PREVENTIVE MEASURES TO CONTROL CLOSTRIDIAL OUTBREAKS OF GANGRENOUS DERMATITIS IN COMMERCIAL BROILER OPERATIONS

A Thesis

by

CASEY RAE WANECK

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

MASTER OF SCIENCE

May 2010

Major Subject: Poultry Science

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ABSTRACT

Preventive Measures to Control Clostridial Outbreaks of Gangrenous Dermatitis in Commercial Broiler Operations. (May 2010)

Casey Rae Waneck, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Morgan B. Farnell
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Gangrenous dermatitis (GD) has become a major health problem among broiler flocks in the United States, resulting in high mortality, carcass condemnations, and trimmed parts. There are large economic losses due to GD. *Clostridium septicum*, *Clostridium perfringens* type A, and *Staphylococcus aureus* are the etiologic agents associated with GD. Gangrenous dermatitis has been associated with birds that have a compromised immune system.

It is known that the gastrointestinal (GI) tract plays a crucial role in animal health and performance. The development of a healthy normal microflora in the GI tract benefits the host by improved resistance to pathogens. Our hypothesis is the application of commercial disinfectants, probiotics, vitamins, acidifiers, and windrowing technologies will reduce *Clostridium* levels in poultry operations. The objective of the first study was to administer probiotics to commercial broilers on three farms periodically throughout the grow-out cycle to conclude if bird health and performance was improved. The objective of the second study was to use commercial disinfectants, vitamins, acidifiers, and windrowing technologies on three farms in multiple houses and

determine their effects on broiler production parameters. During grow-out, standard production practices were followed in all experiments and standard production parameters were measured.

On all three farms in this study, the probiotic-treated houses had no mortality due to GD and an increase ($P \leq 0.05$) in body weight gain was observed unlike their respective control houses. These experiments indicate that the application of probiotic in this field trial significantly altered the onset of GD by providing the birds with normal GI flora that contributed to their overall health during a commercial field study.

When evaluating the different products and field technologies to control GD, our laboratory observed that treatment houses that were windrowed and received added vitamins did break with GD. Houses that were treated with peroxymonosulfates and monoglyceride, peroxymonosulfates, or glutaraldehyde litter disinfectants; acidifiers or vitamins had higher gross and net pounds weight gain at processing than their respective control houses. In conclusion, the significance of this work was to determine if products and technologies can be used by growers in commercial broiler houses to eliminate disease.

DEDICATION

I dedicate this thesis to my mother. It is not easy raising three children as a single parent but you made the best of it and always supported us in anything that we wanted to do. I have you to thank for the way that I have turned into a responsible adult. Also, would like to thank you for your unselfish kindness and generosity and how sometimes you went without having things you wanted so I could fulfill my dreams. I know that I have not always been the easiest person to get along with and I may have stubborn ways but you saw through them and only saw the best in me. I can only hope that one day I can be as good of a mother as you have always been to me.

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years, for never doubting me, and always seeing how much better I could do even when I was doing my best.

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I have been blessed to have so many great co-workers, friends, and family in my life. Thank you to everyone for everything you have done to help me become the person

I am today and for enabling me to accomplish the goals that I have. Thank all of you for your kindness and support.

NOMENCLATURE

CFU	Colony forming unit
CoA	Coenzyme A
CP	*Clostridium perfringens*
CS	*Clostridium septicum*
d	Day
g	Gram
GA	Glutaraldehydes
gal	Gallons
GALT	Gut-associated lymphoid tissue
GD	Gangrenous dermatitis
GI	Gastrointestinal
GTP	Guanosine triphosphate
h	Hour
IgA	Immunoglobulin A
min	Minute
mL	Milliliter
NE	Necrotic enteritis
oz	Ounce
POXM	Peroxymonosulfates
SA	*Staphylococcus aureus*
TCA	Tricarboxylic acid cycle
wk	Week

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

Page

Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

Commercial broilers are reared in large flocks consisting of approximately 13,000-27,500 birds per house. Poultry integrators continually look for ways to influence and improve management practices. Even with the best management, pathogens are still present in the environment, and when given the opportunity will flourish and often cause disease. These environmental pathogens include viruses, bacteria, and parasites. Reducing pathogens in poultry houses has been attempted with a wide variety of management tools. Most pathogens seen in the poultry industry typically produce subclinical infections; however, inducing immunosuppression provides a niche for infections with opportunistic pathogens such as *Clostridium*. *Clostridium* is a potential pathogen that has been historically controlled with sub-therapeutic levels of antibiotics that target Gram-positive bacteria in the digestive tract. *Clostridium* is one of the two of the etiologic agents causing Gangrenous dermatitis (GD). Recently, there has been an increase of this disease in commercial broiler operations across the United States and has become a significant economic problem for the industry.

Understanding the disease progression of GD has been very difficult due to its complexity and predisposing factors (dietary components, immunosuppression,

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This thesis follows the style of Poultry Science.

gut health, and management practices) that contribute to this disease. The microbial ecology of the gastrointestinal (GI) tract is important as one of the first lines of defense against invading pathogenic bacteria (Fuller, 1989). Administration of probiotics has shown to be important to establish the beneficial bacteria in the GI tract within the first few days of life. Other products or technologies that reduce the pathogen load within the environment are important to keep pathogenic bacteria from becoming established in the bird throughout the grow-out period. The goals of these studies are to establish beneficial bacteria within the GI tract of broilers by administering a probiotic and to be able to reduce clostridia in the litter and waterlines by using disinfectants, litter amendments, and composting litter. By preventing these pathogenic bacteria from becoming established in the host we hope it reduce the onset of GD. Research in this thesis is focused on the prevention and control of *Clostridium* and GD.

CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

Certain microorganisms cause disease and are known as pathogens; these microorganisms include parasites, viruses, and bacteria. Several bacterial pathogens are in the genus *Clostridium*, that can cause serious illness in humans, such as *Clostridium botulinum, C.difficile*, *C. tetani,* and *C. perfringens* (Allen et al., 1999). Most clostridia are opportunistic pathogens that when provided with the appropriate local environmental conditions they will flourish (Allen et al., 1999). The normal intestinal microflora of poultry protects the host from these bacteria (Fuller, 1989). However, if the ecology of the gut is disturbed these pathogens can grow and cause diseases such as Necrotic enteritis (NE) and Gangrenous dermatitis (GD).

CLOSTRIDIUM

Both *Clostridium septicum* (CS) and *Clostridium perfringens* (CP) type A are spore-forming, Gram-positive, rod-shaped bacteria that grow in anaerobic conditions and are found in many areas of the environment. *Clostridium perfringens* forms large, round, slightly opaque, and shiny colonies when grown anaerobically on agar. Theses colonies typically have a double-zone of hemolysis on blood agar plates that has a clear inner theta-toxin zone and an outer zone caused by alpha-toxin production. *Clostridium septicum* is a motile bacterium that swarms on agar plates and induces hemolysis on blood plates. The optimum temperature of growth of CP is 45°C, but the bacterium can grow between 15 and 50°C. The average generation time for most CP strains is an

average of 30.8 min, but times as low as 9 min have been reported (Labbe, 2000). Some of the primary host reservoirs of *Clostridium* include humans, cats, cows, pigs, sheep, and chickens (Maier et al., 2000).

Toxins

Clostridium perfringens produces a large variety of biologically active toxins that play a significant role in its pathogenicity. There are five extracellular toxins including toxin types A through E and four major toxins: α, β, ε, and ι represented in Table 1. The β-2-toxin is the most recently discovered toxin (Hatheway, 1990). The α, β, and ε-toxins are extracellular and disrupt cell membranes by forming pores. The ι-toxin acts intercellularly. *Clostridium perfringens* is ubiquitous in nature and type A is most commonly found in the environment and digestive tract of most animals. The other types of CP are more host specific: CP type D is commonly isolated from ruminants, typically sheep; CP type C is found mainly in pigs; and CP type E is found in calves (Hatheway, 1990; Songer, 1996). *Clostridium perfringens* type A causes NE in poultry while types B, D, and E do not cause disease in poultry (Immerseel et al., 2004).

Table 1: *Clostridium perfringens* Types A through E and their corresponding toxins.

	Type A	Type B	Type C	Type D	Type E
α (Alpha)	+	+	+	+	+
β (Beta)		+	+		
ε (Epsilon)		+		+	
ι (Iota)					+

Enterotoxin. *Clostridium perfringens* also produces a CP enterotoxin (CPE) that is released at the completion of sporulation. Once the CPE is released into the luminal contents, it binds to the epithelial cells, which causes characteristic symptoms such as diarrhea and abdominal cramps. Over the last 15 years, CPE is found in the GI flora and has become a major factor in non-foodborne GI diseases.

Sporulation

When bacteria are subjected to harsh natural environmental conditions they must adapt quickly. The optimal temperature range for CP to sporulate is 35 to 40ºC (Garcia-Alvarado et al., 1992). Clostridia are very good at adapting to their environmental conditions as demonstrated by their ability to be ubiquitous in nature. When clostridia are in favorable conditions, they maintain normal cellular activity and reproductive functions. However, in response to nutrient deprivation, this bacteria has alternative mechanisms which aid in its search of nutrients. Clostridia can synthesize a flagella that aids in the search for metabolizable carbon, nitrogen and phosphorous compounds (Bahl and Durre, 2001). Key enzymes of the tricarboxylic acid cycle (TCA cycle) and other carbon utilization enzymes are expressed, giving the bacteria a wider range of energy metabolism. The bacteria also increase production of their extracellular enzymes including proteases, nucleases, amylases, phosphorlyases, and other hydrolytic enzymes that aid in energy acquisition. If all of these fundamental changes do not result in adequate uptake of energy to support cellular function, then the bacteria will enter into a stage known as sporulation (Rood et al., 1997; Bahl and Durre, 2001).

Clostridium's sporulation is generally compared to that of *Bacillus spp.*, which is more recognized and studied. During the sporulation process, bacteria go through morphological, physiological, and biochemical changes (Errington, 1993). *Bacillus* and *Clostridium spp.* species sporulate by incorporating a wide range of environmental and physiological signals that occur from nutrient depletion, cell density, and the Krebs cycle (Stragier and Losick, 1996). Sporulation is not only a basis of survival in unfavorable environmental conditions, but is also a key component for the induction of CPE synthesis which is a major virulence factor released when the mother cell is lysed (McClane, 2007; Paredes-Sabja and Sarker, 2009).

In the *Bacillus spp.* the regulatory protein Spo0A controls the initiation of sporulation and promotes changes in gene expression. *Clostridium perfringens* also has the same regulatory protein that is required for spore formation (Dillon and Labbe, 1989). It is believed that in each species of *Clostridium* the difference in environmental niches might result in different signals required for the initiation of sporulation (Paredes-Sabja and Sarker, 2009). Sporulation is divided into seven stages (I-VII) and is initiated by nutritionally deprived conditions (Paredes-Sabja and Sarker, 2009) which causes a drop in the guanosine triphosphate (GTP) pool (Marks and Freese, 1987). Stage 0 can be described as vegetative cells that proliferate like normal rod-shaped cells that double in length and divide in the middle to produce two identical daughter cells (Ryter, 1965). The beginning of sporulation is referred to as stage I, an asymmetric division resulting in sister cells that differ in size (Warth and Strominger, 1972). Stage II results when a spore septum is complete and the prespore is engulfed by the mother cell (Warth and

Strominger, 1972). Stage III occurs when the engulfment is complete and the membrane around the cytoplasm of the prespore (now named forespore) does not have a layer of peptidoglycan to have a defined shape (Warth and Strominger, 1972). The spore begins to mature in stage IV and takes an oval shape as the cortex or a modified cell wall (Warth and Strominger, 1972) is produced between the prespore membranes. Stage V is recognized by a proteinaceous spore coat that begins to be deposited on the outside surface of the spore. Maturation, stage VI, has little change in morphology but is characterized by properties such as resistance, dormancy, and germinability (Dion and Mandelstam, 1980; Jenkinson et al., 1980). Stage VII is defined when the mother cell lyses and releases a mature spore (Errington, 1993). Through the sporulation process clostridia maintains its vitality in nature for an unknown length of time. When the spore is ingested and given optimal environmental conditions, a mature vegetative cell will grow and proliferate as an active part of the host microbial flora. In the right environment, a new vegetative cell has the potential to become a pathogen and cause diseases such as GD.

The induction of sporulation has been extensively studied *in vitro*. Starch and dextrin are used as a carbohydrate sources in sporulation media of CP (Duncan and Strong, 1968; Sacks and Thompson, 1978). Amylolytic action during sporulation in some media promotes cell growth and sporulation by providing metabolizable, short-chain carbon sources. Synthesis of high levels of α-amalyase requires a small amount (6-10 mM) of a simple sugar (Shih and Labbe, 1994). *Clostridium perfringens* sporulation is inhibited by high concentrations (greater than 15 mM) of glucose, maltose,

mannose, lactose, and sucrose but is unaltered by the presence of high amounts (greater than 15 mM) of ribose, galactose, and fructose (Shih and Labbe, 1996). The absence of inorganic phosphate induces CP sporulation (Duncan and Strong, 1968).

Food Safety

The focus of this discussion will be on CP and how it relates to foodborne illness. *Clostridium perfringens* is ranked forth for most estimated cases of bacterial illness and the third for foodborne illness between years 1983 through 1997 in the United States (Mead et al., 1999). The number of cases is greatly underestimated with outbreaks due to CP representing one of the most common foodborne diseases in industrialized nations (McClane, 1997). The number of cases reported in the United States between 1983 to 1994, has varied between 202 and 1240 (Labbe, 2000). One way this enteric pathogen can be transmitted to humans is through consumption of contaminated poultry products (Labbe, 1991; Immerseel et al., 2004). *Clostridium perfringens* does not have the ability to generate 13 of the 20 essential amino acids; thus it is associated with foods that are high in protein. Of foodborne outbreaks due to CP, 75% can be traced back to meat and processed meat products (Johnson and Gerding, 1997).

Food poisoning is not caused by the bacterium itself, but by the toxins that CP release during early sporulation. A small number of enterotoxigenic cells of CP exist with a large number of nonenterotoxigenic CP cells in the same intestinal sample. Of the fifty samples that were taken from cattle, swine, and broiler chickens, 22 to 40% were positive for enterotoxigenic CP (Miwa et al., 1997). The high percentage of intestinal samples that are positive for this type of CP will likely result in the

contamination of carcasses and processed meat at the slaughterhouse or poultry processing plant (Miwa et al., 1997).

Food poisoning that results from CP is likely due to the presence of heat-resistant spores of enterotoxigenic isolates (McClane, 2007). After consumption of CP-contaminated food, some vegetative cells survive the stomach's acidity and remain viable when entering the small intestine where the cells multiply and sporulate releasing harmful toxins (McClane, 2007; Paredes-Sabja and Sarker, 2009). The CPE also has devastating effects on the mucosal lining of the intestine inhibiting glucose absorption and the release of large amounts of intestinal fluid and electrolyte loss, as well as extensive histopathological damage (Rood et al., 1997). *Clostridium perfringens* enterotoxin causes Type A food poisoning which results from the consumption of at least 10^7 CP. The incubation time is between 6-24 h after ingesting contaminated food and symptoms includes acute abdominal pain, nausea, and diarrhea (Andersson et al., 1995). Illness typically lasts 24 h, death is rare but does occur due to dehydration in the elderly and very young (Brynestad and Granum, 2002).

Clostridium perfringens spores can survive for one h or longer at boiling temperatures in a relatively protective medium (Labbe, 1989; Sarker et al., 2000). Different CP strains show substantial variation in heat resistance in food isolates that cause food poisoning in humans. The CP spores that cause food poisoning have greater heat resistance than spores of CP that cause non-foodborne gastrointestinal diseases (Sarker et al., 2000). It is important to note that incomplete cooking and inadequate heating may not kill CP spores in foods but may actually induce spore germination that

causes food poisoning (Paredes-Sabja et al., 2008). By being tolerant of low temperatures (Li and McClane, 2006) spores may germinate and cause food poisoning when the food is warmed for serving after being refrigerated or frozen (Paredes-Sabja and Sarker, 2009).

