list from a much larger manifest of the roles of minerals in both broiler performance and meat quality. Typically sold in salt form, these minerals are relatively affordable for the producer. As a result, it is common practice to formulate broiler feed to contain mineral levels exceeding the NRC (1994) recommendations to ensure that each bird absorbs adequate levels for maximum performance. Though this is common practice, overfeeding minerals presents three primary obstacles according to a review by Ao and Pierce (2013): 1) as a result of inter-mineral relationships the excess of one mineral may result in inadequate levels of another essential mineral (Suttle, 2010), 2) minerals may form a chelate with phytate and thereby hinder the activity of phytase (Maenz et al., 1999; Schlegel et al., 2012)and 3) excess minerals excreted in to poultry manure can increase environmental burden and could be a possible source of phytotoxicity (Blanco-Penedo et al., 2006).

A solution to these obstacles would be a source of trace mineral that is more bioavailable to the broiler and could therefore be administered in lower quantities. Trace minerals as organic metal complexes in the form of chelates or proteinates have been examined in depth and found to be more bioavailable than inorganic salts due to their increased neutrality, solubility and ability to pass through the intestinal wall via amino acid transport mechanisms (Nollet et al., 2007; Miles and Henry, 1999; Aldridge et al., 2007).

Performance

Several trials have been carried out to investigate the effects of organic minerals on performance, Studies on bioavailability have shown that in organic form Cu, Mn, and Zn were absorbed at 138%, 139% and 157-183% of their inorganic form, respectively (Pierce et al., 2005; Brooks et al., 2012; Ao et al., 2006). Briens et al. (2013) showed that apparent digestibility of organic selenium was twice that of the inorganic counterpart. The increased bioavailability aligns with findings from feeding organic trace minerals (Zn, Mn and Cu) at 1/3 NRC recommendations had no negative effects on broiler performance and decreased levels of mineral excretion (Asku et al., 2011; Peric et al., 2007). Rama Rao et al. (2016) found that in addition to increased deposition in the muscles, supplementing organic trace minerals (Se, Cr and Zn) significantly increased body mass gain (BMG) and feed intake compared to inorganic control. The same study found organic Cr supplementation to increase feed efficiency. Similarly, organic Cu has been found to positively affect BWG, nutrient utilization and FCR as well as sustain comparable growth and performance when used as a replacement for avilamycin (AGP used in broilers) (Das et al., 2010; Kim et al., 2011).

Oxidative Stability

The antioxidant defense is crucial in a lipid dense protein such as chicken. Trace minerals play a critical role as catalysts and cofactors of many antioxidant enzymes, including the 3 major cytoplasmic enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Poultry Federation, 2012). Within the cytoplasm these antioxidants represent a first line of defense deactivating free radicals and their precursors, and without sufficient trace minerals their activity will not effectively deter oxidative stress. GPx activation by selenoproteins and Cu/Zn-SOD and Mn-SOD represent specific mineral-antioxidant relationships that are crucial to the cell's redox stability. Moreover, the activity of these antioxidant enzymes is believed to be inducible upon increased deposition of trace mineral proteinates (Al-Qudah et al., 2010). For instance, organic Zn improved the activity of Cu/Zn SOD and GPx, and chelated Mn was reported to increase activation of Mn-containing SOD expression at the point of both transcription and translation (Ma et al., 2011; Li et al., 2011). Organic Zn was also found to reduce oxidative stress and positively affect immune indices in both healthy and challenged broilers, which could positively affect oxidative stability of the meat (Bun et al., 2011). Similarly, several studies have found that feeding organic minerals inhibits lipid oxidation in broiler meat and increases activity of antioxidant enzymes. (Rama Rao et al., 2016; Asku et al., 2011). As external stressors often manifest on the cellular level as oxidative stress, the bioavailability and oxidative stability offered by organic trace minerals could be particularly significant in challenged birds. Heat stress, for instance, is known to result in oxidative damage as well as mineral excretion; whether these issues are causal or compounding, an increase in trace mineral deposition to compensate for this depletion is critical for bird welfare and meat quality (Altan et al., 2003; Akbarian et al., 2016; Rama Rao et al., 2016).

Meat Quality

Organic minerals via their impact on oxidative stability and interaction with myofibrillar proteins have an impact on meat quality. Organic minerals can be fed in lower quantities than inorganic minerals without altering the breast weight or ultimate pH. (Petrovic et al., 2009; Rama Rao et al., 2016). Feeding organic Cu, Zn and Mn was also found to increase the brightness of the meat which is a trait desired by the consumer in some regions (Rama Rao et al., 2016). Peric et al. (2009) also evidenced that feeding organic forms of Se reduces drip loss. Whereas, Saenmahayak et al., (2012) found that organic Zn had no effect on meat quality and increased drip loss; though in earlier studies organic Zn increased the amount of breast meat and decreased cook loss (Saenmahayak 2010; 2007). In other species organic Se has been shown to decrease myofibrillar protein oxidation as well as water loss in comparison to control (Calvo et al., 2016). This effect is conceivably due to the role of Se in the activity of the antioxidant glutathione peroxidase (GPx) (Rotruck et al., 1973). Increased Se has been previously identified in meat with greater oxidative stability, which could result in provision of myofibirllary protein integrity(Delles et al., 2014). The structure and integrity of the myofibrillar proteins is essential to maintaining the desired WHC (Liu et al., 2010). Calvo et al. (2016) also postulated that increased Se improved early proteolysis which increased opportunity for water binding and therefore WHC.

2.6.2. Vitamin E

Vitamin E, a concentration of tocopherol and tocotrienal, is a lipid soluble nutrient vital to the broilers' circulatory, reproductive, immune, nervous and muscular systems (Habibian et al., 2014; Dalolio et al., 2015). Within the cell's lipid membrane Vitamin E serves as a potent antioxidant, scavenging peroxy radicals. While Vitamin E is essential in a basic broiler diet, it is also fed as a means to increase the animal's antioxidant capacity and mitigate industry practices that result in additional oxidative stress.

Poultry production in hot, arid regions and the feeding of oxidized oil as an affordable, energy dense feedstuff each result in an increased susceptibility to oxidative damage for an already fast-growing, vulnerable broiler strain (Altan et al., 2003; Delles et al., 2014). As previously discussed oxidative stress during production can result in unwanted conditions during the transition from muscle to meat or in a predisposal to lipid oxidation (Zhang et al., 2012; Gray and Pearson, 1987). Increasing antioxidant capacity by feeding Vitamin E in high doses has the potential to manage oxidative stress in the bird and reduce loss of exudate and stabilize fat deposits to deter lipid oxidation of the meat (Li et al., 2009; Dalolio et al., 2015; Dikeman, 2007). Moreover, with supplementation the meat becomes enriched with Vitamin E, and thus is attractive as a functional food with increased shelf life to the end consumer (Dalólio et al., 2015).

Vitamin E's protective effect is thought to occur via increasing the peroxide and radical scavenging potential within the membrane and also by a sparing effect with Se. In the latter, increased Vitamin E decreases the demand on GPx activity for lipid peroxide removal in the membrane (Surai, 2000). Based on these principles many trials have been conducted to examine the effects of Vitamin E supplementation on both performance and meat quality with particular interest in stressed or challenged broilers. Heat stress can significantly alter the shear force values of meat so that texure changes are perceivable to the consumer. However, supplementation with Vitamin E counters the effects of heat stress so that shear force values are restored to the level of an unchallenged or thermoneutral bird (Hashizawa *et al*., 2013). Similarly, Imik et al. (2012) found that Vitamin E was able to alleviate the negative effects of heat stress on final body weight of the bird and lipid oxidation of the meat. A review by Dalolio et al. (2015) concluded that the effects of Vitamin E on broiler meat are more often qualitative than quantitative, meaning that while breast weight does not increase, studies have shown an improvement in quality parameters. However, a recent study combining Vitamin E and alpha-lipoic supplementation resulted in increased daily gain and BWG in addition to improving meat quality parameters (Yoo et al., 2016). This combination may be similar to the sparing effect between vitamin E and selenium, as alpha-lipoic acid has been credited with inducing synthesis of endogenous enzymes such as glutathione (Shay et al., 2008). Habibian et al. (2015) showed that combined supplementation of Vitamin E and Se actually had no effect on oxidative stability of thermoneutral broilers, but was able to mitigate oxidative stress and improve TBARs in heat stressed birds. Beyond heat stress, Vitamin E has also been found to have a mild prevention of muscle damage due to nutritional myopathies such as white striping (Guetchom et al., 2012).

2.6.3. Algae

Supplementing microalgae in broiler diets represents a nutritional strategy aimed at improving the meat quality as well as the nutritional content for the end consumer. The recommended level of polyunsaturated fatty acids (PUFA) is not being met for the majority of the adult population (Givens and Gibbs, 2006). Therefore, Omega-3, long chain PUFA (LCPUFAn-3), such as DHA, which are essential for infant development, brain growth, cognitive maintenance, and cranial grey matter as well as protective against cardiovascular morbidity and inflammatory response, are also not being consumed in sufficient quantity (Horrocks and Yeo, 1999). While oily marine fish is a good source of DHA, the rate of consumption in the typical western diet is too low to meet the body’s essential fatty acid requirements. The pursuit of an alternative source of protein with higher LCPUFAn-3 has long been underway. Unfortunately, there is limited opportunity for this in beef as biohydrogenation in the rumen prevents the deposition of PUFA into the muscle cells (Van Elswyk, 1993). PUFA supplementation is more effective in pork, but there still speculation as to whether porcine tissue FA composition can be modified without accruing undesirable changes within the meat (Van Elswyk, 1993; Vossen et al., 2016a). Poultry have shown the most promise in fatty acid modification, as supplementation with fish oil successfully increased LCPUFAn-3 deposition in the thigh and breast with limited impacts on flavor and texture. Fish oil, however, is expensive due to limited quantities and high demand in the human supplement market; therefore, it is not a sustainable feed additive. Attempts to increase LCPUFAn-3 deposition by feeding broilers shorter chain precursors like flaxseed oil have not proved to be an effective alternative to fish oils (Rymer et al., 2010; Shin et al., 2012). Heterotrophic production of marine algae, of which many strains are exclusively abundant in DHA, has been evaluated as a sustainable alternative to fish oil (Yaakob et al., 2014).

Algae supplementation can also have a beneficial effect on the nutritional content of broiler meat. Yan and Kim (2013) found that supplementation with 0.1-0.2% Schizochytrium improved the fatty acid profile of breast meat by decreasing the ratio of n-6 to n-3 PUFA and the ration of saturated fatty acids to PUFA. Evans et al. (2015) showed that dried Spirulina can be successfully fed at up to 16% and successfully change the FA composition of the breast meat without having a negative effect on broiler performance. Other studies have evidenced performance benefits in addition to FA modification, such as increased BWG, FCR, and villus length (Shanmugapriya et al., 2015).

Algae is also a valuable source of fat soluble carotenoids which have a beneficial effect on broiler meat quality as well as performance. Carotenoids, such as lycopene or beta-carotene, are natural coloring agents found in plants that increase the yellowness and redness of broiler meat (Rajput et al., 2014). Toyomizu et al. (2001) evidenced that using *Spirulina* as a source of B-carotene increased the yellowness of the skin, muscle and fat making the meat more attractive to consumer. Carotenoids have also been shown to improve WHC in challenged birds and contribute to an increase in overall oxidative stability of the meat (Rajput et al., 2014; An et al., 2004). A study in minced pork meat reported that samples with increased levels of caratenoids also showed lower TBARs values and improved sensory evaluations over time. These results allude to an increased oxidative stability and shelf-life (Kim et al., 2013). An et al. (2014) found that supplementation of broilers with the carotenoid astaxanthin decreased lipid peroxides found in the skin and ultimately had the potential to deter oxidation in the broiler carcass. An early study comparing the efficacy of algal biomass to fish oil supplementation evidenced that lipid oxidation was delayed in algae treatment groups due to the encapsulation of the PUFA rich oil within the cell (Mooney et al., 1998). Supplementing the algae itself with key minerals such as organic selenium may also increase an animal's capacity to deter oxidation, as increased selenium is key in the function of antioxidant enzymes such as GPx (Skrivan et al., 2010).

2.6.4. Mannose Rich Fraction

Mannose rich fractions (MRF) are a purified carbohydrate derived from the mannon oligosaccharide matrix of the yeast cell wall. A wide variety of studies have investigated the impact of MRF of animal performance, but there is very little known about their effect on meat quality. MRF can cause the agglutination of undesirable enterobacteria, specifically those with Type 1 fimbriae, by offering an alternative binding site to the mannose fractions lining the gastrointestinal tract (GIT) (Ganner & Schatzmayr, 2012). Performance trials have shown that supplementation with MRF can effectively restore gut health as reducing pathogenic populations allows for the flourishing of beneficial microbial populations. Furthermore, challenge trials in broilers have shown that MRF supplementation results in improved immunomodulatory activity and thus better defense against the pathogens (Agunos et al., 2007). In both porcine and poultry MRF has been investigated and suggested as an alternative to antibiotic growth promoters (AGP) as they decrease pathogen load without hindering performance traits (Edwards et al., 2014; Matthis et al., 2012). MRF has also been found to modify broiler reponse to heat stress. A study by Edens et al. (2014) showed that broilers fed MRF exhibited a downregulation of genes within the heat shock family. Heat shock proteins are known to be expressed as a pro-survival response to oxidative stress (Lomiwes et al., 2014). Decreasing these genes from within the transcriptomes would suggest an increased oxidative stability which could have a direct effect on shelf-life and oxidative stability of the meat. Another study by Nolin et al. (2013) observed differential genetic expression in broilers under heat stress. Those supplemented with MRF showed a downregulation in inflammatory genes and an upregulation in those genes associated with nutrient absorption. The potential for increased nutrient absorption lends to the possibility that MRF would allow for broilers to better absorb essential minerals from the diet, many of which are necessary for antioxidant activity. If this were the case, MRF could have an indirect role in improving the antioxidant capacity during the transition from muscle to meat.

