# EVALUATION OF CHROMIUM PROPIONATE AND A BUTYRIC ACID-ZINC COMPLEX ON BROILER GROWTH PERFORMANCE, CORTICOSTERONE LEVEL AND YIELD

#### A Thesis

by

#### THOMAS EUGENE LESTER III

Submitted to the Office of Graduate and Professional Studies of Texas A&M University in partial fulfillment of the requirements for the degree of

#### MASTER OF SCIENCE

Chair of Committee, Christine Alvarado

Co-Chair of Committee, Jason Lee

Committee Member, Gregory Archer

Head of Department, David Caldwell

December 2018

Major Subject: Poultry Science

Copyright 2018 Thomas Eugene Lester III

#### **ABSTRACT**

Two experiments were conducted to evaluate the effects of chromium propionate (CP) and butyric acid-zinc complex (BAZ) on broiler performance and processing yields. Experiment 1 evaluated CP inclusion on broiler growth performance when reared on used litter. A total of 36 pens with 720 chicks were randomly assigned to 3 treatments (12 reps/treatment; 20 birds/ treatment): control (no CP), 100 ppb, and 200 ppb CP. Experiment 2 evaluated CP combined with BAZ on performance, carcass yield, and meat quality when fed to broilers reared in simulated heat stress. A total of 1680 broilers (15 replicates per treatment and 28 bird/replicate) were raised in a completely randomized 2x2 block design (CRBD) fed CP at (0 or 200 ppb) and BAZ at 0 or 132 g butyric acid and 55 g of zinc. . Chicks were placed into 60 pens based on body weight. Broilers were kept in a tunnel-ventilated house during July and August to simulate summer heat stress. For both experiments, feed intake and body weight were collected biweekly, and at the termination of the trial a subsample of birds were processed to determine processing weights, yield percentage, and woody breast/white striping presence and severity scoring. On d 42 in experiment 2, 30 birds per treatment/2 birds per pen were sampled to determine plasma corticosterone levels. Results from experiment 1 indicate the addition of 200 ppb CP significantly improved feed conversion ratio (FCR) through d 42 (1.61 vs 1.58) and carcass yield by 1.48% (P=0.006) compared with the control diet. In addition, the inclusion of 200 ppb of CP significantly increased breast weigh by 41g (P=0.029). Therefore, the dietary inclusion of CP can be advantageous on broiler growth performance and processing yields. Results from experiment 2 indicated improvements (P<0.05) with the addition of CP at 200ppb in feed conversion ratio, increased breast meat yield, and decreased total mortality. The diet containing the combination of BAZ and CP reduced (p<0.05) average woody breast score. In conclusion, these data confirm

the ability	of CP to	o improve	performance	and yiel	d in bro	ilers expo	sed to env	vironmental	and l	heat
stress.										

# **DEDICATION**

This thesis is dedicated to my family and everyone that has helped me become who I am today. Without each of you motivating and supporting me, I would not have been able to achieved this accomplishment.

#### **ACKNOWLEDGEMENTS**

I would like to thank my chair and co-chair of my committee Dr. Christine Alvarado and Dr. Jason Lee for this awesome opportunity. Coming into the program without an academic background in poultry or science, my advisors created a great research program for me where the knowledge, experiences, and friendships I gained are now irreplaceable. I am extremely lucky to have had them create such a great research program for me in the nutrition and processing side of poultry science that I can apply to the industry. To my other committee member, Dr. Archer, thank you for your guidance and support throughout the course of my research program. I was challenged in the course work, but because of the research you each provided me, I was able to apply it to situations and learn a great amount. Thank you each so much for everything you did for me throughout this program.

I would also like to thank my lab mates and the undergraduates that were a part of my career as a Master's student, as well as the entire poultry science department. The past two years you all were my family and helped me adjust to the graduate studies lifestyle. Without the help from all of you, I would not have been able to of done what I did. You have been great coworkers and I am lucky to have some of you as my best friends now. I am looking forward to working together and seeing where our careers take us in the industry.

# CONTRIBUTORS AND FUNDING SOURCES

This work was supported by a thesis committee consisting of Professor Christine

Alvarado (advisor) and Professor Jason Lee (co-advisor), and Professor Gregory Archer of the

Department of Poultry Science.

The data analyzed in chapter II was provided by Professor Jason Lee. Graduate study was supported by a fellowship from Texas A&M University and funding for this research was made possible by Kemin Industries.

# **NOMENCLATURE**

BW Body Weight

d Day

DDGS Dried Distillers Grain

FCR Feed Conversion Ratio

FC Feed Consumption (gram/bird/day)

FSIS Food Safety Inspection Services

ft Foot

g Gram

hr Hours

IACUC Institutional Animal Care and Use Committee

kcal Kilocalorie

kg Kilogram

m Meters

MBM Meat and bone meal

mg Milligram

PC Positive control

ppm Parts per million

SBM Soybean Meal

WB Woody Breast

WS White Striping

# TABLE OF CONTENTS

Pag	,e
ABSTRACTii	
DEDICATIONiv	
ACKNOWLEDGEMENTSv	
CONTRIBUTORS AND FUNDING SOURCESvi	
NOMENCLATURE vii	
TABLE OF CONTENTSviii	
LIST OF TABLESix	
CHAPTER I INTRODUCTION AND LITERATURE REVIEW1	
Introduction	
ALONE AND IN COMBINATION WITH A BUTYRIC ACID ZINC COMPLEX FOR BROILER GROWTH PERFORMANCE, CORTICOSTERONE LEVEL, AND YIELD	
UNDER SIMULATED STRESS CONDITIONS12	
Introduction12Materials and Methods Experiment 115Experiment 1 Results20Materials and Methods Experiment 227Experiment 2 Results35Discussion48	
CHAPTER III CONCLUSION53	
REFERENCES 55	

# LIST OF TABLES

	Page
Table 1. Basal Profile and Nutrients of Diets for Experiment 1, Evaluating the Inclusion of Chromium Propionate at 100ppb, 200ppb, and 300ppb as well as a Control	16
Table 2. Evaluation of Body Weight of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	21
Table 3. FCR of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	22
Table 4. Feed Consumption (G/Bird/Day) of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	22
Table 5. Mortality Percentage (%) of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	22
Table 6. Processing Parameters (grams) of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	23
Table 7. Processing Parameters of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	24
Table 8. Processing Parameters of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	25
Table 9. Processing Yields & Average Woody Breast, White Striping Scores of Cocci.  Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter	26
Table 10. Dietary Formulations (lb/ton) and Calculated Nutrient Content of the Basal Diet for Experiment 2. The addition of ButiPearl Z and KemTRACE Chromium, or corn starch were added to achieve final experimental treatments.	
Table 11. Average Body Weight (kg) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions	37

Table 12. Mortality Corrected Feed Conversion Ratio of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in
Combination Reared to 52 Days in Heat Stressed Conditions
Table 13. Mortality Corrected Feed Conversion Ratio (FCR) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions
Table 14. Mortality Percentage (%) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions
Table 15. Mortality Percentage (%) Phases of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.
Table 16. Feed Consumption (Grams/Bird/Day) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.
Table 17. Feed Consumption (grams/bird/day) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.
Table 18. Processing Traits of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions
Table 19. Processing Traits of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions
Table 20. Woody Breast Profile of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions
Table 21. Day 42 Corticosterone Levels (pictogram/mL) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared in Heat Stressed Conditions

#### CHAPTER I

#### INTRODUCTION AND LITERATURE REVIEW

#### **INTRODUCTION**

Poultry consumption has almost tripled in the past 50 years as the amount of time and the costs associated in producing broilers has become more efficient. Poultry integrators focus on ways to efficiently produce mass quantities of poultry without impacting quality. Compared to broilers 50 years ago, today's broilers have a 300% improvement in growth rate, feed conversion has been reduced, and body weight has increased. Numerous research trials are conducted every year to investigate additional ways to continue to improve broiler performance, health, and their economic efficiency. Advances in broiler genetics, nutrition, and management have resulted in the overall improved performance of broilers (Owens, et al., 2008). These improvements have been due to broiler genetic selection, combined with beneficial feed additives (Zuidhof, et al., 2014).

A broiler today has many of these nutritional, genetic, and managerial applications in place for it to perform greatly. However, chickens are prone to external factors that can alter their welfare, productivity, and health (Nain, et al., 2008). Broilers are challenged with diseases, infections, nutrition variation, poor management, and environmental change. In the industry, broilers are exposed to many stressors. These stressors can be classified as short-term stressors, such as acute heat stress, or long-term stressors, both of which can have detrimental effects on production efficiency. A definition of stress is any situation in which the biological stress mechanisms of an animal is elicited. A more in depth definition of stress is any biological

response elicited when an animal perceives a threat to its homeostasis (Virden and Kidd, 2009). Stress can impair poultry performance, ultimately leading to the loss of profitability for the integrator.

#### **STRESSORS**

There are numerous definitions of stress and how animals perceive stress. Generally, stress denotes a real or perceived activation to an organism's physiological homeostasis or psychological well-being. Events that cause stress are called stressors. These stressors elicit different coping mechanisms or adaptive changes, including behavioral reactions, and the activation of the nervous system or the secretion of certain hormones (Council, 2010). Stress responses in broilers can be categorized as a short term (specific) stress or a long term (non specific) stress. Short term stressors activate the neurogenic system leading to an increase in blood pressure, muscle tone, nerve sensitivity, blood sugar, and respiration. This response is due to the release of epinephrine and norepinephrine (Virden and Kidd, 2009). Failure to combat or flee from the stressor, from the initials stress response, is distress (Council, 2010). Distress can lead to acute and chronic stress (Moberg, 2000). When a broiler is introduced to distress, this results in the activation of the hypothalamic-pituitary-adrenal cortical system(Virden and Kidd, 2009). The transition of stress to distress depends on several factors, stressor intensity and duration, either of which is likely to produce behavior or physical signs of distress (Council, 2010). It is important to investigate new strategies to alleviate the negative effects associated with stress and distress.

A common stress for broilers is the stress associated with raising broilers on used litter that is not managed properly. It is very important to have an adequate environment to allow

broilers to perform successfully. Gases such as methane, ammonia, and carbon dioxide can reach toxic levels if adequate ventilation and litter management are not maintained (Quarles and Kling, 1974). It is important to control these gases as it is common to reuse litter in order to reduce costs and decrease environmental waste (Huff, et al., 1984). Ammonia is the result of microbial degradation of poultry waste and can reach levels that limit poultry performance; high levels can decrease growth rate and increase feed conversion (Huff, et al., 1984). As the microbial degradation within the litter increases, broilers footpads are also negatively affected. Therefore, raising broilers on used litter is common, but can affect the life and meat quality of the broiler as damage to the respiratory tract can potentially occur from the high ammonia levels if the litter is not managed properly (Quarles and Kling, 1974).

Another stressor is coccidiosis, a common disease among broilers, which causes mortality, morbidity, diarrhea or bloody feces, as well as adverse effects on economic performance. Today, this disease is highly controlled by the use of anticoccidial drugs administered as live, multivalent vaccines (Williams, 2002). These vaccines trigger an immune reaction and can negatively affect intestinal health causing a rise in broiler stress levels (Yegani and Korver, 2008).

A common distress, especially in the southeastern part of the United States, are periods of temporary heat stress during the summer months. If broilers are exposed to heat stress, it can negatively affect cellular energy, reduce performance, and negatively influence intestinal permeability (Yegani and Korver, 2008). Chronic heat exposure and heat stress are causing increasing concern to poultry producers in hot climates as well as temperate countries due to reduced growth performance and increased mortality. During heat stress, birds are unable to

maintain a near-constant body temperature. To achieve a constant body temperature, heat produced by metabolism must equal heat lost. Heat losses are limited by feathering and by the absence of sweat glands in birds (Geraert, et al., 1996). The main consequences of heat exposure is a reduction in feed intake, an effect of trying to reduce metabolic heat production. This decrease in feed intake leads to a poor growth performance and poor feed efficiency (Howlider and Rose, 1987). This reduction in performance can be attributed to the reduction in the integrity of the intestinal system of broilers (Quinteiro-Filho, et al., 2010). As the permeability of the ileum increases, "leaky gut" syndrome develops, which hinders the broiler from absorbing certain nutrients and can cause poor performance (Uerlings, et al., 2018). Numerous studies agree that as broilers stress levels increase, their performance decreases (Geraert, et al., 1996; Lara and Rostagno, 2013; Quinteiro-Filho, et al., 2010)

Heat stress is caused by an imbalance of environmental factors such as, sunlight, thermal radiation, air temperature, and humidity (Lara and Rostagno, 2013). Broilers seem to be more challenged by temperature in times of heat, when compared to livestock. When a broiler is introduced to any stress, the hypothalamic-pituitary adrenal cortical system is activated. The flow of the cortical system begins once the bird is introduced to stress. As the hypothalamic-pituitary adrenal cortical system is activated, the proliferation of the adrenal cortical tissue takes place. The adrenal cortical tissue then leads to the production and secretion of the stress hormone, corticosterone. Corticosterone will produce negative impacts on several biological factors of the broiler, such as feed intake, metabolism, and growth (Siegel, 1980; Virden and Kidd, 2009). It is important to investigate new nutritional strategies to help reduce the impact of stress on the animal, which could potentially mitigate production losses.