Microbial Ecology

Clostridium perfringens not only affects humans, but it also has the potential to adversely affect poultry. Understanding the disease progression of clostridia in poultry has been very difficult due to its complexity and several predisposing factors such as diet, immuno-suppression, mechanical irritation of the gut, and sudden gut microflora changes (Smith, 1965; Elwinger et al., 1992; Calnek, 1997). Bacteria in the GI tract derive most of their nutritional requirements for reproduction and growth from dietary components. These nutritional components are either not broken down by digestive fluids or are absorbed slowly enough that bacterial populations can compete for them. Since many bacteria utilize different substrates for growth, it is important to understand that the dietary composition largely determines the microbial make-up of the GI tract (Apajalahti and Bedford, 2000). Specific species of bacteria, including lactic acid producing bacteria, can be selected by administering certain feed ingredients that are specifically utilized by the bacteria and not by the host. Some of these ingredients include prebiotics, such as dietary fiber and oligosaccarides. Sudden changes in rations can alter the native microbial population and give rise to opportunistic bacteria such as clostridia (Apajalahti and Bedford, 2000). Investigations evaluating the alimentary tract of the chicken during onset of NE, a clostridial disease, can be attributed to the diet fed

to birds (Smith, 1965; Nairn and Bramford, 1967). It has also been shown that high levels of fish meal and wheat in the diet exacerbate outbreaks of NE (Johnson and Pinedo, 1971; Truscott and Al-Sheikhly, 1977; Branton et al. 1987; Riddell and Kong, 1992). Increased disease prevalence could be associated with the high protein levels in the fish meal that cause a shift in the microbiota, or in the case of the wheat diet may be associated with the high levels of non-starch polysaccharides such as hexose and pentose that are resistant to digestive enzymes. When working with clostridial related diseases such as NE or GD, understanding the effects of dietary components in maintaining the homeostatic microbial ecology of the GI tract is an important consideration.

GANGRENOUS DERMATITIS

Gangrenous dermatitis has become a major health problem among broiler flocks in the United States and is accompanied by high mortality, carcass condemnations, and trimmed parts. Economic losses are estimated to be as much as $1.31 per affected bird (Cocci Forum, 2008). There are also large economic losses involved in antibiotic therapy associated with treatment of GD. The known etiologic agents of the disease are CS, CP type A, and *Staphylococcus aureus* (SA), either individually or in combination (Ficken and Wages, 1997). While natural outbreaks of the disease have been reported in chickens from 17 to 140d-of-age, the majority are reported in 4-to 8-wk-old broilers (Damerow, 1994). Clinical signs of GD are limited because the period of illness is generally short (less than 24h) prior to birds being found dead and mortality observed can be between 60-100% (Damerow, 1994). Post-mortem observations include: air in the subcutis with underlying hemorrhagic musculature and lesions found on the

abdomen and legs (Hofarce et al., 1986). The bird's skin often feels "spongy" due to gas production from accumulating bacteria between the muscle and dermis.

Understanding the disease progression of GD has been difficult due to the biological complexities of the disease and diverse predisposing factors that are thought to give CS, CP, and SA an opportunity to cause disease. In a commercial setting there are several time-points when GD occurs. Outbreaks of GD are associated with vaccination, viral infections, immunosuppression (Rosenberger et al., 1975), coccidial infections (Baba et al., 1996), dietary changes (Kahn, 2005), sudden gut microflora changes, poor management practices, and standard production grow-out stresses. Gangrenous dermatitis is often referred to as necrotic dermatitis, gangrenous cellulitis, gangrenous dermatomyositis, avian malignant edema, gas edema disease, wing rot, and blue wing disease in turkeys (Flicken and Wages, 1997). In chickens, blue wing disease is caused by chicken infectious anemia virus (Engstrom and Luthman, 1984).

The current theory of GD is that the etiological agents are obtained from the environment and results in dermal lesions. These lesions are believed to be contributed to overcrowding in broiler houses. The dermal scratches from toenails contain pathogenic bacteria from the high loads of bacteria in the litter (Ritter, 2008). The scratches allow for an entryway for the bacteria into the dermis to proliferate inside the bird, thus causing gangrenous-type lesions on the skin and disease (Willoughby et al., 1996; Ritter, 2008).

Other theories include this disease beginning in the GI tract with the pathogenic bacteria overtaking the beneficial bacteria and translocating through the mucosal layer to

other organs including the dermis. Bacterial translocation is defined as "the passage of certain indigenous bacteria from the GI tract to the mesenteric-lymph-node-complex and other extraintestinal organs" (Berg and Garlington, 1979). There are multiple factors that contribute to bacterial translocation including bacterial overgrowth in the intestine, insufficient host defense, increased permeability, or damage of the intestinal mucosal barrier (Berg, 1995). This theory of pathogenic bacteria translocating to the dermis has yet to be reproduced in an experimental setting, but could explain why some birds found dead with GD do not have any skin abrasions or dermal lesions (Fowler and Hussaini, 1975).

Etiologic Agents

The etiologic agents known to cause this disease are CS, CP type A, and SA, either individually or in combination (Ficken and Wages, 1997). All of these opportunistic pathogens and reside in the GI tract of host animals and in nature. Given the right conditions these bacteria will flourish, potentially giving rise to disease (Miliotis and Bier, 2003).

Staphylococcus aureus is a Gram-positive cocci, found in grape-like clusters, and is a facultative anaerobe that causes a variety of suppurative infections in humans and domestic animals. *Staphylococcus aureus* also causes superficial skin lesions such as boils, styes, and urinary tract infections in humans and bumblefoot, osteomylitis, arthritis-synovitis, and GD in commercial poultry (Kloos and Bannerman, 1999). This bacterium is commonly found on the skin and in mucous membranes of poultry (Flicken and Wages, 1997).

Causative Factors

Gangrenous dermatitis is associated with birds that have a compromised immune system and occurs as a sequela to disease produced by other infectious agents such as infectious bursal disease virus, chicken anemia virus, avian adenovirus infections, Marek's disease, reovirus, and mycotoxins (Rosenberger et al., 1975; Hagood et al., 2000; Ritter, 2008). Gangrenous dermatitis often occurs secondary to skin hemorrhages caused by viral infections (Ficken and Wages, 1997).

Additional factors that exacerbate GD include vaccination programs for coccidia and chicken anemia virus (Hagood et al., 2000; Cocci Forum, 2008). Interestingly, some outbreaks of GD are parental related. A specific broiler-breeder flock's progeny can repetitively break with GD (Gerdon, 1973). For example, lack of antibodies in parental lines to a particular virus, like infectious bursal disease virus, appear to make the offspring more susceptible to early infection of infectious bursal disease virus. This early immune competency predisposes the progeny to other infectious agents like *Clostridium* and *Staphylococcus* and these birds then break with GD (Gerdon, 1973). The lack of antibodies to infectious bursal disease in breeder flocks is related to increased susceptibility of progeny to chicken anemia virus so that when birds are infected with the virus it leaves them immunosuppressed and more likely to get GD (Rosenberger et al., 1975).

In the commercial broiler industry, birds are fed strict diets that are designed specifically to address the nutritional requirements for each stage of life. Typically, commercial integrators will change these basal rations four to five times during the six

week grow out cycle. Because of these feed changes, birds are stressed and the ecology of the GI tract is altered (Helfer et al., 1969). At feed changes (starter, grower, and finisher diets) concentrations of dietary components are suddenly altered including protein, carbohydrates, and vitamins. Historically, outbreaks of GD occur at the transitions between grower and finisher rations. The purpose of feed changes is to be economically efficient for corporations while improving livability for the birds.

Through our industry relations and reviewing previous literature we determined that one factor that may be involved in the increased susceptibility to GD is the reduction in vitamins from a starter to finisher diet. Previous observations from Kahn (2005) indicate that vitamin deficiencies can play an integral role in the development of dermatitis. Vitamin B_5, also known as pantothenic acid, is essential for all forms of life, including chickens. Pantothenic acid is found in living cells in the form of coenzyme A (CoA), a vital coenzyme in numerous chemical reactions that aid in the digestion of fats, proteins, and carbohydrates for energy and the production of cholesterol and steroids. In poultry, there are many symptoms characteristic of a pantothenic acid deficiency including reduced growth, feed consumption, poor feather growth, and rapidly developing dermatitis (Kahn, 2005).

The primary broiler diet is corn- and soy-based. However, it is possible that a dietary ration's main components can change to what ingredient is economically efficient and available, at that time especially if a commercial integrator uses a grain source such as wheat, barley, or rye. This dietary change may also cause a shift in the intestinal microbiota. It has been shown that wheat can act as the sole source of dietary

protein in chicken diets and can contribute to increased numbers of *Clostridium* in the gut (Jeppesen and Grau 1948). This increased prevalence of *Clostridium* in the GI tract is associated with the high levels of non-starch polysaccharides such as hexose and pentose that are resistant to digestive enzymes. Sudden changes in the main nutritional component of a feed can also contribute to GD by increasing the number of CP, CS, and SA in the GI tract.

Coccidiosis, an enteric parasitic disease is caused by the protozoa *Eimeria*. Coccidia infections can lead to tissue damage, poor nutrient absorption, dehydration, blood loss, and increase the development of GD (Williams, 2005). When chickens are infected with different *Eimeria spp*., the clostridial population in specific regions of the GI tract increase (Baba et al., 1996; Collier et al., 2007). Gastrointestinal lesions caused by *Eimeria maxima* provide a point of entry for clostridia (Cocci Forum, 2008). A coccidial infection increases the mucus production of the gut, clostridial populations, and the opportunity for bacterial translocation (Deplancke et al., 2002).

The poultry industry has strict grow-out protocols that producers follow. These grow-out procedures are not only for the health and welfare of the birds but also for controlling the rate of early growth to reduce stress. By restricting early growth, feed conversion and livability are improved. There are multiple conditions that the industry uses to reduce growth and stress on birds including temperature, lighting, feed changes, density, and litter moisture. From our on-farm experiences and industry sources, it was determined that improper house temperature, litter moisture, and most importantly not removing dead birds could result in GD. Since the pathogens associated with GD are

ubiquitous in the environment, inadequate litter moisture and extreme high and low temperatures in poultry houses with dead birds will give these bacteria a chance to flourish and cause GD.

There is broad host/pathogen dynamics and possible mechanisms that contribute to the dramatic changes in the resistance of broilers to clostridial infections during a grow-out period. Alterations in intestinal microflora, intestinal physiology, and host defenses all contribute to decreased immune resistance of broilers (Fuller, 1989). Different stresses on birds, including sudden changes in gut ecology, will weaken tight junctions in the intestinal epithelial and increase the chance for bacterial translocation from the GI tract into systemic circulation (Fuller, 1989). These two changes may allow the bacteria to move to alternate areas of the body and increase the chance of a GD outbreak.

Preventative Measures

There are multiple preventive measures that a grower can enforce to prevent their farm from breaking with GD. As demand for antibiotic-free food products increase and antibiotic-resistance also increase, it is important to develop alternative methods of prevention and treatment. The best way to prevent GD starts with the management of a broiler farm. There is proven research regarding beneficial use of litter disinfectants, composting, litter amendments, and probiotics to prevent GD which will be discussed later (Dvorak, 2005; Lung et al., 2001; Macklin et al., 2007; Liao, 2009; Pope and Cherry, 2000; Nurmi et al., 1992). If the industry still cannot prevent disease from occurring, new methods will be required.

Outbreaks of this disease are sporadic, and good management practices are the best preventive measure. Removing old litter and disinfecting a broiler house is ideal but are not always practical if there is only a few days of down-time available between flocks. Regular maintenance of a farm is required by every grower. Small problems can rapidly turn into large ones very quickly. For example, leaky waterlines contribute to wet litter, broken feedlines can create moldy feed, holes in curtain walls prevents adequate ventilation, and even malfunction of lighting, heating, and cooling systems can stress the birds. From our experiences on the farm, stocking densities are also very important. Typically, if one end of a broiler house contains a higher density of birds, it is believed to more likely to break with GD due to birds piling up and causing dermal lesions.

Litter Disinfectants. In the commercial broiler industry, bedding material is one of the major expenses in production. To alleviate some of these incurred costs, litter is typically recycled from flock to flock, sometimes for upward of a year and a half. Under these conditions, litter may harbor high levels of CP, CS, and SA; therefore, increasing the likelihood of a GD outbreak. Entire house clean-out is not always practical, so evaluation of alternative approaches to reduce these bacteria would be beneficial to the commercial poultry industry. One possible alternative measure is the use of chemical disinfectants.

Glutaraldehydes (GA) are a type of aldehyde disinfectant that can reduce bacteria, fungi, viruses, mycobacteria, and spores (Jeffrey, 1995). Glutaraldehydes accomplish sterilization by denaturing proteins and disrupting nucleic acids (Maris,

1995; Ewart, 2001). This disinfectant works best at a pH above 7, at high temperatures, and are more effective in the presence of organic matter than other aldehyde disinfectants (Greene, 1998; Quinn and Markey, 2001). Glutaraldehydes are non-corrosive to metals, rubber, plastic, and cement (Morley, 2002). Therefore, GA would be a practical disinfectant for use by the poultry industry.

Another disinfectant that can be applied to used litter is peroxymonosulfates (POXM), which works as an oxidizer (Dvorak, 2005). Peroxymonosulfates are broad-spectrum disinfectants used on hard surfaces and equipment. These peroxide-based compounds function by denaturing the proteins and lipids of microorganisms (Maris, 1995). This disinfectant has a broad microbial spectrum of activity and some efficacy in the presence of organic material; therefore, it is also appropriate for use in a poultry facility (Shulaw and Bowman, 2001).

Iodine-based compounds are a halogen type of disinfectant. Iodine compounds are wide spectrum compounds, affordable, and are easy to use. They are also less toxic compared to other disinfectants, yet are considered efficient for a wide range of bacteria, mycobacteria, fungi, and viruses (Jeffrey, 1995). Iodine-based compounds denature proteins to hinder the enzymatic systems of microorganisms (Maris, 1995). Concentrated iodine compounds can damage rubber and some metals and are inactivated by organic debris (Shulaw and Bowman, 2001). For these reasons, iodine-based products are applied prior to another disinfectant that works well in the presence of organic material.

Monoglyceride fatty acids are another type of disinfectant that are commonly used by the poultry industry. However, there is much yet to be discovered with this type of disinfectant, but it is used in a wide array of livestock facilities. The use of these products in a commercial poultry management should be considered because it could potentially reduce high levels of pathogenic bacteria, which could decrease mortality associated with GD.

Composting. Composting litter is another viable approach to reduce the etiologic agents of GD and the overall microbial load in litter. Composting is a cost-effective way to reduce pathogens by pasteurization. This term is commonly used interchangeably with composting because it is a process of using heat to kill microbial organisms that can potentially cause disease. Composting also uses ammonia to kill pathogens in the litter. The target temperature desired for the inside of the compost pile is 135˚F, but temperatures as high as 130˚F will reduce pathogens in the litter (Macklin et al., 2007). In-house composting is carried out for 5-10 d and the litter may be turned one or more times to efficiently compost all of the litter in the house (Macklin et al., 2007). Composting cow manure for 48 h and 72 h eliminates all *Samonella enteritidis* and *Escherichia coli* 0157:H7 (Lung et al., 2001), and *Clostridium* can be reduced by 99% in composted litter compared to non-composted litter (Macklin et al., 2007). In a recent study involving three foodborne pathogens in composted and uncomposted litter, *Salmonella* was entirely eliminated; *Campylobacter* was unrecoverable in both samples, and CP had a slight (less than one log) reduction in composted litter. Even the slightest

reduction of CP may to be economically important to the broiler industry because of its disease-causing potential (Macklin et al., 2008).

