WOODY BREAST IMPLICATION ON BROILER MEAT QUALITY: A PIECE OF THE PUZZLE

A Dissertation

by

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ABSTRACT

Woody Breast condition (WB) is detrimental for water holding capacity of the meat (WHC). Two dietary experiments were conducted by varying the ingredients used to formulate traditional corn-soybean meal diets (SBM) in the attempt to minimize the negative impact of WB. In the first study, fast growing, high breast yielding broiler chicks were grown to d 49 and d 56 of age under simulated industry parameters. The birds were fed an iso-nitrogenous and iso-energetic traditional SBM diet, or a diet where the SBM was fully replaced by canola meal (CM), with *ad libitum* access to feed and water. After processing, the carcass yield (CY), breast yield (BY), drip loss (DL), pH, meat color (L*, a*, b*), and cook loss (CL) were determined to assess WHC. The incidence of WB severity of the fillets was also determined. In the second study, the same parameters were measured on d 42 birds fed four different diets. The diets consisted of a corn-soybean meal control (Ctrl), a Ctrl + β-mannanase enzyme (Ctrl+Mannan), a diet reduced in soybean meal and replaced with canola meal (CM), and a diet formulated to reduce corn and soybean meal with various ingredients (Mix). A third study was conducted to reduce the cooking times of the bigger breast fillets used to determine CL values using the oven method (OM), by using 3-D printed cutting molds to standardize the meat sample size. The data were subjected to χ^2 analysis for WB incidence, and ranked analysis of variance at an $\alpha=0.05$. In experiment 1, the CM at d 49 reduced ($p<0.05$) the % incidence of WB severity and minimized meat quality parameters issues. In experiment 2, the Mix diet produced the lowest ($p<0.05$) % incidence and severity of WB, but the harvested meat had a higher ($p<0.05$) pH value. In contrast, when analyzing WB severity, pH was highest ($p<0.05$) in the moderately affected fillets. In both studies, the reduction in WB was at the cost of BY. The modified OM was able to reduce cooking time and produce similar CL values to the OM.

Keywords: Woody breast, Water Holding Capacity, Cook Loss, Broiler Diets, Soybean Meal

DEDICATION

To my fiancé, and soon to be wife Daad Abighanem, who's love I will always be grateful for and appreciate. Without her kind words and support I would have lack the strength to push forth.

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NOMENCLATURE

$\chi 2$	Chi Square
a*	Redness
AA	Amino Acids
ADP	Adenosine Di-Phosphate
ANOVA	Analysis Of Variance
Arg	Arginine
ATP	Adenosine Tri-Phosphate
b*	Yellowness
BW	Body Weight
BY	Breast Yield
C	Celsius
Cal	Calories
CI	Critical Limit
CL	Cook Loss
CM	Canola Meal
CP	Crude Protein
Ctrl	Control
CV	Coefficient of Variation
CY	Carcass Yield
Cys	Cysteine

d	Days
DFD	Dark, Firm, Dry
DL	Drip Loss
DPM	Deep Pectoral Myopathy
FCL	Fiber Change Length
FCR	Food Conversion Ratio
FSIS	Food Safety and Inspection Service
g	Gram
GAA	Guanidine Acetic Acid
GLM	General Linear Model
Gly	Glycine
H	Height
h	Hours
K	Kelvin
Kg	Kilogram
L	Length
L*	Lightness
lb	Pounds
Lys	Lysine
Mannan	Mannanase
max	Maximum
MBM	Meat Bone Meal

Met	Methionine
min	Minimum
mm	Millimeter
MOM	Modified Oven Method
n	sample size
NSP	Non-Starch Polysaccharides
OM	Oven Method
OR	Odds Ratio
P	Phosphorus
p	Probability Value
P.minor	Pectoralis Minor
PBP	Broiler By-Product
PER	Protein Efficiency Ratio
PSE	Pale, Soft, and Exudative
ROS	Reactive Oxygen Species
SAA	Sulfur Amino Acids
SBM	Soybean Meal
sd	Standard Deviation
se	Standard Error
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine

v	Version
W	Width
WB	Woody Breast
WHC	Water Holding Capacity
wk	Week
WOG	Without Giblets
WS	White Striping
Wt	Weight
α	Alpha
T	Tao

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1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

In the last five years, the global poultry industry has witnessed the emergence of a breast meat condition known as woody breast (WB) that negatively affects the quality of the broiler meat. The condition has been identified as a myopathy that elicits multiple symptoms, but no direct cause has been identified (Trocino et al., 2015). Tijare et al. (2016) developed a categorization method commonly used in research to identify the different stages of WB onset in breast fillets. The main categories are unaffected a.k.a. "normal" (0), mild (1), moderate (2), and severe (4) WB. In severe cases, the breast fillet affected by WB presents a pale appearance, petechial hemorrhaging, accumulation of exudate on the surface, and a hard cranial and caudal portion of the breast (Sihvo et al., 2014; de Brot et al., 2016). The scattered, small, pinpoint blood spots and gelatinous exudate portions of the fillets are trimmed and represent an economic loss to companies (FSIS, 2018). Additionally, the WB condition has also been observed to impact the water holding capacity (WHC) of the breast fillets. The WHC of the meat is essential for determining the functionality of the meat for further processed products, and customer acceptability. Thus, determining ways to mitigate the effect of a muscle condition such as WB on the WHC is essential to minimize economic loss (Mudalal et al., 2015; Sihvo et al., 2016; Soglia et al., 2016a).

Experiments that manipulate the diet formulation and management of broilers have been proposed as interventions to reduce the incidence and severity of WB (Trocino et al., 2015; Bordignon et al., 2017; Cruz et al., 2017; Simoes et al., 2017; Cemin et al., 2018; Livingston et al., 2018b; Bodle et al., 2018; Meloche et al., 2018). The efficacy of these numerous manipulations has varied. A commonality among the previous research is the use of feed composed of similar ingredients: corn-wheat, soybean meal, distillers dried grains with solubles, and supplemental amino acids and minerals, which are comparable to the diets suggested by the primary breeders (Aviagen, 2014). The most ubiquitous source of protein in broiler diets is soybean meal (SBM), as it provides a high content of protein (>36%), and contains a desirable conformation of required amino acids (NRC, 1994; Baker, 2009). However, other sources of proteins (canola, blood, and corn meals) are available and are used sparingly depending on availability, cost, digestibility, and amino acid composition (Baker, 2009; Córdova-Noboa et al., 2018). The purpose of this study was to test new sources of proteins and carbohydrates from feed ingredients not commonly used in the U.S.A. for poultry diet formulation, and to determine how they impact the WHC of the breast meat in relation to the WB severity and incidence (%). A secondary objective was to develop an improved method to test cook loss (%), a WHC parameter, that would aid in the determination of WHC in future research.

1.2. Water Holding Capacity of the Breast Meat

Water holding capacity (WHC) is a measurement of meat quality and it is defined as the ability of the muscle to retain free water in the system (Fennema et al., 1996; Lee et al., 2008). The higher the content of water in the meat, the more tender and juicy the product is, which is related to higher consumer acceptability (Alvarado and Sams, 2004a; Saha et al., 2009; Kuttappan et al., 2012b; Petracci et al., 2012).

Water is found in three states in muscle: bound, immobilized, and free (Fennema et al., 1996). Water found in its bound state in food is chemically attached to the food structure and does not interact with other biochemical processes (Fennema et al., 1996). In the immobilized state, water is involved in biochemical activity occurring around the meat proteins, and is held in close vicinity of the proteins through hydrogen bonds and ionic charges (Fennema et al., 1996; Barbanti and Pasquini, 2005). Free water is usually the majority of the water found in the muscle and is weakly held in the muscle by physical forces (i.e. capillary forces, hydrogen bonds) (Fennema et al., 1996; Tornberg, 2005).

The free water and immobilized water content in the meat constitute the main portion of the water that is measured when WHC tests are performed. These water states can be affected by the transportation and processing of the live birds, as well as by the handling and occurrence of meat myopathies in the broilers (Owens et al., 2000; Barbut, 2015a). Inappropriate transportation and processing of the birds leads to breast meat with lower WHC and in some cases can induce pale, soft, and exudative (PSE), or dark, firm, and dry (DFD) meat (Alvarado and Sams, 1995, 2002; Owens et al., 2000; Barbut, 2009). Similarly, breast myopathies such as green muscle disease, white striping (WS), spaghetti breast, and WB, are reported to lower WHC of the meat. Understanding and measuring the impact all of these variables have on the water content in breast meat is of interest to the industry and the research communities.

There is no direct way of measuring WHC, as multiple factors impact the ability of the muscle to retain water (Gorsuch and Alvarado, 2010; Petracci and Baéza, 2011). However, multiple measurements can be used to assess the interaction of water with proteins, thus establishing a complex assessment of the quality state of the meat proteins. These measurements can involve determining the impact of final pH, drip loss, cook loss (CL), and the color of the meat matrix. The ability to acquire fast, consistent, and accurate measurements of the WHC parameters allows the researchers to more readily generate an overall assessment of the quality of meat.

1.2.1. Final pH in the breast meat

The final pH of the meat is positively correlated with WHC (Alvarado and Sams, 2004a; Fanatico et al., 2007; Tijare et al., 2016). During exsanguination, the start of *rigor mortis* disrupts oxygen and nutrient supply to the muscle. The muscle's cells continue to function after exsanguination and demand ATP for their metabolism and muscle contraction (Owens et al., 2000). In order to supply the ATP, the cell switches from aerobic to anaerobic respiration which depends on the glycolysis cycle. Muscle glycogen is broken down into glucose that is utilized by the cell to generate ATP needed by the muscle. During this process, lactic acid accumulates in the tissue and the cells inability to remove the lactic acid causes a drop in pH. The end of *rigor mortis* is marked by reduction of the initial pH of the muscle (~7.2) to a final pH of 5.8 - 6.00 (Owens et al., 2000; Barbut, 2015a). However, environmental factors can cause the meat proteins to be closer to their iso-electric point (~5.1-5.2), thus lowering protein-water interaction due to the loss of ionic charges (Fennema et al., 1996; Owens et al., 2000).

Proteins have a net positive or negative charge depending on the surface amino acid residues that conform them (Fennema et al., 1996). When the negative and positive charges are present in equal amounts a net zero charge is obtained, known as the isoelectric point. It is at this point that protein-water interaction is almost nonexistent which explains the loss in WHC (Fennema et al., 1996).

There are multiple pre- and post- slaughter events which affect the final pH of the meat and the WHC. Birds that are stressed prior to slaughter can develop a pale, soft, and exudative-like condition (PSE-like) causing the pH to drop due to rapid glycogen depletion and accumulation of lactic acid (Alvarado and Sams, 1995). The contrary can occur when prolonged stress prior to slaughter occurs, in which breast meat develops dark, firm, and dry (DFD) meat. This is a condition where insufficient amounts of glycogen stores are present in the muscle during rigor, preventing anaerobic glycolysis and lactic acid formation, which leads to higher pH values (>6.00) (Barbut, 2015a). Beauclerq et al. (2017) compared two genetic lines of broilers, one selected for higher muscle pH (+pH) and one for lower pH (-pH). Genes related to glycogen pathway were upregulated in the -pH line, causing the final pH to drop to 5.55, compared to 6.34 final pH for the +pH meat. Moreover, improper processing practices during the onset of rigor can also impact the final pH. Slow chilling of the carcass may lead to lower final pH due to high carcass temperatures that induce rapid glycolysis in the muscle, leading to increased accumulation of lactic acid which induce PSE-like conditions (Alvarado and Sams, 2002). Thus, proper handling and processing techniques are required to allow for proper muscle conversion to meat resulting in improved meat quality.

1.2.2. Drip loss percentage

Drip loss percent (DL) represents the moisture loss from gravitational forces that meat experiences during storage. There are multiple variations to the method proposed to assess this measurement (Pearson and Dutson, 1995), all of which include holding the breast meat under refrigeration for 24 h. The weight loss difference is then calculated to determine the % of DL. The higher the DL, the lower the WHC of the meat. Multiple factors can affect the drip loss values and contribute to experimental variation. The sample mass and shape of the fillet can change the force being applied to the individual fillet increasing variation within samples (Pearson and Dutson, 1995). The meat's age post-mortem together with the time the sample is left to drip under refrigeration can affect the results, with older meat requiring longer drip time to produce reliable data (Pearson and Dutson, 1995). Additionally, warmer storage temperatures (>4 C) will produce higher DL.

Muscle composition can also produce differences in DL results. Tissue that has higher concentration of collagen and fat (e.g. thigh meat) will have higher DL compared to cuts (e.g. breast meat) with higher protein content (Wyrwisz et al., 2012). Sun et al. (2018) compared the DL of unaffected fillets to mild or severe WB fillets over 8 days of storage, and reported that unaffected fillets had the lowest DL of the three groups across all time points. This is likely due to the increase in fat and collagen of WB fillets, as these components do not retain or bind water.

Additionally, a negative correlation between DL and pH values exists. As explained in section 1.2.1, as pH nears the iso-electric point of myosin and actin, there are fewer charges to bind water, which leads to an increase in the DL of the meat. Thus, DL in meat samples can be partially explained by changes in pH. In Beauclerq et al. (2017), a broiler line selected for lower pH (5.55) had higher DL % (5.1%) compared to the DL (1.80 %) of a line selected for higher pH (6.34). The results of DL results observed under laboratory conditions provide a guidance of the possible difference that may be observed between one treatment vs. another under actual processing and handling conditions.

1.2.3. Color in poultry meat

The human eye can observe the electromagnetic radiation between 390 nm and 700 nm in wavelength, also known as the visible spectrum of light, with lower wavelengths rendering the blue color and higher wavelengths the red color. Color can be measured using a color scale such as the CIELAB. This scale determines the lightness in the gray scale (L*), the red and green hue (a*), and the yellow and blue hue (b*). The actual color of an object will depend on many factors, including composition, shape, texture, and illuminant. Higher concentration of fat and lower WHC in the meat can alter the measureable lightness of the meat (Harford et al., 2014; Tomažin et al., 2018). Similarly, slow-twitch muscles with higher concentration of myoglobin will appear redder than myoglobin-poor fast twitch muscle (Lilburn et al., 2018). The source of light, known as the illuminant (e.g. sun light, yellow sodium lamps), can also interfere with color observation. For example, sodium lamps can make red color appear dark brown (Hunt and

Pointer, 2011). Considering that the perception of the human eye can vary across individuals, an objective method needs to be used when assessing the color of meat. Such a method is well described by the International Commission on Illumination (CIE, following the French initialism) and is based on the color space. The color space is a numerical arrangement of different colors that can be measured within a scale. Multiple scales exist, the most common one used in meat is the CIE 1976 (L*, a*, b*) color space, which determines the color of the sample in relation to a three-point measurement of the sample in association with the lightness of the colors (Hunt et al., 1999). The measurements represent the white to black in the y axis (L*) and the rest of the colors in the x (a*) and z (b*) axis (red to green, and blue to yellow, respectively), with every color found within the scale being relative to the lightness. Moreover, these measurements can be impacted by the color being reflected around the sample, as well as the source of light used.

Sunlight contains the full spectrum of visible light, but can be affected by atmospheric changes, thus making it an inconsistent source of illumination. On the other hand, artificial sources of light vary in spectrum conformation depending on the method of producing light and their chemical composition. Xenon lights are commonly used as a source of light in colorimetric equipment, as they provide similar spectrum power levels across all the viewable spectrum length (Konica Minolta Inc., 2017). Both sunlight and artificial sources of light are dependent on the spectral power of each source, which is determined by the color temperature in kelvin (K). Color determination is relative to the color temperature of the illuminant, thus standardizing the illuminant used in measuring is necessary to obtain reproducible results.

In measuring the color of poultry meat, the most common illuminant cited in the literature is the illuminant C (Hunt and Pointer, 2011). Although the C illuminant continues to be used in poultry research, a more relevant illuminant is the D65, as promoted by the CIE (Petracci and Baéza, 2011). This illuminant provides a closer representation of daylight illumination. More importantly, and in order to obtain comparable results, studies on meat quality need to name the illuminant used to measure the color of the meat (Petracci and Baéza, 2011). Additionally, by selecting appropriate illuminants retailers can showcase the meat appropriately to consumers.

The particular conformation of a muscle can impact the measured color. A muscle's conformation can change according to the muscle group and level of activity (Qiao et al., 2001; Gorsuch and Alvarado, 2010). Muscles engaged in constant movement have larger amounts of Type I muscles fibers that require oxygen to produce ATP. These muscles also have larger quantities of myoglobin, the heme chromophore group concentration of which produces a red coloration of the tissue. Additionally, these muscles can have extracellular fat storage which may impact the color of the sample. In contrast, muscles with large quantities of Type II fibers rely on the glycolytic potential of the muscle for energy, which does not require oxygen for energy production, thus less myoglobin is required. In poultry, the breast meat is primordially composed of Type II fibers, which have less red color compared to the leg quarters, which are mainly Type I fibers that produce a red color.

Aside from conformation of the muscle, the dimensions, biochemical and biological conditions can cause changes in meat color. Bianchi and Fletcher (2002) reported changes in color result from chicken breast samples that were thinner than 1 cm, due to the background color effect. Thus, to reduce the background interference, color measurements must be performed on breast fillets that are thicker than 1 cm and with a white background (Bianchi and Fletcher, 2002). The pH of the meat can also affect the L* values, lower WHC caused by lower final pH values (<5.8) in the meat lead to higher L* values (Alvarado and Sams, 2004a; Beauclercq et al., 2017). The increases of L* response occur due to the denaturing of sarcoplasmic proteins which increase water on the muscle surface at the time of color measurement causes light scattering. (Casco et al., 2013). The contrary occurs when DFD meat is present, where water is tightly held between the muscle fibers and more light is absorbed by the tissue, producing a lower L* response (Fennema et al., 1996). Moreover, spoilage bacteria can also impact the color of the meat. Spoilage bacteria can degrade proteins in the muscle tissue and cause color changes by increasing the concentration of hydrogen sulfide or peroxide, which generates a green color in the meat at high pH values (>6.0) (Pearson and Dutson, 1995).

The color values along the a* and b* axis are less likely to fluctuate for breast meat, but improper processing (e.g. bruising during catching) or diets can change the proximal composition of the meat (Heath and Wabeck, 1975; Perez-Vendrell et al., 2001; Lyon and Lyon, 2002; Bowker and Zhuang, 2013; Barbut, 2015b). The a* value can be affected by any change in myoglobin and hemoglobin concentrations in the muscle. Improper stunning and bleed out of the birds can lead to higher hemoglobin concentration in the muscles, increasing the a* values (Barbut, 2015a). Changes in diet formulation, particularly of the ingredients high in carotenoids, may lead to the deposition of a different pigmentation in the meat tissue, thus changing the b* values (Córdova-Noboa et al., 2018).

In summary, color values provide an interpretation of the WHC of the muscle and other possible changes in tissue brought by conformation, processing practices, and diet formulations. Additionally, color measurements also provide a non-destructive sampling method that is correlated with consumer acceptance and WHC of the meat (Qiao et al., 2001).

1.2.4. Cook loss of broiler meat

Cooking of animal proteins serves as a method to assess the WHC of meat by establishing an association of the state and quality of the meat proteins with their ability to retain the natural water of the muscle. The cook loss (CL) method aims at determining the difference between the raw weight of the meat and its cooked weight in order to obtain a percentage of weight loss. The higher the percentage of weight loss, the lower the WHC of the meat, which is related to undesirable meat quality (Owens et al., 2000; Barbut, 2015a). The differences in CL observed between tested samples can help indicate the impact of diet change, growing environment, breed, processing methods, or marination ingredient on the WHC of the meat, and ultimately on the quality of the sample.

The process of cooking consists of applying energy to a food substance, which induces a chemical change in the structure of the food. In particular for meat proteins, the increase of energy from outside sources breaks hydrogen bonds, van der Waal's forces, and electrostatic interactions between the proteins, allowing the proteins to aggregate and form a meat gel (Box et al., 1972; Tornberg, 2005). A meat gel consists of randomly organized protein structures that have created stronger covalent bonds between each other. During the process of protein-protein association the hydrophobic and hydrophilic regions of the proteins obtain an equilibrium that allows for the creation of a matrix where water can be held (Tornberg, 2005). If, in the process of forming the meat gel, the association of proteins is larger than desired, an imbalance of the colloidal gel can occur leading to precipitation of the protein. This is due to rapid aggregation of the meat proteins. A meat gel that has rapid aggregation creates larger spaces in the matrix and longer protein strands, thus reducing the capillary forces, and causing a lower WHC (Van Der Sman, 2013; Deb-Choudhury et al., 2014). In contrast, a balanced associated gel will have smaller spaces and shorter, less organized, aggregated protein strands. During the cooking process, the aggregation of meat proteins can be influenced by multiple extrinsic and intrinsic factors that can affect the CL measurements.

The extrinsic factors are the conditions outside of the meat that can impart variation on the cooking process. These factors include the method of cooking, the source of energy, and environmental conditions. The three main cooking methods for animal proteins are grilling, oven roasting, and water bath (Sous-vide), with the latter two methods being most commonly used to determine the CL of meat samples.

The cooking equipment used in the oven and water bath methods transfer the energy from their heat source in a direct or indirect form, respectively. If the heating process from these methods is not controlled excessive protein aggregation can occur.

When direct heat (i.e. grill) is used to cook the meat, high temperature (>200 C) is directly applied to the tissue, causing the proteins on the surface of the meat to denature at a faster rate than the proteins in the middle (Van Der Sman, 2013; Barbut, 2015a). In contrast, an indirect heating source (i.e. oven) has to convey the energy through the air or other fluid to reach the tissue and takes longer to cook (Pearson and Dutson, 1995). The fluid dynamics play an important role in dispersing heat evenly to minimize the cold and hot spots that can occur in ovens (Verboven et al., 2000). It has been shown that meat cooked to the same internal temperature with an indirect heat source has a lower cook loss value when compared to direct heating (Pearson and Dutson, 1995; Aguirre et al., 2018). Similarly to direct heating, the outer part of the meat tissue cooked with an indirect heat source will always be further denatured than the center. This is due to the heat transfer coefficient, which is related to the environment's higher temperature setting (e.g. 177 C) in relation to the temperature of the meat (e.g. 4 C) (Honikel, 1998; Van Der Sman, 2013). The larger the differences between these two temperatures, the larger the amount of protein aggregation occurring at the surface of the meat will be by the time the innermost layer of proteins gel. Research has shown that the two factors that affect CL the most are heating rates (temperature/time) and the ultimate final temperature of the meat (Pearson and Dutson, 1995).

The environment also has an effect on the transfer of heat to the meat, and can impact the rate of protein denaturation and water loss. When air is used as the medium to transfer heat, forced air from convection-type ovens can disperse heat evenly across the surface of the meat by disturbing the insulating layer of air over the meat sample, increasing the heat transfer rate on to the product (Van Der Sman, 2013). Higher airflow directly over the meat samples increases evaporation rates, which leads to higher CL. This loss of water vapor can be minimized by saturating the air with water, thus increasing the relative humidity. The saturated environment will limit the amount of water that can be drawn from the meat into the air, which in turns improves the heat transfer rate of the air due to the water's higher efficiency at conveying the energy.

The CL values of a meat product can be affected by intrinsic factors such as genetics, diets, and physiological conformation of the meat. The physiological conformation of the meat consists of proteins, collagen, fat, and structural dimension of the sample (Scheuermann et al., 2003). The proteins actin and myosin, found in the myofibril, are the most functional proteins of the meat in regards to WHC as they can interact with the water molecules through ionic charges. Any changes in the content or functionality of actin and myosin proteins will affect the WHC. Protein content of the meat can be lower when higher percentages of fat and collagen are found in the meat sample. The fat in broiler breast muscle typically averages between 1 and 2 %, but can be affected by bird genotype and production systems, as well as by diet composition (Qiao et al., 2002; Fanatico et al., 2005; Husak et al., 2008). The diet can impact the fatty acid composition of the fat which affects its melting point (Wood and Enser, 1997). Fats with lower melting points cause a higher fat loss during the cooking process thus producing higher CL values (Wood et al., 2003). Collagen, a protein with low functionality with regards to WHC, can also be impacted by the heating process and environment. Collagen is a small fraction of the breast meat (1% - 2% by wet weight of the breast) and consists of proteins that are not soluble in water or brine solutions. Collagen proteins are denatured at high temperatures (<60° C) and gelatinize at 80° C under high humidity.

The dimensional (length x width x height) differences observed between breast fillets in the field can impact the rate at which heat travels through the meat. In both processing plant and research applications, the differences in dimensional height can increase the cook times of the breast fillet in order to reach the desired internal temperature at the center most point of the cranial area. It has been shown that the longer a meat sample cooks, independently of the heating temperatures, the higher the observed CL (Pearson and Dutson, 1995). Additionally, the breast fillets with taller/thicker profiles have not been shown to contain an increase in mass compared to lower-wider fillets; however, these taller fillets may have differing muscle density leading to changes in cooking time and loss of water.

Market preferences for larger bird weights and higher breast yields have been tightly correlated to taller, wider, and longer breast fillets (Scheuermann et al., 2003; Kuttappan et al., 2017). This increase in larger birds has also increased the incidence of WB, a condition that has changed the compositional and dimensional features of the breast muscle compared to non-WB fillets (Mallmann et al., 2016; Tijare et al., 2016; Kuttappan et al., 2017). The reported physiological changes in WB afflicted fillets include the increase in hardened texture and changes in the dimensions of the fillet such as the height of the cranial region. In addition, the proximate analysis or composition of the fillets differ with WB fillets having lower protein (22.06% vs. 24.66%), higher collagen (1.42% vs. 0.85%), lower fat (1.07% vs. 1.15%), and higher moisture (77.26% vs. 73.78%) and hardened tissue, when compared to unaffected fillets (Soglia et al., 2016a; Cai et al., 2018). The changes are more evident on the severe WB compared to the mild and moderate severities, but all can impact the CL results of the meat samples due to the physical changes.

To assess the CL, two main methodologies have been used. The first method, water bath, consists of taking a piece of meat with known dimensions and weight, vacuum packing it, and cooking it in a water bath at a temperature not higher than 80 C (Pearson and Dutson, 1995; Tornberg, 2005; Van Der Sman, 2013). The second cooking method, oven methods (OM), consists of placing the whole breast fillet on a metal grill within an aluminum cover metal pan in a convection oven at 177° C (Sams, 1990; Casco et al., 2013; Tijare et al., 2016). Both methods cook the fillets to a predetermined internal temperature and integrate different aspects of the extrinsic factors that impact CL results. As long as the extrinsic factors are controlled, the method of cooking will produce reproducible CL values, but it is important to establish the heating rate and end internal temperature of the meat more so than the heating method (Pearson and Dutson, 1995; Petracci and Baéza, 2011; Van Der Sman, 2013). Broiler meat is typically cooked to the internal temperature (73 C) known to eliminate the pathogens of concern, Salmonella *spp.* (Casco et al., 2015). Cooking above this temperature causes increase water loss and affects tenderness and juiciness of the cooked product (Bouhrara et al., 2011; Van Der Sman, 2013). Heating rates establish the speed at which protein denaturation occur within the meat structure. Slow heating rates increase the time needed to reach the internal temperature and cause higher water loss, conversely, fast heating rates denature the protein to a tight arrangement of the meat gel increasing water loss (Bouhrara et al., 2011; Van Der Sman, 2013).

The OM uses the heat and air velocity generated from gas or electric convection ovens to heat, as evenly as possible, up to six aluminum covered steam table metal pans(dependent on oven and pan size), in which the fillets are placed. The amount of fillets in the pans varies considerably depending on dimensions of the fillets. The meat is heated gradually as the metal pans radiate heat inward, causing the fillets to lose water through evaporation and drip. The ambient air within the pan saturates with water, as the evaporation is limited by the covered pans, increasing the heat transfer rate into the meat (Lowe, 1964). A higher heat transfer coefficient can shorten cook times of the fillets, which can limit over cooking of the surface layers of proteins (Van Der Sman, 2013).

Over the last couple of years, bird breast size has increased, thus extending the cook times. Cook times used to range between 40 and 60 min (Casco et al., 2013), and currently range between 60 and 100 min, when the end internal temperature of the fillets is 73° C at an oven temperature of 177° C. In addition, the number of pans and oven cycles required to cook an experimentally and statistically sound (n) number of fillets have also increased since the larger fillets take up more space within the pan. Aside from the possible impact on cooking rates, the increase in cooking time presents a logistical issue from the experimental perspective. The OM is used on large sample sizes (>100 experimental units) that are processed at a specific time point post-mortem. Based on the oven capacity and the cooking time frames mentioned above, this equates to 12.5 pans or three cooking cycles lasting between 1.3 h - 2 h. The bigger fillets would equate to 16.6 pans or three cook cycles lasting between 2.7 h – 4.6 h. These time frames are in addition to the sample preparation. The addition of extra pans and multiple oven cycles further adds variation to the experiment.