#### **CHROMIUM**

Dietary inclusions with various nutrients and feed additives is a common method used to alleviate the effect of certain stressors. Proteins, amino acids, vitamins, and minerals are among some of the most common nutritional supplements shown to alleviate stress from broilers. Chromium is an essential element required for carbohydrate, fat, and protein metabolism (Anderson, 1997). The NRC has a recommended chromium inclusion level of 300 ug/kg in diets of laboratory animals; however, currently there are no NRC recommendations for chromium in poultry. Chromium has been considered by many nutritionists as an essential nutrient for animals and humans (Jackson, et al., 2008). Several studies show evidence for improved broiler performance with the supplementation of chromium (Sands and Smith, 1999; Toghyani, et al., 2012).

Studies have shown that dietary chromium supplementation improves physiological functions such as cell preservation, antioxidant activity and immune response that are especially important for animal homeostasis and thermoregulatory capacity under heat stressed conditions (Dalolio, et al., 2017). When broilers are exposed to heat stress, they maintain their body temperature through thermoregulatory responses that negatively impact growth performance and metabolism, which results in poor performance and losses for the integrator (Renaudeau, et al., 2012).

Over the years as broilers have been genetically selected to improve growth and muscle deposition the broiler has become more susceptible to higher temperatures as their metabolic heat production increases (Brossi, et al., 2009). Numerous solutions to alleviate heat stress have been investigated over the last couple of years, as heat stress is becoming a larger issue. The use

of air conditioning, ventilation and evaporative cooling systems, management of drinking water, vitamin and mineral supplementation and variation of the dietary electrolyte balance have been evaluated in order to reduce broilers heat stress levels. As birds experience heat stress, a reduction in the circulating levels of vitamins and minerals is observed, which may be associated with low feed intake and high water consumption (Altan, et al., 2003). Chromium supplementation as a nutritional strategy has been observed to improve growth performance (Ghazi, et al., 2012), nutrient metabolism (Sahin, et al., 2003), immune responses (Oba, et al., 2012), antioxidant function and stress response (Ghazi, et al., 2012) in broiler chickens exposed to heat stress.

Chromium supplementation has been shown to alleviate the negative effects of stress by improving the health and performance of livestock. When an animal is stressed, the neurogenic system is activated, resulting in the release of cortisol. Cortisol release causes changes in behavioral, metabolic, immunological, and intestinal changes (Virden and Kidd, 2009). At temperatures above or below the thermoneutral zone, corticosteroid secretion increases as a response to stress (Sahin, et al., 2003). The inclusion of chromium in broiler feed has been shown to reduce the levels of corticosteroids, lessening the negative impact of stress. The primary role of chromium in metabolism is to potentiate the action of insulin through its presence in organometallic molecules called glucose tolerance factors. Research has shown that insulin metabolism influences lipid peroxidation. Chromium (insulin cofactor) is postulated to function as an antioxidant (Sahin, et al., 2003). Chromium is thought to be essential for activating certain types of enzymes and for stabilizing proteins and nucleic acids. Besides acting as an antioxidant, chromium plays a crucial role in metabolism by potentiating the action of

insulin through its presence in an organometallic molecule called glucose tolerance factor. The oligopeptide low-molecular-weight chromium-binding protein (chromodulin) tightly binds four chromic ions before the oligopeptide obtains a conformation required for binding to the tyrosine kinase active site of the insulin receptor. The oligopeptide chomodulin binds chromic ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide can bind to an insulin-stimulated insulin receptor, activating the receptor's tyrosine kinase activity. Thus, chromodulin appears to play a role in an auto amplification mechanism in insulin signaling. In addition, release of chromium from chromium propionate for use in cells requires reduction of the chromic center, a process that can lead potentially to the production of harmful hydroxyl radicals (Sahin, et al., 2003).

Overall, chromium can influence carbohydrate and protein metabolism, insulin sensitivity in peripheral tissues, and growth rate. Chromium is essential for normal glucose metabolism, working with insulin to move glucose into cells for glucose utilization (Sahin, et al., 2003). Chromium has been used in pork and beef diets for several years. For the poultry industry however, chromium has only recently been approved to be added into poultry diets in the form of chromium propionate.

Chromium propionate is the first trace mineral approved for broiler diets in more than 40 years. Chromium propionate is a safe and highly bioavailable source of organic chromium for broilers (Rajalekshmi, et al., 2014). Chromium propionate is shown to reduce levels of corticosterone, which allows for more nutrients to be used for growth instead of being used to fight the negative side effects associated with corticosterone production (Samanta, et al., 2008). Chromium propionate has also shown to improve overall performance and carcass quality

(Toghyani, et al., 2012). Research suggests that the chromium propionate inclusion improves feed conversion ratio, growth rate, as well as carcass and breast yields (Rajalekshmi, et al., 2014). Seeing the positive roles chromium propionate can play in broiler production leads to the idea that supplementing diets with another additive in conjunction with chromium propionate could provide synergistic effects with other compounds that also show positive effects on stress and broiler performance.

#### **BUTYRIC ACID**

Short chained fatty acids are considered a potential alternative to antibiotic growth promoters (Van Immerseel, et al., 2005). Butyric acid is a short chained fatty acid that has a higher bactericidal activity when the acid is undissociated (Lesson, 2007). Studies show that the inclusion of butyric acid into broiler diets changes the intracellular pH of the GI tract, which leads to the death of bacterial cells. Butyric acid also plays role in developing the intestinal epithelium. Butyric acid that is derived from the fermentation of non-starch polysaccharides is considered to be important for the normal development of epithelial cells, leading to an improvement of gastrointestinal health (Brouns, et al., 2002). Studies show that butyric acid supplemented broilers show higher body weights when compared to birds without butyric acid in the diet (Leeson, et al., 2005). Additionally, the inclusion of butyric acid showed a reduction of pH in the upper gastrointestinal tract (crop, proventiculus and gizzard), allowing for the bird to perform better, and absorb nutrients more efficiently (Panda, et al., 2009).

Butyric acid is a short-chained fatty acid that is produced during microbial fermentation, primarily in the large intestine. It allows for an increase in antioxidant levels to protect against pathogens and promote healing in the gastro intestinal tract by upregulating tight junction

proteins in the intestine, which ultimately improves intestinal performance (Kaczmarek, et al., 2016).

#### **ZINC**

Zinc is required for normal growth and development and is involved in a variety of metabolic processes, including synthesis of nucleic acids and proteins. In chickens, zinc is necessary for immunity, skin quality, bone development, feathering, and optimum growth (Hess, et al., 2001). When not enough zinc is introduced into broiler diets, a decrease in growth and feathering is observed, which increases mortality rate, and causes leg abnormalities (Young, et al., 1958). Zinc is an integral player in a variety of physiological systems. Studies show that supplementing broiler diets with zinc may increase live performance, and reduce product quality losses associated with different environmental conditions, such as high temperatures, humidity, or placement density (Hess, et al., 2001).

#### **BUTYRIC ACID-ZINC COMPLEX**

Today's consumers are typically more conscience of what goes into their products and where their products come from. Historically, poultry integrators have included antibiotics in feed to prevent disease, improve feed performance and reduce cost; however, recent customer trends and demands have resulted in an increase in poultry meat raised without antibiotics.

Research suggests that organic acid could potentially replace antibiotics in broiler diets, allowing optimal bird performance. Panda's study shows that the inclusion of butyric acid into broiler diets could offer an alternative to certain antibiotics, allowing for body weight gain, and feed conversion improvements (Panda, et al., 2009). Zinc combined with butyric acid has been shown to reduce illeal permeability during heat stress (Zdunczyk, et al., 2010). Combined with the

benefits from organic zinc, a butyric acid zinc complex feed additive could be an organic feed additive alternative, allowing for performance improvements and cost savings for the integrator.

A common breast meat quality issue in the industry is woody breast. Woody breast is characterized by its notable pale complexion and rigid breast fillets which are typically firmer upon palpation compared to normal fillets (Sihvo, et al., 2014). Woody breast can vary in degrees of severity and is scored on a scale of 0-3. With 0 representing a non-affected or normal breast fillet and a 3 representing the most severe case (Tijare, et al., 2016). There is research that states that the addition of a butyric acid zinc complex may reduce woody breast prevalence and illeal viscosity, as well as permeability (Kaczmarek, et al., 2016).

#### CHROMIUM PROPIONATE COMBINED WITH A BUTYRIC ACID-ZINC COMPLEX

Chromium can compete with other minerals such as zinc and iron for the same absorption sites, which influences its absorption within the intestinal lining (Chen, et al., 1973). Research has shown that butyric acid is quickly absorbed within the upper digestive tract, such as the crop, which makes it not an ideal feed additive (Snel, et al., 2002). However, butyric acid efficacy has been shown to increase when it is fed in a protected form such as encapsulation (Smith, et al., 2012). Encapsulating the butyric acid-zinc complex allows for the feed additive to bypass degradation in the crop and proventriculus, so the epithelial cells in the small intestine can utilize the butyric acid, allowing for the chromium and zinc to no longer be in competition for absorption (Pryde, et al., 2002).

#### **CONCLUSION**

It is well documented that chromium fed in broiler diets allows for multiple benefits in growth performance and carcass traits (Toghyani, et al., 2012). Chromium inclusion into broiler diets has also shown to alleviate the negative signs associated with heat stress (Sahin, et al., 2017). Diets containing a butyric acid-zinc complex combined with chromium could allow for major beneficial results for the integrator, as it has the potential to mitigate several issues. Butyric acid is necessary for epithelial cell development, so bypassing degradation early on in the crop or proventriculus by encapsulating the butyric acid-zinc complex, allows for the epithelial cells in the small intestine to utilize the butyric acid more efficiently (Pryde, et al., 2002). The chromium addition into the diet would allow for lower corticosterone levels, which would allow the intestinal tract to be less stressed, causing a synergistic effect, allowing the encapsulated butyric acid-zinc complex to maximize its efficiency while being absorbed in the small intestine. When a broiler is under stress, and would typically perform poorly, the inclusion of these additives show evidence for increases in the broilers intestinal integrity, which would allow for higher growth performance, and carcass quality. Therefore, the objective of the current research program is to evaluate the use of a chromium propionate in broiler diets in the absence and presence of butyric acid zinc complex in broilers reared in conditions mimicking multiple environmental stressors.

#### CHAPTER II

EVALUATION OF THE IMPACT OF CHROMIUM PROPIONATE ALONE AND IN

COMBINATION WITH A BUTYRIC ACID ZINC COMPLEX FOR BROILER

GROWTH PERFORMANCE, CORTICOSTERONE LEVEL, AND YIELD UNDER

SIMULATED STRESS CONDITIONS

#### **INTRODUCTION**

Chromium is an essential element required for carbohydrate, fat, and protein metabolism (Anderson, 1997). Chromium has been considered by many nutritionists as an essential nutrient for animals and humans (Jackson, et al., 2008). Several studies have shown evidence of improved performance with the inclusion of chromium (Sands and Smith, 1999). Chromium supplementation has been shown to also alleviate the negative effects of stress by improving the health and performance of livestock, leading to the increased profitability for the producer. When an animal is introduced to stress, its neurogenic system is activated, resulting in the release of corticosterone. Corticosterone release causes behavioral, metabolical, immunological, and intestinal changes (Virden and Kidd, 2009). The inclusion of chromium in broiler feed has been shown to reduce the levels of corticosteroids, lessening the negative impact of stress.

Today, broiler houses are built to be as environmentally controlled and efficient as possible, focusing on broilers safety and health. Two common challenges that exist in operating a broiler farm are the seasonal changes in weather and temperature. As birds experience an

increase in temperatures, especially during the summer months or come in contact with pathogenic organisms, corticosterone levels increase. This response to stress includes the production of corticosterone and the potential behavioral changes such as panting to try to return to homeostasis. Panting requires energy; as birds are under high ambient temperatures, this energy being produced is being "wasted" as it is being diverted from growth, as well as decreasing broiler feed intake in order to reduce metabolic heat production. Lower growth rate as well as a reduction in feed efficiency are commonly observed in birds experiencing heat stress (Toghyani, et al., 2012). Chromium addition into animal diets help to alleviate the negative impacts corticosterone produces (Dalolio, et al., 2017).

