2015

IMPACT OF ALGAE SUPPLEMENTED DIETS COMBINED WITH ANTIOXIDANTS ON THE NUTRITIONAL PROFILE, QUALITY ATTRIBUTES, AND STORAGE STABILITY OF CHICKEN BREAST MEAT

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IMPACT OF ALGAE SUPPLEMENTED DIETS COMBINED WITH ANTIOXIDANTS ON THE NUTRITIONAL PROFILE, QUALITY ATTRIBUTES, AND STORAGE STABILITY OF CHICKEN BREAST MEAT

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and Environment at the University of Kentucky

By

Rebecca Grace Norcross

Lexington, Kentucky

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Lexington, Kentucky

2015

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ABSTRACT OF THESIS

IMPACT OF ALGAE SUPPLEMENTED DIETS COMBINED WITH ANTIOXIDANTS ON THE NUTRITION PROFILE, QUALITY ATTRIBUTES, AND STORAGE STABILITY OF CHICKEN WHITE MEAT

Consumers’ demands for ω-3 polyunsaturated fatty acids (PUFAs) are at all-time high. Algae, a common source of PUFAs, and antioxidants are both used as supplements in livestock feeds, are known to affect the overall quality of meat. To implement PUFA deposits into broiler meat, this study evaluated combining antioxidants and algae in broiler feed to enhance the breast meat quality. Broilers were fed diets supplemented with 50 IU Vitamin E or 200 g/ton EconomasE (EcoE, an antioxidant pack) plus 10 IU Vitamin E, with or without 0.5% algae extract (SP-1). The feed oil was partially oxidized soybean oil (POV: 86 mEq of O₂/kg). The feed supplementation with combined SP-1 and EcoE increased meat lipid oxidation but had no effect on protein. This combination supplement substantially reduced \( P < 0.05 \) meat exudation during refrigerated storage while no evident differences were seen on cooking loss or tenderness between diets. Meat from SP-1 supplemented diets was found less acceptable than meat from other diets due to detected off-flavors. The results indicate that EcoE at a supplementation level other than 200 g/ton may be required to overcome off-flavors of broiler meat due to feed incorporation of 0.5% SP-1 with oxidized oil.

KEYWORDS: Oxidative stability, supplement, meat quality, antioxidant

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IMPACT OF ALGAE SUPPLEMENTED DIETS COMBINED WITH ANTIOXIDANTS ON THE NUTRITION PROFILE, QUALITY ATTRIBUTES, AND STORAGE STABILITY OF CHICKEN WHITE MEAT

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DEDICATION

To my parents who have taught me the most valuable information: To God be the glory
I am extending my sincerest gratitude towards my advisor Dr. Youling Xiong for his dedicated effort, valuable time, and shared technical knowledge. Without his diligence and perseverance to help me enter and complete this program I would not be as professionally advanced. Thank you to my committee members, Dr. William Boatright and Dr. Greg Rentfrow for your time and instruction.

I am thankful for Alltech’s® financial support as well as Dr. Tuoying Ao and Dr. Karl Dawson’s support. Rebecca Delles was immensely helpful with my laboratory training, final data analyses and everything in-between. I appreciate her freely extended guidance and time. Thank you to Dani True for her boundless technical help as well as general lab advice– it was tremendously valuable. My lab mates, Xu Wang, Jiayi Yang, Yanyun Cao, and Yungang Cao were also of unsurpassable help.

Additionally, thank you to Kelsey Lamb as without her help and support the lab work would have seemed unmanageable. I am also grateful to Leeann Slaughter, Katelyn Hawkins, Jennifer McNeil, Mahesh Nair, Hayriye Cetin-Karace, RuthMarie Sarros, Jenny Holleman, Kristen Ray, and Megan Dudley for their shared technical skills, advice, and/or general support; you kept me mostly sane during this journey.

Finally, but very importantly, thank you Emily and Jessica Norcross for your endless love, support, and care – without it I would not be who or where I am today.
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Exudative Loss

Textural Properties

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CHAPTER 1
INTRODUCTION

The demand for animal derived food has been steadily increasing over the past several decades due to consumer preference (Bou, Condony, Tres, Decker, & Guardiola, 2009; Henchion, 2014). The market for meat is especially growing in countries that are rapidly developing such as India, China, the Middle East, Africa, and Latin America. (Daniel, Cross, Koebnick, & Sinha, 2010; Holman & Malau-Aduli, 2013; Trostle & Seeley 2013). For example, by 2020 it is predicted that developing countries will have increased their meat consumption from 107 million metric tons in 1996 to 177 million metric tons (Delgado, 2003). Additionally, the world population is forecasted to reach 9.1 billion people by 2050 (Food and Agriculture Organization, 2009). This numerical increase in consumers directly impacts the needed quantity of required meat. Therefore, establishing a reliable meat supply is of importance to meet the ever growing demand for high quality meat (Henchion, McCarthy, & Resconi, 2014).

Consumers highly value the quality of the meat product when selecting their purchase (Henchion et al., 2014; Rijswijk & Frewer, 2008; Troy & Kerry, 2010). In addition to high quality meat, consumers are demanding nutritious meat and meat products. As the deleterious effects of too many saturated fats in diets become more widely known for example the increased risk for heart disease, obesity, and the numerous negative health issues that can arise from those, consumers are developing an adamancy for healthier foods (Trienekens, Wognum, Beulens, & Van Der Vorst, 2012; Mozaffarian, Micha, & Wallace, 2010; Verbeke, Perez-Cueto, Barcellos, Krystallis, & Grunert, 2010).
Research is paralleling these consumers’ demands. As meat quality and its nutritive value are understandably of utmost importance and a growing concern among consumers, data is indicating that food quality is one of the central issues in food economics; it is no longer sufficient or accepted for suppliers to provide consumers with meat of inferior quality because the consumer drives the market for food (Grunert, 2005).

Before meat reaches demanding consumers at the market, it undergoes a specific route. The meat quality chain relationship begins at the farm, expands through processing, retail, the consumers’ perception of a high quality meat, and ends with consumers’ mandate of a high quality meat (Grunert, 2005; Grunert, 2006; Henchion et al., 2014). Therefore, to fulfill consumers’ rapidly growing demand for high quality meat, science and technology can and should continue to be utilized at the beginning stage at the farm which will increase the quality of the meat, and thereby mutually benefit both consumers and producers (Swaminathan & Bhavani, 2013).

To this end, research in the past few decades has explored the impacts of supplementing livestock feeds with antioxidants, minerals, and other additives to produce high quality and nutritional meat (Bou, Codonym, Tres, Decker, & Guardiola, 2009; Bou, Gaurdiola, Tres, Barroeta, & Codony, 2004; Bou et al., 2006; Decker, Faustman, Lopex-Bote, & Xiong, 2000). Quality aspects that have been studied include meat’s shelf life (which is closely tied to protein and lipid oxidative stability), color, tenderness, and water binding properties of the meat. It is widely known that proteins and lipids can be oxidized resulting in low quality, rancid meat (Kanner, 1994; Xiong, 2000). Altering meat’s nutritional factors, such as the degree of lipid saturation in fatty acid tissue deposits directly from supplementing feeds with various lipids, has also been and continues to be
researched (Barton, Marounek, Kudrna, Bures, & Zahradkova, 2007; Habeanu, Durand, Gobert, & Bauchard, 2008; McNiven, Duynisveld, Charmley, & Mitchelo, 2004). It is widely known that supplementing animal feeds can have significant quality and nutritional impacts on the end meat product (Bou et al., 2009). For example, using antioxidants as supplements in feeds can decrease lipid oxidation which increases the stability of the meat and thereby improves the meat’s shelf life (Arshad, Anjum, Khan, Shahid, & Sohaib, 2013). Therefore, this literature review will unveil the effects of oxidation on proteins and lipids in meat as well as other quality measurements which heavily impact the desirability of meat. Furthermore, feed supplements and their impacts on producing high quality, as well as nutritional meat, will also be discussed.

The purpose of this research was to work in collaboration with Alltech® to optimize a chicken feed for the production of high quality meat with the following attributes: good nutritional value, acceptable palatability (flavor, juiciness, and tenderness), and lengthened shelf stability. Utilizing previous research pertaining to antioxidant supplementation, the optimization of creating a feed mixture for producing high quality meat was continued to reach these objectives:

1. To further the understanding of the effects of antioxidant supplementation (Alltech® algae) and other antioxidants (vitamin E and EconomasE, etc.) on the quality of chicken meat.

2. To understand the molecular mechanism of how algae and EconomasE antioxidants interact on meat.

3. To determine if the storage stability of the meat is impacted by algae supplemented diets.

4. To conclude if the sensory properties of the meat are altered due to algae based supplementation (with or without other antioxidant supplements).
CHAPTER 2

LITERATURE REVIEW

Introduction

The demand for high quality meat is rapidly expanding. As a result, a significant amount of research is being conducted studying the effect of livestock feed supplements on the finished meat product. This review will discuss the matter of protein and lipid oxidation in meat, the methods of determining meats oxidation, the necessity and benefits of using livestock feed supplementation in aid of meeting the increasing demand for the production of high quality meat (tender, juicy, flavorful, desired color, long shelf life), as well as communicate the increasing need to improve the oxidative stability and nutrition of meat. The necessity of effectively utilizing livestock feed supplementations to produce nutritious, high quality meat results from consumers’ altered food choices; they are demanding high quality meat. Benefits of using various feed additives include increasing meat availability, improving the meat quality (oxidative stability, color water retention, and tenderness), and providing healthier meat.

Oxidation in Meat: A General Concept of Oxidation’s Impact on Meat Quality

Oxidation is a common chemical reaction that involves the loss of an electron or hydrogen atom, or the addition of an oxygen atom to a compound, widely considered as one of the main causes for lowered meat quality during processing and storage (Estevez, 2011; Shahidi & Zhong, 2010; Xiong, 2000). The consequences of oxidation in meat are the production of off-flavors, rancidity, discoloration, loss of nutrition, textural changes such as hardening or softening of muscle tissue (known commonly as tenderness), and
the production of toxins. Some of the well-known factors which influence lipid and protein oxidation include the degree of lipid unsaturation, exposure to light and oxygen, the presence/absence of antioxidants, metal ions such as iron and copper, heme protein, cell wall disruption, temperature, pH, moisture, and salt (Shahidi & Zhong, 2010; Xiong, 2000). Additionally, it is known that postmortem, animal muscle tissue and fat continues to undergo oxidative reactions (Smet, Raes, Huyghebaert, Haak, Arnouts, & De Smet, 2008). Protein and lipid oxidation impacts aspects of the meat which consumers highly value such as color, texture, and shelf life (Grunert, 2006; Henchion et al., 2014; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998; Smet et al., 2008). Furthermore, lipid oxidation can cause hazardous biological effects which have been linked to certain diseases such as neurodegenerative and cardiovascular diseases and cancer (Bou et al., 2009). Therefore, there is significant value in researching methods to produce and maintain the highest quality of meat possible for human safety, consumer satisfaction, and for optimization of producers’ economic gain.

**Protein Oxidation**

Oxidation occurs naturally in biological muscle tissue due to natural metabolic pathways (Kohen & Nyska, 2002). However, byproducts of metabolic oxidation such as reactive oxygen species (ROS) (hydroxyl free radicals and superoxide) can cause damage to both the lipids and proteins in the tissue (Smet et al., 2008). As proteins are inherently complex macromolecules, they are susceptible toward oxidation through a number of different avenues. Proteins are composed of 20 amino acids which are characterized by their varying side chains. Amino acids are susceptible for oxidation on their side chains...
while proteins are susceptible on their peptide backbone as well as on amino acid side chains. The diversity of the side chains further increases the complexity of the oxidation reactions on proteins and amino acids as well as hinders our complete understanding of the exact oxidation and cross-linking mechanisms (Davies, 2005).