Litter Amendments. There are several factors that contribute to a pathogens' ability to colonize the litter including: moisture, litter pH, temperature, and environmental oxygen levels in the litter. Litter amendments are another viable alternative to reduce pathogenic bacteria in litter (Macklin et al., 2007). Currently, there are several compounds commonly used in the poultry industry to decrease litter pH and reduce ammonia levels in the houses. Addition of an acidifier can reduce *Salmonella* on alfalfa seed by 3.9 colony forming unit (CFU) log units (Liao, 2009). Currently, commercial products are widely used as acidifiers to reduce the microbiota load in litter. Previous work shows that acidifiers may be useful for on-farm pathogen reduction (Pope and Cherry, 2000). The use of acidifiers to reduce the etiologic agents of GD should be evaluated.

Probiotics. It has long been known that the GI tract is composed of a wide array of bacteria that play a crucial role in animal health and performance. The GI microbial community is a sophisticated network of numerous species of bacteria that differs from host to host. There are many factors that play a vital role in the development of a microbial population and include geographical location, age, health status, diet, and type of animal (Savage, 1977). Normal microbial populations develop on the mucosal surfaces which line the nose, mouth, stomach, GI tract, respiratory tract, urinary tract, vagina, and the skin (Klaenhammer, 2001). These bacteria can be classified as commensal bacteria and start to develop at birth. It has been shown that neonatal

children can shed facultative anaerobes reaching concentrations of 10^8 to 10^{10} /g of feces within 2 d of age (IIentges, 1993). As the child develops the microbial populations will change. The adult human body contains 10^{14} cells and of these only 10% are derived from host cells of the body and 90% are derived from the microbial population (Savage, 1977). Indigenous microflora should be able to: (a) grow anaerobically, (b) found in normal adults, (c) able to colonize particular areas of their respective tracts, (d) to colonize their niche during succession in infant animals, (e) maintain stable population levels, and (f) have complex interactions with the mucosal epithelium (Savage, 1977).

A probiotic has been defined as a "live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance" (Fuller, 1991). It was later redefined as "a live microbial food ingredient that is beneficial to health" (Salminen et al., 1998). For a microorganism to be characterized as a probiotic, it must be from the host species it is to be consumed by, safe for the designated species, be able to withstand acid and bile, and be able to be attached to the intestinal mucosa (Ouwehand et al., 1998).

The ability of probiotic microorganisms to colonize the GI tract is not well known (Barrow, 1992). Since microorganisms have a constant turnover rate in the GI tract, it is unknown if a probiotic can establish permanently or for any length of time in the gut. Another factor to consider is adherence to the GI tract wallThe probiotic microorganisms must be able to inhabit the GI tract to be able to benefit the host and combat pathogenic bacteria (Fuller, 1999).

Probiotics are expected to enhance animals' health and growth rates. In poultry that includes feed conversion, digestion and absorption of nutrients, egg production, egg quality, carcass quality, and less carcass contamination of pathogenic bacteria (Fuller, 1999). There are several important practicalities when considering the use of probiotics in the field. Newly hatched chickens may respond better than older chickens because in older birds, the microflora has become more established and is more difficult to influence. Oral dosing of chickens is best but not always practical in the field, so spraying eggs or injecting probiotics into the air sacs has been used (Fuller, 1999). Probiotics, composed of beneficial intestinal microflora from healthy adult chickens can be administered to neonatal chickens for the successful prevention of intestinal colonization by pathogens (Nurmi and Rantala, 1973).

Probiotics, also referred to as competitive exclusion cultures, and are non-pathogenic bacteria that reduce pathogen colonization in the GI tract of animals (Mead, 2002). There are several mechanisms by which probiotics can alter the environment in the GI tract to make it more favorable for beneficial bacteria and adverse for pathogenic bacteria. One mechanism involves competition for intestinal attachment sites on the mucosa of the intestine (Nurmi et al., 1992). It is beneficial for microorganisms of a competitive exclusion culture to fill all available intestinal attachment sites before challenge with a pathogen; thus, the pathogen will pass through the animal. Another method of excluding pathogens is competition for nutrients in the intestine of chickens. If a pathogen does not have the appropriate nutrients for growth, it will not establish in the host (Nurmi et al., 1992). Another suggested method to prevent pathogen

establishment is through the production of compounds that are toxic to invading pathogens. These compounds are primarily the volatile fatty acids (VFA): acetic, propionic, and butyric acids (Nisbet et al., 1996).

Probiotics also stimulate the immune system including enhancement of the humoral immune response, contribution to the intestine's immune barrier (Kaila et al., 1992; Isolauri et al., 1993), stimulation of non-specific host defense to bacterial pathogens (Perdigon et al., 1986), and to assist the intestinal inflammatory response (Isolauri et al., 2001). Probiotics can also alleviate the intestinal inflammation by enhancing the immunoglobulin A (IgA) response which has a stabilizing effect on the GI tract (Isolauri et al., 2001). All of these mechanisms aid the host in fighting off invading microorganisms in the GI tract.

The most common bacterial species in probiotics are *Lactobacillus* and *Bifidobacterium* (Isolauri et al., 2001). Studies performed on *Lactobacilli spp.* shows it increases the humoral immune response (Ogawa et al., 2006), stimulates the mucosal immune system by secreting IgA (Nahashon et al., 1994), and excludes pathogens in the GI tract by improving nonspecific host defense to bacterial pathogens (Perdigon et al., 1986). Many of the commensal bacteria produce compounds known as bacteriocins that effect both Gram-positive and Gram-negative bacteria. Reuterin, a bacteriocin produced by *Lactobacilli*, has been shown *in vitro* to be inhibitory against *Salmonella*, *Shigella*, *Clostridium* and *Listeria* (Naido et al., 1999). *Lactobacilli* also produce lactic acid which also has inhibitory effects on *Salmonella* in the crops of broiler chickens. Corrier and colleagues (1999) investigated the effects of feed withdrawal on crop pH, lactic acid

concentrations, and *Salmonella* concentrations in broiler chickens and showed decreased lactic acid concentrations and increased pH during an 8 h withdrawal period. These results show the importance of the normal microflora in the host animal and further support the use of probiotics.

Hydrogen peroxide is also produced by commensal bacteria which results in the peroxidation of lipid membranes and increased membrane permeability (Nisbet et al., 1996). Other protective products are short chain fatty acids which are generated by the commensal bacteria as an end product of microbial fermentation. These compounds are predominately the VFA, acetic, propionic, and butyric acids, and are to be biological indicators of a healthy microbial ecosystem, as well as having inhibitory effects on *Salmonella* colonization in chickens (Nisbet et al., 1996).

In rats, beneficial bacteria enhance mucosal defense in the GI tract against pathogenic bacteria even when the host is under stress it can cause a disruption of the microbial populations (Zareie et al., 2006). Although not known in birds, this could be important throughout the grow-out period when birds experience multiple stresses including feed changes, vaccinations, and fluctuations in temperature. When young animals are subjected to stressful environments, changes in the structure and activity of the GI microflora occur. The task of probiotic supplementation is to restore these imperfections and provide microflora that reside in undomesticated animals that are unaffected by modern rearing methods (Fuller, 1999). Anytime an animal is stressed their immune defense is weakened leaving it more vulnerable to infection from opportunistic pathogens (Zareie et al., 2006). Oral administration of probiotic isolates

stimulates the innate immune functions of oxidative burst and degranulation in heterophils isolated from chickens (Farnell et al., 2006). Heterophils, part of the innate immune system, are vital component for a chicken's ability to defend itself against foreign invaders. If probiotics stimulate heterophil function, then birds that receive a probiotic may have an improved immune response to bacterial pathogens that cause GD.

There are many benefits to using probiotics in the poultry industry. Through our experiences, the most important for the coporate integrators is decreased feed conversion which translates to increased weight gain. Additionally, there may be an enhanced immune response in birds given probiotics.

CONCLUSION

The United States commercial poultry industry produces 9 billion birds annually (National Chicken Council, 2008). It is important for scientists to develop new technologies to aid in the prevention of enteric diseases. One disease that is very likely to affect the industry over the next several years is GD. When consumers demand antibiotic-free birds in the market place; the poultry industry will be forced to react with new innovative technologies.

This thesis evaluates the effects of commercial disinfectants, vitamins, acidifiers, windrowing technologies, and probiotics and their effects on GD. The research objective of this thesis is to take a multifaceted approach to evaluate several commercial products to help control the etiologic agents of GD in broiler chickens undergoing a field challenge of GD. The working hypothesis is birds receiving these products will potentially exhibit improved animal health, welfare by reducing disease, and production

parameters due to the enhancing of microbial populations within the GI tract and reducing pathogenic bacteria in the environment. The goal is to increase growth parameters of the broiler production by improving production, reducing mortality due to GD, and reducing the administration of antibiotics. The following chapters of this thesis will provide data from these areas of research and will provide the industry with several alternative technologies for the reduction of *Clostridium*.

CHAPTER III

REDUCING GANGRENOUS DERMATITIS THROUGH PROBIOTIC ADMINISTRATION IN COMMERCIAL POULTRY

INTRODUCTION

The etiologic agents of Gangrenous dermatitis (GD) are *Clostridium septicum* (CS), *Clostridium perfringens* (CP) type A, and *Staphylococcus aureus* (SA), either individually or in combination. *Clostridium* is a spore-forming, Gram-positive, rod-shaped bacterium that grows in anaerobic conditions and is found in virtually all areas of our environment. *Clostridium perfringens* is ranked forth for most estimated cases of bacterial illness and the third for foodborne illness between years 1983 through 1997 in the United States (Mead et al., 1999). *Staphylococcus aureus* is a facultative anaerobe, non motile, Gram-positive cocci (Loir et al., 2003) that is commonly found on the skin and in mucous membranes of poultry and can result in bumblefoot, osteomylitis, arthritis-synovitis, and GD in commercial poultry (Ficken and Wages, 1997; Kloos and Bannerman, 1999).

Gangrenous dermatitis is a major health problem among broiler flocks in the United States resulting in high mortality, carcass condemnations, and trimmed parts. Economic losses are estimated to be as much as $1.31 per affected bird. There are also large economic losses involved in the antibiotic therapy associated with treatment of GD (Cocci Forum, 2008). While natural outbreaks of GD have been reported in chickens from 17 to 140d-of-age, the majority of cases are reported in 4-to 8-wk-old broilers. Clinical signs of GD are limited because the period of illness is generally short (less than

24h) prior to birds being found dead and mortality observed can be between 60-100% (Damerow, 1994). Post-mortem observations include spongy-air-filled subcutis with underlying hemorrhagic musculature, and lesions on the abdomen and legs (Hofarce et al., 1986; Wilder et al., 2000; Ritter, 2008).

The GI microbial community is a sophisticated association of many species of bacteria. Probiotics, composed of beneficial intestinal microflora from healthy adult chickens, can be administered to neonatal chickens for the prevention of intestinal colonization by pathogens (Nurmi and Rantala, 1973). Probiotics are non-pathogenic bacteria that reduce pathogen colonization in the GI tract of animals (Mead, 2002). There are several mechanisms by which probiotics can alter the environment in the GI tract to make it more favorable for beneficial bacteria and less so for pathogenic bacteria, such as competition for mucosal attachment sites and nutrients (Nurmi et al., 1992) and production of toxic compounds including volatile fatty acids (Nisbet et al., 1996).

The current dogma of GD is that the etiological agents are obtained from the environment, resulting in dermal lesions. Our laboratory believes that the normal gut micro-flora has the potential to become pathogenic and translocates from the GI tract to the dermis and other organs via the circulatory or lymphatic systems resulting in disease. There are multiple causes of bacterial translocation including bacterial overgrowth in the intestine, insufficient host defense, increased intestinal permeability, or damage to the intestinal mucosal barrier (Berg, 1995). This hypothesis has yet to be proven in an experimental setting, but could explain why some birds are found dead with intact dermal integument. The present investigation was designed to evaluate a probiotic in a

commercial setting during a field outbreak of GD. If our theory is true the administration of probiotics will reduce or eliminate mortality associated with an outbreak of GD.

MATERIALS AND METHODS

Experimental Design

In the present investigation, three commercial poultry Farms (1, 2, and 3) were selected based on their history of GD outbreaks in previous flocks. Prior to placement of birds, flocks were chosen and bird distribution was uniform in both probiotic-treated and control houses. In all studies, commercial practices including heating, cooling, lighting, vaccination, feeding regime, and therapeutic administration of antibiotics were followed according to the producers normal routine. During a mild outbreak of GD in a house of 27,500 broilers (mortality between 50-99 birds/day) the producer treated infected control houses with Linxmed (64mg/gal) or Pen-Aqua-Sol (340,000units/gal) during a severe outbreak (mortality ≥100 birds/day).

Probiotic Administration

The probiotic, Biomin® PoultryStar (Biomin GmbH, Herzogneburg, Austria), contains 2.3×10^{12} CFU per pound of lactic acid producing bacteria including: *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, and *Lactobacillus rueteri*. The probiotic was administered through the drinking water at a concentration of 20g/1000 birds/day which delivers 1×10^{8} CFU/mL. Stock concentration was adjusted to meet the appropriate demands so that 1×10^{8} CFU/mL was delivered to the birds as water consumption increased. The calculated water

consumption was based on the National Research Council guidelines and averaged 225, 480, 725, 1,000, 1,250, and 1,500 mL/bird/week (1994). Probiotics were given during periods of stress and days the ecology of the gut would be altered throughout the grow-out period, including: day of placement, vaccination, feed changes, and before catch. Birds on Farms 1 and 2 were administered the probiotic on d1-3, d10, d13-15, d27-29, d34-36, and d40-42. For experimental Farm 3 the frequency of administration was reduced, and the probiotic was administered on d1-3, d27-29, and d34-36.

Parameters Measured

Throughout the grow-out period, daily mortality was recorded at least twice a day by the grower and averaged for each week. Morbidity was also monitored and birds appearing ill were periodically euthanized and necropsied. During spikes in mortality, birds were also necropsied and examined for the presence of GD. The average weekly weights of 300 birds were recorded per house (reared in the brood area). We compared processing parameters of gross and net kilograms from all three experimental farms.

Statistical Analysis

Average weekly mortality and weights were analyzed using a One-Way ANOVA (Analysis of Variance), significant differences shown at ($P \leq 0.05$) using the SAS program. Average weekly mortality was analyzed by day and grouped by week. Average weekly treatment weights were analyzed by 300 birds for each treatment and grouped per week. Multiple comparison procedures (Tukey Test) were used to further analyze the mortality data. If ANOVA was significant ($P \leq 0.05$), Tukey's Test was used to further analyze control and treatment significance in mortality.

RESULTS

Experimental Farm 1

The control, non-probiotic-treated house broke with GD at week five, at which time antibiotics were administered, yet mortality continued to increase. Mortality in the probiotic-treated house remained at normal levels and the house did not break with GD. At no time during the experimental study were antibiotics administered to the probiotic-treated house. At week seven, the control house had a significantly ($P \leq 0.05$) higher mortality rate than probiotic-treated house (Figure 1). The probiotic-treated house had heavier birds ($P \leq 0.05$) compared to the control house (Figure 2).