Vitamins, minerals, amino acids, proteins, and many other agents are also used to alleviate broiler stressors, and allow for maximum broiler efficiency. Butyric acid is a short chained fatty acid that is produced during microbial fermentation, primarily in the large intestine. It allows for an increase in antioxidant levels to protect against pathogens and promote healing in the gastro intestinal tract by upregulating tight junction proteins in the intestine, which ultimately improves intestinal performance (Kaczmarek, et al., 2016). Zinc also plays an important role in the biological process of health and performance in animals. Zinc allows an increase in microbial diversity, which upregulates tight junction proteins in the intestine (Hess, et al., 2001). This study sought to determine if a synergistic effect would be observed with co-administration of a butyric acid-zinc complex with chromium propionate.

Chromium has been supplemented into beef and pork diets for many years. For the poultry industry however, chromium has only recently been approved to be added into poultry diets in the form of chromium propionate. Chromium propionate is a safe and highly

bioavailable source of organic chromium for broilers (Rajalekshmi, et al., 2014). Chromium propionate has shown to reduce levels of corticosterone, which reduces the amount of nutrients that are diverted to managing stress in the animal and allow for improved growth performance (Samanta, et al., 2008). Chromium propionate supplemented diets can positively affect broiler body weight, carcass yield and feed conversion ratio compared to birds that were not fed chromium (Haq, et al., 2016). U.S. literature is limited in recent research data regarding the impact of chromium inclusion on the modern commercial broiler. Therefore, the objective of the current experiments were to evaluate the use of chromium propionate in broilers reared in environments which simulated industry stressors. Experiment 1 evaluated multiple levels of chromium in broilers reared on used litter while experiment 2 investigated the impact of chromium when fed to broilers reared during the summer months which experienced heat stress and fed in combinations with a butyric acid zinc complex.

#### MATERIALS AND METHODS EXPERIMENT 1

# Experimental Design and Diets

This study evaluated three diets, consisting of 36 pens containing 20 Ross 708 broilers (10 male, 10 female), for a total of 720 chicks. A standard U.S. corn-soybean diet was formulated and manufactured to act as a basal diet. Three dietary treatments were manufactured from the one basal; a control diet, a diet with 100ppb of chromium propionate<sup>1</sup>, and a diet with 200 ppb of chromium propionate. Diets were pelleted at 85°C and were fed in four dietary phases. The starter phase (d 1-14) which was fed as a crumble, the grower phase from (d 14-28) fed as a pellet, the finisher phase from (d28-42), and the withdrawal phase from (d 42-49). The three treatments were randomized and replicated within 12 blocks.

-

<sup>&</sup>lt;sup>1</sup> KemTRACE® Chromium, Kemin Industries, Des Moines, IA

TABLE 1. BASAL PROFILE AND NUTRIENTS OF DIETS FOR EXPERIMENT 1, EVALUATING THE INCLUSION OF CHROMIUM PROPIONATE AT 100PPB, 200PPB, AND 300PPB AS WELL AS A CONTROL

Ingredient	Starter	Grower	Finisher	Withdrawal
Corn	61.00	66.201	67.85	69.10
Soybean	25.65	20.45	18.90	17.70
DL-MET	0.30	0.28	0.24	0.21
Lysine HCL	0.31	0.31	0.25	0.23
L-Threonine	0.10	0.10	0.08	0.07
Soy Oil	1.12	1.36	2.28	2.49
Limestone	0.60	0.52	0.57	0.61
BIOFOS 16/21P	0.12	0.02	0.00	0.00
Sodium Chloride	0.33	0.15	0.12	0.14
Sodium Bicarbonate	0.09	0.26	0.32	0.36
Trace Minerals	0.05	0.05	0.05	0.05
Vitamins	0.25	0.25	0.25	0.25
BMD 50	0.05	0.05	0.05	0.00
LO-DDGS	5.00	5.00	5.00	5.00
pork MBM	5.00	5.00	4.02	3.77
OptiPhos Phytase	0.01	0.01	0.01	0.01
	Calculat	ed Nutrients		
ME kcal/kg	3036	3101	3168	3190
Protein	22.21	20.10	18.90	18.25
Crude Fat	4.49	4.87	5.71	5.93
Calcium	0.90	0.84	0.76	0.75
Phosphorus	0.57	0.53	0.49	0.48
AV Phosphorus	0.45	0.42	0.38	0.37
Methionine	0.64	0.59	0.54	0.50
Lysine HCL	1.32	1.17	1.07	1.01
Threonine	0.88	0.80	0.75	0.71
Sodium	0.20	0.18	0.18	0.19
-	Analyze	ed Nutrients		
Moisture	14.08	13.89	13.64	13.6

TABLE 1 CONTINUED.

	Starter	Grower	Finisher	Withdrawal					
Analyzed Nutrients									
Crude Protein	20.7	19.9	18.8	17.2					
Crude Fat	4.51	4.43	4.38	5.42					
Acid Detergent Fiber	3.1	4.4	3.2	2.4					
Ash	4.95	4.36	4.35	4.57					
Calcium	1.19	0.85	0.9	0.93					
Phosphorus	0.72	0.66	0.66	0.57					

<sup>&</sup>lt;sup>1</sup> Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin D<sup>3</sup>, 91 IU vitamin E, 0.04 mg B12, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls.

#### **Animals and Management Practices**

The study consisted of a total of 36 pens with 12 blocks of 3 contiguous pens, each containing 20 chicks at d 1 of age (Ross 708 x Ross). Chicks were feather sexed upon arrival at the research center. All broilers received a coccidiosis vaccination (Advent)<sup>2</sup> via spray cabinet upon arrival at the research center and allowed to preen for 1 hr. prior to randomization. Chicks were allocated to the pens and treatments based on initial body weight to ensure statistically equivalent weights across all treatments. Pens contained 5 inches of built up litter (4 previous flocks reared on it). Bird weights (kg) by pen were recorded at study initiation, d 14, d 28, d 42 and on termination on day 49 which corresponded with dietary changes. Feed and water was

\_

<sup>&</sup>lt;sup>2</sup> Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, 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.

<sup>&</sup>lt;sup>2</sup> ADVENT®, Huvepharma®, Inc., Peachtree, GA

available *ad libitum* Animal care was provided in accordance with a protocol approved by Texas A&M IACUC (REF #045470).

#### Parameters Evaluated

All broilers and feed was weighed by pen biweekly on d 14, d 28, d 42, and d 49 to determine average body weight (BW), mortality adjusted feed conversion ratio (FCR), feed consumption (FC), mortality percentage, and cumulative FCR. Following an 8 hr feed withdrawal period on day 49, eight broilers from each pen were randomly selected for processing from each replicate pen. Fasted live weight, chilled carcass weight, and the weight of cut up parts (skinless boneless breast (pectoralis major), and tenders (pectoralis minor) were measured. The weighs were averaged to calculate the total carcass yield, the white meat yield, and the average body weight, as well as woody breast and white striping scores using the pen as the experimental unit.

# **Processing Parameters**

On day 50, eight broilers (4 male and 4 female) from each pen were selected for processing from each replicate pen (288 birds in total based on 3 treatments; 12 replicates/ treatment; 96 birds/treatment). Upon completion of the trial on d 49, broilers within each pen were weighed. Birds were placed on an 8 hr feed withdrawal period prior to processing on day 49. All birds were conventionally processed in a pilot scale processing facility at Texas A&M University. Birds were stunned (Model SF-7000, Simmons Engineering Corp., Dallas, GA) in a 1% saline bath, 13 mA, 7 s, 500 Hz, DC and bled using a unilateral neck cut. The exsanguinated birds were allowed to bleed for 90 s. All birds were conventionally scalded (61°C, 45 s), picked in a rotary drum picker (Model sp30ss, Bower Corp., Houghton, LA 52, 631) for 25 s, and

manually eviscerated. Birds were then chilled to 4°C within 80 min. The following parameters were measured on the day of processing: fasted live weight, chilled carcass weight, and the weight of cut-up parts (skinless boneless breast (Pectoralis major), and tenders (Pectoralis minor). After chilling, carcasses were deboned (breast fillets and tenders) and weighed. Weights were recorded and averaged to calculate the total carcass yield and the breast and tender yield, along with white meat yield consisting of breast and tenders. After weighing, the left and right P.Major filets were palpated and scored for white striping based on the scoring system of Kuttapan et al. and woody breast based on the scoring system of Tijare et al. (Kuttappan, et al., 2012; Tijare, et al., 2016).

### Statistical Analysis

All performance data were analyzed via a One-Way Analysis of Variance using the General Linear Model of SAS. Treatments means were significant at P≤0.05 and separated using Duncan's Multiple Range Test. All processing data was subjected to a 3 (TRT) x 2 (Sex) factorial. Main effect means were deemed significantly different at P≤0.05 and separated using Duncan's Multiple Range Test. Percentage data were transformed using arc sin transformation prior to analysis. Statistics were calculated sex separately and as an average pen (male & female). However, no significant differences were observed during in the sex separated, so all data presented in experiment 1 is with averaged pen weights and yields.

#### **EXPERIMENT 1 RESULTS**

The inclusion of chromium propionate at 100ppb and 200ppb did not show a significant difference in body weight or body weight gain of straight run broilers throughout the 48 days of the trial. (Table 2). As for feed conversion, during the starter and grower phases, the addition of 200ppb of chromium propionate significantly reduced FCR compared to the control diet; the results from the addition of 100ppb of chromium propionate was intermediate compared to the control. Cumulatively through d 28, the inclusion of 200ppb of chromium propionate significantly reduced FCR compared to the control (Table 3). Similar results were observed cumulatively from d 1-49, where the inclusion of 200ppb of chromium propionate significantly reduced FCR compared to the control diet (1.72 vs. 1.68) (Table 3). When adjusting for weight at the conclusion of the trial the inclusion of 200ppb of chromium significantly reduced FCR compared to the control (1.72 vs. 1.66) with the inclusion of 100ppb of chromium being intermediate. (Table 3). According to the Ross 708 Broiler Guide, feed conversion ratios improved in experiment 2 from d-48 compared to those of the Ross guide (1.78 vs. 1.683) (Aviagen, 2014). There was no significant difference observed in feed consumption or mortality throughout the duration of the trial (Table 4 & 5). Evaluating the subset of birds sampled for processing, the inclusion of 200ppb of chromium propionate significantly increased average WOG carcass yield compared to the control diet with the inclusion of 100ppb of chromium being intermediate (Table 6, 7, 8, & 9). As expected, male broilers had a significantly higher average WOG weight compared to female broilers within treatments. An interaction was observed between diet treatment and sex; the inclusion of 200ppb of chromium propionate increased average carcass yield in males compared to males fed 100ppb of chromium propionate and males

fed the control diet (Table 6, 7, 8, & 9). The inclusion of chromium at 100ppb and 200ppb significantly increased average breast, tender, and total white meat weight compared to the control diet. Male broilers had a significantly higher average breast, tender, and total white meat weight compared to female broilers (Table 6, 7, 8, & 9). In conclusion, the addition of chromium propionate improved feed conversion ratio, increased white meat weight, and increased carcass yield compared to non-chromium propionate supplemented diets.

Table 2. Evaluation of Body Weight of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

	D14 (g)	D28 (kg)	D42 (kg)	D48 (kg)
TRT 1 - Control	409.2	1.365	2.670	3.245
TRT 2 - 100ppb Chromium	415.9	1.377	2.678	3.267
TRT 3 - 200ppb Chromium	427.2	1.417	2.739	3.323
p-value	0.344	0.461	0.546	0.509
pooled CV	7.2	7.500	5.907	5.407
pooled SEM	4.9	0.017	0.027	0.03

No significant differences were observed in BW throughout the duration of the trial

Table 3. FCR of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium

Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

(0, _ 0 )			D1-28		D1-42		D1-48	Wt. Adj. <sup>1</sup>
	Starter	Grower	FCR	Finisher	FCR	WD	FCR	FCR
TRT 1 -								
Control	1.442 <sup>a</sup>	1.507 <sup>a</sup>	1.489 <sup>a</sup>	1.793	1.612 <sup>a</sup>	2.120	1.719 <sup>a</sup>	1.721 <sup>a</sup>
TRT 2 -								
100ppb								
Chromium	1.405 <sup>ab</sup>	1.501 <sup>ab</sup>	1.474 <sup>a</sup>	1.802	1.613 <sup>a</sup>	2.010	1.701 <sup>ab</sup>	$1.697^{ab}$
TRT 3 -								
200ppb								
Chromium	1.380 <sup>b</sup>	1.482 <sup>b</sup>	1.453 <sup>b</sup>	1.737	1.581 <sup>b</sup>	2.087	1.683 <sup>b</sup>	1.663 <sup>b</sup>
p-value	0.038	0.019	0.001	0.076	0.019	0.233	0.059	0.057
pooled CV	4.133	1.812	1.553	4.677	2.116	8.166	2.240	3.908
pooled SEM	0.010	0.005	0.004	0.014	0.006	0.029	0.006	0.011

a,b Means within column with different superscripts differ significantly at  $P \le 0.05$ .

