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The Effect of Spirulina platensis Algae Inclusion in Feed of Commercial Broilers Subjected to Cyclic Heat Stress

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The Effect of *Spirulina platensis* Algae Inclusion in Feed of Commercial Broilers Subjected to Cyclic Heat Stress

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Abstract

The effect of *Spirulina platensis* inclusion in feed of commercial broilers was evaluated over one experiment. The study aimed to investigate the effects of *Spirulina platensis* freshwater algae inclusion on live performance and processing characteristics of commercial broilers subjected to daily cyclic heat stress. Day old Ross 708 male broilers were placed into 8 environmentally controlled chambers. At d 21, four chambers remained on a control diet containing no algae (CON) while 4 chambers received a diet that included algae at an inclusion rate of 2.5% (ALG). An 8 hour daily cyclic heat stress (24°C to 36°C) was applied to the chambers from d 22 to processing at d 48. Twenty-one broilers were sampled for breast tissue on d 45. The remaining broilers were processed at d 48 and meat quality attributes were assessed. No differences were observed for feed conversion ratio, water conversion ratio or live weight throughout the grow-out period. Additionally, mortality did not differ between the treatments (ALG=5.8%, CON=3%). From sampling there was no significant difference between ALG and CON birds for SOD1, SOD2, GPx, HSP70 and HSP90 regulation. At processing, no differences were observed between the groups for carcass weight, wing, breast, tender, or leg quarter yield, breast pH, breast L* or drip loss. The ALG birds had a higher a* value and b* value than CON birds. It appears that inclusion of freshwater algae at a level of 2.5% did not have an impact on live performance, processing parameters or meat quality of commercial broilers subjected to cyclic heat stress. Higher levels of algae inclusion need to be evaluated.

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Chapter 1 Introduction

Introduction

The world is experiencing an increase in the global population. By 2100, the global population is expected to reach 10.9 billion people (Cilluffo and Ruizy, 2021). As the population increases, so has the need for animal protein, with a projected 73% increase in demand needed by 2050(OECD/FAO, 2018). One of the most abundant animal proteins is poultry, with consumption reaching 129 million tons globally in 2021 (Shahbandeh, 2022). Global consumption of pork comes in at only 113 million tons and beef at 70 million tons in 2021 (Shahbandeh, 2022). In the United States the per capita consumption of poultry has increased from 94.7 lbs. in 2000 to a forecasted 113.7 lbs. in the year 2022 (USDA, 2021). As the poultry industry grows to meet market demands, birds have been selected for faster growth rates, lower feed conversion ratios (FCR), and increased breast yield. Havenstein and colleagues (2003) compared broilers from the 1950's to broilers commercially available in 2001. This study determined that 90% of the improvements to the modern broiler resulted from genetic selection with the remaining improvements coming from changes in management and nutrition (Havenstein et al., 2003). Though there is an increasing demand for poultry products, finding ways to maximize performance under non-ideal conditions is imperative.

One way that the poultry industry experiences loss in performance is through heat stress. Broiler performance is affected by genetics, feed, management, and environmental factors. Heat stress has become more prominent over the years as a result of global warming, with global temperatures increasing ~1.3°-1.9° F each year (NOAA, 2021). The rising global temperatures creates the potential for long bouts of heat stress, causing issues when managing broiler flocks for peak performance.

Spirulina algae, originally presented as a dietary substitute for soybean meal, has increasingly been evaluated for other dietary substitutions, and impact on broiler performance. Its high nutrient content and superior amino acid profile makes algae a possible key to broiler nutrition (Barka and Blecker, 2016). Inclusion of algae in broiler diets has exhibited reduced oxidative stress (Abdel-Daim et al., 2013), a potential side effect of chronic heat stress. A major site of ROS production is in the mitochondria (Mujahid et al., 2007). It is possible that algae supplementation in the diet can alter the negative effects of birds subjected to bouts of heat stress and help manage potential economic loss.

Maintenance of live bird performance is important, but it is also necessary to consider alteration to meat quality. As customer acceptance of a product is directly tied to meat quality, a small improvement in meat quality can lead to increased profitability. Meat quality evaluation includes water holding capacity, pH, color, and woody breast. The breast muscle myopathy, woody breast, is a top concern for the industry as it results in substantial economic losses and downgraded products.

The current study aimed to investigate the effects of *Spirulina platensis*, freshwater algae, inclusion on the live performance, processing characteristics, antioxidant gene expression, and heat shock protein gene expression of commercial broilers subjected to daily cyclic heat stress

Chapter 2: Literature Review

Heat stress

Heat stress occurs when the amount of heat generated endogenously and entering the animal exogenously exceeds the amount of heat dissipation (Lara and Rostagno, 2013). Broilers are more susceptible to heat stress than red meat species due to their high metabolic rate, an extensive amount of feather coverage, and lack of sweat glands (Loyau et al., 2013). Heat stress is classified into acute and chronic heat stress based on exposure durations. Acute heat stress refers to short bouts of heat exposure and chronic heat stress refers to extended periods of heat exposure (Siddiqui et al., 2020). The ability to keep the birds at constant and comfortable temperature is important when considering the birds' overall performance. Poultry subjected to chronic heat stress have significantly reduced feed intake, lower body weights, higher feed conversion ratios, and higher corticosterone concentrations (Sohail et al., 2012). Increased feed conversion for broilers subjected to heat stress can be a result of poor gut health, with decreasing digestibility. In extreme cases, flocks subjected to chronic heat stress can also experience increased mortality (Lara and Rostagno, 2013). The economic impact of heat stress in the US poultry industry amounts to $128 to $165 million annually (St-Pierre et al., 2003). With such a large loss annually, and rising global temperatures, finding ways to mitigate the negative effects of heat stress is of great concern.

Broiler integrators rely on birds to grow efficiently and reach peak performance to meet market demand. Broilers with faster growth rates and larger body weights are known to have a higher sensitivity to increases in temperature (Orlowski et al., 2020; Tabler et al., 2020). The modern broiler has been genetically selected to have a faster growth rate, higher yields, and lower feed conversion ratio. Unfortunately, this selection pressure for efficiency related traits makes the commercial broiler more susceptible to the negative effects of heat stress. Broilers subjected to heat stress spend less time feeding and more time drinking and panting (Mack et al., 2013; Tabler et al., 2020). This change in behavior is the bird's response to homeostasis, the tendency toward a relatively stable equilibrium, however, performance is reduced. As feed accounts for 60% or more of the total cost of production, demand for lower feed conversions and maintaining proper gut health remain crucial to the industry's success. The gastrointestinal tract is very responsive and vulnerable to heat stress (Zeng et al., 2014; Huang et al., 2015). Heat stress alters gut morphology by lowering crypt depth, mucous area, and villus height ultimately reducing nutrient absorption (Marchini et al., 2016). The susceptibility of the gastrointestinal tract allows for a multitude of metabolic problems under heat stress, such as leaky gut syndrome, increased feed conversion, high mortality, immunosuppression, and bacteria translocation (Zeng et al., 2014; Huang et al., 2015). Changes in the gastrointestinal tract are caused by oxidative stress that leads to the denaturation of proteins and enzymes utilized in the gut. Exposure to chronic heat stress also negatively affects fat deposition and meat quality in broilers (Lu et al., 2007). Changes in carcass composition and meat quality alters the marketability of the bird and contributes to the annual loss attributed to heat stress.