As mentioned previously, oven design plays a major role in even heat distribution and loading causes cold and hot spots within the oven. Thus, each pan and oven cycle presents an inherent source of variation within the CL measurements (Verboven et al., 2000). In an attempt to control the variation of the fillets, the fillets are segregated into groups of 100g increments. This preparation ensures fillets within a pan cook at similar rates. With the increasing demand for larger birds and increased incidence of WB-fillets and associated changes in length, width, and height of the breast fillets of modern broilers, a modification to the OM that reduces cook times and possibly limits the experimental sources of variation should be pursued.

1.3. The Woody Breast Condition

1.3.1. Breast meat abnormalities

Poultry continues to grow as the main meat source of animal protein globally. The U.S.A. per capita consumption of chicken reached 93 lb per person in 2018 (National Chicken Council, 2019a). Broiler slaughter volumes have grown by an annual average of 2.0% from the year 2000 to 2018 volumes, and live broiler weights have also increase annually in average by 1.65% in the same time period (National Chicken Council, 2019b). The increase in weight has been achieved through a combination of methods to optimize the broilers' genetic, feed conversion, and environment, allowing the industry to meet demands and optimize economic gains (Petracci et al., 2013).

The demand for chicken meat, and in particular breast meat, continues to increase. To meet this demand, the live bird's genetic traits continue to be advanced through genetic selection programs that focus on higher breast yields, larger birds, improved feed efficiencies, and faster grow-out periods (Fanatico et al., 2007; Petracci et al., 2015; Russo et al., 2015). Physiological abnormalities that may impact the quality of meat have appeared with the continuous pursuit of improved bird performance. In the past three decades, several muscle conditions have surfaced in the breast meat of broiler chickens. These abnormalities in broiler breast meat include: Deep Pectoral Myopathy (DPM), Pale, Soft, and Exudative meat (PSE), White Striping (WS), and Woody/Wooden Breast (WB) (Richardson et al., 1980; Alvarado and Sams, 2004b; Bianchi et al., 2006; Kuttappan et al., 2012a, 2013; Lien et al., 2012; Petracci et al., 2013; Mazzoni et al., 2015).

Deep pectoral myopathy, also known as "green muscle disease", was discovered about 30 years ago (Siller, 1985), and still occurs in birds that reach processing weight within 42-56d, have high breast meat yield, and are considered "fast-growing" broilers (Fanatico et al., 2007; Tijare et al., 2016). This pathology has not been observed in slow or medium growing broiler strains, but can be induced in model controlled grow-out conditions (Lien et al., 2012). The cause of the abnormality has been associated with a constriction of the vascular system in the pectoralis minor (P. minor) muscle caused by exercise (i.e. wing flapping) (Lien et al., 2012). The P. minor lies within the fascia membrane between the sternum and the Pectoralis major. Wing flapping causes exercise-induced inflammation of the muscle exerts pressure into the P. minor contained within the fascia. With little room for expansion, the increased pressure occludes the vascular system, and eventual blood supply subsides, inducing a necrotic state in the muscle cells (Bianchi et al., 2006; Lien et al., 2012; Petracci et al., 2015). Minimizing stress that would induce wing flapping during grow-out can diminish the occurrence of DPM at the processing plant (Lien et al., 2012).

Pale, soft, and exudative meat is a condition in which a faster decline of pH in the muscle occurs during slaughter and prior to chilling (Barbut, 2009). The occurrence of PSE in broilers is mainly due to environmental stressors such as ante-mortem stress (i.e. heat stress, handling, and transportation), and post-mortem processing conditions (i.e. stunning and rate of chilling), which cause a rapid glycogen consumption in the muscle increasing the concentration of lactic acid (Alvarado and Sams, 1999; Owens et al., 2009). When these factors are not managed, higher incidences of PSE are observed at the processing plant. The meat from PSE birds is characterized as having a lower pH, softer texture, and high amounts of surface water that contributes to the paler appearance of the meat. (Alvarado and Sams, 1999, 2002, 2004b; Owens et al., 2009). Consumers are less likely to purchase meat that is lighter in color and which exudes water (Barbut, 2009). Lower functional properties (e.g. increased DL, CL, poor protein functionality and toughness) of the meat are observed in PSE-like affected meat (Alvarado and Sams, 1999; Barbut, 2009; Owens et al., 2009; Gorsuch and Alvarado, 2010).

White striping (WS) in broiler muscle is identified as white lines of adipose tissue that run parallel to the muscle fibers. A classification scale categorizes WS into three categories (normal, moderate, and severe) (Kuttappan et al., 2012a). Severe WS breast fillets have detrimental effects on consumer acceptability and desire to purchase in retail settings (Kuttappan et al., 2016). In addition, lower WHC and higher fat content have been observed in the breast muscle with severe WS (Petracci et al., 2013, 2015; Tijare et al., 2016). In the live bird, WS is categorized as a muscle degenerative abnormality in which higher rates of muscle fiber degeneration, inflammation, and fibrosis are observed (Petracci et al., 2013, 2015). Higher incidences of the WS abnormality are observed in male broilers that are selected for high breast yield, fast growth rates, and heavier weights (Kuttappan et al., 2012a).

Woody breast (WB) is a muscle condition that has surfaced recently in commercial genetic lines of high breast yield and fast-growing broilers (Sihvo et al., 2014). Woody breast is described as having a rigid feel when handled, with visual ridges along the length of the breast fillet, and a pale colored surface with a wet appearance (Sihvo et al., 2014; Tijare et al., 2016). As with WS, WB has been categorized depending on the hardness and flexibility of the muscle (0 = unaffected a.k.a. “normal”, 1 = mild, 2= moderate, 3 = severe) (Tijare et al., 2016). The incidence of WB has been reported from 10% up to 50% in commercial flocks, and it has been observed to be present with WS (Sihvo et al., 2014; Petracci et al., 2015; Tijare et al., 2016). Woody breast has been described as a muscle degenerative condition, with increased inflammation and fibrosis. In addition, some muscle regeneration has been observed with changes to gene expression indicative of upregulated fiber type switching gene expression (Sihvo et al., 2014; Mutryn et al., 2015; Tijare et al., 2016). The WB condition is still not well understood as to how or what causes these physiological conditions to appear selectively in the breast muscle and not in other muscle groups.

A common trend observed in the appearance of DPM, WS, and WB in broilers is a state of muscle degradation. The degradation of the meat causes disruption of the protein structure that affects WHC which leads to an increase of DL and CL. In addition, conformational changes in protein, fat, and collagen have also been observed (Sihvo et al., 2014; Mutryn et al., 2015; Tijare et al., 2016). With changing lean muscle protein content, less functional protein is available to hold water. Selection of birds that are fast growing, heavier final weights, and larger breasts are also commonalities within the muscle abnormalities mentioned above.

1.3.2. Histology of the woody breast condition

The first mention of WB as a muscle condition was by Shivo et al. (2014), where the macroscopic and microscopic aspects were described. Macroscopically, the muscle structure has a hardened appearance that was also identifiable through palpation. This hardness allowed Trocino et al. (2015) to classify the WB condition into four categories (0 = unaffected a.k.a. “normal” fillets, 1 = mild WB, 2 = Moderate WB, and 3 = severe). In addition to the hardness, the WB fillets have a superficial accumulation of semi-viscous fluid underneath the fascia, and superficial petechial hemorrhages in the moderate and severe cases. The presence of WS has also been observed when WB is detected, leading to the speculation that they are intra-related conditions (Zotte et al., 2017), but no direct correlation has been reported (Sihvo et al., 2014, 2016; Mutryn et al., 2015; Lilburn et al., 2018).

Microscopically, the tissue of WB fillets has several etiologies being manifested. The myofibers of WB fillets have a smaller diameter than unaffected fillets, and the organization of the myofibrils and bundles is disrupted (Velleman, 2015; Zotte et al., 2017). There is evidence of cell damage leading to a state of tissue degeneration with evidence of necrosis (Sihvo et al., 2014). A surge of inflammatory cells (macrophages and heterophils) has been reported around capillaries and the damaged tissue, most probably present to remove injured cells and debris (Laskin et al., 2011). These macrophages can be evolved based on inflammatory mediator signals to carry out different functions, including tissue repair, healing, T cell regulation, and other functions (Laskin et al., 2011). Imbalances in the immune response may lead the immune system to over express modulating signals that cause the macrophages to damage tissue or promote fibrosis (Laskin et al., 2011). In moderate and severe WB fillets, fibrosis leads to an increase in collagen and fat (Soglia et al., 2016b). In addition to the macrophages possibly causing cell damage, an increase of reactive oxygen species (ROS) has been reported (Sihvo et al., 2016).

The ROS are molecules used by the innate immune system to control pathogens. They are also a by-product of the electron transport chain in the mitochondria (Lauridsen, 2018). These molecules play several roles. The presence of ROS also acts as a signaling mediator to macrophages, allowing them to react to toxic chemicals in tissues, particularly in the liver (Laskin et al., 2011). Considering that ROS are oxidative molecules that have the capacity to donate an electron and cause molecular structural change, excesses can be detrimental to the cell/organism (Lauridsen, 2018; Surai et al., 2019). The presence of tissue damage observed in histological samples suggest that ROS may leak outside the cells and exacerbate oxidative stress in the WB tissue. Applying interventions that address the oxidative state of the meat may provide an alleviation of the WB conditions.

Reactive oxygen species and their intermediates are regulated in the cells by several mechanisms. Enzymes such as superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase, and glutathione transferase, allow the cell to regulate ROS around the mitochondria (Zotte et al., 2017; Lauridsen, 2018; Surai et al., 2019). These enzymes break down the ROS into less reactive molecules, but the enzymes also need the presence of micronutrients such as copper, selenium, and zinc (Lauridsen, 2018). Lipid and water soluble vitamins E and C are also antioxidants that can neutralize ROS (Zempleni et al., 2007). Vitamin E is found intercalated within the phospholipid bilayer of cell membranes protecting the polyunsaturated fatty acids from free peroxyl radicals (Zempleni et al., 2007). The free radical end of the oxidized vitamin E can be regenerated by vitamin C and continues to function (Zempleni et al., 2007). Other plant-based molecules such as tannins, carotenoids, and isoflavones can also act as antioxidants by sequestering free radicals (Cherian et al., 2002; Wadas and Mioduszewska, 2011; OECD, 2012). Supplementing the diet with vitamins or varying the diet’s ingredients to yield other antioxidant molecules and micronutrients may provide a practical intervention for addressing the increase in oxidative stress observed in WB muscle (Zotte et al., 2017; Bottje, 2018).

The vascular system transports nutrients (e.g. vitamins, oxygen, minerals, and immune cells) and eliminates by-products of the cell's respiration. In DPM, breast yield increase in selected broiler breeds causes capillaries to constrict upon physical activity of the P. minor. This lead to necrosis onset in the muscle due to the decrease of nutrient and oxygen supply (Bilgili and Hess, 2002). Although a different mechanism is present in WB, diminished tissue vascularization and damage are histological characteristics also observed in WB (Joiner et al., 2014; Soglia et al., 2017; Zotte et al., 2017). Soglia et al. (2017) suggested that the diminished amount of capillaries may contribute to the myodegeneration in the breast fillet, and increase the fibrosis of the lost tissue as it is regenerated. Bautista-Ortega and Ruiz-Feria (2010) reported that the inclusion of arginine (Arg) and vitamins E and C improved pulmonary vascular performance in hypoxic chickens. Thus, it is possible to partially limit the extent of vascular damage in WB meat. However, Zampiga et al., (2019) did not observe a significant reduction in WB incidence when supplementing broiler diets with 30% above current requirements of Arg:Lys ratios, but it did reduce WS and spaghetti breast abnormalities.

The muscle cell undergoes a process of regeneration in an attempt to mitigate the damage from the myofibril and connective tissue injury (Velleman, 2015). This regenerative process occurs as the breast muscle undergoes hyperplasia due to muscle growth. In both situations, satellite cells are recruited as a source of nucleic acid material that would allow the cells to increase protein production and accretion (Powell et al., 2014; Bailey et al., 2015a; Velleman, 2015). Satellite cells are progenitor cells that undergo a process of proliferation and differentiation upon recruitment into the cell. This process occurs near a capillary, as both angiogenesis and myogenesis occur during satellite cell activation (Velleman, 2015). In cases where capillaries are not present, fibrosis onset is likely to occur (Markley and Faulkner, 1978). Baily et al. (2015a) mentioned that satellite cell modulation can be achieved by manipulating egg hatching conditions and dietary practices. Powell et al. (2014) reported that nutrient restriction of Met/Cys concentrations altered adipogenic gene expression which led to an increase of satellite cells differentiation to adepogenic tissue providing an explanation to the increase in fat of severe WB. It is evident that the restorative capabilities of the muscle cell are being affected by many intra-related mechanisms that present a challenge when addressing the WB condition.

1.3.3. Genetic and metabolic facets of the woody breast condition

The appearance of WB has been associated with broiler breeds that have been selected for higher feed efficiency (FCR), heavier birds at slaughter, higher breast yields (BY), and faster growth rates (Mudalal et al., 2015; Mutryn et al., 2015; Velleman and Clark, 2015). The role of genetic selection in this muscle condition was brought into question by Bailey et al. (2015a), in an extensive study comparing multiple flocks of broiler lines selected for high BY and another line unselected for BY. The study concluded that although selection to remove WB could be achieved, it would take a long time to accomplish due to the low heritability of the muscle condition. In addition, the authors proposed that proper management (feeding practices, diet formulation, welfare, hatchery practices) of growth-selected broiler lines can mitigate WB. Although such interventions have seen limited success, there is evidence that these interventions come at the cost of BY, feed efficiency, and time to slaughter weight (Trocino et al., 2015; Zhou et al., 2015; Tijare et al., 2016; Kuttappan et al., 2017; Livingston et al., 2018a; b; Córdova-Noboa et al., 2018; Bodle et al., 2018). Proteomic studies haves shown differences between unaffected and WB fillets in the increase of oxidative stress compounds and glycolytic proteins (Cai et al., 2018).

The accretion of breast muscle requires satellite cells to proliferate and differentiate into myotubes under the order of myogenic regulatory molecules (e.g. myogenic factor 5, myogenic determination factor 1, myogenin). The genes that encode these factors have been found to be overexpressed in WB meat compared to unaffected breast fillets (Velleman and Clark, 2015; Clark and Velleman, 2017), indicating a constant state of regeneration as a method to repair the damaged cell structures observed in WB. However, this state of proliferation and differentiation is also mediated by the expression of extrinsic factors such as the extracellular matrix, decorin, and myostatin (Velleman and Clark, 2015). Decorin, a small leucine rich proteoglycan, is implicated in the proper growth, density, and size of the collagen fibrils in animal tissues (Dunkman et al., 2013). Decorin is expressed in higher amounts in WB fillets compared to a unaffected breast fillet, and the presence of this proteoglycan is linked to an increase in collagen formation and crosslinking (Weber et al., 1996; Velleman and Clark, 2015). Velleman and Clark (2015) postulated that the increase in decorin content hinders satellite cell proliferation, which foments fibrosis in the form of highly cross-linked collagen fibers, thus increasing the textural hardness of the fillet (Aguirre et al., 2018).

Broiler breast meat affected by the WB and WS anomalies have higher pH values of at least 0.1 than unaffected breast fillets (Aguirre et al., 2018; Sun et al., 2018). Although an increase in final meat pH is usually associated with improved WHC, in these anomalies a lower WHC is observed (Barbut, 2002; Tijare et al., 2016; Kuttappan et al., 2017). The high pH of the meat was originally speculated to be related to the increase in adipose tissue content (Meisner and Tenney, 1977; Barbut, 2015a; Zotte et al., 2017). However, Mutryn et al. (2015) proposed that the lack of pH decline may be in part caused by the disruption in the glycogen metabolism in the cell due to the modified expression of several genes. These results were later supported by Abasht et al. (2016), where muscle glycogen and the glycolytic intermediates (e.g. glucose-6-phosphate and fructose-6-phosphate) of WB afflicted meat were found to be reduced. Glycogen in the muscle cells acts as a reserve source of glucose, and is used in the glycolysis process to generate ATP and lactic acid. Thus, reduced levels of glycogen and glycogen regulatory intermediates may be the cause of the higher pH in WB meat, as there would not be enough lactic acid accumulation in the muscle during the rigor process (Berri et al., 2008; Abasht et al., 2016).

Another common aspect of the WB condition is the state of oxidation of the muscle. Abasht et al. (2016) reported elevated levels of histamine metabolites in WB tissue. Histamines are immunostimulating molecules that can elicit inflammation and pain (Macglashan and Baltimore, 2003). These molecules act as cell signals that are involved in multiple reactions which can even cause protein breakdown or affect the extracellular matrix (Abasht et al., 2016). An increase of inflammation and protein breakdown would cause an increase in ROS, as supported by the increase of glutathione compounds that are used to regulate oxidative stress (Abasht et al., 2016). However, in WB meat, the amount of ROS surpasses the cellular availability of glutathione compounds, and increases damage from oxidation (Zotte et al., 2017; Lilburn et al., 2018). Improving the antioxidant potential in the breast muscle may provide an alleviation of the oxidative stress in broilers affected by WB.

Although low heritability of the WB condition was reported, modifications in gene expression occur in the WB muscle tissue that cause an overexpression of undesirable molecules which exacerbate the condition. It is possible that by modifying the feeding program of the birds (e.g. consumption rates, amino acid levels, or antioxidants), a reduction of the WB incidence or severity can occur.

1.4. Dietary Intervention to Mitigate Woody Breast Incidence

Poultry diets, in particular those formulated for broiler chickens, have evolved over time to ensure proper nutrition is met to allow for maximal growth. This focus on bird growth was due to the lower price and increased demand for broiler products across the world (National Chicken Council, 2015). The national research council (NRC, 1960, 1966, 1994) provides the basic nutritional requirements of broilers based on published data and this report is used as the basis for any diet formulation. Early in the 20th century, essential nutritional compounds, such as vitamins, minerals, and essential amino acids (AA), were in their early discovery stages, placing the initial research effort on understanding the effects of macronutrients rations in the diets (Hill and Dansky, 1953; NRC, 1960). In order to meet the nutritional requirements needed for early rapid growth of broilers, researchers formulated diets that included a variety of ingredients, such as fish meal, bone meal, dried whey, dried brewer's yeast, alfalfa meal, crude casein, and soybean meals (Hill and Dansky, 1953; Donaldson et al., 1956; NRC, 1960). These early studies assessed the balance of the diet's productive energy, Cal/lb and crude protein % (CP) content in order to establish minimum requirements for optimal growth. Optimal growth was described by a combination of factors that assessed growth rate, feed consumption, and body composition of the broilers at a set age. In the study by Donaldson et al., (1956), a diet formulated to contain a high productive energy increased the carcass fat deposition and lowered the water content. Hill and Dansky (1953) reported on iso-nitorgenous diets formulated with increasing energy content and observed that feed consumption was depressed as energy levels of the diets increased ; however, the density and palatability of the diets could have impacted feed consumption. In the same study, varying the CP content of iso-energetic diets did not change feed consumption. A proper ratio of energy and CP is needed in order to ensure the broilers consume the proper amount of feed needed to allow for maximal growth.

Diet formulation for broilers is constantly changing to accommodate the selection programs for broiler strains that can grow to the desired target weight faster (Pym and Nicholls, 1979). This selection program for fast growth rates is possible due to the high heritability of body weight (BW) and conformation of the broiler breeds, which allow breed selection for heavier BW (Pym and Nicholls, 1979). This increase in BW comes at the expense of higher feed consumption, with feed accounting for 70 % of the husbandry cost. Thus, improving feed conversion ratio (FCR) for each g of weight gain, becomes essential for the industry. The breed selection programs also targets other parameters of interest to the industry, which include percentage of carcass (CY) and breast (BY) yield (Tesseraud et al., 2003; Baker, 2009).

The ultimate goal of a well-balanced diet in broiler operations is supplying adequate amounts of AA in the most economic form that allows for maximal muscle accretion. Feedstuffs that supply proteins (e.g. oilseeds) have varying AA compositions which impede the use of single source ingredients; yet 90% of the current broiler diets are mainly composed of two ingredients: corn and soybean meal (Baker, 2009). In these corn/soy diets, Fernandez et al., (1994) observed that the limiting AAs are methionine (Met), lysine (Lys), and threonine (Thr), which need to be supplied through other ingredients. Supplementation with industrially produced amino acid has proved an economical strategy to balancing AA ratios. Nevertheless, full replacement of the CP by artificial AA has not been successful (Edmonds and Baker, 1987; Baker, 2009).

The AA Lys has become the reference for all rations being formulated. The remaining essential AA are included as a ratio based on Lys (Fernandez et al., 1994; Baker, 2009). This Lys ration based inclusion is used because Lys is one of the main AA involved in protein accretion. The estimated Lys requirements for broilers is between 1.28% to 1.12% of the diet (Mehri et al., 2012). There is little effect of broiler breed and dietary energy levels on the ideal ratios of the AA to Lys (Baker, 2009). However, bird age, gender, and housing temperatures have an impact on AA ratio requirements that may warrant specific diet formulations.

The national research council organization suggests formulating three different diets (starter, grower, and finisher) because nutritional requirements change with broiler age (0 - 3 wk, 3 - 6 wk, and 6 - 8wk, respectively). As the bird ages, growth rates decline and so BW and muscle gain slows, which causes a decrease in CP needs but a slight increase in sulfur amino acids (SAA), Thr, and tryptophan (Trp) (Warren and Emmert, 2000; Pope and Emmert, 2001). It is important to understand that meeting the ideal protein content of a diet formulation is a challenge, and the AA availability can be affected by the interaction of other AA, or over-supplementation may present toxicity effect on the birds.

Male broilers have greater BW and better FCR compared to female birds, which causes the males to have an increase in CP and AA requirements. A reduction of 10% in Lys requirements, but no other AA, was observed between sexes (Rosa et al., 2001). Different diet formulations can be used for female broilers due to the difference in AA ratios, but it is far more practical to feed both sexes the same diet formulations when reared as mixed sex groups (a.k.a. “straight run”) at the same nutritional levels (Baker, 2009). Environmental temperature and infectious disease also impact the nutritional requirements of broilers, mainly through the reduction of feed consumption caused by heat stress or increased inflammatory processes (Baker, 2009). Perez-Carbajal et al. (2010) reported that increasing the supplementation of arginine and vitamin E (>0.3% and 80IU/kg, respectively) above NRC requirements improved the immune response of vaccinated birds to a coccidiosis challenge. Additionally, studies by Murray and Murray (1979) showed that increasing nutrient density, in order to compensate the loss of consumption from disease stressed animals may not be beneficial to the animals overall health. Warren and

Emmert (2000) and Pope and Emmert (2001) observed that SAA, Thr, and Tyr demands increased as birds grow, suggesting that there is an economic and nutritional benefit to adjusting diet formulations as birds age.

Supplementation with AA elicits a multi-level response in BW, BY, FCR, and meat quality parameters. The AA dietary formulation is based on the ideal protein content for the bird, a concept founded on obtaining maximal muscle accretion by providing the precise amount of all AA. Lys is used as the reference AA to which all other AA are fed as a ratio in order to formulate diets on the ideal protein content.

The presence of adequate amounts of SAA (cysteine (Cys) and Met) is essential for the proper growth and performance of fast growing birds. The supplementation of SAA is highly dependent on the initial concentration of these AA in the feedstuffs used to formulate the rations. In broilers, Met is an essential AA but is highly dependent on the concentration of Cys. In diets balanced for Cys, incremental doses of Met cause an increase in weight gain in broilers (Dilger and Baker, 2007). However, when Cys is deficient, the increase in Met supplementation over NRC levels decreases weight gain by half; when both amino acids are deficient, supplementation of only Cys does not produce a positive response in weight gain. Thus, Cys levels in diets must be adequate for proper utilization of proteins by broilers (Baker, 2009). Moreover, Edmonds and Baker (1987) observed that over-supplementation of Met (4%) above dietary requirements can cause growth depression through toxicity. Similarly, supplementation with L-Cys above 10% of dietary requirements induced high toxicity. No other AA caused toxicity as long as they were fed at <3% of the diet, over NRC recommended AA levels in diets formulated to ≥19% CP (Edmonds and Baker, 1987).

Mehri et al. (2012) observed that estimated digestible-Met and digestible-Thr for optimal BW gain were 48% and 70% based on 100% Lys, respectively, but for optimal FCR, the ratios were 47% and 66%, respectively. In white Pekin ducks fed until d 21 of age a basal diets containing 0.71 % of Arg and supplemented with L-Arg up to 1.71% from a basal diet showed, that estimate requirements of Arg for optimum BW gain, FCR, and BY were 0.95%, 1.16%, 0.99% of the diet total, respectively (Wang et al., 2013). Overall, supplementation of sythetic AA's is used to ensure that the formulated diets provide adequate amounts and ratios of AA to provide optimum nutrition for growth, FCR, and BY. Understanding the AA sources is necessary, as these may also contain other components that may affect nutrient efficiency or inhibit growth. Baker (2009) presented a comprehensive review of the protein quality from multiple sources, adapted from Boling-Frankenbach et al. (2001), and showed that the highest protein efficiency ratio (PER) was that of the Canola meal (CM, 4.00 PER and 37.1% CP) followed in descending order by soybean meal (SBM, 3.75 PER and 48.4% CP), casein (3.01 PER and 88.3% CP), cottonseed meal (2.41 PER and 44.1% CP), peanut meal (2.24 PER and 43% CP), meat and bone meal (2.03 PER and 49.0% CP), and corn gluten meal (1.51 PER and 62.9%), each with their own limiting AA (Lys, SAA, Arg, and Try, and Lys, respectively). The PER assay is a sensitive method used for detecting differences in protein utilization by calculating the grams of weight gained divided by the weight of protein consumed. It is important to mention that all these sources of proteins have different methods of extraction (e.g. pressure, heat, organic solvents) that impact not only the quality of the protein but also the CP content (Toghyani et al., 2014). Even though CM has the highest PER compared to SBM, the use of CM is limited to 10 % to 30 % by weight of the diet (Canola Council of Canada, 2016). The reasons for the limitation include its lower contents of energy, CP, and Lys content. Additionally, CM may have other anti-nutritive components, such as erucic acid, which causes health issues and depressed growth (Khajali and Slominski, 2012). Soybean meal also contains non- nutrient components, such as phytate, non-starch polysaccharides (NSP), and phytoestrogens that may hinder the efficient use of nutrients.

Considering that the majority of the diets are based on corn-SBM, a series of enzymatic additives have been used to improve the nutrient availability of the diets. Phytate, a ubiquitous source of trapped phosphorus (P) in plant feedstuff has been of interest to broiler operations (Tahir et al., 2012). Inorganic phosphorus is commonly added to ensure diets provide the required amount of P for biological reactions in the body, but it is an expensive source. Phytase, an enzyme, is added to liberate these trapped phosphate sources, and reduce ingredient supplementation and environmental impact from excreted phosphorus (Tahir et al., 2012; Munoz et al., 2018). The NSP are indigestible long chain polysaccharides (commonly termed dietary fiber), which, although non-toxic, can inhibit growth through caloric dilution, interference with nutrient availability and possibly by imparting a feeling of fullness. (Zou et al., 2006; Klein et al., 2015). To reduce the effect of NSP, the ß-mannanase enzyme has been incorporated into diets to break down the glucomannan and galactomannan carbohydrate linkage found in plant cell walls (Zou et al., 2006). The incorporation of this enzyme improved BW gains and FCR in broilers by reducing the viscosity of the feed in the broiler gut, thus improving nutrient availability. Additionally, by reducing the mannan chemical linkages, mannano oligosaccharides are generated which provide an immune modulating benefit to broilers (Klein, 2013; Liu et al., 2014; Klein et al., 2015). The use of enzymes in corn-SBM diets provide a new technique to improve nutrient availability, reduce feed cost, limit environmental impact, increase broiler performance, and improve undesirable traits of feedstuff. Although these non-nutrient feed components are present in every plant based-feedstuff, their effects can be altered through the use of specific enzymes. Following enzyme addition unique components may arise from each feedstuff that may elicit an effect on growth performance of broilers.

The physiological effects of a high content of phytoestrogens in SBM can vary and create controversy in how they possibly behave in broiler diets. Phytoestrogens (genistein and daidzein) are flavonoid compounds that play multiple roles in plants, but the similarity to estrogen allows them to elicit estrogen-like responses in avian and mammalian species, by binding to estrogen receptors in cells (Grippo et al., 2007). There is no consensus in the literature on the beneficial or detrimental quality of these components relating to BW gain and FCR (Patisaul and Jefferson, 2010; OECD, 2012). However, they can modulate the reproductive processes in avian species (Grippo et al., 2007; Xiao et al., 2018). Compared to SBM, CM contains low levels of genistein and daidzein, but contains higher amounts of tannins and erucic acid, which are known to depress growth in broilers (Arntfield, 2011; Arntfield and Maskus, 2011; Chen et al., 2015). The presence of these compounds and the nutritional composition of the CM limit the use of this feedstuff in broiler diets to no more than 10% in the starter phase, and up to 30% in the grower phase, without any negative effect on growth performance when compared to a pure corn-SBM diet (Canola Council of Canada, 2016). Oilseed selection has not only focused on productivity, but also on improving the composition of the AA profile as well as the reduction of erucic acid content in CM to improve its desirability as a feedstuff (Torki and Chegeni, 2002; Chen et al., 2015).