Table 4. Feed Consumption (G/Bird/Day) of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

n Osca Ditter			D1-28		D1-42		
	Starter	Grower	FC	Finisher	FC	WD	D1-48 FC
TRT 1 - Control	37.10	102.87	69.67	165.74	101.20	195.58	113.02
TRT 2 - 100ppb							
Chromium	36.82	101.04	68.32	167.02	100.80	194.30	112.67
TRT 3 - 200ppb							
Chromium	37.32	104.73	70.51	166.70	101.81	197.58	113.70
p-value	0.881	0.620	0.672	0.945	0.938	0.831	0.968
pooled CV	5.87	8.86	8.08	5.80	6.57	6.32	6.43
pooled SEM	0.36	1.51	0.93	1.63	1.14	2.09	1.23

No significant differences were observed in FC throughout the duration of the trial.

<sup>&</sup>lt;sup>1</sup>Wt. Adjustment calculated on 36 g of BW equivalent to 1 pt of FCR.

Table 5. Mortality Percentage (%) of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

	Starter	Grower	Finisher	Withdrawal	Total
TRT 1 - Control	0.417	0.417	0.439	0.417	2.105
TRT 2 - 100ppb Chromium	1.210	0.00	0.833	0.00	2.044
TRT 3 - 200ppb Chromium	0.833	1.272	1.754	0.417	4.169
p-value	0.588	0.173	0.531	0.626	0.379
pooled SEM	0.310	0.284	0.464	0.199	0.657

No significant differences were observed in mortality throughout the duration of the trial.

Table 6. Processing Parameters (grams) of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

	Live Wt.	Carcass Wt.	Breast Wt.	Tender Wt.	White Wt.
TRT 1 - Control	3165.88	2496.50	0.72	0.141	0.860
TRT 2 - 100ppb Chromium	3215.36	2559.49	0.753	0.145	0.898
TRT 3 - 200ppb Chromium	3243.19	2596.63	0.761	0.147	0.908
p-value	0.515	0.153	0.091	0.201	0.088
pooled CV	6.06	6.080	7.396	6.004	6.991
pooled SEM	32.38	25.860	0.009	0.001	0.01

a,b Means within column with different superscripts differ significantly at P ≤0.05.

Table 7. Processing Parameters of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

	Carcass Yield (g)	Breast Yield (g)	White Yield (%)	Woody Breast	White Striping
TRT 1 - Control	78.88 <sup>b</sup>	28.79	34.46	1.06	1.22
TRT 2 - 100ppb Chromium	79.66 <sup>ab</sup>	29.35	35.03	1.40	1.36
TRT 3 - 200ppb Chromium	80.05 <sup>a</sup>	29.30	34.98	1.13	1.35
p-value	0.018	0.185	0.198	0.071	0.181
pooled CV	1.430	2.670	2.360		
pooled SEM	0.190	0.130	0.140	0.060	0.040

a,b Means within column with different superscripts differ significantly at  $P \le 0.05$ .

Table 8. Processing Parameters of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate (0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

		Carcass Wt.	Breast Wt.	Tender Wt.	White Wt.
Unit		kg	kg	kg	kg
TRT	SEX				
Control	Female	2.277	0.658	0.135	0.793
Control	Male	2.715	0.781	0.147	0.928
100ppb Chromium	Female	2.323	0.679	0.136	0.815
100ppb Chromium	Male	2.796	0.827	0.153	0.98
200ppb Chromium	Female	2.352	0.696	0.141	0.837
200ppb Chromium	Male	2.84	0.827	0.153	0.979
Main Effect Means					
TRT					
Control		2496.50 <sup>b</sup>	0.720 <sup>b</sup>	0.141	$0.860^{b}$
100 ppb Chromium Prop		2559.49 <sup>ab</sup>	0.753ª	0.145	0.898 <sup>a</sup>
200 Chromium Prop		2596.63ª	0.761 <sup>a</sup>	0.147	0.908 <sup>a</sup>
	SEX				
	Female	2317.82 <sup>b</sup>	0.678 <sup>b</sup>	0.137 <sup>b</sup>	0.815 <sup>b</sup>
	Male	2783.92ª	0.812ª	0.151 <sup>a</sup>	0.962ª
	p-value				
TRT		0.043	0.029	0.12	0.028
SEX		< 0.001	< 0.001	< 0.001	< 0.001
TRT x SEX		0.817	0.729	0.557	0.689
SEM		0.026	0.009	0.001	0.01

a,b Means within column with different superscripts differ significantly at  $P \le 0.05$ .

Table 9. Processing Yields & Average Woody Breast, White Striping Scores of Cocci. Vaccinated Straight Run Broilers Fed Increasing Levels of Chromium Propionate

(0, 100, 200ppb) Throughout 48d Trial When Reared on Used Litter

		Carcass Yield	Breast Yield	White Meat Yield	Woody Breast	White Striping			
Unit		%							
TRT	SEX								
Control	Female	79.32 <sup>abc</sup>	28.9	34.83	0.96	1.25			
Control	Male	78.44°	28.69	34.1	1.17	1.19			
100ppb Chromium	Female	80.16 <sup>ab</sup>	29.18	35.04	1.23	1.39			
100ppb Chromium	Male	79.15 <sup>bc</sup>	29.53	35.01	1.56	1.34			
200ppb Chromium	Female	79.72 <sup>ab</sup>	29.46	35.45	1.04	1.36			
200ppb Chromium	Male	80.38ª	29.13	34.52	1.21	1.33			
	p-value	0.004							
Main Effect Means									
TRT									
Control		78.88 <sup>b</sup>	28.79	34.46	1.06 <sup>b</sup>	1.22			
100 ppb Chromium Prop		79.66ª	29.35	35.03	1.40 <sup>a</sup>	1.36			
200 Chromium Prop		80.05ª	29.3	34.98	1.13 <sup>b</sup>	1.35			
		79.73	29.18	35.10 <sup>a</sup>	1.08 <sup>b</sup>	1.33			
		79.32	29.12	34.54 <sup>b</sup>	1.31 <sup>a</sup>	1.29			
TRT		0.006	0.137	0.168	0.021	0.173			
SEX		0.165	0.797	0.04	0.022	0.514			
TRT x SEX		0.043	0.496	0.366	0.781	0.982			
SEM		0.19	0.13	0.14	0.06	0.04			

<sup>&</sup>lt;sup>a,b</sup> Means within column with different superscripts differ significantly at  $P \le 0.05$ .

### MATERIALS AND METHODS EXPERIMENT 2

# Experimental Design and Diets

This trial was a 2 (chromium propionate<sup>1</sup> at 200 ppb/ton) x 2 (butyric acid zinc complex<sup>3</sup> at 132 g of butyric acid and 55 g of zinc) factorial consisting of 60 pens in a randomized block design. This study evaluated four diets in 15 replications, containing 28 male broilers for a total of 1,680 Cobb 700 broiler chicks during a 52 assay period at a stocking density of 1.25 sq. ft. per bird. Prior to placement, all broilers were vaccinated with a coccidiosis vaccine<sup>4</sup> in a commercial spray cabinet and allowed to preen for 1 hr prior to placement. The diet manufactured was a standard U.S. corn-soybean based diet, which generated four dietary treatments. There was a common basal diet manufactured for all treatments and then chromium propionate, butyric acid zinc complex, and corn starch added to generate treatments. The dietary program consisted of four phases; the starter phase fed as a crumble from d 1 to 14, and the grower phase fed as a pellet from d 14 to 28, the finisher phase fed as a pellet from d 28 to 42, and the withdrawal phase from d 42 to 52 also fed as a pellet. The diets included corn, soybean meal, meat and bone meal and Dried Distilled Grains with solubles. The conditioning time was 12 seconds and the pelleting temperature was around 78 to 80°C. The feed was pelleted; with a target moisture content of 13% and a PDI of 85%.

<sup>&</sup>lt;sup>1</sup> KemTRACE® Chromium, Kemin Industries, Des Moines, IA

<sup>&</sup>lt;sup>3</sup> ButiPEARL<sup>TM</sup> Z, Kemin Industries, Des Moines, IA

<sup>&</sup>lt;sup>4</sup> ADVENT®, Huvepharma®, Inc. Peachtree City, GA

Table 10. Dietary Formulations (lb/ton) and Calculated Nutrient Content of the Basal Diet for Experiment 2. The addition of ButiPearl Z and KemTRACE Chromium, or corn starch were added to achieve final experimental treatments.

		Starter	Grower	Finisher	Withdrawal
Ingredient Name					
CORN		1163	1249	1318	1331
SOYBEAN ML48%		632	516	460	394
DL-MET98		6.7	5.75	4.6	3.55
LYSINE HCL		4.75	5.25	4.7	4.3
L-THREONINE 98.5%		3.05	2.6	1.85	1.05
SOY OIL		34.91	33.88	49.72	60.17
LIMESTONE		17.4	17.9	17	17.7
SALT		5.5	5.35	6	5.95
SODIUM BICARB		3.8	3.9	3.45	3.35
TAMU TRACE MINERALS <sup>1</sup>		1	1	1	1
TAMU VITAMINS <sup>2</sup>		2.5	2.5	2.5	2.5
MEAT and BONE MEAL		64.24	55.94	30.2	24.41
DDGs		60	100	100	150
Phytase <sup>3</sup>		0.25	0.25	0.25	0.25
Salinomycin <sup>4</sup>		1	1	1	1
Ingredient Total:		2000.1	2000.32	2000.27	2000.23
Formula Dry Matter %:		88.17	88.13	87.29	86.76
Nutrient Name	Units				
DRY MATTER	РСТ	88.169	88.128	87.289	86.764
MOISTURE	РСТ	11.831	11.872	12.711	13.236
PROTEIN	РСТ	23.408	21.294	19.446	18.392
CRUDE FAT	РСТ	4.708	4.819	5.582	6.185
CRUDE FIBER	РСТ	2.75	2.726	2.667	2.692
CALCIUM	РСТ	0.9	0.859	0.719	0.7

Table 10 Continued.