It is known that some amino acid side chains are more susceptible to oxidation than others (Davies, 2005; Traore et al., 2012; Xiong, 2000). Amino acids with side chains composed of more reactive groups such as sulfhydryl, thioether, amino groups and imidazole or indole rings, have a higher risk of being oxidized (Xiong, 2000; Zhang, Xiao, Lee, & Ahn, 2011). For example, as seen in Table 1, the aromatic amino acids (phenylalanine, tryptophan, and tyrosine) have a rate constant (at pH 7) with a factor of $10^{10}$ when reacted with a hydroxyl radical (HO•) indicating that the oxidation reaction occurs relatively quickly when compared to the same reaction with other amino acids. Residues with side chains containing a sulfur atom (cysteine and methionine) have a rate constant of $10^{10}$ and $10^{9}$ respectively under the same conditions. Histidine’s structure with an imidazole structure possesses a rate constant factor of $10^{10}$ while proline has an indole ring with a rate constant of $10^{8}$ under the previously stated reaction conditions (Buxton, Greenstocke, Helmand, & Ross; 1988).

Some of the known protein oxidation reactions include the formation of disulfide bonds between cysteine residues, insoluble protein aggregates forming from cross-linking of lysine residues, or lysine resides with an available aldehyde groups, hydrophobic interactions as the protein unfolds, the addition of oxygen atoms to methionine residues, and a fractured protein backbone structure, (Esteves, 2015; Traore et al., 2012; Xiong,
2000). Although many of these oxidations can occur from components that are not free radicals, a protein’s backbone is rarely fragmented by non-free radical oxidants (Davies, 2005).

**Table 1.** Rate constants for amino acid reactions with HO• at pH 7.0

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rate constant (dm$^3$ mol$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>$7.7 \times 10^7$</td>
</tr>
<tr>
<td>Arginine</td>
<td>$3.5 \times 10^9$</td>
</tr>
<tr>
<td>Asparagine</td>
<td>$4.9 \times 10^7$</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>$7.5 \times 10^7$</td>
</tr>
<tr>
<td>Cysteine</td>
<td>$3.4 \times 10^{10}$</td>
</tr>
<tr>
<td>Cystine</td>
<td>$2.1 \times 10^9$</td>
</tr>
<tr>
<td>Glutamine</td>
<td>$5.4 \times 10^8$</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>$2.3 \times 10^8$</td>
</tr>
<tr>
<td>Glycine</td>
<td>$1.7 \times 10^7$</td>
</tr>
<tr>
<td>Histidine</td>
<td>$1.3 \times 10^{10}$</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>$1.8 \times 10^9$</td>
</tr>
<tr>
<td>Leucine</td>
<td>$1.7 \times 10^9$</td>
</tr>
<tr>
<td>Lysine</td>
<td>$3.4 \times 10^8$</td>
</tr>
<tr>
<td>Methionine</td>
<td>$8.3 \times 10^9$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>$6.5 \times 10^9$</td>
</tr>
<tr>
<td>Proline</td>
<td>$4.8 \times 10^8$</td>
</tr>
<tr>
<td>Serine</td>
<td>$3.2 \times 10^8$</td>
</tr>
<tr>
<td>Threonine</td>
<td>$5.1 \times 10^8$</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>$1.3 \times 10^{10}$</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>$1.3 \times 10^{10}$</td>
</tr>
<tr>
<td>Valine</td>
<td>$7.6 \times 10^8$</td>
</tr>
<tr>
<td>Cyclo(Gly)$_2$ (backbone attack)</td>
<td>$7.8 \times 10^8$</td>
</tr>
<tr>
<td>$N$-Ac-Gly–Gly (backbone attack)</td>
<td>$7.8 \times 10^8$</td>
</tr>
</tbody>
</table>

Adapted from: Buxton, Greenstocke, Helman, & Ross (1988).
Step one in Figure 1 shows the alpha carbon’s hydrogen being removed leaving a reactive free radical carbon. In the presence of oxygen, a peroxo group bonds to an alpha carbon (step 2). It is usually necessary for the stable oxygen to be present for backbone fragmentation (Davies, 2005). The exact mechanism of the protein backbone fragmentation is not fully clear. Two possibilities are presented in steps 3 and 4 both of which cause the same end result: a division of the protein’s carbon nitrogen backbone. Further discussion of protein oxidation mechanisms can be found in Davies (1996), Davies (2005), Estevez (2011), Garrison (1987), and Xiong (2000).
The extent of oxidation that can occur on a sample is dependent upon the following: 1) Where on the sample the oxidation occurs; 2) Any subsequent reactions produced or catalyzed by the primary oxidation; 3) Any repair mechanisms in the system such as endogenous antioxidant enzymes. The rate of the oxidation is dependent upon the concentration of susceptible targets, and the oxidation reactions rate constant for reacting with the sample (Davies, 2005). With these determined considerations, it is easier to understand how susceptible certain targets i.e.: muscle tissues are towards being oxidized. As muscle tissue is full of compounds such as lipids, heme pigments, transitional metal ions, and oxidative enzymes which are all precursors or catalysts for the production of reactive oxygen species, muscle tissue is very susceptible to oxidation. Reactive oxygen species are often free radicals including $\cdot$OH, $O_2\cdot^*$, RS•, and ROO• (where R is any organic structure, S is sulfur, and O is oxygen). However, some oxidative catalysts are not free radicals such as $H_2O_2$, ROOH, and carbonyls. Therefore, oxidation may occur due to free radical compounds or other prooxidative compounds (Xiong, 2000). Protein oxidation usually occurs through a mechanism composed of sequential series of free radical reactions. For example, one protein oxidation mechanism initiated by an oxidative lipid could proceed as shown in Figure 2.

In addition to lipid free radical initiated mechanism, proteins can be oxidized by any number of reactive oxygen species. For example, as shown in the reaction steps listed in Figure 3, a protein radical ($P\cdot$) can be created from a loss of a hydrogen atom (1). The protein radical can then be reduced with oxygen to become a peroxyl radical (POO•) (2) which can then be converted to an alkyl peroxided (POOH) (3) through an oxidation of
another molecule. Through either a reaction with a reactive oxygen species (4) or through a reaction with reduced transition metals (M$^{n+}$) (5) an alcoxy radical (PO•) is produced. The PO• can react with water (6) or another transitional metal (7) to produce a protein hydroxyl produce (Estevez, 2011).

<table>
<thead>
<tr>
<th>Initiation</th>
<th>L → L•</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propagation</td>
<td>L• + O$_2$ → LOO•</td>
</tr>
<tr>
<td>Hydrogen Abstraction</td>
<td>LOO• + P → LOOH + P• (-H)</td>
</tr>
<tr>
<td>Addition</td>
<td>LOO• + P → •LOOP</td>
</tr>
<tr>
<td>Complex</td>
<td>•LOOP + P + O$_2$ → •POOLOOP</td>
</tr>
<tr>
<td>Polymerization</td>
<td>P-P• + P• + P → P-P-P• + P-P-P</td>
</tr>
</tbody>
</table>

**Figure 2.** A protein oxidation mechanism initiated by an oxidized lipid. L is lipid and P is protein. Adapted from: Xiong (2000).

1) PH + HO• → P• + H$_2$O  
2) P• + O$_2$ → POO•  
3) POO• + PH → POOH + P•  
4) POOH + HO$_2$• → PO• + O$_2$ + H$_2$O  
5) POOH + M$^{n+}$ → PH• + HO$^-$ + M$^{(n+1)}$  
6) PO• + HO$_2$• → POH + O$_2$  
7) PO + H$^+$ + M$^{n+}$ → POH + M$^{(n+1)+}$

**Figure 3.** A protein oxidation mechanism initiated by a reactive oxygen species. P is protein, L is lipid, and M is a transitional metal. Adapted from: Estevez (2011).

Protein oxidation in muscle foods targets amino acid side groups which directly impact the proteins’ secondary and tertiary protein structures. As a protein’s structure dictates its functions and characteristics, as it is modified by oxidation, its function is
thereby altered. As a result, oxidation directly affects the quality of meat. This will be discussed later in further detail.

**Lipid Oxidation**

Lipid oxidation is widely known to have negative effects directly on animals’ growth performance, the meats’ quality, and eventually also on humans’ health once the lipids in the meat are consumed (Tavarez et al., 2011). Lipid oxidation causes off-flavors, loss of nutrients, and can form toxic products (Avila-Ramos et al., 2013; Estevez, 2011; Gobert et al., 2014; Pignoli, Bou, Rodriguez-Estrada, & Decker, 2009; Smet et al., 2008; Zhang et al., 2011). It has been reported that malondialdehyde (MDA) a primary byproduct of lipid oxidation is carcinogenic and mutagenic (Botsoglou, Fletouris, Papageorgiou, Vassilopoulos, Mantis, & Trakatellis, 1994). Therefore, heavily lipid oxidized meats are not suitable for consumption. As a result of their negative health contributions, the topic of lipid oxidation has been significantly researched in the past decades. The extent of oxidation that may occur on lipids is dependent upon the degree of lipid saturation, environmental conditions, and the use/type of antioxidants, if any, in the feed or in the meat (Estevez, 2011; Estevez, 2015; Li, Zhao, Chen, Zheng, & When, 2009; Shahidi & Zhong, 2010; Smet et al., 2008). Similar to the previously listed protein oxidation mechanism, lipid oxidation has three steps: initiation, propagation, and termination. These three steps, which are initiated from a reactive oxygen species, are a complex series of chemical reactions which form the mechanism of lipid oxidation. Figure 4 illustrates these three steps of lipid oxidation (Shahidi & Zhong, 2010).
Figure 4. A lipid oxidation mechanism. Adapted from: Shahidi & Zhong, 2010

Figure 4, unsaturated fatty acids can donate a hydrogen atom to another molecule producing a free radical on the lipid. This is often accelerated due to heat, light, or the presence of metal ions. The newly formed lipid radical reacts with oxygen producing peroxyl radicals (ROO•). The peroxyl radicals continue the reaction chain in the propagation step by attacking other non-radical lipids to create more free radicals. This may continue hundreds of times until the chain mechanism is ended by a radical quenching compound. Therefore, because the propagation step is self-reliant, lipid oxidation can occur easily and exponentially given an appropriate environment (Shahidi & Zhong, 2010)
Table 2. The energy required for H disassociation

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\Delta E$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}$</td>
<td>103</td>
</tr>
<tr>
<td>$\text{H}_2$</td>
<td>100</td>
</tr>
<tr>
<td>$\text{H}_3$</td>
<td>85</td>
</tr>
<tr>
<td>$\text{H}_4$</td>
<td>77</td>
</tr>
<tr>
<td>$\text{H}_5$</td>
<td>65</td>
</tr>
<tr>
<td>H--OO--R</td>
<td>90</td>
</tr>
</tbody>
</table>

Adapted from: Shahidi & Zhong, 2010

Table 2 shows the energy required to dissociate the marked hydrogen (Shahidi & Zhong, 2010). Based on Table 2, it is understood that because polyunsaturated fatty acids (PUFAs) require less energy to be removed, hydrogen on compounds with double bonds (unsaturated compounds) are most easily oxidized. Therefore, the more unsaturated a compound, the more readily it will be oxidized (Delles, Xiong, True, Ao, & Dawson, 2015). As a result, polyunsaturated fatty acids are the most susceptible fatty acids to being oxidized followed by monounsaturated fatty acids.