A health concern for the broiler caused by heat stress is oxidative stress. Oxidative stress is caused by the body’s inability to regulate the oxidation-antioxidation pathway. Oxidative stress can negatively affect multiple organs and tissues within the bird. The tissues include but are not limited to the gut, muscle, and liver. During heat stress nuclear factor, erythyroid 2-like 2 (NrF2) is activated and is a master regulator of the oxidation-antioxidation pathway (Chan et al., 2001; Jin et al., 2016). Nrf2 trans-activates multiple antioxidant proteins such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). During oxidative stress, electrons are leaked and combine with oxygen (O_2) which forms superoxide (O_2^-) (Turrens, 2003).

SOD converts superoxide, a free radical, to a non-radical hydrogen peroxide (H_2O_2) (Velayutham and Zweier, 2013). Hydrogen peroxide can be converted into hydroxyl radical (OH^-) which is highly reactive and dangerous (Halliwell, 2001). GPx works with glutathione (GSH) to convert the hydroxyl radical into H_2O (Ng, 2007). After the conversion of OH^- to H_2O, GSH is converted into glutathione disulfide (GSSG) by GPx. GSSG can be reduced again into GSH by glutathione reductase (GR) allowing GSH and GPx to remove multiple reactive oxygen species (ROS) (Droge, 2002). Alternative to the use of GSH and GPx to eliminate ROS in the body is catalase (CAT), CAT can convert H_2O_2 created by SOD into H_2O (Al-Abrash et al., 2000). The exposure of birds to heat stress after 12 days resulted in an increase of mRNA expression of SOD1, though there is no change in mRNA expression of SOD1 after 1 day in the liver tissue (Habashy et al., 2018). Habashy and colleagues (2018) determined that an increase in H_2O_2, produced due to the up regulation of mRNA expression of SOD1, resulted in an increase in the mRNA expression of CAT. As the expression of SOD1 and CAT increased, there becomes a down regulation of mRNA expression of GPx. Catalase has a much higher km for H_2O_2 than GPx. Therefore, the use of catalase is only during excessive oxidative stress. When exposed to prolonged bouts of heat stress poultry use CAT and SOD1 more than the use of GPx and GSH (Habashy et al. 2018).

Heat shock proteins (HSPs) become an important factor in the maintenance of homeostasis under heat stress. During heat stress, proteins produced by cells can be damaged and denatured leading to apoptosis. HSPs help to reduce the amount of damage to cells based on the type caused by heat stress. The upregulation of HSPs increases cell stability and develops thermotolerance during heat stress conditions (Shehata et al., 2020). It has been determined that HSPs are significantly upregulated in broilers subjected to heat stress (Tabler et al., 2020). Heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90) work on proteins that are classified according to molecular weights (Shehata et al.,2020). HSP70 attaches to newly synthesized polypeptides and prevents aggregation (Hartel and Hayer-Hartel, 2002; Hartel et al.,1994). HSP70 maintains polypeptide and structure folding at the native state (Hartel and Hayer-Hartel, 2002; Hartel et al.,1994). HSP70 also increases antioxidant status, prevents lipid peroxidation, and increases gut enzyme activity (Zhao et al., 2014). Heat shock protein 90 (HSP90) works on older proteins and modifies the tertiary structure of a protein to prevent denaturization (Wegele et al., 2004). HSP's ability to prevent further damage caused by oxidative stress makes them key factors when evaluating heat stress.

Spirulina platensis **Algae in Poultry Feed**

Spirulina platensis is an alternative feed ingredient that is rich in protein, vitamins, and minerals. Thirty percent of the world's algae production is used in animal feeds with the target animal of poultry (Becker, 2007). The nutrient density of *Spirulina* algae has allowed it to be evaluated as an effective alternative dietary source to substitute the costly ingredient of soybean meal, fishmeal, and fish oil required as a protein source in poultry diet. The potential of algae as a dietary supplement is by its similar amino acid profile and high crude protein concentration (43-70%) to that of soybean meal (Barka and Blecker, 2016; Gouveia et al., 2008). Though it is presented as an alternative to different feed stuffs the inclusion of algae must be evaluated to determine any potential effects on poultry performance.

Inclusion of algae at levels over 20% has exhibited adverse effects on production values, including body weight (Ross and Dominy, 1990). By three weeks of age an inclusion of 10% algae reduced growth rate (Ross and Dominy, 1990). Ross and Dominy (1990) exhibited that the quality of algae used in the feed may be a factor in performance. On the other hand, Yoshida and Hoshii (1980) observed no adverse effects at 20% algae inclusion, however inclusions as low as 5-12% exhibited no significant difference in feed efficiency (Ross and Dominy, 1990). Ross and Dominy (1990) was able to derive the conclusion through comparison with the previous study by Yoshida and Hoshii (1980) that exhibited no significant growth depression to birds fed a diet with a spirulina inclusion of over 20%. Birds grown to 42 days and provided a diet with a 12% inclusion had no significant difference in FCR when compared to those feed a control diet (Ross and Dominy,1990). The inclusion of *Spirulina platensis* of 10% in low crude protein diets responds differently based on sex (Mullenix, 2021a). Mullenix (2021a) found that in Ross 708 females fed low crude protein diet with algae inclusion of 10% feed conversion increased, while in males feed conversion decreased. Furthermore, Mullenix (2021a) evaluated an inclusion level of *Spirulina platensis* at 2.5% that resulted in increased feed conversion ratio but no effect on body weight gain. Moustafa and colleagues (2021) found that the inclusion of algae at one percent significantly increased the average daily gain and final body weight in birds subjected to heat stress. It has been determined that algae inclusion in feed results in an upregulation of SOD (Mullenix et al., 2021b; Moustafa et al., 2021). SOD plays a crucial role in oxidative stress regulation; an upregulation has the potential to alleviate the effect of ROS that accumulate in birds subjected to heat stress.

Spirulina has two distinctive natural colors, carotenoids and C-phycocyanin. These natural color agents have shown increased importance due to their health-related benefits. These benefits allow for the possibility of nutritionists to add algae to diets to increase birds' overall health. Carotenoids and C-phycocyanin both exhibit the ability to reduce oxidative stress that occurs in the body (Abdel-Daim et al., 2013). C-phycocyanin has exhibited antioxidant, antibacterial, and anti-inflammatory properties (Abd El-Baky et al., 2012; Liao et al., 2016;

Manconia et al., 2009; Sonani et al., 2017). These properties being present in *Spirulina platensis* supports the hypothesis that algae inclusion in diets could mitigate negative effects of heat stress. The color agents can degenerate during the production process of heat drying or sunlight. This makes freeze-dried algae a higher nutrient dense and higher quality product to use as a feed stuff (Park et al., 2018). The natural color agents in *Spirulina* cause an increase in the yellowness (b*) of the breast meat (Moustafa et al.,2021; Pestana et al., 2020, Mullenix, 2021a). Though the yellowness is increased, with a trained sensory panel of meat from birds fed a diet with algae inclusion of 15% exhibited no significant difference in overall acceptability of product compared to birds fed a common diet (Pestana et al., 2020).