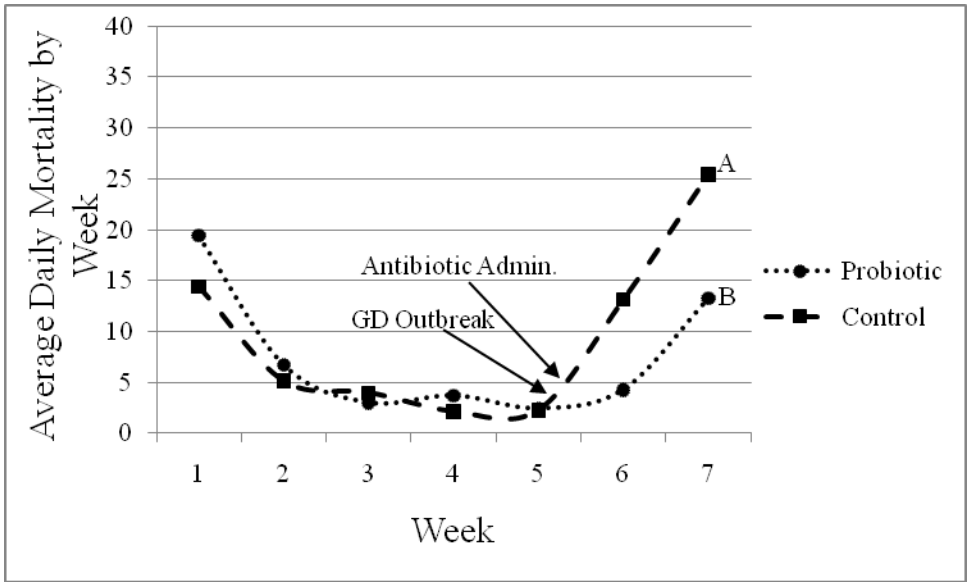

Figure 1: Average daily mortality by week on Farm 1 in control and probiotic-treated houses. $^{A\text{-}B}$Means with no common letters differ significantly ($P \leq 0.05$).

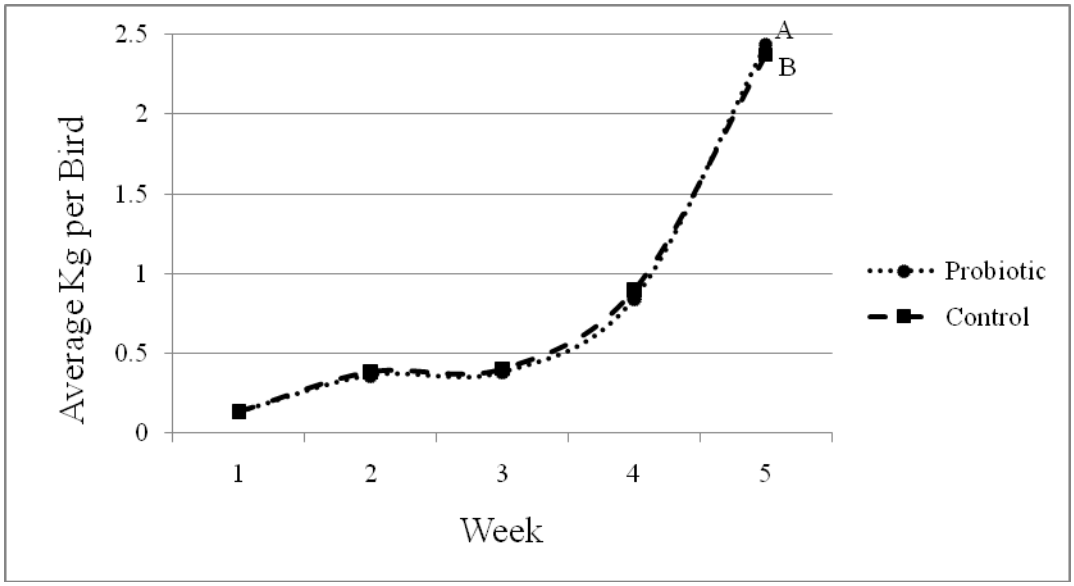

Figure 2: Weight gain in kilograms on a weekly basis on Farm 1 in control and probiotic-treated houses. Comparing collected data from 300 birds per house. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

Experimental Farm 2

Experimental Farm 2 showed similar results to those observed on Farm 1. The control house broke with GD at week four (Figure 3). Antibiotics were administered and on this farm the mortality dropped to normal the following week. At week four the control house had a significantly ($P \leq 0.05$) higher mortality than the probiotic-treated house. During week six, a normal mortality increase was observed for both houses. This was consistent with the birds being reared during the hot summer months in the southern region of the United States. Weights were comparable in control and probiotic-treated houses until week 5 at which point the probiotic-treated birds were heavier ($P \leq$

0.05) (Figure 4). The probiotic-treated house did not break with GD at any time during the grow-out.

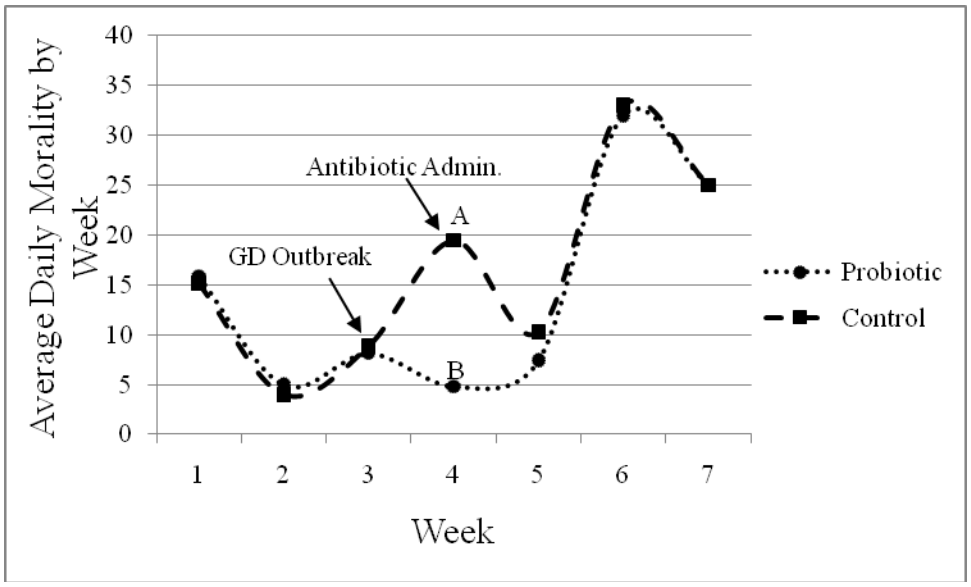

Figure 3: Average daily mortality by week on Farm 2 in control and probiotic-treated houses. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

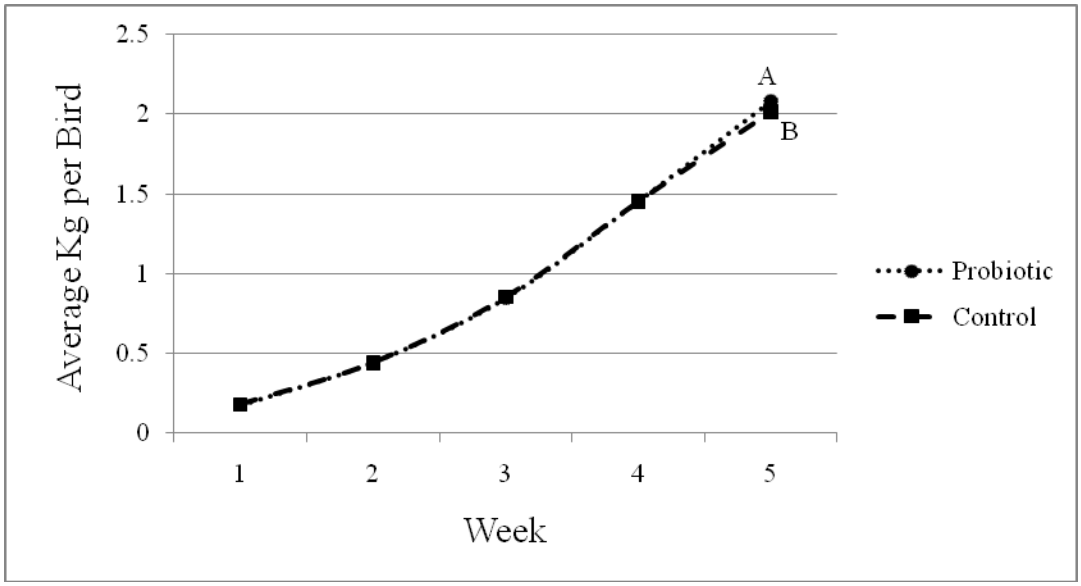

Figure 4: Weight gain in kilograms on a weekly basis on Farm 2 in control and probiotic-treated houses. Comparing collected data from 300 birds per house. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

Experimental Farm 3

It is important to note that the protocol for Experimental Farm 3 was altered and the probiotic was administered on d1-3, d27-29, and d34-36. The difference between Farm 3 and Farms 1 and 2 is that there was no administration of the probiotic on d10, d13-15, and d40-42. The control house broke with GD at week four, at which point antibiotics were administered and mortality returned to normal (Figure 5). The probiotic-treated house did not break with GD until the product was removed on d36. The large increase in mortality in the probiotic-treated house is partially due to GD, but was also influenced by damages due to a weather-related loss of power and services to the complex. At week seven the control house had a significantly ($P \leq 0.05$) higher mortality than the probiotic-treated house. Weights were comparable in control and

probiotic-treated houses until week four and five at which point the probiotic-treated birds were heavier ($P \leq 0.05$); (Figure 6).

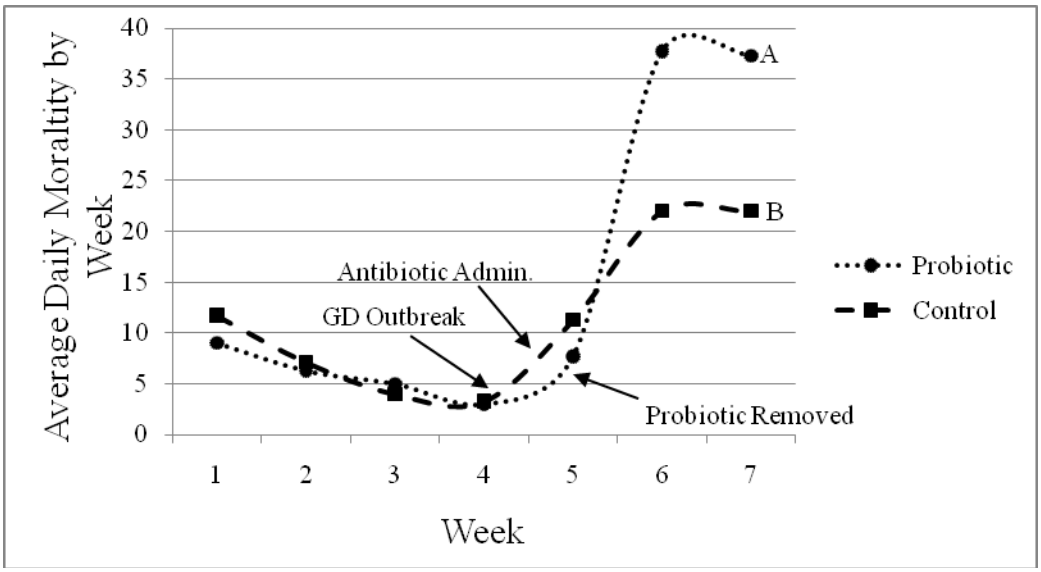

Figure 5: Average daily mortality by week on Farm 3 in control and probiotic-treated houses. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

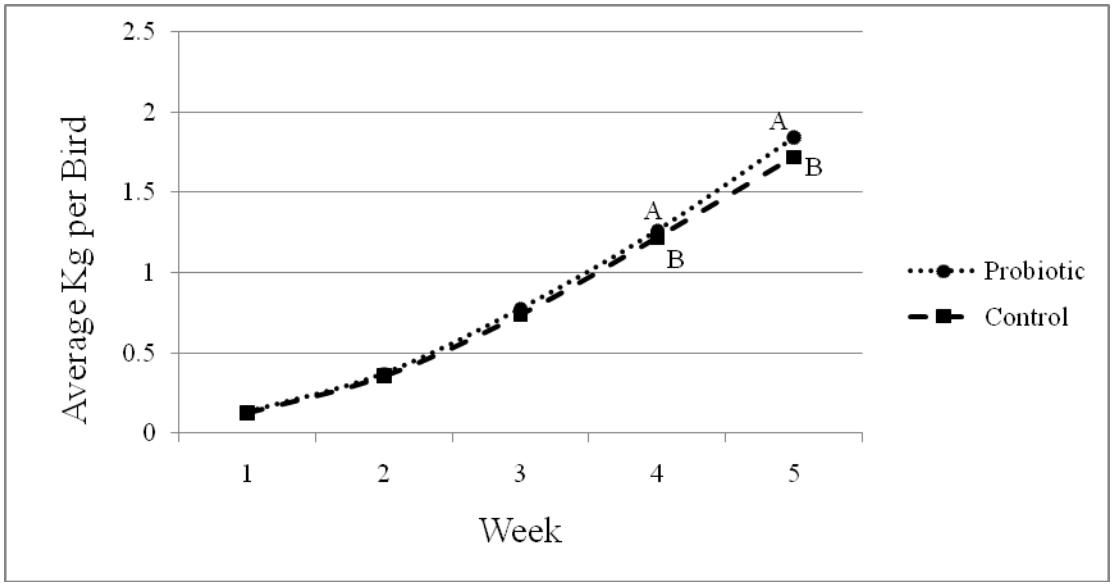

Figure 6: Weight gain in kilograms on a weekly basis on Farm 3 in control and probiotic-treated houses. Comparing collected data from 300 birds per house. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

Processing Data

Processing data was supplied by a commercial processor and reflects their methods of record keeping and analysis. The processing plant was unaware of the different treated houses. Gross kilograms are weights of birds on the truck when they arrive at the processing plant and net kilograms are weights of the total processed carcasses. The probiotic-treated houses had an increase in total net kilograms when compared to the control houses, with eexception to Experimental Farm 1 (Table 2). The processing data of Experimental Farms 2 and 3 reflect weekly average weighs of live birds by having heavier birds at processing of probiotic treated houses compared to

control houses. The net kilograms compared to gross pounds follow a similar pattern, with net kilograms being 78-111 kilograms less than gross kilograms.

Table 2: Gross kilograms and net kilograms at processing following the administration of probiotics on three commercial Farms 1, 2, and 3. Data collected at processing plant after a 50 day grow-out. Gross Kilograms (weight of birds on truck at arrival to plant); Net Kilograms (weight of total carcasses processed); and Difference between probiotic-treated house and control house per farm are shown in the last column.

Experimental Farm	Treatment	Gross Kilograms	Net Kilograms	Kilograms Difference
1	Probiotic	72,393.283	72,303.472	-1,118.558
1	Control	73,509.12	73,422.03	
2	Probiotic	73,894.673	73,792.161	+2,004.877
2	Control	71,898.868	71,787.284	
3	Probiotic	75,352.971	75,274.953	+1,037.365
3	Control	74,318.842	74,237.588	

DISCUSSION

Gangrenous dermatitis causes significant economic losses to the commercial poultry industry. Only in the last two years has the prevalence of this disease increased and caused major concerns for the industry. Although there are multiple known etiological agents that contribute to an outbreak of GD, a definitive cause is unknown. A

better understanding of both the cause(s) of GD and the host-pathogen interactions will facilitate development of preventative measures in the future.

Previous research involving on-farm reduction of disease is minimal. There are many on-farm case studies to determine the cause of the GD outbreak, but in some cases the actual cause is still undetermined. Numerous factors that contribute to GD outbreaks include high levels of environmental contamination of pathogens, immunosuppression of the birds, and overcrowding (Willoughby et al., 1996). Known causes of GD in field case studies include infectious bursal disease virus, chicken anemia virus, avian adenovirus infections, Marek's disease, reovirus, mycotoxins (Rosenberger et al., 1975; Hofacre et al., 1986; Ritter, 2008) and coccidial infection (Baba et al., 1996; Collier et al., 2007). Reproduction of GD in experimental condition relies on immunosuppression via administration of cyclophosphamide and calcium chloride (Kaul et al., 2000) or by hyperimmunizing birds with infectous bursal disease virus or chicken anemia virus (Rosenberger et al., 1975; Hagood et al., 2000).