The oxidative instability of polyunsaturated fatty acids produces a challenge in the food production industry because it is widely recognized that saturated fats are linked to heart disease although saturated fats are the least susceptible to oxidation (Addis, 1986). A trend is increasing among consumers desiring foods with healthier fats, (polyunsaturated) that promote good heart health (Malayoglu, Ozkan, Kocturk, Oktay, & Ergul, 2009). There have been numerous studies completed recommending that the
consumption of saturated fats be decreased to reduce the risk of developing coronary heart disease (Mozaffarian et al., 2010). Driving the demand for meat containing healthier fats corresponds to the average consumer’s increasing knowledge.

As the science behind human health expands, the general public becomes more educated about diets, fat consumption, and overall health awareness. Humans cannot synthesis polyunsaturated fatty acids with double bonds at the third and sixth carbon atoms, n-3 and n-6 fatty acids respectively. Examples of these fats are α-linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), more commonly known as simply omega-3 fatty acids.

As human bodies cannot synthesis LNA, EPA, and DHA, they are essential dietary nutrients which benefit brain and heart health (Academy of Nutrition and Dietetics, 2014). It is also recommended that while the consumption of saturated fats should decrease, the consumption of PUFA should increase to promote good heart and brain health. Western diets are known to be particularly low in PUFAs (Riberio et al., 2013; Rymer, Gibbs, & Givens, 2010). Therefore, convenient and perceived natural methods to increase PUFA in diets, especially methods that replace saturated fats with PUFAs, would be highly marketable and also beneficial to human health. However, because of the undesirability of lipid oxidation, a balance between modifying meat to contain PUFA rather than saturated fats, and decreasing the oxidative stability of meat must be carefully considered and researched before implementing changes.
Measurements of Oxidation

As previously established, in the past few decades consumers’ preference for high quality meat has and continues to rapidly increase. This trend has led scientists to pursue the development of a wide variety of accurate measurements to quantify the degree of oxidation that has occurred in meat. It is widely known that methods to determine lipid oxidation include chromatography, a sensory panel, peroxide value, and one of the most common methods for determining lipid oxidation: 2-thiobarbituric acid-reactive substances (TBARS) assay.

Similarly, there are many methods for determining protein oxidation. Changes in primary, secondary, and tertiary protein structure are all methods of evaluating protein oxidation to determine aggregates or cross-links have formed. Methods for determining primary structure changes include quantifying sulfhydryl concentrations, and identifying any protein oxidation derivatives such as carbonyls. Secondary structure changes can be detected via Raman infrared spectroscopy, Fourier transform infrared spectroscopy, and circular dichroism. Tertiary structure changes can be measured using methods such as differential scanning calorimetry, hydrophobicity measurements, and X-ray crystallography. Aggregates and cross-links can be identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), gel filtration and high-performance liquid chromatography (HPLC), atomic force microscopy and transmission electron microscopy (TEM) (Xiong, 2013).

In addition to determining any known or potential oxidation that has occurred, compounds and enzymes in meat can be evaluated to understand the meats’ degree of susceptibility towards postmortem oxidation reactions. As oxidation in meat is caused by
reactive oxygen species, if these compounds are nullified, the meat will have significantly higher oxidative stability. Therefore, various radical scavenging activity assays as well as antioxidant enzyme activity can be determined to predict the samples’ oxidative stability or susceptibility.

Determining any secondary structure protein alterations is one of the simplest and most common ways to establish protein oxidation. As protein oxidation occurs, the number of free sulfhydryl groups from the amino acid cysteine decreases as they are oxidized forming disulfide bonds with another previously free sulfhydryl group. The compound, 5,5’-dithiobis-(2-nitrobenzoic acid), DTNB reagent, is used to quantify any free sulfhydryl groups in the sample by oxidizing the free sulfhydryl group and bind to the sulfur atom forming a disulfide bond between the cysteine and DTNB compound. This complex produces a yellow color which is measurable via a photospectrometer at 412nm. It can therefore be expected that over time the quantity of free sulfhydryls will decrease as protein oxidation occurs on the sample (Ellman, 1959; Hultin, 1997).

Another primary structure method commonly used to determine the degree of protein oxidation is the measurement of carbonyls present. The production of carbonyls on amino acids is the primary change to proteins due to oxidation (Wang, Pan, Peng, Zhao, & Zhou, 2009). 2,4-dinitrophenylhydrazine (DNPH) is a compound commonly used to quantitate the carbonyl concentration in a sample. Through a condensation mechanism, the amine functional group on a DNPH compound binds to the α carbon of the carbonyl group of the oxidized amino acid. Besides its primary structure, the protein is then entirely denatured by a high concentration of guanidine hydrochloride. The
complex of DNPH and protein is measured photospectromically and the carbonyl concentration calculated (Levine, 1990; Liu, Xiong, & Chen, 2009).

An informative, yet technically demanding and more complex method of determining protein oxidation is through electrophoresis. Using polyacrylamide gels as the separating matrix, charged, denatured myofibril proteins can be separated based upon their size using an electrical field. Cross-linking from oxidation can be relatively determined by sample comparison through adding a reducing agent (mercaptoethanol or dithiothreitol) to a duplicate sample. The reducing agent cleaves any formed disulfide bonds and polymers from protein oxidation allowing the bands to be recovered and appear as relatively darker bands in sodium dodecyl sulfate the gel (Nelson & Cox, 2008; Nielsen, 2010). Commonly known as SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), this method separates proteins and works well for a visual aid when determining protein oxidation.

Chromatography, a method used for separating and quantifying compounds, provides a procedure for detecting protein and lipid oxidation as well as providing the fatty acid profile of samples. Gas and high performance liquid chromatography are not as commonly used for determining lipid and protein oxidation due to its expense and required technical knowledge to operate the equipment. However, once a protocol has been established for specific types of sample on a machine, data collection is relatively labor-free. Utilizing a mobile and stationary phase, chromatography allows samples to separate based on their physical compositions to identify compounds in a sample. Chromatography can detect and determine the extent of the oxidation of the meat sample because of highly reactive compounds (such as polyunsaturated fatty acids) that further
react to form detectable compounds such as aldehydes and ketones (Ajuyah, Fenton, Hardin, & Sim, 1993; Ladeira et al., 2014; Pignoli et al., 2009).

Both lipid and protein oxidation produce hydroperoxides ultimately react to become peroxide (Nielsen, 2010; Yi, Haug, Nyquist, & Egelandsdal, 2013). Peroxides are harmful to cells in living tissue as well as present health issues for a consumer because peroxides can easily react to produce free radicals, known carcinogens. Furthermore, polyunsaturated fatty acids, more common in poultry and lamb meat, are more easily oxidized causing certain types of meat to be more prone towards being peroxide rich (Raes, De Smet, & Demyer, 2004). Thus, particularly in such samples, monitoring the generated peroxides can be beneficial for meat production’s quality control. To complete the peroxide value (POV) method, the lipid dissolved in acetic acid and potassium iodide is added in excess. The peroxides react with the iodine leaving an excess of iodine. Starch is then added to react with iodine and standardized sodium thiosulfate allowing for a back calculation of the hydroperoxides concentration in the lipid sample (Damodaran, Parkin, & Fennema, 2008; Feiner, 2008). A downfall of this method is that a relatively large lipid sample is required. Additionally, as hydroperoxides degrade to become peroxides, a low POV value can indicate either high quality lipids or low quality lipids in a sample (Nielsen, 2010). Thus, some researchers recommend pairing the POV method for determining lipid oxidation with a second method such as TBARS.

The TBARS assay is one of the most common and potentially the most popular method to determine the degree of lipid oxidation. Due to its cheap, easy, and convenient technique and methodology it has become a standard method when conducting lipid
oxidation analyses. Reactive oxygen species act on polyunsaturated fatty acids in meat to produce a byproduct of lipid oxidation, malondialdehyde (MDA), a major product of lipid hydroperoxides from lipid oxidation. During lipid oxidation, hydroperoxides are formed from which aldehydes and ketones are produced. A large number of these byproducts are volatile giving the rancid meat off-flavors and aromas (Botsoglou et al., 1994; Calkins & Hodgen, 2007; Pignoli et al., 2009). The TBARS assay determines the amount of malondialdehyde in a sample spectrophotometrically by reacting thiobarbituric acid (TBA) with the MDA already present in the sample. The reaction between TBA and MDA produces an absorbable pink color which is read at 532 nm (Sinnhuber & Yu, 1977; Yu & Sinnhuber, 1957).

To gain a consumer’s perspective of the edibility and quality sensory panels can be performed. Although the judgment of meats’ sensory qualities is subjective and open to large experimental error, when properly conducted, sensory panel data on meat samples can be highly informative about the overall acceptability of a meat or a meat product (Font-i-Furnols & Geurrero, 2014). Specifically, sensory panels can be used to detect oxidation as certain odors and flavors will arise from protein, and lipid oxidation that are often unfavorable among consumers (Mottram, 1998; Resconi, Campo, Font-i-Furnols, Montossi, & Sanduo, 2009). Furthermore, if the levels of polyunsaturated fats (PUFAs) are altered by any means, this can cause the meat to develop new flavors in addition to causing a more oxidized taste and odor to the meat (Calkins & Hodgen, 2007).

Clearly, consumers will not be content with meat and meat products that emit odors and taste unsatisfactory. Therefore, as another means to create and maintain a high
quality meat, endogenous aspects such as antioxidant enzymes should be considered (Decker & Xu, 1998). The internal oxidative stability of meat is determined by a multifaceted system composed of antioxidants, prooxidants, fatty acid profiles, types of phospholipids, metal ions present, other chemical elements, as well as active enzymes in the tissue (Bou, 2009; Decker & Xu, 1998; Pignoli et al., 2009). Research is concluding there is a significant nutrigenomics effect on endogenous enzymes in the meat (Delles, Xiong, True, Ao, & Dawson, 2014; Li et al., 2009; Hesketh, 2008; Xiao et al., 2011).

Before uncooked meat reaches a consumer or even a sensory panel, it can be tested in the production stage for various enzymes that are known to provide increased oxidative stability of muscle tissue. Endogenous muscle enzymes such as catalase, glutathione peroxidase, and superoxide dismutase by various mechanisms act as oxidation stabilizers. They are a way to predict the relative oxidative stability of a meat sample. Catalase and superoxide dismutase have similar functions in that they are endogenous antioxidant enzymes active in muscle tissue (Delles et al., 2014). However, catalase and superoxide function differently yet in cooperation with each other to maintain the oxidative stability and quality of meat. For example, superoxide dismutase acts by scavenging superoxide, a byproduct of normal metabolism. It then catalyzes the conversion of the superoxide into a stable form of oxygen or to hydrogen peroxide. Catalase converts the formed hydrogen peroxide into water or a stable form of oxygen. Similar to catalase, glutathione peroxidase also acts eliminate hydrogen peroxides. It catalyzes the conversion of hydrogen peroxides to less harmful compounds as well as renews antioxidants that have been oxidized (Delles et al., 2014; Chan, Decker, & Feustman, 1994). Thus, these enzymes provide natural protection against oxidation. To
minimize meat from turning rancid, producers can optimize their product by selecting to breed animals with naturally higher muscle enzymes (Decker et al., 1998).

**Meat Quality Attributes**

*Consumer Demands*

Increasingly so, consumers desire to buy high quality meat (Papanagiotou, Tzimitra-Kalogianni, & Kelfou, 2013). However, when asked to explain what they consider “high quality” meat, consumers typically give varying and ambiguous descriptions (Becker, 2000). Although meat’s color is the attribute that consumers will first judge when considering a fresh meat purchase, after the procurement and consumption, a consumer will evaluate a number of other quality attributes such as the meat’s juiciness, tenderness, flavor and aroma as part of their judgement for a potential repeat purchase (Hughes, Oiseth, Purslow, & Warner, 2014; Papanagiotou et al., 2013; Suman, Hunt, Nair, & Rentfrow, 2014; Troy & Kerry, 2010).