Meat Quality

Consumer preference is a key driver in poultry marketing and management as minor improvements in meat quality can improve consumer acceptability. Appearance plays the largest role in consumer acceptability (Allen et al., 1998). Factors such as drip loss, pH, color, and woody breast scores can highly impact palatability and ultimately consumer acceptability of a poultry product (Anthony, 1998; Barbut, 1996, 1997, 1998; Kuttappan et al., 2016).

pH

Postmortem, muscle is converted to meat through a muscle stiffening process called rigor mortis (Aberle et al., 2012). Rigor mortis is a Latin word that translates to "the stiffness of death". Rigor mortis begins at the time of death (Lawrie, 1998). Postmortem, an animal's muscle metabolism switches from aerobic (with oxygen) to anaerobic (without oxygen) metabolism. This process is aided by the process of exsanguination, which is considered to be the most crucial step in the conversion of muscle to meat (Lawrie, 1998). With the decrease in blood flow and the utilization of cellular energy stores having been expended, lactic acid increases in the muscle (Lawrie, 1998; Aberle et al., 2012). The increase in lactic acid causes the pH to drop and muscle characteristics to change. The rate of pH decline and the ultimate pH that the meat reaches has a large impact on meat quality. Poultry rigor mortis is completed between two to eight hours postmortem (de Fermery and Pool, 1959; Wiskus et al., 1976; Addis, 1986; Hedrick et al., 1994). The normal ultimate pH for poultry meat is a pH of 5.6 to 6.2, with breast fillets having a normal pH of 5.8 through 5.9 (Dransfield and Sosnicki, 1999; Pearson and Dutson, 1999; Qiao et al., 2001; Duclos et al., 2007). Broilers fed diets with algae inclusion of 15% exhibited no significant difference in 24-hour pH compared to those being fed a common control diet (Pestana et al., 2020). Though broilers subjected to cyclic heat stress and acute heat stress had lower ultimate pH (Feng et al., 2006; Wang et al., 2009). Muscle pH has been highly correlated to other meat quality traits such as color, water-holding capacity, tenderness, and shelf life (Allen et al.,1998; Qioa et al., 2001).

Color

Meat color is a highly important attribute in terms of consumer acceptability and willingness to purchase. Consumers often make purchase decisions solely based on product appearance and packaging (Allen et al., 1998). Meat color can be assessed using a colorimeter that uses 3 different wavelengths designated as L*, a*, and b*. L* is defined by the lightness or darkness of a product with higher values representing lightness, a* is a bilateral scale with positive representing a red color and a negative value representing a green color, b* is also a bilateral scale with positive values correlating to yellow and negative values correlating to blue color. Broilers fed diets with *Spirulina* inclusions experience an increase in yellowness (b*), while lightness (L*) remains unaffected compared to those fed a common control diet (Pestana et al., 2020; Moustafa et al., 2021). Meat pH and color have a strong negative correlation, with breast fillets that have a low ultimate pH will have a high L* or a pale breast. High L* value has lower water retention in both raw and cooked states (Fletcher, 2002). Lighter fillets also have softer textures, as muscle proteins are denatured through rapid glycolysis and pH decline (Owens et al., 2000; Woelfel and Sams, 2001; Woelfel et al., 2002). On the other hand, darker fillets in further processed products exhibit higher marinade uptake and moisture, and lower cook-loss and drip-loss (Allen et al., 1998). In terms of shelf life, darker fillets have a better performance and increased in one day shelf stability, while lower L* fillets have decreased shelf life (Allen et al., 1997, 1998).

Water Holding Capacity

Water holding capacity (WHC) affects multiple meat quality attributes such as texture, juiciness, and color (Brewer et al., 2012). Water holding capacity is positively correlated to muscle pH. It can be measured by many different methods such as drip loss, cook loss or expressible moisture. Broilers fed diets with algae inclusion had no significant difference in cook loss compared to those fed a control diet, however when presented to a trained sensory panel meat juiciness was less intense in algae fed birds then birds given a control diet (Pestana et al., 2020). Pestana and colleagues (2020) also found that meat tenderness was not affected by diet, algae or control, when evaluated by trained sensory panel. Wang and colleagues (2009) found that exposure to heat stress resulted in increased drip loss and cook loss suggesting lower water holding capacity. The decrease in water holding capacity of heat stress birds is caused by the damage of proteins in the muscle due to oxidative stress. Breast fillets with high pH have an increased ability to hold water, improving water holding capacity (Bowker and Zhuang, 2015). Water is present in three different forms throughout the meat and attributes to seventy-five percent of total muscle weight (Pearson and Duston, 1999; Alvarado and Owens, 2005). The three water forms are bound water, immobilized water, and free water. Bound water is tightly bound to the interfilamental spaces and can only be removed by ashing and is attributed to only one percent of water in muscle (Alvarado and Owens, 2005). Immobilized water is the intermediate state and is held in place by ionic forces, this attributes fifteen percent of total water (Bechtel, 1986; Alvarado and Owens, 2005). Free water accounts for 85-89% of water in meat and is located extracellularly and can be easily extracted (Pearson and Dutson, 1999). There are two different determinants that affect WHC, they are the ionic and steric effect. The ionic effect is related to muscle pH and influences one-third of WHC. Steric effect is related to the orientation of the muscle proteins and accounts for two-thirds of WHC. Thus, it is important to consider WHC when assessing meat quality.

Woody Breast

As the industry has moved towards higher yielding, faster growing broilers, an unwanted consequence in the form of muscle myopathies has occurred. The muscle myopathy that has produced cause for the greatest concern for both producers and consumers is woody breast. Woody breast first appeared in the industry in 2014 (Sihvo et al., 2014). It is characterized as natural stiffness to the muscle fibers from palpation techniques along with noticeable “ridges” throughout the breast, these breasts can be scored mild, moderate, or severe (Sihvo et al., 2014; Tijare et al., 2016). Breasts exhibiting woody breast have an increased split fibers, fraying from polygonal fiber shape, loss of striated muscle structure and increase in inflammatory cells (Sihvo et al., 2014). A yellow inflammatory exudate can be found on breasts exhibiting severe woody breast. This exudate can lead to trimmed condemnations at the plant per USDA (USDA: FSIS, 2017). It is estimated that woody breast has caused over $200 million in economic loss in the broiler industry (Kuttappan et al., 2016).

Woody breast can be evaluated by hand palpation. The former can be subjective to each individual performing the palpation. To decrease the variation and subjectivity grading scales are used. A broadly used scale is from 0-3 with increments of 0.5 created by Tijare et al. (2016). This method scored breast as follows. Fillets that remained flexible throughout are given a score of 0. Fillets with localized hardness in the cranial regions and maintain flexibility throughout are given a score of 1. Fillets with hardness throughout and maintain flexibility through the middle and caudal regions of the breast received a score of 2. Fillets exhibiting hardness and rigidity throughout the breast are given a score of 3. In research analysis the scoring scales are consolidated into subgroups. The subgroups are 0-0.5 as normal, 1-1.5 as mild, and 2-3 as moderate/severe (Maynard et al., 2019).

Woody breast not only lacks visual appeal to the consumer but results in poor functionality of the fillet for further processing and cooking. Fillets with woody breast exhibit impaired marinade uptake, and decreased water retention in both raw and cooked states (Soglia et al., 2016; Tijare et al., 2016; Petraccis et al., 2019). Woody breast fillets also have decreased protein content (Soglia et al., 2016), increased fat (Dalle Zotte et al., 2020), and increased insoluble collagen (Petracci et al., 2019). This change in the protein and molecular content of the fillet due to woody breast reduces water holding capacity (Soglia et al., 2016). The reduction in water holding capacity results in fillets cooking faster, dry out faster, and fillets with excessive cooking loss (Soglia et al., 2016; Tijare et al., 2016). This reduction in functionality results in a fillet that is lower quality and limited use in further processing.

Summary

As the demand for poultry increases the need for efficiency remains important to provide an affordable and healthy protein source to the growing populations. Any challenges the industry is facing must be addressed, specifically heat stress as a result of rising global temperatures. In addition, meat quality and the ability to produce a higher quality product will play a part in producing and meeting the demand driven by the consumers. Feed additives such as freshwater algae may help alleviate the stressor put on the birds by increasing availability of antioxidants. The current study aimed to investigate the effects of *Spirulina platensis* freshwater algae inclusion of 2.5% on the live performance and processing characteristics of commercial broilers subjected to daily cyclic heat stress. The potential benefit of reduced oxidative stress in broilers fed *Spirulina platensis* in their diet, may have the potential to mitigate the negative effect brought by cyclic heat stress.