Probiotics enhance animals' health and growth rate. In poultry benefits of probiotics include feed conversion, digestion, absorption of nutrients, carcass quality, and less carcass contamination (Fuller, 1999). However, little is known about the role of probiotics on actual diseases. The results from this study showed that administration of a commercial probiotic helped reduce and/or prevent the onset of GD in three separate studies. When an outbreak of GD occurs in commercial operations, mortality can become quite high; however, the probiotic-treated houses on Farm 1 and 2 maintained normal flock mortality and morbidity and produced birds with heavier body weights

compared to control houses that were not administered the probiotic. To our knowledge this study was the first to show that probiotics can prevent the onset of the disease and increase body weights of birds reared under normal management practices at the time of a GD outbreak.

As pressure mounts to discontinue the use of antibiotics in the poultry industry due to increased consumer demands for antibiotic-free products, it is increasingly important to develop new strategies to combat costly enteric pathogens. Additionally, antibiotics are likely to become less effective due to a dramatic increase in antibiotic-resistant bacteria that also cause GD (Bedford, 2000). In the present investigation, all control houses were administered therapeutic antibiotics to control GD during an outbreak. However, no antibiotics were used in any of the probiotic-treated houses, indicating that the addition of these beneficial bacteria was sufficient to reduce the clinical effects of this disease.

It has been shown in mice that stress aids in the disruption of the microbial population and translocation of pathogenic bacteria out of the GI tract to other parts of the body (Zareie et al., 2006). Birds raised under commercial conditions are frequently exposed to many stressors including overcrowding, heat, cold, and other environmental stresses. From our experiences on farms any stress, vaccination, feed changes, and fluctuations in temperature or lighting, could possibly give rise to opportunistic pathogen bacterial translocation. During periods of stress, the immune response is depressed, providing an opportunity for a GD outbreak. In this study probiotics were given before

vaccination, during feed changes, and stressful times during the grow-out. The probiotic was efficient in preventing the onset of GD on all farms.

Previous studies show probiotics stimulate the immune response by increasing cytokine gene expression (Delneste et al., 1998) and increased phagocytosis of bacteria by peripheral blood leucocytes (Schiffrin et al., 1995). Therefore, the use of probiotics during periods of stress and subsequent immunosuppression may enhance the immune response and prevent disease, particularly of the intestine (Fernandes et al., 1987). Probiotics also provide a protective role at the mucosal level of the GI tract (Delneste et al., 1998). Based on those studies, we hypothesize that probiotics prevent bacterial translocation through the mucosa of the GI tract to the dermis therefore preventing the onset of GD.

In the present study, the probiotic was administered for three consecutive days. It is unknown how long the probiotic bacteria actually inhabit the GI tract or if they become established in the host. Further research would be beneficial to determine how long these probiotic bacteria reside in the host. Further studies are underway to determine the ecology of the GI tract during outbreaks of GD and during disease-free time points and how variations in GI microflora and host health status relate to the GD disease process.

Our study shows administration of probiotics is a beneficial and/or alternative management tool in flocks or farms that have a history of GD outbreaks. Birds that received this product in the present investigation had improved health and production parameters due to enhancing the microbial populations within the GI tract. A benefit of

providing a probiotic is that it restores beneficial bacteria in the gut (Nurmi and Rantala, 1973; Mead, 2002). Using this information, we believe the probiotic provides the bird with an enhanced ability to combat opportunistic pathogens such as CS, CP, and SA thereby reducing outbreaks of GD.

CHAPTER IV

CONTROLLING GANGRENOUS DERMATITIS WITH COMMERCIAL PRODUCTS

INTRODUCTION

Gangrenous dermatitis (GD) is a major health problem among broiler flocks in the United States resulting in high mortality, carcass condemnations, and trimmed parts. Economic losses are estimated to be as much as $1.31 per affected bird (Cocci Forum, 2008). The etiologic agents of GD are *Clostridium septicum* (CS), *Clostridium perfringens* (CP) type A, and *Staphylococcus aureus* (SA), either individually or in combination (Ficken and Wages, 1997). While natural outbreaks of the disease have been reported in chickens from 17 to 140d of age, the majority of cases are reported in 4-to-8-wk old broilers (Damerow, 1994). Clinical signs of GD are limited because the period of illness is generally short (less than 24h) prior to birds being found dead and mortality observed can be between 60-100% (Damerow, 1994). Post mortem observations include air in the subcutis with underlying hemorrhagic musculature, and lesions on the abdomen and legs (Hofarce et al., 1986; Wilder et al., 2000; Ritter, 2008). The bird's skin commonly has a "spongy" feeling due to gas production from accumulating bacteria between the muscle and dermis.

Understanding the disease progression has been difficult due to the biological complexity and diverse predisposing factors that are thought to give opportunistic pathogens a chance to cause GD. When evaluating this disease in the commercial setting, there are several time points during grow-out when GD is historically observed.

Outbreaks of GD are known to be associated with vaccination, virus infections, immunosuppression, (Rosenberger et al., 1975; Hagood et al., 2000; Ritter, 2008), coccidial infection (Baba et al., 1996; Collier et al., 2007), antibiotic growth promoters (Fowler and Hussaini, 1975), dietary changes (Kahn, 2005), sudden gut microflora changes, poor management practices, and standard production grow-out stresses.

In the commercial broiler industry, bedding material is a major expense. Litter is typically recycled from flock to flock, sometimes from upward of a year and a half, to alleviate some of these incurred costs. When litter is reused it may harbor high levels of CS, CP, and SA; thereby, increasing the likelihood of a GD outbreak. Clean-out of entire houses is not always practical, so evaluation of alternative approaches to reduce these bacteria would be beneficial to the commercial poultry industry. Two possible alternatives could be the utilization of chemical disinfectants and composting of litter.

Many disinfectants are commercially available to poultry producers. Glutaraldehydes (GA) are a type of aldehyde disinfectant reduces bacteria, fungi, viruses, mycobacteria, and spores (Jeffrey, 1995). Peroxymonosulfates (POXM) disinfectants work as an oxidizer (Dvorak, 2005). Iodine-based compounds are a halogen-type of disinfectant and are considered efficient for a wide range of bacteria, mycobacteria, fungi, and viruses (Jeffrey, 1995). A monoglyceride fatty acid-type of disinfectant that is commonly used in livestock facilities was used as a treatment also. The use of these products in a commercial poultry operation could potentially reduce high levels of pathogenic bacteria in the litter which could improve mortality associated with GD.

Composting litter is another viable approach to reduce the etiologic agents of GD as well as the overall microbial load. Composting is a process of using heat to kill microbial organisms that can potentially cause disease. Composting cow manure for 48 and 72h all *Samonella enteritidis* and *Escherichia coli* 0157:H7 is eliminated (Lung et al., 2001), and *Clostridium* can be reduced by 99% in composted litter compared to non-composted litter (Macklin et al., 2007).

Litter amendments are another viable alternative to reduce pathogenic bacteria in litter (Macklin et al., 2007). Currently, there are several compounds commonly used in the poultry industry to decrease litter pH and reduce ammonia levels in the houses. Previous work shows that a litter acidifier may be useful for on-farm pathogen reduction (Pope and Cherry, 2000).

In the commercial broiler industry birds are fed strict diets that are designed specifically to address the nutritional requirements for each stage of life; with rations being changed four to five times during the six week grow-out cycle. Historically, outbreaks of GD occur at the transition between grower and finisher; the reduction in vitamins as the birds get older and shifts from diet to diet could be a factor that contributes to disease outbreaks. In poultry, there are many symptoms characteristic of a vitamin B_5 or pantothenic acid deficiency including: reduced growth and feed consumption, poor feather growth, and rapidly developing dermatitis (Kahn, 2005). Therefore, we are proposing to increase the levels of vitamins in the rations as a measure to eliminate GD.

The use of commercial disinfectants, acidifiers, vitamins, and windrowing technologies in a commercial poultry operation can potentially reduce the onset of GD by reducing high levels of *Clostridium*. Over the course of the last several years the commercial poultry industry has seen a sharp increase of GD. This research is important because there is a need for products or technologies that can be utilized by the grower to reduce clostridial numbers therefore minimizing the possibility of a GD outbreak. The objectives of this research were to reduce *Clostridium* in the litter by utilizing litter disinfectants, composting, and liter amendments and to improve bird health by increasing the vitamin concentration during feed changes. The overall goal was to eliminate the onset of GD on commercial poultry farms by using commercially available products and technologies.

MATERIALS AND METHODS

Experimental Design

In the present investigation, three commercial poultry Farms (A, B, and C) were chosen based on their previous history of breaking with GD. All farms chosen for this study had GD at significant levels in previous flocks. Prior to placement of birds, flocks were chosen and bird distribution was uniform in treated and control houses. In all studies, commercial practices were followed according to the producers normal routine including but not limited to: heating, cooling, lighting, vaccination, feeding regime, and therapeutic administration of antibiotics if needed. During a mild outbreak of GD in a house of 27,500 broilers (mortality ≥50 birds/day) the producer treated infected control

houses with Linxmed (64mg/gal) and Pen-Aqua-Sol (340,000units/gal), on all farms, during a severe outbreak (mortality ≥100 birds/day).

Product Administration

All waterlines in treated and control houses were cleaned with a commercial disinfectant that is potable for birds on the day prior to placement. This step was performed to remove any vegetative or spore forms of *Clostridium* and other pathogens and microorganisms in the waterline, including the removal of any biofilm. A two solution disinfectant was added to the drinking system using a quick mix station. Per manufacturer's recommendation, 12.8 fl. oz. of solution 1 and 12.8 fl. oz. of solution 2 were added to one gal of tap water in separate buckets. The solutions were fed simultaneously through the waterlines and allowed to sit for one h then flushed for 15 min with clean water. Four drinking water samples were taken at the end of the waterlines to determine CFU of CP pre- and post- disinfection. All disinfectants that were applied through waterlines, were applied through a standard administration pump at an application rate of 1:128.

Farm A Product Administration

Litter on Farm A was over a year old and had been used to rear eight flocks prior to product application. Normal cake-out procedures were performed in every house before treatments were applied. Litter acidifiers and disinfectants were applied using a pull-behind sprayer and were sprayed on the litter and chain walls of each treatment house. House numbers and treatment information are provided in Table 2.

Iodine- and GA-based disinfectants were the litter disinfectants used on Farm A, house one. Prior to placement of birds, the iodine-based disinfectant was applied at a concentration of 15 gal per 100 gal of water, and was applied at a concentration of 15% using a total application of 30 gal of product. After 24 h of contact time, the GA disinfectant was then applied at a concentration of 15 gal per 100 gal of water, applying a total of 30 gal of product.

Monoglyceride- and POXM-based disinfectants were the litter disinfectants used on Farm A, house two. Before birds were placed in the house, the POXM disinfectant was applied at a rate of 30 lbs in 200 gal of water and allowed 24 h of contact time. The monoglyceride disinfectant was applied the following day at a rate of 10 gal per 100 gal of water. Twenty gal of product was applied.

Houses three and four, on Farm A, were windrowed. Litter was piled in two rows the length of the house and allowed to compost for ten d and reached a maximum temperature of 130˚F. The piles were turned on d five and then allowed to compost an additional five d. Temperature was monitored at the very center and half way up the pile to insure appropriate temperatures were reached. Piles need to reach 130˚F to fully decompose the organics and waste material so pathogen load will be reduced in the litter.

Two other treatment houses, five and six, on Farm A were used to evaluate the effects of vitamins on the development of GD. The concentration of vitamins in the feed, for both houses, remained at 100g/ton throughout all feed changes during the grow-out from starter to the final finisher diet. The control house maintained vitamin levels at

standard concentrations, with normal fluctuations in the vitamin levels throughout feed changes.

The control house on Farm A only received waterline disinfectant before placement of the birds. No treatment was applied to this house at any time during the duration of the grow-out. Standard management procedures were followed for this house by the grower.

Farm B Product Administration

Prior to placement of birds, on farm B, all houses were cleaned out and fresh litter applied to all houses on Farm B. The purpose of this trial was to evaluate two types of litter disinfectants and determine if they were beneficial in preventing an outbreak of GD. Disinfectants were only applied to the floors of the treatment houses before new litter was distributed. House numbers and their treatment regime are shown in Table 3.

Treatment house one on this farm received a POXM-based disinfectant that was applied four d prior to placement of birds at a concentration of 40 lbs (dry weight) to 200 gal of water. The second house was treated with a GA-based disinfectant and was applied three to four d prior to bird placement a concentration of 3.125 gal per 200 gal of water. The GA disinfectant was applied using a sprayer (2gal/min) 50 gal at a time by making several passes throughout the length of the house.

Farm C Product Administration

The litter on Farm C was approximately one year old and sustained eight flocks. A litter acidifier was applied 24h before flock placement at a concentration of 100 lbs

per 1,000 sq ft using a tractor and fertilizer spreader. The mid-flock litter treatment was applied at the same application rate using manual push spreaders and was distributed throughout the entire house on d 30 which is three to five d before a typical outbreak of GD occurs. Additionally, a water acidifier was applied in the drinking water at the nipple drinkers at a rate of one package (16oz.) mixed with five gal of water daily throughout the grow-out period. Refer to Table 2 for house numbers and their designated treatment.

Table 3: Products applied to designated house numbers on Farms A, B, and C.

Farm	House	Water Disinfectant	Iodine	GA	POXM	Monoglyceride	Windrow	Vitamin	Acidifiers
A	Control	X							
A	1	X	X	X					
A	2	X			X	X			
A	3	X					X		
A	4	X					X		
A	5	X						X	
A	6	X						X	
B	Control	X							
B	1	X			X				
B	2	X		X					
C	Control	X							
C	1	X							X

Parameters Measured

Mortality, Morbidity, and Processing. Throughout the rearing period, daily mortality was recorded and averaged for each week during the grow-out period. Morbidity was monitored and birds appearing ill were euthanized and periodically necropsied. During spikes in mortality, birds were also necropsied and examined for the presence of GD. Average weekly weights were taken on 300 birds reared in the brood area per house. Processing parameters from all three experimental farms were evaluated.

Microbiology. To quantitatively measure populations of CP, litter samples were taken from the houses treated with the disinfectant and acidifier products. A total of 6-8 samples were taken per house in a uniform fashion, concentrating on the areas between the waterlines and feedlines because these are the areas of high traffic and heavy contamination. Litter samples were taken before treatment and one h post treatment to allow the products to take effect. A litter sample of 25 g was placed in 75 mL of anaerobic peptone water, stomached for 30 s, and 1.0 mL of sample contents was removed and placed into 9 mL of thioglycollate media. Ten-fold serial dilutions were performed and plated on Shahidi Ferguson Perfringens Agar and incubated (24h at 37°C). All microbiota culturing was conducted under anaerobic conditions in an anaerobic hood. Colonies exhibiting typical colony morphology for CP were counted and recorded for comparison.

Sample Collection. In Figure 7, the post samples were taken after the waterlines were flushed clean of disinfectant. In Figure 8, T1 represents litter samples taken prior

to the application of iodine- and POXM-based disinfectants and T2 describes samples take one h post the same disinfectants. Sample T3 was taken 24h post iodine- and POXM-based disinfectants and prior to GA- and monoglyceride-based disinfectants. Sample T4 was taken one h after application of the GA- and monoglyceride-based disinfectants.