*Tenderness and Water-Holding Capacity*

Consumers worldwide readily agree that tenderness (closely linked to meat’s juiciness) is the most important sensory aspect of meat consumption (Gobert et al., 2014). Producers are at risk of huge economic losses if their product is not satisfactorily juicy and tender. It has been found that 51% of consumers are willing to pay an average of $1.83/lb for tenderer beef and research has been conducted which proposes a new method for communicating the degree of beef tenderness to consumers which could directly increase profits (Riley, Schroeder, Wheeler, Shackelford, & Koohmaraie, 2009). Additionally, proof on concept research propositions through genetic selecting for cattle
with properties attributing to meat’s tenderness, profits for feeder and fed cattle could increase $9.60 and $1.23 per head respectively resulting in a $7.6 billion economic increase (Weaber & Lusk, 2010). Conversely, production of tough meat will result in financial loss. The knowledge of consumers’ desire for tender meat paired with the amplified demand for high quality meat has spurred producers and researchers to increase their efforts to fully comprehend the systems that cause meat to become more tender and moist.

It is reported by many that protein denaturation (caused by oxidative changes to protein) significantly harms the water holding capacity of meat; the effects of protein oxidation prevent protein and water interactions such as hydrogen, electrostatic, and capillary bonds (Hughes et al., 2014; Liu, Xiong, Chen, 2011; Zakrys-Waliwande, O’Sullivan, O’Neill, & Kerry, 2009). As previously explained, among many other outcomes, oxidation of meat also causes proteins to aggregate and become less soluble (Xiong, 1997). This may decrease the protein’s surface area that would normally be available for interaction with water molecules. Water within meat is located in one of two places: 1) associated through ionic bonds and polar attractions to amino acids, or 2) associated among the protein structure through surface tension otherwise known as capillary forces. Regardless, water within meat can only remain captive if the structure of the protein contains it (Xiong, 1997).

The composition of muscle is 55-60% myofibrils – the muscle proteins responsible to form gel matrices in meat products through protein interactions, bind to water for water holding capacity, and function in emulsion systems to surround fat globules (Xiong, 2000). It is also known that post-rigor meat is composed of
approximately 75% water (Hughes et al., 2014; Traore et al., 2012). The proportions of myofibrils and water that compose meat is key to understanding how to increase meat juiciness and tenderness because it has been reported that actin and myosin are the primary muscle tissue targets of protein oxidation (Traore et al., 2012). If myofibril proteins are no longer capable of performing their water retaining functions due to their structure loss, meat loses much of its desirability for consumers.

Livestock Feed Supplements: Strategies to Improve Meat Quality

Altering Meat’s Fatty Acid Profile

The growth of an animal and ultimately the meat’s characteristics are based upon an animal’s diet. Huge changes in meat quality such as water holding capacity, tenderness, and flavor result from small diet modifications. Indeed, it is reported that diet is the primary factor to control the quality of meat (Anderson, Oksbjerg, Young, & Therkildsen, 2005). Pertaining to improving meat quality via feed control, this section will focus specifically on altering fatty acid profiles of muscle by feed additives.

Altering the type of fatty acids in livestock feed can modify the fatty acid deposits in the tissue. For example, this was shown by adding algal biomass into broilers’ diets for six weeks. The breast meat’s fatty acid profile was modified by the addition of PUFA deposits into the meat (Rymer et al., 2010). The fatty acid profile of salmon was found to be altered when an algae source was added into fish feed (Norambuena et al., 2015). Another feed study with similar fatty acid modification goals as the previously listed found that adding up to 10% flaxseed meal into broilers’ diets increased PUFA content
without negatively impacting the sensory acceptability due to lipid oxidation causing off-flavors and odors (Ajum, Haider, Khan, Sohaib, & Arshad, 2013).

In non-ruminant animals, fatty acids are absorbed directly through the small intestine without first being hydrolyzed. This allows these non-ruminant animals such as poultry and swine to relatively easily implement polyunsaturated fatty acids into their tissues without modifications to the dietary supplemented fatty acids incorporated in the feeds (Preedy, Srirajaskanthan, & Patel, 2013). However, this can lead to off-flavors in the meat as polyunsaturated fatty acids are less shelf-stable. For example, pigs’ fatty acid profile can be modified to replace saturated fatty acid tissue deposits with the more desirable healthier fats; however off odors and flavors often result (Woods & Fearon, 2009).

In opposition to swine or poultry digestion and fatty acid absorption, ruminants’, such as cattle, bacterial flora in the animals’ guts hydrolysis the fatty acids. This results in higher levels of saturated fatty acid deposits in the animal’s tissue than what would be expected for a non-ruminant animal (Preedy et al., 2013). Therefore, it can be more difficult to incorporate polyunsaturated fatty acids into ruminants’ meat.

Nevertheless, some studies have shown success for implementing polyunsaturated fatty acids into beef. One study conducted to enrich beef with PUFA utilized the finishing period for cattle (Gatellier, Kondjoyan, Portanguen, & Sante-Lhoutellier, 2010). During the finishing period animals were given a diet supplemented with extruded linseed and rapeseed – both of which are rich in PUFA. The results indicated no difference in carbonyl formation over an 11 day storage study. Therefore, it was concluded that linseed and rapeseed extrude can be used as a supplement to increase PUFA in beef without
increasing protein oxidation. Additionally, it was found that fatty acid profiles can be changed in beef by adding yellow grease to alfalfa in a corn-based cattle diet (Nelson, Bushbloom, Ross, & O’Fallon, 2008).

Specifically, fish or fish oil is the most efficient method to increase the amount of DHA in meat (Raes et al., 2004). Although increasing the amount of PUFAs in meat is both a relatively easy and healthy attainment, as noted earlier, the double bonds in unsaturated fatty acids are more easily oxidized introducing a number of offsetting factors that must be accounted for (Anderson et al., 2005; Raes et al., 2004). As the concentration of PUFA deposits increases in meat, the propensity of lipid oxidation also increases. In addition to the type of fatty acids supplemented into the feed, the quality of oil added into the feed also impacts the oxidative stability of the meat. The more oxidized the feed oil is, the more susceptible meat may be towards oxidation. Delles et al. (2014) found that broilers fed diets containing either high (121 meq O₂/kg) or low (23 meq O₂/kg) oxidized oil resulted in a significant difference of oxidation stability within the meat during a poultry dietary study.

Unfortunately, higher quality oil is more expensive. Of the many costs associated with animal production in the transition from the farm to consumers’ plates, the largest cost is the feed stuff (Tahir & Pesti, 2012). Rising prices have caused farmers to seek innovative methods to combat the costs of feed stuff. To compensate for this expense, producers commonly use vegetable oils to create a high energy feed. Unfortunately, these vegetable oils are often lower quality (more oxidized). This introduces reactive oxygen species into the animal which may hinder their growth and performance, and ultimately produce meat that is more readily oxidized and thereby less attractive to consumers (Liu,
Chen, Kerr, Weber, Johnston, & Shurson, 2014). Therefore, a balance between supplementing livestock feeds with PUFA as well as considering the quality of oil used in feeds must be reached to produce an optimum muscle to meat product.

*Optimizing Meat Quality Via Antioxidant Supplementation*

Exploration of potential antioxidants to counteract the oxidative species in meat is being researched and applied to reach the goal of producing highly nutritious, yet oxidatively stable meat. Antioxidants provide many health benefits in biological systems by being reacted upon themselves rather than any other biological tissue being damaged by free radicals or oxygen reactive species. Numerous synthetic antioxidant supplements have been explored and are used as supplements to prolong meat’s shelf life as well as preserve the flavor, color, and safety of the meat (Olsson & Pickova, 2005). These include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquine (TBHQ), and propyl gallate (PG) (Karre, Lopez, & Gette, 2013). However, these antioxidative supplements are government regulated as they are known to be toxins in relatively high concentrations (Karre et al., 2013). Recently, as health related scientific discoveries continue to grow, and as consumers become more health conscientious, there is a growing demand for the inclusion of natural antioxidants in meat and meat products to provide oxidative stability. Such natural antioxidants can be found in many fruits such as plums, cranberries and pomegranates, or in other plants such as herbs and spices – rosemary, oregano, garlic, and sage. Additionally Vitamin E and honey are both known as antioxidants (Karre et al., 2013; Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010).
Within the meat production industry, significantly higher levels of Vitamin E are implemented into livestock feeds to promote animals’ performance and the quality of meat (Xiao et al., 2011). For example, the current Vitamin E NRC for meat-type chickens is 10 IU for 0-8 weeks of life (kg of diet, 90% dry matter). However, levels as high as 50 and 100 IU Vitamin E have been used in feed studies as antioxidative supplements.

The use of antioxidants has been tested for effectiveness in both dietary and postmortem meat. For example, a study was conducted on turkeys and the impact of oregano oil and/or α-tocopherol acetate on the oxidative stability of the meat. Dietary treatments incorporated the oregano oil and α-tocopherol directly in the feed whereas the some treatments were supplements mixed directly into the postmortem meat. Meat patties were formed and TBARS measured over a span of 9 days. Researchers found that although both the dietary and postmortem treatment had relatively less lipid oxidation compared to the control, the dietary treatment underwent less oxidation than the postmortem treatment (Govaris, Botsoglou, Papageorgiou, Botsoglous, & Ambrosiadi, 2004).

An additional study was conducted on beef also using Vitamin E or a white mineral oil containing D-α-tocopherol as a feed or postmortem additive. Researchers found that the dietary supplementation with Vitamin E significantly hindered the oxidation of the meat (TBARS determined), whereas the postmortem addition of Vitamin E only slightly prevented oxidation. Therefore, this study found that Vitamin E as an endogenous antioxidant was more effective than Vitamin E utilized as a postmortem additive (Mitsumoto, Arnold, Schaefer, & Cassens, 1993). These studies provide convincing evidence to continue seeking dietary treatments to increase oxidative stability.
As opposed to focusing on postmortem treatments such as additives in packaged lunch meats.

However, an additional study preformed dietary versus postmortem supplementation using broilers, Vitamin E and oregano oil. The study utilized two concentrations of Vitamin E as well as oregano oil (100 mg/kg of feed). The postmortem groups were treated with 3% honey or BHT. The meat was harvested, formed into patties, and cooked. The results show that the lower dietary treatment of Vitamin E had the most oxidation occur. Unlike the previously described turkey study, there was no other significant oxidative difference between the dietary and postmortem treatments. However, the higher level of dietary Vitamin E, oregano oil, honey, and BHT all had a positive impact in preventing oxidation (Avila-Ramos et al., 2013)

These studies demonstrate that many factors must be considered when designing a feed system that will produce oxidatively stable meat. They furthermore provide evidence that the biological system, such as the animal species as well as the specific antioxidants, must be closely studied and evaluated before a dietary treatment system is assigned for meat production purposes. Haphazardly providing livestock with feed not scientifically researched is not economical, and will likely result in producer monetary losses as well as a decreased client database due to consumers’ rejection of poor quality.