Animal sources of CP can also be used in broiler diet formulation. Animal sources, include fish meal, meat and bone meal, blood meal, and broiler by-products. These ingredients contain various materials and can vary by the type of product or production method used to generate them (Angkanaporn et al., 1996; Kim et al., 2012). The consistency in the composition of these products can be a concern at the time of formulation design (Kirby et al., 1993). Animal protein sources are generally higher in CP content than plant sources but vary in AA digestibility and content across products (Kim et al., 2012). They also contain creatinine (Tossenberger et al., 2016), phosphorus, and calcium (Sell, 1996; Munoz et al., 2018), which can make them a desirable ingredient in broiler diets because they lower the needed for other supplements. The cost of animal protein sources can be prohibitive and limit their use. Sell (1996) reported that diets formulated with up to 10% of the diet in meat and bone meal (MBM) can be used in turkey diets with similar performance to diets without MBM. In the same study, the authors suggested that the level of replacement in inorganic phosphorus and fat justified the cost of the MBM.

A multitude of ingredients from agricultural production that can be used to formulate diets will not be covered in this literature review. There are, however, chemical precursor additives that can improve muscle accretion and performance. Of these, one worth mentioning is guanidine acetic acid (GAA), a precursor to muscle creatinine. Creatinine acts as an energy balancing agent in the muscle to ensure proper balance of ATP and ADP, and can be found in MBM or other animal proteins sources (Tossenberger et al., 2016), although the low inclusion levels of MBM in broiler diets does not provide sufficient amounts of creatinine, and creatinine is lost during heating in the feed manufacturing process. The body can generate creatinine through the use of Gly and Arg. Providing a precursor such as GAA can thus spare the AA's for growth development (Tossenberger et al., 2016).

The complex relationship of diet formulation and nutrient requirements of broiler breeds that rapidly acquire high BW and BY, begs the question as to how much can dietary intervention be used to address muscle conditions. In a study of the genetic basis of breast muscle myopathies in modern broiler breeds, a larger component related to the incidence of breast myopathies such as WB was attributed to environmental factors (Bailey et al., 2015a). These results have led to a series of published articles that focused on the manipulation of the broiler diet and feeding practices in an attempt to reduce the incidence of WB in broiler operations (Trocino et al., 2015; Simoes et al., 2017; Cemin et al., 2018; Livingston et al., 2018a; b; Córdova-Noboa et al., 2018; Bodle et al., 2018; Meloche et al., 2018). These articles based their research ideas on trying to find different growth patterns that will elicit similar growth rates with same end weights, while reducing the incidence and severity of WB in broilers. In Trocino et al. (2015), researchers compared the effect of feed restriction during a specific growth period (13 to 21 d of age) on performance, meat quality, and the occurrence of WB in two broiler genetic lines (high BY and standard BY) compared to an un-restricted feeding program. Birds were fed a corn-SBM diet in 4 stages (0 d - 12 d, 13 d - 21 d, 22 d - 35 d, and 36 d - 46 d, with a group having feed access *ad libitum* vs. another group having restricted feed intake during the second growth stage. The results of the study showed that restricting the feed intake during one of the feeding stages did not cause a significant reduction in WB incidence. Moreover, male broilers had a higher incidence of WB than female broilers, which was attributed to a faster growth rate, heavier BW, higher BY, and lower FCR. In another study that examined feed restriction, broilers of two genetic lines (high BY or high BW) fed a corn-SBM diet in two stages (0 d -14 d and 15 d - 42 d) were analyzed to determine the impact that genetic lines and feeding program had on growth, processing and meat quality parameters, as well as WB severity and incidence (Livingston et al., 2018b). The results showed that limiting feed intake by time from 8- 42 d produced broilers that were lighter in BW, consumed less feed, had better FCR, and had lower carcass yield (CY) and BY than the birds fed *ad libitum*. Ultimately in the latter study, the feed restriction did have a significant reduction in the severity of WB compared to the *ad libitum* feeding program, but the incidence of WB was still around 91.0% of the sampled fillets. The difference between the feeding programs of the Trocino et al. (2015) and the Livingston et al. (2018b) studies was that the latter sustained the feed restrictions until the day of slaughter and feed was not restricted by amount consumed. These studies showed that by restricting feed, by either consumption amount or access time, it is possible to reduce the severity of WB at the cost of lower BY, BW, or FCR. However, incidence levels still remained around 90% of the sampled fillets.

Other approaches to eliminate the incidence of WB include manipulating the AA contents of the diets or incorporating supplements such as GAA and vitamin C. The approach of two digestible Lys studies (Cruz et al., 2017; Meloche et al., 2018) was to reduce the concentration of Lys in one or two growth phases of the broilers feeding program, based on the recommended amount of Lys for corn-SBM diets. In both studies, the incidence of WB and WS was reduced in the diets that provided less Lys but at the expense of growth or processing performance. This suggests that it may be possible to mitigate WB incidence by manipulating the growth rate at a particular feeding stage of the broilers, through the use of AA.

Other studies have looked into addressing some of the underlying histological symptoms of WB through the use of additives or AA that are known to modulate metabolic pathways. In the study of Cordova-Noboa et al., (2018), a corn-SBM diet was formulated to compare the effect of incorporating broiler by-product (PBP) at 5% of the diet as a source of creatinine, and GAA (0.06% of the diet) as a precursor of creatinine would have on performance and WB incidence. The concept behind this study was that by providing creatinine or its precursor, enough energy would be available to the muscle cell to undergo repair of the tissue. The study's results showed that incorporating PBP reduced growth performance while GAA improved it, without either diet negatively impacting processing yields. Interestingly, the GAA and PBP individual diets had a higher incidence of mild

WB, but WB incidence was still above 90.0% of the sampled fillets. In the study of Bodle et al. (2018), several corn-SBM diets interventions were formulated to 1) increase Arg to Lys ratio by 10%, 2) increase the antioxidant potential of the diets by incorporating vitamin C, 3) double the vitamin premix content, 4) reduce the AA density of the diet by 15% at the grower phase, and 5) combine all the previous strategies. Increasing Arg to Lys ratios, vitamin C supplementation, or a combination of all the interventions reduced the WB severity compared to the control diet. The authors concluded that providing supplementation that mitigated oxidative stress or reduced the density of AA at the expense of reduced FCR may improve WB severity.

There are still many questions remaining on the effects of dietary interventions on WB severity and incidence, especially in regards to the actual role that the diet composition has on WB vs. the relationship with growth performance. Considering that most of the diets used so far in research are based on corn-SBM, there may be a value in exploring varying the diet's ingredients to formulate a nutritional composition similar to the traditional diets.

2. WATER HOLDING CAPACITY ATTRIBUTES OF WOODY BREAST MEAT FROM BROILERS FED A SOYBEAN OR CANOLA MEAL DIET

2.1. Synopsis

Woody breast (WB) is a condition that has been reported to negatively impact the water holding capacity (WHC) of broiler meat. The objective of this study was to evaluate WHC and incidence of WB from high yielding broilers fed either a soybean meal (SBM), or canola meal (CM) based commercial-type diets of equivalent nutritional composition. Day-old broiler chicks were reared in 5 pens (n=25/pen) under two dietary treatments at a stocking density of 0.08 m^2./ bird over 2 trials. Birds had free access to feed and water. Conventional methods were used to harvest and debone half of the birds on day 49 and the remaining half on d 59 in order to determine broiler without giblets (WOG) quality parameters (WOG and breast yields; WB scores). Breast meat was evaluated at 72 h for drip loss (DL), pH, color, and cook loss (CL). Categorical data were analyzed by $\boldsymbol{\chi}^2$ (WB), the remaining variables tests using a GLM of ANOVA. On day 49 severe WB incidence was reduced by 83.34% in birds fed the CM diet ($p<0.05$) compared to birds fed SBM diet. The CM was lower ($p<0.05$) compared to the SBM diet in WOG yield, breast yield, DL, pH, and CL (77.48%±2.04 vs 78.75±3.55, 21.42±1.32 vs 22.85±1.72, 3.01±3.27 vs 3.64±3.44, 6.11±0.13 vs 6.20±0.13, 21.09±4.83 vs 24.32±4.72; respectively). Dietary effects on day 59 had similar incidence of severe WB in birds fed CM vs. SBM fed birds; respectively. The CM WOG yield, breast yield, DL, and pH were lower ($p<0.05$) when compared to the SBM diet. CL at 59 d was similar between the dietary groups. Meat color was not affected (p>0.05) by diet at any age. In conclusion, the incidence of WB increased with the age of harvest of the birds, independent of the diet fed. CM may minimize WB negative effects on meat quality when birds are harvested on or before day 49.

Key words: Canola meal, Soybean meal, Woody breast, Water holding capacity

2.2. Introduction

The increased demand for broiler products led the industry to raise heavier birds and use broilers breeds with higher breast yields and faster growth rates to meet the demand. The increased size of the faster growing breeds with high breast yields have been associated with an increased in WB incidence and severity (Velleman and Clark, 2015). Decreasing the growth rate of birds can lower incidence of WB in broilers. In order to achieve such genetic performance, broiler diets need to be highly nutritious and of low cost, considering that feed is the main cost of broiler production. Diet formulation for broilers are performed on the least cost basis to allow maximum performance of the fast growing lines and to minimize feed costs to producers (Vieira et al., 2014). The availability, cost, and nutritional composition of the ingredients are of importance during the formulation process. Soybean meal (SBM) is an ideal feed ingredient for use in broiler diet formulation due to the desirable amino acid content and composition, low cost, and its ready availability (Abdallh et al., 2017; Erdaw et al., 2017). The availability of SBM has allowed it to become the main source of protein for most broiler diets (Hill and

Dansky, 1953; Donaldson et al., 1956; Hurwitz et al., 1978; Reece et al., 1984; Avanzo et al., 2001; Meng and Slominski, 2005; Chen et al., 2015). Other oilseed meals such as canola meal (CM), cottonseed meal, and sunflower meal have also been researched to evaluate their use and inclusion in broiler diet formulations (Abdallh et al., 2017). Howerver, the availability of these other oilseeds may be limited due to geographic growing conditions and nutrient compostion. Canola oil is produced widely in Canada and northern states of the U.S.A., and due to it low meal cost, is used to replace up to 20% SBM in the diet (Khajali and Slominski, 2012). This inclusion rate is recommended by the Canola Council of Canada (2016) to cope with CM's lower energy and antinutrative factors when compared to the SBM. A common trait of all oilseed meals is the presence of antinutritive molecules. SBM is known to contain phytic acid and non-starch polysaccharides (NSP), which can cause antinutritive effects and hinder broiler performance (Jackson et al., 1999; Meng and Slominski, 2005). Soy is also known to be a rich source of isoflavones (genistein and daidzein) that have received mixed reviews in the literature for their health benefits in multiple species (Grippo et al., 2007; Patisaul and Jefferson, 2010; Ekİncİ and Erkan, 2012; Sirotkin et al., 2016). Canola meal contains glucosinolates, sinapine, and tannins as antinutritive components, in addition to the phytic acid and NSP (Khajali and Slominski, 2012). Glucosinolates have been shown to deteriorate broiler health by affecting hepatic and thyroid function, while high sinapine content can lead to fishy smell through the breakdown of the compound into trimethylamine (McNeill et al., 2004). However, advancements in feed additives or crop varietals have improved or reduced the negative effects caused by these ingredients. The antinutritive effects of SBM have been reduced by incorporating enzymes that breakdown phytic acid and NSP to increase nutrient availability for the broilers (Klein et al., 2015). Isoflavones have not been shown to produce any negative impact on growth (Shutt, 1976). Improved varieties of commercial canola seeds have reduced concentrations of antinutritive molecules and improved the nutritional composition. Even improved canola varieties cannot fully replace SBM due to its lower energy and lysine contents compared to SBM. Due to these limitations, CM is typically included at 10% in the starter phase and 20% in the grower phase of the total diet (Khajali and Slominski, 2012; McNaughton et al., 2014; Chen et al., 2015; Canola Council of Canada, 2016).

Current research on WB has focused on different nutritional and husbandry approaches to lower the incidence of WB in heavy, high breast yielding broiler lines (Powell et al., 2014; Bailey et al., 2015b). Increasing the levels of potassium and available phosphorus reduced the occurrence of WB at 42 d (Livingston et al., 2016). Powell et al. (2014) reported an increase in protein degradation through the high inclusion levels of amino and fatty acids during the first 8 d post hatch of broilers. Schlumbohm et al. (2016) reported that no effects on WB incidence were observed between birds from two genetic lines, fed usual or lower levels of ideal protein, and varying levels (11,723 – 13,816) of TME *kJ/kg*. Kidd and Choct (2017) fed a diet balanced on all essential amino acids, and a diet balanced to all limiting amino acids, and observed that the diets did not impact body weight (BW), feed conversion ratio (FCR) at 42 d, or WB incidence.

A common feature of experimental diets in research is the use of corn-SBM based diet or wheat-SBM diets as these are the most common. To the authors' knowledge, no research has been conducted to determine the impact of WHC of breast meat in broilers fed diets formulated with CM as the primary protein source, compared to a traditional corn-SBM diet. In light of previous reports on WB, the objective of this study was to assess the incidence of WB and WHC severity in broilers raised on a diet with corn-SBM or corn-CM

2.3. Materials and Methods

The experiment was designed to determine the impact of WB severity on WHC parameters of broiler breast meat from a fast growth commercial breed, harvested at day 49 and day 56, fed either a SBM or a CM based diet. The experimental design consisted of two experimental trials each containing five pens per dietary treatment and 25 birds per pen (n=125 birds/diet/harvest day). The birds were reared under Texas A&M University's animal use protocol # 2014-0202 on concrete floors lined with new pine shavings as bedding material. Hatch-day broilers were stocked at a density of 0.08 m2/bird and were placed on an *ad libitum* feed of assigned diet with age-appropriate supplemental heating. The animals were raised with four dietary phases during their growth: a starter (1 d -14 d), grower (15 *d* – 28 d), finisher (29 *d* – 42 d), and a withdrawal diet (43 d – 56 d). The lighting schedule was adjusted according to their dietary phases: starter (23L:1D), grower (20L:4D), and ended with a 16L:3D:2L:3D light cycle. The dietary groups consisted of an industry control (SBM) formulated with corn and soybean meal compared to a canola (CM) diet consisting of corn and canola meal and were formulated to be isonitrogenous and isoenergetic (Table 1). The starter diet was fed as a crumble and all other phases fed as pellets. At day 49 and 56, feed was withdrawn eight hours prior to transport to slaughter and the birds were processed at the Texas A&M University Broiler Research Center, in College Station, TX. Birds were electrically stunned at 13 to 15 V for 7 to 10 s with an electrified knife (Cervin Electrical Systems, Minneapolis, MN) with an AC/DC converter set at 500*Hz* prior to exsanguination. The jugular vein and carotid artery were severed in a unilateral cut and birds were allowed to bleed for ~90 *s* before scalding at 60°*C* for 10 *s* (model SS-30-SS, Bower Corp., Haughton, IA) prior to feather removal using a rotary drum picker (model SP-30-SS, Bower Corp.) for 25 *s*. Neck and hocks were removed before manually eviscerating the birds. The eviscerated carcasses were chilled in an agitated 4°*C* ice water bath for 1.5 hours prior to deboning. The weights of live bird, chilled carcass, and breast with rib meat were recorded to calculate carcass yield % and breast yield %. The breast was scored for WB (Sihvo et al., 2014; Russo et al., 2015; Kuttappan et al., 2016) immediately after deboning (~ 3 h PM).

Table 1. Dietary formulation and calculated nutrient content of control (SBM) and canola (CM) starter, grower, finisher and withdrawal diets fed to market broilers.

	Starter		**Grower**		**Finisher**		**Withdrawl**	
	CM	**SBM**	**CM**	**SBM**	**CM**	**SBM**	**CM**	**SBM**
Ingredients								
Corn	38.72	54.53	35.24	60.02	44.17	63.34	49.88	66.86
Soy Bean Meal	0.00	37.66	0.00	32.79	0.00	29.55	0.00	26.17
Corn Gluten 60%	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DL-Methionine 98%	0.12	0.32	0.05	0.30	0.09	0.28	0.05	0.22
L-Lysine HCL	0.47	0.12	0.29	0.14	0.32	0.16	0.28	0.14
L-Threonine 98%	0.05	0.04	0.00	0.05	0.05	0.08	0.02	0.05
L-Arginine 98%	0.00	0.00	0.00	0.00	0.07	0.00	0.08	0.00
Soy oil	6.66	3.47	7.99	2.96	7.26	3.24	6.97	3.42
Limestone	0.00	1.46	0.77	1.41	0.75	1.25	0.67	1.14
Monocalcium phosphate	2.02	1.59	1.27	1.52	1.06	1.26	0.96	1.14
Salt	0.29	0.46	0.34	0.46	0.31	0.38	0.24	0.31
Sodium Bicarb	0.09	0.00	0.00	0.00	0.08	0.11	0.19	0.22
Vitamins[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Canola Meal	47.24	0.00	53.69	0.00	45.52	0.00	40.30	0.00
Salinomycin[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated Nutrients								
Dry Matter	82.36	86.70	80.95	87.10	81.72	86.78	82.05	86.55
Moisture	17.64	13.30	19.05	12.90	18.28	13.22	17.95	13.45
Protein	23.00	23.23	22.26	21.35	20.21	20.09	18.78	18.67
Dig-Lysine	1.22	1.22	1.14	1.12	1.05	1.05	0.95	0.95
Crude Fat	9.82	5.88	11.14	5.53	10.46	5.91	10.22	6.19
Crude Fiber	0.90	2.67	0.77	2.60	0.97	2.55	1.10	2.49
Calcium	0.95	0.92	0.88	0.88	0.78	0.77	0.70	0.70
Av Phosphate	0.46	0.46	0.44	0.44	0.38	0.38	0.35	0.35
Ash	6.03	6.29	6.54	5.99	5.87	5.40	5.36	4.97
ME Kcal/Kg	3058.0	3058.0	3080.0	3080.0	3137.0	3137.0	3183.0	3183.0

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadion 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient salinomycin sodium (500 g/ton inclusion: Sacox® 120 microgranulated, Huvepharma, Sofia, Bulgaria). As an aid in the prevention of coccidiosis caused by *Eimeria necarix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria mivati, and Eimeria maxima*.

At 72 *h* post mortem, breast weights were recorded to determine drip loss % (DL) from the deboned weight, followed by pH measurements (model 205, Testo AG, Sparta, NJ). Color of the meat was measured at a 0º angle with a C-illuminant colorimeter (CR400, Konica Minolta, Ramsey, NJ). Cook loss % (CL) was determined by the oven method from the right breast fillet (Sams, 1990). At 88 *h post-mortem*, the left fillets were multi needle injected (Type BI-88 P-VSP, Inject Star of the Americas Inc., Mountain View, AR) to a target of 15% with a solution consisting of water (92.49%), refined salt (4.22%; Formtech Solutions, College Station, TX), and a phosphate blend (3.30%, Blend 100, Formtech Solution) following industry formulations, for a final concentration of 0.55% salt and 0.43% phosphate in the product. The pick-up yield percentages were recorded immediately after injection, followed by a 20 min and 24 h post injection retention (Casco et al., 2013).

2.3.1. Statistical Analysis

In this study, the experimental unit used for analysis was the broiler. To ensure that the comparisons of carcass yield (CY), breast yield (BY), DL, CL, pH, color, and marinade retention at 20 min and 24 h were not biased by possible differences in live performance, the breast meat weight data were normalized to one standard deviation from the population mean. The breast weight at day 49 had a mean of 680.05 g and a standard deviation of 100.38 g. After normalization a total of 132 birds were considered for further analysis. At day 56, the mean breast weight was 928.28 g with a standard deviation of 154.45 g, resulting in 148 birds for analysis after normalization.

Dietary groups (SBM and CM) and WB scores (0 = unaffected a.k.a. “normal”, 1 = mild, 2 = moderate, and 3 = severe) were analyzed with the general linear model (GLM) of a 2-way analysis of variance (ANOVA) on the R statistical platform (v. 3.3.2), using RStudio (v.1.0.136, ©2009-2016 RStudio, Inc.), and post-hoc means were separated by Tukey’s HSD. The categorical data of WB were analyzed through Pearson’s χ^2 test to determine the percentage of WB severity within the two dietary groups.

2.4. Results and Discussion

Regardless of severity, the incidence of WB at day 49 was lower ($p<0.05$) for the CM fed birds compared to the SBM birds (Table 2). Within the CM diet birds, 41.94% of the fillets were scored as unaffected, in contrast ($p<0.05$) to the SBM fed birds (5.71%). When observing the impact of the diets within a score of unaffected fillets, the CM diet accounted for 86.67% vs. 13.33% from the SBM. The majority of the fillets were moderate to severe WB (n = 49 and 41, respectively) for both dietary groups. The CM diet had 51.02% of moderate fillets and 24.39% of severe WB fillets, slightly more ($p<0.05$) than the 48.98% moderate and 75.61% severe WB fillets from the SBM diet. Of the 12 fillets that had extremely severe WB, 91.67% were from the SBM diet.

Table 2. Woody breast incidence and odds ratio on breast meat harvested at d 49 from broilers fed a Canola (CM) or Soybean meal (SBM) based diet.[1]

		Normal		**Mild**		**Moderate**		**Severe**		
Within[2]	**Diet**	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**	**Total n**
Column	CM	26.00	86.67	25.00	51.02	10.00	24.39	1.00	8.33	
	SBM	4.00	13.33	24.00	48.98	31.00	75.61	11.00	91.67	
	Total	30.00	100.00	49.00	100.00	41.00	100.00	12.00	100.00	
Row	CM	26.00	41.94	25.00	40.32	10.00	16.13	1.00	1.61	62.00
	SBM	4.00	5.71	24.00	34.29	31.00	44.29	11.00	15.71	70.00

	Odds Ratio	95% C.I.	p-value
CM – SBM	0.05	0.01 – 0.17	<0.0001

[1] Significant differences between diets within woody breast severity were observed (p-value =1.2874^{-7}) through Pearson's Chi-squared test (X^2=34.88,df=3,n=132)

[2] Within column compares the results between diets for each of the woody breast (WB) severity levels. Within row compares the results between the WB severity levels within each diet.

However, within each of the CM and SBM diets, these extremely severe WB fillets only accounted for 1.61% and 15.71%, respectively, of the total amount of fillets sampled within each dietary group. The odds ratio indicated that exposure to the CM diet lowered (p<0.0001) the total incidence of WB by 95%. Previous studies have reported lower incidence of severe WB in 42 d vs 54 d birds (Kuttappan et al., 2017). The results show a significant reduction of WB incidence when birds are raised to 49 day of age and fed a commercially available CM as the main source of protein when compared to birds fed a SBM diet. In order to meet the isonitrogenous and iso-energetic study design for both diets and remedy the lower energy and lysine content of the CM diet, amounts of supplemental soy oil and synthetic lysine were adjusted to match energy and lysine amounts provided by the SBM-diet (Table 1). It is possible that the compositional changes of protein source, oil content, and synthetic amino acids could impact the incidence of the WB condition.

Enrique et al (2017) reported that feeding broilers a corn-SBM based diet containing digestable lysine above 0.77% was associated with higher incidences of white striping and WB conditions, together with higher breast weights. This report is in contrast with the results from the current study, as the CM diet had similar levels of digestable lysine to the SBM diets across growing stages, ranging from 0.95% to 1.22%, yet the WB incidence was lower in the CM diet compared to the SBM diets. Overall, the CM fed birds had a lower incidence of severe and extremely severe WB fillets, and the majority of the SBM fed birds had a higher incidence of severe and moderate WB fillets.

Table 3. Woody breast incidence and odds ratio on breast meat harvested at d 56 from broilers fed a Canola (CM) or Soybean meal (SBM) based diet.[1]

		Normal		**Moderate**		**Severe**		**Extremely Severe**		
Within[2]	**Diet**	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**	
Column	CM	0.00	0.00	14.00	35.00	34.00	47.89	17.00	51.52	
	SBM	4.00	100.00	26.00	65.00	37.00	52.11	16.00	48.48	
	Total	4.00	100	40.00	100	71.00	100	33.00	100	
										Total n
Row	CM	0.00	0.00	14.00	21.54	34.00	52.31	17.00	26.15	65.00
	SBM	4.00	4.82	26.00	31.33	37.00	44.58	16.00	19.28	83.00
		Odds Ratio	95% C.I.		p-value					
CM - SBM		1.94	0.23 -56.87		0.2999					

[1] No significant differences between diets within woody breast severity were observed (p-value =0.1598) through Pearson's Chi-squared test (X^2=5.65, df=3, n=148)

[2] Within column compares the results between diets for each of the woody breast (WB) severity levels. Within row compares the results between the WB severity levels within each diet.

By day 56, the differences in incidence of WB severity by diet observed on day 49 were not present (p>0.05; Table 3). This suggests that factors other than diet protein source are regulating the incidence and severity of WB as birds age past day 49. The majority of the 148 fillets analyzed at day 56 had a moderate to severe WB incidence (n = 40 and 71, respectively). No unaffected fillets were observed for the CM diet, and only 4 fillets were unaffected for the SBM fed birds. The odds ratio that the CM diet could cause a higher incidence of WB was not significant. These results are in agreement with those obtained by Kuttappan et al., (2017) who observed an increased in WB severity and incidence between broilers fed the same diet and raised to 6 and 9 weeks of age. The same authors reported that at week 9, the severity of WB reached a plateau in incidence from the sampled birds. The observation of incidence within the current project suggests that CM played a significant role in reducing the severity of WB within day 49 but the mechanisms by which this diet reduced WB are not yet understood.

2.4.1. Breast meat WHC

The final pH of the meat is determined through resolution of rigor mortis. The pH of meat is an indicator of meat functionality and is related to higher WHC: the higher the pH (>6.00), the more water can be held within the muscle. Normally, lower meat pH values are observed in birds that have low glycogen deposits due to environmental and management factors. The increase in WHC in meat is attributed to high pH and the improved steric effect in the muscle resulting in increased negative charges that hold water within the muscle (Owens et al., 2000; Alvarado and Sams, 2004a; Tijare et al., 2016). If the pH of the meat approximates the iso-electric point of the actin and myosin proteins (~5.1), more water is lost causing an increase in drip loss, lightness, and cook loss, which in turn decreases the WHC (Owens et al., 2000). However, this is not what happens in WB. The higher pH values of the WB meat have been associated with a combination of physiological changes that include a lower glycogen and protein content of muscle with a higher amount of connective and fatty tissue (Berri et al., 2001; Petracci et al., 2013). Thus, the regular assumptions of WHC of the meat and pH are not te same.

2.4.2. pH and color of the meat

All the pH values observed in the results fall within expected values for meat that has been processed under commercial conditions (Alvarado and Sams, 2002; Casco et al., 2013). The means, standard deviation, and sample sizes for pH, and color values L*, a*, and b* are shown in Table 4. The results for day 49 indicate that birds fed the CM diet had a lower pH (6.11 vs. 6.20, $p<0.05$) compared to the SBM fed birds. No differences ($p>0.05$) were observed between all the WB severity levels at day 49. By day 56, the same results were observed for the dietary effects: CM diet had the lower ($p<0.05$) pH (5.97 vs. 6.03) when compared to the SBM diet. However, differences in WB severity levels were observed. The unaffected and moderate WB had similar ($p>0.05$) pH values (5.87 and 5.96, respectively) to each other but were lower ($p<0.05$) than the extremely severe WB fillets. The pH of severe WB fillets (6.01) was not different ($p>0.05$) from the other levels of WB severity.