CHAPTER 3

MATERIALS AND METHODS

3.1. Broiler Production

All procedures used in the study herein were approved by the University of Kentucky Animal Care and Use Committee. A total of 1200 1-day old Cobb 500 chicks were fed one of five dietary treatments from 1 to 42d: (1) a corn-soy commercial level diet with 100% inorganic minerals; (2) a corn-soy diet with 100% organic minerals (Bioplex + EconomasE); (3) Diet 2 + mannose rich fraction (MRF) (Actigen); (4) Diet 2 + algae/DHA (1% Forplus; (5) Diet 2 + MRF + Algae/DHA. Details of the basal diet are shown in Table 1, while specifications for each treatment are shown in Table 2. Each dietary treatment was carried out in 10 replicate pens containing 24 birds each. Each pen with litter of soft wood shavings was equipped with a feeder and a nipple drinker line. Water was available on an *ad libitum* basis and broilers consumed feed in mash form.

Table 1: Nutrient levels of corn-soy basal diet fed to all broilers in the trial

	Starter	Grower	Finisher
AME_n, kcal/kg	3050	3100	3150
Protein, %	22	20	18
Ca, %	1.00	0.90	0.89
available P, %	0.45	0.41	0.38
TSAA, %	0.97	0.88	0.75
Lysine, %	1.32	1.16	0.95
Na, %	0.20	0.20	0.20

Table 2: Inclusion rate of nutritional supplements for formulation of treatment diets.

Ingredient	Control	Organic Minerals	Actigen	Forplus	Actigen + Forplus
	%	%	%	%	%
Inorganic mineral premix	0.25				
Organic mineral premix*		0.25	0.25	0.25	0.25
Forplus				1.00	1.00
Actigen			0.04		0.04

*Organic mineral premix included BioPlex minerals along with EconomaseE, an algae-based antioxidant containing Se that can be used as a replacement for Vitamin E

Table 3: Concentrations (ppm) of individual minerals within inorganic and organic premix

Mineral	ppm	
	Inorganic	Organic
Zn	72	40
Mn	100	40
Cu	10	5
Fe	45	20
Se	0.3	0.3

All broilers were reared in the same thermoneutral environment up to day 19, at which point heat stress commenced. From day 19 to day 42 all birds were subject to cyclical heat stress which entailed 6 hours (10:00 - 16:00) at 33.3°C and 18 hours (16:00 – 10:00) at 21.1°C. Temperature increase took place from 9:00 to 10:00 and temperature decrease from 16:00 to 17:00. Relative humidity peaked at approximately 70% during heat stress and decreased to approximately 50% during the thermoneutral part of the heat stress cycle. Data loggers were used to measure humidity and temperature within the pen, and an evaporative cooler fan was used to manage moisture levels in the pen. The photoperiod for the experiment was 22 h of light and 2 h of dark.

3.2. Slaughter method and sampling

On day 42 five broilers per treatment (5 diets x 5 birds) were randomly selected, humanely harvested by stunning. Immediately following exsanguination, aliquots of

Pectoralis major muscle samples (approximately 10 g each) were removed from each broiler and flash frozen at -196°C in liquid N_2. Samples were stored at -80°C until use in antioxidant activity studies.

On day 43 four broilers were randomly selected from each of the 50 pens (5 diets x 10 replicates). The birds were humanely harvested, de-feathered and chilled in ice slurries for 3 h. Then, both sides of the *Pectoralis major* were removed and skinned. Breasts were vacuum packaged using a FoodSaver® V3240 vacuum sealing system and stored at -80°C for use in oxidative stability and meat quality assays.

3.3. White Striping and Wooden Breast Scoring

After chilling and before deboning the *Pectoralis major* of each bird (n = 50) was evaluated by two scorers. Scorers evaluated each bird for both the degree of WS and degree of WB and assigned one of the following scores based on the scale established by Kuttapan and colleagues (2009): normal (1), moderate (2), or severe (3).

On day 43 one additional bird from each pen was selected and humanely harvested. The bird was immediately skinned and the hot carcass was scored for WS and WB (Kuttapan et al., 2009). There was only one scorer evaluating the hot carcass samples.

3.4. Meat Preparation, Packaging and Storage

In preparation of oxidation and quality studies, breasts were thawed at 4°C for 48 hours and packaged by placing on a #2 supermarket white polystyrene trays (20.8×14.5×2.3 cm in dimension; Pactive LLC; Lake Forest, IL) and overwrapping with an air-permeable polyvinylchloride (PVC) film (15,500–16,275 $cm^3/m^2/24$ h oxygen transmission rate at 23 °C; E-Z Wrap Crystal Clear PVC Wrap, Koch Supplies, North Kansas City, MO). PVC packaged breasts were stored in a retail display cooler at 2-4°C until assayed on day 0, day 4 or day 7. In order to simulate retail conditions, all samples received approximately 1076 lux of warm white fluorescent light.

3.5. Lipid Oxidation

Lipid oxidation within the breast meat was measured according to Witte et al. (1970) Method. MDA was extracted from the sample using 11% trichloroacetic acid (TCA) and a Warring™ blender on low speed (18,000 rpm) for 60 sec. A 20 mM sample of thiobarbituric acid (TBA) was added to each sample and allowed to incubate for 20 hours. The pink complex that forms when MDA is reacted with two molecules of TBA can be spectrophotometrically quantified at 532nm. The MDA content was then expressed in terms of mg of MDA using a standard curve. .

3.6. Protein Oxidation

Myofibrillar proteins were isolated from the chicken breast on days 0, 4 and 7 of storage using a rigor buffer (0.1 M NaCl and 10 mM Na2HPO4, pH 7.0). The protein concentration was then quantified using the Biuret method. Carbonyl concentration was evaluated within 24 hours of extraction using the 2,4-dinitrophenylhydrazine (DNPH) colorimetric method (Levine et al., 1990). The protein hydrazones were measured at 370 nm and using an absorption coefficient of 22,000 M^{-1} cm^{-1} the carbonyl content was expressed as nmol per mg of protein.

3.7. Antioxidant Enzymes

3.7.1. Catalase

For analysis of catalase enzyme activity approximately 1g of partially thawed muscle sample was mixed with ~10 ml of chilled buffer (50 mM Potassium Phosphate, pH 7.0 and 0.5 mM EDTA) and homogenized for 30 s at 9500 min^{-1} with an Ultra-Turrax homogenizer, model T25. The homogenate was centrifuged for 15 minutes at 10,000 x g at 4°C. The supernatant was then assayed according to Cayman Chemical Catalase Assay Kit (Ann Arbor, MI). Catalase activity was determined by the rate of H_2O_2 disappearance via measuring absorbance at 540 nm.

3.7.2. Glutathione Peroxidase

For analysis of GPx activity approximately 1g of partially thawed muscle sample was homogenized in ~10 ml of chilled buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT) for 30s at 9500 min^{-1} with an Ultra-Turrax homogenizer, model T25. The homogenate was centrifuged for 15 minutes at 10,000 x g at 4°C. The supernatant was then assayed according to Cayman Chemical Glutathione Peroxidase Assay Kit (Ann Arbor, MI). GPx activity was determined by measuring the decrease in absorbance at 340nm over 5 minutes. This decrease was representative of the oxidation of NADPH to $NADP^+$, and therefore the activity of GPx under conditions where GPx activity is rate limiting.

3.8. Mineral Content

Samples were prepared using a microwave acid digestion following AOAC Method 968.08 Minerals in Animal Feed (1995, 16th Edition). Mineral content (selenium and iron) of the samples was analyzed using an Agilent 7700x inductively coupled plasma mass spectrometer (ICP-MS) instrument (Agilent Technologies, Inc., Japan) equipped with an octopole cell in hydrogen gas mode.

3.9. Fatty Acid Analysis

The fatty acid quantification in chicken breast was performed according to the method M-P528, which is the Merieux NutriSciences implementation of the AOAC method 996.06 that has been validated according to the ISO17025 standards. Breast samples were ground and freeze-dried prior to extraction and analysis. Fats and fatty acids were extracted from the breast tissue by acidic hydrolytic methods to release fat from bound protein and carbohydrates. Pyrogallic acid was added to minimize oxidative degradation of fatty acids. Fat was extracted into ether followed by methylation resulting in fatty acid methyl esters (FAMEs) using Boron Trifluoride (BF3) in methanol. FAMEs were separated and quantitatively measured by Gas Chromatography - Flame ionization Detector (GC-FID using a Supelco SP-2560 column, 100m x 0.25 mm i.d., 0.20um) against Triundecanoin (C11:0) internal standard. Concentrations of C16:0, C18:1C, C18:1T, C18:2C, C18:2T, C20:5 (EPA), C22:6 (DHA) were determined using this method. The response factors was determined by analyzing known reference standard mixtures of FAMEs (GLC85 or 463). The sum of the individual fatty acids expressed as triglyceride equivalents were used to calculate total fat.

3.10. Meat Quality

Cook loss, purge loss, color and puncture force were all measurements taken to evaluate the effect of dietary interventions on meat quality. Chicken breasts were weighed prior to packaging, prior to cooking and after cooking as a means to calculate percent cook loss and percent purge loss. Prior to cooking surface color (*L**, *a**, *b**) of the raw chicken breast was assessed using a Chroma Meter CR-300 colorimeter, equipped with a 1-cm aperture, Illuminant C (Minolate, Osaka, Japan). Colourimetric measurements were taken at random locations, in triplicate, on each breast fillet.

One breast per bird was cooked on day 0 and the other stored in the retail display cooler before cooking on day 7. Breasts were cooked at 176.7°C so that thickest part of the breast reached 72°C. The cooked chicken breasts were stored overnight at 4°C before assessing puncture force. Utilizing the EZ-Test Model Instron Instrument (Shimadzu Corporation, Kyoto, Japan) and a blunt, wedged probe was used to determine the kg force necessary to reach the point of fiber rupture. The Instron was operated using a 10N load cell and a

50mm/min crosshead speed. On each breast four punctures were made perpendicular to the fiber direction based on recommendations by (Lee et al., 2008).

CHAPTER 4

RESULTS & DISCUSSION

4.1. Visual Scoring

Fifty hot carcasses (10 per treatment) were evaluated for degree of WB. Results are shown in Figure 1 with no significant difference between treatments. Thus, nutritional intervention appeared to have minimal effects on WB development. The inorganic control did show a numerically greater score, and it is worth noting that more than half of the broilers scored for each treatment showed at least moderate WB. Owens (2014) also found that affected flocks exhibited some degree of WB at an incidence rate of approximately 50%.

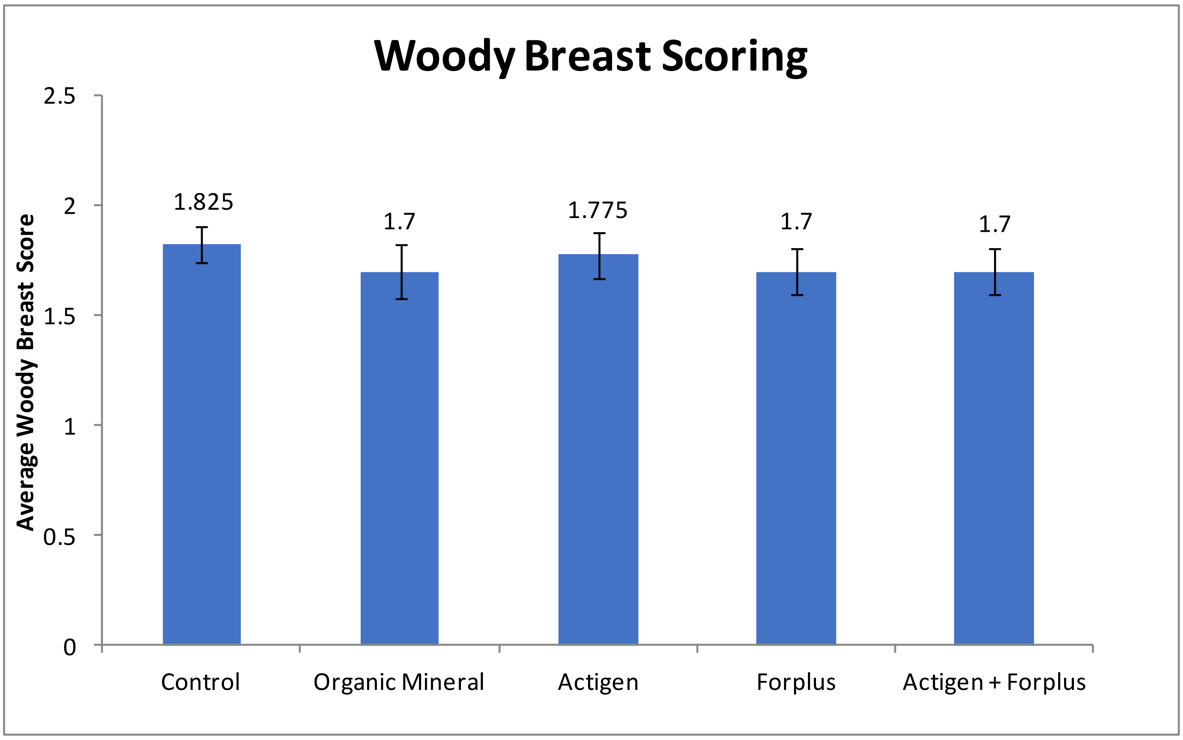

Figure 1: Average scores of chilled (*n* = 10)) carcasses for Wooded breast syndrome. Breasts were scored normal (1), moderate (2) or severe (3) by two scorers.

Scores for white striping of both hot and chilled carcasses are shown in Figure 2. There was no significant difference between treatments regardless of when scoring took place. All chilled carcasses were graded as normal (1) by both scorers while there was a greater incidence of WS when the carcass was scored while hot. Hot carcasses from all treatments were scored as moderate (2) WS or greater on average. Hot carcass scoring values for WS and WB coincide as the two conditions are believed to share a common etiology or even represent a disease spectrum where WS precedes WB and WB affected breasts exhibit severe WS (Sihvo et al., 2014; Mutryn et al., 2015).

However, chilled carcasses were found to be more straightforward to score, while it was more difficult to differentiate between scores on a hot carcass. Moreover, scoring done on the chilled carcass provides information relevant to the industry as it presents the breast as it would be sold to the consumer. Thus, future scoring would be best conducted within a processing plant with the capacity to score a large number of breasts post chilling.