**Concluding Remarks**

The demand for healthy, high quality meat is increasing for many reasons. However, it has generated some issues within the feed and meat production processes as PUFAs are known to promote lipid oxidation and thereby foster protein oxidation. The
alteration of proteins (from oxidation) deteriorates its structure and decreases the overall quality of the meat. Nevertheless, the growing trend for healthier, PUFA enriched meat is an understandable and commendable goal. Therefore, to meet the demand for healthy, high quality meat, producers must first understand what quality means to consumers as well as the aspects controlling the overall quality of meat. As it is known that the oxidative stability of meat, or lack of, is closely linked to quality aspects such as flavor, color, aroma, tenderness, and juiciness, producers and meat science researchers intently focus on the comprehension, prevention, and detection of lipid and protein oxidation. Although antioxidants are widely used for this, optimizing the oxidative stability of an animal, and eventually the meat through utilizing antioxidants as dietary supplements is a complex undertaking that requires vast knowledge of the lipid and protein oxidation process, as well as the mechanisms of the antioxidant/s chosen for a feeding system. Paired with application, viewing meat quality as a large, comprehensive, chain-reaction system, the attainable goal of producing high quality, nutritious meat to expectant consumers can continue to be met.
CHAPTER 3
CHEMICAL ANALYSIS OF MEAT FROM BROILERS FED ANTIOXIDANT SUPPLEMENTED DIETS

Introduction

Consumers are more health conscientious about the quality of meat they purchase than previous generations (Jensen, Lauridsen, & Bertelsen 1998). They are seeking tender, flavorful meat in addition to making progressive strides towards healthier diet choices (Ruiz, Guerrero, Arnaú, Guardia, & Esteve-Garcia, 2001). They are becoming more conscientious about nutrition – specifically pertaining to their fat intake (Theobald & Lunn, 2006). Poultry is known to have less saturated fat relative to beef or pork. As a result, the growing trend of poultry consumption has and is predicted to continue increasing due to its affordability, convenience, and healthier fatty acid profile (Thornton, 2010). However, due to the expanding knowledge about the significance of incorporating polyunsaturated fatty acids into our diets (for numerous health benefits such as brain and heart health) animal feed specialists have been exploring efficient and fiscal methods for modifying livestock feeds to incorporate essential fatty acid deposits into meat (Mozaffarian et al., 2010; Raes et al., 2002).

Unfortunately, polyunsaturated fatty acids are more easily oxidized than saturated fat. It is widely known and accepted that the primary cause of meat rancidity (besides bacterial spoilage) is oxidation of the meat’s lipids and proteins. When macromolecules within meat are oxidized, off-flavors and odors are produced. Furthermore, as meat is oxidized, it also undergoes discoloration and decreased water holding capacity due to the disruption of myofibrils – the primary component in muscles responsible for water-
binding (Lu, Harper, Zhao, & Dalloul, 2014; Sheldon, Curtis, Dawson, & Ferket, 1997; Xiong, 2000). As tenderness is closely linked to water holding capacity, the disruption of myofibrils (decreased water-holding capacity) also inclines the tenderness of the meat to be lowered causing the meat to have a tougher characteristic. The deterioration of the meat is obviously unfavorable among consumers. Therefore, as oxidative reactions on muscle lipids and proteins cause a detrimental drop in the meat quality, there is a need to extensively study oxidation in meat to prevent, detect, and quantify the degree of degradation due to oxidative mechanisms.

Vitamin E is commonly used in commercial animal feeds at much higher levels than the NRC recommendations to promote animal health and performance through its antioxidative properties (Xiao et al., 2011). However, due to the increasing cost of raw materials such as Vitamin E, Alltech® has been investigating an algae-based antioxidative pack, EconomaseE (Alltech®, Nicholasville, KY; EcoE) as a cheaper antioxidative alternative than Vitamin E.

Alltech® researchers have reported that EcoE can replace up to 80% of vitamin E in poultry feed. Therefore, Alltech® conducted a study to compare the effects of EcoE (200 g/ton) feed supplementation to high levels of Vitamin E (50 and 100 IU/kg feed) supplemented in broiler feed (Xiao et al, 2011). Researchers desired to determine any gene differences in the breast meat between the dietary treatments as well as any corresponding, underlying molecular mechanisms. Through the use of relatively new microarray and gene expression technologies, molecular mechanisms within the breast muscle tissue were evaluated and matched with their gene ontology (Xiao et al, 2011). When compared to the control meat (lacking high levels of Vitamin E and EcoE) it was
found that vitamin E and EcoE have similar impacts on the breast meats’ nutriphysiological related gene regulation. Therefore, researchers concluded that EcoE is a suitable and less expensive replacement product as opposed to Vitamin E for broiler antioxidative gene regulation and oxidative protection (Xiao et al, 2011). Furthermore, additional work completed in collaboration with Alltech® found that EcoE can be used as an antioxidative supplement to prolong the shelf life of broiler breast meat (Delles, Xiong, True, Ao, & Dawson, 2015). The research provided applicable data that breast meat supplemented with EcoE was more oxidatively stable over a storage study than meat without EcoE (Delles et al, 2015.)

Collaborative Alltech® and University of Kentucky researchers were primarily interested in understanding any interactions and differences between broilers’ feed dietary treatments with SP-1 and antioxidants (Vitamin E and EcoE.) Therefore, to further the knowledge of EcoE’s effect on broiler breast meat oxidative stability and to produce nutritious, high quality meat, the objective of this present study focuses on implementing an proprietary algae (SP-1), known to be rich in polyunsaturated fatty acids, in various levels into broilers’ feed to modify the birds’ muscle fatty acid profile, with the overall goals to: 1) enhance the nutritional value of the meat, and 2) explore using the proprietary pack blend (EcoE) to replace Vitamin E as the typical incorporated feed antioxidant. Therefore, Vitamin E, as well as an algae-based, selenium and SP-1 containing, natural antioxidant mixture were implemented at various levels to theoretically compensate for the potentially more oxidatively vulnerable meat samples. Moreover, partially oxidized oil (soybean) was used as a main energy source in the dietary formulations to mimic commercial feeding operations where oxidized oils (from
dining services and the rendering industry) are widely used. Methods to determine lipid and protein oxidation were selected to quantify the byproducts of the chemical reactions such as the generated carbonyls, disulfide bonds (myosin cross-linking) from the modification of amino acid side chains and malondialdehyde, a product of lipid oxidation.

Materials and Methods

Materials

Alltech® (Nicholasville, KY) supplied a proprietary algae mix (SP-1) as well as an algae-based antioxidant pack blend (EconomaseE, EcoE) containing selenium and SP-1 as additives to the diets. Food grade soybean oil (peroxide 2 mEq of O₂/kg) was obtained from a local source. Prior to being blended into the feed, the soy oil was first oxidized as low quality recovered oils are often used in commercial poultry production. To create low quality oil, the oil was poured into aluminum pans (41 × 14 × 4 cm) and placed in a convection oven set at 95-100 °C for 5-7 days. The peroxide value (AOCS, 2007) of the oil was measured periodically until the final pooled POV reached peroxide 86 mEq of O₂/kg. The oil was removed from the ovens, cooled at room temperature, and immediately used in the preparation of the broilers’ diets. For all of the following methods, chemicals were procured from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Broiler Production

All broilers were treated humanely and slaughtered according to USDA regulations and in agreement with the University of Kentucky’s Institutional Animal Care
and Use Committee (IACUC #01004A2006). A total of 1035 1-day old chicks were randomly assigned to different 2 x 2.5 meter pens (23 birds per pen). A dietary treatment was randomly assigned to each pen (total of 45 pens) thereby allowing 9 replicate pens per diet. The experimental design was a randomized complete block design using the pens as the blocks. Each pen had a feeder, nipple drinker line, and contained wood shavings. The diets were all formulated using the low quality oxidized oil (POV = 86 mEq of O₂/kg). The four diets were as follows:

Diet 1: Basal diet 1 + 50 IU Vitamin E
Diet 2: Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E
Diet 3: Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E
Diet 4: Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E

Birds consumed feed ad libitum for seven weeks (49 days) according to the following schedule: Starter diet days 1-18; grower diet days 19-30; finisher diet days 31-49. Table 3 provides the nutrient summary of the starter, grower, and finisher diets and was provided by Alltech® researchers. (The mixing sheets for the basal and treatment diets, provided by Alltech® researchers, are included in the appendix.) It is worth noting that the NRC Vitamin E requirement for (meat-type) chickens is 10 IU (as kg of diet, 90% dry matter) for birds 0-8 weeks of age (National Research Council, 1994). Therefore, birds in this study received levels of vitamin E considerably higher than the minimum Vitamin E requirement for acceptable animal growth and performance. The broilers’ pectoralis major (breast meat) was selected as the meat sample because it is the most valued and demanded cut of meat from a chicken.
Table 3. Nutrient composition of treatment diets.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Starter 0 -18d</th>
<th>Grower 19 - 30d</th>
<th>Finisher 31 – 49d</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMEn kcal/kg</td>
<td>3050</td>
<td>3100</td>
<td>3150</td>
</tr>
<tr>
<td>Protein %</td>
<td>22</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Ca %</td>
<td>1.00</td>
<td>0.90</td>
<td>0.89</td>
</tr>
<tr>
<td>Available P %</td>
<td>0.45</td>
<td>0.41</td>
<td>0.38</td>
</tr>
<tr>
<td>TSAA %</td>
<td>0.97</td>
<td>0.88</td>
<td>0.75</td>
</tr>
<tr>
<td>Lysine %</td>
<td>1.32</td>
<td>1.16</td>
<td>1.00</td>
</tr>
<tr>
<td>Na %</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

AMEn: Apparent metabolizable energy
TSAA: Total sulfur amino acids

Meat Sampling and Storage

After 49 days of dietary treatment and growth, eight broilers from randomly selected pens (4 pens per diet) were randomly selected, harvested according to human practices, gutted, and chilled in ice slurry for 1.5 h to undergo rigor mortis. Birds were removed from the ice baths, and both the right and the left pectoralis major (white meat) were removed and skinned. Breasts were then placed in self-sealing bags and transported on ice to University of Kentucky. Within 3 hours the skinless breasts were vacuum packaged. The vacuum packaged breast meat was stored in a –30 °C freezer for less than 1 month. Breast meat was thawed in a refrigerator (4±1 °C) for 24 hours. On day 0 (the day of complete thawing), all breast fillets were placed on individual polystyrene trays and covered with polyvinylchloride (PVC) film, and then subjected to retail display at 4±1 °C for 0, 2, 4, and 6 days.
Lipid Oxidation

Lipid oxidation of stored meat was measured by the detection of thiobarbituric acid-reactive substances (TBARS) using the method from Sinnhuber and Yu (1977). Three birds (6 samples) were tested for each diet on each day of the analysis. Each bird was from a different pen so that potential pen-to-pen variations were absorbed in the experimental design. On day 0, a ~15 g sample was excised from the posterior end of the breast meat. The remaining breast meat was then repackaged on the polystyrene tray, rewrapped in PVC wrap, and returned to the retail cooler for additional storage. The aliquot of removed breast meat sample was ground via a Ninja® Express Chopper, vacuum packaged (95.5% oxygen removal) and stored in a -80 °C freezer. The above sample collection and handling was repeated on days 2, 4, and 6. After the 6 day storage time, sample collection was completed, all the ground and frozen samples were thawed and analyzed at the same time to eliminate error and variation between test days. Thus, the TBARS assay was completed on all the samples on the same day. The results were expressed as milligrams of MDA per kg of muscle sample.

Protein Oxidation

Myofibrillar protein was isolated from the breast meat on the predetermined test days (0, 2, 4 and 6) and used for the analyses. Myofibrillar proteins from the breast meat were isolated for the protein oxidation analysis because they are the most impactful proteins on meat quality (Xiong, 2000). The extraction buffer was comprised of 10 mM disodium phosphate, 2 mM magnesium chloride, and 0.1 M sodium chloride (pH 7.0). The extraction was done as described by Xiong (2000). The myofibrillar protein extract was kept on ice to minimize oxidation throughout the process. The Biuret method was
used to determine the protein concentration of all samples. A small sample of the extract (~2 grams) was frozen in a microcentrifuge tube in a -80 °C freezer for completing electrophoresis when convenient.