Table 4. Means, standard deviation, and sample size for the quality parameters of breast meat pH, lightness (L*), redness (a*), and yellowness (b*) from birds fed a canola meal (CM) or soybean meal (SBM) diet and graded for woody breast at harvest days 49 and 56.

		pH		**L***		**a***		**b***	
	Levels	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**
Day 49	CM	6.11^{y}±0.13	58	51.96±1.87	58	1.77±0.66	55	5.19±1.06	58
	SBM	6.20^{z}±0.13	69	52.52±2.06	69	1.63±0.94	66	4.95±1.26	69
	Normal	6.10±0.15	27	52.02±1.78	27	1.80±0.76	27	4.94±0.93	27
	Moderate	6.15±0.12	48	51.82±2.01	48	1.76±0.65	48	5.03±0.90	48
	Severe	6.20±0.14	40	52.96±1.76	40	1.50±1.01	40	4.84±0.94	40
	Extremely Severe	6.20±0.11	12	52.25±2.61	12	1.82±0.91	12	4.86±0.95	12
	Factor Effects[1]	p-value		p-value		p-value		p-value	
	Diet	0.0003		0.1140		0.3460		0.2528	
	Woody Breast	0.4624		0.1290		0.4910		0.6877	
	Diet X Woody Breast	0.2293		0.9640		0.6800		0.0612	
	Levels								
Day 56	CM	5.97^{y}±0.11	80	54.35±2.13	80	1.95±0.78	80	5.57±1.19	80
	SBM	6.03^{z}±0.12	65	54.44±3.06	65	1.89±0.95	65	5.32±1.36	65
	Normal	5.87^{b}±0.13	4	53.62±1.35	4	2.43±0.96	4	5.17±0.63	4
	Moderate	5.96^{b}±0.11	39	54.44±2.27	39	1.79±0.66	39	5.74±1.32	39
	Severe	6.01^{ab}±0.12	69	54.49±2.59	69	1.97±0.97	69	5.36±1.21	69
	Extremely Severe	6.04^{a}±0.09	33	54.21±3.06	33	1.92±0.80	33	5.36±1.38	33
	Factor Effects	p-value		p-value		p-value		p-value	
	Diet	0.0010		0.8470		0.6870		0.257	
	Woody Breast	0.0200		0.9860		0.4970		0.498	
	Diet X Woody Breast	0.9909		0.8470		0.7380		0.725	

[1] Factor effects for diet, woody breast incidence, and interaction between diet and woody breast were considered significant when p-value <0.05

$^{x\text{-}z}$ Means with different superscripts for diet levels within a column are significantly different when p-value <0.05

$^{a\text{-}d}$ Means with different superscripts for woody breast levels within a column are significantly different when p-value <0.05

These results are in contrast to published reports that found significant differences in pH and b* values of unaffected vs. extremely severe WB fillets in birds harvested at d 42 but not different than the pH reported for birds harvested at d 63 (Kuttappan et al., 2017). However, differences in the diet, processing conditions, and experimental unit size of the current research compared to those published by Kuttappan et al., (2017) may provide a possible explanation of the differences observed between the two studies. It is unclear why the CM diet was able to produce lower pH values compared to the SBM diet at both harvesting days, aside from the difference in incidence of WB difference at both days. Other breast meat conditions that have similar characteristics to those of WB (fibrosis, higher fat and collagen deposition) have been reported to contain a lower glycolytic potential, which could explain the increase of pH at the time of harvesting on day 49 for the SBM meal (Berri et al., 2001; El Rammouz et al., 2004; Kuttappan et al., 2017), which had the highest WB incidence. However, at day 56, there were no differences in WB incidence, yet the CM had a lower pH than the SBM.

Meat that has high L* values is associated with lower pH and higher DL and CL percent (Alvarado and Sams, 2002; Barbut, 2009). Results for L* values indicate a range of 51.86 – 52.96 at day 49, and 53.62 – 54.49 at day 56 with no significant differences ($p > 0.05$ and 0.05, respectively) observed at any of the dietary or WB severity factors. These results are in agreement with the values reported in the literature. Mazzoni et al., (2015) reported no difference on L* values, with a range of 54.7 – 55.1, on breast meat from 4 broilers breeds weighing 3.6 *kg* and scored according to muscle degeneration scores based on histological lesions. Kuttappan et al., (Kuttappan et al., 2013) observed the meat quality of WS afflicted breast meat and showed that L* values range between 52.63 and 53.08, and did not show any changes between WS severity levels.

The a* values are associated with higher red pigmentation originating from myoglobin or hemoglobin as well as the oxidative state of the heme group (Hunt et al., 1999). The present results show no difference ($p>0.05$) due to the CM vs. SBM diets (1.69 vs.1.52, respectively), or WB score severity (1.68, 1.73, 1.35, and 1.67; respectively). Similar results were observed ($p>0.05$) for a* values at day 56 of CM vs. SBM (1.92 vs. 1.69, respectively) and WB score severity levels (2.43, 1.73, 1.83, and 1.85; respectively). Mazzoni et al., (2015) reported no differences in a* values from 4 broilers breeds weighing 3.6 kg and graded according to muscle degeneration scores based on histological lesions. Kuttappan et al., (Kuttappan et al., 2013) found that WS did not impact a* values in breast meat.

The breast meat was also analyzed for b* color values. Kuttappan et al. (Kuttappan et al., 2013, 2017) reported that b* values were a possible tool for identifying extremely severe WS and WB afflicted breast meat. However, the results from the current experiment show that no differences ($p>0.05$) were observed in b* values at day 49 for the dietary effect (CM = 5.19 vs. SBM = 4.95) or the WB severity level (Normal= 4.94, moderate=5.03, severe=4.84, and extremely severe=4.86). The same results were observed on day 56 fillets, with no differences ($p>0.05$) found for the diet or WB severity effects. The positive b* values are an objective measurement of yellowness in the color spectrum. The amounts of fat and fatty acid composition are known to cause changes in color (Qiao et al., 2002). It is possible that the high soybean oil used in the manufacturing of the CM diet may have affected the b* color in the meat.

Overall, the experimental diets did not pose a negative impact on the physicochemical state of the meat on either harvesting day. Moreover, the CM fed birds did have a lower pH in the breast fillet compared to the SBM fed birds at day 49 and 56. In addition, no interactions were found between diet and WB severity. Considering that the data were normalized to control for growth rate differences, it is possible that the CM diet presents an ability to modulate the final pH in WB afflicted meat. While the WB severity effect on pH was only observed at day 56 and not on day 49, these results coincide with the incidence percentage observed for the respective days. The absence of any significant difference in L*, and b* values attributes that have been reported to vary between unaffected and severe WB fillets by multiple authors (Kuttappan et al., 2013; Mudalal et al., 2014; Tijare et al., 2016), may be explained by the normalization of the data. Normalizing the data to similar breast weights may control the source of variation caused by growth rate and processing, thus minimizing color differences in the breast meat.

2.4.3. Carcass Performance

For a processor, the carcass yield (CY) represents the amount of sellable broiler product after the live bird is harvested. If a diet or a breast meat abnormality affects the carcass yield, processors are willing to adjust processes to improve CY. The results show that diets at either 49 d or 56 d differ significantly (p-value < 0.05 and 0.05;respectively) in CY percentages (Table 5). The severity of WB had no effect on CY for either 49 d or 56 d (p-value = 0.6703 and 0.4765, respectively). This lack of difference may be in part due to the normalization of the data. However, independent of the normalization, the CM fed birds produced a lower CY compared to the SBM fed birds on both harvest days. To the author's knowledge, there has been no recent published research where a diet comprised of only CM as the main source of protein was compared to another source.

The breast meat represents the portion of meat with the highest demand from broiler carcasses (Wells, 2015, 2016). The BY results are shown in table 5. At day 49, BY values between the diets were different (p-value <0.05; (CM = 21.42% vs. SBM = 22.85%), while the effect of WB severity on BY showed that the unaffected (20.83%) fillets were lower (p-value < 0.05) in yield compared to the moderate, severe, and extremely severe WB afflicted fillets (22.18%, 22.87%, and 23.14%, respectively) which did not differ from each other. By day 56 the dietary effect on BY, showed that the CM diet (23.42%) was lower in yield (p-value <0.05) than the SBM diet yields (24.08%), but WB severity did not negatively affect (p>0.05) the BY.

Table 5. Means, standard deviation, and sample size for the quality parameters of carcass yield, breast yield, drip loss %, and cook loss % from birds fed a canola meal (CM) or soybean meal (SBM) diet and graded for woody breast at harvest days 49 and 56.

		Carcass Yield %		**Breast Yield %**		**Drip Loss %**		**Cook Loss %**	
	Levels	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**
Day 49	CM	$77.48^{y}\pm2.04$	58	$21.42^{y}\pm1.32$	62	$-3.01^{y}\pm3.27$	59	$-21.09^{y}\pm4.83$	45
	SBM	$78.75^{z}\pm3.55$	69	$22.85^{z}\pm1.72$	70	$-3.64^{z}\pm3.44$	68	$-24.32^{z}\pm4.72$	42
	Normal	77.28±2.19	27	$20.83^{b}\pm1.16$	30	$-2.24^{b}\pm1.18$	26	-19.80±3.74	19
	Moderate	78.08±3.58	48	$22.18^{a}\pm1.69$	49	$-2.51^{b}\pm1.27$	42	-21.95±4.97	33
	Severe	78.87±3.00	40	$22.87^{a}\pm1.61$	41	$-3.22^{ba}\pm1.53$	41	-24.62±4.33	27
	Extremely Severe	78.25±1.48	12	$23.14^{a}\pm1.08$	12	$-4.25^{a}\pm1.95$	10	-25.70±6.41	8
	Factor Effects[1]	p-value		p-value		p-value		p-value	
	Diet	0.0186		<0.0003		0.0163		0.0018	
	Woody Breast	0.6703		0.0016		0.0045		0.0691	
	Diet X Woody Breast	0.6299		0.2538		0.8723		0.4137	
	Levels								
Day 56	CM	$79.32^{y}\pm1.69$	77	$23.42^{y}\pm1.38$	83	$-2.09^{y}\pm1.16$	76	-25.78±3.83	58
	SBM	$80.24^{z}\pm1.55$	63	$24.08^{z}\pm1.77$	65	$-2.53^{z}\pm1.26$	57	-25.36±4.12	30
	Normal	79.74±2.39	4	22.31±0.42	4	$-1.01^{b}\pm0.22$	4	$-19.28^{b}\pm3.17$	2
	Moderate	79.36±3.77	36	23.35±1.85	40	$-1.59^{b}\pm0.74$	38	$-23.50^{b}\pm2.39$	25
	Severe	79.78±2.66	68	23.81±1.50	71	$-2.43^{b}\pm1.16$	66	$-26.03^{a}\pm3.93$	45
	Extremely Severe	80.06±1.93	32	24.10±1.38	33	$-3.13^{a}\pm1.35$	25	$-28.64^{a}\pm3.27$	16
	Factor Effects	p-value		p-value		p-value		p-value	
	Diet	0.0010		0.0112		0.0178		0.595	
	Woody Breast	0.4765		0.1394		<0.0001		<0.0001	
	Diet X Woody Breast	0.0289		0.1766		0.0468		0.856	

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[1] Factor effects for diet, woody breast incidence, and interaction between diet and woody breast were considered significant when p-value <0.05

[x-z] Means with different superscripts for diet levels within a column are significantly different when p-value <0.05

[a-d] Means with different superscripts for woody breast levels within a column are significantly different when p-value <0.05

On day 49 of harvest, the CM was able to reduce the incidence of WB (Table 2), but this reduction may be in relation to the lower BY of the fast-growing broilers. This inference is further supported by the observed results of lower BY on unaffected fillets at day 49. When the birds were harvested at day 56, the CM diet continued to have a lower BY compared to the SBM, but the incidence of WB was not different between the two diets (Table 3). The WB severity levels did not show any differences ($p>0.05$) between each other at day 56, in contrast to day 49. Overall, it was observed that as BY increases, so does the severity of WB, independent of diet and low CY. The results published by Kuttappan et al. (2017) report similar finding to the current study in regards to BY of broiler birds harvested at d 42, were birds fed a corn-SBM diet showed higher BY for extremely severe WB compared to unaffected fillets. However, at d 63, the researchers also observed differences between extremely severe WB and unaffected fillets contrasting the observed results of the current study. The difference between the studies may be explained by the experimental design, diet composition, and harvesting day.

2.4.4. Drip loss percentage

Drip loss serves as an indirect measurement of WHC. The theory is based on the ability of the proteins and physical forces (stearic effect, net charges, protein quality, and protein ratio) to retain the existing water in the muscle. At day 49, the dietary effects were noticeable ($p<0.05$) with the CM fed birds having the least amount of DL (3.01 %) compared to the SBM fed birds (3.64 %). This observed diet effect was expected, as WB incidence was lower in the CM fed than the SBM fed birds (Table 2). The effect of WB severity on DL was also significant (p<0.05). The unaffected fillets and moderate WB fillets (2.24% and 2.51%, respectively) had a lower DL compared to the severe and extremely severe fillets (3.22% and 4.25%, respectively). By day 56, there were also differences (p<0.05) in the diets and the WB severity factors. Even though the incidence of WB at day 56 was not different between the diets (Table 3), the CM diet was able to reduce the DL (2.09%) compared to the SBM diet (2.53%). The extremely severe WB fillets had the highest DL (3.13%) compared to the unaffected, moderate, and severe WB fillets (1.01%, 1.59%, and 2.43%; respectively). The results show that the CM fed birds had lower drip loss in their breast fillets on both day 49 and 56, but the incidence of WB was only lower on day 49 compared to day 56 fillets, leading to the assumption that the CM diet may reduce the negative DL effects of WB afflicted fillets. It is not fully understood whether the mechanisms that caused a lower DL by the CM are due to lower CY and BY performance compared to the SBM diet, or a nutritional component of the CM diet. The differences observed in DL for the WB severity are in agreement with those reported by Kuttappan et al., (2017) where higher DL was observed in fillets grading to Severe WB when compared to unaffected fillets (2.14% vs. 0.49%, respectively) at d 42 and d 63 (0.51% vs. 2.06%, respectively) of harvest. Overall, the CM diet may prevent higher DL on fillets afflicted by WB.

2.4.5. Cook loss percentage

The use of CL as a WHC measurement is based on the assumption that the quality of the proteins and their ability to hold water can be assessed when the meat is cooked under controlled conditions. Lower CL % would indicate better WHC of the meat. At day 49, differences ($p<0.05$) between the diets were observed. The CM diet (-21.09%) was able to reduce the CL by at least 3.00% over the SBM diet (-24.32%). This result coincides with the CM diet having a lower DL and incidence of WB fillets at day 49. The different levels of WB severity did not cause an apparent reduction ($p=0.0691$) of CL at day 49, thus indicating that the CM diet was the main factor leading to an improvement in CL of the fillets together with the lower incidence of WB. By day 56, the results observed on day 49 were reversed. The main factor affecting CL was the severity of WB ($p<0.05$), where the unaffected (19.28%) and the moderately afflicted WB fillets (23.50%) had a lower CL% compared to the severe (26.03%) and extremely severe (28.64%) afflicted WB fillets. The diet had less ($p>0.05$) of an effect on CL than it did on day 49. The effect of WB severity observed at day 56 is in agreement with the results reported for WB incidence at day 56, were no differences in WB frequency across severity level and diet level were observed (Table 3). Mudalal et al. (2015) reported higher CL % on breast meat affected by WB compared to meat that was considered unaffected, in birds harvested at d 52 from a commercial facility. Soglia et al. (2016a) used proton transverse relaxation decay curves (T_2) to assess the bound, intra-myofibrillar, and extra-myofibrillar proton pools to determine the effects of cooking on unaffected and WB afflicted fillets, and found that there was a higher level of extra-myofibrillar water in WB afflicted fillets obtained from a commercial facility compared to unaffected fillets. Overall, The CL % of breast meat seems to be impacted greatly by the incidence of WB. At earlier ages, it appears that changes in diets may provide an avenue for reducing CL, as long as the WB incidence is also reduced. For birds harvested past day 49, the effects of diets are lost and the severity of WB plays a bigger role in the percentage of CL observed in the breast meat.

2.4.6. Inject marination

The ability of meat to retain brines right after injection and over time serves as an indication of WHC. The breast fillets harvested at day 49 (Table 6) indicate that the CM (13.18%) diet was able to pick-up a higher ($p<0.05$) level of brine compared to the SBM (12.32%) diet, right after injecting to a target of 15%. Although the WB severity factor showed no difference with a p-value of 0.0503, it is still considerably close to the statistical significance threshold of alpha = 0.05. In the WB effect, it can be observed that the unaffected fillets picked-up 13.05% ± 1.57%, a larger amount of the brine compared to the extremely severe WB fillets that were only able to pick-up 10.65% ± 2.45%. By day 56, the differences in pick-up between diets ($p>0.05$) were lost, coinciding with the equal incidence of WB in the diets at this age. The observed differences in pick-up between the WB severities show that unaffected fillets (12.02%) and moderately (12.15%) affected WB fillets were similar ($p>0.05$) to each other, but unaffected fillets did not differ ($p>0.05$) to the severe (10.83%) and extremely severe (9.68%) affected WB fillets, which were not different to each other. It is possible that the low number of unaffected fillets used in the analysis, after normalization, may have caused this discrepancy. Overall, the brine pick-up results seem to match the results observed for the incidence of WB and are in agreement with other published results that show extremely severe WB having lower ability to pick-up brine compared to unaffected fillets (Mudalal et al., 2015; Kuttappan et al., 2016).

Table 6. Means, standard deviation, and sample size for brine pick-up %, 20 min retention yield, and 24 h retention yield from woody breast graded1 breast meat harvested on day 49 and 56 of birds fed a canola meal (CM) or soybean meal (SBM) diet.

		Pick up %		**20 min Retention %**		**24 h Retention %**	
Day 49	*Levels*	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**
	CM	13.18^{y}±1.82	48	98.03±1.29	41	97.85±1.23	56
	SBM	12.32^{z}±2.39	59	97.83±1.42	38	97.81±1.37	60
	Normal	13.05±1.57	23	98.36±1.12	19	97.91±1.47	25
	Moderate	12.98±2.19	41	98.04±0.95	31	97.94±1.15	43
	Severe	12.74±2.21	33	97.70±1.65	24	97.74±1.42	37
	Extremely Severe	10.65±2.45	10	96.76±2.05	5	97.51±1.10	11
	Factor Effects[2]	p-value		p-value		p-value	
	Diet	0.0406		0.4821		0.8810	
	Woody Breast	0.0503		0.0679		0.7280	
	Diet X Woody Breast	0.6415		0.0081		0.8720	
Day 56	*Levels*						
	CM	11.17±2.51	73	99.18±0.59	81	98.23±1.16	76
	SBM	10.79±2.50	59	99.09±0.52	63	98.23±1.26	62
	Normal	12.02^{ab}±2.30	4	99.56±0.35	4	99.13^{a}±0.45	3
	Moderate	12.15^{a}±2.46	37	99.13±0.60	40	98.46^{a}±0.77	38
	Severe	10.83^{b}±2.53	64	99.15±0.52	68	98.13^{a}±0.76	67
	Extremely Severe	9.68^{b}±1.80	27	99.09±0.61	32	98.07^{a}±0.81	30
	Factor Effects	p-value		p-value		p-value	
	Diet	0.3698		0.3422		0.9878	
	Woody Breast	0.0010		0.5678		0.0197	
	Diet X Woody Breast	0.4313		0.0924		0.1346	

[1] Woody breast grades = unaffected (a.k.a. “normal”), moderate, severe, and extremely severe

[2] Factor effects for diet, woody breast incidence, and interaction between diet and woody breast were considered significant when p-value <0.05

[x-z] Means with different superscript for diet levels within a column are significantly different when p-value <0.05

[a-d] Means with different superscript for woody breast levels within a column are significantly different when p-value <0.05

The results for the retention of the brine 20 min after injection at day 49 shows no difference ($p>0.05$) from the diet or WB severity factors. However, an interaction ($p<0.05$) between the diet and WB severity was observed. When the CM and SBM diet were analyzed independently for the WB severity factor, no differences ($p>0.05$) were detected on each of the severities within the diets. The possible interaction observed at 20min retention may have occurred from the lower incidence of WB in the CM diet compared to the SBM. At 56 days, there was no difference ($p>0.05$) for all the factors and interactions. This effect may be caused by the lower injection levels, even though differences in pick up % were observed for the WB levels at day 56. After 24 h from injection of the brine, the retention for birds processed at day 49 showed no difference ($p>0.05$) at any of the factors levels and interaction. For day 56 processed birds, the model found differences ($p<0.05$) for the WB levels factor, but after a post-hoc analysis, it was determined that there were no differences between the WB levels.

Overall, the injection and retention of brine below 15% pick up were not affected by the diet assignment or WB severity levels when birds were harvested at day 49, even though the CM diet was undoubtedly able to affect the pick up of brine. By day 56, the effects of WB severity were more evident on brine pick up %, but no other effects were noted. Tijare et al., (2016) reported on vacuum tumbled, horizontally portioned, unaffected and WB breast fillets from broilers harvested at 6 and 9 wk, that were marinated to a target of 15% up-take, and found that the unaffected fillets had a higher brine up-take (10.6%) than that of the WB fillets (6.3%) of 61 day old broilers; when comparing the effect of marination up-take % between 6 and 9 wk old birds, it was observed that older birds had a lower up-take than the younger birds. The main differences between the current study and the outcomes reported by Tijare et al. (2016) is the method of marination and the brine retention was reported in the current article. Inject marination allows the brine to come into contact with proteins located on the inside of the breast meat, while vacuum tumbling allows only the surface of the portion to come in contact with the brine and relies on time, physical force, and vacuum to aid in the absorption (Owens et al., 2000; Barbut, 2015a).

2.5. Conclusions

In conclusion, the incidence of WB increased with the age of harvest of the birds, independent of the diet fed. However, it was also observed that dietary effects may have had a higher impact on minimizing WB negative effects on meat quality when birds were harvested on or before day 49. It was evident that the CM based diet was able to lower the incidence of WB at day 49 compared to the SBM based diet. Although birds fed the CM based diet had a lower carcass and breast yield then the SBM fed birds, the reduction of WB incidence in CM diet fed birds improved the WHC of the meat by normalizing the pH, lowering drip loss and cook loss, and improving brine pick up. The positive effects of the CM diet disappeared by day 56. The incidence of WB was similar to the SBM diets, and the WHC capacity was slightly impacted by an improved drip loss of the breast meat, but at the cost of lower CY and BY.

The woody breast severity effect on WHC was notable on breast yield, pH, drip loss, and cook loss. The unaffected and moderate WB fillets seemed to be similar to each other in the ability to retain water, as were the severe and extremely severed WB fillets. The inject marination would be negatively affected by severe and extremely severe WB fillets, making the consistency of the injection process a challenge. At low injection levels of 10% or below, the severe and extremely severe WB fillets were able to retain the marination 20 *min* and 24 *h* after injection, at similar retention yields of the unaffected fillets.

3. WATER HOLDING ATTRIBUTES OF WOODY BREAST MEAT FROM BROILERS FED DIETS FORMULATED WITH INCREASING INGREDIENT COMPLEXITY

3.1. Synopsis

The woody breast condition (WB) affects the water holding capacity (WHC) of broiler meat. This study was focused on evaluating the WHC and WB incidence in fast-growth, high breast yielding (BY) broiler breeds fed a corn-soybean meal control (Ctrl), a Ctrl + β-mannanase enzyme (Ctrl+Mannan), a diet reduced in soybean meal and replaced with canola meal (CM), and a diet formulated to reduce corn and soybean meal with various ingredients (Mix). In two trials, day-old chicks were reared under industry simulated conditions in 8 concrete pens (n=100birds/diet) lined with pine shavings and with *ad libitum* access to water and feed. At d 42, the fasted bird BW (BW) was measured and the birds were processed following industry practice. The chilled carcasses were weighed to determine carcass yield (CY), manually deboned to extract the breast meat to establish BY and grade for WB severity (unaffected a.k.a. "normal", mild, moderate, and severe). At 24h, the pH, drip loss % (DL), and color (L*, a*, and b*) meat quality parameters were measured. The results for WB incidence cross tables were statistically analyzed with χ^2, and the response variables (CY, BY, pH, DL, and color) were ranked transformed before analyzing them with an analysis of covariance with the BW as a covariate and diet and WB severity as independent variables. Statistical difference was stablished at an $\alpha = 0.05$. The results showed that the Mix diet reduced ($p<0.05$) the incidence and severity of WB. However, the pH was inexplicably higher ($p<0.05$) in the Mix diet, contradicting the WB severity effect on pH values. The effect of WB severity was marked ($p<0.05$) on BY, DL, and pH as expected. In conclusion, within the current study of the WB condition, it is impossible to assess the effect of dietary intervention on any given meat quality parameter without knowledge of the effect of the WB severity on said parameter, the incidence of WB, and the relationship between WB incidence and severity.

Keywords: Woody Breast, Water Holding Capacity, Soybean meal, Diet Formulation

3.2. Introduction

The poultry industry is interested in addressing the issues of woody breast (WB) muscle condition, which causes economic loss from undesirable texture attributes and lower water holding capacity (WHC) (Kuttappan et al., 2017; Aguirre et al., 2018; Sun et al., 2018). In severe cases of WB, edemas and spotted petechial hemorrhage have been reported (Sihvo et al., 2014), and the Food Safety and Inspection Service (FSIS) of the U.S. has deemed the presence of these histologies in severe WB fillets as an adulterant that processors have to condemn at the slaughterhouse (FSIS, 2018). The presence of moderate and severe WB causes the breast meat to be downgraded to further processed products due to the undesirable attributes to consumers (Soglia et al., 2016a).

Woody breast in broiler meat is characterized by an increase in the meat's hardness when palpated (Velleman and Clark, 2015; Tijare et al., 2016). Though the source of this hardness is yet to be determined, other unique physiological parameters have been associated with WB. The muscle of WB meat undergoes a continuous state of fiber degeneration and regeneration, and presents a higher inflammatory response compared to unaffected breast meat (Sihvo et al., 2014; Soglia et al., 2016b). This degeneration causes the tissue to undergo fibrosis, which increases the collagen and fat deposition in the meat (Trocino et al., 2015; Soglia et al., 2016a; Papah et al., 2017). The WB muscle has a different metabolic state characterized by lower glycogen potential and deposition, higher state of oxidation, and an imbalance in the Krebs cycle when compared to unaffected fillets (Abasht et al., 2016). All these histological factors have a negative impact on the quality of the breast fillet. Breast fillets with severe WB are known to have less WHC, which impacts the functionality of the meat in value-added products (Kuttappan et al., 2017; Aguirre et al., 2018; Sun et al., 2018).

The most ubiquitous source of protein in broiler diets is the soybean meal (SBM), as it provides a high content of protein (>36%), and contains proper amounts of required amino acids. However, other sources of proteins (canola, blood, and corn meals) are available and are used sparingly depending on availability, cost, digestibility, and amino acid composition (Baker, 2009; Córdova-Noboa et al., 2018). The effect of varying the source of proteins and the complexity of the dietary feed formulation and how these formulas impact WB has not been previously evaluated. This study focused on evaluating how diets with an increasing variety of ingredients, that are iso-energetic, affect the incidence and severity of WB, and other meat quality parameters from fast-growing broilers. In addition, the WB severity levels were assessed independently regarding their effect on meat quality and their influence on the diets.

3.3. Materials and Methods

The experiment was designed to measure the influence of four experimental diets on WB severity and WHC of the breast meat from commercial fast growth broilers harvested at d 42. The experimental design consisted of two experimental trials that contained two pens per dietary treatment and 50 birds per pen (n=100 birds/dietary treatment). The birds were reared under Texas A&M University's animal use protocol #2014-0202 in an open-sided house with solid hinged side panels for ventilation on concrete floors lined with fresh pine shavings as bedding material. Hatch-day broilers were stocked at a density of 0.08 m2/bird and were placed on an *ad libitum* feed of the assigned diet and continuous access to water with age-appropriate supplemental heating. The animals were raised with three dietary phases during their growth: a starter (1 d -14 d), grower (15 d – 28 d), and finisher diet (29 d – 42 d). The lighting schedule was adjusted according to their dietary phases: starter (23L: 1D), grower (20L: 4D), and ended with a 16L: 3D: 2L: 3D light cycle. Dietary groups consisted of 1) a control diet (Ctrl) with phytase to represent a traditional commercial diet; 2) a control diet supplemented with β-mannanase enzyme (Ctrl+Mannan); 3) a canola meal (CM) diet that replaced 10% of the SBM with CM in the starter phase, and 20% through the remaining phases; and 4) a mixed diet (Mix) comprised of multiple sources of proteins and carbohydrates (Tables 7 - 9). The CM diet was based on the recommendation from the Canola Council of Canada (2016) that limited the amount of CM that can be incorporated to diets, with the modification that birds were never removed from the CM diet prior to slaughter. Starter diet was fed as a crumble and all other phases were fed as pellets. At day 42, the feed was withdrawn eight hours prior to slaughter and the birds were processed at the Texas A&M University Poultry Research Center in College Station, TX.

Birds were transported to the facility in seven batches of 28 randomly selected birds from each treatment and the fasted BWs of the birds were recorded. Birds were electrically stunned at 13 to 15 V for 7 to 10 s with an electrified knife (Cervin Electrical Systems, Minneapolis, MN) with an AC/DC converter set at 500 Hz prior to exsanguination. The jugular vein and carotid artery were severed in a unilateral cut, and birds were allowed to bleed for ~90 s prior to scalding at 60°C for 10 s (model SS-30-SS, Bower Corp., Haughton, IA) and feather removal using a rotary drum picker (model SP-30-SS, Bower Corp.) for 25 s. Neck and hocks were removed before manually eviscerating the birds. The eviscerated carcasses were chilled in an agitated 4°C ice water bath for 1.5 h prior to deboning. The weights of the chilled carcasses and whole breasts with rib meat were recorded to calculate carcass yield % (CY) from BW, and breast yield % (BY) from the chilled carcass weight. The breasts were graded (0 = unaffected a.k.a. "normal", 1 = mild, 2 = moderate, 3 = severe) for WB (Tijare et al., 2016) immediately after deboning, and placed in plastic ziploc bags in a walk-in cooler (Hobart, Model W, Troy, OH) for 24 h at 4C, before re-weighing to determine drip loss % (DL). Thereafter, the pH of the breast was measured from the centermost point in the cranial region of the breast (model 205, Testo AG, Sparta, NJ). The color (L*, a*, and b*) of the meat was measured at a 0^{o} angle with a C-illuminant colorimeter at the bone side of the breast (CR400, Konica Minolta, Ramsey, NJ).

Table 7. Starter male broiler diet (1 - 14 d of age) percentage formulation and calculated nutrient content for each experimental treatment.