PHOSPHORUS         PCT         0.526         0.501         0.437           AV PHOSPHATE         PCT         0.45         0.43         0.37           ASH         PCT         4.117         3.956         3.826           ADF         PCT         3.565         3.526         3.472           NDF         PCT         8.394         8.29         8.372           ME POULTRY kcal/kg         KCAL/KG         3046.884         3079.923         3157.058         31           AV-MET         PCT         0.651         0.583         0.507         AV-MET         AV-MET         PCT         0.941         0.85         0.761           AV-TSAA         PCT         0.941         0.85         0.761         AV-LYS         PCT         1.249         1.12         0.999           AV-TRP         PCT         0.224         0.195         0.177         AV-TRP         PCT         0.88         0.78         0.689           AV-ARG         PCT         1.357         1.181         1.06         AV-VAL         PCT         0.94         0.85         0.779           AV-LEU         PCT         1.775         1.642         1.543         AV-PHE         PCT         0.881	Nutrient Name	Units	Starter	Grower	Finisher	Withdrawal
ASH PCT 4.117 3.956 3.826  ADF PCT 3.565 3.526 3.472  NDF PCT 8.394 8.29 8.372  ME POULTRY kcal/kg KCAL/KG 3046.884 3079.923 3157.058 31  AV-MET PCT 0.651 0.583 0.507  AV-TSAA PCT 0.941 0.85 0.761  AV-LYS PCT 1.249 1.12 0.999  AV-TRP PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.887 0.745 0.682  AV-PHE PCT 0.887 0.771 0.723  CYSTINE PCT 0.888 0.614 0.536  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.501 1.315 1.18  HISTIDINE PCT 1.505 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	PHOSPHORUS	РСТ				0.427
ADF PCT 3.565 3.526 3.472  NDF PCT 8.394 8.29 8.372  ME POULTRY kcal/kg KCAL/KG 3046.884 3079.923 3157.058 31  AV-MET PCT 0.651 0.583 0.507  AV-TSAA PCT 0.941 0.85 0.761  AV-LYS PCT 1.249 1.12 0.999  AV-TRP PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.881 0.771 0.723  CYSTINE PCT 0.881 0.771 0.723  CYSTINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.998 0.891 0.79  ARGININE PCT 0.558 0.507 0.479  VALINE PCT 0.558 0.507 0.479  VALINE PCT 1.505 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV PHOSPHATE	РСТ	0.45	0.43	0.37	0.36
NDF         PCT         8.394         8.29         8.372           ME POULTRY kcal/kg         KCAL/KG         3046.884         3079.923         3157.058         31           AV-MET         PCT         0.651         0.583         0.507         AV-TSAA         PCT         0.941         0.85         0.761           AV-LYS         PCT         1.249         1.12         0.999         AV-TRP         AV-TRP         PCT         0.224         0.195         0.177           AV-TRP         PCT         0.88         0.78         0.689         AV-TRP         AV-TRP         PCT         0.88         0.78         0.689           AV-ARG         PCT         1.357         1.181         1.06         0.689         AV-ARG         PCT         0.94         0.85         0.779           AV-LEU         PCT         0.94         0.85         0.779         0.779         0.682         0.779         0.682         0.779         0.682         0.779         0.682         0.779         0.682         0.745         0.682         0.682         0.771         0.723         0.723         0.745         0.682         0.682         0.771         0.723         0.723         0.724         0.683         0	ASH	РСТ	4.117	3.956	3.826	3.797
ME POULTRY kcal/kg         KCAL/KG         3046.884         3079.923         3157.058         31           AV-MET         PCT         0.651         0.583         0.507           AV-TSAA         PCT         0.941         0.85         0.761           AV-LYS         PCT         1.249         1.12         0.999           AV-TRP         PCT         0.224         0.195         0.177           AV-TRP         PCT         0.88         0.78         0.689           AV-TRP         PCT         0.88         0.78         0.689           AV-TRP         PCT         1.357         1.181         1.06           AV-ARG         PCT         1.357         1.181         1.06           AV-VAL         PCT         0.94         0.85         0.779           AV-LEU         PCT         1.775         1.642         1.543           AV-PHE         PCT         0.881         0.771         0.723           CYSTINE         PCT         0.357         0.329         0.31           METHIONINE         PCT         0.683         0.614         0.536           TSAA         PCT         1.047         0.951         0.853	ADF	РСТ	3.565	3.526	3.472	3.504
AV-MET PCT 0.651 0.583 0.507  AV-TSAA PCT 0.941 0.85 0.761  AV-LYS PCT 1.249 1.12 0.999  AV-TRP PCT 0.224 0.195 0.177  AV-THR PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.934 1.79 1.677	NDF	PCT	8.394	8.29	8.372	8.141
AV-TSAA PCT 0.941 0.85 0.761  AV-LYS PCT 1.249 1.12 0.999  AV-TRP PCT 0.224 0.195 0.177  AV-THR PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-IEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	ME POULTRY kcal/kg	KCAL/KG	3046.884	3079.923	3157.058	3189.867
AV-LYS PCT 1.249 1.12 0.999  AV-TRP PCT 0.224 0.195 0.177  AV-THR PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV-MET	PCT	0.651	0.583	0.507	0.447
AV-TRP PCT 0.224 0.195 0.177  AV-THR PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV-TSAA	PCT	0.941	0.85	0.761	0.689
AV-THR PCT 0.88 0.78 0.689  AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	\V-LYS	PCT	1.249	1.12	0.999	0.901
AV-ARG PCT 1.357 1.181 1.06  AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV-TRP	PCT	0.224	0.195	0.177	0.161
AV-VAL PCT 0.94 0.85 0.779  AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV-THR	PCT	0.88	0.78	0.689	0.609
AV-LEU PCT 1.775 1.642 1.543  AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV-ARG	PCT	1.357	1.181	1.06	0.96
AV-ILE PCT 0.837 0.745 0.682  AV-PHE PCT 0.881 0.771 0.723  CYSTINE PCT 0.357 0.329 0.31  METHIONINE PCT 0.683 0.614 0.536  TSAA PCT 1.047 0.951 0.853  LYSINE PCT 1.389 1.249 1.114  TRYPTOPHAN PCT 0.26 0.227 0.207  THREONINE PCT 0.998 0.891 0.79  ARGININE PCT 1.501 1.315 1.18  HISTIDINE PCT 0.558 0.507 0.479  VALINE PCT 1.05 0.954 0.875  LEUCINE PCT 1.934 1.79 1.677	AV-VAL	PCT	0.94	0.85	0.779	0.735
AV-PHE         PCT         0.881         0.771         0.723           CYSTINE         PCT         0.357         0.329         0.31           METHIONINE         PCT         0.683         0.614         0.536           TSAA         PCT         1.047         0.951         0.853           LYSINE         PCT         1.389         1.249         1.114           TRYPTOPHAN         PCT         0.26         0.227         0.207           THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	AV-LEU	PCT	1.775	1.642	1.543	1.476
CYSTINE         PCT         0.357         0.329         0.31           METHIONINE         PCT         0.683         0.614         0.536           TSAA         PCT         1.047         0.951         0.853           LYSINE         PCT         1.389         1.249         1.114           TRYPTOPHAN         PCT         0.26         0.227         0.207           THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	AV-ILE	PCT	0.837	0.745	0.682	0.635
METHIONINE         PCT         0.683         0.614         0.536           TSAA         PCT         1.047         0.951         0.853           LYSINE         PCT         1.389         1.249         1.114           TRYPTOPHAN         PCT         0.26         0.227         0.207           THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	AV-PHE	PCT	0.881	0.771	0.723	0.654
TSAA         PCT         1.047         0.951         0.853           LYSINE         PCT         1.389         1.249         1.114           TRYPTOPHAN         PCT         0.26         0.227         0.207           THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	CYSTINE	PCT	0.357	0.329	0.31	0.296
LYSINE         PCT         1.389         1.249         1.114           TRYPTOPHAN         PCT         0.26         0.227         0.207           THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	METHIONINE	PCT	0.683	0.614	0.536	0.477
TRYPTOPHAN         PCT         0.26         0.227         0.207           THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	rsaa	PCT	1.047	0.951	0.853	0.78
THREONINE         PCT         0.998         0.891         0.79           ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	.YSINE	PCT	1.389	1.249	1.114	1.012
ARGININE         PCT         1.501         1.315         1.18           HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	FRYPTOPHAN	PCT	0.26	0.227	0.207	0.189
HISTIDINE         PCT         0.558         0.507         0.479           VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	THREONINE	PCT	0.998	0.891	0.79	0.709
VALINE         PCT         1.05         0.954         0.875           LEUCINE         PCT         1.934         1.79         1.677	ARGININE	PCT	1.501	1.315	1.18	1.079
LEUCINE PCT 1.934 1.79 1.677	HISTIDINE	PCT	0.558	0.507	0.479	0.454
	/ALINE	РСТ	1.05	0.954	0.875	0.83
	.EUCINE	РСТ	1.934	1.79	1.677	1.606
SOLEUCINE  PCT   0.918  0.82  0.751	SOLEUCINE	PCT	0.918	0.82	0.751	0.703

Table 10 Continued.

Nutrient Name	Units	Starter	Grower	Finisher	Withdrawal
PHENYLALANINE	РСТ	0.96	0.841	0.789	0.714
GLYCINE	PCT	0.856	0.763	0.717	0.665
PHE+TYR	PCT	2.079	1.813	1.66	1.471
VITAMIN D3 IU/kg	IU/KG	3857.836	3857.411	3857.51	3857.59
VITAMIN K mg/kg	MG/KG	1.587	1.596	1.603	1.604
VITAMIN A KIU/kg	KIU/KG	11.603	11.645	11.68	11.687
VITAMIN E IU/kg	MG/KG	59.666	60.431	61.107	61.153
THIAMIN	MG/KG	5.984	5.948	5.979	5.896
RIBOFLAVIN	MG/KG	7.466	7.34	7.294	7.205
PANTOTHENIC ACID	MG/KG	27.275	26.574	26.292	25.824
NIACIN	MG/KG	66.832	66.581	66.795	66.226
CHOLINE	MG/KG	1354.035	1222.169	1167.129	1081.07
BIOTIN	MG/KG	0.686	0.67	0.663	0.653
FOLIC ACID	MG/KG	2.391	2.332	2.31	2.27
VITAMIN B12 mgr/kg	Mgr/KG	16.249	16.247	16.248	16.248
VITAMIN B6	MG/KG	12.815	12.824	12.926	12.807
LINOLEIC ACID	РСТ	1.406	1.477	1.542	1.543
ELECTROLYTES	MEQ/KG	225.923	204.857	189.954	181.726
SODIUM	РСТ	0.2	0.2	0.2	0.2
POTASSIUM	РСТ	0.851	0.769	0.717	0.679
CHLORIDE	РСТ	0.28	0.28	0.285	0.281
MAGNESIUM	РСТ	0.183	0.177	0.169	0.168
SULPHUR	РСТ	0.226	0.217	0.201	0.206
(Na+K)-(Cl+S)	MEQ/KG	8.548	6.947	6.452	5.342
IRON	MG/KG	153.28	147.882	141.461	139.898
COPPER	MG/KG	14.527	13.841	13.203	12.855
ZINC	MG/KG	99.518	101.551	98.962	103.139

**Table 10 Continued.** 

Nutrient Name	Units	Starter	Grower	Finisher	Withdrawal
MANGANESE	MG/KG	78.136	75.873	74.72	73.304
COBALT	MG/KG	0	0	0	0
IODINE	MG/KG	0.4	0.4	0.4	0.4
SELENIUM	MG/KG	0.049	0.045	0.043	0.04
Analy	yzed Nutrien	t Content %			
Treatment 1 Control Basal	Starter	Grower	Finisher	Withdrawal	
Protein	20.50	20.60	18.30	16.80	
Crude Fat	5.04	4.39	11.40	7.28	
Crude Fiber	2.60	3.00	3.30	3.10	
Ash	4.37	4.48	3.56	3.86	
Calcium	0.94	1.03	0.61	0.69	
Phosphorus	0.52	0.54	0.44	0.44	
Sodium	0.17	0.21	0.20	0.17	
Manganese	78.60	81.70	65.80	60.50	
Copper	13.80	13.40	13.70	13.20	

Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, 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.

 $<sup>^2</sup>$  Vitamin premix added at this rate yields 11,000 IU vitamin A, 3,850 IU vitamin D<sub>3</sub>, 45 IU vitamin E, 0.02 mg B<sub>12</sub>, 6.0 mg riboflavin, 46 mg niacin, 20.2 mg d-pantothenic acid,131.1 mg choline, 1.5 mg menadione, 1.75 mg folic acid, 7.1 mg pyroxidine, 2.9 mg thiamine, 0.60 mg biotin per kg diet. The carrier is ground rice hulls.

<sup>&</sup>lt;sup>3</sup> OptiPhos 2000 PF, Huvepharma, Peachtree City, GA.

<sup>&</sup>lt;sup>4</sup> Sacox, Huvepharma, Peachtree City, GA.

## Animals and Management Practice

This study consisted of a total of 60 pens with 15 blocks of 4 contiguous treatments, each containing 28 chicks at day 1 of age for a total placement of 1,680 Cobb 700 male broilers. Bird weights by pen were recorded at the study initiation, d 14, d 28, d 42, and on d 52. Feed and water was available *ad libitum* and provided age appropriate supplemental heat. Animal care was provided by and in accordance with the protocol approved by the Texas A&M IACUC (REF #053962).

# Heat Stress Exposure

This trial focused on the effects of the inclusion of chromium propionate fed separately and in combination with a butyric acid-zinc complex on broilers reared to 52 days. Industry rearing conditions were replicated during the summer months in Texas. Temperatures ranged at/or above 100°F each day. Starting at two weeks of age, visible panting was observed throughout the house as temperatures within the house reached 8 to 10 degrees above set temperature. Temperatures were recorded at multiple times throughout each day to accurately determine the length of time heat stress was potentially experienced. Visible signs of panting were observed around 8 to 12 hours per day in the afternoon/early evening hours. No signs of panting were observed during the late evening/ early morning hours as the house temperature returned to levels similar to target temperature.

# Parameters Evaluated

All broilers and feed were weighed by pen biweekly on d 14, 28, 42, and 52 to determine average body weight (BW), mortality adjusted feed conversion ratio (FCR), feed consumption

(FC), mortality percentage, and cumulative FCR. On d 42, blood was drawn from 2 birds per pen, 30 birds per treatment, for plasma corticosterone determination. Following an 8 hr feed withdrawal period on d 53, six broilers from each pen were randomly selected for processing from each replicate pen. Fasted live weight, chilled carcass weight, and the weight of cut up parts (skinless boneless breast (pectoralis major), and tenders (pectoralis minor) were measured. The weighs were averaged to calculate the total carcass yield, the white meat yield, and the average body weight, as well as woody breast and white striping scores using the pen as the experimental unit.

## **Processing Parameters**

Upon completion of the trial on d 49, broilers within each pen were weighed for live weight determination, and were placed on an 8 hr feed withdrawal period prior to processing. All birds were conventionally processed in a pilot scale processing facility at Texas A&M University. Birds were stunned (Model SF-7000, Simmons Engineering Corp., Dallas, GA) in a 1% saline bath, 13 mA, 7 s, 500 Hz, DC and bled using a unilateral neck cut. The exsanguinated birds were allowed to bleed for 90 s. All birds were conventionally scalded (61°C, 45 s), picked in a rotary drum picker (Model sp30ss, Bower Corp., Houghton, LA 52, 631) for 25 s, and manually eviscerated. Birds were then chilled to 4°C within 80 min. The following parameters were measured on the day of processing: fasted live weight, chilled carcass weight, and the skinless boneless breast (Pectoralis major), and tenders (Pectoralis minor). After chilling, carcasses were deboned (breast fillets and tenders) and weighed. Weights were recorded and averaged to calculate the total carcass yield and the breast and tender yield, along with white meat yield consisting of breast and tenders. After weighing, the left and right P.Major filets were

palpated and scored for white striping based on the scoring system of Kuttapan et al. and woody breast based on the scoring system of Tijare et al. (Kuttappan, et al., 2012; Tijare, et al., 2016).