Total free sulphydryl concentration was determined on fresh, diluted myofibrillar protein (2 mg/mL) on each test day via 5,5’ dithio-bis-2-nitrobenzoic acid using the Ellman (1959) method. Using an extinction coefficient of 13,600 M/cm, the results were reported in nmol/mg protein.

Carbonyl formation was determined according to Levine et al. (1990) using 2,4-dinitrophenylhydrazine. The molar absorption coefficient of 22,000 M/cm was used and the carbonyl concentration was reported in nm/mg of protein. Protein oxidation was further evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to determine whether protein covalent cross-linking was prevalent among any of the treatments. Samples were run in duplicate: two samples per diet (each sample was from a different pen). The gels were made and run according to the method of Laemmli (1970) and were composed of 3% acrylamide (stacking gel) and 12% (resolving gel). The protein samples were prepared to contain 2 mg/mL protein and 25 μL were loaded into each well resulting in 25 μg per well.

Statistical Analysis

One trial was completed with triplicate sample analyses. Data was analyzed in Statistix software 9.0 (Analytical Software, Tallahassee, FL) via analysis of variance (ANOVA) using the general linear model’s procedure and a randomized block design with each pen being a block to determine storage time effect. When a storage dietary treatment effect was significant, data was subject to Tukey’s testing to determine the
difference between the individual days considering $P < 0.05$ significant. Likewise, when the ANOVA using a completely randomized design detected a diet effect, the means between samples for each day were separated by the Tukey’s test.

**Results and Discussion**

*Lipid Oxidation*

As seen in Figure 5, the general trend of lipid oxidation increased over the 6 day storage time. The standard error bars are shown. Among the treatment groups on the specific days, there were no statistical differences except diet 4 which had significantly more lipid oxidation than the other three treatment groups. Table 4 shows the total unsaturated fatty acids as well as the content of the measured unsaturated fatty acid types within the breast meat. (These values were determined via a GS/MS method and supplied directly from Alltech® researchers.)

It is evident that diet 4, containing both EcoE and SP-1, has the highest amount of unsaturated fat deposits followed by meat from treatment 2 which also contains SP-1. Diets 1 and 3 which contain no SP-1 are remarkable lower than diets 2 and 4 in these fatty acids. Since diet 4 contains the greatest amount of SP-1 (mostly from direct SP-1 supplementation although some from the EcoE pack), the presence of a relatively high concentration of PUFAs probably contribute to the oxidative instability of the muscle tissue.
Table 4. Unsaturated fatty acid profile (mg/g muscle tissue) from dietary treatments*

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Diet</th>
</tr>
</thead>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total unsaturated</td>
<td>11.860</td>
</tr>
<tr>
<td>DHA</td>
<td>0.078</td>
</tr>
<tr>
<td>EPA</td>
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</tr>
<tr>
<td>Myristoleic acid</td>
<td>0.044</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>0.783</td>
</tr>
<tr>
<td>Cis-10-Heptadecanoic acid</td>
<td>-</td>
</tr>
<tr>
<td>Oleic acid/Elaidic acid</td>
<td>5.471</td>
</tr>
<tr>
<td>Linoleic acid/Linolelaic acid</td>
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</tr>
<tr>
<td>γ-Linolenic acid</td>
<td>0.038</td>
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<tr>
<td>Cis-11-Eicosenoic acid</td>
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</tr>
<tr>
<td>Cis-11,14,17-Eicosatrienoic acid</td>
<td>0.738</td>
</tr>
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</table>

*Values provided by Alltech®. Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomaseE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomaseE + 10 IU Vitamin E.

It has been found that poultry feed supplemented with Vitamin E in combination with either fresh fish oil (not oxidized oil) or a marine algae source, formulated to contain equal amounts of DHA, provides sufficient oxidative protection against lipid oxidation in poultry breast meat as concluded by total aldehyde formation determination. (Rymer et al., 2010). However, feeds containing the marine algae source had statistically higher amounts of hexanal, the most abundant aldehyde detected (Rymer et al., 2010). Vitamin E has been used as an effective dietary treatment in turkeys to prevent lipid oxidation measured by TBARS (Govaris et al., 2004). In light of previously conducted research, it appears that higher levels of antioxidant/s or packs need to be utilized to overcompensate the oxidative lipid instability that SP-1 causes.
Figure 5. Lipid oxidation (formation of TBARS) in broiler breast meat from different diets during refrigerated storage (3–6 °C). Same capital letters (A,B) denote no difference between the treatment groups on a specific day; same lowercase letters (a,b) represent no difference within the same treatment over the storage period ($P < 0.05$). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

Protein Oxidation

Myofibrillar proteins from the breast meat were isolated for the protein oxidation analysis because they are the most impactful proteins on meat quality (Xiong, 2000). The proteolysis of the myofibrils is the main contributor to post-mortem tenderness (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). Similarly, the actin and myosin lattice and spacing controls the water-holding capacity of the meat (Zayas, 2012).
As tenderness and juiciness are closely linked, using the myofibrillar protein extraction from the breast meat for the chemical analysis, a connection between the chemical and quality analysis data is produced.

There was no evident trend in the free sulfhydryl concentration change during storage (Figure 6). Nevertheless, there was a significant difference between the dietary treatments on day 4; diet 3, containing EcoE and Vitamin E supplements, caused the meat proteins to maintain their free SH groups indicating less oxidation. It is known (and thereby expected) that over time the quantity of free SH functional groups will decrease in meat as disulfide bonds are formed due to the oxidation of cystine as well as other cross-linking that occurs among other amino acids (Ellman, 1959). However, as seen in Figure 6 there was much variation and large standard errors. Three dietary studies (Haak, Raes, Smet, Claeys, Paelinck, & De Smet, 2006; Mercier, Gatellier, Viau, Remignon, & Renerre, 1998; Petron, Raes, Claeys, Lourengo, Fremaut, & De Smete, 2007) evaluating meat quality and oxidative stability also noticed unremarkable differences in protein oxidation in terms of free sulfhydryl losses over a storage time. Unexpected, sulfhydryl results are indefinite; oxidative conclusions cannot be drawn from these results. This may be due to low protein oxidation and difficulty determining values. Bird to bird variation may contribute to the large standard error.
Figure 6. Free sulphydryl (SH) groups in broiler breast meat from different diets during refrigerated storage (3–6 °C). There was no storage time effect nor dietary treatment effect except for day 4 where significant difference ($P < 0.05$) was indicated by different letters (A,B). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

As anticipated, a general trend of increasing carbonyl group formation can be observed throughout the storage time. However, there was no significant difference between treatment groups of the formation of carbonyls except for diet 4 on day 4 (Figure 7). A different study fed birds feeds formulated with oil of either a POV value of 1 or 156 meq O2/kg oil. Results found birds fed diets with the oxidized oil had significantly lower levels of Vitamin E in their blood plasma and body fat (Engerg, Lauridsen, Jensen, & Jakobsen, 1996.) Moreover, nutrition studies with rat models have shown Vitamin E
deposits in rats’ blood and tissue vary when the experimental animals were fed diets supplemented with Vitamin E in feeds formed with low or high quality oil (Liu & Huang, 1996). Their results concluded that more Vitamin E was metabolized in the rats from diets prepared with higher quality oil. This indicates that Vitamin E and other phenolic compounds may not retain their antioxidative efficacy in diets formed with highly oxidized oil which can explain no significant differences between the dietary treatments in the present study. Thus, low quality oxidized oil may have prevented the antioxidants from preforming at their full capacity within the birds resulting in no significant muscle difference on days 0, 2, and 6. There is, however, a significant trend of increasing carbonlys throughout the storage study for diets 2 and 4, both containing SP-1. Three possible explanations to these results are: 1) the oxidized oil could have prevented the expected and necessary oxidative protection for the meat, 2) the SP-1 supplement has an unknown underlying mechanism preventing meat oxidative stability, 3) the higher levels of PUFAs deposits in the meat have overwhelmed the abilities of the antioxidants’ capacity.
Figure 7. Myofibrillar protein oxidation (formation of carbonyls) in broiler breast meat from different diets during refrigerated storage (3–6 °C). There was no dietary treatment effect except for day 4 where significant difference ($P < 0.05$) was indicated by different letters (A,B). For storage time effect, significant differences ($P < 0.05$) within the same diet are indicated by lowercase letters (a,b). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

Figure 8 is a representation of SDS-PAGE gels completed for the analysis of protein aggregation due to protein oxidation. However, no visual differences among the diets can be distinguished between the gels with the reducing agent ($\beta$ME) and those without indicating little protein oxidation occurred. Therefore, this suggests that there were no differences of disulfide bond formation among the dietary treatments. This is in
concordance with the previously described sulfhydryl results indicating no significant protein cross-linking differences due to low oxidation values. As described in the carbonyl results, the oxidized oil used to formulate the diets could have reduced antioxidants’ ability to act resulting in no measurable difference of protein cross-linking.

**Figure 8.** Typical SDS-PAGE profiles of myofibrillar protein extracted from broiler breast meat stored at 3–6 °C for 0 and 6 days. The diets (1 through 4) are labeled at the top of each well. BME: β-mercaptoethanol. MHC: myosin heavy chain. Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.
**Conclusion**

The results indicate a general trend of lipid oxidation increases among all of the diets throughout the storage time although the change was significant only for diet 2 which contains SP-1. This is suggestive of the algae oil (PUFA) to oxidation. As EconomasE also contains algae, its presence in diet 4 actually causes more lipid instability (TBARS). Conversely, protein cross-linking via disulfide bond formation is not apparent as concluded from sulfhydryl and SDS-PAGE gel results. Similarly, protein carbonyl formation during storage was remarkable in diet 4 but less appreciable in other diets. The oxidized oil used to formula the diets may be an explanation of the general statistical insignificants of protein cross-linking throughout the storage study. The chemical analysis suggests that treatments that contain SP-1 (diets 2 and 4) are less effective than treatments without algae (diets 1 and 3) for maintaining the quality of broiler breast meat during refrigerated storage.
CHAPTER 4
QUALITY ANALYSIS OF MEAT FROM BROILERS FED ANTIOXIDANT-SUPPLEMENTED DIETS

Introduction

Consumers are interested in purchasing meat that is tender and juicy (Wang et al., 2009). Post-rigor meat, primarily formed of myofibril proteins, is composed of approximately 75% water (Traore et al., 2012; Hughes, Oiseth, Purslow, & Warner, 2014; Xiong, 2000). Water associated with myofibrils is contained in myofibrils (intra-myofibrillar water) or around myofibrils (inter-myofibrillar water) (Pearce, Rosenvold, Anderson, & Hopkins, 2011) (Figure 9). Of the water associated with myofibrils, 85% is intra-myofibrillar (Huff-Lonergan & Lonergan; 2005). This water is captured by hydrophilic interaction with amino acids, physical entrapment, or held by capillary forces (Pearce et al., 2011).

Figure 9. Illustration of myofibril-associated water location. Adapted from: Pearce et al., (2011).
Any alterations to myofibrillar proteins (such as oxidation) can cause significant changes to the water-holding capacity (WHC) of the meat (Huff-Lonergan & Lonergan; 2005). It is widely accepted that oxidation is a primary cause of lowering meat’s quality including WHC and tenderness (Xiong & Decker, 1995). As these two quality aspects (WHC and tenderness) are closely linked to each other, the myofibrils’ protein integrity is relevant to the production of high quality meat; specifically, meat that is both juicy and tender.