Statistical Analysis

Data was analyzed using a One-Way ANOVA (Analysis of Variance), significant differences shown at ($P \leq 0.05$) using the SAS program. Average weekly observed mortality was analyzed by day and grouped by week. Average weekly treatment weights were analyzed by 300 birds for each treatment and grouped per week. Multiple comparison procedures (Tukey's Test) were used to further analyze the mortality data. If ANOVA was significant ($P \leq 0.05$), Tukey's Test was used to compare control and treatment significance with respect to mortality.

RESULTS

Water consumption was monitored and no treatments evaluated in this study had any effect.

Farm A

The number of CP-positive samples recovered from waterlines pre- and post-treated with a two solution disinfectant are shown in Figure 7. All of the pre samples taken were enriched and tested positive for CP. The second bar, that is not present in Figure 7, is the post samples taken and showed none of the samples were positive for CP.

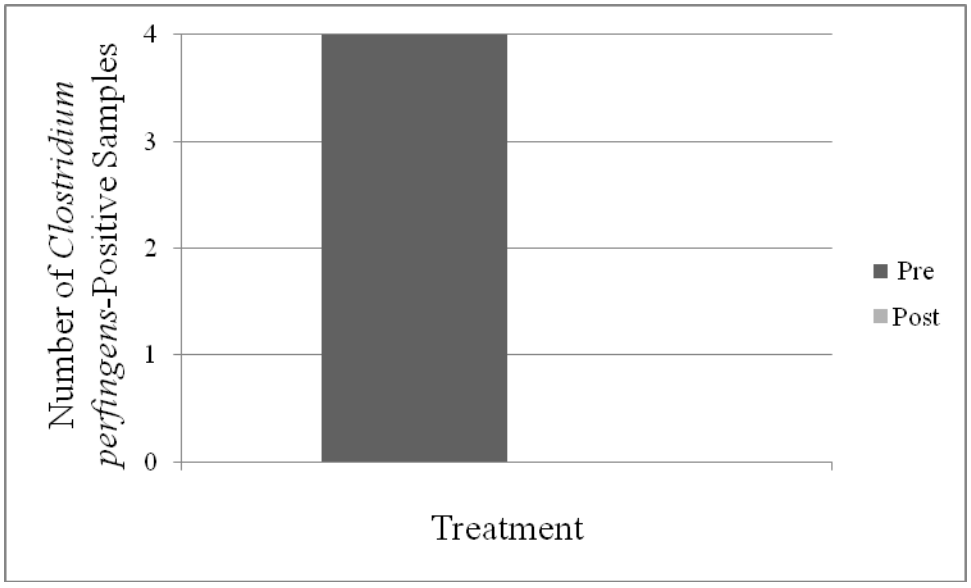

Figure 7: An evaluation of a waterline disinfectant on Farm A showing water samples positive for CP. Positive enriched samples of *Clostridium perfringens* from waterline samples taken on Farm A, comparing four samples collected from the ends of waterlines pre- and post-treatment from treatment house one on Farm A.

On Farm A there is a reduction of CP in the litter shown in Figure 8. Litter samples were collected at four times on Farm A. Time point, T1, is the sample taken before the first litter disinfectant was applied to the litter and chain walls of the house. At time T2 the next sample was taken one h after the first disinfectant was applied. At time T3, this sample was taken 24 h after the first disinfectant was applied but before the second one was administered. At the T4 time point, a sample was taken one h after the second disinfectant was applied. At each time the number of CP was determined. There is a reduction with both of the disinfectant treated houses, one and two, shown in Figure 8. The reductions of CP in the litter of the Iodine and GA house was comparable to the POXM and monoglyceride treated house. A reduction of .6 to 1 log of CP at T2 time

point, is observed with an even greater reduction between T2 and T3 time point by .7 to 2.3 log of CP, also shown in Figure 8. There was an increase of .8 to .9 log of CP from between samples that were taken at T3 and T4 time points.

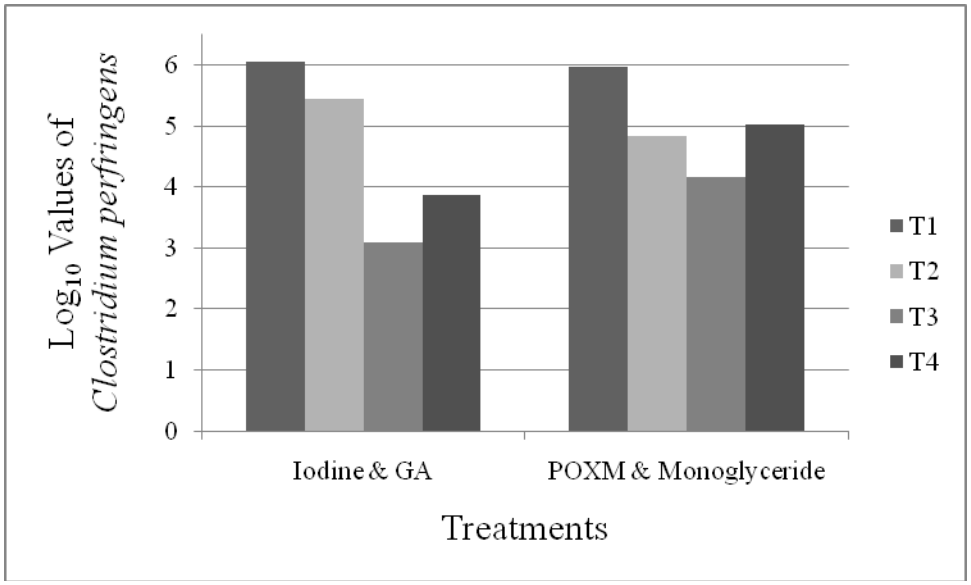

Figure 8: An evaluation of disinfectants on Farm A on litter concentrations of CP. Log_{10} values of *Clostridium perfringens* in litter samples on Farm A. At each timepoint eight samples were collected from areas throughout the house of each disinfectant-treated house. Litter samples taken at T1 were previous to the application of iodine- and POXM-based disinfectant and T2 describes samples take an hour post the same disinfectant. Samples taken at T3 were 24h post the iodine- and POXM-based disinfectant and previous to GA- and monoglyceride-based disinfectant and T4 is samples taken an hour post the GA- and monoglyceride-based disinfectant application.

On Farm A, treatment house one received treatments of iodine-based and GA litter disinfectants. This house was subjected to ammonia burn during week one and

spiking syndrome early in week two during the grow-out. Treatment house one was one of two houses that broke with GD on Farm A. House one broke with GD on week five at which time antibiotics were administered and the mortality continued to increase due to disease. This increase in mortality during week six was significantly ($P \leq 0.05$) greater when compared to the mortality in the control house. The control house broke with GD at the end of week six, at which time antibiotics were administered and the mortality continued to increase until day of catch. The slight increase in mortality of treated house one at the end of six weeks was due to birds being reared during hot summer months in the southern geographical region of the United States. This data is summarized in Figure 9. Neither of the windrowed and vitamin houses Farm A broke with GD. There were no significant ($P \leq 0.05$) differences of mortality in vitamin houses compared to the control house at any time during the grow-out (Figure 10). The difference in mortality of the control house compared to the windrowed houses was significantly higher ($P \leq 0.05$) at week six. There was a significant ($P \leq 0.05$) difference between both windrowed houses compared to each other and also compared to the control house, with the control house having a higher mortality than both windrowed houses at week seven (Figure 11).

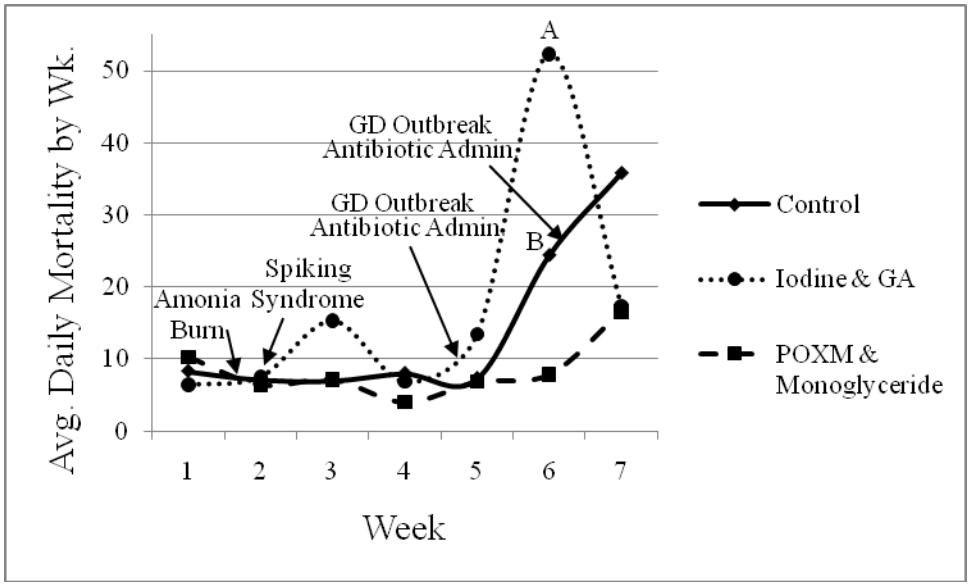

Figure 9: Average daily mortality by week on Farm A in control and litter disinfectant-treated houses. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

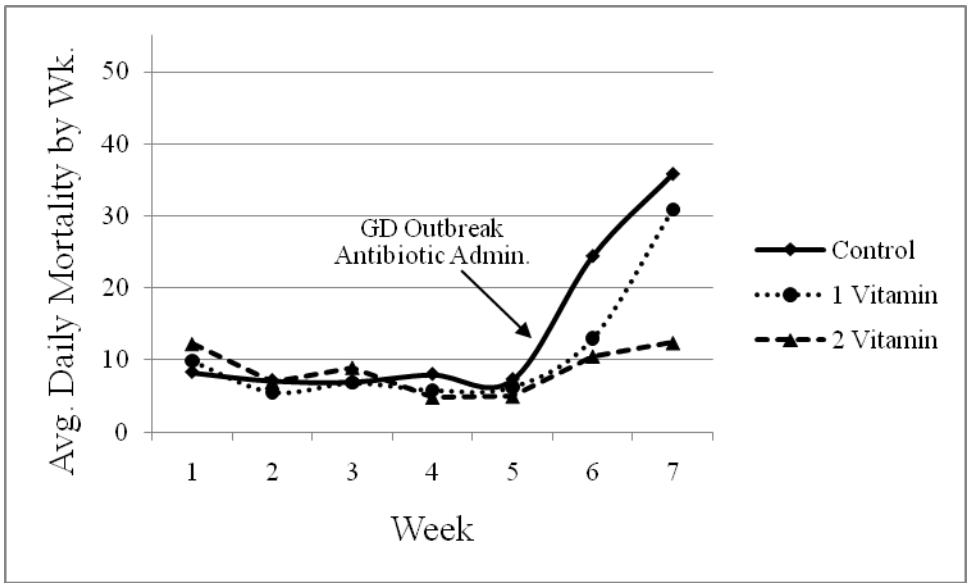

Figure 10: Average daily mortality by week on Farm A in control and vitamin-treated houses. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

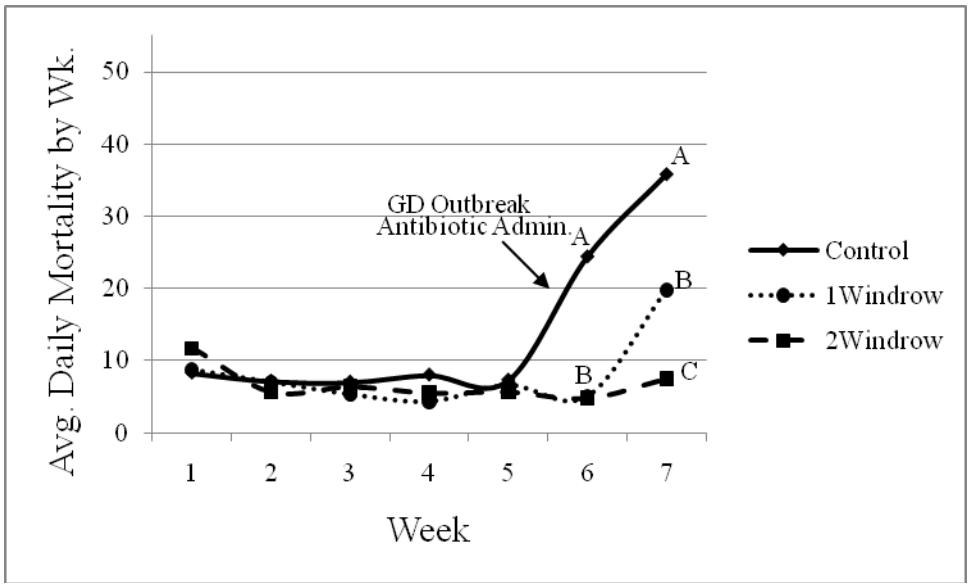

Figure 11: Average daily mortality by week on Farm A in control and windrow-treated houses. $^{A\text{-}C}$Means with no common letters differ significantly ($P \leq 0.05$).

The data in the average weekly weight (Figures 12, 13, and 14) shows significant ($P \leq 0.05$) differences throughout the grow-out period, however differences are variable and change from week to week. No treatment house had higher weights than the control house at week six. Processing data for Farm A is presented in Table 3. All treatments, except the treatment house one that broke with GD, had higher gross and net pounds when compared to the control house. Differences from the control house to each of the treated houses were between 662 and 7120 kg higher for the treated houses.

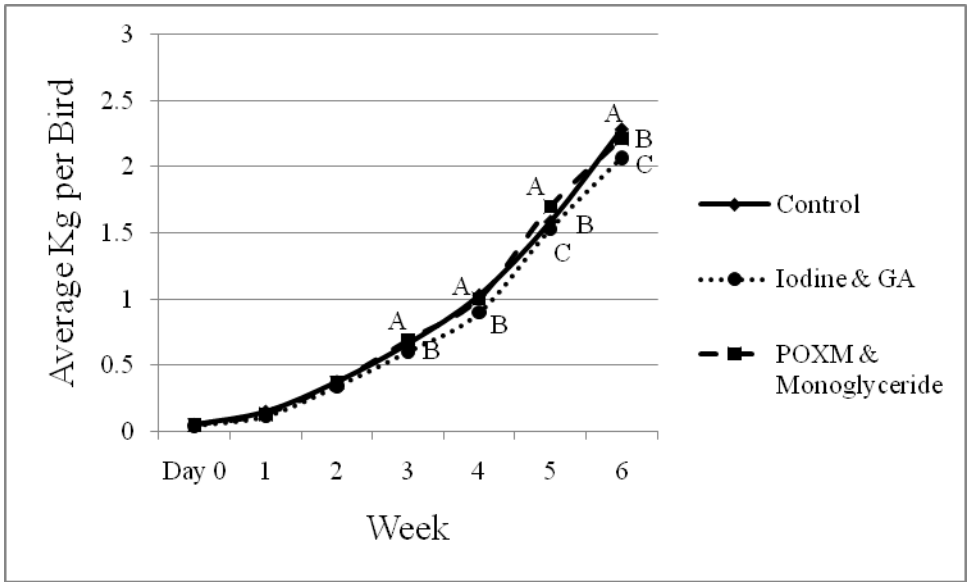

Figure 12: Weight gain in kilograms on a weekly basis on Farm A in control and litter disinfectant-treated houses. Comparing collected data from 300 birds per house. [A-C]Means with no common letters differ significantly ($P \leq 0.05$).