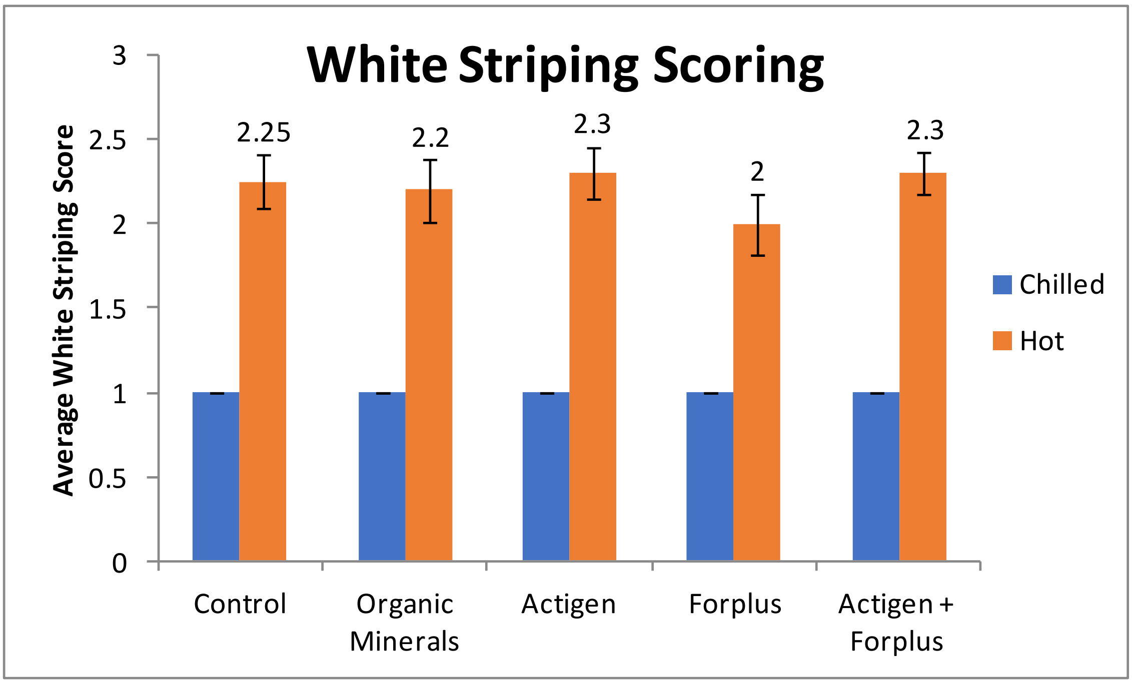

Figure 2: Average scores of chilled (*n* = 10)) and hot (*n* = 10) carcasses for White Striping. Breasts were scored normal (1), moderate (2) or severe (3) by two scorers.

4.2. Oxidative Stability

4.2.1. Lipid Oxidation

In the present study TBA-reactive values measured as mg MDA/mass in kg of chicken breast were used as a measurement of lipid oxidation within the breast meat (Figure 3). Lipid oxidation increased with retail storage for all dietary treatments ($P < 0.05$) except for the organic trace mineral control, but this treatment group did show a similar trend ($P < 0.1$). These findings align with previous work on lipid oxidation in chicken breast over time (Delles et al., 2014; Viana et al., 2017).

There was also a significant treatment effect on day 7. Forplus supplementation resulted in a dramatic increase in MDA content, which is likely attributed to the increase in more readily oxidizable LC-PUFA (Morrissey et al., 1998). Similarly, Mooney et al. (1998) found that supplementing marine algae or marine fish oil resulted in an increase in the degree of lipid oxidation and a decreased sensory acceptability. Ribeiro et al. (2013) found that supplementing broilers with 7.4% DHA negatively impacted the acceptability of the meat via increased substrate for lipid oxidation followed by secondary lipid oxidation products and therefore, rancidity. An increase in TBARS values in response to algae supplementation was also found in other species such as pork (Vossen et al., 2016b) and lamb (Urrutia et al., 2016). It is important to note that in this study even those samples showing higher TBARS values were well below the threshold of negative sensory attributes and rancidity (1 mg/kg MDA).

The combination of Forplus and Actigen would suggest that Actigen offers a protective effect as the TBARS values for the Forplus + Actigen were not significantly different from the control. As Actigen is known to improve gut morphology and absorptive capacity (Lea et al., 2011; Baurhoo et al., 2009), it is speculated that the protective effect is related to the uptake and availability of minerals. A separate study supplementing mannonoligosaccarides (MOS) to heat stressed broilers found that the MOS treatment group resulted in an increased concentration of serum trace minerals (Cu, Zn and Mn), but muscle deposition was not investigated (Sohail et al., 2011). Many trace minerals are essential to the functionality of antioxidant enzymes, thus this may be one mechanism by which Actigen bolsters oxidative stability.

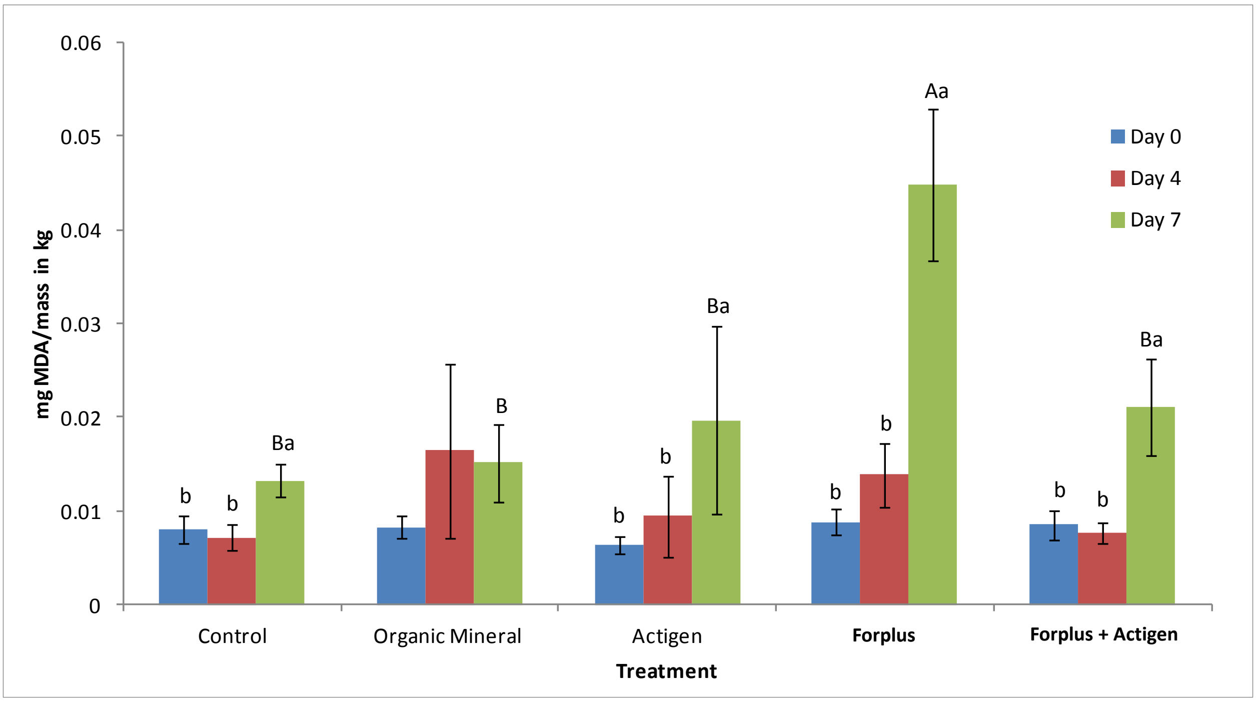

Figure 3: Effects of diets on lipid oxidation (TBARS, mg MDA/ kg meat) in broiler breast meat packaged in air-permeable polyvinylchloride (PVC) during refrigerated storage at 2–4 °C for up to 7 days. Different uppercase letters (A,B) indicate significant difference ($P < 0.05$) of means ($n = 12$) between between dietary treatments. Different lowercase letters (a,b) indicate significant difference ($P < 0.05$) of means ($n = 12$) between storage days within the same dietary treatment.

4.2.2. Protein Oxidation

Protein carbonyl content increased from storage days 0 to 7 in all dietary treatments except for Actigen, where there was a 43% decrease in nnmol carbonyl/mg of meat from day 4 to day 7. On days 0 and 7 there was also a significant treatment effect.

On day 0 Actigen showed significantly higher carbonyl content compared to the control and organic mineral treatments. By day 7, however, the treatment effect aligned with lipid oxidation findings (Figure 1). Actigen supplementation alone resulted in a significantly lower (P<0.5) carbonyl content on day 7 and Forplus + Actigen was numerically lower than the organic and inorganic controls and Forplus alone.

A recent study evaluating the effect of commercial, carbohydrate based prebiotics (used for selectivity in the gut) on the quality of broiler meat found that feeding indigestible oligosaccharides did result in a decrease in oxidative stability of breast meat. Based on evidence that larger, domestic birds are more susceptible to oxidation, it was suggested that increased muscle size as a result of feeding prebiotics may leave the same muscles more susceptible to oxidation (Maiorano et al., 2017). Breast weight data from the day of slaughter is not available for this study, but previous studies have found mannon oligosaccharides to increase bodyweight and cold carcass yield (Attia et al., 2014; Ao et al., 2016). Moreover, Actigen supplemented breasts were scored as 1.78 for average degree of Wooden Breast (Figure 3) which suggests that the majority of the birds were affected at least moderately by this myopathy that has previously been correlated to large birds (Kuttappan et al., 2013a; Petracci et al., 2013b). Thus, the early oxidative damage to the myofibrillar proteins may be a result of increased muscle size in the broilers being examined. Carcass and breast weight prove to be a valuable inquiry in further studies on Actigen and meat quality.

These results, again, suggest that Actigen, even after exhibiting lower protein oxidative stability on day 0, had a protective effect against oxidative damage with time. Therefore, Actigen, overall, has a stabilizing effect and the role of Actigen in extending shelf life could hold promising results.

Other studies (Ventanas et al, 2006; Estevez et al., 2008; Mercier et al., 1998) have shown that increased TBARS is correlated to an increase in carbonyl content. Thus, increased lipid oxidation may have contributed to the higher carbonyl content in Forplus.

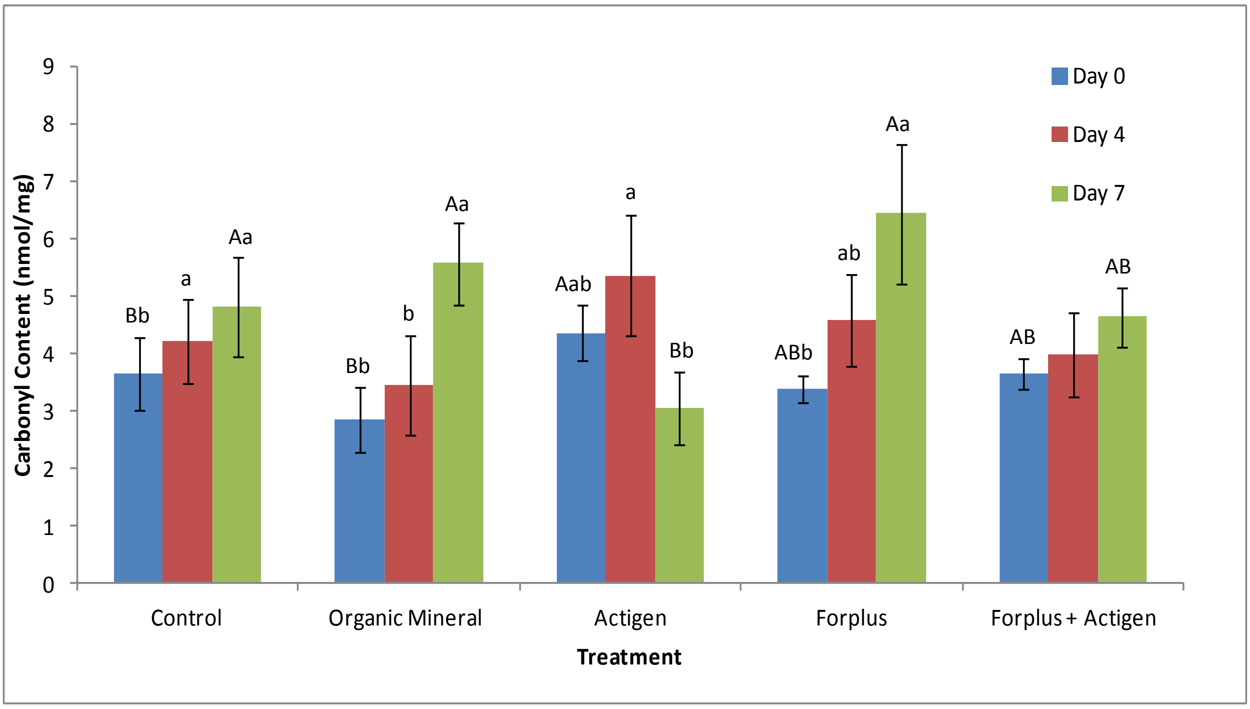

Figure 4: Effects of diets on protein carbonyl formation (nmol/ mg protein) in broiler breast meat packaged in air-permeable polyvinylchloride (PVC) during refrigerated storage at 2–4 °C for up to 7 days. Different uppercase letters (A,B) indicate significant difference ($P < 0.05$) of means ($n = 12$) between dietary treatments. Different lowercase letters (a,b) indicate significant difference ($P < 0.05$) of means ($n = 12$) between storage days within the same dietary treatment.

4.3. Antioxidant Enzyme Activity

In order to explain the variations in oxidative stability among treatments the activity of antioxidant enzymes known to be important in meat were measured. Catalase activity was measured (Figure 5) in nmol/min/ml. Forplus supplementation showed the greatest catalase activity, though it was not significantly greater than the organic mineral. However, a similar study by Delles and colleagues (2014) supplementing organic trace minerals and algae-based Se yeast, supplements used in all dietary treatments except for control found that catalase enzyme activity was significantly increased in the treatment group contributing to oxidative stability. Because a similar feeding regimen in the current study did not increase catalase activity there may have been another limiting factor, such as iron concentration (Switala and Loewen, 2002).