In addition to high quality, consumers are increasingly demanding nutritious meat that is low in saturated fats and contains polyunsaturated fatty acid deposits (Academy of Nutrition and Dietetics, 2014). It is known that livestock and poultry dietary treatments can alter the fatty acid profile. By modifying broiler diets, an increase of polyunsaturated fatty acid deposits can be obtained (Delles et al., 2014; Malayogul et al., 2009; Ruiz et al., 2001). As unsaturated fats are more easily oxidized, supplementations can increase the susceptibility of the myofibrils to oxidation through lipid oxidation byproducts thereby decreasing the quality of meat by negatively impacting the meats’ WHC and tenderness (Estevez, 2015).

Dietary supplementation, if not carefully controlled, can also easily cause off-flavors in meat. Thus, undesired off-flavors in meat can come from: 1) The oxidation of lipids and proteins, and 2) Directly from feed supplementation. Therefore, it is important to monitor the flavor of the finished meat.

To investigate the quality attributes that consumers widely recognize (flavor, juiciness, and tenderness) the breast meat from the broilers in this dietary study were analyzed. WHC and tenderness measurements were completed and an Institutional
Review Board (IRB) approved sensory panel was conducted (Exemption Certification for Protocol No. 14-0817-X4B).

**Materials and Methods**

*Materials, Broiler Production, Meat Sampling, and Storage*

Dietary treatments were produced as previously described in Chapter 3 sections Materials and Broiler Production. Broilers were produced, harvested, stored, and thawed as previously described in Chapter 3 sections Broiler Production and Storage.

*Exudative Loss*

Exudative loss between days 0-2, 2-4, and 4-6 was measured on the samples that were also used for cooking loss and tenderness measurements. After thawing, 12 birds per diet, 24 breasts, were individually weighed. The breasts came from 3 different pens per diet; 4 birds from each pen to eliminate pen to pen variation. They were then placed on a polystyrene tray with an absorptive pad, covered with PVC wrap and stored in a retail cooler (4 ± 1 °C). On days 2, 4 and 6 the breasts were blotted dry to remove exudate and weighed again. The weight difference between the original weight on day 0 and the subsequent measurements from days 2, 4, and 6 was calculated and converted to a percentage. This numerical percentage is considered the exudative loss.

*Cooking Loss*

Cooking loss was determined over a 6 day storage time. Approximately 24-48 hours before day 0, 12 birds per diet (4 birds from 3 different pens to eliminate pen to pen variation) were thawed in a refrigerator (4 ± 1 °C). The breasts used for cooking loss were also used for exudative loss and were therefore weighed prior to being baked on the
test days. On days 0, 2, 4, and 6, six breasts per diet (one bird per pen) were baked at 190 °C on a metal pan under an aluminum foil covering for approximately 35 minutes until the internal temperature reached 76 °C, which was checked with a thermocouple. Breasts were cooled to room temperature and blotted to remove excess moisture. The breasts were then weighed and their cooked weight subtracted from their initial weights. The weight lost during baking was considered cooking loss. Cooking loss results were calculated and reported as a percentage of weight lost.

Textural Properties

On days 0, 2, 4, and 6 during storage, six breasts per diet were cooked then subjected to textural analysis using an EZ-Test Model Instron Instrument (Shimadzu Corporation, Kyoto, Japan) with a 10 N load cell and a crosshead speed of 50 mm/min. A wedged probe with a 12-mm cross-length (or diameter) specifically designed for this study was attached to the cell. Tenderness was measured by the compression of the cooked muscle tissue with the probe whose wedge was aligned perpendicular to the breast muscle fibers’ orientation. Two sets of data were obtained by manipulation of the compression: 1) a 5-mm depression of the muscle tissue where the maximum force registered was expressed as “firmness”, and 2) deep penetration of the probe into the muscle tissue until the muscle fibers were disrupted and the force required was defined as the “rupture force”. The rupture force measurements were performed on the same contact points of the muscle tissue where firmness measurements were done. For both firmness and puncture force five measurements were taken on each breast.
Sensory Panel Evaluation

The trained sensory panel was conducted to determine whether consumers could detect any difference among dietary treatments. An IRB exemption certification request was submitted and approved to complete a sensory panel for this study. The study involved 17 consensual participants ranging 21–70 years old. The age categories were as follows: six females < 40; four females > 40; three males < 40; four males > 40. To prime and heighten the participants’ senses, participants were given brief training prior to contact with the study’s samples. They were allowed to taste a control chicken breast, an over-cooked, dry and tough chicken breast, smell oxidized oil, and smell grass clippings. This training familiarized the participants with potential sensory properties the breast meat may have contained.

A hedonic rating scale (Figure 10) for sensory evaluation ranging from 1-7 was used to score the following organoleptic traits: chicken flavor, rancidity, off-flavors, tenderness, juiciness, and overall acceptability. To eliminate pen to pen variation, sampling breast meat for each diet was from birds originating from three different pens per diet. The breast meat was baked as previously described for cooking loss. A random three digit number was assigned to each sample. Participants were served two cubes of breast meat for each sample (3 birds per diet) identified only by the random three digit number on a paper plate. Samples were served warm to room temperature under a red light at individual stations.
Figure 10. The hedonic rating scale for the six sensory categories evaluated for the chicken breasts.

**Statistical Analysis**

One trial was completed with triplicate sample analyses. Data was analyzed in Statistix software 9.0 (Analytical Software, Tallahassee, FL) via analysis of variance (ANOVA) using the general linear model’s procedure and a randomized block design with each pen being a block to determine storage time effect. When a storage dietary treatment effect was significant, data was subjected to Tukey’s testing to determine the difference between the individual days considering $P < 0.05$ significant. Likewise, when the ANOVA using a completely randomized design detected a diet effect, the means between samples for each day were separated by the Tukey’s test.
Results and Discussion

Exudative Loss

Figure 11 shows the exudative loss of the breast meat over a 6 day study. Vitamin E has previously been used as an antioxidant feed supplement in both pork and broilers and shown to increase the oxidative stability of meat without impacting the water-holding capacity (Asghar, Gray, Booren, Gomaa, Abouzied, Miller, & Buckley, 1991; Li et al., 2009; Zhang et al., 2011). It was further reported that Vitamin E can reduce the exudative loss from breast fillets without affecting any other quality attributes (O’Neill, Galvin, Morrissey, & Buckley, 1998.) Thus, it is interesting that dietary treatment 1 (no SP-1 or EcoE but formulated with oxidized soybean oil) had higher exudative loss on all days which was statistically significant on days 4 and 6. Furthermore, diet 3, containing no SP-1, had numerically higher (yet not significantly) exudative loss on days 2 and 4.

It is worth noting that (as observable on day 6) the standard error bar is larger for diets 1 and 3, which appears to contribute to the lack of statistical significance from diet 4 on day 6. Therefore, both diets with SP-1 (diets 2 and 4) had less exudative loss than diets without SP-1 ($P < 0.05$). Breasts from dietary treatment 4 had lower exudative loss all three measurement days although only different ($P < 0.05$) on day 2 from diet 1. It is therefore concluded that broiler diets with SP-1 supplementation (diets 2 and 4) undergo less exudative loss than the diets with EcoE and SP-1 supplementation. However, diet 3 (with EcoE but no SP-1) also had a lower exudative loss than diet 1 (with only Vitamin E). This is remarkable because as previously stated, Vitamin E is capable of reducing the exudative loss from broiler breast meat during storage studies (O’Neill et al., 1998.).
Utilizing the combination of these supplements may be a novel method to preserve intermuscular water within the myofibrils to produce juicy, moist meat.

Figure 11. The exudative loss of broiler breast meat from different diets after storage at 4±1 °C for different periods. Same capital letters (A,B) denote no difference between the treatment groups on a specific day; same lowercase letters (a,b) represent no difference within the same treatment over the storage period \((P < 0.05)\). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

*Cooking Loss*

Figure 12 shows the cooking loss that the meat underwent over the 6 day storage period. Samples from dietary treatment 3 (containing no SP-1 but EcoE) had higher cooking loss on days 0, 2, and 6 \((P < 0.05)\) on days 0 and 2). This is consistent with the
exudative loss suggesting breast meat from broilers fed diets with SP-1 supplementation are capable of maintaining their myofibrillar water. Similarly, samples from diet 1 had higher cooking loss on days 0 and 6. Although there is less statistical evidence than exudative loss, a lower cooking loss may result when SP-1 is included in the diet.

These finding were consistent with the reports on broiler meat quality from diets supplemented with different fat sources (Pekel, Demirel, Midilli, Yalcintan, Ekiz, & Alp, 2012). A decrease in cooking loss with an increased percentage of incorporated dietary fat was observed for both fat sources. Pertaining to this study, as SP-1 incorporates more fatty acid deposits into the meat, a decrease in cooking loss is consistent with pervious results. Thus, more fat in diets may decrease the cooking loss. A possible explanation is the hydrophobic effect of inner and intra-myofibrillar water associations within meat. It is possible that additional fat drives water molecules to association together thereby preventing the meat from moisture riddance. An additional explanation is that fat may have an insulation effect on the water preventing it from oozing from the meat during cooking.
Figure 12. Cooking loss of broiler breast meat from different diets after storage at 4±1 °C for different periods. Same capital letters (A–C) denote no difference between the treatment groups on a specific day; same lowercase letters (a,b) represent no difference within the same treatment over the storage period ($P < 0.05$). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

**Textural Properties**

Results from the meat firmness tests are summarized in Figure 13, namely, the force (N) required for the probe to press into the breast meat for a 5-mm distance. On day 0 there is a markedly evident difference ($P < 0.05$) between the diets with EcoE (diets 3 and 4) than dietary treatments without EcoE (diets 1 and 2). However, that difference is not statistically different on day 2 and not apparent on days 4 and 6. As the firmness data...
was being collected, it appeared that the location of the applied force on the breast caused variation on the end force result.

Firmness can be described as the mechanical stability and the structural integrity of the meat (Belk, 2001; Delles & Xiong, 2014). If the myofibrillar proteins are denatured, juiciness and therefore tenderness of the meat may be affected (Delles et al., 2015; Liu et al., 2011) It is known that juiciness and tenderness of cooked meat are closely related (Huges et al., 2014; Otremba, Dikeman, Milliken, Stroda, Chambers, & Chambers, 2000). It is evident in Figure 13 that the firmness of the meat containing EcoE significantly decreased over the storage study \((P < 0.05)\) suggesting the activation of muscle endogenous proteases such as calpain (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010). There is also a non-significant decreasing trend on days 0, 2, and 4 for the other diets without EcoE. This is inconsistent with the trends from exudative loss and cooking loss. As water is lost from the meat, it is expected that more force would be required to depress 5 mm into the meat. This inconsistency could be caused by the structural integrity of the proteins altering as inter and intra myofibrillar muscle fibers lose water. The perception of a softer, tenderer mouth feel can occur as protein structure is degraded. Thus, the data suggests that the meat firmness for all diets decreases over the storage study although exudative and cooking loss increase.
Figure 13. Firmness (force registered upon a 5-mm deformation of the samples) of cooked broiler breast meat from different diets after storage at 4±1 °C for different periods. Same capital letters (A,B) denote no difference between the treatment groups on a specific day; same lowercase letters (a–c) represent no difference within the same treatment over the storage period ($P < 0.05$). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

The force (N) required for the probe to puncture through the myofibrils of the breast meat (“rupture force”) is shown in Figure 14. As with the firmness measurement, the rupture force has a trend of decreasing throughout the time study. This is consistent with results from a beef storage study (Grobbel, Dikeman, Hunt, Milliken, 2008), indicating the causative effect of muscle endogenous proteases irrespective of dietary treatments. Consistent with the muscle firmness testing result (Figure 13), breast meat from dietary treatment 1 (no SP-1 or EcoE) was ruptured at a smaller force than the other
three diets on days except day 0 where it is not significantly higher than diet 2. Although diet 1 is not significantly lower than the other diets on all of the days, data does suggest that the EcoE and SP-1 supplementation may cause the meat to be tougher. It has been reported that under oxidative conditions, calpain loses its activity rather quickly, therefore, is unable to tenderize meat during postmortem aging of meat (Carlin, Maddock, Lonergan, Rowe, & Huff-Lonergan, 2006; Kim, Huff-Lonergan, & Lonergan, 2012; Lund, Lametsch, Hviid, Jenson, & Skibsted, 2007). This might explain the high rupture force for dietary 4 meat that exhibits a relatively high level of oxidative potential (Figure 5).