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Chapter 3: The Effect of *Spirulina platensis* Algae Inclusion in Feed of Commercial Broilers Subjected to Cyclic Heat Stress

Abstract

Broiler growth and performance can be affected by a multitude of factors including genetics, management, and environment. With rising global temperatures, the potential for long bouts of heat stress may become an issue when managing broilers for peak performance. Finding ways to mitigate the negative effects of heat stress is imperative. The current study aimed to investigate the effects of *Spirulina platensis* freshwater algae inclusion on live performance and processing characteristics of commercial broilers subjected to daily cyclic heat stress. Day old Ross 708 male broilers were placed into 8 environmentally controlled chambers (n=30 birds/chamber). Broilers were fed a common starter diet from d 0 to d 21 and were reared under common conditions. At d 21, four chambers remained on a control diet containing no algae (CON) while 4 chambers received a diet that included algae at an inclusion rate of 2.5% (ALG). An 8 hour daily cyclic heat stress (24°C to 36°C) was applied to the chambers from d 22 to processing at d 48. Daily feed and water intake were recorded as well as weekly body weights. Twenty-one broilers were sampled for breast tissue on d 45. Broilers were processed at d 48 and meat quality was assessed. No differences were observed for FCR, WCR or live weight throughout the grow-out period. Additionally, mortality did not differ between the treatments (ALG=5.8%, CON=3%). There was no significant difference between ALG and CON birds for SOD1, SOD2, GPx, HSP70 and HSP90 regulation. At processing, no differences were observed between the groups for carcass weight, wing, breast, tender, or leg quarter yield, breast pH, breast L* or drip loss. The ALG birds had a higher a* value and b* value than CON birds. Inclusion of *Spirulina platensis* algae at an inclusion level of 2.5% had minimal effect on live performance and meat quality of broilers subjected to cyclic heat stress. Higher levels of algae inclusion may need to be evaluated to elucidate potential positive effects under heat stress.

Introduction

The world is experiencing unprecedented growth with the population expected to reach 10.9 billion people by 2100 (Cilluffo et al., 2021). This increase in the world population has resulted in a subsequent increase in the demand for animal protein. By 2050, current animal protein production must increase by 73% to feed the population (OECD/FAO, 2018). Poultry is one of the most widely used animal proteins surpassing that of beef and pork (Shahbandeh, 2021). Because of its efficiency, poultry production will help to meet the rising demand for animal protein. In the United States alone, poultry demand has increased from 94.7 lbs per capita consumption in 2000 to a forecasted 113.7 lbs per capita in 2022 (USDA, 2021). Genetic selection of the broiler for faster growth rates, higher yields, and lower feed conversions has helped meet the demand and will contribute to helping feed the growing population.

The selection pressures for commercial broilers have come with some inadvertent issues such as susceptibility to heat stress. Broilers are more susceptible to heat stress than red meat species due to their high metabolic rate, extensive feather coverage, and lack of sweat glands (Loyau et al., 2013). It has been observed that broilers with faster growth rates are more susceptible to heat stress through higher mortalities and decreased yields (Orlowski et al., 2020; Tabler et al., 2020). It is estimated that heat stress has cost the poultry industry in the US $128 to $165 million annually (St-Pierre et al., 2003). As global temperatures continue to rise the potential for long bouts of heat stress increases as well as the potential for greater economic losses. When heat stress occurs, broilers begin a change in behavior to maintain optimal core body temperatures. They spend less time feeding and more time drinking and panting (Mack et al., 2013). This change in behavior negatively impacts feed conversion and reduces growth rate, conflicting with industry expectations for peak performance and welfare. A possible solution to mitigate the negative effects caused by heat stress can be found in the inclusion of *Spirulina platensis* in the feed. Spirulina algae is a high protein, vitamin, and mineral rich feed stuff. Due to its nutrient richness, it has been presented as an effective alternative to other feed stuffs such as soybean meal, fishmeal, and fish oil. Moustafa and colleagues (2021) found that the inclusion of *Spirulina* at 1% resulted in a significant increase in average daily gain and final body wight in birds subjected to heat stress. Algae inclusion has the potential to mitigate some of the negative effects caused by heat stress in commercial broiler production.

With changes in overall performance, birds subjected to heat stress also experience changes within body systems. One of the factors attributing to changes within systems is oxidative stress caused by heat stress. Oxidative stress is caused by the body's inability to regulate the oxidation-antioxidation pathways affecting multiple organs including the liver, muscle, and gastrointestinal system. *Spirulina platensis* algae's distinctive natural colors, carotenoids and C-phycocyanin, are key to its ability to alleviate the negative effect of heat stress by reducing oxidative stress (Abdel-Daim et al., 2013). C-phycocyanin exhibits antioxidant and anti-inflammatory properties (Abd El-Baky et al., 2012; Liao et al., 2016; Manconia et al., 2009; Sonani et al.,2017). Heat shock proteins play a large role in homeostasis regulation during heat stress. During heat stress, proteins produced by cells can be damaged and denatured leading to apoptosis. Heat shock proteins help to reduce the amount of damage to cells. The upregulation of heat shock proteins increases cell stability and develops thermotolerance during heat stress conditions (Shehata et al., 2020). The ability of these proteins to prevent denaturation of the polypeptides increases the performance of broilers subjected to heat stress conditions.

Visual meats appearance plays the largest role in consumer acceptability (Allen et al., 1998). The addition of algae in fed at an inclusion of 15% results in an increase in yellowness (b*) compared to those not fed algae, though there is no significant difference in products acceptability when evaluated by a trained sensory panel (Pestana et al, 2020). This change in color is brought on by the natural colors within *Spirulina platensis.* Factors such as drip loss, pH, color, and woody breast scores can highly impact the palatability and marketability of the product (Anthony, 1998; Barbut, 1996, 1997, 1998; Kuttappan et al., 2016). Heat stress can change the composition of the carcass and negatively impact meat quality of a carcass (Lu et al., 2007). Algae inclusion in broiler diets is still being evaluated for the potential effect on meat quality and bird health.

The current study aimed to investigate the effects of *Spirulina platensis* freshwater algae inclusion on the live performance and processing characteristics of commercial broilers subjected to daily cyclic heat stress. The potential benefit of reduced oxidative stress in broilers fed *Spirulina platensis* in their diet, may have the potential to mitigate the negative effect brought by cyclic heat stress.

Materials and Methods

This study was approved by the University of Arkansas animal care and use committee (protocol #21123). Day-old Ross 708 male broiler chicks placed into 12 environmentally controlled chambers at the density of 40 birds per chamber. Densities were reduced to 30 birds per chamber by 21 days of age. Chambers were equipped with two hanging feeders at the front of the chamber and a five-gallon plastic poultry bell waterer was used to provide water in the center of the pen. Feed and water were provided ab libitum throughout the study and daily feed and water intake were recorded for each pen at the same time every day. Pen weights were recorded weekly and individual bird weights were taken on d 21 and d 48. Pen temperatures were set to 36°C from d 0-2. Temperature was reduced to 31°C from d 3-5, 29°C from d 6-9, 27°C from 10-13, and 24°C from d 14-21. During the first week, d 0-7, birds were provided with 23-hour light: 1-hour dark lighting program. For the remainders of the trial, d 8-48, birds were provided with 18-hour light: 6-hour dark lighting program

All pens were fed a common starter diet from d 0-21. On d 22, chambers were assigned to one of two dietary treatment groups. The two diets were a control diet (CON) and a diet supplemented with freshwater algae (ALG). The ALG diet contained a 2.5% inclusion of *Spirulina platensis* in the diet while meeting energy and nutrient requirements set by Ross broiler nutrition guide. The control diet contained no algae and met the same energy and nutrient requirements of the algae diet. The birds remained on their experimental grower diets (Control or Algae) from d 22-35. The birds transitioned to their experimental finisher (Control or Algae) diets on d 36 till the end of trial. The diet specifications are provided in Table 1.