Ingredients	Control	Control+ Mannase	Canola meal	Mix
Corn	57.20	57.20	52.99	51.39
Soy Bean Meal (48%)	36.40	36.40	29.94	21.30
DL-Methionine 98%	0.31	0.31	0.27	0.27
L-Lysine HCL	0.19	0.19	0.22	0.41
L-Threonine 98%	0.07	0.07	0.06	0.10
L-Arginine 98%	0.00	0.00	0.00	0.10
Soy oil	2.60	2.60	3.47	1.50
Limestone	1.50	1.50	1.38	0.68
Monocalcium phosphate	0.95	0.95	0.90	0.00
Salt	0.46	0.46	0.44	0.11
Sodium bicarb	0.00	0.00	0.00	0.24
Vitamins[1]	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05
Canola meal	0.00	0.00	10.00	0.00
Wheat middlings	0.00	0.00	0.00	5.15
Fish Meal	0.00	0.00	0.00	2.00
Corn DDGS	0.00	0.00	0.00	3.00
Pork meat and bone meal	0.00	0.00	0.00	3.42
Bakery by-products	0.00	0.00	0.00	5.00
Enspira[6]	0.00	0.00	0.00	0.01
Phytase[4]	0.01	0.01	0.01	0.01
β-mannan[5]	0.00	0.05	0.00	0.00
Calculated Nutrients				
Dry Matter	87.44	87.44	86.35	88.22
Moisture	12.56	12.56	13.65	11.78
Protein	22.93	22.93	22.97	23.70
Crude Fat	5.11	5.11	6.11	4.87
Available-Lysine	1.249	1.249	1.249	1.25
Available-Arginine	1.388	1.388	1.367	1.31
Available-Methionine	0.622	0.622	0.6	0.635
ME Kcal/Kg	3047.01	3047.01	3047.00	3047.03

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadion 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient salinomycin sodium (500 g/ton inclusion: Sacox® 120 microgranulated, Huvepharma, Sofia, Bulgaria). As an aid in the prevention of coccidiosis caused by *Eimeria necarix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria mivati, and Eimeria maxima*.

[4]Optiphos® PF, Enzyvia LLC Sheridan, IN

[5]Hemicell® HT, Elanco, Greenfield, IN

6Enspira, United Animal Health, Sheridan, IN

Table 8. Grower male broiler diet (15 - 28 d of age) percentage formulation and calculated nutrient content for each experimental treatment.

Ingredients	Control	Control+ Mannase	Canola meal	Mix
Corn	63.86	63.86	55.54	57.45
Soy Bean Meal (48%)	30.26	30.26	17.25	16.95
DL-Methionine 98%	0.28	0.28	0.19	0.22
L-Lysine HCL	0.20	0.20	0.27	0.39
L-Threonine 98%	0.07	0.07	0.06	0.09
L-Arginine 98%	0.00	0.00	0.00	0.10
Soy oil	2.40	2.40	4.13	1.50
Limestone	1.35	1.35	1.11	0.68
Monocalcium phosphate	0.80	0.80	0.71	0.00
Salt	0.39	0.39	0.36	0.00
Sodium bicarb	0.10	0.10	0.08	0.42
Vitamins[1]	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05
Canola meal	0.00	0.00	20.00	0.00
Corn gluten (60%)	0.00	0.00	0.00	5.00
Corn DDGS	0.00	0.00	0.00	3.00
Pork meat and bone meal	0.00	0.00	0.00	2.48
Bakery by-products	0.00	0.00	0.00	5.00
Wheat middlings	0.00	0.00	0.00	4.40
Fish Meal	0.00	0.00	0.00	2.00
Enspira[6]	0.00	0.00	0.00	0.01
Phytase[4]	0.01	0.01	0.01	0.01
β-mannan[5]	0.00	0.05	0.00	0.00
Calculated Nutrients				
Dry Matter	87.528	87.528	85.361	88.119
Moisture	12.472	12.472	14.639	11.881
Protein	20.5	20.5	20.552	21.44
Crude Fat	5.102	5.102	7.074	4.93
Available-Lysine	1.1	1.1	1.1	1.1
Available-Arginine	1.211	1.211	1.165	1.154
Available-Methionine	0.561	0.561	0.516	0.565
ME Kcal/Kg	3101.96	3101.956	3101.967	3101.93

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadion 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient salinomycin sodium (500 g/ton inclusion: Sacox® 120 microgranulated, Huvepharma, Sofia, Bulgaria). As an aid in the prevention of coccidiosis caused by *Eimeria necarix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria mivati, and Eimeria maxima*.

[4] Optiphos® PF, Enzyvia LLC Sheridan, IN

[5] Hemicell® HT, Elanco, Greenfield, IN

[6] Enspira, United Animal Health, Sheridan, IN

Table 9. Finisher male broiler diet (29 - 42 d of age) percentage formulation and calculated nutrient content for each experimental treatment.

Ingredients	Control	Control+ Mannase	Canola meal	Mix
Corn	69.57	69.57	58.14	59.40
Soy Bean Meal (48%)	24.11	24.11	13.95	19.45
DL-Methionine 98%	0.25	0.25	0.13	0.16
L-Lysine HCL	0.21	0.21	0.19	0.26
L-Threonine 98%	0.08	0.08	0.03	0.03
L-Arginine 98%	0.00	0.00	0.00	0.00
Soy oil	2.95	2.95	5.17	2.23
Limestone	1.27	1.27	1.02	0.88
Monocalcium phosphate	0.70	0.70	0.59	0.34
Salt	0.23	0.23	0.29	0.00
Sodium bicarb	0.32	0.32	0.17	0.42
Vitamins[1]	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05
Canola meal	0.00	0.00	20.00	0.00
Corn gluten (60%)	0.00	0.00	0.00	5.00
Corn DDGS	0.00	0.00	0.00	2.00
Pork meat and bone meal	0.00	0.00	0.00	2.00
Bakery by-products	0.00	0.00	0.00	7.50
Enspira[6]	0.00	0.00	0.00	0.01
Phytase[4]	0.01	0.01	0.01	0.01
β-mannan[5]	0.00	0.05	0.00	0.05
Calculated Nutrients				
Dry Matter	86.96	86.96	84.36	87.54
Moisture	13.03	13.03	15.63	12.45
Protein	18.00	18.00	19.05	20.46
Crude Fat	5.80	5.80	8.16	5.53
Available-Lysine	0.959	0.959	0.959	0.961
Available-Arginine	1.03	1.03	1.067	1.048
Available-Methionine	0.501	0.501	0.444	0.475
ME Kcal/Kg	3190.10	3191.10	3189.85	3189.78

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D3, 46 IU vitamin E, 0.0165 mg B12, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadion 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3] Active drug ingredient salinomycin sodium (500 g/ton inclusion: Sacox® 120 microgranulated, Huvepharma, Sofia, Bulgaria). As an aid in the prevention of coccidiosis caused by *Eimeria necarix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria mivati, and Eimeria maxima*.

[4] Optiphos® PF, Enzyvia LLC Sheridan, IN

[5] Hemicell® HT, Elanco, Greenfield, IN

6Enspira, United Animal Health, Sheridan, IN

3.3.1. Statistical Analysis

In this study, the experimental unit used for analysis was the broiler and the dependent variables of CY, BY, DL, pH, color, WB and the bird's BW (covariate) were rank transformed (Conover and Iman, 1982) prior to analysis. The independent variables of dietary groups (Ctrl, Ctrl+Mannan, CM, and Mix), WB scores (0 = unaffected a.k.a. "normal", 1 = mild, 2 = moderate, and 3 = severe), and their interactions were analyzed in a non-parametric independent unbalanced randomized analysis of covariance. Correlation between the covariate and the dependent variables was performed by Kendall's correlation. In addition, the incidence % of WB was analyzed by Pearson's χ^2 test to determine the % of WB severity within the dietary groups, and odds ratio was used to determine the likelihood of a diet minimizing the incidence of WB. All the data analyses were performed on the R statistical platform (v. 3.3.2), using RStudio (v.1.0.136, ©2009-2016 RStudio, Inc.) with significance established when $\alpha < 0.05$ and post-hoc rank transformed marginal means separated by Tukey's HSD, with the multicomps (v.1.4-8) and emmeans (v.1.2.4) packages.

3.4. Results

Initial test of assumptions for the dependent variables showed that the data were not normally distributed, thus non-parametric analysis was performed on the dependent variables and the covariate following the method described by Conover and Iman (1982). An analysis of covariance was chosen to control for BW of the broiler's growth difference between the dietary treatments (Table 10), since growth rate and final bird weight impact the incidence of WB

(Kuttappan et al., 2017). Thus, controlling for bird BW on the dependent variables ensures that a true effect from the diet and WB is assessed. The dataset's statistical analysis (median ± standard error) results are shown in table 11 and discussed below.

3.4.1. Processing performance and meat quality

Processing performances are of importance to producers due to the economic impact on profitability. Thus, the effect of the dietary treatments and WB on performance yields was measured. As expected the BW (Table 11) of the birds and the dietary treatments had an effect ($p<0.05$) on CY, with CY % increasing as BW increases. The Mix diet had an overall lower ($p<0.05$) CY than the Ctrl diet, but both Mix and Ctrl diets were not different ($p>0.05$) to the other two dietary treatments which were similar to each other ($p>0.05$). The WB severity levels had no ($p>0.05$) effect on the CY % of the sampled birds, and no interaction effects were observed on CY results between the diets and WB severity. Regarding the correlation between the CY and control variables of BW ($\tau = 0.29$, 95% CI= 0.22 - 0.35), dietary treatments ($\tau = -0.11$, 95% CI= -0.19 - -0.03), and WB severity levels ($\tau = 0.10$, 95% CI= 0. 2 - 0.18), it was observed that all variables were associated ($p<0.05$) to the CY, but the BW of the birds had a larger correlation ratio. As for the BY, all the control variables had an effect ($p<0.05$) on the BY, but no interaction ($p>0.05$) between the Trt and WB were observed.

Similarly to the CY, the BY % increased with the BW of the birds for the dietary treatments, with the CM diet having the highest ($p<0.05$) median (28.38 ± 0.21) yield compared to the Mix diet (26.94 ± 0.21). Both CM and Mix diets were not different ($p>0.05$) to the Ctrl (28.13 ± 0.23) and Ctrl+Mannan (27.50 ± 0.22), which were similar ($p>0.05$) to each other. An interesting relationship was observed for the WB severity levels and the covariate. A clear stratification among the four WB levels was evident ($p<0.05$), with the moderate mean rank (30.82 ± 0.29) having the highest BY % rank, with a slight tendency to increase in yield as birds get heavier. The unaffected (26.21 ± 0.19) fillets had the lowest BY % of all the levels and showed an almost 0 slope in relation to BW of the birds. While the mild (28.04 ± 0.12) WB severity level mean ranks had an increasing slope as bird BW got heavier. The correlation data showed that BW, dietary treatments, and WB severity levels (unaffected, mild, and moderate) were all associated ($p<0.007$) with BY data ($\tau = 0.18$, 95% CI= 0.12 – 0.24; $\tau = -0.10$, 95% CI= -0.17 – -0.02; $\tau = 0.42$, 95% CI= 0.36 – 0.48; respectively), in particular the severe WB severity had a high ratio of association ($\tau = 0.42$) to BY %.

Table 10. Dietary treatments and woody breast (WB) processing yields and meat quality parameters (median ± standard error) for 42 d old male broiler birds and the statistical model effect.[1]

	Dietary Treatments[2]				WB Severity[3]		
	Ctrl	Ctrl+Mannan	CM	Mix	Normal	Mild	Moderate
BW (g)	2346.00 ± 25.03	2436.00 ± 28.94	2327.00 ± 26.88	2276.00 ± 26.52	2160.00 ± 32.23	2359.00 ± 15.58	2450.00 ± 43.48
Carcass Yield	74.99 ± 0.21^{a}	75.12 ± 0.20^{ab}	74.74 ± 0.24^{ab}	73.97 ± 0.27^{b}	74.39 ± 0.25	74.77 ± 0.14	75.80 ± 0.34
Breast Yield	28.13 ± 0.23^{ab}	27.50 ± 0.22^{ab}	28.38 ± 0.23^{a}	26.94 ± 0.21^{b}	26.21 ± 0.19^{c}	28.04 ± 0.12^{b}	30.82 ± 0.29^{a}
Drip Loss	2.60 ± 0.18	2.31 ± 0.22	2.39 ± 0.16	2.04 ± 0.14	1.83 ± 0.09^{c}	2.38 ± 0.09^{b}	4.93 ± 0.43^{a}
pH	5.95 ± 0.01^{b}	5.95 ± 0.02^{b}	6.04 ± 0.02^{a}	6.00 ± 0.01^{a}	5.89 ± 0.02^{b}	5.99 ± 0.01^{a}	6.11 ± 0.03^{a}
L*	52.64 ± 0.29	52.57 ± 0.34	52.38 ± 0.34	52.51 ± 0.25	52.87 ± 0.37^{a}	52.38 ± 0.17^{b}	53.73 ± 0.62^{ab}
a*	2.61 ± 0.09	2.50 ± 0.09	2.20 ± 0.10	2.40 ± 0.10	2.44 ± 0.10	2.50 ± 0.05	2.21 ± 0.18
b*	4.56 ± 0.14^{ab}	5.48 ± 0.19^{a}	4.38 ± 0.18^{b}	4.29 ± 0.18^{b}	4.57 ± 0.22	4.69 ± 0.10	4.42 ± 0.33
WBⱡ	200.05 ±7.15^{a}	183.04 ±7.12^{ab}	197.84 ±7.12^{a}	166.40 ±7.32^{b}	-	-	-

	Model Effect			
Effects	Live Wt.	Diet	WB Severity	Diet x WB
Carcass Yield	<0.0500	<0.0500	>0.0500	>0.0500
Breast Yield	<0.0500	<0.0500	<0.0001	>0.0500
Drip Loss	<0.0500	>0.0500	<0.0500	>0.0500
pH	>0.0500	<0.0500	<0.0500	>0.0500
L*	<0.0500	>0.0500	<0.0500	>0.0500
a*	<0.0500	>0.0500	>0.0500	>0.0500
b*	>0.0500	<0.0500	>0.0500	>0.0500
WB	<0.0001	<0.0500	-	-

[1] Statistical comparisons was established by an independent unbalanced randomized analysis of covariance on rank transformed dependent and covariate variables (Y ~ Covariate + Dietary Treatments*WB, n=373)

[2] Crtl = Control Corn-Soybean meal diet, Ctrl+Mannan = control diet supplemented with β-mannase, CM = canola meal used as a replacer of soybean meal at 10% in the starter and 20% in grower and finisher dietary phases, Mix = variety of ingredients compared to other diets.

[3] No severe WB were observed during the process of scoring the breast fillets.

$^{a-c}$ Different letters within a row and factor indicate statistical differences between means ± standard error when $p<0.05$

ⱡMean ranks ± standard deviation of the WB scores

Breast meat quality can be determined through WHC parameters that assess the loss of water over time. Drip loss, pH, and CIELAB color scale are WHC measurements that are correlated with the capillary strengths of the muscle, internal ionic strengths, and color parameters of the meat (Pearson and Dutson, 1995). The DL results serve as an indication of the capillary forces present in the muscle to hold water. As observed in the results (Table 10), it is evidenced that the BW of the birds and the WB severity levels had an effect ($p<0.05$) on the meat's DL %, but the dietary treatments did not affect ($p>0.05$) the DL %. The WB severity levels differed among each other with the unaffected fillets (1.83 ± 0.09) having the lowest DL %, followed by the mild (2.38 ± 0.09) WB fillets, and the moderate (4.93 ± 0.43) WB fillets having the highest mean rank DL %. However, in relation to the BW of the birds the moderate WB fillets tended to slightly increase in DL % as the birds got heavier while the mild and unaffected fillets tended to slightly decrease in DL %. No effect ($p>0.05$) was observed in the interaction between the diets and the WB severity, but a correlation effect was observed between the DL % and the WB severity levels. The increase in WB severity was related to the increase in DL % ($\tau = 0.29$, 95% CI= 0.21 – 0.35, $p<0.0001$) observed on the breast fillets.

Dietary treatments ($p<0.0001$) and the WB severity ($p<0.0001$) affected the results for pH values but did not ($p>0.05$) interact with each other. The CM and Mix treatments had similar ($p>0.05$) pH mean rank values to each other (6.04 ± 0.02 and 6.00 ± 0.01, respectively) but were higher ($p<0.05$) than the Ctrl+Mannan (5.95 ± 0.02) and Ctrl (5.95 ± 0.01) treatments which had the same ($p>0.05$) pH values. Regarding the pH values for the WB severity levels, the moderate (6.11 ± 0.03) and mild (5.99 ± 0.01) WB breast fillet had the highest ($p<0.05$) pH values compared to the unaffected (5.89 ± 0.02) fillets and were similar ($p<0.05$) to each other. No effect on the pH ($p>0.05$) values were observed by the control variable but a correlation ($\tau = 0.28$, 95% CI= 0.19 – 0.36, $p<0.0001$) between the WB severity levels and the pH values was observed, and showed that as WB severity increases so do the pH of the breast meat.

The color measurements are divided into three parameters that determine the objective lightness (L^*), redness (a^*), and yellowness (b^*) of the meat sample. Higher levels of L^* in breast fillets have been associated with lower WHC, additionally, the L^*, a^*, and b^* values can impact the color of the meat which is related to customer acceptability. The results for L^* show that the covariate and the WB severity levels had an effect ($p<0.05$) on lightness, but no effects ($p>0.05$) were observed from the dietary treatments or the interaction of the diets and the WB levels. As the birds increased in weight the meat increased in L^* for both the diets and the WB severity levels. However, the effects of the WB severity levels on L^* of the meat indicated that the unaffected fillets (52.87 ± 0.37) were higher ($p<0.05$) compared to mild fillet's (52.38 ± 0.17) values, but both levels did not differ ($p>0.05$) from the moderate WB level (52.73 ± 0.62). The correlation results show a strong association ($\tau = 0.13$, 95% CI= 0.07 – 0.20, $p<0.0001$) between the BW of the birds and the L^* values, but no relationship ($p>0.05$) in the L^* values was observed for the WB severity levels. Regarding the a^* values, no differences were discerned for the effects of treatments ($p>0.05$), WB severity ($p>0.05$), or their interaction effect ($p>0.05$), but the covariate had an effect ($p<0.05$) on the redness of the meat. The a^* tended to decrease as the BW of the bird increased. Although a correlation between the a* value and the BW of the bird was present ($p<0.05$) this relationship was not strong (τ = -0.02, 95% CI= -0.11 – 0.05). Moreover, the b* of the breast meat was affected ($p<0.05$) by the dietary treatments, particularly by the Ctrl+Mannan which had the highest ($p<0.05$) b* value (5.48 ± 0.19) compared to the CM (4.38 ± 0.18) and Mix (4.29 ± 0.18) diets which did not differ ($p>0.05$) from each other. The Ctrl (4.56 ± 0.14) diet was similar ($p<0.05$) to all the other dietary treatments. No other confounding effects were observed between the b* values and the WB severity levels ($p>0.05$), interaction effect ($p>0.05$), or from the covariate ($p>0.05$). No considerable correlation ($p>0.05$ and τ = -0.07) was observed between the b* values and the covariate or the WB severity levels.

In regards to the effect that the dietary treatments had on the severity level of WB in the breast fillets, The Mix diet had the lowest ($p<0.05$) mean rank WB severity (166.40 ± 7.32) compared to all the dietary treatments. The Ctrl (200.05 ± 7.15) and CM (197.84 ± 7.12) diets, together, had the highest mean rank compared to the rest of the treatments, while the Ctrl + Mannan (183.04 ± 7.12) was not different ($p>0.05$) to any of the diets. The heaviest birds manifested the most severe WB scores. As a result, BW had a covariate effect ($p <0.05$) on WB severity levels; indeed BW correlated with WB score (τ = -0.11, 95% CI= -0.19 – 0.03, $p < 0.05$). Overall, the diets that had the lowest BY coincided with having the lowest WB severity mean ranks without affecting negatively the meat quality parameters. However, it is important to relate these results with the level of WB incidence observed in the experiment.

3.4.2. Incidence and odds ratio of WB on diets

The frequency results (Table 11) of the 373 breast fillets analyzed in the Diet x WB cross table showed differences (χ^2=31.18, df=6) within the table. The WB had counts mainly clustered around the mild severity (73.19%) followed by the normal (19.03%) and moderate (7.77%) levels. Within each of the levels, the unaffected fillets were distributed mainly in the Mix diet (42.25%) with the Ctrl+Mannan (26.76%) and the CM (22.54%) both holding the second and third majority in incidence, respectively. The Ctrl diet had the lowest amount of unaffected fillets (8.45%) of all the diets. In the mild severity, the Ctrl accounted for 29.67% of the afflicted fillets while the Ctr+Mannan, CM, and Mix had gradually reducing values of 24.91%, 23.81%, and 21.61% respectively. For the moderately afflicted fillets, the CM had more than a third of the counts (44.83%), whereas the Mix diet only accounted for 3.45% of the affected fillets and the Ctrl+Mannan (31.03%) and Ctrl (20.69%) had the remaining counts. Within each dietary treatment the frequency in the counts of WB were distributed in a similar manner across all treatments, with the Ctrl+Mannan having 25.74% of the counts followed by the CM, Ctrl, and Mix (25.20%, 24.93%, and 24.13%, respectively). The Ctrl's counts had 87.10% of the mild severity and equal amounts (6.45%) of unaffected and moderate WB fillets. The rest of the diets, when compared independently to the Ctrl diet, had lower amounts of mild WB severity (Ctrl+mannan = 70.83% v. CM = 69.15% v. Mix = 65.56%) and higher counts of unaffected fillets (Ctrl+mannan = 19.79% v. CM = 17.02% v. Mix = 33.33%). Interestingly, the CM had the highest amounts (13.83%) of moderate WB fillets compared to the rest of the diets.

Table 11. Woody breast incidence on breast meat harvested at d 42 from broilers fed a control (Ctrl), control supplemented with beta-mannase (Ctrl+Mannan), canola meal (CM),), or a variety of ingredients (Mix) based diet.[1]

		Normal		**Mild**		**Moderate**		**Total n**	**Total %**
Within[2]	**Diet**	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**		
Column	Ctrl	6	8.45%	81	29.67%	6	20.69%		
	Ctrl+Mannan	19	26.76%	68	24.91%	9	31.03%		
	CM	16	22.54%	65	23.81%	13	44.83%		
	Mix	30	42.25%	59	21.61%	1	3.45%		
	Total	71	19.03%	273	73.19%	29	7.77%	373	
Row	Ctrl	6	6.45%	81	87.10%	6	6.45%	71	24.93%
	Ctrl+Mannan	19	19.79%	68	70.83%	9	9.38%	61	25.74%
	CM	16	17.02%	65	69.15%	13	13.83%	63	25.20%
	Mix	30	33.33%	59	65.56%	1	1.11%	62	24.13%

[1] Significant differences between diets within woody breast severity were observed (p-value <0.0001) through Pearson's Chi-squared test (X2=31.18, df=6, n=373)

[2] Within column compares the results between diets for each of the woody breast (WB) severity levels. Within row compares the results between the WB severity levels within each diet.

The odds ratio (Table 12) provide a better understanding on how the diets had a chance of reducing or increasing the severity of WB compared to unaffected fillets. Within the fillets that were afflicted by the mild WB severity level, the Ctrl+Mannan and Canola were 3.7 and 3.3 times more likely in having unaffected fillets ($p<0.05$) compared to the Ctrl diets. Although, the Mix dietary treatment was 6.8 times more likely at having unaffected fillets over the Ctrl diet. Additionally, the Mix diet was 2.0 time more likely ($p<0.05$) at having more unaffected fillets than the CM diet. When the diets odds at reducing the amount of moderately afflicted fillets over unaffected fillets was accessed, it was observed that only the Mix diets had better odds ($p<0.05$) of having unaffected fillets over moderate WB fillets (Ctrl = 30.0 , Ctrl+Mannan = 14.2, and CM = 24.3), across all diets.

Table 12. Odds Ratio (OR) between the selected control diets to the treatment diets in relation to the incidence of woody breast (WB) severity from broiler breast meat harvested at d 42. [3]

Level A	Level B	OR	95% CI	P-Value
WB: Normal – Mild[1]				
Ctrl+Mannan	Crtl	3.7721	1.4259 – 9.9786	0.0075*
CM	Ctrl	3.3231	1.2306 – 8.9739	0.0178*
Mix	Crtl	6.8644	2.6853 – 17.5471	0.0001*
Crt+Mannan	Canola	1.1351	0.5378 - 2.3956	0.7395
Mix	Crt+Mannan	1.8198	0.9292 – 3.5642	0.0808
Mix	CM	2.0657	1.0241 – 4.1668	0.0427*
WB: Normal – Moderate[1]				
Ctrl	Crtl+Mannan	0.4737	0.1189 - 1.8864	0.2892
Ctrl	CM	0.8125	0.2111 - 3.1268	0.7627
Mix	Crtl	30.000	3.0340 – 296.64	0.0036*
Crt+Mannan	Canola	1.7153	0.5830 - 5.0468	0.3271
Mix	Crt+Mannan	14.210	1.6645 – 121.32	0.0153*
Mix	CM	24.375	2.9183 – 203.59	0.0032*
Normal – WB[2]				
Ctrl+Mannan	Crtl	3.5779	1.3594 – 9.4170	0.0098*
CM	Ctrl	2.9744	1.1087 – 7.9791	0.0304*
Mix	Crtl	7.2500	2.8428 – 18.489	0.0001*
Crt+Mannan	Canola	1.2029	0.5763 - 2.5107	0.6226
Mix	Crt+Mannan	2.0263	1.0406 – 3.9459	0.0378*
Mix	CM	2.4375	1.2179 – 4.8784	0.0118*

[1] Odds Ratio within each WB severity (Mild or Moderate) compared to the unaffected a.k.a. “normal” breast fillet

[2] Odds Ratio within the total amount of breast fillets afflicted by WB to the unaffected a.k.a. “normal” fillets.

[*] Diets that have significantly different ($p<0.05$) OR within their respective WB comparison.

[3] Crtl = Control Corn-Soybean meal diet, Ctrl+Mannan = control diet supplemented with β-mannase, CM = canola meal used as a replacer of soybean meal at 10% in the starter and 20% in grower and finisher dietary phases, Mix = variety of ingredients compared to other diets.

The dietary treatment’s likelihood at reducing the overall amount of WB incidence was accessed and it was concluded that the Ctrl+Mannan (3.5), CM (2.97), and Mix (7.25) diets were more likely ($p<0.05$) at diminishing ($p<0.05$) the incidence of WB over the Ctrl diet, with only the Mix diet having better odds at unaffected fillets over the Ctrl+Mannan (2.0) and CM (2.43). The results coincide with those observed on the BY but in contrast to the observed pH results.

3.5. Discussion

The occurrence and severity of WB have been attributed to the fast growth rate in high breast yielding commercial broiler lines, as well as the age of slaughter (Abasht et al., 2016; Kuttappan et al., 2017). Recent studies showed that restricting the feed not only caused a reduced growth performance, but also resulted in reduction in WB severity (Kuttappan et al., 2017). Other publications have provided a new perspective on diet interventions that target some of the underlying histological issues seen in WB breast, through the supplementation of broiler diets with vitamin C, arginine, guanidinoacetic acid, restricted feeding plans, and lower amino acid content (Trocino et al., 2015; Velleman and Clark, 2015; Cruz et al., 2017; Bodle et al., 2018; Meloche et al., 2018). For instance, diets that increase the supplementation of arginine and vitamin C showed a reduction in the incidence of WB without affecting growth performance (Bodle et al., 2018). This effect was attributed to the reduction of oxidative radicals and the possible improvement in vascularization from the arginine and vitamin C supplements. All of the above studies have reported either a positive or negative effect on WB caused by the dietary supplementation or feeding program interventions on the dependent variables (growth and processing performance, and meat quality), but have not analyzed the WB severity levels as an independent variable. Thus, the approach of the present study was to understand how the dietary treatments, WB severity levels, and BW of the birds, as a growth performance control variable, are intra-related in the explanation of the processing performance and meat quality parameters results in addition to establishing the relation of these effects to the incidence of WB.

3.5.1. Processing Performance

The CY in a slaughter operation serves as a process efficiency marker for the processor (Owens et al., 2000). The final BW of the birds at the day of harvesting can impact CY after chilling, as shown by the correlation factor ($\tau = 0.29$, $p < 0.0001$) and the significant effect of the covariate (BW) on the CY results (Table 10), with CY % increasing as bird BW increases. The median of the dietary treatments had an effect on the CY, but not the WB severity levels ($p <0.05$ and $p >0.05$, respectively). Reports have been published that show no effects of the dietary treatments on CY from broilers harvested at 46, 48, or 56 d (Córdova-Noboa et al., 2018; Bodle et al., 2018; Meloche et al., 2018). Cruz et al. (2017) observed differences in CY, but only when digestible lysine dropped below 0.93% in the diet, not the case for the current study. While differences in pre-chilled CY were also observed when a diet was fed with a time limit vs. *ad libitum* feeding program, no differences were observed between high growth rate or high breast yielding broiler lines (Livingston et al., 2018b). The same authors also reported differences in WB severity levels, where differences ($p<0.05$) in hot CY between the WB severity levels were seen in the moderate and severe fillets compared to the unaffected fillets. The disparity between those results and the present study may be in part due to the body weight differences, broiler breed, and dietary formulation in the published article compared to the current experiment. Additionally, the published articles do not control for BW disparity within the experimental units. Thus, accounting for the BW of the experimental unit may aid in observing dietary effect on CY %.