Figure 14. The rupture force (force required to disrupt muscle fibers) of cooked broiler breast meat from different diets after storage at 4±1 °C for different periods. Same capital letters (A–C) denote no difference between the treatment groups on a specific day; same
lowercase letters (a–c) represent no difference within the same treatment over the storage period ($P < 0.05$). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

**Sensory Score**

Of the six sensory categories evaluated (chicken flavor, juiciness, off-flavors, tenderness, rancidity, and overall acceptability), off-flavors and overall acceptability were the only categories that were found significantly influenced by diets (Table 5). Breast meat from dietary treatments 2 and 4 (containing SP-1 supplement) had a higher rating (unfavorable) for off-flavors than meat from treatments 1 and 3 ($P < 0.05$). Similarly, meat from dietary treatments 2 and 4 received lower scores on overall acceptability than diets 1 and 3.

It's known that to maintain desirable sensory properties, such as flavor and overall acceptability, the concentration of antioxidants added into the feed, the oil quality, and the oil fatty acid profile, are all factors that must be considered and balanced to achieve meat with acceptable sensory outcomes (Bou, Grimpa, Baucells, Codony, & Guardiola, 2006; Jensen, Lauridsen, & Bertelsen, 1998; Jensen, Engberg, Jakobsen, Skibsted, & Bertelsen, 1997.) For example, a study utilizing flaxseed meal to enhance PUFA deposits in broiler breast meat found that the lipid peroxidation caused off-flavors and bad odors (Anjum et al., 2013). Although the current study did not receive comments for bad odors, with consideration of the negative oxidative impacts more unsaturated fatty acid deposits cause in meat, the results are consistent with the fatty acid profile show in Table 4 (found within the Lipid Oxidation section within the Results and Discussion in Chapter 3). The
higher amount of oxidatively unstable unsaturated fatty acids contributed to the lower overall acceptability of the breast meat from diets 2 and 4.

**Table 5.** Sensory panel results of chicken breast meat of the statistically significant categories.*

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-flavors</td>
<td>1.53B</td>
<td>2.17A</td>
<td>1.51B</td>
<td>2.49A</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>5.25A</td>
<td>4.59BC</td>
<td>4.90AB</td>
<td>4.29C</td>
</tr>
</tbody>
</table>

*Values represent mean scores; means without a common letter (A–C) differ significantly (*P* < 0.05). Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.

Some participants did note in the allotted optional section on the assessment forms. Diet 2 received 4 comments stating fishy flavors or aromas were detected including the comment, “Fish oil tastes strong so that I cannot rate it acceptable” and “Tastes like fish oil with chicken.” Diet 2 also received a comment on its “grassy” flavor. Diet 3 received two comments: one pertaining to a rancid odor and the other about a grass or eggy flavor. Similar to dietary treatment 2, meat from diet 4 received four comments about a fishy off-flavor. It is worth noting that diet 1, which is free of SP-1 or EcoE supplements, did not receive such negative comments.

Concluded from the statistics and comments on the sensory evaluation, dietary treatments 2 and 4 produced less acceptable, lower quality meat than the dietary treatments than did not have the added SP-1 supplement. Although meat from dietary treatment 4 (contained EcoE) had a lower overall acceptability than treatment 1 (no SP-1 or EcoE), it was not significantly different. Therefore, SP-1, especially in combination
with EcoE which already contains some SP-1 in its mixture, produces less acceptable meat.

**Conclusion**

The SP-1 and EcoE supplementation, individually or in combination, decrease the exudative loss of raw broiler meat during refrigerated storage over time, but a difference in cooking loss was not observed between diets. Of the four diets, non-stored meat (day 0) from EcoE-supplemented diets is firmer than meat from other diets and shows significant reduction in firmness during storage. This textural change does not correlate with tenderness testing that shows variable fiber-rupture forces across storage time and dietary treatment. The more remarkable effect of the algae dietary treatments (0.5%) is their apparent causation of meat off-flavors. The addition of 200 g/ton EcoE, while improving the texture of meat, was unable to eliminate the algae-originated off-flavor, indicating that more research must be conducted to elucidate the causes as well as to develop processing strategies to overcome the undesirable sensory trait.
CHAPTER 5
OVERALL CONCLUSIONS

Feed supplements applied to livestock feed are known to have profound impacts on the overall quality of meat. These supplements aid in the production of high quality, nutritious meat to fulfill consumers’ growing demand. Added sources of unsaturated fatty acids (such as SP-1, also included in the EcoE pack) increase the quantity of ω-3 polyunsaturated fatty acids deposits within the muscle tissue. Although healthier for consumers, these PUFA deposits can negatively impact the organoleptic quality of meat due to their susceptibility to oxidation, leading to an overall quality reduction and a shortened shelf life due to rancidity. Due to the expense of industry feed antioxidants (used to combat oxidative instability within muscle tissue) cheaper alternatives such as the EcoE pack produced by Alltech® could be a fiscally efficient and effective alternative dietary method to ensure high quality of broiler meat.

This thesis research revealed the impacts of the supplements SP-1, EcoE, and Vitamin E used in various combinations on the quality attributes of broilers’ breast meat subjected to a six day refrigerated retail display storage study. It was found that SP-1 supplementation increased the lipid oxidation of the meat. Furthermore, the dietary treatment containing both SP-1 and EcoE was observed to be the least oxidatively stable as it contained the most SP-1. Little evidence of different protein oxidative stability was observed although SP-1 at the 0.5% level in combination with 200g/ton EcoE may promote protein oxidation as seen via the formation of carbonyls. However, both SP-1 and EcoE supplementation were shown to significantly increase the water holding
capacity of the meat although this was not detected via the sensory panel. Additionally, EcoE pack supplementation increases the firmness of the meat although tenderness was less affected. The sensory panel did not detect a texture difference between the dietary treatments.

Feed supplemented with SP-1 manufactured with low quality oil significantly lowered the overall sensory acceptability of the meat and off-flavors were significantly detected. Possible explanations include the oxidized soy oil deteriorated the antioxidants or prevented Vitamin E deposits in the tissue, the added antioxidants’ failed to counteract rampant lipid oxidation due to the high concentration of PUFA in the meat, or, as the EcoE pack blend already contains some level of SP-1, EcoE in combination with 0.5% SP-1 (or 0.5% SP-1 alone) may contribute to the off-flavors causing the reported fishy taste.

Meat quality attributes such as shelf life stability, water-holding capacity, and tenderness are imperative attributes to consider for producing high quality meat. However, organoleptic properties, such as an apparent off-flavor, are major contributors to the consumer acceptability and ultimately repeat purchases. Therefore, to continue the exploration of utilizing SP-1 as a method to implement PUFAs deposits into the meat, further investigations must be conducted to establish said methods without lowering the overall sensory properties of the meat.
APPENDIX

Table 6. Mixing sheets of starter basal diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal 1</th>
<th>Basal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>56.55</td>
<td>56.30</td>
</tr>
<tr>
<td>Soybean meal (48% cp)</td>
<td>36.00</td>
<td>35.90</td>
</tr>
<tr>
<td>Vegetable oil (normal)</td>
<td>2.95</td>
<td>2.81</td>
</tr>
<tr>
<td>SP-1</td>
<td>0.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.42</td>
<td>1.42</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral premix (no Se)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix (no Vitamin E)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Table 7. Mixing sheet of treatment starter diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal 1</td>
<td>700</td>
<td></td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>Basal 2</td>
<td></td>
<td>700</td>
<td></td>
<td>700</td>
</tr>
<tr>
<td>Selenite premix (300 ppm)</td>
<td>319 g</td>
<td>319 g</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin E (250 IU/g)</td>
<td>63.9 g</td>
<td>63.9 g</td>
<td>12.8 g</td>
<td>12.8 g</td>
</tr>
<tr>
<td>EconomasaE</td>
<td>—</td>
<td>—</td>
<td>63.6 g</td>
<td>63.6 g</td>
</tr>
</tbody>
</table>

Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasaE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasaE + 10 IU Vitamin E.
### Table 8. Mixing sheet of grower basal diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal 1</th>
<th>Basal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>62.23</td>
<td>61.88</td>
</tr>
<tr>
<td>Soybean meal (48% cp)</td>
<td>30.90</td>
<td>30.85</td>
</tr>
<tr>
<td>Vegetable oil (normal)</td>
<td>2.81</td>
<td>2.70</td>
</tr>
<tr>
<td>SP-1</td>
<td>0.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1.58</td>
<td>1.58</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.23</td>
<td>0.24</td>
</tr>
<tr>
<td>Mineral premix (no Se)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix (no Vitamin E)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

### Table 9. Mixing sheet of treatment grower diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal 1</td>
<td>1100</td>
<td>1100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal 2</td>
<td>1100</td>
<td></td>
<td>1100</td>
<td></td>
</tr>
<tr>
<td>Selenite premix (300 ppm)</td>
<td>500 g</td>
<td>500 g</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin E (250 IU/g)</td>
<td>100.2 g</td>
<td>100.2 g</td>
<td>20.1 g</td>
<td>20.1 g</td>
</tr>
<tr>
<td>EconomasE</td>
<td>—</td>
<td>—</td>
<td>100.2 g</td>
<td>100.2 g</td>
</tr>
</tbody>
</table>

Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.
Table 10. Mixing sheet of finisher basal diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal 1</th>
<th>Basal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>67.04</td>
<td>66.61</td>
</tr>
<tr>
<td>Soybean meal (48% cp)</td>
<td>26.20</td>
<td>26.20</td>
</tr>
<tr>
<td>Soy oil (normal)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Soy oil (oxidized)</td>
<td>2.85</td>
<td>2.78</td>
</tr>
<tr>
<td>SP-1</td>
<td>0.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.30</td>
<td>1.30</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.45</td>
<td>1.45</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Mineral premix (no Se)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix (no Vitamin E)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Table 11. Mixing sheet of treatment finisher diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal 1</td>
<td>1740</td>
<td>1740</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal 2</td>
<td></td>
<td>1740</td>
<td>1740</td>
<td></td>
</tr>
<tr>
<td>Selenite premix (300 ppm Se)</td>
<td>791.5 g</td>
<td>791.5 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E (250 IU/g)</td>
<td>158.5 g</td>
<td>158.5 g</td>
<td>31.8 g</td>
<td>31.8 g</td>
</tr>
<tr>
<td>EconomasE</td>
<td></td>
<td></td>
<td>158.5 g</td>
<td>158.5 g</td>
</tr>
</tbody>
</table>

Diet 1 = Basal diet 1 + 50 IU Vitamin E; Diet 2 = Basal diet 2 + 0.5% SP-1 + 50 IU Vitamin E; Diet 3 = Basal diet 1 + 200 g/ton EconomasE + 10 IU Vitamin E; Diet 4 = Basal diet 2 + 0.5% SP-1 + 200 g/ton EconomasE + 10 IU Vitamin E.
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oil, α-tocopheryl acetate, and zinc supplementation on the composition and consumer

rate constants for reaction of hydrated electrons, hydrogen atoms and hydroxyl


oxidation, pH, and ionic strength on calpastatin inhibition of (micro)- and m-calpain.


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VITA

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