On d 22 8 chambers were exposed to cyclic heat stress beginning on day 22. Temperatures were raised to induce heat stress at 36°C from 0800 to 1600. At 1600 chambers were returned to a thermal neutral temperature of 24°C, creating 8 h cyclic heat stress. Within the heat stress chambers the chambers were randomly assigned to feed treatment either algae feed (ALG) at 2.5% inclusion (4 chambers) or a control feed (CON) (4 chambers). The remaining 4 chambers remained in thermoneutral conditions at 24°C and were fed a control diet containing no algae.

On d 45, seven birds were sampled from each treatment group. Sampling began one hour after the initiation of heat stress. Birds were selected randomly for sampling with at least one bird per pen as a representative of the pen. Following cervical dislocation, samples from the breast fillet were collected for evaluation. The samples were placed in snap cap tubes and labeled with band number of the bird from which they came from, flash frozen in liquid nitrogen, then stored at -80°C until further analysis could be conducted.

One µg of total RNA was extracted from the sampled breast tissues via Trizol reagent (Life Technologies) following the manufacturer's recommendations. Ribonucleic acids were then treated with DNAse, and reverse transcribed qScript cDNA SuperMix (Quanta Biosciences). Next, the cDNA was amplified using real-time quantitative PCR (Applied Biosystems 7500 Real-Time PCR system) with Power SYBR green Master Mix (Life Technologies). Oligonucleotide primers for chicken SOD1, SOD2, GPx, HSP70 and HSP90 were used. Primer concentration of 0.5µL and volume of 1µL per sample was used. The qPCR cycling conditions were 50°C for 2 min, 95°C for 10 min followed by 40 cycles of a two-step amplification program, 95°C for 15 s and 58°C for 1 min. At the end of the amplification, melting curve analysis was applied using the dissociation protocol from the 27 Sequence. Relative gene expressions were determined using 2-ΔΔCt method (Schmittgen and Livak, 2008).

On d 48, all remaining birds were cooped and transported to the University of Arkansas pilot processing plant. Feed was removed from the pens on d 47, at 2000, 12 hours prior to processing while access to water remained until loading. On the back dock of the processing plant, a final dock weight was recorded for each individual bird and birds were hung on shackle-line. Using inline equipment, birds were electrically stunned (11V, 11mA, 10) in an inline water bath stunner. Following the stun, they were manually cut through the left carotid artery and jugular vein and exsanguinated for 2 minutes. Carcasses then entered a water bath scalder at 55°C and were picked using an in-line commercial de-feather machine. Feet were then removed using an in-line hock cutter. Following the removal of hocks, head and neck, carcasses were rehung on an evisceration line where an automatic evisceration machine was used. Postevisceration, WOG (without giblets) and fat pad weights were then recorded, and carcasses were placed in an ice bath chill tank. After 3 h chill, carcasses were removed from chill tanks and placed on a hand debone line. On the debone line birds were deboned into parts including wings, breast, tenders and leg quarters. Parts were weighed and woody breast was scored using a 4-point scale. The scale included 0, 1, 2, 3 with 0 being no woody breast, 1 being mild woody breast, and 2 and 3 being severe woody breast. Breast fillets were covered and stored in a refrigerator at 4ºC for 24 hours then remaining meat quality parameters were measured. The remaining parameters included ultimate pH, drip loss and color. Breast pH was taken with a Testo 205 pH probe in the cranial region of the right breast fillet. Breast color was evaluated using a Minolta CR-300 colorimeter and drip loss was calculated by taking the weight of the breast after refrigeration and subtracting the original weight of the breast when deboned.

Data were analyzed using JMP 16.0 (SAS, 2022). Data for all variables were analyzed using one-way analysis of variance (ANOVA) with the main effect of diet. Significant differences between treatment means were determined using LS Means Student T-test. Significant values were determined at $p \leq 0.1$.

Results and Discussion

Live performance

The results for bird live performance are presented in Table 2. There was no significant difference between the heat stress groups, HS ALG and HS CON, in relation to final body weight (FBW), water intake (WI), feed intake (FI), water conversion ratio (WCR), feed conversion ratio (FCR), and average daily gain (ADG). There was a difference in mortality with the HS CON having a lower morality count than the HS ALG (p=0.0633). Between treatment groups, there was a significant difference in FBW (p=0.0021), FCR (p=0.0235), and ADG (p=0.0025). The TN CON birds pens had a higher FBW and ADG than the other two treatments (HS CON and HS ALG). Furthermore, the TN CON group had a lower FCR compared to HS CON and HS ALG. Mortality remained different between treatments with HS CON having a lower amount of mortality than HS ALG and TN CON (p=0.0679). There was no significant difference between treatments in relation to WC, FC, and WE. Daily average water consumption can be observed in Figure 1 and daily feed consumption is presented in Figure 2. Based on these findings between the TN and HS groups a significant level of heat stress was achieved due to the decrease in bird performance.

When comparing the HS ALG to HS CON groups, the results contradict the findings of Moustafa and colleagues (2021) that observed a significant increase in live performance for birds supplemented with algae and subjected to cyclic heat stress over those fed a control diet. Moustafa and colleagues (2021) had the greatest improvement in live performance at an inclusion level of 1%, along with a minor increase in performance with an inclusion of 1.5%. An inclusion of 2.5% might have been too high of an inclusion to see a significant difference between the algae fed and control fed birds subjected to cyclic heat stress. The data did support the findings of Ross and Dominy (1990) with no significant difference observed for FCR of birds fed algae compared to those fed a control diet. With an inclusion of 2.5% based off the findings of Mullenix (2021a) a decrease in FCR for birds fed algae diet should have been observed however, the results of this study showed no difference. The results of live performance exhibited no negative or positive data indicating addition of *Spirulina platensis* at an inclusion of 2.5% to feed of broilers subjected to heat stress. Therefore, the addition of *Spirulina platensis* at 2.5 % can be added to feed without a loss in performance should other protein ingredients such as soybean meal become scarce.

Lara and Rostagno (2013) found that chronic heat stress causes an increase in mortality. The current study, the HS CON that had a lower mortality than the TN CON. This implies that the cyclic heat stress applied was not extreme. In addition, growth rate and feed intake of the birds in the TN CON group may have resulted in an increase in mortality compared to the HS CON.

As birds change behavior to maintain homeostasis it is expected that birds subjected to heat stress will exhibit an increase in water consumption and decrease feed consumption. This concept was exhibited by Sohail and colleagues (2012) in which birds subjected to heat stress had reduced feed intake. In the current study, all treatment groups had similar feed intakes throughout the 48-d period. Furthermore, there was no increase in water consumption of birds subjected to cyclic heat stress when compared to those under thermoneutral conditions. However, the data of the current trial supports the findings of Sohail and colleagues (2012) that birds subjected to heat stress have higher FCR and lower body weights. The increase in performance exhibited by the TN CON birds compared to the heat stress treatments demonstrates the application of stress in relation to heat.