The effect of WB severity on BY was clearly defined across all levels of severity with the normal < mild < moderate fillets being all different ($p<0.05$). Moreover, the observed WB incidence (Table 11) had 73.19% of the total sampled fillets in the mild category, which caused the diet's medians to be around the 28.04 % median observed for the mild WB severity score. The increase in moderate WB severity led the CM diet to have the highest ($p<0.05$) BY, while for the Ctrl diet, both the unaffected and moderate fillets incidence levels were lower and similar ($p>0.05$) in values to all the other diets. Additionally, an effect ($p >0.05$) from the covariate was observed with a linear increase of WB severity levels and dietary treatments in relation to BY, it is possible to infer that the differences observed by the dietary treatments on BY were caused by the incidence of WB rather than the diets. Thus, emphasizing the need to establish rapport between the WB incidence, WB severity level effect, and dietary treatment on dependent variables. This inference is further supported by the correlation factors observed for the BW ($\tau = .18$, $p<0.05$) and the WB severity scores ($\tau = .18$, $p<0.05$) to the BY %. However, it is necessary to establish if the same observation will occur when birds are harvested later than 42 d of age. Livingston et al. (2018b) observed similar results in the effect of WB severity on BY of 42 d old broiler birds from two genetic backgrounds, fed *ad libitum* or on a timed program. Researchers found that the unaffected and mild breast fillets were similar ($p>0.05$) but lower in yield ($p<0.05$) than the moderate and severe WB, which were similar ($p>0.05$). Several studies analyzing the impact of dietary intervention on WB have reported differences in BY between the tested diets, but these studies have not tested the effect of WB severity levels on the BY in relation to the incidence of WB across diets (Cemin et al., 2018; Córdova-Noboa et al., 2018; Bodle et al., 2018; Meloche et al., 2018). In the study from Livingston et al. (2018b), a similar inference, to the one being established in the present study, can be drawn between the incidence % in WB, the effect of WB severity level, and the effect of feeding program difference ($p<0.05$) on pectoralis yield.

Overall for 42 d old broilers, the CY of the broilers was affected by the diets, but was highly dependent on the BW. For BY, even though the diets had an effect on BY % this effect was not a true effect as the differences can be explained by the incidence % of WB and mean effect of the WB severity levels on BY %

3.5.2. Meat quality

Meat quality is a complex assessment of interrelated parameters that affect yields, shelf life, customer complaints, and further processing. Drip loss % is one parameter that can assess the quality of the meat (Pearson and Dutson, 1995). In WB meat the DL is known to be higher ($p<0.05$) in moderate and severe fillets compared to unaffected and mild fillets (Mudalal et al., 2015; Sun et al., 2018). This is to an extent in agreement with the results obtained in this study which show that normal<mild<moderate fillets are different ($p<0.05$) in DL %. Since no severe WB were reported, it is not possible to extrapolate the effect of severe WB on DL %, but it is probable that it would have had similar behavior as to that reported in the literature (Sun et al., 2018). No dietary effect on DL % was observed, even when the response variable of WB severity score effect were lower ($p<0.05$, Table 10) and improved ($p<0.05$, Table 12) odds ratios were observed for the broilers fed the Mix diet. The lack of the dietary treatments effect on DL % may be explained by the incidence frequency (Table 11) seen on the tested fillets, of which 92.22% were unaffected and mild, and a mean difference between the WB severity levels was evident. Livingston et al. (2018b) reported contrasting results in which the DL % among the WB severity levels were not different ($p>0.05$). However, in the same study, it was also observed that birds fed *ad libitum* had lower ($p<0.05$) DL % compared to time limited fed birds. Thus, the discrepancy between the reported results and the current study may be in part due to the analysis that incorporated the WB severity from birds with different genetic backgrounds and were fed with different feeding programs, which had a significant effect on DL %. Cordova-Noboa et al. (2018) reported meat quality parameters on broilers fed a base corn-soy diet and one with lower amount of soy replaced with broiler by-product, both diets were supplemented with or without guanidinoacetic acid, in a 2x2 factorial design as an intervention to reduce WB incidence. The researchers observed that drip loss was not affected ($p>0.05$) by the diets or their interaction. As with BY, to understand the impact of diets on DL % from broiler breast afflicted with WB, it is necessary to observe the effect of the WB incidence and severity levels on the dependent variable.

The meat quality role in the final pH is complex and associated with the internal ionic strength and the effect on capillary forces within the muscle structure (Owens et al., 2000). Lower pH values weaken the forces holding water in the meat and increase DL % (Alvarado and Sams, 2004a). Additionally, phenotypic events involving birds' transportation, processing practices, and dietary practices can impact the final pH (Alvarado and Sams, 1995, 2002; Alnahhas et al., 2016). Early reports on WB have showed no difference in pH values of unaffected fillets vs. severe WB severity (Mudalal et al., 2015; Trocino et al., 2015; Soglia et al., 2016b). Other dietary experiments assessing interventions to mitigate WB have reported no effect on pH values by diet supplementation (Córdova-Noboa et al., 2018), or genetic lines (Livingston et al., 2018b). In the latter study, *ad libitum* feeding programs increased final pH values compared to timed feeding programs. The authors also observed that the unaffected and mild WB fillets had similar ($p>0.05$) pH values that were lower ($p<0.05$) than the moderate and severe WB, which were similar ($p>0.05$). Similarly, Aguirre et al. (2018) reported higher values in pH for severe WB fillets compared to unaffected fillets. This emphasizes the importance in evaluating the impact of WB severity categories on pH values in the process of evaluating dietary treatment intervention for WB. The data obtained from the present study showed that mild and moderate WB fillets had similar ($p>0.05$) pH values that were higher ($p<0.05$) than the unaffected fillet pH values. Regarding the effect of the diets on pH, a confounding observation that is at odds with previous published studies was made. The Mix diet had one of the highest ($p<0.05$) pH values, considering that it also had the lowest ($p<0.05$) WB severity rank and a higher

($p<0.05$) likelihood in having unaffected fillets compared to the other diets. Building on the inference of the BY, where the effect of WB severity level and incidence were interrelated with the dietary effects, it is possible that the observed pH value resulted from the higher amount of Mild WB severity in the Mix diet. Moreover, it would also be expected that the pH mean would be lower due to the higher amount of unaffected fillets of the Mix diet compared to the rest of the diets. As this was not the case and the DL % was not affected negatively, it can be inferred that the Mix diet birds' higher pH is a desirable result independent of the WB severity. However, the Ctrl fed birds had a high ($p<0.05$) WB severity median, the highest ($p<0.05$) incidence of mild WB, and the lowest ($p<0.05$) incidence of unaffected fillets, but had the lowest ($p<0.05$) pH value of the diets. These results contradict the previous inference of the Mix diet and the results observed in other published research (Aguirre et al., 2018; Livingston et al., 2018b). Graphical analysis of the data showed that the Mix and CM diet's pH mean rank values, within each of the WB severity levels were higher than the Ctrl and Ctrl+Mannan diets. This observation was exacerbated by the incidence % of the WB which favored the Mix's higher unaffected fillet counts and CM's higher moderate WB counts. Collectively, the pH data prevent a clear determination of the effect of the different diets on pH, and illustrate the need for further research aimed at studying the effect of WB severity levels and incidence % and their impact on the different diets in pH values.

Color is determined through the CIELAB scale and is divided into three levels L*(0-100), a* (-100 - +100), and b*(-100 - +100). The L* has been associated with WHC because high values are correlated with lower pH values and higher DL and cook loss %

of breast meat (Pearson and Dutson, 1995; Owens et al., 2000). The results show that WB severity levels had an effect ($p>0.05$) on the L* values. Taking into consideration that the effect of WB severity levels on DL% and pH was significant, it would have been expected that the L* results show a similar response. However, the unaffected fillets had the highest ($p<0.05$) L* values and the mild WB fillets the lowest ($p<0.05$), with the moderate WB fillets having similar ($p>0.05$) values to the other diets. No clear explanation to the disparity in the observed results, a similar conclusion can be drawn to that of the pH of WB severity levels. Where, the unaffected fillet means across all treatments were higher ($p<0.05$) than the mild WB fillets, but similar ($p>0.05$) to the moderate WB fillets. Additionally, the covariate had an effect which increased the L* values of the fillets as bird weight increased. Trocino et al. (2015) reported results where no difference ($p = 0.4400$) in L* values were observed between unaffected fillets and fillets that had an uncategorized WB severity. As reported by Aguirre et al (2018), L* values between unaffected and severe WB fillets, categorized with the method described by Tijare et al. (2016), were different ($p = 0.04$). Thus, further research is needed to determine the effect of dietary treatments on broiler birds with similar growth performance, and the effect of WB severity levels on L* values in populations of older birds, known to have higher incidences of WB (Tijare et al., 2016; Kuttappan et al., 2017).

The a* and b* color values are associated with consumer visual acceptability, as these parameters, in their positive values, impact the redness and yellowness of the breast fillet (Owens et al., 2000). Published results comparing the WB severity effect on a* have reported differences ($p<0.05$) between unaffected fillets and fillets afflicted with moderate or severe WB (Chatterjee et al., 2016; Aguirre et al., 2018). These reports contradict the results observed here, which showed no difference in the WB severity effect. This divergence may be due to the disparity in sample units between the experiments, as well as the specific experimental designs that focused on comparing the unaffected vs. severe fillet only, whereas the current study relied on the randomness in WB severity of the selected birds. No ($p>0.05$) differences were observed for the WB severity effect on b* values.

The breast meat of the birds fed the dietary treatments had not effect ($p>0.05$) on the L* and a* values, but did have an effect ($p<0.05$) on the b*. The Ctrl and Ctrl+Mannan diets caused the breast meat to have a slightly higher ($p<0.05$) yellowness color. The higher amounts of corn in these formulations compared to the CM and Mix diets may have increased the carotenoids deposition in the phospholipid layers of the cell membranes. A similar result was reported by Cordova-Noboa et al. (2018), where diets formulated with five percent of broiler by-product, which had higher amounts of corn, caused the b* values in breast meat to be higher ($p<0.05$) than diets that had no by-product and lower concentration of corn.

3.6. Conclusions

In conclusion, it is clear that WB severity levels had a negative effect on DL% and pH meat quality parameters as previously reported, but had no effect on color parameters. The effect of WB, after controlling for body weight of the broiler, showed that breast yield results in the dietary treatments are highly related to the incidence % and WB severity effect. Furthermore, in order to understand how dietary strategies designed to reduce the incidence of WB affect the meat quality parameters, it is essential to relate the diets' effect on meat quality parameters with the observed results from the WB severity effect and incidence % of WB. Additionally, controlling for growth performance of the individual bird in the statistical analysis may provide new results at how the diets are impacting the meat quality parameters of WB afflicted birds. Regarding the dietary treatments' effect on WB incidence, the Mix diet fed birds showed lower incidence and higher likelihood of having less WB incidence when compared to birds that have similar growth performance, without having any clear negative impact on meat quality. Additional studies that control for growth performance parameters are needed to view how dietary treatment interventions aimed at mitigating WB affect processing performance and meat quality parameters in birds harvested later than 42 d.

4. REDUCTION OF COOKING TIMES IN THE OVEN COOK LOSS METHOD THROUGH SAMPLE SIZE REDUCTION USING 3-D PRINTED CUTTING MOLDS

4.1. Synopsis

Breast fillets have increased in weight and dimension changing the oven method (OM) cook times. This study introduced a tool that would standardize the shape and size of the meat, with the objective of reducing cook time while making the meat uniform to improve cooking efficiency. The study was carried out in two parts to 1) establish the dimensional parameters of the breast fillet and design the cutting molds for the modified OM (MOM), and 2) compare the MOM CL results to those of the OM. In three trials, a total of 57 whole breast were split in half and trimmed of excess fat before assigning the left and right fillets to the OM and MOM method, respectively. The breast fillet's length (mm), cranial width (mm), height (mm), fiber change length (mm), pH and weight (g) were measured to design the dimensions of the cutting molds, and ensure that the fillet sides were similar. The cooking process consisted of placing the samples in pre-oiled grill pans covered with aluminum foil in a pre-heated convection oven at 177C until the meat reached 73C. The meat parameters were analyzed using an independent t-test, while the CL difference between methods and trials was analyzed with a non-parametric two-way analyses of variance with alpha at 0.05. As expected, no differences were observed between the dimensional parameters, pH, and weight of the fillets. The 3-D printed mold was designed to allow duplicate samples of 29 mm x 52 mm x 20 mm to be cut and placed next to each other in the pans. The CL results showed that the MOM can reproduce ($p>0.05$) the CL results obtained from the OM at a shorter cooking interval. However, in the presence of WB and WS meat conditions the MOM is less likely ($p<0.05$) to overestimate the CL values of the fillets compared to the OM. In summary, using the cutting molds allowed the MOM to reduce cook time and produce a similar results to those observed by the OM, while increasing the sensitivity of the CL procedure to meat conditions such as WB and WS.

Keywords: Cook Loos, Cooking Method, Breast Meat

4.2. Introduction

The cooking of animal proteins serves as a method to assess the water holding capacity (WHC) of meat by establishing an association of the state and quality of the meat proteins and their ability to retain the natural water of the muscle. The cook loss (CL) method aims at determining the difference between the raw weight of the meat and cooked weight, to obtain a percentage of weight loss. The higher the percentage of weight loss, the lower the WHC of the meat, which is related to undesirable meat quality (Owens et al., 2000; Barbut, 2015a). The differences in CL observed between tested samples can help indicate the impact of diet change, growing environment, breed, processing methods, or marination ingredient on the WHC of the meat and ultimately on quality of the sample.

The process of cooking consists of applying energy, usually caloric, to a food substance which induces a chemical change in the structure of the food. In particular for meat proteins, the increase of heat from outside sources breaks hydrogen bonds, van der Waal’s forces, and electrostatic interactions between the proteins, allowing the proteins to aggregate and form a meat gel (Box et al., 1972; Tornberg, 2005). The meat gel consists of a randomly organized structure that has created stronger covalent bonds between the proteins and found an equilibrium between the hydrophobic and hydrophilic structures during the process of protein association, thus creating a matrix that can hold water mainly through capillary forces (Tornberg, 2005). If, in the process of forming the meat gel, a rapid protein association occurs larger spaces in the matrix and longer protein strands are created. This larger spaces reduce the capillary forces, and cause a lower WHC in the meat (Van Der Sman, 2013).

The aggregation of meat proteins can be influenced by multiple extrinsic and intrinsic factors involved in the cooking processes that can affect the CL measurements. The extrinsic factors are the conditions outside of the meat that can impart variation to the cooking process. These factors include the method of cooking, the source of caloric energy, and environmental conditions. The three main cooking methods for animal proteins are: grilling, oven roasting, and water bath (Sous-vide). The last two methods are most commonly applied to determine the CL of meat samples. In plant or research applications these methods will transfer the caloric energy from their source in a direct or indirect form impacting the cooking process by causing excessive protein aggregation if not applied appropriately. Research has shown that the two factors that affect cook loss are heating rates (temperature/time) and the ultimate final temperature of the meat (Pearson and Dutson, 1995).

The market tendencies for larger bird weights and higher breast yields have been highly correlated to deeper, wider, and longer breast meat (Scheuermann et al., 2003; Kuttappan et al., 2017). Genetic selection allowed the increase in larger birds which has changed the compositional and dimensional size of the breast fillet compared to unaffected breast fillets; these changes have been related to the increased in incidence and severity of WB (Mallmann et al., 2016; Tijare et al., 2016; Kuttappan et al., 2017). The reported physiological changes in WB fillets have been focused on the increase in thickness of the cranial region, protein (22.06%), collagen (1.42%), fat (1.07%), moisture (74.99%) and harden tissue when compared to unaffected fillets (24.66%, 0.85%, 1.15%; respectively), with the differences more evident on severe WB.

All the variables within the physiology of the breast meat described above can impact the CL results of the meat samples. To assess the CL two main methodologies are used in the literature. The first method, water bath, consist of taking a piece of meat with known dimensions and weight, vacuum packing in it, and cooking it in a water bath at temperatures not higher than 80 C (Pearson and Dutson, 1995; Tornberg, 2005; Van Der Sman, 2013). The second cooking method consists in placing the whole breast fillet on a metal grill within an aluminum cover metal pan in a convection oven at 177 C (Sams, 1990; Casco et al., 2013; Tijare et al., 2016). Both methods cook the fillets to a predetermined internal temperature and integrate different aspects of the extrinsic factors that impact CL results, moreover it is important to determine the heating rate and end internal temperature of the meat more so then the heating method (Pearson and Dutson, 1995; Petracci and Baéza, 2011; Van Der Sman, 2013). The focus of this study will be on the latter CL method, hereon known as the oven method (OM).

The current research is proposing a modification to the oven method described by Sams (1990). The modification (MOM) will consist of standardizing the fillet size samples using molds to cut the breast fillets to predetermined sizes and run the CL measurements in duplicates. The objective of this study was to determine if the MOM can produce CL values similar to the current OM while reducing the sample cooking times.

4.3. Materials and Methods

The study was divided into two parts: 1) Determining the dimensions and characteristic of the fillet to develop the mold and 2) test the CL of the meat using the developed mold. To test the objective of the study 57 whole breasts, were sampled in 3 trials. The broiler breast, 24 h post mortem, were random in size and untrimmed when acquired from a local distributor. The whole breasts were split in the middle through the keel line and trimmed of excessive fat and rib meat. The left (OM) and right (MOM) side fillets of each butterfly, determined from the frontal plane, were tagged with an identifier in increasing order. The tags were made of Tyvek® (model S-3552YPW, Uline, Coppell, TX, 75019) and secured with a heavy-duty plastic fastener (model S-19965, Uline). The height (H), width (W), length (L), and fiber change length (FCL) were measured from each of the fillets using a digital caliper (model: KDT3756, Apex Tool Group, MD, USA) as described in figure 1. The FCL is a subjective measurement establish from the start of the cranial section to the point on which the overall fiber direction start to change. The pH (n=30) of the fillets were measured at the cranial area of each fillet (model 205, Testo AG, Sparta, NJ), followed by the raw weight (Wt) using a bench scale (Model D31P15BR Defender®3000, OHAUS Scale Corp., Parsippany, NJ, 07054).

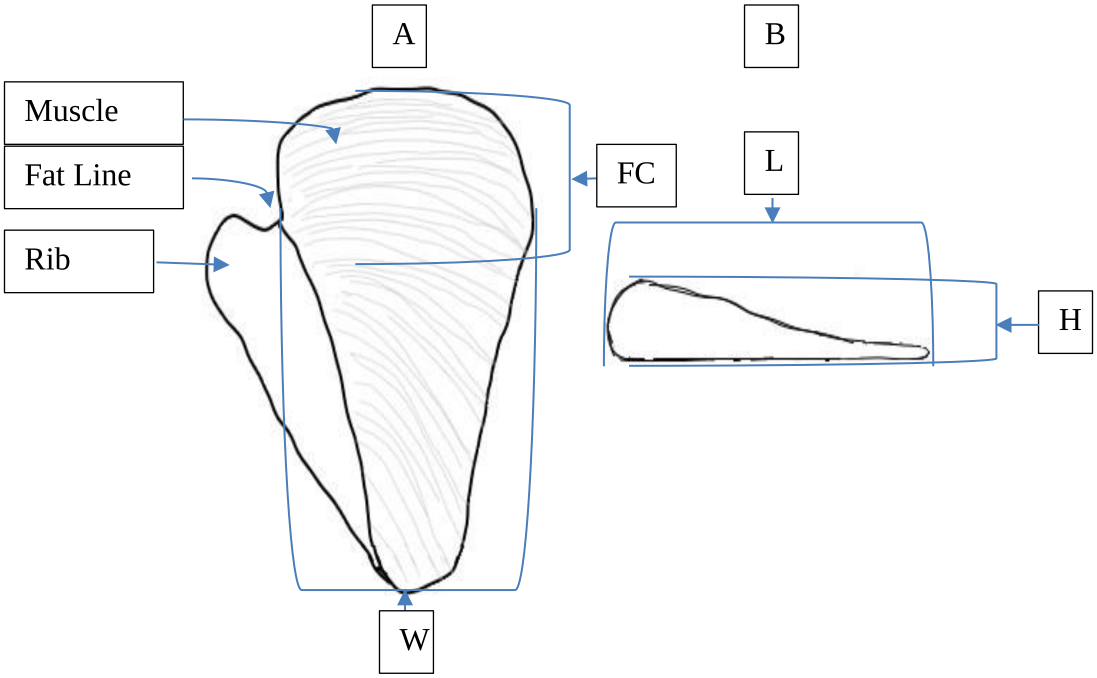

Figure 1. Diagram of the measurements taken on the left breast fillet viewed from the frontal (A) and midsagittal (B) plane. The measurements include: length (L), height (H), width (W), and a measurement of the length from the start of the cranial section to the point at which the fibers change in direction (FCL) in the breast fillet.

Based on the dimension data gathered, two molds were 3D printed on a Fortus 400MC in high fidelity out of ULTEM 9085 resin with 100% in-fill at the Department of Mechanical Engineering at Texas A&M University (College Station, TX; Figure 2). The 1st mold defines the perimeter (L x W) of the meat sample, and the 2nd mold standardizes the sample H and creates a duplicate subsample of the fillet. This process allows for duplicates to run in the cooking process and determine the coefficient of variation (CV) within the sample as a measure to control cooking effect on the meat for the MOM method.

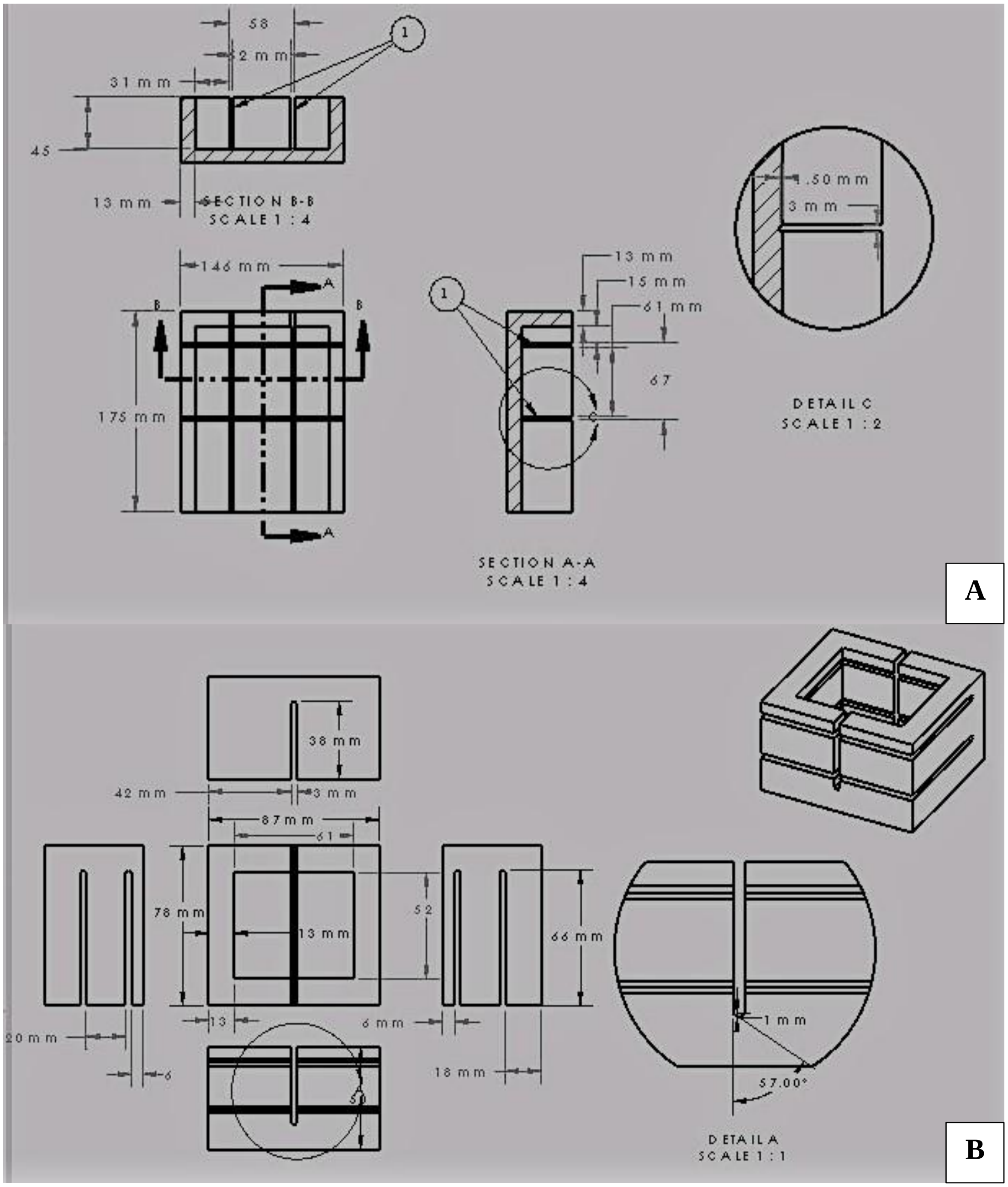

Figure 2. Details drawings of the constructed molds. Mold 1 (A) established the perimeter cuts (L x W) around the meat sample, followed by the cuts to control the sample thickness (H) and created the duplicate samples in mold 2 (B).

A cooking trial was performed according to the procedure for the OM described above. The MOM process consisted of cutting all the sample with the molds and labeling the duplicates appropriately for proper identification. After weighing the cut samples individually with a bench scale (Scout® Model SPX622, OHAUS Scale Corp., OHAUS Scale Corp., Parsippany, NJ, 07054), the samples were placed in metal pans. The metal pans (model: 36633, Hubert Company, Harrison, OH, 45030) were lined with heavy duty aluminum foil before placing a pre-oiled metal wire grate (Model 83757, Hubert Company) in the pans. The duplicate samples were placed next to each other without touching and with the label facing towards the metal grate, this ensures no mass is lost at the time of removal from the grate. A total of 40 duplicated samples were able to fit in one 508 mm x 304 mm x 101 mm 22 gauge 18/8 stainless steel pan. Beaded thermocouple probes (Model 61161-372, VWR, Radnor, PA, 19087) were inserted into the most central location of 2 random samples within each pan used, to track the internal temperature progression with a thermometer (Model 61161-280, VWR). The pans were then covered with heavy duty aluminum foil to ensure a tight seal around the pans and placed in a walk-in cooler (Model W, Hobart, Troy, OH) until all the samples were prepared. The sealed pans were then placed in a gas convection oven preheated to 177 C, and timers used to track the cooking time (Model BDO-100-G-ES, G.S. Blodgett Corporation, Burlington, VT, 05401). The pans were removed from the oven when the internal temperature of the meat reached 73 C and set on tables to cool for 15 min before weighing the samples in a bench scale (Scout® Model SPX622, OHAUS Scale Corp.). The OM CL was calculated by the difference between cooked and raw weights divided by the raw weight of the fillet.

Each fillet represented an experimental unit and was used in the statistical analysis. The MOM, created a raw duplicate from each fillet sample. The duplicates CL was calculated as described for the OM, for each duplicate group a mean and SD were calculated. A CV was calculated (SD ÷ mean) to establish the variation imparted by the oven on the duplicate sample. If the CV value of the sample duplicate was >0.10 the sample was discarded from the statistical analysis.

4.3.1. Statistical Analysis

For this project, the experimental units used in the analysis were the individual fillets. The raw data were analyzed for normality (shapiro-Wilk test) and scalar heterogeneity (Fligner-Killeen test). The mean, standard deviation (sd), median, minimum (min), maximum (max), and standard error (se) were calculated for each of the dimensional parameters of H (n=110), W (n=110), L (n=30), FCL (n = 110), Wt (n = 86), and pH (n=30). The difference in mean between the left and right fillets was tested using an independent Welch's T-test. The CL results (n=52) for each cooking method (levels = 2) and trial (levels = 3) effect were analyzed with a randomized independent non-parametric two-way analysis of variance (ANOVA) with type III sums of the square to account for an unbalanced design, interaction effect, and non-normality of the data. If interaction between the factors were observed then a non-parametric one-way ANOVA was used to analyze the independent effects. The effect size difference between the means was calculated to aid in measuring the strength of the relationship between the measured parameters using Cohen's d (Cohen, 1992), were |d|<0.2 is considered negligible effect,

|d|<0.5 a small effect, |d|<0.8 a medium effect, otherwise a large effect size between the means. All these analysis were performed with the built in formulas on the R statistical platform (v. 3.3.2) in RStudio (v.1.0.136, ©2009-2016 RStudio, Inc.). Additionally, non-parametric analysis were measured with the RFit package (v.0.23.0) and effect size determined by the package effsize (v.0.7.4). Statistical significance was established at an α of 0.05.