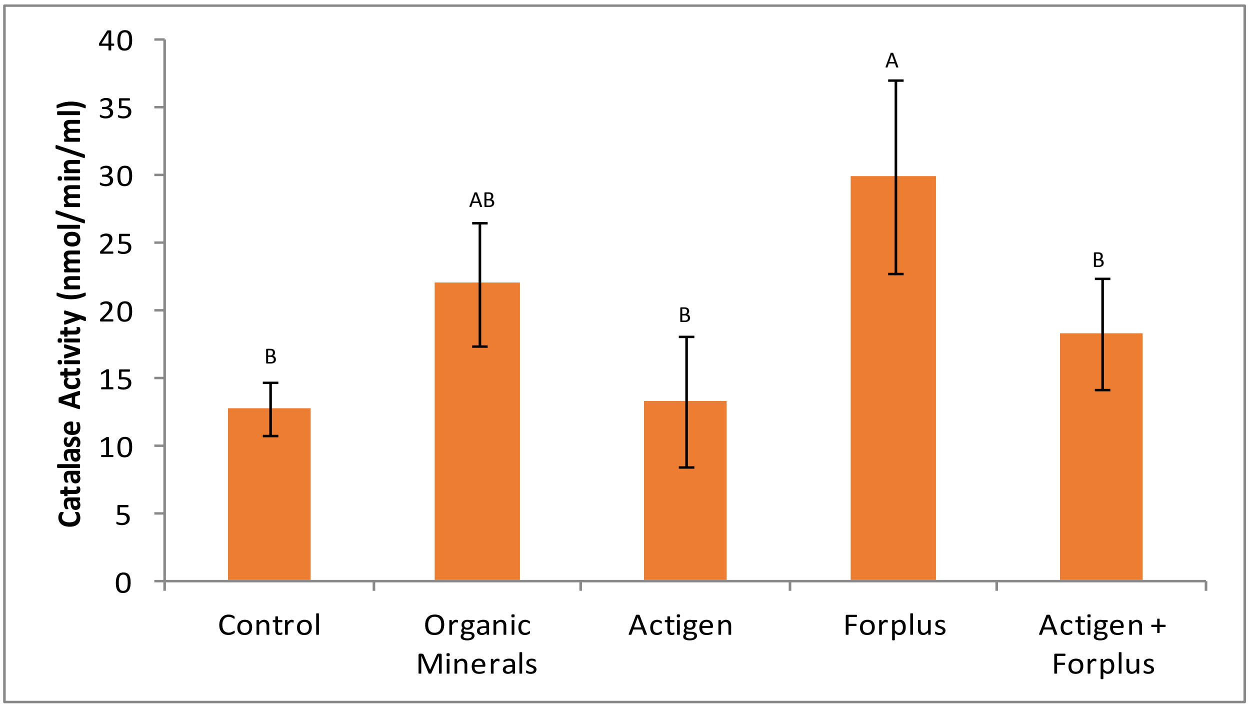

Figure 5: Catalase activity (nmol/min/ml) in Pectoralis major from broilers fed one of five different diets for 42 days. Different uppercase letters (A,B) indicate significant difference (*P* < 0.05) of means (*n* = 10) between dietary treatments.

GPx activity, shown in Figure 6, was significantly affected by dietary treatment. Actigen and Actigen + Forplus showed a significantly higher activity than the other treatments. Based on these results, it is speculated that Actigen may, through its role in gut health and absorptive capacity, play a role in improving GPx activity via a potential enhancement of selenium absorption and retention. The increased activity of GPx substantiates the improved oxidative stability of Actigen supplemented groups (Figures 3 and 4).

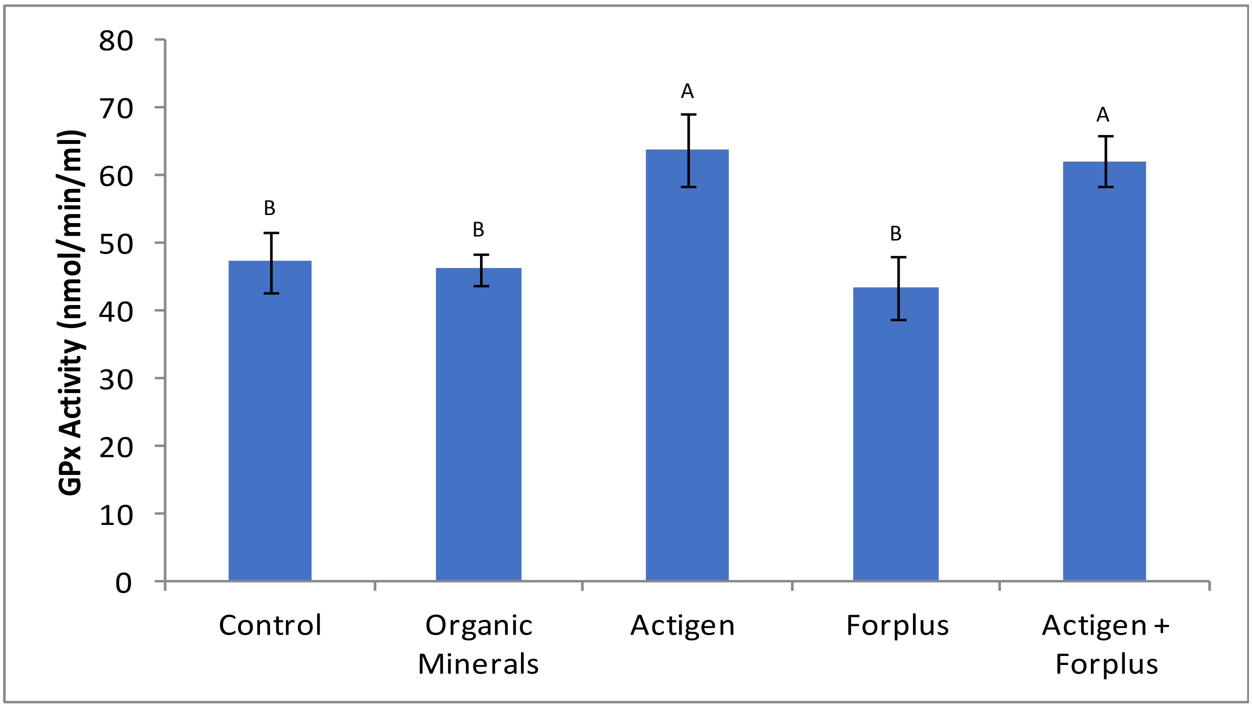

Figure 6: Glutathione peroxidase activity (nmol/min/ml) in Pectoralis major from broilers fed one of five different diets for 42 days. Different uppercase letters (A,B) indicate significant difference ($P < 0.05$) of means ($n = 10$) between dietary treatments.

4.4. Mineral Content

Concentrations of minerals of interest, Se and Fe, within the breast were investigated using Inductively Coupled Plasma Mass Spectrometry. The results for Se deposition in the breast are shown in Table 1. The organic mineral control, Actigen, and Forplus treatment groups all exhibited significantly higher ($P < 0.05$) selenium content than the inorganic control. It was hypothesized that the Actigen's stabilizing effect on the oxidative status of the chicken breast was a result of its facilitation of Se absorption and availability to increase GPx activity. However, GPx activity (Figure 4) was not directly proportional to Se content (Table 1). Thus, Se content likely contributes to the increased GPx activity of the Actigen supplemented group, but it is not the only factor. Further investigation of Se content in other tissues, such as liver, and GPx gene expression may provide greater insight as to the mechanism behind Actigen.

The ability of catalase to react with and decompose hydrogen peroxide is due to the iron within the heme group of the enzyme. All diets fed organic minerals and selenium yeast (organic mineral; Actigen; Forplus; Forplus + Actigen) were expected to exhibit an increase in catalase activity based on findings from previous studies (Delles et al., 2014). Actigen, in particular, was hypothesized to show improved catalase activity based on the increased oxidative stability in Actigen supplemented broilers (Figure 3 and 4). As catalase activity was found to be contrary to the hypothesis, an insufficient iron supply was postulated as a potential reason for the unexpected lack of catalase activity. ICP-MS results for iron content (Table 1) confirmed that the Actigen supplemented group had a significantly lower iron content compared to both inorganic and organic controls as well as Forplus. The Actigen + Forplus broilers also showed numerically lower iron content than the organic mineral control and significantly lower Fe content than the inorganic control. As the Actigen and Actigen + Forplus treatment groups were all fed organic, mineral enriched yeast, these results are in contrast to previous studies that found that organic Fe is more effectively absorbed than the inorganic counterpart (Jia et al., 2014).. The decreased iron content resulting in the Actigen group does not align with previous work and suggests the need for further experimentation. However, it should be noted that the lower iron content in the Actigen treatment may have contributed to improved oxidative stability due to lower amounts of nonheme iron catalyzing the decomposition of preformed lipid hydroperoxides into lipid radicals.

The Forplus supplemented broilers showed a significantly higher catalase activity ($P<0.05$) (Figure 3) and an iron content significantly greater than that of Actigen and Forplus + Actigen. However, iron content was not significantly different from the control or organic mineral treatments. The increase in catalase activity in Forplus broilers may be better explained as a response to increased concentration of oxidants, as catalase velocity is dependent on substrate (H_2O_2) concentration when the oxidant is still present at low or moderate concentrations (Switala and Loewen, 2002).

Table 4: Selenium content (ppb) and iron content (ppm) of *Pectoralis major* determined by ICP-MS.

Mineral	**Control**	**Organic Mineral**	**Actigen**	**Forplus**	**Actigen + Forplus**
Selenium (ppb)	153.5 ± 6.3^{C}	198.5 ± 10.0^{A}	180.0 ± 3.1^{AB}	180.1 ± 5.6^{AB}	172.8 ± 4.8^{BC}
Iron (ppm)	3.86 ± 0.22^{A}	3.5 ± 0.078^{AB}	2.95 ± 0.18^{C}	3.45 ± 0.10^{AB}	3.23 ± 0.055^{BC}

$^{A-C}$ Means (n = 10) between diets without a common uppercase superscript differ significantly ($P < 0.05$).

4.5. Fatty Acid Analysis

In order to determine how each treatment affected fatty acid profile, samples were analyzed for their content of palmitic (16:0), trans-elaidic (18:1), cis-oleic (18:1), trans-linoelaidic acid (18:2), cis-linoleic acid (18:2), eicosapentaenoic acid (EPA) (20:5), and DHA (22:6). The only treatment effects on fatty acid profile were seen in the concentration of EPA and DHA as shown in Table 2. Forplus and Actigen + Forplus exhibited a significantly ($P <$ 0.05) greater concentration of DHA as well as a greater concentration of EPA that was approaching significance (P = 0.07). The distinct increase in PUFA in those groups supplemented with algae substantiates the increased lipid and protein oxidation within the same groups. Previous work confirms that PUFA are more readily oxidizable and their increased concentration within meat results in a decreased oxidative stability (Morrissey et al., 1998; Nute et al., 2007).

Table 5: Effect of dietary treatments on concentrations of EPA (mcg/g) and DHA (mcg/g) of *Pectoralis major* determined by Merieux NutriSciences implementation of the AOAC method 996.06.

Treatment	EPA (mcg/g)	DHA (mcg/g)
Control	63 ± 2.65^{b}	246.7 ± 38.7^{B}
Organic mineral	111 ± 30.5^{ab}	287.7 ± 7.80^{B}
Actigen	68.7 ± 10.3^{b}	235.3 ± 36.2^{B}
Forplus	136.3 ± 29.3^{a}	2081.3 ± 772^{A}
Actigen + Forplus	154.3 ± 29.5^{a}	2513.3 ± 189^{A}

^{A,B} Means ($n = 3$) between diets without a common uppercase superscript differ significantly ($P < 0.05$).
^{a,b} Difference of means (n=3) between diets without a common lowercase superscript is approaching significance ($P < 0.1$)

4.6. Meat quality

4.6.1. Puncture Force

Puncture force is interpreted as the kg of force necessary to rupture the fibers of or puncture the chicken breast. Forplus and Forplus + Actigen treatment exhibited a significant decrease ($P < 0.0001$) in tenderness during storage, while other treatment groups showed no change over time (Figure 8). The change over time may have been more prominent in groups supplemented with Forplus due to increased oxidation (Figure 3 and 4). Increased oxidative damage, particularly of the myofibrillar proteins can lead to disulfide cross-linkages, which has been reported to decrease tenderness of meat (Lamesteschet al., 2007). Moreover, increased carbonyl content as seen in the Forplus treatment has also been reported to correlate with increased instrumental texture values (Rowe et al., 2004).

On day 0, puncture force of the Forplus supplemented group was numerically lower than the organic mineral group and significantly lower ($P = 0.0263$) than all other treatment groups.

The change in fatty acid profile and increases LC-PUFA (Table 2) is a likely cause of decreased hardness of the meat. Moraes et al. (2016) supplemented broilers with canola oil and also found that hardness decreased with increased levels of PUFA, specifically alpha-linonlenic acid, within the fatty acid profile. On day 7, however, the Forplus dietary treatment did not benefit meat texture.

While Actigen nor Forplus had a significant effect on texture, the Forplus + Actigen significantly increased ($P < 0.0001$) puncture force on day 7. These results could be a manifestation of both change in fatty acid profile and increased growth rate.. The increased PUFA content of Actigen + Forplus may indirectly result in increased protein oxidation. Whether through calpain inactivation or cross-linking, protein oxidation has a direct effect on texture (Lund et al., 2011; Rowe et al., 2004). Moreover, Actigen supplementation has also been shown to increase growth rate (Hooge & Connolly, 2011). As growth rate is correlated with development of myopathies it is possible that supplementation with Actigen may result in subcutaneous signs of WB not yet visible to the scorer at 42 days (Kuttappan et al., 2013a; 2012c). A histological investigation is necessary to investigate potential fibrosis within the tissue, or a longer feeding period to allow for the complete manifestation of the WB condition.