Antioxidant gene expression

Gene expression was evaluated in the breast tissue of the birds (Table 3). Gene expression of glutathione peroxidase, SOD1, SOD2 were not significantly different between treatments, contradicting current literature. Moustafa and colleagues (2021) exhibited a significant upregulation of SOD expression in birds fed algae compared to those fed the control diet when subjected to cyclic heat stress ($p=0.004$). Mullenix and colleagues (2021b) found that the inclusion of algae in feed resulted in an upregulation of SOD2. SOD's ability to convert superoxide into a non-radical hydrogen peroxide allows it to begin the mitigation process of reducing oxidative stress. Due to the increased bout of cyclic heat stress, it is expected that there would be an increased expression of SOD1 over GPx (Habashy et al., 2018). This was observed with the ALG group as it has a higher expression of SOD1 than GPx confirming the findings of Habashy and colleagues' (2018). GPx has the ability to remove multiple reactive oxygen species (ROS) as it converts glutathione (GSH) into glutathione disulfide (GSSG) in the muscle. This further confirms the application of cyclic heat stress in oxidative stress. Though it was not significantly different, the inclusion of algae to feed exhibits a potential trend for reduction of oxidative stress. It is possible that different algae inclusion levels might result in significant differences in terms of antioxidant gene expression when subjected to heat stress and should be evaluated in future studies.

Heat Shock Proteins

Heat shock protein (HSP) expression was also evaluated in the breast tissue of the birds (Table 3). HSP70 gene expression between HS ALG and HS CON was not significantly different (p=0.48888). HSP90 was different between HS ALG and HS CON, with an upregulated in the HS ALG birds compared to the HS CON birds (p=0.065677). The upregulation of HSP90 within the HS ALG treatment compared to that of the HS CON further displays algae's ability to mitigate the negative effects of heat stress. HSP's protect proteins produced by cells from being damaged and denatured during heat stress with an upregulation in HSP's increasing cell stability and thermotolerance (Shehata et al., 2020). The upregulation of HSP90 supports the findings of Tabler and colleagues (2020) and shows that algae supplementation may help reduce some of the negative impacts of heat stress on cell and protein structure.

Processing data

The results for processing and meat quality performance are presented in Table 4. The a* value was higher for the HS ALG group than of the HS CON group (p<0.0001). The b* value was significantly higher for the HS ALG birds than that of the HS CON birds (p<0.0001). Furthermore, HS ALG has a higher pH than HS CON (p=0.0624). There was no significant difference between HS ALG and HS CON for fat pad, pH, dripless, wing yield, and tender yield, WOG weight, breast yield, and leg quarter yield. There was a significant difference between treatments in terms WOG weights (p=0.0033), a* (p=0.001), b* (p<0.0001), breast yield (p=0.0016) and leg quarter yield (p=0.0092). TN CON has a higher WOG weight, higher breast meat yield, and lower leg quarter yield than both the HS ALG and HS CON group. HS ALG has higher a* and b* values than HS CON and TN CON. Furthermore, HS CON had a higher fat pad weight than TN CON, however HS ALG is the same as HS CON and TN CON (p=0.00667).

Color plays an important role in consumer acceptability of a fresh product. There is some concern with algae supplementation in broiler diets that is due to its characteristics, consumer acceptability may be limited. When evaluating the color of the breast fillet, there is concern regarding a* and b* values as they vary from the control diet. Breast color evaluation is determined using L*, a*, and b*. With the measurements, a* measurements give an indication of the redness of the fillet while b* indicated fillet yellowness. The inclusion of algae in the diet gives breast meat a more orange color compared to a pinker color that is observed with a breast from a bird fed a diet containing no algae. The increase in b* value, and no difference in L* values match with the findings of Moustafa and colleagues (2021) as well as Pestana and colleagues (2020). The increase in b* and a* supports the finding of Mullenix (2021a), however contradicts with the Mullenix and colleagues' observation of a lower L* in bird fed a diet containing algae.

Variation in color in algae fed birds is a result of the pigmentation of the algae including the two distinctive colors of carotenoids and C-phycocyanin's. Although there is a change in product color of breast fillets from birds fed algae, Pestana and colleagues (2020) found no significant decrease in consumer acceptability. The difference for pH between HS ALG and HS CON contradicts the findings of Pestana and colleagues (2020), however both ultimate pH readings are well within the normal range of breast meat pH. Similarly, Pestana and colleagues exhibited no significant difference between birds fed algae and birds fed a control diet in cook loss while this study found no significant difference in drip loss, both being measures of water holding capacity. Contrary to the findings of Mullenix (2021a) when evaluating algae inclusion of 2.5% there was no difference in breast yield of the HS ALG compared to HS CON. The processing does support the findings of Mullenix (2021a) similar carcass composition of bird fed algae compared to control fed birds, however that study evaluated an algae inclusion of 5%.

When comparing the TN CON, HS CON, and HS ALG groups for processing data, a majority of the differences relate back to growth rate. TN CON had the highest growth rate and highest final body weight when compared to the HS groups. Similar to their improved live performance, TN CON have an improved breast yield and increased WOG weights. Previous studies have found a decrease in breast pH between heat stress and control birds, (Wang et al., 2009; Feng et al., 2006) however there was no decrease in pH between thermoneutral treatment and heat stress treatments observed in the current study. It is known that pH is positively correlated to water holding capacity (Bowker and Zhuang, 2015). Being that there was no difference between treatment groups for pH, a similar trend was observed for drip loss.

Woody breast was categorized into non-woody (score of 0 and 1) and woody breast (score of 2 and 3). A score of 2 or 3 has the potential to be downgraded or condemned at the processing plant. The instance of wood breast and percent woody breast is presented in Figure 3 and Table 5. There was no significant difference in the instance of woody breast between the HS ALG and the HS CON ‘this supports the findings of Mullenix (2021a) (Table 5). TN CON had a significantly higher instance of woody breast compared to HS ALG and HS CON (p=0.0297). As woody breast has many factors that can cause it to show up, it is unknown if woody breast incidence would occur at a high prevalence. Birds with higher growth rates typically have a higher instance of woody breast (Caldas-Cueva and Owens, 2020). The HS ALG and HS CON did not have a significant difference in average daily gain compared to each other which would explain the lack of difference in woody breast. The increase in ADG that TN CON exhibits explains its increased prevalence of woody breast. However, with algae exhibiting anti-inflammatory properties the instance of woody breast had the potential to be reduced as many breasts exhibiting woody breast have an increase in inflammatory cells (Sihvo et al., 2014). Therefore, the addition of *Spirulina platensis* did not alleviate or worsen the frequency of woody breast myopathies in broilers subjected to heat stress.

Conclusion

The inclusion of *Spirulina platensis* freshwater algae in broiler diets of birds subjected to cyclic heat stress exhibited no significant difference than those feed a control diet for live performance or processing characteristics. It was hypothesized that algae inclusion would help combat the oxidative stress imposed by cyclic heat stress, however the effects were not observed in the study besides an upregulation of HSP90. The inclusion level of 2.5% algae did not mitigate the negative effects caused by heat stress and only slightly altered breast color through increased redness and yellowness. Different levels of algae inclusion need to be evaluated in the future to determine if algae could have a positive impact on heat stress regulation and broiler performance under adverse conditions such as heat stress.

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Tables and Figures

Table 1. Diet formulation for experimental diets fed from Day 21-48.