## Plasma Corticosterone Collection and Analysis

On d 42 plasma corticosterone levels were determined from 2 birds per pen. Two mL of blood was collected from each bird and measured using a commercially available ELISA<sup>5</sup> kit (BD Life Sciences, Franklin Lakes, New Jersey). The blood was injected into a plasma separation gel and lithium heparin vacutainer, which was temporarily stored in an ice bath. Once all samples were collected, the vacutainers were centrifuged in a Beckman GS-6R centrifuge<sup>6</sup> (Beckman Coulter, Brea, Ca) for 15 minutes at 4000 RPM to separate the cells from the plasma. The plasma was separated into 2 mL micro centrifuge tubes and stored at -19°C until further analysis. All samples were analyzed in the same assay to avoid inter-assay variability.

# Statistical Analysis

Data was analyzed via a 2x2 factorial using the General Linear Model. Main effects were deemed significantly different at a  $P \le 0.05$ . In instances where there was a significant interaction, a one way ANOVA was conducted with individual treatment means deemed significantly different at a  $P \le 0.05$  and separated using the Duncan's Multiple Range Test. All percentage data was arc sin transformed prior to analysis.

-

<sup>&</sup>lt;sup>5</sup> EnzoLife Sciences, ADI-901-097, Farmingdale, NY

<sup>&</sup>lt;sup>6</sup> Beckman Coulter, Brea, CA

### **EXPERIMENT 2 RESULTS**

Average body weight and average body weight gain was calculated on d 0, 14, 28, 35, 42, and 52 (Table 11). No significant differences were observed throughout the trial with body weight or body weight gain. Mortality corrected feed conversion ratio was evaluated by period and on a cumulative basis. Early impacts on FCR were not observed. As the age of the broiler increased and the level of stress associated with temperature increased, impacts on FCR were observed with the addition of chromium. The addition of chromium significantly reduced FCR during the WD phase and cumulatively (d 1-52). For chromium supplemented birds, the withdrawal phase had a significant reduction in FCR of 18 points, and 2 points cumulatively on FCR when compared to the control diet. The inclusion of the butyric acid-zinc complex did not affect FCR significantly (Table 12 & 13). Mortality percent was evaluated by phases, and a consistent reduction in mortality for each phase was observed throughout the experiment with the addition of chromium reaching the level of significance for cumulative mortality from d 1 to d 52 (Table 14 & 15). Although no significant interactions were observed when mortality percentage was evaluated via a one way ANOVA, there was a 3% reduction in mortality observed in the combination of chromium propionate and butyric acid-zinc compared to the control. (Table 14 & 15). When compared to the Cobb 700 Broiler Performance and Nutrition Supplement Guide, birds in experiment 2 exhibited a reduction in cumulative feed consumption (d1-42) (106g vs. 116g). As a result of the heat stress in experiment 2, broilers consumed less feed compared to the Cobb guide, resulting in a decrease in body weight. Due to the level of mortality associated with the heat stress observed in experiment 2, FCR was lower than that of the Cobb guide. It is possible that the reduction in FCR observed in experiment 2 was not

necessarily an improvement in performance, but a result of the current study having an FCR that was corrected for mortality compared to the Cobb's FCR, which was not mortality corrected (Cobb-Vantress, 2012)(Table 12, 13, 16, & 17). Processing characteristics were evaluated on d 53 of the experiment with fasted body weight, carcass weight, tender weight, breast weight, carcass yield, tender yield, breast yield, as well as woody breast and white striping incidence and severity. Broilers fed chromium had an increased level of breast meat yield as compared to broilers not supplemented with chromium. A significant interaction was observed between the butyric acid zinc complex and chromium regarding average woody breast score. This interaction indicated that a positive impact on woody breast was observed with the addition of both additives, which was not present when each additive was supplemented separately. The addition of the butyric acid zinc complex impacted woody breast profile by shifting a significant percentage of breast fillets from a score of 2 to a score of 1. (Table 18, 19, 20). This shift resulted in significant increases in breasts that were classified as mild compared to those classified as moderate to severe. Additionally, blood plasma corticosterone levels were reduced in broilers fed diets that contained the supplemental chromium propionate. (Table 21). Environmental conditions were monitored daily (F) during the morning, midday, and evening hours. Barn temperature was taken as max and min and averaged for each corresponding day. The average temperatures range from 83°F to around 90°F. To summarize, chromium propionate reduced corticosterone levels, and mortality percentages all while improving feed conversion in the withdrawal phase as well as cumulatively. These improvements yielded an improvement in breast meat yield. The butyric acid zinc complex supplemented birds had an increased percentage of normal and mild woody breast fillet scores, while decreasing the percentage of moderate to severe woody breast fillets. The combination of the chromium propionate and the

butyric acid zinc complex reduced corticosterone levels and average woody breast score when birds were introduced to heat stress.

Table 11. Average Body Weight (kg) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Treatment	D 14	D 28	D 35	D 42	D 52
1	Control	0.407	1.418	2.132	2.820	3.605
2	1 lb. ButiPearl Z	0.413	1.447	2.160	2.843	3.685
3	1 lb. KemTRACE Chromium	0.412	1.437	2.155	2.843	3.693
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	0.404	1.427	2.141	2.818	3.635
	One Way P-value	0.132	0.149	0.600	0.784	0.337
	Ma	in Effects and	Interactions			
ButiPearl	Z					
Control		0.410	1.427	2.144	2.831	3.649
1 lb/ton		0.408	1.437	2.151	2.831	3.659
Chromiun	n					
Control		0.410	1.433	2.146	2.831	3.643
1 lb/ton		0.408	1.432	2.148	2.830	3.644
Probabilit	ies (p values)					
Butyric Ac	id	0.712	0.279	0.664	0.986	0.882
Chromium		0.605	0.949	0.890	0.961	0.588
Butyric Ac	id Z x Chromium	0.024	0.042	0.202	0.307	0.129
PSEM		0.002	0.007	0.010	0.016	0.035

Table 12. Mortality Corrected Feed Conversion Ratio of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Treatment	Starter	Grower	Finisher	WD	Total
						FCR
1	Control	1.271	1.543	1.830	2.772	1.845
2	1 lb. ButiPearl Z	1.258	1.535	1.827	2.640	1.820
3	1 lb. KemTRACE Chromium	1.250	1.530	1.805	2.550	1.808
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	1.265	1.535	1.822	2.508	1.812
	One Way P-Value	0.153	0.481	0.577	0.182	0.096
	Main Et	ffects and Int	teractions			
ButiP	earl Z					
Contro	ol	1.261	1.536	1.818	2.661	1.827
1 lb/to	on	1.261	1.535	1.824	2.569	1.816
Kem	TRACE Chromium					
Contro	ol	1.264	1.539	1.829	2.711 <sup>a</sup>	1.834 <sup>a</sup>
1 lb/to	on	1.258	1.532	1.814	2.529 <sup>b</sup>	1.810 <sup>b</sup>
Proba	abilities (p values)					
Butyri	ic Acid	0.907	0.826	0.625	0.389	0.504
Chron	nium	0.313	0.249	0.278	0.054	0.033
Butyri	ic Acid Z x Chromium	0.040	0.301	0.461	0.679	0.297
PSEM	!	0.005	0.005	0.007	0.071	0.008

a,b Main effect means with non-similar superscripts differ significantly at P≤0.05.

<sup>&</sup>lt;sup>1</sup> FCR was adjusted using 36 grams of body weight equal to 1 point of FCR. All birds were adjusted to 3.65 kg. Significant is loss in this adjustment because of the increase in variability.

Table 13. Mortality Corrected Feed Conversion Ratio (FCR) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TR	Treatment	D 1 to 28	D 28 to 35	D 1 to 35	D 35 to 42	D 1 to 42
T						
1	Control	1.465	1.728	1.552	1.938	1.645
2	1 lb. ButiPearl Z	1.460	1.709	1.542	1.955	1.640
3	1 lb. KemTRACE Chromium	1.455	1.694	1.534	1.905	1.628
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	1.463	1.708	1.545	1.946	1.641
	One Way P-value	0.556	0.492	0.163	0.406	0.214
		Main	Effects and I	nteractions		
Buti	Pearl Z					
Cont	rol	1.460	1.711	1.543	1.921	1.626
1 lb/	ton	1.462	1.709	1.544	1.950	1.641
Kem	TRACE Chromium					
Cont	rol	1.463	1.719	1.547	1.946	1.642
1 lb/	ton	1.459	1.701	1.540	1.926	1.635
Prob	pabilities (p values)					
Buty	ric Acid	0.789	0.884	0.840	0.190	0.456
Chro	mium	0.497	0.252	0.189	0.351	0.203
Buty	ric Acid Z x Chromium	0.218	0.305	0.066	0.578	0.127
PSE	M · · · · · · · · · · · · · · · ·	0.00	0.01	0.00	0.01	0.00

Table 14. Mortality Percentage (%) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TR	Treatment	Starter	Grower	Finisher	WD	Total
<u>T</u>	Control					
1	Control	5.47	1.53	0.29	3.66	10.46
2	1 lb. ButiPearl Z	7.14	0.81	0.49	2.56	10.99
3	1 lb. KemTRACE Chromium	4.52	0.99	1.05	2.62	9.44
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	5.24	0.52	0.52	1.80	7.42
	One Way P- value	0.421	0.622	0.509	0.516	0.193
		Main Ef	fects and Intera	ctions		
Buti	Pearl Z					
Cont	rol	5.00	1.27	0.67	3.14	9.95
1 lb/	ton	6.19	0.66	0.51	2.17	9.20
Kem	TRACE					
	omium					
Cont		6.31	1.17	0.39	3.13	10.71 <sup>a</sup>
1 lb/		4.88	0.76	0.79	2.21	8.47 <sup>b</sup>
	oabilities (p					
valu	,					
	ric Acid	0.266	0.284	0.663	0.235	0.424
	omium	0.184	0.457	0.287	0.273	0.025
	ric Acid Z x	0.654	0.830	0.323	0.853	0.150
	omium	0.50	0.20	0.10	0.46	0.01
PSE	M	0.68	0.30	0.18	0.46	0.81

a,b Main effect means with non-similar superscripts differ significantly at P≤0.05.

Table 15. Mortality Percentage (%) Phases of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Treatment	D 1-28	D 1-35	D 35 to 42	D 1-42
1	Control	6.903	7.141	0.000	7.141
2	2 1 lb. ButiPearl Z		7.856	0.275	8.417
3	1 lb. KemTRACE Chromium	5.475	6.190	0.503	6.666
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	5.713	5.951	0.267	6.189
	One Way P-value	0.528	0.737	0.612	0.643
	Main Effect	s and Interact	tions		
ButiPea	rl Z				
Control		6.189	6.666	0.252	6.904
1 lb/ton		6.785	6.903	0.271	7.265
KemTR	ACE Chromium				
Control		7.380	7.499	0.133	7.757
1 lb/ton		5.594	6.071	0.385	6.428
Probabi	lities (p values)				
Butyric A	Acid	0.599	0.830	0.970	0.657
Chromiu	m	0.120	0.203	0.341	0.202
Butyric A	Acid Z x Chromium	0.752	0.668	0.360	0.388
PSEM		0.741	0.764	0.120	0.761

Table 16. Feed Consumption (Grams/Bird/Day) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Starter	Grower	WD
1	32.2	110.4	187.4
2	32.1	112.9	194.6
3	32.5	111.3	189.7
4	31.9	111.8	189.2
	0.445	0.232	0.912
	Main Effec	l ets	
ButiPearl			
Control	32.4	110.9	188.6
1 lb/ton	32	112.4	191.7
KemTRACE Chromium			
Control	32.1	111.7	190.7
1 lb/ton	32.2	111.6	189.5
Probabilities (p values)			
Butyric Acid	0.162	0.083	0.646
Chromium	0.881	0.93	0.981
Butyric Acid Z x Chromium	0.414	0.259	0.564
PSEM	0.2	0.6	2.7

Table 17. Feed Consumption (grams/bird/day) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	D 1 – 28	D 28 -35	D 35 - 42	D 1 to 42	Total FI D 1-52
1	70.5	171.9	188.9	106.1	121.1
2	71.4	173.5	187.5	106.8	124.0
3	71.3	172.6	186.9	106.7	122.1
4	71.0	174.0	186.9	106.8	122.3
	0.64	0.70	0.874	0.895	0.805
		Main	Effects		
ButiPearl					
Control	70.9	172.3	187.9	106.4	121.6
1 lb/ton	71.2	173.7	187.2	106.8	123.1
KemTRACE Chromium					
Control	70.9	172.7	188.2	106.4	122.5
1 lb/ton	71.2	173.3	186.9	106.8	122.2
Probabilities (p values)					
Butyric Acid	0.601	0.278	0.708	0.58	0.456
Chromium	0.644	0.646	0.515	0.692	0.805
Butyric Acid Z x Chromium	0.278	0.92	0.713	0.726	0.551
PSEM	0.3	1.2	1.4	0.5	0.9