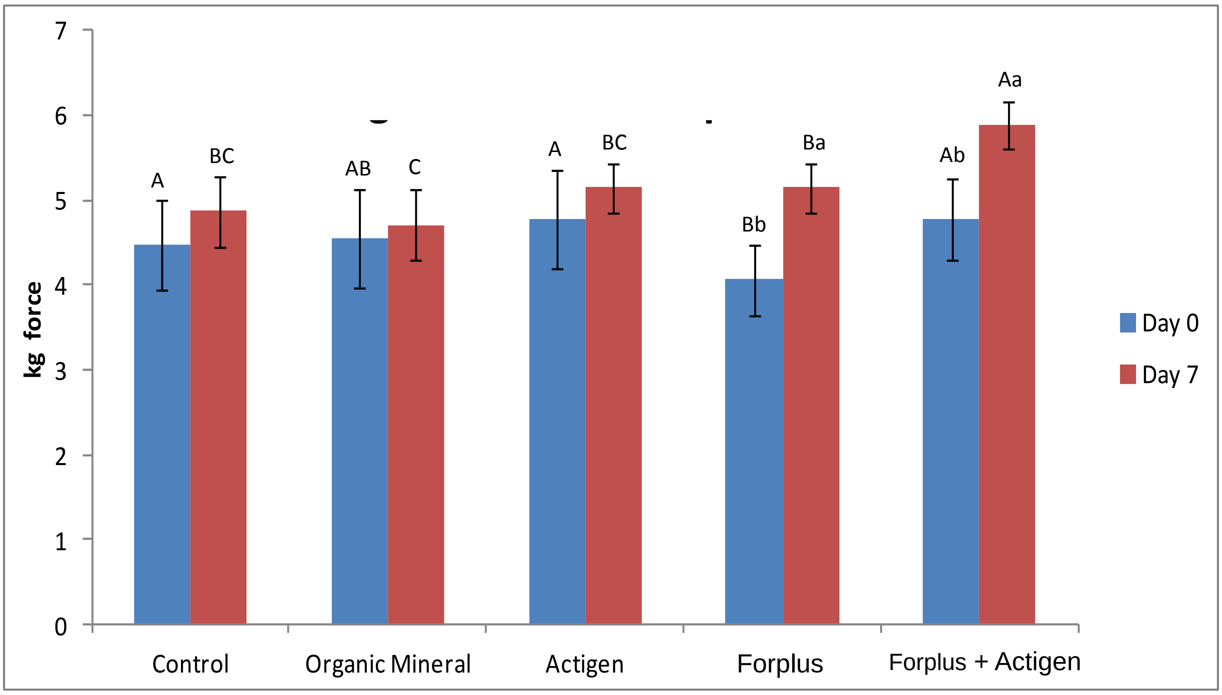

Figure 7: Effect of dietary treatment on puncture force (kg force) of broiler breast meat packaged in polyvinylchloride (PVC) at refrigerated storage at 2–4 °C for up to 7 days. Puncture force or the kg force necessary to rupture the fibers of chicken breasts taken from broilers fed one of five different diets for 42 days. Puncture force was measured after cooking at 0 and 7 days *post mortem*. Different uppercase letters (A–C) indicate significant ($P < 0.05$) difference between dietary treatments within the same day. Different lowercase letters (a,b) indicate significant ($P < 0.05$) difference between storage days within the same dietary treatment.

4.6.2. Water Holding Capacity

Cook loss decreased after 7 days of storage for all treatment groups. This is likely because those samples stored 7 days had lower water content due to purge loss (Figure 9). There was no significant treatment effect on cook loss or purge loss (Figure 8 and 9). However, those groups receiving Actigen supplementation exhibited a numerically lower purge loss which may coincide with the higher degree of oxidative stability (Figure 3 and 4). As oxidative stability is directly related to the integrity of myofibrillar protein matrix, Actigen may thereby improve water holding capacity (Liu et al., 2010).

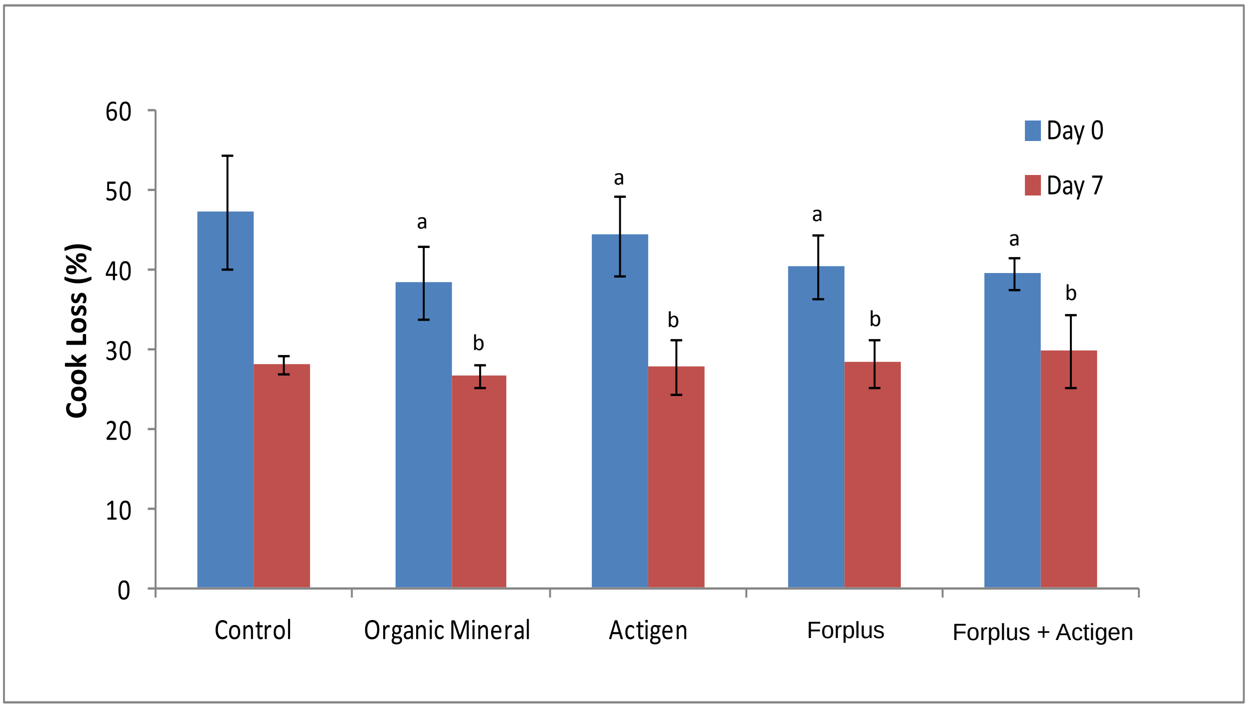

Figure 8: Effect of dietary treatment on cook loss (%) of broiler breast meat packaged in polyvinylchloride (PVC) at refrigerated storage at 2–4 °C for up to 7 days. Different lowercase letters (a,b) indicate significant ($P < 0.05$) difference between storage days within the same dietary treatment.

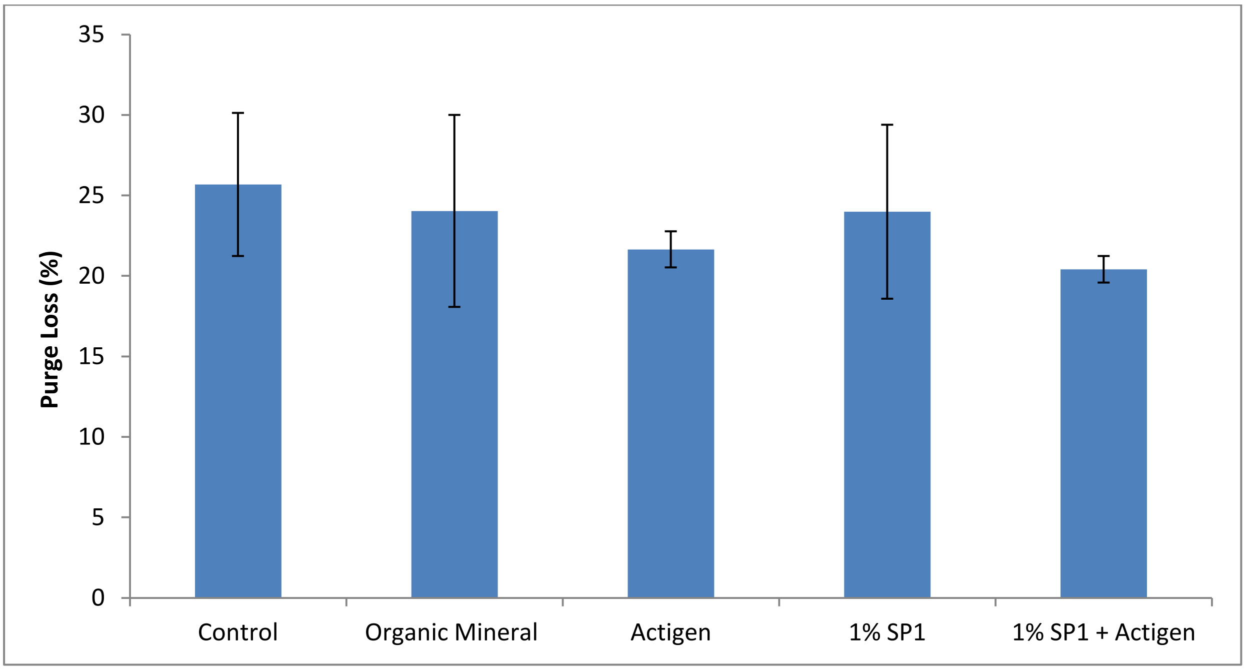

Figure 9: Effect of dietary treatment on purge loss (%) of broiler breast meat packaged in polyvinylchloride (PVC) at refrigerated storage at 2–4 °C for up to 7 days.

4.6.3. Instrumental Color

In Forplus supplemented groups redness ($a*$) was significantly decreased compared to the controls on day 0 as can be seen in Table 2. This may be a result of increased aldehyde byproducts of lipid oxidation (Figure 3) which can covalently bind myoglobin and accelerate metmyoglobin formation and therefore discoloration (Suman & Joseph, 2014). A broiler study supplementing dietary quercetin evidenced that increased redness occurred with increased antioxidant inclusion in the diet (Goliomytis et al., 2014). Thus, oxidative instability as a result of increased PUFA content (Table 2) could be correlated to a decrease in redness. Similarly, Nute et al. (2007) found that broilers fed a diet enriched with PUFA (marine algae or fish oil) were more susceptible to both oxidation and discoloration. Current results suggest that antioxidative and color improving effects of carotenoids within algae are not potent enough to mitigate the oxidative instability and discoloration associated with the increase in PUFA (Figure 3 and Table 2).

Lightness ($L*$) was significantly decreased ($P = 0.0045$) in Forplus samples on day 7 in comparison to controls and Actigen. Improved lightness aligns with previous findings that the carotenoids within algae improve meat color (Toyomizu et al., 2001). Based on the threshold for pale meat set by Petracci et al. (2004) ($L* >56$) all treatment groups, on average, exhibited pale meat. Similarly, Woelfel et al. (2002) found that up to 47% of breasts at a commercial plant were recorded as pale, any of which could exhibit poor water holding capacity. Thus, the lightness of all treatment groups could coincide with the similarities across dietary treatment groups in water holding capacity. As PSE-like characteristics are known to arise due to heat stress, the changes in meat color could be attributed to an external source other than diet (Owens et al., 2000; Zhang et al., 2012). In this case, our results would suggest that dietary intervention did not completely thwart the negative effects of heat stress on meat quality.

There was no significant effect on the yellowness (*b**) of the meat on days 0 or 7. This aligns with Schiavone et al. (2007) who found that supplementing microalgae to increase DHA had no effect on the yellowness of Muscovy duck breast. However, other groups have found that supplementation with algae significantly increases yellowness of the meat (Toyomizu et al., 2001; Venkataraman et al., 1994).

Table 6: Effect of dietary treatments on lightness (*L**), redness (*a**) and yellowness (*b**) surface color values for raw chicken breasts taken on day 0 or day 7 post mortem.

	L*		***a****		***b****	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Control	61.5 ± 0.65	60.9 ± 1.20^{A}	11.6 ± 0.67^{A}	10. 6 ± 0.92	12.1 ± 0.92	15.9 ± 0.48
Organic Mineral	60.6 ± 0.44	59.6 ± 0.33^{AB}	12.0 ± 0.36^{A}	11.0 ± 0.46	12.5 ± 0.90	14.7 ± 0.40
Actigen	61.5 ± 1.10	60.5 ± 1.40^{AB}	11.5 ± 0.69^{AB}	10.7 ± 0.47	12.2 ± 0.81	15.6 ± 0.33
Forplus	61.4 ± 0.81	58.6 ± 0.93^{C}	11.0 ± 0.38^{B}	10.4 ± 0.37	13.2 ± 1.4	15.4 ± 0.39
Forplus + Actigen	61.5 ± 1.20	59.1 ± 0.87^{BC}	11.5 ± 0.03^{AB}	10.6 ± 0.19	12.1 ± 0.37	15.1 ± 0.38

^{A,B,C} Means (n =30) between diets within the same day without a common uppercase superscript differ significantly ($P < 0.05$).

CHAPTER 5

CONCLUSIONS

In conclusion, nutritional intervention did have a direct impact on meat quality. Feeding DHA rich microalgae meal (Forplus) successfully changed the fatty acid profile of the breast muscle, increasing the concentration of PUFA. While increased PUFA has desirable health benefits within the meat, it also increased vulnerability of the meat to lipid and protein oxidation and negatively impacted shelf-life after 7 days. This is likely due to the readily oxidizable nature of the allylic and double allylic bonds within the LC-PUFA. Although the degree of lipid oxidation in the Forplus treatment did not impact olfactory characteristics of the breast meat, even after 7 days of retail storage, there was still a detrimental impact on color through reduced redness. Protein oxidation, however, did affect meat quality parameters such as texture resulting in increased hardness.