	Diet 1 Grower CON	*Diet 2 Grower ALG*	*Diet 1 Finisher CON*	*Diet 2 Finisher ALG*
Ingredients	%	%	%	%
Corn	67.02	68.37	69.31	70.69
Soybean meal	28.09	25.13	25.56	22.57
Spirulina Algae		2.5		2.5
Fat, Poultry Oil	1.92	1.29	2.32	1.67
Limestone	0.98	1.06	0.92	1.00
Dicalcium Phosphate	0.78	0.67	0.7	0.58
Sodium Bicarbonate	0.28	0.09	0.29	0.1
L-Lysine-HCL	0.24	0.25	0.25	0.27
Salt	0.22	0.22	0.22	0.21
DL-Methionine	0.18	0.17	0.17	0.16
Vitamins	0.08	0.08	0.05	0.05
Minerals	0.08	0.08	0.08	0.08
L-Threonine	0.06	0.04	0.06	0.04
Phytase, Optiphos	0.05	0.05	0.05	0.05
Choline Chloride (60%)	0.03	0.03	0.03	0.03
Crude Protein	19.25	19.60	18.17	18.51
ME, kcal/Kg	3100	3100	3150	3150
Ca	0.67	0.67	0.62	0.62
Available P	0.27	0.27	0.25	0.80
Na	0.18	0.18	0.18	0.18
DEB, mEq/Kg	227.83	228.84	215.24	216.10
Digestible Lys	1.05	1.05	1.00	1.00
Digestible, Thr	0.70	0.70	0.67	0.67
Digestible Val	0.82	0.84	0.78	0.79
Digestible Arg	1.07	1.07	1.00	1.00

Table 2. Live performance effects of dietary Spirulina platensis supplementation at 2.5% on growth performance of broiler chickens subjected to cyclic heat stress from 22-48 days of age.

Parameters	*HS CON*	*HS ALG*	*TN CON*	*HS ALG vs. HS CON p-value*	*Treatment p-value*
Mortality	12.5 b	14.75 a	14.75 a	0.0633	0.0679
FBW (Kg)	3.852 b	3.891 b	4.25 a	0.6611	0.0021
WI (Kg)	69.62 a	69.65 a	68.66 a	0.9821	0.5290
FI (Kg)	28.57 a	29.29 a	27.88 a	0.3581	0.1617
WCR	3.78 a	3.92 a	3.77 a	0.7213	0.8666
FCR	1.673 a	1.652 a	1.57 b	0.5569	0.0235
ADG (Kg/day)	0.077 b	0.078 b	0.086 a	0.7101	0.0025

Means within a row with different scripts significantly differ ($p<0.05$). HS CON were fed control diet and underwent cyclic heat stress (n=4); HS ALG were fed algae diet and underwent cyclic heat stress (n=4); TN CON were fed control diet and remained in thermoneutral conditions (n=4). FBW, final body weight; WI, water intake; FI, feed intake; WCR, water conversion ratio; FCR, feed conversion ratio; ADG, average daily gain.

Table 3. Antioxidant and heat shock protein effect of dietary Spirulina platensis supplementation at 2.5% on relative gene expression of broiler chickens subjected to cyclic heat stress from 22-48 days of age.

Parameters	HS *CON*	HS *ALG*	HS *ALG vs.* HS *CON p-value*
GPx	1	1.30	0.4723
SOD1	1	1.44	0.2278
SOD2	1	0.91	0.5511
HSP70	1	1.25	0.4889
HSP90	1	1.54	0.0657

Means within a row with different scripts significantly differ ($p<0.05$). HS CON were fed control diet and underwent cyclic heat stress (n=7); HS ALG were fed algae diet and underwent cyclic heat stress (n=7). GPx, glutathione peroxidase; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; HSP70, heat shock protein 70; HSP90, heat shock protein 90.

Table 4. Processing performance effect of dietary Spirulina platensis supplementation at 2.5% on processing performance of broiler chickens subjected to cyclic heat stress from 22-48 days of age.

Parameters	*HS CON*	*HS ALG*	*TN CON*	*CON vs. ALG p-value*	*Treatment p-value*
Fat (Kg)	0.058 a	0.054 ab	0.052 b	0.1645	0.0667
WOG (Kg)	3.008 b	2.960 b	3.265 a	0.5072	0.0033
pH	5.630 a	5.678 a	5.649 a	0.0624	0.2442
*L**	55.396 a	55.486 a	55.61 a	0.8161	0.8537
*a**	2.824 b	3.462 a	3.102 b	<0.0001	0.0010
*b**	8.726 b	12.798 a	8.723 b	<0.0001	<0.0001
Driploss (%)	0.555 a	0.497 a	0.515 a	0.4562	0.7270
Wing Yield (%)	10.09 a	10.19 a	10.09 a	0.2906	0.3778
Breast Yield (%)	29.05 b	28.79 b	30.64 a	0.5429	0.0016
Tender Yield (%)	5.74 a	5.77 a	56.85 a	0.7032	0.4987
Leg Quarter Yield (%)	29.59 a	29.93 a	28.81 b	0.2500	0.0092

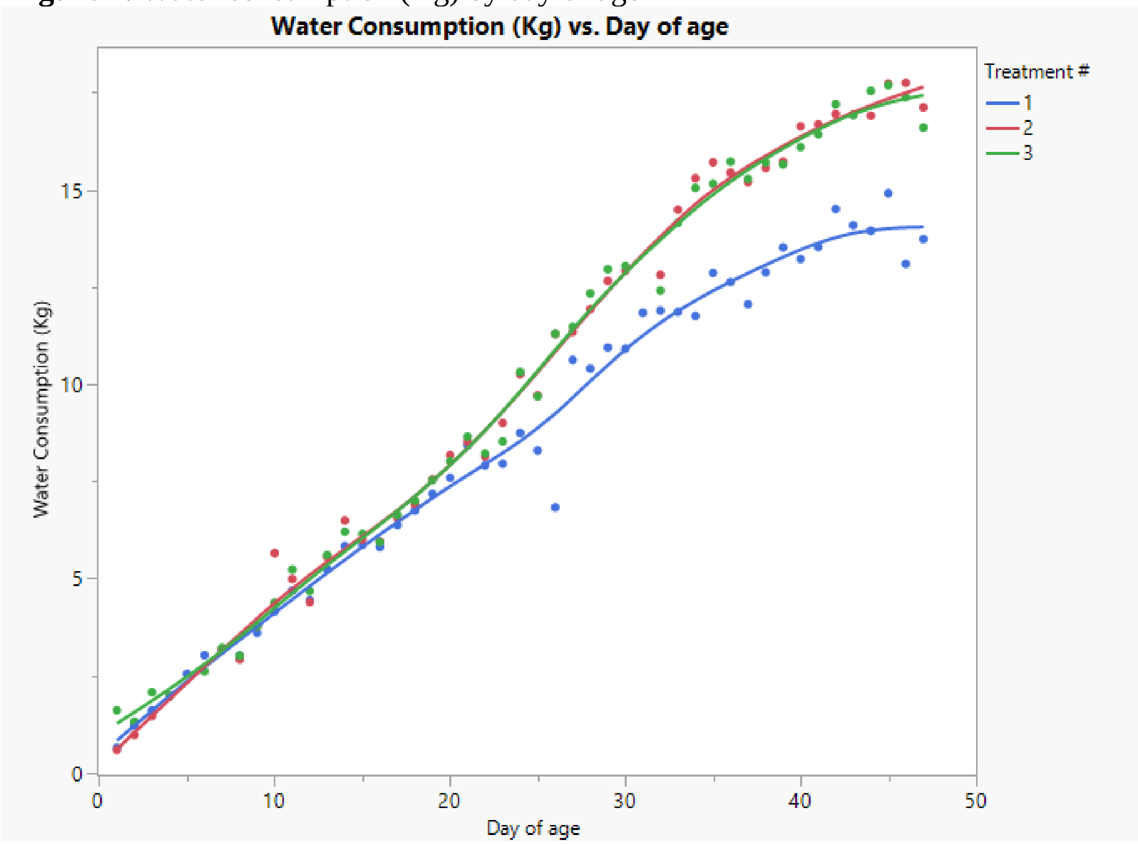
Means within a row with different scripts significantly differ (p<0.05). HS CON were fed control diet and underwent cyclic heat stress (n=4); HS ALG were fed algae diet and underwent cyclic heat stress (n=4); TN CON were fed control diet and remained in thermoneutral conditions (n=4).