4.4. Results and Discussion

In order to avoid differences that are inherent to the meat sample, each butterfly breast was split into left and right fillets, which were then assigned to the OM and MOM, respectively. As expected, the dimensional data (H, W, L, and FCL) showed no differences ($p>0.2000$) between the left and right fillets (Table 13). Furthermore, the effect size observed between the fillet means was small ($d<0.22$) for each of the dimension parameters. The same observations were noted for the Wt and pH of both left and right fillets, where both sides did not differ ($p>0.05$) from one another and had a small effect size ($d<0.26$). After similarity between the fillet sides was established the descriptive statistics (mean, standard deviation, median, minimum, maximum, and standard error) of the whole sample population were calculated to assist in determining the dimensions of molds used to excise the meat sample for the MOM (Table 14).

4.4.1. Descriptive statistics

Dimensional data of broiler fillets are available in the literature but are targeted at establishing differences between broiler genetic lines and meat quality effects (Bird, 1948; Scheuermann et al., 2003; Mehaffey et al., 2006; Brewer et al., 2012a). To the authors' knowledge, there are no published reports that compare the dimensions of the broiler breast fillet's left and right sides. The results observed in this study for the fillets L, W, and H were as follow (Table 13): (L = 175.16 mm ± 9.91, W = 84.29 mm ± 12.53, and H = 39.12 mm ± 6.82 vs L = 176.68 mm ± 11.35, W = 84.36 mm ± 13.19, and H = 37.74 mm ± 5.48 for the left and right sides, respectively). Similar results were reported by Scheuermann et al., (2003). However, these authors were comparing genetic lines, whereas, the descriptive statistics of the current study represented a random sample from a commercial supplier.

Table 13. Mean comparison and descriptive statistics between breast fillet side for the parameters of height, width, length, fiber change length (FCL), weight, and pH[1]

	Height		**Width**		**Length**		**FCL[2]**		**Weight**		**pH**	
Statistics[*]	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
mean	39.12	37.74	84.29	85.36	175.16	176.68	61.91	60.61	342.85	353.20	5.88	5.89
sd	6.82	5.48	12.53	13.19	9.91	11.35	6.69	7.04	40.27	44.68	0.16	0.20
median	39.67	37.47	81.04	81.04	178.52	179.23	62.59	61.39	341.94	356.28	5.95	5.93
min	22.52	22.63	70.01	64.87	157.40	153.91	42.65	46.00	258.00	250.00	5.57	5.48
max	53.57	53.85	114.78	118.96	191.56	193.55	76.90	78.99	423.89	430.00	6.09	6.35
se	0.92	0.74	1.69	1.78	2.56	2.93	0.90	0.95	60.11	7.78	0.04	0.05
Left vs Right												
p-value	0.2454		0.6114		0.6993		0.3256		0.2821		0.8599	

[1] Statistical significance between the left and right fillet within each parameter was determined when p-value <0.05

[2] FCL is a measurement of the length from the start of the cranial section to the point at which the fibers change in direction

[*] sd=standard deviation; min = minimum; max = maximum; se = standard error

Table 14. Descriptive statistics of the population breast fillet for height, width, length, fiber change length (FCL)1, weight, and pH parameters.

Parameters	**Mean**	**sd***	**median**	**min***	**max***	**se***
Height	38.43	6.2	38.87	22.52	53.85	0.59
Width	84.83	12.82	80.86	64.87	118.96	1.22
Length	175.92	10.5	179.11	153.91	193.55	1.92
FCL[1]	61.26	6.87	61.94	42.65	78.66	0.65
Weight	346.82	42.06	343.12	250	430	4.54
pH	5.88	0.18	5.94	5.48	6.35	0.03

[1] FCL is a measurement of the length from the start of the cranial section to the point at which the fibers change in direction

* sd=standard deviation; min = minimum; max = maximum; se = standard error

The L of broiler fillets harvested at wk four have been reported to range between 161.00 and 178.50 mm, and between 173.80 and 220.90 mm for fillets harvested at wks six and seven (Galobart and Moran, 2004; Brewer et al., 2012a). These reported L’s are similar to the ones obtained for this study (175.92 mm ± 10.50). The L of the fillet helped determine the length of the first mold to ensure that the fillet would rest properly on the mold while the meat was being cut. However, the FCL measurement provided the starting point to establish the sample’s length for the first mold. This measurement arbitrarily encompasses the majority of the muscle fibers that run parallel to each other in the cranial area (Figure 1). Fibers that are arranged in the same direction on the MOM meat samples could help minimize between-sample differences at the time of heating. As reported by Van Der Sman, (2013), the meat expands in height, but undergoes shrinkage in the L and W of the fillet during high temperature (above water boiling temperature) or long heating times during the cooking process. This movement of the fibers in the heating process can contribute to CL yields by creating channels where water vapor is lost. To the authors’ knowledge, this is the first reporting of FCL (61.26 ± 6.87 mm) measurement for breast fillets. The W of the fillets is used in published reports as a measurement for carcass quality and breast Wt prediction (Scheuermann et al., 2003; Mehaffey et al., 2006). The W of the fillets can be impacted by the point of measurement performed by the researchers, but the main reason of variation occurs by genetic line and age of harvest of the birds. Birds harvested at wk four averaged 86.40 mm in W (Brewer et al., 2012a) compared to those harvested at wk seven which averaged 125.40 mm (Galobart and Moran, 2004). The wider and longer fillets take up more space in the metal pans used in the conventional OM, thus increasing the number of pans needed to cook the fillets. The measurements from the current study averaged at 84.83 ± 12.82 mm but were taken at the lowest point of the wing cut, and not at the widest point of the fillet. The main reason for this point of measurement was that the area of the cranial section was the main target to obtain the meat sample of the MOM. As observed in figure 1 panel A, the H of the broiler fillet at the thickest part of the cranial region is highly variable, mainly due to genetic lines, rearing conditions, gender, harvest Wt, and the presence of conditions such as PSE, WB, and WS (Fanatico et al., 2005; Brewer et al., 2012b; a; Kuttappan et al., 2013, 2017; Mudalal et al., 2015; Mutryn et al., 2015). In small bird operations, the H averages between 22.10 mm and 26.00 mm (Brewer et al., 2012a), while for big bird operation the H of the fillets from birds harvested at 56 d averages between 28.2 mm and 40.00 mm (Scheuermann et al., 2003; Galobart and Moran, 2004). Controlling this variation in the conventional OM is a challenge, as cook times between pans will vary and internal temperature can differ after the cooling period. In the study, fillets had an average H of 38.43 ± 6.2mm and ranged from 22.52 mm to 53.85 mm, which coincide with the common practice of large bird operations in the industry. The presence of severe and extremely severe WB and WS on breast fillets are also correlated with an increase in cranial H (Kuttappan et al., 2017). For this study, the prevalence of WB and WS was noted (data not shown), but because the cooking methods were compared using the same meat, the variation from these conditions were treated as inherent and not as a factor. Moreover, a χ^2 analysis comparing the prevalence of WB and WS between the left and right fillet, showed that the occurrences of both meat conditions were similar ($p>0.05$) between the sides. The H data was used to determine the second mold's dimensions, thus attempting to standardize the sample H in the MOM process. The Wt of the fillets was used to establish a reference in fillet size that the mold would cut effectively. The fillets weights averaged between 346.82 g ± 42.06 with a range from 250.00 g to 430 g. The results are in agreement with other published data (Scheuermann et al., 2003; Galobart and Moran, 2004), but in contrast to those published by Hickling and Guenter (1990), where a significant difference was observed between mean Wt of the left (250.2 g) and right (244.0 g) fillets of 20 birds harvested at d 64. The calculated effect size from the published study was numerically similar to the one reported in the current research (d = 0.32 vs. 0.25, respectively), which is considered a small effect size (Cohen, 1992). Moreover, the possible reason for the difference between the studies may lie in the sample size, paired vs. independent T-test, and the sex of the studied birds. The pH was used to ensure that the fillet sides did not vary in their biochemical state. The results showed no difference between the fillets sides (Left = 5.88 ± 0.16 vs. Right = 5.89 ± 0.20), with an average for the population pH of 5.88 ± 0.18 and a range between 5.48 and 6.35. Although the average is similar to reported values (Casco et al., 2013), some extreme values were present. Presence of extremely severe WB and WS for the higher pH values and possible PSE condition (not confirmed) for the lower pH values can explain these extremes. All the descriptive stats collected from the fillets were used to develop the MOM's molds from which the meat sample would be prepared.

4.4.2. Mold design

It was important to determine and understand the variability in the dimensions of the fillets in order to design the molds in a way that would maximize the meat sample for the MOM. Based on the FCL measurements, the sample length was established at 61 mm after the first cut of the fillets cranial section was removed at 15 mm and the caudal section was cut off at 83 mm. A 3mm gap was left at all cutting grooves to allow the blade of a knife to slice through the mold. The W of the sample was established at 52 mm, well below the minimum W of the sampled fillets. To perform this cut, the fillet was centered within the outline boundary of the first mold's (Figure 2, frame A, section B-B) cutting grooves. After the L and W cuts were performed, a rectangular sample was extracted from the fillet. The skin side of the fillet was placed facing downward in the second mold and pressed firmly to ensure the sample fit around the mold as it was being cut. The first cut removed 5 mm of the sample skin side and the second cut removed any excess meat, standardizing the sample H to 20mm. A final cut was performed to split the meat sample in half and create the duplicate sample with L of 29 mm, W of 52mm, and a H of 20 mm. During the process of making the cuts, the following observations were noted. In cases where the fillet was on the upper end of the H range, usually due to severe WB, the tissue surface was rounder and hardened. This rounded surfaces caused miss cuts to occur and an extra 5 mm of sample needed to be removed from the skin side in order to obtain a uniformly cut surface. Breast meat that had a H of less than 1.0 SD from the mean would not allow the cuts to be performed correctly in the 2nd mold. The lower H fillets also coincided with the Wt being 1 SD less than the mean of the fillets, which for the majority of cases were birds that had no WB and WS. Overall the molds of the MOM are ideal for breast fillets that weigh more than 310 g and have a H of 32 mm or more, which coincide with fillets obtained from birds >3.0 kg (i.e. “big-bird operations”) (Scheuermann et al., 2003; Brewer et al., 2012b).

4.4.3. Cooking process

Cook times for the OM method ranged from 71 min to 95 min for all the trials, with the heavier (>350 g) fillets taking 95 min to reach the 73 C in internal temperature. The MOM cooking time ranged between 27 min and 40 min. The CV results >0.10 for each sample of the MOM fillets were not included in the analysis. This sample rejection process resulted in a 40.0% of the MOM fillets in trial one, 62.0% in trial two, and 53.0% of trial three not being used in the statistical analysis. This high rejection of samples illustrates the complexities of thermal distribution within the pans, which may have led to the high variance of the duplicates. However, the duplicates created by the MOM help reduce the within sample variation caused by the cooking process.

After initial assumption checks, the data were observed to be not normally distributed. Additionally, outliers (Figure 3) were observed that could not be explained, and could influence the results. Thus, to determine the effects and interaction of the trials and the cooking methods on the CL results, a rank based two-way non-parametric ANOVA was used to analyze the data. The results comparing both methods and trials are shown in table 15.

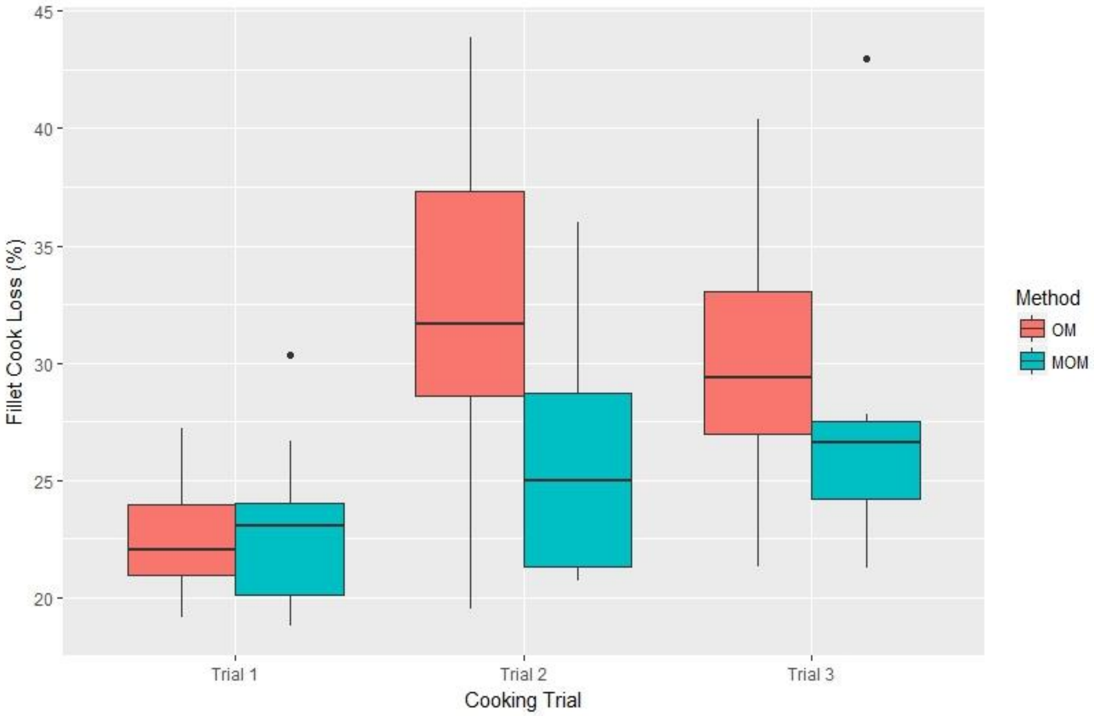

Figure 3. Boxplot of the cook loss results by oven method (OM) and modified oven method (MOM) within each trial.

A difference ($p<0.0001$) in what was observed between the three trials, which led to a significant interaction ($p<0.0427$) between the trials and between the methods. A one-way non-parametric ANOVA of the trials effect on CL (results not shown) confirmed that trial one was different ($p<0.05$) from trials two and three, which did not differ ($p>0.05$) between each other (as observed in Figure 3). There was no apparent reason as to why the commercial fillets varied over the three trials, but the presence of WB and WS was noted and recorded at the time of sample collection. It is well documented that the presence of these two meat conditions causes an increase in CL compared to fillets that are considered unaffected (Petracci et al., 2013; Aguirre et al., 2018). A cross table analysis with χ^2 showed that the presence of WB and WS was lower ($p<0.0005$ and $p<0.0001$, respectively) in trial one, with 72.22% and 86.67% (respectively) of the fillets being considered unaffected, compared to the other trials. In contrast, trial two and three altogether contained 79.31% mild, 84.00% moderate, and 88.89% severe WB and 73.17% mild, 100.00% moderate, and 100.00% severe WS. There was no incidence of moderate or severe WS in trial one. This incidence of WB and WS provides an explanation for the difference observed between the trials. In order to ensure that the cooking methods were being compared within the constraints of trials differences, a non-parametric two-sample test between the OM and MOM was performed for trial one, and for the pooled data of trials two and three (Table 15).

Table 15 Comparison of the cook loss percent (CL) median, standard error, and statistical model between cooking methods for each experiment.

Cooking Method[1]	**Trial 1**[3]	**Trial 2 & 3**[3]	**Pooled Experiments**
OM	22.05 ± 0.59	30.96 ± 0.85	28.58 ± 0.82
MOM	23.08 ± 1.22	26.63 ± 1.40	24.12 ± 1.05
Model (p-value)[2]			
Method	0.8713	0.0018	0.0023
Trial	-	-	<0.0001
Method x Trial	-	-	0.0427

[1] OM = oven method; MOM = modified oven method

[2] Non-parametric rank based analysis of variance

[3] Trial interaction effect (Trial 1 ≠ Trial 2 = Trial3)

The WB and WS effects on the results of the two cooking methods were analyzed using a χ^2 test. The results show that the outcomes from the two cooking methods were not affected by the occurrence of WB (p>0.4574) and WS (p>0.3185) in any trials. These results were expected as the fillets used in the comparison between methods originated from the same whole breasts. In trial one, both the OM (22.05 % ± 0.59) and MOM (23.08% ± 1.22) had similar (p=0.8713) CL median and SE, indicating that both methods have the ability to perform similarly with meat that has low incidence of WB and WS. In trials two and three, the OM (30.96 % ± 0.85) had a higher CL (p<0.0018) than the MOM (26.63 % ± 1.40). This observation suggests that in the presence of higher incidence of WB and WS, the OM would overestimate the CL values compared to the MOM. Larger breast fillets, commonly observed in fillets with WB, increase the time needed to reach the desired internal temperature of the meat. The increase in time is attributed to the increase in cranial height of the fillets, which is correlated (r = 0.39, p = 0.0002) with higher CL (figure 4).

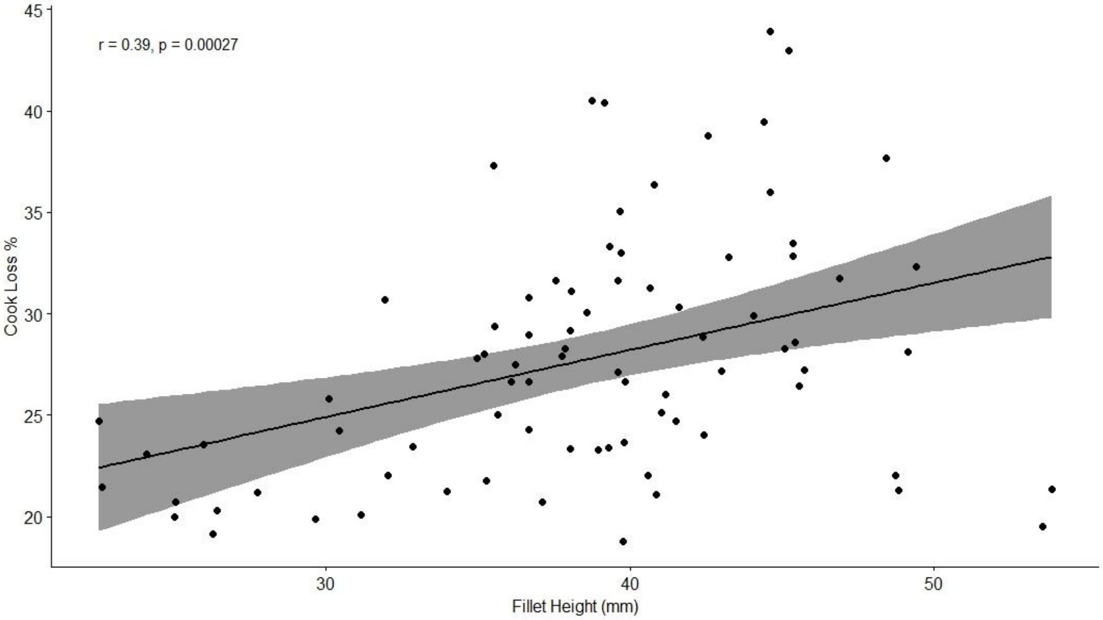

Figure 4. Relationship of breast fillets cook loss % and their cranial height for each sampled fillet within the oven cooking method (OM) and the modified oven method (MOM). The solid line is a regression line of the cook loss and hieght data with the standard error represented by the gray shadow.

4.5. Conclusion

To determine CL, it is evident that the MOM can reproduce the CL results obtained from the OM at a shorter cooking interval. However, in the presence of WB and WS meat conditions, the MOM appears to be less likely to overestimate the CL values of the fillets compared to the OM. In addition, preliminary tests show that the cooked duplicate samples can be used to perform a texture profile analysis without any further sample preparation in comparison to the method describe by Aguirre (2018). An appropriate experiment needs to be designed to test this hypothesis, which could lead to further time-saving for researchers and more consistent textural test.

5. CONCLUSIONS

Addressing the incidence and severity of the WB condition is important in order to improve consumer acceptability, meat functionality, and reduce economic loss due to lower WHC. The incidence and severity of WB can be mitigated by reducing growth performance. Thus, the challenge lies in finding interventions (e.g. feeding practices, diet formulae, and ingredients) that will reduce WB while minimizing the impact on the bird's growth performance.

The two dietary intervention studies conducted here showed that using non-conventional diets formulated with varying ingredients are able to reduce the severity of the WB condition in the breast meat. Diets formulated with canola meal as the sole protein source and diets formulated with varying sources of nutrients have a higher likelihood of reducing the incidence of the WB condition. Within any particular study of the WB condition, it is impossible to assess the effect of dietary intervention on any given meat quality parameter without knowledge of the effect of the WB severity on said parameter, the incidence of WB, and the relationship between WB incidence and severity.

To improve the CL determination in breast fillets of ever increasing weights and dimensions, the cutting molds proved to be a useful tool for the current oven method. The modification allowed for a reduction in the variation coefficient and produced similar cook loss % results as to the uncut fillets, while reducing cooking times.

Future research trends into ways to mitigate the negative impact of the WB condition thru dietary interventions need to be rethought. The growth performance of the birds needs to be established on the birds as the experimental unit rather than the pens, in order to make a direct comparison between the birds growth performance, the WB condition, and meat quality parameters. In addition, a study that completely eliminates the use of soybean meal and replaces it with animal and insect protein sources is needed to evaluate the effect of eliminating vegetable protein sources in rations has on meat quality and WB.

For the cook loss method, there is a need for studies that allow for taking the cut samples after cooking directly into texture and sensory studies without any further preparation.

6. REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PLoS One 11:e0153750.

Abdallh, M. E., M. M. Bhuiyan, D. J. Cadogan, and P. A. Iji. 2017. Response of broiler chickens to high inclusion levels of cotton seed meal supplemented with composite microbial enzymes.Page 231 in 28th Annual Australian Poultry Science Symposium.

Aguirre, M. E., C. M. Owens, R. K. Miller, and C. Z. Alvarado. 2018. Descriptive sensory and instrumental texture profile analysis of woody breast in marinated chicken. Poult. Sci. 97:1456–1461.

Alnahhas, N., C. Berri, M. Chabault, P. Chartrin, M. Boulay, M. C. Bourin, and E. Le Bihan-Duval. 2016. Genetic parameters of white striping in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. BMC Genet. 17:61.

Alvarado, C. Z., and A. R. Sams. 1995. Rigor mortis development in turkey breast muscle and the effect of electrical stunning. 79:1694–1698.

Alvarado, C. Z., and A. R. Sams. 1999. Injection marination strategies for remediation of pale , exudative broiler breast meat. 82:1332–1336.

Alvarado, C. Z., and A. R. Sams. 2002. The role of carcass chilling rate in the development of pale, exudative turkey pectoralis. Poult. Sci. 81:1365–1370.

Alvarado, C. Z., and A. R. Sams. 2004a. Turkey carcass chilling and protein denaturation in the development of pale, soft, and exudative meat. Poult. Sci. 83:1039–1046.

Alvarado, C. Z., and A. R. Sams. 2004b. Early postmortem injection and tumble marination effects on broiler breast meat tenderness. Poult. Sci. 83:1035–1038.

Angkanaporn, K., V. Ravindran, and W. L. Bryden. 1996. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal, and meat and bone meal for broilers. Poult. Sci. 75:1098–1103.

Arntfield, S. D. 2011. Canola and other oilseed proteins.Pages 289–315 in Handbook of Food Proteins. Limited, W.P., ed.

Arntfield, S. D., and H. D. Maskus. 2011. Peas and other legume proteins.Pages 233–266 in Handbook of food proteins.

Avanzo, J. L., C. X. De Mendonça, S. M. Piccoli Pugine, and M. De Cerqueira Cesar. 2001. Effect of vitamin E and selenium on resistance to oxidative stress in chicken superficial pectoralis muscle. Comp. Biochem. Physiol. Part C 129:163–173.

Aviagen. 2014. ROSS 708: Nutrition Specifications 2014.

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015a. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870–2879.

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015b. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870–2879.

Baker, D. H. 2009. Advances in protein–amino acid nutrition of poultry. Amino Acids 37:29–41.

Barbanti, D., and M. Pasquini. 2005. Influence of cooking conditions on cooking loss and tenderness of raw and marinated chicken breast meat. LWT - Food Sci. Technol. 38:895–901.

Barbut, S. 2002. Poultry product processing: an industry guide. CRC Press, Boca Raton, FL.

Barbut, S. 2009. Pale, soft, and exudative poultry meat--Reviewing ways to manage at the processing plant. Poult. Sci. 88:1506–1512.

Barbut, S. 2015a. The science of poultry and meat processing. University of Guelph, Guelph, Ontario, Canada.

Barbut, S. 2015b. Structure and muscle physiology.Pages 1–38 in The Science of Poultry and Meat Processing. Guelph, Ontario, Canada.

Bautista-Ortega, J., and C. A. Ruiz-Feria. 2010. L-Arginine and antioxidant vitamins E and C improve the cardiovascular performance of broiler chickens grown under chronic hypobaric hypoxia. Poult. Sci. 89:2141–2146.

Beauclercq, S., C. Hennequet-Antier, C. Praud, E. Godet, A. Collin, S. Tesseraud, S. Métayer-Coustard, M. Bourin, M. Moroldo, F. Martins, S. Lagarrigue, E. Le Bihan-Duval, and C. Berri. 2017. Muscle transcriptome analysis reveals molecular pathways and biomarkers involved in extreme ultimate pH and meat defect occurrence in chicken. Sci. Rep. 7:6447.

Berri, C., J. Besnard, and C. Relandeau. 2008. Increasing dietary lysine increases final pH and decreases drip loss of broiler breast meat. Poult. Sci. 87:480–484.

Berri, C., N. Wacrenier, N. Millet, and E. Le Bihan-Duval. 2001. Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines. Poult. Sci. 80:833–838.

Bianchi, M., and D. L. Fletcher. 2002. Effects of broiler breast meat thickness and background on color measurements. Poult. Sci. 81:1766–1769.

Bianchi, M., M. Petracci, a Franchini, and C. Cavani. 2006. The occurrence of deep pectoral myopathy in roaster chickens. Poult. Sci. 85:1843–1846.

Bilgili, S. F., and J. B. Hess. 2002. Green muscle disease in broilers increasing. World Poult. 18:42–43.

Bird, S. 1948. Quantitative determination and segregation of breast conformation in poultry. Poult. Sci. 27:506–508.

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, and J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. Poult. Sci. 97:3298–3310.

Boling-Frankenbach, S. D., C. M. Peter, M. W. Douglas, J. L. Snow, C. M. Parsons, and D. Baker. 2001. Efficacy of phytase for increasing protein efficiency ratio values of feed ingredients. Poult. Sci. 80:1578–1584.

Bordignon, H. F., A. Rorig, E. Vanroo, S. De Castro Palma, D. A. Pazdiora, and J. I. Muller Fernandes. 2017. Effect of supplementing organic selenium on occurrence and severity of white striping.Pages 52–56 in Student Competition: Muscle Myopathies—Multidiscipline Approach Poult. Sci. 96(E-Suppl. 1).

Bottje, W. G. 2018. Oxidative metabolism and efficiency: the delicate balancing act of mitochondria. Poult. Sci. 0:1–7.

Bouhrara, M., S. Clerjon, J.-L. Damez, C. Chevarin, S. Portanguen, A. Kondjoyan, and J.-M. Bonny. 2011. Dynamic MRI and thermal simulation to interpret deformation and water transfer in meat during heating. J. Agric. Food Chem. 59:1229–1235.

Bowker, B. C., and H. Zhuang. 2013. Relationship between muscle exudate protein composition and broiler breast meat quality. Poult. Sci. 92:1385–1392.

Box, P. O., P. E. Bouton, P. V Harris, and C. Hill. 1972. The effects of cooking temperature and time on some mechanical properties of meat. J. Food Sci. 37:140–144.

Brewer, V. B., J. L. Emmert, J. F. C. Meullenet, and C. M. Owens. 2012a. Small bird programs: Effect of phase-feeding, strain, sex, and debone time on meat quality of broilers. Poult. Sci. 91:499–504.

Brewer, V. B., V. A. Kuttappan, J. L. Emmert, J.-F. C. Meullenet, and C. M. Owens. 2012b. Big-bird programs: Effect of strain, sex, and debone time on meat quality of broilers. Poult. Sci. 91:248–254.

de Brot, S., S. Perez, H. L. Shivaprasad, K. Baiker, L. Polledo, M. Clark, and L. Grau-Roma. 2016. Wooden breast lesions in broiler chickens in the UK. Vet. Rec.:178.

Cai, K., W. Shao, X. Chen, Y. L. Campbell, M. N. Nair, S. P. Suman, C. M. Beach, M. C. Guyton, and M. W. Schilling. 2018. Meat quality traits and proteome profile of woody broiler breast (pectoralis major) meat. Poult. Sci. 97:337–346.

Canola Council of Canada. 2016. Poultry Diets Using Canola Meal. Available at http://www.canolacouncil.org/oil-and-meal/canola-meal/poultry-diets-using-canola-meal/ (verified 12 June 2016).

Casco, G., T. M. Taylor, and A. N. D. C. Z. Alvarado. 2015. Evaluation of novel micronized encapsulated essential oil-containing phosphate and lactate blends for growth inhibition of Listeria monocytogenes and Salmonella on poultry bologna , pork ham , and roast beef ready-to-eat deli loaves. 78:698–706.