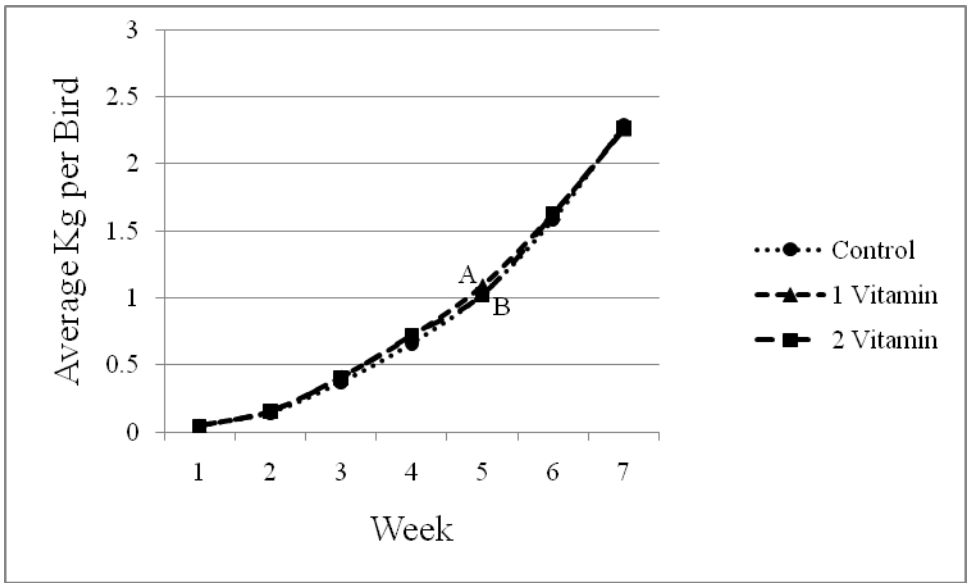

Figure 13: Weight gain in kilograms on a weekly basis on Farm A in control and vitamin-treated houses. Comparing collected data from 300 birds per house. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

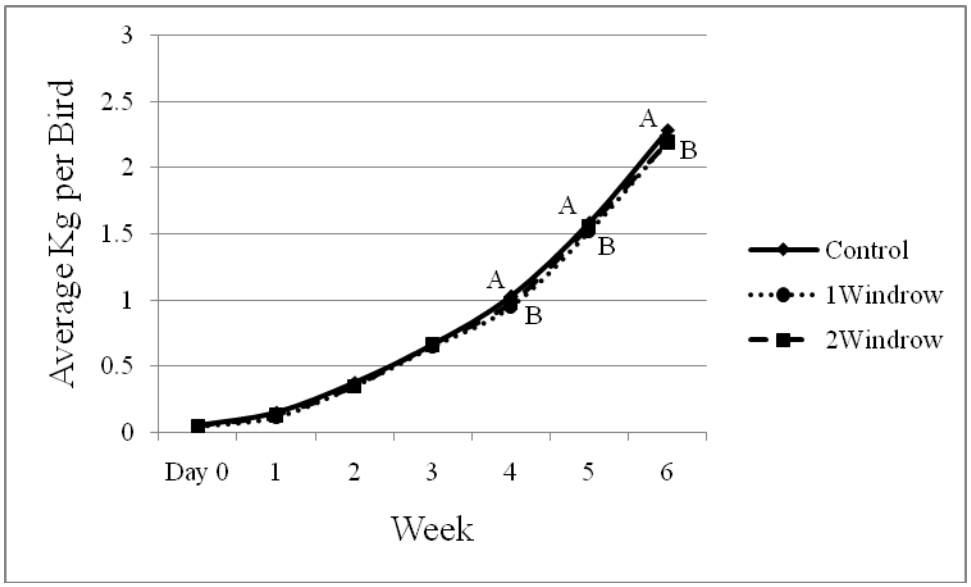

Figure 14: Weight gain in kilograms on a weekly basis on Farm A in control and windrow-treated houses. Comparing collected data from 300 birds per house. [A-B]Means with no common letters differ significantly ($P \leq 0.05$).

Farm B

Litter disinfectant pre- and post-samples of on experimental Farm B are shown in Figure 15. The two disinfectants, POXM and GA, used on Farm B exhibited comparable results in CP reductions. The post-samples taken one h after application of disinfectant showed a 0.2 to 0.5 log reduction of CP.

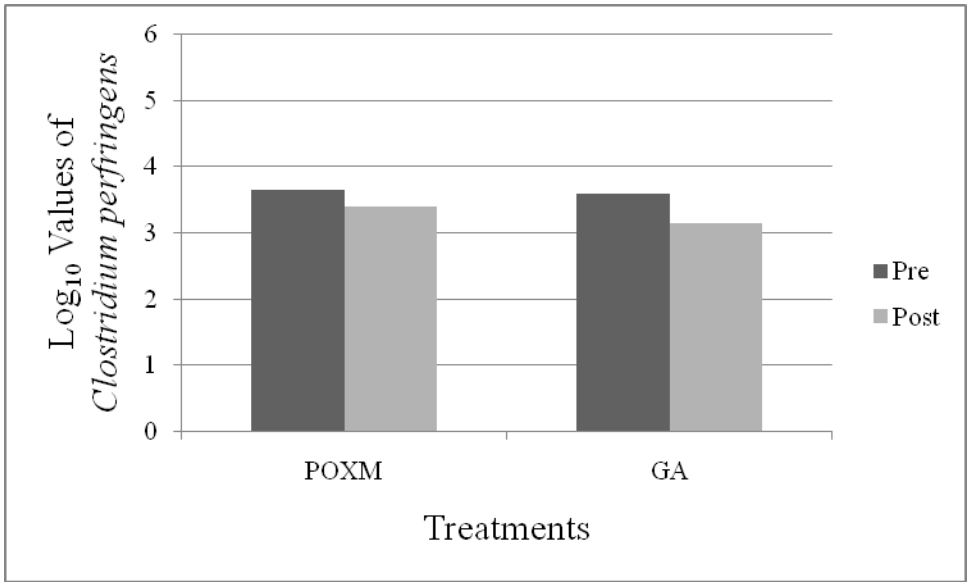

Figure 15: An evaluation of disinfectants on Farm B on litter concentrations of CP. Log_{10} values of *Clostridium perfringens* in litter samples on Farm B. Comparing eight samples collected data from areas, at each time point, throughout the house of disinfectant treated houses.

Farm B did not break with GD at any time. Mortality was highest during week one with some variation in mortality and treatments but they were not significant (Figure 16). There were differences between average weekly weights at week four and week six between treatment houses and the control house shown in Figure 17. A significant ($P \leq 0.05$) difference at week four with both treatments houses compared to the control house and also at week six with a difference between each treatment and the control house, with the control house having the lowest average weekly weights before catch. When compared to the control house, both disinfectant-treated houses on Farm B had higher gross and net pounds at processing (Table 4). When compared to the control house, the

POXM disinfectant treated house one on Farm B was 557 lbs greater, and the GA disinfectant treated house two was 1372 kg greater.

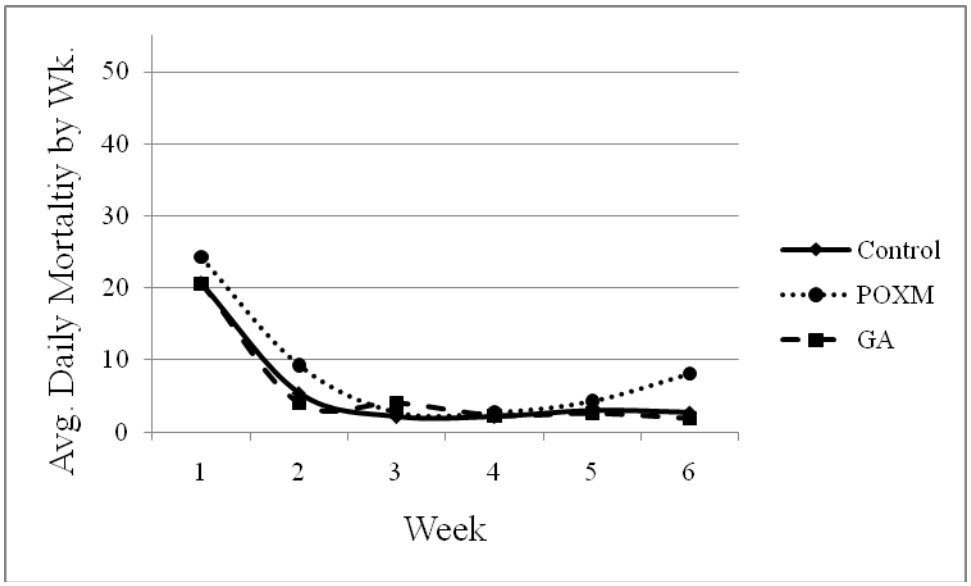

Figure 16: Average daily mortality by week on Farm B in control and litter disinfectant-treated houses. Means with no common letters differ significantly ($P \leq 0.05$).

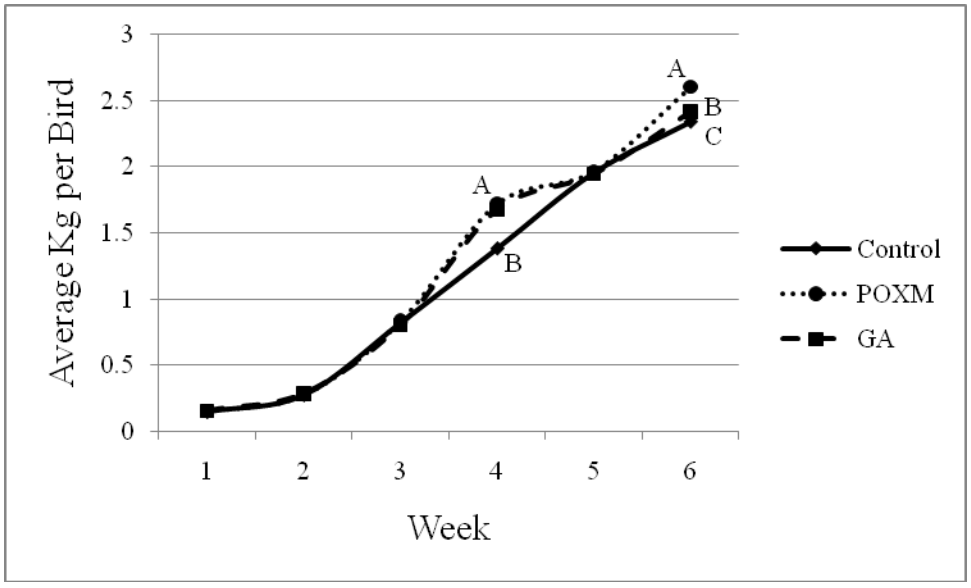

Figure 17: Weight gain in kilograms on a weekly basis on Farm B in control and litter disinfectant-treated houses. Comparing collected data from 300 birds per house. [A-C]Means with no common letters differ significantly ($P \leq 0.05$).

Table 4: Gross kilograms and net kilograms at processing following the administration of disinfectants, acidifiers, vitamins, and windrowing technologies on three commercial Farms, A, B, and C. Data collected at processing plant after a 50 day grow-out. Gross Kilograms (weight of birds on truck at arrival to plant); Net Kilograms (weight of total carcasses processed); and Difference between treated house and control house per farm are represented in the last column.

Experimental Farm	Treatment	Gross Kilograms	Net Kilograms	Kilograms diff.
1	Control	157970	157846	
1	Iodine & GA	150870	150729	-7117
1	POXM & Monoglyceride	165150	164966	+7120
1	1 Vitamin	158650	158508	+662
1	2 Vitamin	163030	162874	+5028
1	1 Windrow	161050	160906	+3060
1	2 Windrow	163670	163550	+5704
2	Control	171830	171557	
2	POXM	172310	172114	+557
2	GA	173130	172929	+1372
3	Control	163845	163666	
3	Acidifier	169425	169241	+5575

Farm C

Figure 18 shows the log value of CP pre- and post-samples from the acidified-treated house. No difference of the log value of CP recovered between the pre- and post-samples was observed.

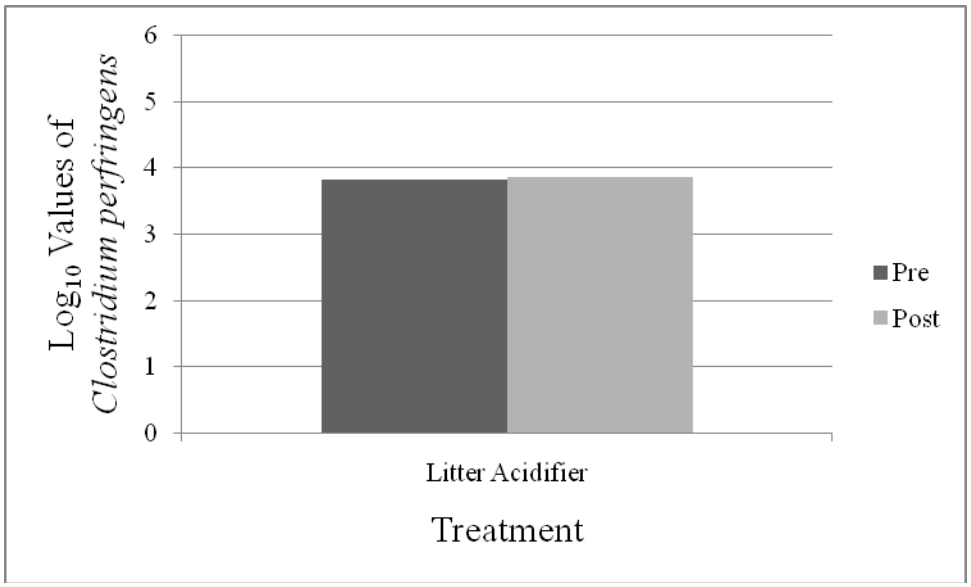

Figure 18: An evaluation of an acidifier on Farm C on litter concentrations of CP. Log_{10} values of *Clostridium perfringens* in litter samples on Farm C. Comparing eight samples collected data from areas, at each time point, throughout the house of the acidified litter treated house.

The control house on Farm C broke with GD at week four, at which time antibiotics were administered and the mortality returned to normal (Figure 19). The large increase in mortality in the acidified-treated house on Farm C was partially (25%) due to GD, but also reflects damages due to a weather-related loss of power and services to the complex (75%).

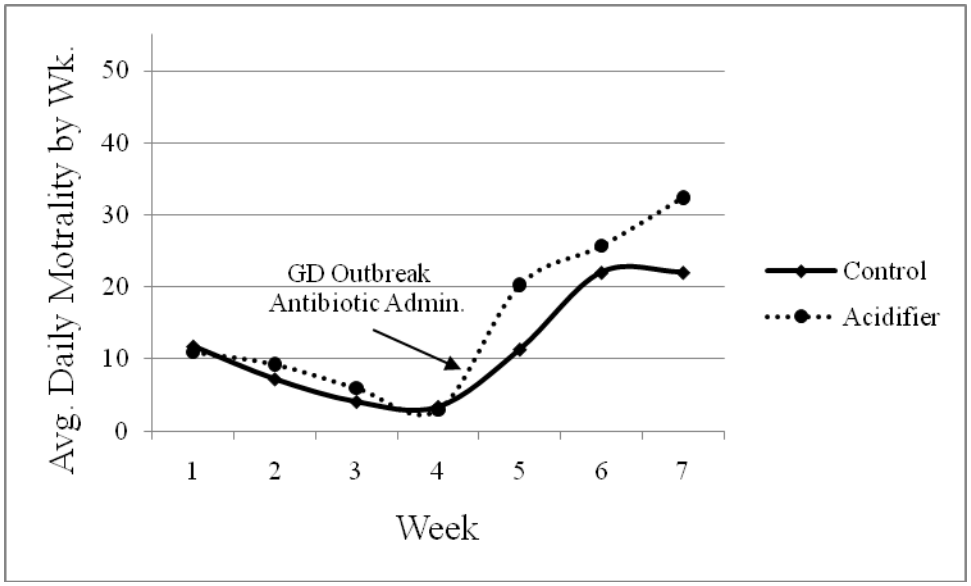

Figure 19: Average daily mortality by week on Farm C in control and litter/water amendment-treated houses. Means with no common letters differ significantly ($P \leq 0.05$).