Table 5. Number of Breast Fillets Exhibiting Woody breast and Non-Woody breasts

Parameters	*HS CON*	*HS ALG*	*TN CON*	*CON vs ALG value*	*Treatment p-value*
Woody	1.25 b	1.00 b	4.5 a	0.8394	0.0297
Non-Woody	25.25 a	25.5 a	20.75 b	0.8648	0.0216

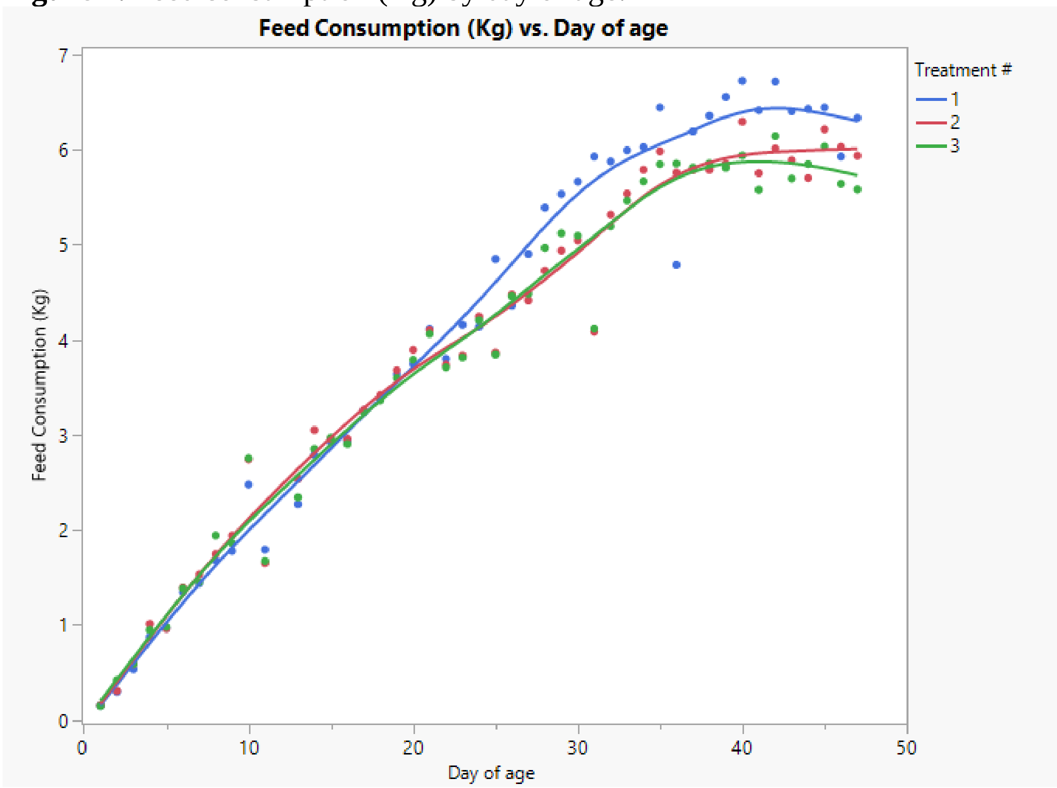
Means within a row with different scripts significantly differ ($p<0.05$). HS CON were fed control diet and underwent cyclic heat stress (n=4); HS ALG were fed algae diet and underwent cyclic heat stress (n=4); TN CON were fed control diet and remained in thermoneutral conditions (n=4).

Figure 1. Water consumption (Kg) by day of age



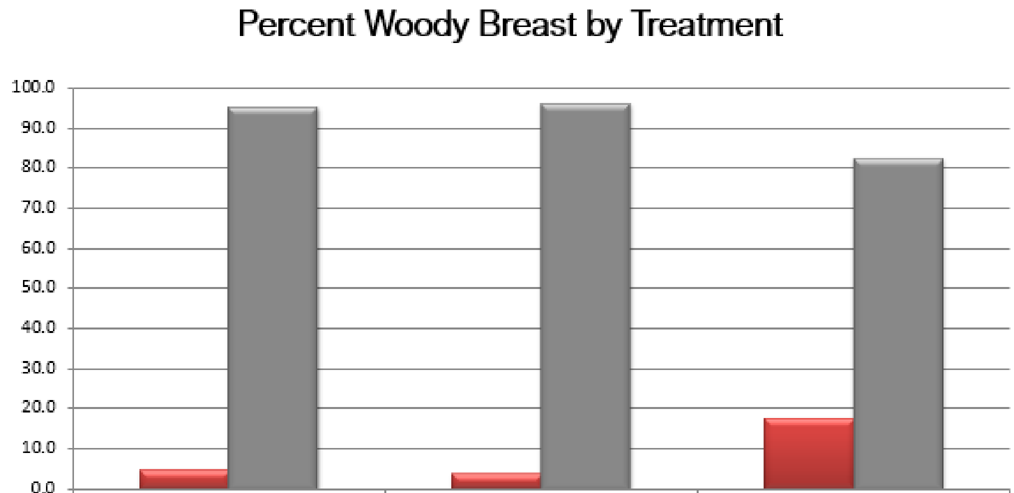
CON, birds fed control diet and subjected to cyclic heat stress; ALG, birds fed algae diet and subjected to cyclic heat stress. The p-value for the difference between the treatments is p=0.9821

Figure 2. Feed consumption (Kg) by day of age.



CON, birds fed control diet and subjected to cyclic heat stress; ALG, birds fed algae diet and subjected to cyclic heat stress. The p-value for the difference between the treatments is p=0.5569

Figure 3. Percent and Instance of Woody Breast per Treatment



	HS CON	HS ALG	TN CON
Woody	4.7	3.8	17.5
Non Woody	95	96	83
Score 0	88	80	47
Score 1	13	22	36
Score 2	4	3	6
Score 3	1	1	12

HS CON were fed control diet and underwent cyclic heat stress (n=4); HS ALG were fed algae diet and underwent cyclic heat stress (n=4); TN CON were fed control diet and remained in thermoneutral conditions (n=4).

Chapter 4 Conclusion

Conclusion

Algae has been used in other research studies as an alternative protein source in broiler feed or a way to mitigate the negative effects of heat stress. This study did not exhibit any benefits to adding *Spirulina platensis* into the feed of birds subjected to heat stress, though there were no negative effects with its addition. Furthermore, algae inclusion of 2.5% in fed did not mitigate the negative effects of heat stress. It also did not alleviate the instance of woody breast, another issue that is being seen in the poultry industry. At the temperature set point of this research, bird performance was not affected by diet. Therefore, more research should be done to see if inclusion level when subjected to heat stress is different that the optimal level in normal growing conditions.

Appendix

DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

To: Sara Orlowski
Fr: Billy Hargis - Ag-IACUC Chair
Date: June 21st, 2021
Subject: IACUC Approval
Expiration Date: June 18th, 2022

The Division of Agriculture Institutional Animal Care and Use Committee (Ag-IACUC) has APPROVED your protocol # 21123 *Evaluation of commercial broilers fed diets supplemented with algae under either Thermal Neutral or acute and chronic heat stress conditions.*

In granting its approval, the Ag-IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the Ag-IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond June 18th, 2022 you can submit a modification to extend project up to 3 years from the orginal date approved, or submit a new protocol. By policy, the Ag-IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Sara Orlowski, Sami Dridi, Walter Bottje, Michael Kidd, Joseph Hiltz, Kentu Lassiter, Liz Greene, Marisela Maqueda, Travis Tabler, Kristina Shafer, Craig Maynard, and Garrett Mullenix. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The Ag-IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

BMH/tmp