Supplementing broilers with MRF (Actigen) resulted in an increase in oxidative stability of meat. Actigen alone was able to subdue oxidative stress brought on by rearing birds under heat stress, and when supplemented in combination with Forplus, Actigen was able to mitigate the vulnerabilities seen in Forplus alone. As Actigen supplementation was accompanied by increased GPx activity, it is likely that increased antioxidant enzyme activity is at least partially responsible for the increased stability of Actigen supplementation. However, the concentration of minerals key for antioxidant activities was relatively low in Actigen supplemented group. Thus, further investigation of liver and serum mineral content is necessary to determine the mechanism by which Actigen increases antioxidant activity.

As this study was also meant to serve as a continuation to Delles et al. (2014), the birds were fed algae-based antioxidant, containing Se yeast as a replacement for Vitamin E and organic minerals. Results from this study conclude that while these supplementations did significantly increase Se deposition, there was no significant effect on oxidative stability or meat quality. These interventions alone are unable to thwart the damages to meat quality that arise under increased environmental stress. However, these supplements do successfully replace inorganic minerals without detriment to meat quality.

Overall, the results show that nutritional intervention can be used to positively influence meat quality and attributes. Including Actigen in the nutritional regimen has previously been intended to improve broiler performance, but results conclude that it can also be fed in order to extend oxidative stability of the meat and deter negative effects of environmental stress. Actigen also proves to offer a way of making increased PUFA content in the meat more sustainable by inhibiting negative effects on oxidative stability and meat quality during retail storage. The mode of action by which Actigen improves meat quality is likely related to the activity of antioxidant enzymes such as glutathione peroxidase. Further investigations are needed to determine how Actigen supplementation and modulation within the gut directly or indirectly affect mineral deposition and antioxidant enzyme activity.

REFERENCES

Abasht, B., Mutryn, M. F., Michalek, R. D., & Lee, W. R. (2016). Oxidative Stress and Metabolic Perturbations in Wooden Breast Disorder in Chickens. *Plos One,11*(4). doi:10.1371/journal.pone.0153750

Agunos, A., Ibuki, M., Yokomizo, F., & Mine, Y. (2007). Effect of dietary β1–4 mannobiose in the prevention of Salmonella enteritidis infection in broilers. *British Poultry Science, 48*(3), 331-341. doi:10.1080/00071660701370442

Ajuyah, A., Fenton, T., Hardin, R., & Sim, J. (1993). Measuring Lipid Oxidation Volatiles in Meats. *Journal of Food Science,58*(2), 270-273. doi:10.1111/j.1365-2621.1993.tb04253.x

Akbarian, A., Michiels, J., Degroote, J., Majdeddin, M., Golian, A., & Smet, S. D. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology J Animal Sci Biotechnol, 7*(1). doi:10.1186/s40104-016-0097-5

Aksu, T., Aksu, M., Yoruk, M., & Karaoglu, M. (2011). Effects of organically-complexed minerals on meat quality in chickens. *British Poultry Science, 52*(5), 558-563. doi:10.1080/00071668.2011.606800

Aldridge, K., Saddoris, L., & Radcliffe, J. (2007). Copper can be absorbed as a Cu-peptide chelate through the PepT1 transporter in the jejunum of weanling pigs. *Journal of Animal Science,85*, 154.

Al-Qudah, K. M., Gharaibeh, A. A., & Al-Shyyab, M. M. (2010). Trace Minerals Status and Antioxidant Enzymes Activities in Calves with Dermatophytosis. *Biological Trace Element Research,136*(1), 40-47. doi:10.1007/s12011-009-8525-4

Altan, O., Altan, A., Oguz, I., Pabuçcuoglu, A., & Konyalioglu, S. (2000). Effects of heat stress on growth, some blood variables and lipid oxidation in broilers exposed to high temperature at an early age. *British Poultry Science,41*(4), 489-493. doi:10.1080/713654965

Altan,O., Pabuçcuoğlu, A., Altan, A., Konyalioğlu, S., & Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science,44*(4), 545-550. doi:10.1080/00071660310001618334

An, G., Song, J., Chang, K., Lee, B., Chae, H., & Jang, B. (2004). Pigmentation and Delayed Oxidation of Broiler Chickens by the Red Carotenoid, Astaxanthin, from Chemical Synthesis and the Yeast, Xanthophyllomyces dendrorhous. *Asian-Australasian Journal of Animal Sciences,17*(9), 1309-1314. doi:10.5713/ajas.2004.1309

Ao, T., & Pierce, J. (2013). The replacement of inorganic mineral salts with mineral proteinates in poultry diets. *World's Poultry Science Journal, 69*(01), 5-16. doi:10.1017/s0043933913000019

Ao, T., Macalintal, L.M., Paul, M.A., Pescatore, A.J., Cantor, A.H., Ford M.J. & Dawson, K.A. (2016). Dietary Actigen® and Allzyme SSF® improved performance and carcass yield of broiler chicks. *International Poultry Science Federation*, Atlanta, GA

Ao, T., Pierce, J., Power, R. P., Dawson, K., Pescatore, A., Cantor, A., & Ford, M. (2006). Evaluation of Bioplex Zn® as an Organic Zinc Source for Chicks. *International Journal of Poultry Science, 5*(9), 808-811. doi:10.3923/ijps.2006.808.811

Attia, Y., Al-Hamid, A. A., Ibrahim, M., Al-Harthi, M., Bovera, F., & Elnaggar, A. (2014). Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. *Livestock Science, 164*, 87-95. doi:10.1016/j.livsci.2014.03.005

Baurhoo, B., Ferket, P. R., & Zhao, X. (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poultry Science, 88*(11), 2262-2272. doi:10.3382/ps.2008-00562

Bekhit, A. E., Hopkins, D. L., Fahri, F. T., & Ponnampalam, E. N. (2013). Oxidative Processes in Muscle Systems and Fresh Meat: Sources, Markers, and Remedies. *Comprehensive Reviews in Food Science and Food Safety, 12*(5), 565-597. doi:10.1111/1541-4337.12027

Blanco-Penedo, I., Cruz, J., López-Alonso, M., Miranda, M., Castillo, C., Hernández, J., & Benedito, J. (2006). Influence of copper status on the accumulation of toxic and essential metals in cattle. *Environment International,32*(7), 901-906. doi:10.1016/j.envint.2006.05.012

Briens, M., Mercier, Y., Rouffineau, F., Vacchina, V., & Geraert, P. (2013). Comparative study of a new organic selenium source v. seleno-yeast and mineral selenium sources on muscle selenium enrichment and selenium digestibility in broiler chickens. *British Journal of Nutrition, 110*(04), 617-624. doi:10.1017/s0007114512005545

Brooks, M.A., Grimes, J.L., Lloyd, K.E., Valdez, F., & Spears, J.W. (2012). Relative bioavailability in chicks of manganese from manganese propionate. *The Journal of Applied Poultry Research, 21*, 126-130.

Bun, S. D., Guo, Y. M., Guo, F. C., Ji, F. J., & Cao, H. (2011). Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. *Poultry Science, 90*(6), 1220-1226. doi:10.3382/ps.2010-01308

Calvo, L., Toldrá, F., Aristoy, M., López-Bote, C., & Rey, A. (2016). Effect of dietary organic selenium on muscle proteolytic activity and water-holding capacity in pork. *Meat Science, 121*, 1-11. doi:10.1016/j.meatsci.2016.05.006

Castegna, A., Drake, J., Pocernich, C., & Butterfield, D. A. (2000). Protein Carbonyl Levels—An Assessment of Protein Oxidation. In *Methods in Pharmacology and Toxicology: Methods in Biological Oxidative Stress* (pp. 161-169). Totowa, NJ: Humana Press Inc.

Cavitt, L. C., Hargis, B. M., & Owens, C. M. (2004). The use of halothane and succinylcholine to identify broilers prone to developing pale, soft, exudative meat. *Poultry Science, 83*(8), 1440-1444. doi:10.1093/ps/83.8.1440

Coetzee, G., & Hoffman L. C. (2001). Effect of dietary vitamin E on the performance of broilers and quality of broiler meat during refrigerated and frozen storage. *South African Journal of Animal. Science, 31*, 158–173.

Dalólio, F. S., Albino, L. F., Lima, H. J., Silva, J. N., & Moreira, J. (2015). Heat stress and vitamin E in diets for broilers as a mitigating measure. *Acta Scientiarum. Animal Sciences,37*(4), 419. doi:10.4025/actascianimsci.v37i4.27456

Das, T.K., Mondal, M.K., Biswas, P., Bairagi, B. & Samanta, C.C. (2010). Influence of level of dietary inorganic and organic copper and energy level on the performance and nutrient utilization of broiler chickens. *Asian-Australian Journal of Animal Science, 23,* 82-89.

Davies, K., Delsignore, M., & Lin, S. (1987). Protein damage and degradation by oxygen radicals. II. Modification of amino acids. *Journal of Biological Chemistry, 262*(20), 9902-9907.

Decker, E., Elias, R. J., & McClements, D. E. (2010). *Oxidation in Foods and Beverages and Antioxidant Applications: Management In Different Industry Sectors*. Cambridge: Woodhead Publishing.

Delles, R. M., Xiong, Y. L., True, A. D., Ao, T., & Dawson, K. A. (2014). Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. *Poultry Science, 93*(6), 1561-1570. doi:10.3382/ps.2013-03682

Dikeman, M. (2007). Effects of metabolic modifiers on carcass traits and meat quality. *Meat Science, 77*(1), 121-135. doi:10.1016/j.meatsci.2007.04.011

Edens, F., Lowman, Z., Ashwell, C., & Nolin, S. (2014). Actigen Influence on the Gene Expression of Heat Shock Proteins in Ross 708 Broiler Chickens. *International Journal of Poultry Science, 13*(2), 114-123. doi:10.3923/ijps.2014.114.123

Edwards, M., Edwards, A., Millard, P., & Kocher, A. (2014). Mannose rich fraction of Saccharomyces cerevisiae promotes growth and enhances carcass yield in commercially housed grower–finisher pigs. *Animal Feed Science and Technology, 197*, 227-232. doi:10.1016/j.anifeedsci.2014.08.004

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science, 89*(3), 259-279. doi:10.1016/j.meatsci.2011.04.025

Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., & Heinonen, M. (2008). Oxidation of skeletal muscle myofibrillar proteins in oil-in-water emulsions: interaction with lipids and effect of selected phenolic compounds. *Journal of Agricultural and Food Chemistry, 56*(22), 10933-10940. doi:10.1021/jf801784h

Evans, A. M., Smith, D. L., & Moritz, J. S. (2015). Effects of algae incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance. *The Journal of Applied Poultry Research, 24*(2), 206-214. doi:10.3382/japr/pfv027

FAO. (2016, April). FAO's Animal Production and Health Division: Meat & Meat Products. Retrieved January 04, 2017, from http://www.fao.org/Ag/againfo/themes/en/meat/home.html

Fedorova, M., Bollineni, R. C., & Hoffmann, R. (2014). Protein carbonylation as a major hallmark of oxidative damage: Update of analytical strategies. *Mass Spectrometry Reviews, 33*(2), 79-97. doi:10.1002/mas.21381

Fujii, J., Otsu, K., Zorzato, F., Leon, S. D., Khanna, V., Weiler, J., . . . Maclennan, D. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science, 253*(5018), 448-451. doi:10.1126/science.1862346

Ganner, A., & Schatzmayr, G. (2012). Capability of yeast derivatives to adhere enteropathogenic bacteria and to modulate cells of the innate immune system. *Applied Microbiology and Biotechnology, 95*(2), 289-297. doi:10.1007/s00253-012-4140-y

Gao, J., Yin, D. H., Yao, Y., Sun, H., Qin, Z., Schoneich, C., Williams, T. D. & Squier, T. C. (1998). Loss of conformational stability in calmodulin upon methionine oxidation. *Biophysical Journal, 74*, 1115–1134.

Garrison, W. M. (1987). Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chemical Reviews, 87*, 381–398.

Geraert, P. A., Padilha, J. C., & Guillaumin, S. (1996). Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: Biological and endocrinological variables. *British Journal of Nutrition, 75*(2), 205-216. doi:10.1079/bjn19960125

Goliomytis, M., Tsoureki, D., Simitzis, P. E., Charismiadou, M. A., Hager-Theodorides, A. L., & Deligeorgis, S. G. (2014). The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. *Poultry Science, 93*(8), 1957-1962. doi:10.3382/ps.2013-03585

Gray, J.I., & Pearson, A.M. (1987). Rancidity and warmed-over flavor. In A.M. Pearson & T.R. Dutson (Eds.) *Advances in Meat Research, Vol. 3 Restructured Meat and Poultry Products (*pp. 221-269). Reinhold, New York: AVI, Van Nostrand

Guetchom, B., Venne, D., Chenier, S., & Chorfi, Y. (2012). Effect of extra dietary vitamin E on preventing nutritional myopathy in broiler chickens. *The Journal of Applied Poultry Research, 21*(3), 548-555. doi:10.3382/japr.2011-00440

Habibian, M., Ghazi, S., & Moeini, M. M. (2015). Effects of dietary selenium and vitamin E on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. *Biological Trace Element Research, 169*(1), 142-152. doi:10.1007/s12011-015-0404-6

Habibian, M., Ghazi, S., Moeini, M. M., & Abdolmohammadi, A. (2014). Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. *International Journal of Biometeorology, 58*(5), 741-752. doi:10.1007/s00484-013-0654-y

Halliwell, B., & Gutteridge, J. M. (1995). The definition and measurement of antioxidants in biological systems. *Free Radical Biology and Medicine, 18*(1), 125-126. doi:10.1016/0891-5849(95)91457-3

Hashizawa, Y., Kubota, M., Kadowaki, M., & Fujimura, S. (2013). Effect of dietary vitamin E on broiler meat qualities, color, water-holding capacity and shear force value, under heat stress conditions. *Animal Science Journal, 84*(11), 732-736. doi:10.1111/asj.12079

Headlam, H. A., & Davies, M. J. (2004). Markers of protein oxidation: different oxidants give rise to variable yields of bound and released carbonyl products. *Free Radical Biology and Medicine, 36*(9), 1175-1184. doi:10.1016/j.freeradbiomed.2004.02.017

Hooge, D. M., & Connolly, A. (2011). Meta-Analysis Summary of Broiler Chicken Trials with Dietary Actigen® (2009-2011). *International Journal of Poultry Science, 10*(10), 819-824. doi:10.3923/ijps.2011.819.824

Horrocks, L. A., & Yeo, Y. K. (1999). Health Benefits Of Docosahexaenoic Acid (DHA). *Pharmacological Research, 40*(3), 211-225. doi:10.1006/phrs.1999.0495

Imik, H., Atasever, M. A., Urcar, S., Ozlu, H., Gumus, R., & Atasever, M. (2012). Meat quality of heat stress exposed broilers and effect of protein and vitamin E. *British Poultry Science, 53*(5), 689-698. doi:10.1080/00071668.2012.736609

Jayasena, D. D., Ahn, D. U., Nam, K. C., & Jo, C. (2013). Flavour Chemistry of Chicken Meat: A Review. *Asian-Australian Journal of Animal Science, 26*(5), 73242-742.