Table 18. Processing Traits of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Treatment	Fasted Body Weight	Carcass	Tender	Wooden Breast	White Striping
		<b>'</b>	Weights (kg)		Breast	Quality
1	Control	3.649	2.915	0.157	1.296	1.204
2	1 lb. ButiPearl Z	3.650	2.929	0.163	1.346	1.115
3	1 lb. KemTRACE Chromium	3.651	2.923	0.157	1.378	1.133
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	3.642	2.901	0.155	1.153	1.071
	One Way P-value	0.961	0.999	0.754	0.063	0.464
			Main Eff	ects and Interac	ctions	
ButiPo	earl Z					
Contro	ol	3.650	2.919	0.157	1.337	1.168
1 lb/to	n	3.646	2.915	0.159	1.246	1.093
KemT	RACE Chromium					
Contro	ol	3.650	2.922	0.159	1.320	1.161
1 lb/to	n	3.647	2.912	0.156	1.265	1.102
Proba	bilities (p values)					
Butyrio	c Acid	0.972	0.984	0.385	0.123	0.219
Chrom	nium	0.686	0.874	0.712	0.431	0.339
Butyrio	c Acid Z x Chromium	0.713	0.955	0.584	0.038	0.822
PSEM	····	0.034	0.027	0.003	0.039	0.031

Table 19. Processing Traits of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Γ Treatment		Carcass	Tender	Breast Yield	Breast Wt
			Yield	Yield	(%)	(kg)
			(%)	(%)		
1	Control		79.90	5.37	29.71	0.868
2	1 lb. ButiPearl Z		80.31	5.54	29.25	0.859
3	1 lb. Chromium		80.07	5.39	30.02	0.878
4	1 lb. ButiPearl Z + 1 lb. Chromium		79.68	5.35	29.63	0.861
	One Way P-valu	e	0.5.23	0.737	0.108	0.577
		Main Effect	s and Interac	ctions	<u> </u>	
ButiPear	l Z					
Control			79.98	5.38	29.86	0.873
1 lb/ton			80.00	5.45	29.43	0.860
KemTRA	CE Chromium					
Control			80.10	5.46	29.48 <sup>b</sup>	0.864
1 lb/ton			79.90	5.37	29.88a	0.870
Probabili	ities (p values)					
Butyric Acid			0.963	0.384	0.109	0.393
Chromium			0.436	0.673	0.056	0.260
Butyric Acid Z x Chromium			0.098	0.566	0.504	0.741
PSEM			0.001	0.001	0.002	0.011

a,b Main effect means with non-similar superscripts differ significantly at P≤0.05.

Table 20. Woody Breast Profile of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared to 52 Days in Heat Stressed Conditions.

TRT	Treatment	WB Zero	WB One	WB Two	WB Three	Mild	Moder ate and Severe	
1	Control	5.1	54.6	36.4	4.0	59.7	40.3	
2	1 lb. ButiPearl Z	1.9	61.0	28.3	7.0	62.9	37.1	
3	1 lb. KemTRACE Chromium	1.9	53.8	38.4	4.3	55.7	42.4	
4	1 lb. ButiPearl Z + 1 lb. KemTRACE Chromium	1.0	67.9	29.1	2.1	68.9	31.1	
	One Way P-value	0.209	0.085	0.141	0.330	0.232	0.214	
	Main Effects and Interactions							
ButiPear	ButiPearl Z							
Control		3.5	54.2 <sup>b</sup>	37.4 <sup>a</sup>	4.1	57.7 <sup>b</sup>	42.3a	
1 lb/ton		1.4	64.4 <sup>a</sup>	28.7 <sup>b</sup>	4.5	65.9ª	34.1 <sup>b</sup>	
KemTRA								
Chromiu	m							
Control		3.5	57.8	32.3	5.5	61.3	38.7	
1 lb/ton		1.4	60.9	33.9	3.1	62.3	36.7	
	ities (p values)		0.04.1		0.00	0.074		
Butyric Acid		0.171	0.016	0.025	0.987	0.053	0.057	
Chromium		0.126	0.586	0.591	0.305	0.988	0.879	
Butyric Acid Z x Chromium		0.588	0.465	0.973	0.123	0.376	0.328	
PSEM								

a,b Main effect means with non-similar superscripts differ significantly at P≤0.05

Table 21. Day 42 Corticosterone Levels (pictogram/mL) of Male Broilers Fed Diets Supplemented with ButiPearl Z and KemTRACE Chromium Independently and in Combination Reared in Heat Stressed Conditions

TRT	Treatment	Corticosterone		
1	Control	2715		
2	1 lb. ButiPearl Z	3389		
3	1 lb. Chromium	2193		
4	1 lb. ButiPearl Z + 1 lb. Chromium	1149		
	Main Effects And	Interactions		
ButiPear	rl Z			
Control		2439		
1 lb/ton		2290		
KemTR	ACE Chromium			
Control		3071 <sup>a</sup>		
1 lb/ton		1681 <sup>b</sup>		
Probabil	lities (p values)			
Butyric A	Acid	0.839		
Chromiu	m	0.057		
Butyric A	Acid Z x Chromium	0.368		
PSEM		390		

a,b Main effect means with non-similar superscripts differ significantly at P≤0.05.

### DISCUSSION

The objective of these experiments was to determine the effects of feeding chromium propionate separately and in combination with an encapsulated source of butyric acid-zinc complex on broiler growth and processing performance. In experiment 1 and experiment 2, body weight gain and feed consumption were not significantly different. However, there is evidence from several other studies that included chromium propionate into diets, which showed an increase in body weight gain (Eze, et al., 2014; Kim, et al., 1996; Lien, et al., 2005). Experiment 1 and 2 agreed with Jackson et al's study showing chromium having no effect on body weight gain (Jackson, et al., 2008). Kim et al, observed an increase in crude protein content of the broilers carcass, agreeing with experiment 1 and 2, as the chromium inclusion resulted in an increase in carcass yields (Kim, et al., 1996). Other research has shown that the effect of chromium on growth performance and carcass traits in broilers have been variable in thermal neutral situations, but while experiencing heat stress, chromium supplemented birds have shown to improve in growth performance (Jackson, et al., 2008). However, in each experiment discussed, there was no difference in body weight observed.

Sahin et al, reported that chromium supplementation has been reported to have a positive effect on broiler growth rate, and feed efficiency when introduced to stressful environments (Sahin, et al., 2003). The addition of chromium at 200 ppb consistently reduced feed conversion ratio throughout both experiments beginning during the starter phase and continuing through the duration of the experiment (3.6 points representing 2.1% reduction). Reductions in feed conversion ratio in chromium propionate supplemented birds did not significantly change in the starter to finisher

phases, but there were significant differences observed in the withdrawal phase, as well as cumulatively at ~2 points.

Experiment 2 shows that when introduced to heat stress, birds supplemented with chromium propionate do have an increased performance in breast meat yield compared to birds without supplementation, agreeing with Sahin's research as well as Lien's (Lien, et al., 2005; Sahin, et al., 2003). Experiment 1 and 2 produced carcass yield percentage increases, when compared to birds not supplemented with chromium propionate. When introduced to heat stress, experiment 2 solidifies Sahin's study with the depression in feed intake, weight gain, carcass weight, nutrient digestibility and deterioration in feed efficiency and carcass quality (Sahin, et al., 2017). Heat stress causes economic losses for integrators associated with poor performance and product quality, suppressing immune potency, and increasing the susceptibility to infectious diseases. Studies have shown that heat stress causes corticosterone levels to rise, which leads to poor performance (Geraert, et al., 1996). In experiment 1 and 2, there was a reduced mortality percentage, and reduced feed conversion ratio. This may be due to the effect chromium propionate and combined additives have on reducing corticosterone levels in broilers, therefore allowing them to perform more efficiently, which could potentially mitigate the amount of economic loss associated in heat stressed birds.

In a study conducted by Thaxton et al., plasma corticosterone levels for 192 broilers across eight farms under thermal neutral rearing conditions were evaluated. This study demonstrated that broilers blood corticosterone levels that are classified as "unstressed" range from 400pg/mL to 1,100 pg/mL (Thaxton, et al., 2005). In experiment 2, when chromium propionate was fed in addition with the butyric acid-zinc complex, the analyzed blood plasma

corticosterone level was 1149pg/mL compared to the control at 2715pg/mL. This reduction in plasma corticosterone recorded in experiment 2 is similar to the levels observed in Thaxtons study, deeming this reading nearly "unstressed" (Table 22). Experiment 2 agrees with Dalolio's study, that chromium propionate reduces the severity of corticosterone production, as corticosterone levels in the control diet were 2715pg/mL, 2193 pg/mL in diets fed only chromium propionate, and 1149 pg/mL in the diet containing chromium propionate with the butyric acid-zinc complex as an additive effect was observed. This reduction in corticosterone observed in the diets containing chromium propionate essentially reduces stress in the broilers. Heat and other types of stress cause circulating concentrations of corticosterone, and corticosterone reduces insulin sensitivity (Sahin, et al., 2017). Chromium supplementation is known to enhance insulin sensitivity in animals, therefore reducing plasma glucose and nonesterified fatty acid concentrations (Lien, et al., 2005). Experiment 2 agrees with Dalolio's study that chromium supplementation shows to decrease levels of undesirable hormones such as corticosterone (Dalolio, et al., 2017). Chromium propionate offers an organic alternative to antibiotics in order to assist with negative effects of corticosterone allowing for maximum broiler efficiency (Sahin, et al., 2002). The reduction in observed in corticosterone/stress levels then leads to intestinal improvements allowing for better nutrient digestibility, leading to higher performance and processing yields, as well as lower mortality percentages which is represented in experiments 1 and 2 (Table 6, 8,15,16, & 20).

Studies show that butyric acid included into broiler diets have numerical effects on body weight gain, and show significantly positive effects on feed conversion ratio (Kaczmarek, et al., 2016). Leeson et al reported that there is an indication that unlike antibiotics, butyric acid helps

in the maintenance of intestinal villi structure (Leeson, et al., 2005). Although experiment 1 or 2 did not evaluate intestinal integrity, we can conclude that the combination of the butyric acid-zinc complex with chromium propionate allows for a synergistic effect as mortality, feed conversion ratio, and corticosterone levels were all impacted by chromium propionate. The chromium propionate reduced corticosterone levels, allowing the butyric acid-zinc complex to maximize intestinal absorption and performance for the broiler. Experiment 2 did not show any statistical differences in growth parameters with diets that included the butyric acid zinc complex. However, experiment 2 did show that a butyric acid-zinc complex reduced the severity of woody breast scores that were above a two. Similar to Panda's study, the carcass yield was impacted without negatively impacting the quality of the breast meat (Panda, et al., 2009).

Opportunities for additive effects exist for meat quality and other stress related measurements. Through the genetic advances observed in today's modern broilers, such as weight gain and feed conversion ratio, there has also been an increased in the degree of myodegeneration, fibrosis, lipidosis, and regenerative changes within the breast muscle (Kuttappan, et al., 2012). These issues have resulted in consumers, producers, and customers observing a texture and visual quality issue of the breast muscle known as woody breast and white striping (Gee, 2016). Studies have shown that the inclusion of various vitamins and minerals show a potential impact on the severity of woody breast/white striping (Hess, et al., 2001). In experiment 2, the inclusion of a butyric acid-zinc complex combined with the benefits from chromium propionate produced a positive impact on the severity of woody breast when compared to broilers not fed the additives (Table 8 & 19). In experiment 2, although there was an increase in breast yield of 0.5% in broilers supplemented with chromium propionate compared to

the control, the increase in breast yield did not increase the relevance of woody breast.

Additionally, broilers supplemented with chromium propionate combined with the butyric acidzinc complex, showed a significant reduction in woody breast score by 0.3 to 0.5% compared to
all other treatments.

In this study, chromium propionate and butyric acid zinc demonstrated an additive effect in reducing corticosterone levels. The butyric acid zinc improves intestinal integrity, and by doing so, produced less stress in the gut (less alkalosis going into the blood from the gut) hence the release of corticosterone (Kaczmarek, et al., 2016). This would explain the additive effect observed in the reduction of mortality (Table 15). When evaluating mortality percentage in individual treatment means, although no significant interactions were observed, there was a 2-3% reduction in mortality in birds fed chromium propionate combined with the butyric acid-zinc. This trend directly corresponds to the reduction of corticosterone levels observed in broilers fed the combination of the products. In conclusion, benefits of dietary supplementation of chromium propionate separately and in combination with a butyric acid-zinc complex can be observed through improvements in performance and potential reductions in woody breast severity.