Casco, G., G. a Veluz, and C. Z. Alvarado. 2013. SavorPhos as an all-natural phosphate replacer in water- and oil-based marinades for rotisserie birds and boneless-skinless breast. Poult. Sci. 92:3236–3243.

Cemin, H. S., S. L. Vieira, C. Stefanello, L. Kindlein, T. Z. Ferreira, and A. K. Fireman. 2018. Broiler responses to increasing selenium supplementation using Zn-L-selenomethionine with special attention to breast myopathies. Poult. Sci. 97:1832–1840.

Chatterjee, D., H. Zhuang, B. C. Bowker, A. M. Rincon, and G. Sanchez-Brambila. 2016. Instrumental texture characteristics of broiler pectoralis major with the wooden breast condition. Poult. Sci. 95:2449–2454.

Chen, X., C. Parr, P. Utterback, and C. M. Parsons. 2015. Nutritional evaluation of canola meals produced from new varieties of canola seeds for poultry. Poult. Sci. 94:984–991.

Cherian, G., R. Selvaraj, M. Goeger, and P. Stitt. 2002. Muscle fatty acid composition and thiobarbituric acid-reactive substances of broilers fed different cultivars of sorghum. Poult. Sci. 81:1415–1420.

Clark, D. L., and S. G. Velleman. 2017. Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. Poult. Sci. 95:2930–2945.

Cohen, J. 1992. A power primer. Psychol. Bull. PsycARTICLES 112:157–159.

Conover, W. J., and R. L. Iman. 1982. Analysis of covariance using the rank transformation. Biometrics 38:715.

Córdova-Noboa, H. A., E. O. Oviedo-Rondón, A. H. Sarsour, J. Barnes, D. Sapcota, D. López, L. Gross, M. Rademacher-Heilshorn, and U. Braun. 2018. Effect of guanidinoacetic acid supplementation on live performance, meat quality, pectoral myopathies and blood parameters of male broilers fed corn-based diets with or without poultry by-products. Poult. Sci. 97:2494–2505.

Cruz, R. F. A., S. L. Vieira, L. Kindlein, M. Kipper, H. S. Cemin, and S. M. Rauber. 2017. Occurrence of white striping and wooden breast in broilers fed diets with increasing levels of lysine in grower and finisher phases. Poult. Sci.:501–510.

Deb-Choudhury, S., S. Haines, D. Harland, S. Clerens, C. Van Koten, and J. Dyer. 2014. Effect of cooking on meat proteins: mapping hydrothermal protein modification as a Potential Indicator of Bioavailability. J. Agric. Food Chem 62:8187–8196.

Dilger, R. N., and D. H. Baker. 2007. DL-methionine is as efficacious as L-methionine, but modest L-cystine excesses are anorexigenic in sulfur amino acid-deficient purified and practical-type diets fed to chicks. Poult. Sci. 86:2367–2374.

Donaldson, W. E., G. F. Combs, and G. L. Romoser. 1956. Studies on energy levels in poultry rations. 1. The effect of calorie-protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poult. Sci. 35:1100–1105.

Dunkman, A. A., M. R. Buckley, M. J. Mienaltowski, S. M. Adams, S. J. Thomas, L.

Satchell, A. Kumar, L. Pathmanathan, D. P. Beason, R. V Iozzo, D. E. Birk, and L. J. Soslowsky. 2013. Decorin expression is important for age-related changes in tendon structure and mechanical properties. Matrix Biol. 32:3–13.

Edmonds, M. S., and D. H. Baker. 1987. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet: effects on growth and dietary choice in the chick. J. Anim. Sci. 65:699–705.

EkİncĪ, S., and M. Erkan. 2012. Short term effects of genistein on the testes of quail (Coturnix coturnix). Turk. J. Vet. Anim. Sci. 36:251–257.

Erdaw, M. M., R. A. Perez-Maldonado, and P. A. Iji. 2017. Apparent and standardized ileal nutrient digestibility of broiler diets containing varying levels of raw full-fat soybean and microbial protease. J. Anim. Sci. Technol. 59:23.

Fanatico, A. C., L. C. Cavitt, P. B. Pillai, J. L. Emmert, and C. M. Owens. 2005. Evaluation of slower-growing broiler genotypes grown with and without outdoor access: meat quality. Poult. Sci. 84:1785–1790.

Fanatico, A. C., P. B. Pillai, J. L. Emmert, and C. M. Owens. 2007. Meat quality of slow- and fast-growing chicken genotypes fed low- nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 86:2245–2255.

Fennema, O. R., S. Damodaran, and K. L. Parkin (Eds). 1996. Fennemas’s Food Chemestry. 4th ed. CRC Press, Boca Raton, FL.

Fernandez, S. R., S. Aoyagi, Y. Han, C. M. Parsons, and D. H. Baker. 1994. Limiting order of amino acids in corn and soybean meal for growth of the chick. Poult. Sci. 73:1887–1896.

FSIS. 2018. Disposition instructions for “woody breast” and “white striping” poultry conditions. Washington, D.C., United States.

Galobart, J., and E. T. Moran. 2004. Refrigeration and freeze-thaw effects on broiler fillets having extreme L* values. Poult. Sci. 83:1433–1439.

Gorsuch, V., and C. Z. Alvarado. 2010. Postrigor tumble marination strategies for improving color and water-holding capacity in normal and pale broiler breast fillets. Poult. Sci. 89:1002–1008.

Grippo, A. A., K. Capps, B. Rougeau, and B. J. Gurley. 2007. Analysis of flavonoid phytoestrogens in botanical and ephedra-containing dietary supplements. Ann. Pharmacother. 41:1375–1382.

Harford, I. D., H. O. Pavlidis, and N. B. Anthony. 2014. Divergent selection for muscle color in broilers. Poult. Sci. 93:1059–1066.

Heath, J. L., and C. J. Wabeck. 1975. The effect on xanthophyll content and color of broiler skin when scald additive concentration was measured by pH,. Poult. Sci. 54:1288–1292.

Hickling, D., and W. Guenter. 1990. Comparison of weights for left and right breast muscle of broilers. Poult. Sci. 69:711–713.

Hill, F. W., and L. M. Dansky. 1953. Studies of the energy requirements of chickens 1. The effect of dietary energy level on growth and feed consumption. Poult. Sci. 33:112–119.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447–457.

Hunt, R. W. G., and M. R. Pointer. 2011. Relations between colour stimuli.Pages 41–72 in Measuring Colour. 4th ed. John Wiley & Sons, Incorporated.

Hunt, M. C., O. Sørheim, and E. Slinde. 1999. Color and heat denaturation of myoglobin forms in ground beef. J. Food Sci. 64:847–851.

Hurwitz, S., D. Sklan, and I. Bartov. 1978. New formal approaches to the determination of energy and amino acid requirements of chicks. Poult. Sci. 57:197–205.

Husak, R. L., J. G. Sebranek, and K. Bregendahl. 2008. A survey of commercially available broilers marketed as organic, free-range, and conventional broilers for cooked meat yields, meat composition, and relative value. Poult. Sci. 87:2367–2376.

Jackson, M. E., D. W. Fodge, and H. Y. Hsiao. 1999. Effects of β-mannanase in corn-soybean meal diets. Poult. Sci. 78:1737–1741.

Joiner, K. S., G. A. Hamlin, R. J. Lien, and S. F. Bilgili. 2014. Evaluation of capillary and myofiber density in the pectoralis major muscles of rapidly growing, high-yield broiler chickens during increased heat stress. Avian Dis. 58:377–382.

Khajali, F., and B. a. Slominski. 2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91:2564–2575.

Kidd, M. T., and M. Choct. 2017. Assessment of nutritional strategies to reduce dietary crude protein in commercial broilers.Pages 175–180 in 28th Annual Australian Poultry Science Symposium.

Kim, E. J., P. L. Utterback, and C. M. Parsons. 2012. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult. Sci. 91:1350–1355.

Kirby, S. R., G. M. Pesti, and J. H. Dorfman. 1993. An investigation of the distribution of the protein content of samples of corn, meat and bone meal, and soybean meal. Poult. Sci. 72:2294–2298.

Klein, J. T. 2013. Polysaccharides in reduced energy diets on broilers. MSc Diss. Texas A&M Univ, College Station.

Klein, J., M. Williams, B. Brown, S. Rao, and J. T. Lee. 2015. Effects of dietary inclusion of a cocktail NSPase and β-mannanase separately and in combination in low energy diets on broiler performance and processing parameters. J. Appl. Poult. Res. 24:489–501.

Konica Minolta Inc. 2017. L*a*b* Color space. Precise Color Commun.:7 Available at https://www.konicaminolta.com/instruments/knowledge/color/part1/07.html (verified 16 June 2017).

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, and C. M. Owens. 2013. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult. Sci. 92:811–819.

Kuttappan, V. A., S. D. Goodgame, C. D. Bradley, A. Mauromoustakos, B. M. Hargis, P. W. Waldroup, and C. M. Owens. 2012a. Effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the occurence of various degrees of white striping on broiler breast fillets. Poult. Sci. 91:3230–3335.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry : a review. Poult. Sci. 95:2724–2733.

Kuttappan, V. a., Y. S. Lee, G. F. Erf, J.-F. C. Meullenet, S. R. McKee, and C. M. Owens.

2012b. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poult. Sci. 91:1240–1247.

Kuttappan, V. A., C. M. Owens, C. N. Coon, and B. M. Hargis. 2017. Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. Poult. Sci. 00:1–5.

Laskin, D. L., V. R. Sunil, C. R. Gardner, and J. D. Laskin. 2011. Macrophages and tissue injury: agents of defense or destruction? Annu. Rev. Pharmacol. Toxicol 51:267–288.

Lauridsen, C. 2018. From oxidative stress to inflammation: redox balance and immune system. Poult. Sci. 0:1–7.

Lee, Y. S., C. M. Owens, and J. F. Meullenet. 2008. On the quality of commercial boneless skinless broiler breast meat. J. Food Sci. 73:253–261.

Lien, R. J., S. F. Bilgili, J. B. Hess, and K. S. Joiner. 2012. Induction of deep pectoral myopathy in broiler chickens via encouraged wing flapping. J. Appl. Poult. Res. 21:556–562.

Lilburn, M. S., J. R. Griffin, and M. Wick. 2018. From muscle to food: oxidative challenges and developmental anomalies in poultry breast muscle. Poult. Sci.

Liu, X., J. A. Byrd, M. Farnell, and C. A. Ruiz-Feria. 2014. Arginine and vitamin E improve the immune response after a Salmonella challenge in broiler chicks. Poult. Sci. 93:882–890.

Livingston, M. L., P. R. Ferket, J. Brake, and K. A. Livingston. 2018a. Dietary amino acids under hypoxic conditions exacerbates muscle myopathies including wooden breast and white stripping. Poult. Sci. 98:1517–1527.

Livingston, M. L., C. D. Landon, H. J. Barnes, and J. T. Brake. 2016. Wooden breast muscle myopathies are reduced with increased dietary potassium and available phosphorous.Page Poult. Sci. 95(E-Suppl. 1) in Poultry Science Association Annual Meeting Abstracts.

Livingston, M. L., C. Landon, H. J. Barnes, and J. Brake. 2018b. White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. Poult. Sci. 98:217–226.

Lowe, B. 1964. Experimental cookery from the chemical and physical standpoint. 4th ed. John Wiley & Sons, Inc., New York.

Lyon, B., and C. Lyon. 2002. Color of uncooked and cooked broiler leg quarters associated with chilling temperature and holding time. Poult. Sci. 81:1916–1920.

Macglashan, D., and P. Baltimore. 2003. Histamine: A mediator of inflammation. J Allergy Clin Immunol.

Mallmann, B. A., J. L. Solo, X. Sun, F. L. Yang, and C. M. Owens. 2016. Effect of broiler age and woody breast condition on breast meat quality.Page Poult. Sci. 95(E-Suppl. 1) in Poultry Science Association Annual Meeting Abstracts.

Markley, J. M., and J. A. Faulkner. 1978. Regeneration of skeletal muscle after grafting in monkeys. Plast. Reconstr. Surg. 62:415–422.

Mazzoni, M., M. Petracci, A. Meluzzi, C. Cavani, P. Clavenzani, and F. Sirri. 2015. Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poult. Sci. 94:123–130.

McNaughton, J., M. Roberts, D. Rice, B. Smith, B. Hong, B. Delaney, and C. Iiams. 2014. Comparison of broiler performance and carcass yields when fed diets containing genetically modified canola meal from event DP-Ø73496-4, near-isogenic canola meal, or commercial canola meals. Poult. Sci. 93:1713–23.

McNeill, L., K. Bernard, and M. G. MacLeod. 2004. Food intake, growth rate, food conversion and food choice in broilers fed on diets high in rapeseed meal and pea meal, with observations on sensory evaluation of the resulting poultry meat. Br. Poult. Sci. 45:519–523.

Mehaffey, J. M., S. P. Pradhan, J. F. Meullenet, J. L. Emmert, S. R. Mckee, and C. M. Owens. 2006. Meat quality evaluation of minimally aged broiler breast fillets from five commercial genetic strains. Poult. Sci. 85:902–908.

Mehri, M., A. A. Davarpanah, and H. R. Mirzaei. 2012. Estimation of ideal ratios of methionine and threonine to lysine in starting broiler chicks using response surface methodology. Poult. Sci. 91:771–777.

Meisner, H., and K. Tenney. 1977. pH as an indicator of free fatty acid release from adipocytes. J. Lipid Res. 18:774–776.

Meloche, K. J., B. I. Fancher, D. A. Emmerson, S. F. Bilgili, and W. A. Dozier. 2018. Effects of reduced digestible lysine density on myopathies of the Pectoralis major muscles in broiler chickens at 48 and 62 days of age1. Poult. Sci. 97:3311–3324.

Meng, X., and B. a Slominski. 2005. Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poult. Sci. 84:1242–1251.

Mudalal, S., E. Babini, C. Cavani, and M. Petracci. 2014. Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. Poult. Sci. 93:2108–16.

Mudalal, S., M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci. 2015. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal 9:728–734.

Munoz, J. A., C. D. Hanna, P. L. Utterback, and C. M. Parsons. 2018. Phosphorus retention in corn, spray dried plasma protein, soybean meal, meat and bone meal, and canola meal using a precision-fed rooster assay. Poult. Sci. 97:4324–4329.

Murray, M. J., and A. B. Murray. 1979. Anorexia of infection as a mechanism of host defense. Am. J. Clin. Nutr. 32:593–596.

Mutryn, M. F., E. M. Brannick, W. Fu, W. R. Lee, and B. Abasht. 2015. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genomics 16:399–418.

National Chicken Council. 2015. Chicken (broiler and other) production and BW. Available at http://www.nationalchickencouncil.org/about-the-industry/statistics/chicken-broiler-and-other-production-head-and-live-weight/ (verified 1 January 2016).

National Chicken Council. 2019a. Per Capita Consumption of Poultry and Livestock, 1965 to estimated 2019, in pounds. Available at https://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/ (verified

6 April 2019).

National Chicken Council. 2019b. U.S. broiler production. Available at https://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-production/ (verified 6 May 2019).

NRC. 1960. Nutrient requirements of poultry. National Academy of Sciences.

NRC. 1966. Nutrient requirements of poultry.Page 36 in Nutrient Requirements of Poultry. Nutrition, S. on P., ed. 5th rev. National Academy of Sciences, Washington, D.C.

NRC. 1994. Nutrient requirements of poultry.Pages 1–145 in Nutrient Requirements of Poultry. Subcommittee on Poultry Nutrition, ed. 9th rev. National Academy Press, Washington, D.C.

OECD. 2012. Revised consensus document on compositional considerations for new varieties of soybean [Glycine max (L.) Merr.]: key food and fees nutrients, anti-nutrients, toxicants and allergens.

Owens, C. M., C. Alvarado, and A. R. Sams. 2000. Poultry meat processing (CRC Press, Ed.). Boca Raton, FL.

Owens, C. M., C. Z. Alvarado, and a R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. Poult. Sci. 88:1513–1517.

Papah, M. B., E. M. Brannick, C. J. Schmidt, and B. Abasht. 2017. Evidence and role of phlebitis and lipid infiltration in the onset and pathogenesis of Wooden Breast Disease in modern broiler chickens. Avian Pathol. 0:1–21.

Patisaul, H. B., and W. Jefferson. 2010. The pros and cons of phytoestrogens. Front.

Neuroendocrinol. 31:400–419.

Pearson, A. M., and T. R. Dutson. 1995. Quality attributes and their measurment in meat, poultry, and fish products. 1st. ed. Chapman & Hall, Gaithersburg.

Perez-Carbajal, C., D. Caldwell, M. Farnell, K. Stringfellow, S. Pohl, G. Casco, A. Pro-Martinez, and C. A. Ruiz-Feria. 2010. Immune response of broiler chickens fed different levels of arginine and vitamin E to a coccidiosis vaccine and Eimeria challenge. Poult. Sci. 89:1870–1877.

Perez-Vendrell, A. M., J. M. Hernandez, L. Llaurado, J. Schierle, and J. Brufau. 2001. Influence of source and ratio of xanthophyll pigments on broiler chicken pigmentation and performance. Poult. Sci. 80:320–326.

Petracci, M., and E. Baéza. 2011. Harmonization of methodology of assessment of poultry meat quality features. Worlds. Poult. Sci. J. 67:137–153.

Petracci, M., L. Laghi, P. Rocculi, S. Rimini, V. Panarese, M. a. Cremonini, and C. Cavani. 2012. The use of sodium bicarbonate for marination of broiler breast meat. Poult. Sci. 91:526–534.

Petracci, M., S. Mudalal, a Bonfiglio, and C. Cavani. 2013. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult. Sci. 92:1670–5.

Petracci, M., S. Mudalal, F. Soglia, and C. Cavani. 2015. Meat quality in fast-growing broiler chickens. Worlds. Poult. Sci. J. 71:363–374.

Pope, T., and J. L. Emmert. 2001. Phase-feeding supports maximum growth performance of broiler chicks from forty-three to seventy-one days of age. Poult. Sci. 80:345–352.

Powell, D. J., D. C. Mcfarland, A. J. Cowieson, W. I. Muir, and S. G. Velleman. 2014. The effect of nutritional status and muscle fiber type on myogenic satellite cell fate and apoptosis 1. Poult. Sci. 93:163–173.

Pym, R. A. E., and P. J. Nicholls. 1979. Selection for food conversion in broilers: Direct and correlated responses to selection for body-weight gain, food consumption and food conversion ratio. Br. Poult. Sci. 20:73–86.

Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH , moisture , water-holding capacity , and emulsification capacity. Poult. Sci. 80:676–680.

Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2002. Effects of raw broiler breast meat color variation on marination and cooked meat quality. Poult. Sci. 81:276–280.

El Rammouz, R., C. Berri, E. Le Bihan-Duval, R. Babilé, and X. Fernandez. 2004. Breed differences in the biochemical determinism of ultimate pH in breast muscles of broiler chickens-A key role of AMP deaminase? Poult. Sci. 83:1445–51.

Reece, F. N., B. D. Lott, and J. W. Deaton. 1984. The effects of feed form, protein profile, energy level, and gender on broiler performance in warm (26.7 C) environments. Poult. Sci. 63:1906–1911.

Richardson, J. A., J. Burgener, R. W. Winterfield, and A. S. Dhillon. 1980. Deep pectoral myopathy in seven-week-old broiler chickens. Avian Dis. 24:1054–1059.

Rosa, A. P., G. M. Pesti, H. M. Edwards, and R. Bakalli. 2001. Tryptophan Requirements of Different Broiler Genotypes. Poult. Sci. 80:1719–1722.

Russo, E., M. Drigo, C. Longoni, R. Pezzotti, P. Fasoli, and C. Recordati. 2015. Evaluation of white striping prevalence and predisposing factors in broilers at slaughter. Poult. Sci. 94:1843–1848.

Saha, A., A. V. S. Perumalla, Y. Lee, J. F. Meullenet, and C. M. Owens. 2009. Tenderness , moistness , and flavor of pre- and postrigor marinated broiler breast fillets evaluated by consumer sensory panel. 88:1250–1256.

Sams, A. R. 1990. Electrical stimulation and high temperature conditioning of broiler carcasses. Poult. Sci. 69:1781–1786.

Scheuermann, G. N., S. F. Bilgili, J. B. Hess, and D. R. Mulvaney. 2003. Breast muscle development in commercial broiler chickens. Poult. Sci. 82:1648–1658.

Schlumbohm, M. J., K. M. Hilton, G. J. Mullenix, J. V. Caldas, B. A. Mallmann, J. A. England, A. Kalinowski, C. M. Owens, and C. N. Coon. 2016. The effects of dietary amino acids, dietary metabolizable energy, and broiler strain on incidence of breast muscle myopathy at 42 and 58 days.Page Poult. Sci. 95(E-Suppl. 1) in Poultry Science Association Annual Meeting Abstracts.

Sell, J. L. 1996. Influence of dietary concentration and source of meat and bone meal on performance of turkeys. Poult. Sci. 75:1076–1079.

Shutt, D. A. 1976. The effects of plant oestrogens on animal reproduction. Endeavour 35:110–113.

Sihvo, H. K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

Sihvo, H. K., J. Lindén, N. Airas, K. Immonen, J. Valaja, and E. Puolanne. 2016. Wooden breast myodegeneration of pectoralis major muscle over the growth period in broilers. Vet. Pathol. 54:119–128.

Siller, W. G. 1985. Deep pectoral myopathy: a penalty of successful selection for muscle growth. Poult. Sci. 64:1591–1595.

Simoes, C., S. Vieira, L. Kindlein, T. Ferreira, C. Stefanello, G. Santiago, M. Rohr, and S. Caldas. 2017. Ultrasonography patterns in the developing breast muscle of broilers fed at 70% restriction or low density diets.Pages 52–56 in Student Competition: Muscle Myopathies—Multidiscipline Approach Poult. Sci. 96(E-Suppl. 1).

Sirotkin, A. V., R. Alexa, A. Kádasi, A. Štochmaľová, M. Morovič, J. Laurinčik, A. H. Harrath, and R. Grossmann. 2016. The isoflavone daidzein directly affects porcine ovarian cell functions and modifies the effect of follicle-stimulating hormone. J. Anim. Physiol. Anim. Nutr. (Berl). 101:127–135.

Van Der Sman, R. G. M. 2013. Modeling cooking of chicken meat in industrial tunnel ovens with the Flory–Rehner theory. MESC 95:940–957.

Soglia, F., J. Gao, M. Mazzoni, E. Puolanne, C. Cavani, M. Petracci, and P. Ertbjerg. 2017. Superficial and deep changes of histology, texture and particle size distribution in broiler wooden breast muscle during refrigerated storage. Poult. Sci. 96:3465–3472.

Soglia, F., L. Laghi, L. Canonico, C. Cavani, and M. Petracci. 2016a. Functional property issues in broiler breast meat related to emerging muscle abnormalities. Food Res. Int. 89:1071–1076.

Soglia, F., S. Mudalal, E. Babini, M. Di Nunzio, M. Mazzoni, F. Sirri, C. Cavani, and M. Petracci. 2016b. Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. Poult. Sci. 95:651–659.

Sun, X., D. A. Koltes, C. N. Coon, K. Chen, and C. M. Owens. 2018. Instrumental compression force and meat attribute changes in woody broiler breast fillets during short-term storage. Poult. Sci. 97:2600–2606.

Surai, P. F., I. I. Kochish, M. N. Romanov, and D. K. Griffin. 2019. Nutritional modulation of the antioxidant capacities in poultry: the case of selenium. 0:1–12.

Tahir, M., M. Y. Shim, N. E. Ward, C. Smith, E. Foster, A. C. Guney, and G. M. Pesti. 2012. Phytate and other nutrient components of feed ingredients for poultry. Poult. Sci. 91:928–935.

Tesseraud, S., R. A. E. Pym, E. Le Bihan-Duval, and M. J. Duclos. 2003. Response of broilers selected on carcass quality to dietary protein supply: live performance, muscle development, and circulating insulin-like growth factors (IGF-I and-II). Poult. Sci. 82:1011–1016.

Tijare, V. V., F. L. Yang, V. A. Kuttappan, C. Z. Alvarado, C. N. Coon, and C. M. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult. Sci. 95:2167–2173.

Toghyani, M., N. Rodgers, M. R. Barekatain, P. A. Iji, and R. A. Swick. 2014. Apparent metabolizable energy value of expeller-extracted canola meal subjected to different processing conditions for growing broiler chickens. Poult. Sci. 93:2227–2236.

Tomažin, U., N. Batorek-Lukač, M. Škrlep, M. Prevolnik-Povše, and M. Čandek-Potokar. 2018. Meat and fat quality of Krškopolje pigs reared in conventional and organic production systems. Animal:1–8.

Torki, M., and a Chegeni. 2002. Evaluation of dietary replacement of soybean meal by canola meal supplemented by -mannanase (Hemicell) on performance of broiler chicks. Nutrition:637–640.

Tornberg, E. 2005. Effects of heat on meat proteins - Implications on structure and quality of meat products. Meat Sci. 70:493–508.

Tossenberger, J., M. Rademacher, K. Németh, V. Halas, and A. Lemme. 2016. Digestibility and metabolism of dietary guanidino acetic acid fed to broilers. Poult. Sci. 95:2058–2067.

Trocino, A., A. Piccirillo, M. Birolo, G. Radaelli, D. Bertotto, E. Filiou, and M. Petracci. 2015. Effect of genotype , gender and feed restriction on growth , meat quality and the occurrence of white striping and wooden breast in broiler chickens Animals , Experimental Groups , and In Vivo. Poult. Sci. 94:2996–3004.

Velleman, S. G. 2015. Relationship of skeletal muscle development and growth to breast muscle myopathies: a review. Avian Dis. 59.

Velleman, S. G., and D. L. Clark. 2015. Histopathologic and myogenic gene expression changes associated with wooden breast in broiler breast muscles. Avian Dis. 59:410–418.

Verboven, P., N. Scheerlinck, J. De Baerdemaeker, and B. M. Nicola. 2000. Computational fluid dynamics modelling and validation of the temperature distribution in a forced convection oven. J. food:61–73.

Vieira, S. L., C. Stefanello, and J. O. B. Sorbara. 2014. Formulating poultry diets based on their indigestible components. Poult. Sci. 93:2411–6.

Wadas, W., and H. Mioduszewska. 2011. The effect of the sowing date on the content of carotenoids and L-ascorbic acid in spaghetti squash (Cucurbita Pepo L .). Acta Sci. Pol., Hortorum Cultus 10:41–48.

Wang, C., M. Xie, W. Huang, J. J. Xie, J. Tang, and S. S. Hou. 2013. Arginine requirements of White Pekin ducks from 1 to 21 days of age. Poult. Sci. 92:1007–1010.

Warren, W. A., and J. L. Emmert. 2000. Efficacy of phase-feeding in supporting growth performance of broiler chicks during the starter and finisher phases 1. Poult. Sci. 79:764–770.

Weber, I. T., R. W. Harrison, and R. V Iozzo. 1996. Model structure of decorin and implications for collagen fibrillogenesis. J. Biol. Chem. 271:31767–70.

Wells, T. 2015. Supply vs. demand: a game worth watching. Urner Barry's Report. 10:20–21.

Wells, T. 2016. Supply: about the only thing that's guaranteed anymore. Urner Barry's Report.:20–20.

Wood, J. D., and D. M. Enser. 1997. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. Br. J. Nutr. 78:49–60.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2003. Effects of fatty acids on meat quality: a review. Meat Sci. 66:21–32.

Wyrwisz, J., A. Półtorak, M. Zalewska, R. Zaremba, and A. Wierzbicka. 2012. Analysis of relationship between basic composition, pH, and physical properties of selected bovine muscles. Bull Vet Inst Pulawy 56:403–409.

Xiao, Y. Q., D. Shao, H. B. Tong, and S. R. Shi. 2018. Genistein increases progesterone secretion by elevating related enzymes in chicken granulosa cells. Poult. Sci.

Zampiga, M., F. Soglia, M. Petracci, A. Meluzzi, and F. Sirri. 2019. Effect of different arginine-to-lysine ratios in broiler chicken diets on the occurrence of breast myopathies and meat quality attributes. Poult. Sci. 98:2691–2697.

Zempleni, J., R. B. Rucker, D. B. McCormick, and J. W. Suttie. 2007. Handbook of vitamins. 4th ed. CRC Press, Boca Raton, FL.

Zhou, N., W. R. Lee, and B. Abasht. 2015. Messenger RNA sequencing and pathway analysis provide novel insights into the biological basis of chickens' feed efficiency. BMC Genomics 16:195.

Zotte, A. D., G. Tasoniero, E. Puolanne, H. Remignon, M. Cecchinato, E. Catelli, and M. Cullere. 2017. Effect of "wooden breast" appearance on poultry meat quality, histological traits, and lesions characterization. Czech J. Anim. Sci 62:51–57.

Zou, X. T., X. J. Qiao, and Z. R. Xu. 2006. Effect of β-mannanase (Hemicell) on growth performance and immunity of broilers. Poult. Sci. 85:2176–2179.