Jia, Y. F., Jiang, M. M., Sun, J., Shi, R. B., & Liu, D. S. (2014). Studies on Different Iron Source Absorption by in Situ Ligated Intestinal Loops of Broilers. *Biological Trace Element Research, 163*(1-2), 154-161. doi:10.1007/s12011-014-0179-1

Kanner, J. (1994). Oxidative processes in meat and meat products: Quality implications. *Meat Science, 36*(1-2), 169-189. doi:10.1016/0309-1740(94)90040-x

Keen, C.L., Enunsa, J.L. And Clegg, M.S. (2000) Manganese metabolism in animals and humans including the toxicity of manganese. *Metal Ions in Biological Systems, 37*, 89-121.

Kim, G.B., Seo, Y.M., Shin, K.S., Rhee, A.R., Han, J. & Paik, I.K. (2011). Effects of supplemental copper-methionine chelate and copper-soy proteinate on the performance, blood parameters, liver mineral content, and intestinal microflora of broiler chickens. *The Journal of Applied Poultry Research, 20,* 21-32.

Kim, I. S., Jin, S. K., Yang, M. R., Chu, G. M., Park, J. H., Rashid, R. H., . . . Kang, S. N. (2013). Efficacy of Tomato Powder as Antioxidant in Cooked Pork Patties. *Asian-Australasian Journal of Animal Sciences*, *26*(9), 1339-1346. doi:10.5713/ajas.2013.13079

Kuttappan, V. A., Brewer, V. B., Mauromoustakos, A., Mckee, S. R., Emmert, J. L., Meullenet, J. F., & Owens, C. M. (2013a). Estimation of factors associated with the occurrence of white striping in broiler breast fillets. *Poultry Science, 92*(3), 811-819. doi:10.3382/ps.2012-02506

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W., & Owens, C. M. (2012c). Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science, 91*(10), 2677-2685. doi:10.3382/ps.2012-02259

Kuttappan, V. A., Goodgame, S. D., Bradley, C. D., Mauromoustakos, A., Hargis, B. M., Waldroup, P. W., & Owens, C. M. (2012a). Effect of different levels of dietary vitamin E (DL-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. *Poultry Science, 91*(12), 3230-3235. doi:10.3382/ps.2012-02397

Kuttappan, V. A., Hargis, B. M., & Owens, C. M. (2016). White striping and woody breast myopathies in the modern poultry industry: A review. *Poultry Science, 95* (11), 2724-2733. doi:10.3382/ps/pew216

Kuttappan, V. A., Lee, Y. S., Erf, G. F., Meullenet, J. F., Mckee, S. R., & Owens, C. M. (2012b). Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. *Poultry Science, 91*(5), 1240-1247. doi:10.3382/ps.2011-01947

Kuttappan, V. A., Shivaprasad, H. L., Shaw, D. P., Valentine, B. A., Hargis, B. M., Clark, F. D., . . . Owens, C. M. (2013b). Pathological changes associated with white striping in broiler breast muscles. *Poultry Science*, *92*(2), 331-338. doi:10.3382/ps.2012-02646

Lametsch, M. L. (2007). Protein oxidation in meat during chill storage. Copenhagen: Center for Skov, Landskab og Planlægning/Københavns Universitet.

Lan, Y., Verstegen, M., Tamminga, S., & Williams, B. (2005). The role of the commensal gut microbial community in broiler chickens. *Worlds Poultry Science Journal, 61*(01), 95-104. doi:10.1079/wps200445

Lara, L., & Rostagno, M. (2013). Impact of Heat Stress on Poultry Production. *Animals, 3*(2), 356-369. doi:10.3390/ani3020356

Lea, H.K., Kay, Z., & Burton, E.J. (2011). Performance and gut health of poultry in the post-antibiotic era when feeding a novel yeast cell wall technology. [Abstract]. *British Poultry Science*, *7*, 1-2.

Lee, Y., Owens, C., & Meullenet, J. F. (2008). The Meullenet-Owens Razor Shear (Mors) For Predicting Poultry Meat Tenderness: Its Applications And Optimization. *Journal of Texture Studies, 39*(6), 655-672. doi:10.1111/j.1745-4603.2008.00165.x

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, L., Lenz, A. C., Ahn, B. W., Shaltiel, S., & Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology, 186*, 464–478.

Li, S., Lu, L., Hao, S., Wang, Y., Zhang, L., Liu, S., . . . Luo, X. (2011). Dietary Manganese Modulates Expression of the Manganese-Containing Superoxide Dismutase Gene in Chickens. *Journal of Nutrition, 141*(2), 189-194. doi:10.3945/jn.110.126680

Li, W., Zhao, G., Chen, J., Zheng, M., & Wen, J. (2009). Influence of dietary vitamin E supplementation on meat quality traits and gene expression related to lipid metabolism in the Beijing-you chicken. *British Poultry Science, 50*(2), 188-198. doi:10.1080/00071660902755409

Lin, H., Jiao, H., Buyse, J., & Decuypere, E. (2006). Strategies for preventing heat stress in poultry. *Worlds Poultry Science Journal, 62*(01), 71-86. doi:10.1079/wps200585

Liu, G. and Xiong, Y. L. (1996). Contribution of lipid and protein oxidation to rheological differences between chicken white and red muscle myofibrillar proteins. *Journal of Agriculture and Food Chemistry, 44*, 779–784.

Liu, Z., Xiong, Y. L., & Chen, J. (2010). Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. *Journal of Agricultural and Food Chemistry, 58*(19), 10697-10704. doi:10.1021/jf102043k

Lomiwes, D., Farouk, M., Wiklund, E., & Young, O. (2014). Small heat shock proteins and their role in meat tenderness: A review. *Meat Science, 96*(1), 26-40. doi:10.1016/j.meatsci.2013.06.008

Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research, 55*(1), 83-95. doi:10.1002/mnfr.201000453

Ma, W., Niu, H., Feng, J., Wang, Y., & Feng, J. (2011). Effects of Zinc Glycine Chelate on Oxidative Stress, Contents of Trace Elements, and Intestinal Morphology in Broilers. *Biological Trace Element Research, 142*(3), 546-556. doi:10.1007/s12011-010-8824-9

Maenz, D. D., Engele-Schaan, C. M., Newkirk, R. W., & Classen, H. L. (1999). The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. *Animal Feed Science and Technology, 81*(3-4), 177-192. doi:10.1016/s0377-8401(99)00085-1

Mager, W., & De Kruijff, A. (1995). Stress Induced Transcriptional Activation. *Microbiological Reviews, 59*(3), 506-531. doi:10.7717/peerj.59/fig-1

Maiorano, G., Stadnicka, K., Tavaniello, S., Abiuso, C., Bogucka, J., & Bednarczyk, M. (2017). In ovo validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. *Poultry Science, 96*(2), 511-518. doi:10.3382/ps/pew311

Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., & Renerre, M. (1997). Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry, 45*(7), 2481-2487. doi:10.1021/jf960977g

Mathis, G. F., Lumpkins, B., Pierce, J. L., & Hooge, D. M. (2012). Effects of Dietary Antibiotics, Actigen® Yeast Cell Wall Derivative, or Both on Broiler Chicken Live Performance in a Fifty-Two Day Pen Trial on Built-up Litter. *The Journal of Poultry Science, 49*(4), 313-318. doi:10.2141/jpsa.0110175

McKee, S., & Sams, A. (1997). The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. *Poultry Science, 76*(11), 1616-1620. doi:10.1093/ps/76.11.1616

Mercier, Y., Gatellier, P., Renerre, M. (1995). Relationships between lipid and protein oxidation in different beef muscles. In *Proceedings E10* (p. vol. 2 ; 562-563). Presented at 41. ICoMST, San Antonio, 1995

Milan, D. (2000). A Mutation in PRKAG3 Associated with Excess Glycogen Content in Pig Skeletal Muscle. *Science, 288*(5469), 1248-1251. doi:10.1126/science.288.5469.1248

Miles, R.D. & Henry, P.R. (1999). Relative trace mineral bioavailability. *Proceeding California Animal Nutrition Conference*, Fresno, CA, pp. 1-24.

Min, B., Nam, K., Cordray, J., & Ahn, D. (2008). Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *Journal of Food Science, 73*(6), 439-446. doi:10.1111/j.1750-3841.2008.00805.x

Mooney, J. W., Hirschler, E. M., Kennedy, A. K., Sams, A. R., & Elswyk, M. E. (1998). Lipid and flavour quality of stored breast meat from broilers fed marine algae. *Journal of the Science of Food and Agriculture, 78*(1), 134-140. doi:10.1002/(sici)1097-0010(199809)78:1<134::aid-jsfa96>3.3.co;2-s

Moraes, P., Novelini, L., Lemes, J. S., Santos, M. A., Pereira, C. M., & Xavier, E. G. (2016). Carcass yield, sensory analysis and meat quality of broilers fed canola meal. *Acta Scientiarum. Animal Sciences, 38*(3), 267. doi:10.4025/actascianimsci.v38i3.31247

Morrissey, P., Sheehy, P., Galvin, K., Kerry, J., & Buckley, D. (1998). Lipid stability in meat and meat products. *Meat Science, 49*. doi:10.1016/s0309-1740(98)90039-0

Mujahid, A., Yoshiki, Y., Akiba, Y., & Toyomizu, M. (2005). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poultry Science, 84*(2), 307-314. doi:10.1093/ps/84.2.307

Mutryn, M. F., Brannick, E. M., Fu, W., Lee, W. R., & Abasht, B. (2015). Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. *BMC Genomics, 16*(1). doi:10.1186/s12864-015-1623-0

National Chicken Council. (2016a). Broiler Chicken Industry Key Facts 2016. Retrieved September 15, 2016, from http://www.nationalchickencouncil.org/about-the-industry/statistics/broiler-chicken-industry-key-facts/

National Chicken Council. (2016b) Per Capita Consumption of Poultry and Livestock, 1965 to Estimated 2016, in Pounds. Retrieved September 15, 2016, from http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/

National Research Council (1994) *Nutrient Requirements of Poultry. 9th rev. ed*. National Academy Press, Washington, DC.

Nolin, S. J., C. M. Ashwell, & F. W. Edens. (2015). Comparative transcriptome analyses of ileal samples from broilers exposed to acute heat stress and supplemented with dietary Actigen®. [Abstract]. *Poultry Science, 94* (E-Supplement1), 17.

Nollet, L., Klis, J. D., Lensing, M., & Spring, P. (2007). The Effect of Replacing Inorganic With Organic Trace Minerals in Broiler Diets on Productive Performance and Mineral Excretion. *The Journal of Applied Poultry Research*, *16*(4), 592-597. doi:10.3382/japr.2006-00115

Nute, G., Richardson, R., Wood, J., Hughes, S., Wilkinson, R., Cooper, S., & Sinclair, L. (2007). Effect of dietary oil source on the flavour and the colour and lipid stability of lamb meat. *Meat Science,77*(4), 547-555. doi:10.1016/j.meatsci.2007.05.003

Owens, C. M. (2014). Identifying quality defects in poultry processing. *Watt Poult USA*, 42–50.

Owens, C. M., Mckee, S. R., Matthews, N. S., & Sams, A. R. (2000). The development of pale, exudative meat in two genetic lines of turkeys subjected to heat stress and its prediction by halothane screening. *Poultry Science, 79*(3), 430-435. doi:10.1093/ps/79.3.430

Pearce, S. C., Mani, V., Boddicker, R. L., Johnson, J. S., Weber, T. E., Ross, J. W., . . . Gabler, N. K. (2013). Heat Stress Reduces Intestinal Barrier Integrity and Favors Intestinal Glucose Transport in Growing Pigs. *PLoS ONE ,8*(8). doi:10.1371/journal.pone.0070215

Peric, L., Milosevic, N. And Zikic, D. (2007) Effect of Bioplex and Sel-Plex substituting inorganic trace mineral sources on performance of broilers. *Archiv für Geflügelkunde, 71*(3), 122-129.

Peric, L., Milosevic, N., Zikic, D., Kanacki, Z., Dzinic, N., Nollet, L., & Spring, P. (2009). Effect of selenium sources on performance and meat characteristics of broiler chickens. *The Journal of Applied Poultry Research, 18*(3), 403-409. doi:10.3382/japr.2008-00017

Petracci, M., & Cavani, C. (2011). Muscle Growth and Poultry Meat Quality Issues. *Nutrients, 4*(12), 1-12. doi:10.3390/nu4010001

Petracci, M., Betti, M., Bianchi, M., & Cavani, C. (2004). Color variation and characterization of broiler breast meat during processing in Italy. *Poultry Science, 83*(12), 2086-2092. doi:10.1093/ps/83.12.2086

Petracci, M., Bianchi, M., Mudalal, S., & Cavani, C. (2013a). Functional ingredients for poultry meat products. *Trends in Food Science & Technology, 33*(1), 27-39. doi:10.1016/j.tifs.2013.06.004

Petracci, M., Mudalal, S., Bonfiglio, A., & Cavani, C. (2013b). Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science, 92*(6), 1670-1675. doi:10.3382/ps.2012-03001

Petracci, M., Mudalal, S., Soglia, F., & Cavani, C. (2015). 'Meat quality in fast-growing broiler chickens', *World's Poultry Science Journal*, *71*(2), 363–374. doi: 10.1017/S0043933915000367.