### **CHAPTER III**

## **CONCLUSION**

Broilers are introduced to numerous stressors every day, producing negative economic impacts for producers, as the broilers do not efficiently perform. Finding alternative ways to alleviate these stressors are important. Environmental stress is one of the leading causes of broiler inefficiency observed throughout the industry. Numerous alternative feed ingredients and animal practices have been evaluated to reduce the negative impact associated with environmental stress.

When broilers are reared on old litter and are supplemented with a diet containing chromium propionate, there is advantages detected in feed conversion, carcass yield, as well as total white meat weight. Observed in experiment 1, chromium propionate allows the broiler to perform more efficiently when introduced to typical environmental stressors such as being reared on used litter that is not managed correctly, and being exposed to a high bird stocking density.

Another type of environmental stress that takes a toll on poultry integrators is heat stress. When broilers are introduced to heat stress, they spend more energy trying to return to homeostasis, and do not spend their energy on performing well for the producer. As the broilers experience heat stress, their blood plasma corticosterone levels rise and the broilers do not consume feed efficiently, or efficiently develop muscle. Numerous articles have proved that the addition of chromium propionate into a diet can reduce corticosterone levels in broilers.

Through the genetic advances observed in today's modern broilers, such as weight gain and feed conversion ratio, there has also been an increased in the degree of muscle myopathies. These issues have resulted in producers and customers observing a texture and visual quality issue of the breast muscle known as woody breast and white striping.

Numerous studies have investigated alternative methods to reduce the severity of woody breast and white striping. In experiment 2, the inclusion of the butyric acid-zinc complex combined with the benefits from chromium propionate produce a positive impact on the severity of woody breast when compared to broilers not fed the additives. Although breast meat yield is increasing in experiment 1 and 2, the additive effect of the butyric acid-zinc and chromium propionate, produce a reduction in the severity if woody breast when compared to broilers not supplemented with the products.

Current research is limited on the inclusion of chromium propionate in poultry diets, so many opportunities still exist to determine the benefits associated with the inclusion of chromium propionate. Experiment 1 and experiment 2 show that there is a corticosterone reduction observed in broilers, which leads to improvements observed in broiler performance and yields. Experiment 2 shows that chromium propionate and a butyric acid zinc complex have an additive effect in reducing corticosterone, and the butyric acid-zinc complex may reduce woody breast. Although these trials show many improvements with the inclusion of chromium propionate and the butyric acid-zinc complex, many opportunities still exist to further investigate the additive effects on meat quality and stress related measurements.

## REFRENCES

- Altan, Ö., A. Pabuçcuoğlu, A. Altan, S. Konyalioğlu, and H. Bayraktar. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. British poultry science 44:545-550.
- Anderson, R. A. 1997. Chromium as an essential nutrient for humans. Regulatory toxicology and pharmacology 26:S35-S41.
- Aviagen. 2014. Ross 708 Broiler Performance Objectives.
- Brossi, C., C. J. Contreras-Castillo, E. d. A. Amazonas, and J. F. M. Menten. 2009. Estresse térmico durante o pré-abate em frangos de corte. Ciência Rural 39:1284-1293.
- Brouns, F., B. Kettlitz, and E. Arrigoni. 2002. Resistant starch and "the butyrate revolution". Trends in Food Science & Technology 13:251-261.
- Chen, N. S., A. Tsai, and I. Dyer. 1973. Effect of chelating agents on chromium absorption in rats. The Journal of nutrition 103:1182-1186.
- Cobb-Vantress, I. 2012. Cobb700 Broiler Performance & Nutrition Supplement Guide.
- Council, N. R. 2010. Recognition and alleviation of pain in laboratory animals. National Academies Press.
- Dalolio, F., L. ALBINO, J. SILVA, P. CAMPOS, H. LIMA, J. MOREIRA, and V. R. JUNIOR. 2017. Dietary chromium supplementation for heat-stressed broilers. World's Poultry Science Journal:1-16.
- Eze, D., E. Okwor, W. Anike, H. Kazeem, and K. Majiyagbe. 2014. Effect of chromium propionate on the humoral immune response and performance of broilers vaccinated against Newcastle disease in the tropics.
- Gee, K. 2016. Poultry's tough new problem: 'Woody Breast'. Wall Street Journal. Sect. Business and Tech 267:B1.
- Geraert, P., J. Padilha, and S. Guillaumin. 1996. Metabolic and endocrine changes induced by chronic heatexposure in broiler chickens: growth performance, body composition and energy retention. British Journal of Nutrition 75:195-204.
- Ghazi, S., M. Habibian, M. Moeini, and A. Abdolmohammadi. 2012. Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biological trace element research 146:309-317.
- Haq, Z., R. Jain, N. Khan, M. Dar, S. Ali, M. Gupta, and T. Varun. 2016. Recent advances in role of chromium and its antioxidant combinations in poultry nutrition: A review. Veterinary world 9:1392.
- Hess, J., S. Bilgili, A. Parson, and K. Downs. 2001. Influence of complexed zinc products on live performance and carcass grade of broilers. Journal of Applied Animal Research 19:49-60. Howlider, M., and S. Rose. 1987. Temperature and the growth of broilers. World's Poultry Science Journal 43:228-237.
- Huff, W. E., G. W. Malone, and G. W. Chaloupka. 1984. Effect of Litter Treatment on Broiler Performance and Certain Litter Quality Parameters 1. Poultry Science 63:2167-2171. doi 10.3382/ps.0632167
- Jackson, A., S. Powell, S. Johnston, J. Shelton, T. Bidner, F. Valdez, and L. Southern. 2008. The effect of chromium propionate on growth performance and carcass traits in broilers. Journal of Applied Poultry Research 17:476-481.

- Kaczmarek, S., A. Barri, M. Hejdysz, and A. Rutkowski. 2016. Effect of different doses of coated butyric acid on growth performance and energy utilization in broilers. Poultry science 95:851-859.
- Kim, Y., I. K. Han, Y. Choi, I. Shin, B. Chae, and T. Kang. 1996. Effects of dietary levels of chromium picolinate on growth performance, carcass quality and serum traits in broiler chicks. Asian-Australasian Journal of Animal Sciences 9:341-347.
- Kuttappan, V. A., Y. S. Lee, G. F. Erf, J. F. C. Meullenet, S. R. McKee, and C. M. Owens. 2012. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poultry Science 91:1240-1247. doi 10.3382/ps.2011-01947
- Lara, L. J., and M. H. Rostagno. 2013. Impact of heat stress on poultry production. Animals 3:356-369.
- Leeson, S., H. Namkung, M. Antongiovanni, and E. H. Lee. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poultry Science 84:1418-1422. doi 10.1093/ps/84.9.1418
- Lesson, S. Year. Butyrate lancing science versus societal issues in poultry nutrition. Proc. Nutr Abstr Rev.
- Lien, T., K. Yang, and K. Lin. 2005. Effects of chromium propionate supplementation on growth performance, serum traits and immune response in weaned pigs. Asian-Aust. J. Anim. Sci 18:403-408.
- Moberg, G. P. 2000. Biological response to stress: implications for animal welfare. The biology of animal stress: basic principles and implications for animal welfare:1-21.
- Nain, S., B. Ling, B. Bandy, J. Alcorn, C. Wojnarowicz, B. Laarveld, and A. Olkowski. 2008. The role of oxidative stress in the development of congestive heart failure in a chicken genotype selected for rapid growth. Avian pathology 37:367-373.
- Oba, A., P. C. F. Lopes, M. M. Boiago, A. M. S. Silva, H. J. Montassier, and P. A. d. Souza. 2012. Características produtivas e imunológicas de frangos de corte submetidos a dietas suplementadas com cromo, criados sob diferentes condições de ambiente. Revista Brasileira de Zootecnia:1186-1192.
- Owens, B., L. Tucker, M. Collins, and K. McCracken. 2008. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. British poultry science 49:202-212.
- Panda, A., S. R. Rao, M. Raju, and G. S. Sunder. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Australasian Journal of Animal Sciences 22:1026-1031.
- Pryde, S. E., S. H. Duncan, G. L. Hold, C. S. Stewart, and H. J. Flint. 2002. The microbiology of butyrate formation in the human colon. FEMS microbiology letters 217:133-139.
- Quarles, C. L., and H. F. Kling. 1974. Evaluation of Ammonia and Infectious Bronchitis Vaccination Stress on Broiler Performance and Carcass Quality1. Poultry Science 53:1592-1596. doi 10.3382/ps.0531592
- Quinteiro-Filho, W. M., A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, M. Sakai, L. R. M. Sá, A. J. P. Ferreira, and J. Palermo-Neto. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poultry Science 89:1905-1914. doi 10.3382/ps.2010-00812

- Rajalekshmi, M., C. Sugumar, H. Chirakkal, and S. Ramarao. 2014. Influence of chromium propionate on the carcass characteristics and immune response of commercial broiler birds under normal rearing conditions. Poultry science 93:574-580.
- Renaudeau, D., A. Collin, S. Yahav, V. De Basilio, J. Gourdine, and R. Collier. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6:707-728.
- Sahin, K., N. Sahin, and O. Kucuk. 2003. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 C). Nutrition Research 23:225-238.
- Sahin, K., N. Sahin, M. Onderci, F. Gursu, and G. Cikim. 2002. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. Biological Trace Element Research 89:53-64.
- Sahin, N., A. Hayirli, C. Orhan, M. Tuzcu, F. Akdemir, J. Komorowski, and K. Sahin. 2017. Effects of the supplemental chromium form on performance and oxidative stress in broilers exposed to heat stress. Poultry Science.
- Samanta, S., S. Haldar, V. Bahadur, and T. K. Ghosh. 2008. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. Journal of the Science of Food and Agriculture 88:787-796. doi doi:10.1002/jsfa.3146
- Sands, J. S., and M. O. Smith. 1999. Broilers in Heat Stress Conditions: Effects of Dietary Manganese Proteinate or Chromium Picolinate Supplementation. The Journal of Applied Poultry Research 8:280-287. doi 10.1093/japr/8.3.280
- Siegel, H. 1980. Physiological stress in birds. Bioscience 30:529-534.
- Sihvo, H.-K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Veterinary Pathology 51:619-623.
- Smith, D. J., A. Barri, G. Herges, J. Hahn, A. G. Yersin, and A. Jourdan. 2012. In vitro dissolution and in vivo absorption of calcium [1-14C] butyrate in free or protected forms. Journal of agricultural and food chemistry 60:3151-3157.
- Snel, J., H. Harmsen, P. Van der Wielen, and B. Williams. 2002. Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. Nutrition and health on the gastrointestinal tract:37-69.
- Thaxton, J., P. Stayer, M. Ewing, and J. Rice. 2005. Corticosterone in commercial broilers. Journal of applied poultry research 14:745-749.
- 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. doi 10.3382/ps/pew129
- Toghyani, M., M. Toghyani, M. Shivazad, A. Gheisari, and R. Bahadoran. 2012. Chromium supplementation can alleviate the negative effects of heat stress on growth performance, carcass traits, and meat lipid oxidation of broiler chicks without any adverse impacts on blood constituents. Biological trace element research 146:171-180.
- Uerlings, J., Z. G. Song, X. Y. Hu, S. K. Wang, H. Lin, J. Buyse, and N. Everaert. 2018. Heat exposure affects jejunal tight junction remodeling independently of adenosine monophosphate-activated protein kinase in 9-day-old broiler chicks. Poultry Science:pey229-pey229. doi 10.3382/ps/pey229

Van Immerseel, F., F. Boyen, I. Gantois, L. Timbermont, L. Bohez, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2005. Supplementation of coated butyric acid in the feed reduces colonization and shedding of Salmonella in poultry. Poultry science 84:1851-1856.

Virden, W., and M. Kidd. 2009. Physiological stress in broilers: Ramifications on nutrient digestibility and responses. Journal of Applied Poultry Research 18:338-347.

Williams, R. 2002. Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathology 31:317-353.

Yegani, M., and D. R. Korver. 2008. Factors Affecting Intestinal Health in Poultry. Poultry Science 87:2052-2063. doi 10.3382/ps.2008-00091

Young, R., H. Edwards Jr, and M. Gillis. 1958. Studies on zinc in poultry nutrition: 2. zinc requirement and deficiency symptoms of chicks. Poultry Science 37:1100-1107.

Zdunczyk, Z., R. Gruzauskas, J. Juskiewicz, A. Semaskaite, J. Jankowski, I. Godycka-Klos, V. Jarule, A. Mieželiene, and G. Alencikiene. 2010. Growth performance, gastrointestinal tract responses, and meat characteristics of broiler chickens fed a diet containing the natural alkaloid sanguinarine from Macleaya cordata1. The Journal of Applied Poultry Research 19:393-400. doi 10.3382/japr.2009-00114

Zuidhof, M., B. Schneider, V. Carney, D. Korver, and F. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poultry Science 93:2970-2982.