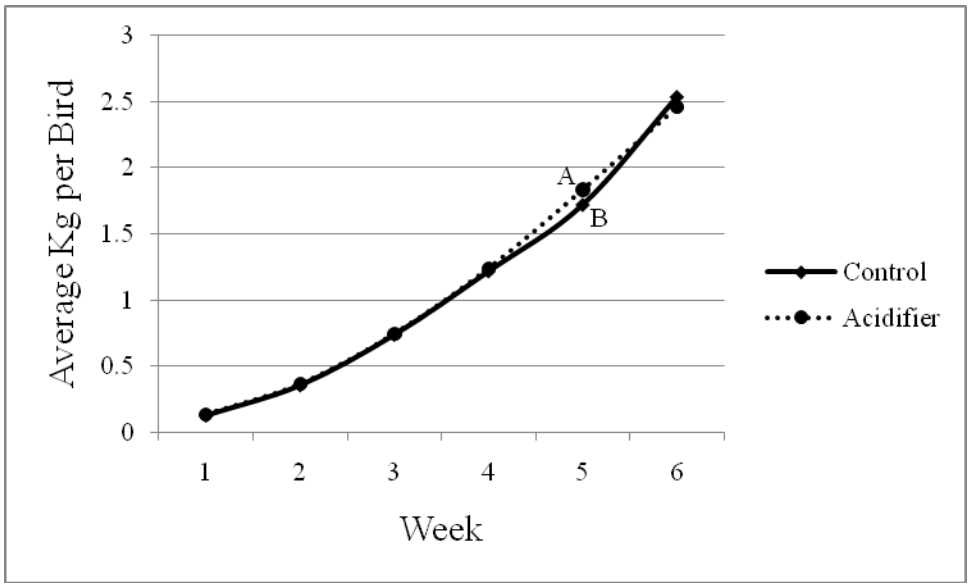

Figure 20: Weight gain in kilograms on a weekly basis on Farm C in control and litter/water amendment-treated houses. Comparing collected data from 300 birds per house. $^{A-B}$Means with no common letters differ significantly ($P \leq 0.05$).

The average weekly weights on Farm C of the treated house has significantly ($P \leq 0.05$) heavier birds at week five when compared to the control house (Figure 20). Processing data for this farm is presented in Table 3 and showed the treated house had heavier gross and total net lbs when compared to the control house, with a difference of 5575 kg at processing.

DISCUSSION

For any product or procedure to be implemented in the poultry industry, it must be both practical and economical. Numerous technologies and parameters were evaluated in this study. The use of commercial disinfectants, acidifiers, vitamins, and windrowing technologies in a commercial poultry operation may reduce high levels of pathogenic bacteria; therefore, contributing to the overall health and wellbeing of chickens which could improve mortality associated with GD. The basis for our investigations was focused on reducing *Clostridium* and eliminating the onset of GD on a commercial poultry farm.

There are many varieties of disinfectants that are available and serve multiple purposes including antimicrobial, anti-viral, and anti-fungal. These chemicals could be beneficial to an industry that reuses litter to minimize cost. Most disinfectants function by denaturing nucleic acids, proteins, or lipids of microorganisms (Maris, 1995). Poultry litter harbors many pathogenic bacteria including clostridia and by reducing these bacteria in the litter one log value demonstrates an importance to prevent these bacteria from causing foodborne illnesses (Macklin et al., 2008). On Farm A, both treated houses of disinfectants (the iodine- and GA-treated house and the POXM- and

monoglyceride-treated house) were beneficial to the reduction of CP in the litter but only one demonstrated a prevention of disease, disinfectant treated house two that received POXM and monoglyceride disinfectants. On Farm B both disinfectants reduced CP in the litter, even though there was no disease outbreaks on this farm both of the disinfectant treated houses, the iodine- and GA-treated house and the POXM- and monoglyceride-treated house, had improved processing data when compared to the control house. Disinfectants should be considered to reduce the pathogenic bacteria in the litter to disrupt the cycle of recurring outbreaks of GD on a farm. Some products may corrode metal and rubber compounds and become inactive in the presence of organic material (Dvorak, 2005). It is beneficial to determine the appropriate type of disinfectant prior to application.

In the treatment houses that were windrowed on Farm A, neither house broke with GD nor were their weights heavier than the control house at processing. Poultry litter not only harbors many pathogenic bacteria; including *Salmonella*, *Campylobacter*, *E. coli*, and CP, it also has high levels of organic matter that allows these bacteria to grow and flourish (Macklin et al., 2008; Lung et al., 2001). Windrowing is composting that contributes to the recycling of litter by reaching high temperatures, using ammonia levels, and other organisms to kill many bacterial pathogens in the litter (Macklin et al., 2007). In a recent study involving three foodborne pathogens in comparing composted and uncomposted litter *Salmonella* was entirely eliminated, *Campylobacter* was unrecoverable in both types of samples, and *Clostridium perfringens* had a slight (less than one log) reduction in composted litter. Even the slightest reduction of CP is

believed to be economically important to the broiler industry due to its disease causing ability (Macklin et al., 2008). Previous research demonstrates that windrowing reduces CP in the litter (Macklin et al., 2007; Macklin et al., 2008). Our research shows that in-house composting improves bird weight, eliminates morbidity, and improves overall production parameters that reflects the higher processing data compared to the control house. Overall, the research performed in this study demonstrates that windrowing is beneficial to the poultry producer and previous work shows that the method of composting is a way to decrease foodborne pathogens in a poultry house (Macklin et al., 2008).

Vitamins are important to the immune system from a nutritional stand point. If too much or too little vitamins are applied to the feed ration, birds may be immunosuppressed (Latshaw, 1991; Aburto and Britton, 1998; Leshchinsky and Klasing, 2001). When birds receive the accurate amount of vitamins, they are better able to fight off bacterial invaders, infection, and disease; as well as, have improved weight gain, lower feed conversion, and overall improved livability because of optimal function of the reproductive, muscular, circulatory, nervous, and immune systems (Wilgus, 1977; Latshaw, 1991; Leshchinsky and Klasing, 2001). Both of the houses that received a constant amount of vitamins in their feed rations on Farm A showed improved animal health, decreased mortality caused by disease, and overall improved bird health by having higher processing weights compared to the control house on this farm. Vitamins are important for the optimal function of the reproductive, muscular, circulatory, nervous, and immune systems (Gershwin et al., 1985). For this reason vitamins are

significant due to their impact in all of the body systems in poultry and reflect on production parameters such as egg production and feed conversion. Vitamins are important to improve animal health and are effective when administered in the physiologically relevant amounts.

On Farm C the house that received acidifiers on the litter and in the drinking water did not have a reduction in CP in the litter prior to flock placement. Previous work contradicts our study and shows that when an acidifier is applied to the litter, it reduces ammonia levels, ascities in broilers, and pathogens in poultry houses (Terzich et al., 1997; Pope and Cherry, 2000). Comparing the litter samples taken from Farm A to samples taken from Farm C one way to possibly take a more accurate litter sample to test for the bacteria is to take the sample 24h post application, this may give more time for the product to take effect and may have a more accurate count of bacteria. A different strategy to apply a litter acidifier is from previous literature that shows litter acidifiers work best when tilled in the litter (Macklin et al., 2007). For the next experiment, there are several options to improve our experimental design to allow for the best opportunities for this product to cause an effect and have a longer amount of time to work efficiently.

Many factors should be considered when choosing a product or procedure to implement into a poultry farm to either reduce pathogens that cause disease or to improve bird parameters by reducing morbidity and mortality as well as increasing weight gain by reducing feed conversion on the farm. Cost is the main factor to consider, but other important aspects when considering implementing a new product or

technology include: practicality, application process, equipment needed, and down time that is available between flocks. In this study, products that we found to lower the amount of CP in the litter are disinfectants (with the exception of iodine-based disinfectants). The product and procedure that improved overall animal health and increased weights at processing were keeping the level of vitamins in the feed rations constant throughout the age of the flock and windrowing.

CHAPTER V

CONCLUSION

The use of probiotics, commercial disinfectants, acidifiers, vitamins, and windrowing technologies in a commercial poultry operation can potentially reduce the onset of Gangrenous dermatitis (GD) by reducing high levels of *Clostridium*. Over the course of the last several years the commercial poultry industry has seen a sharp increase of GD. There are also large economic losses associated with GD (Cocci Forum, 2008). There are numerous circumstances that seem to exacerbate this disease, including vaccination programs, environmental/management practices, standard production grow-out stresses, and diseases that affect the intestine give these opportunistic pathogens a favorable environment to flourish in the bird's gastrointestinal (GI) tract (Fuller, 1989). This research presented in this thesis is important because there is a need for products or technologies that can be utilized by the grower to reduce clostridial numbers which, in turn, may minimize the onset of GD.

The GI microbial community is a sophisticated association of many different species of bacteria that are one of the bird's first lines of defense (Fuller, 1989). Probiotics are known to maintain a stable GI microflora, improve bird feed conversion, digestion, and absorption of nutrients (Fuller, 1999). To our knowledge, our study is the first to prove that probiotics can reduce diseases, including GD, associated with commercial poultry.

In the commercial poultry industry litter can harbor high levels of the pathogenic bacteria known to cause GD. Entire clean-out of houses is not always practical, so

alternative approaches to reduce these bacteria would be beneficial to the commercial poultry industry. Many disinfectants are commercially available to poultry producers and the use of these products in a commercial poultry operation could potentially reduce high levels of pathogenic bacteria, which could reduce mortality and morbidity associated with GD. Composting litter and litter amendments are other viable approaches to reduce the etiologic agents of GD, as well as, the overall microbial load in litter.

In the commercial industry, birds are fed strict diets and from our experiences transitions between grower and finisher rations can cause to the outbreaks of GD. The reduction in vitamins as the birds get older and shifts from diet to diet could be a potential factor that contributes to disease. We believe that by increasing the levels of vitamins in the rations will minimize GD because historically one of the symptoms of a pantothenic acid deficiency is rapidly developing dermatitis (Kahn, 2005).

Poultry integrators currently implement disinfectants, acidifiers, and vitamin supplement protocols for regular on-farm applications. It is unknown if or what kind of benefit these products have, and if they even cause any effect. With the implication of probiotics, disinfectants, acidifiers, vitamins and the technology of windrowing on different farms that have had a history with previous flocks breaking with GD, these experiments were designed to determine if these products reduced *Clostridium* with the expectation to reduce GD while improving bird weekly weight and processing data.

For experiment one, three commercial poultry Farms (1, 2, and 3) were chosen based on their previous history with GD. All farms chosen for this study had GD at

significant levels in previous flocks. Probiotics were given during periods of stress and days the ecology of the gut would be altered throughout the grow-out period, including: day of placement, vaccination, feed changes, typical days of a GD outbreak, and before catch. Parameters observed were: mortality, morbidity, average weekly weights, and processing data.

The results from this experiment show that the commercial probiotic used was beneficial against the development of GD on three different farms. When an outbreak of GD occurs in commercial operations, mortality can become quite high; however, on Farm 1 and 2 the probiotic treated groups maintained normal flock mortality and morbidity, and produced birds with heavier body weights when compared to their respected control house. This product should be considered as an alternative management tool in flocks or farms that have a history with GD in the poultry industry. Birds that received this product in the present investigation appeared to have improved animal health and production parameters. Using this information, we believe the probiotic provides the bird with an enhanced ability to combat opportunistic pathogens such as CS, CP, and SA in the GI tract.

For experiment two a waterline cleaning program was implemented to every house and glutaraldehyde (GA), peroxymonosulfate (POXM), iodine-based, and monoglyceride disinfectants were applied to the litter either individually or 24h after another was applied to their respected treatment house. In two treatment houses litter was windrowed and allowed to compost for a total of ten days. During this experiment the effects of vitamins were also monitored. The amount of vitamins in the feed, for

both of these treated houses, remained at a concentration of 100g/ton throughout all feed changes from starter to the final finisher diet while the control house maintained vitamin levels at standard concentrations. A litter acidifier was also applied to a treatment house previous to flock placement and on day 30, a mid-flock treatment. Parameters measured were mortality, morbidity, average weekly weights, and CFU counts of *Clostridium perfringens* (CP) in treatment houses that received litter disinfectants and acidifiers.

On Farm A, the waterline disinfectant resulted in all four samples were negative for CP after treatment. Both treated houses of litter disinfectants on Farm A were beneficial to the reduction of CP in the litter but only one demonstrated a prevention of disease, disinfectant treated house that received POXM and monoglyceride disinfectants. On Farm B both disinfectants reduced CP in the litter, even though there was no disease outbreaks on this farm, both of the disinfectant treated houses had improved processing data when compared to the control house. This study also shows that in-house composting improves bird weight, eliminates morbidity, and improves overall production parameters that reflects the higher processing data compared to the control house. Both of the houses, treated houses five and six, that received a constant amount of vitamins in their feed rations on Farm A showed improved animal health, decreased mortality caused by disease, and overall improved bird health by having higher processing weights compared to the control house on this farm. On Farm C, although the house that was treated with litter and water amendments had no change of CP in the litter, it did have improved processing data when compared to its respected control house.

Disinfectants that are GA-, POXM-, and monoglyceride-based should be considered to reduce the pathogenic bacteria CP in the litter to disrupt the cycle of recurring outbreaks of GD on a broiler farm. Overall, this study demonstrated that windrowing is beneficial to the poultry producer because it shows that this method of composting as a way to decrease foodborne pathogens in a poultry house (Macklin et al., 2008) while improving bird weights and eliminating GD. When birds do not experience a change in vitamin levels throughout feed changes they are better able to fight off bacterial invaders, infection, and disease; as well as, have improved weight gain, lower feed conversion, and overall improved livability because of optimal function of the reproductive, muscular, circulatory, nervous, and immune systems (Wilgus, 1977; Latshaw, 1991; Leshchinsky and Klasing, 2001).

Many factors should be considered when choosing a product or procedure to implement into a poultry farm to either reduce pathogens that cause disease or to improve bird parameters by reducing morbidity and mortality, as well as, increasing weight gain by reducing feed conversion on the farm. From the data represented in these experiments and data shown from previous literature, probiotics will benefit many parameters of the grow-out process while having improved production parameters. To our knowledge, there is no past literature that presents probiotics effect on disease, this is the first study to determine that probiotics will eliminate the onset of GD when birds are undergoing a field outbreak. We would also recommend the use of waterline disinfectants to get the best water available to chickens. Waterline disinfectants remove scale, biofilm, and bacteria (as shown in this paper by eliminating CP). These

disinfectants also prevent other products that are administered to birds through the waterlines from being deactivated by contaminants in the waterlines. Windrowing has also proven beneficial to the poultry industry. We would highly recommend windrowing to compost litter and reduce pathogenic bacteria. If there is not enough down-time available to windrow houses for the correct amount of time, we would then recommend the use of litter disinfectants such as GA, POXM, and monoglycerides. Even though iodine-based disinfectants may be beneficial for other sanitation purposes, and did show decreased CP in the litter, we would not recommend the use of this type of disinfectant for poultry operations because it did not eliminate disease. The acidified-treated house did have improved production parameters when compared to its control house but did not have a change the concentration of CP in the litter; so this treatment is not recommended for pathogen reduction or disease elimination.

The data presented in this thesis shows how probiotics, vitamins added to the feed, windrowing, GA, POXM, and monoglyceride disinfectants can be used as a tool for the reduction of foodborne pathogens such as CP in a poultry farm. Theses products not only have the potential to reduce these foodborne pathogens but also show promising results in the reduction of clinical signs associated with GD. Reducing the effects of GD will help the poultry industry produce a better, more economically available product for the consumer. This research will have a positive impact on the development of new technologies and the combination of these technologies will further reduce the potential of diseased flocks and contaminated food products.

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