Petrovič, V., Marcinčák, S., Popelka, P., Nollet, L., & Kováč, G. (2009). Effect of dietary supplementation of trace elements on the lipid peroxidation in broiler meat assessed after a refrigerated and frozen storage. *Journal of Animal and Feed Sciences, 18*(3), 499-507. doi:10.22358/jafs/66424/2009

Pierce, J.L., Shafer, B.L., Power, R., & Dawson, K.A. (2005). Nutritional means to lower trace mineral excretion from poultry without compromising performance. *Poultry Science, 84* (Suppl. 1), 11.

The Poultry Federation. (2012). *Trace Minerals in Animal Nutrition*. Lecture presented at 2012 Arkansas Nutrition Conference, Springdale, Arkansas.

Qiao, Y., Sun, J., Ding, Y., Le, G., & Shi, Y. (2012). Alterations of the gut microbiota in high-fat diet mice is strongly linked to oxidative stress. *Applied Microbiology and Biotechnology, 97*(4), 1689-1697. doi:10.1007/s00253-012-4323-6

Quinteiro-Filho, W. M., Rodrigues, M. V., Ribeiro, A., Ferraz-De-Paula, V., Pinheiro, M. L., Sa, L. R., . . . Palermo-Neto, J. (2012). Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute hypothalamic-pituitary-adrenal axis activation. *Journal of Animal Science, 90*(6), 1986-1994. doi:10.2527/jas.2011-3949

Rajput, N., Naeem, M., Ali, S., Zhang, J. F., Zhang, L., & Wang, T. (2013). The effect of dietary supplementation with the natural carotenoids curcumin and lutein on broiler pigmentation and immunity. *Poultry Science, 92*(5), 1177-1185. doi:10.3382/ps.2012-02853

Rao, S. V., Prakash, B., Raju, M. V., Panda, A. K., Kumari, R. K., & Reddy, E. P. (2016). Effect of Supplementing Organic Forms of Zinc, Selenium and Chromium on Performance, Anti-Oxidant and Immune Responses in Broiler Chicken Reared in Tropical Summer. *Biological Trace Element Research, 172*(2), 511-520. doi:10.1007/s12011-015-0587-x

Refsgaard, H. H., Tsai, L., & Stadtman, E. R. (2000). Modifications of proteins by polyunsaturated fatty acid peroxidation products. *Proceedings of the National Academy of Sciences, 97*(2), 611-616. doi:10.1073/pnas.97.2.611

Ribeiro, T., Lordelo, M., Alves, S., Bessa, R., Costa, P., Lemos, J., . . . Prates, J. (2013). Direct supplementation of diet is the most efficient way of enriching broiler meat with n-3 long-chain polyunsaturated fatty acids. *British Poultry Science, 54*(6), 753-765. doi:10.1080/00071668.2013.841861

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science, 179*, 588–590.

Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. *Journal of Animal Science, 82*(11), 3254. doi:10.2527/2004.82113254x

Rymer, C., Gibbs, R. A., & Givens, D. I. (2010). Comparison of algal and fish sources on the oxidative stability of poultry meat and its enrichment with omega-3 polyunsaturated fatty acids. *Poultry Science, 89*(1), 150-159. doi:10.3382/ps.2009-00232

Saenmahayak, B., Bilgili, S. F., Hess, J. B., & Singh, M. (2010). Live and processing performance of broiler chickens fed diets supplemented with complexed zinc. *The Journal of Applied Poultry Research, 19*(4), 334-340. doi:10.3382/japr.2010-00166

Saenmahayak, B., Bilgili, S., & Hess, J. (2007). Influence of complexed trace mineral supplementation on carcass grade and meat quality of broilers processed at 42 an 56 days of age. *Poultry Science, 86*, 278.

Saenmahayak, B., Singh, M., Bilgili, S., & Hess, J. (2012). Influence of dietary supplementation with complexed zinc on meat quality and shelf life of broilers. *International Journal of Poultry Science, 11*(1), 28-32. doi:10.3923/ijps.2012.28.32

Sandercock, D. A., Barker, Z. E., Mitchell, M. A., & Hocking, P. M. (2009). Changes in muscle cell cation regulation and meat quality traits are associated with genetic selection for high body weight and meat yield in broiler chickens. *Genetics Selection Evolution, 41*(1), 8. doi:10.1186/1297-9686-41-8

Sandercock, D., Hunter, R., Mitchell, M., & Hocking, P. (2006). Thermoregulatory capacity and muscle membrane integrity are compromised in broilers compared with layers at the same age or body weight. *British Poultry Science, 47*(3), 322-329. doi:10.1080/00071660600732346

Schiavone, A., Chiarini, R., Marzoni, M., Castillo, A., Tassone, S., & Romboli, I. (2007). Breast meat traits of Muscovy ducks fed on a microalga (Crypthecodinium cohnii) meal supplemented diet. *British Poultry Science, 48*(5), 573-579. doi:10.1080/00071660701615796

Schlegel, P., Sauvant, D., & Jondreville, C. (2012). Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. *Animal, 7*(01), 47-59. doi:10.1017/s1751731112001000

Shacter, E. (2000). Quantification and significance of protein oxidation in biological samples. *Drug Metab. Rev. 32*, 307–326

Shay, K. P., Moreau, R. F., Smith, E. J., & Hagen, T. M. (2008). Is α-lipoic acid a scavenger of reactive oxygen speciesin vivo? Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity. *IUBMB Life, 60*(6), 362-367. doi:10.1002/iub.40

Shin, D., Choi, S. H., Go, G., Park, J. H., Narciso-Gaytan, C., Morgan, C. A., . . . Ruiz-Feria, C. A. (2012). Effects of dietary combination of n-3 and n-9 fatty acids on the deposition of linoleic and arachidonic acid in broiler chicken meats. *Poultry Science, 91*(4), 1009-1017. doi:10.3382/ps.2011-01836

Sihvo, H. K., Immonen, K., & Puolanne, E. (2014). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Veterinary Pathology, 51*(3), 619-623. doi:10.1177/0300985813497488

Skřivan, Skřivanová, M., Dlouhá, V., Dlouhá, G., Brányiková, I., Zachleder, V., & Vítová, M. (2010). The use of selenium-enriched alga Scenedesmus quadricauda in a chicken diet. *Czech Journal of Animal Science,55*(12), 565-571.

Smet, K., Raes, K., Huyghebaert, G., Haak, L., Arnouts, S., & Smet, S. D. (2008). Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry Science, 87*(8), 1682-1688. doi:10.3382/ps.2007-00384

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., . . . Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry Science, 91*(9), 2235-2240. doi:10.3382/ps.2012-02182

Soladoye, O., Juárez, M., Aalhus, J., Shand, P., & Estévez, M. (2015). Protein Oxidation in Processed Meat: Mechanisms and Potential Implications on Human Health. *Comprehensive Reviews in Food Science and Food Safety, 14*(2), 106-122. doi:10.1111/1541-4337.12127

Soleimani, A. F., Zulkifli, I., Omar, A. R., & Raha, A. R. (2011). Physiological responses of 3 chicken breeds to acute heat stress. *Poultry Science, 90*(7), 1435-1440. doi:10.3382/ps.2011-01381

St-Pierre, N. (2013). Current and Future Economic Impact of Heat Stress in the U.S. Livestock and Poultry Sectors. Retrieved February 03, 2017, from http://articles.extension.org/pages/67799/current-and-future-economic-impact-of-heat-stress-in-the-us-livestock-and-poultry-sectors

St-Pierre, N., Cobanov, B., & Schnitkey, G. (2003). Economic Losses from Heat Stress by US Livestock Industries. *Journal of Dairy Science, 86*. doi:10.3168/jds.s0022-0302(03)74040-5

Suman, S. P., & Joseph, P. (2014). Color and Pigment. In *Encyclopedia of Meat Sciences* (2nd ed., pp. 244-251). Amsterdam, Netherlands: Elsevier.

Surai, P. F. (2000). Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. *British Poultry Science, 41*(2), 235-243. doi:10.1080/713654909

Suttle, N. F. (2010). *Mineral nutrition of livestock* (4th ed.). Wallingford, Oxfordshire, UK: CABI.

Switala, J., & Loewen, P. C. (2002). Diversity of properties among catalases. *Archives of Biochemistry and Biophysics, 401*(2), 145-154. doi:10.1016/s0003-9861(02)00049-8

Tijare, V. V., Yang, F. L., Kuttappan, V. A., Alvarado, C. Z., Coon, C. N., & Owens, C. M. (2016). Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. *Poultry Science, 95*(9), 2167-2173. doi:10.3382/ps/pew129

Toyomizu, M., Sato, K., Taroda, H., Kato, T., & Akiba, Y. (2001). Effects of dietary Spirulina on meat colour in muscle of broiler chickens. *British Poultry Science, 42*(2), 197-202. doi:10.1080/00071660120048447

Urrutia, O., Mendizabal, J. A., Insausti, K., Soret, B., Purroy, A., & Arana, A. (2016). Effects of Addition of Linseed and Marine Algae to the Diet on Adipose Tissue Development, Fatty Acid Profile, Lipogenic Gene Expression, and Meat Quality in Lambs. *Plos One, 11*(6). doi:10.1371/journal.pone.0156765

USDA ERS. (2017). Meat Price Spreads. Retrieved May 19, 2017, from https://www.ers.usda.gov/data-products/meat-price-spreads/

Van Elswyk, M. E. (1993). Designer Foods. *Nutrition Today, 28*(2), 21-28. doi:10.1097/00017285-199303000-00004

Venkataraman, L. V., Somasekaran, T., & Becker, E. W. (1994). Replacement value of blue-green alga (spirulina platensis)for fishmeal and a vitamin-mineral premix for broiler chicks. *British Poultry Science, 35*(3), 373-381. doi:10.1080/00071669408417702

Ventanas, S., Estevez, M., Tejeda, J. F., & Ruiz, J. (2006). Protein and lipid oxidation in Longissimus dorsi and dry cured loin from Iberian pigs as affected by crossbreeding and diet. *Meat Science, 72*(4), 647-655. doi:10.1016/j.meatsci.2005.09.011

Viana, F. M., A. C. V. C. S. Canto, Costa-Lima, B. R., Salim, A. P., & Conte-Junior, C. A. (2017). Color stability and lipid oxidation of broiler breast meat from animals raised on organic versus non-organic production systems. *Poultry Science, 96*(3), 747-753. doi:10.3382/ps/pew331

Vossen, E., Claeys, E., Raes, K., Mullem, D. V., & Smet, S. D. (2016a). Supra-nutritional levels of α-tocopherol maintain the oxidative stability ofn-3 long-chain fatty acid enriched subcutaneous fat and frozen loin, but not of dry fermented sausage. *Journal of the Science of Food and Agriculture, 96*(13), 4523-4530. doi:10.1002/jsfa.7668

Vossen, E., Raes, K., Mullem, D. V., & Smet, S. D. (2016b). Production of docosahexaenoic acid (DHA) enriched loin and dry cured ham from pigs fed algae: Nutritional and sensory quality. *European Journal of Lipid Science and Technology*. doi:10.1002/ejlt.201600144

Wang, S., Zhang, L., Li, J., Cong, J., Gao, F., & Zhou, G. (2017). Effects of dietary marigold extract supplementation on growth performance, pigmentation, antioxidant capacity and meat quality in broiler chickens. *Asian-Australasian Journal of Animal Sciences, 30*(1), 71-77. doi:10.5713/ajas.16.0075

Witte, V. C., Krause, G. F., & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science, 35*(5), 582-585. doi:10.1111/j.1365-2621.1970.tb04815.x

Woelfel, R. L., Owens, C. M., Hirschler, E. M., Martinez-Dawson, R., & Sams, A. R. (2002). The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poultry Science, 81*(4), 579-584. doi:10.1093/ps/81.4.579

Xiao, R., Power, R. F., Mallonee, D., Crowdus, C., Brennan, K. M., Ao, T., . . . Dawson, K. A. (2010). A comparative transcriptomic study of vitamin E and an algae-based antioxidant as antioxidative agents: Investigation of replacing vitamin E with the algae-based antioxidant in broiler diets. *Poultry Science,90*(1), 136-146. doi:10.3382/ps.2010-01018

Xiao, S., Zhang, W. G., Lee, E. J., Ma, C. W., & Ahn, D. U. (2011). Effects of diet, packaging, and irradiation on protein oxidation, lipid oxidation, and color of raw broiler thigh meat during refrigerated storage. *Poultry Science, 90*(6), 1348-1357. doi:10.3382/ps.2010-01244

Xiong, Y. L., (2000). Protein oxidation and implications for muscle food quality. In Decker, E. A., C. Faustman, & C. J. Lopez-Bote (Eds), *Antioxidants in muscle foods: nutritional strategies to improve quality* (pp. 85-111). New York: Wiley.

Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., & Takriff, M. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research-Thessaloniki, 21*(1), 6. doi:10.1186/2241-5793-21-6

Yan, L. And Kim, I.H. (2013). Effects of dietary ω-3 fatty acid-enriched microalgae supplementation on growth performance, blood profiles, meat quality, and fatty acid composition of meat in broilers. *Journal of Applied Animal Research, 41*, 392-397.

Yoo, J., Yi, Y. J., Koo, B., Jung, S., Yoon, J. U., Kang, H. B., . . . Heo, J. M. (2016). Growth performance, intestinal morphology, and meat quality in relation to alpha-lipoic acid associated with vitamin C and E in broiler chickens under tropical conditions. *Revista Brasileira de Zootecnia, 45*(3), 113-120. doi:10.1590/s1806-92902016000300005

Zahoor, I., Mitchell, M., Hall, S., Beard, P., Gous, R., Koning, D. D., & Hocking, P. (2016). Predicted optimum ambient temperatures for broiler chickens to dissipate metabolic heat do not affect performance or improve breast muscle quality. *British Poultry Science, 57*(1), 134-141. doi:10.1080/00071668.2015.1124067

Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition, 53*(11), 1191-1201. doi:10.1080/10408398.2011.577540

Zhang, Z. Y., Jia, G. Q., Zuo, J. J., Zhang, Y., Lei, J., Ren, L., & Feng, D. Y. (2012). Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poultry Science*, *91*(11), 2931-2937. doi:10.3382/ps.2012-02255