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Application of botanicals in poultry production to improve microbiological quality

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I am submitting herewith a thesis written by Francisco Javier Gonzalez-Gil entitled "Application of botanicals in poultry production to improve microbiological quality." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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Application of botanicals in poultry production to improve microbiological quality

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ABSTRACT

Foodborne illness and outbreaks associated with poultry products are commonly caused by *Campylobacter jejuni* or *Salmonella enterica*. These pathogens colonize the bird intestines during rearing, and if processing, handling or cooking is not done properly, contamination and human illness can occur. Probiotics, prebiotics and botanicals are being evaluated as novel feed additives to reduce pathogen colonization and serve as growth promoter additives in poultry production. Some botanicals are of industrial interest because they are natural antimicrobials or possess beneficial effects on human health. In this research, the application of a botanical (yerba mate) and a probiotic were evaluated as feed additives for broiler chickens to reduce *Salmonella* colonization. First, the antimicrobial activity of yerba mate extract was evaluated *in vitro* against *Salmonella* Enteritidis (SE) and lactic acid bacteria (LAB). Then, *in vivo* evaluations were conducted. Day-of-hatch chicks were treated with of the following 1) no treatment (control); 2) ground yerba mate in feed; 3) probiotic treatment (*Lactobacillus acidophilus* and *Pediococcus*; 9:1 administered once on day-of-hatch by gavage) or 4) both yerba mate and probiotic treatments. At day 3, all chicks were challenged with SE and at day 10, all birds were euthanized and cecal contents enumerated for *Salmonella*. For the *in vitro* evaluation, antimicrobial activity was observed against *Salmonella*, while the same treatment enhanced growth of LAB. For *in vivo* evaluations, the probiotic treatment significantly reduced *Salmonella* colonization in the horizontal transmission experiment while none of the yerba mate treatments significantly reduced SE colonization. Yerba mate decreased chicken body weight and decreased the performance of the probiotic treatment when used in combination. It is important to evaluate the use of novel probiotics, prebiotics or botanicals for poultry production. Bird

health, growth promoter effects or antinutritional factors of botanicals should be considered before designing diets.

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INTRODUCTION

The Centers for Disease Control and Prevention (CDC) estimates that 48 million people get sick, 128,000 are hospitalized and 3,000 die of foodborne illness in the United States each year (CDC, 2011). Consumption of meat and meat products contaminated with enteric pathogens has been identified as the source of several foodborne outbreaks, which is a big concern for animal producers, authorities and consumers. *Campylobacter jejuni* is associated with consumption of raw or undercooked poultry and poultry products and *Salmonella enterica* outbreaks commonly involve poultry and produce. Extensive research is being conducted to evaluate the use of probiotics, prebiotics and botanicals in chicken performance and microbiological quality. The main target is to find a feed additive that provides an ideal flora that allows optimum growth performance while inhibiting the colonization of pathogenic bacteria in the GI tract to reduce the number of foodborne illness related to poultry consumption. Chapter I will discuss the use of botanicals in poultry production and advantages and disadvantages of the use of botanicals with high polyphenol content. Chapter II provides information of an *in vivo* and *in vitro* research conducted with the use of yerba mate, a botanical feed additive with antimicrobial activity against *Salmonella* Enteritidis and *Campylobacter jejuni in vitro*.

CHAPTER I:

LITERATURE REVIEW

Abstract

Campylobacter jejuni and *Salmonella enterica* are common pathogens associated with poultry. They both cause approximately 2.5 million cases of foodborne illness each year in the United States. Probiotics, prebiotics and botanicals are being evaluated to find novel feed additives that reduce pathogen colonization and serve as growth promoter additives in poultry production. The objective is to decrease foodborne illness and outbreaks related to poultry products and serve as an alternative to antibiotic growth promoters. An important form of botanical feed additives is essential oils (EOs). EOs is secondary metabolites that contain most of the active substances of the plant, including polyphenols. EOs work as antimicrobial because they target the cell membrane of microorganisms and disintegrate it. EOs can increase performance and productivity when administrated at the optimum inclusion rate. Polyphenols present in EOs have several beneficial effects in hu

man and animal health for their antioxidant capacity. They possess biological properties including anti-aging, anti-cancinogen, anti-atherosclerosis, cardiovascular protection and anti-inflammation. In some cases, polyphenols can have a detrimental effect in the consumer. They could decrease protein and lipid digestibility, they can be toxic to liver and kidney or alter spermatic activity. These antinutritional factors are attributed at that polyphenols are produced by plants as natural defense mechanism. There is scare information about if polyphenols are beneficial or detrimental to the consumer but what is sure is that at high concentrations they represent a risk to the consumer. Antinutritional factors of botanicals should be taken in consideration before designing diets for poultry production.

Introduction

Foodborne Pathogens

The Centers for Disease Control and Prevention (CDC) estimates that 48 million people get sick, 128,000 are hospitalized and 3,000 die of foodborne illness in the United States each year (CDC, 2011). Foodborne illness is caused mainly by enteric bacteria, viral pathogens, and parasites, but also can be caused by marine dinoflagellates, bacteria that produce biotoxins and the self-inducing prions of the transmissible encephalopathies (Tauxe, 2002). The most common route of contamination and illness is the consumption of contaminated food with pathogens, microorganisms or toxins (Doyle and Erickson, 2006). Scharff (2010) estimated that the total cost of foodborne illness in the US is $152 billion per year, suggesting that foodborne illness continues to be a significant problem that needs to be addressed.

Consumption of meat and meat products contaminated with enteric pathogens has been identified as the source of several foodborne outbreaks, which is a big concern for animal producers, authorities and consumers. For example, *E. coli* O157:H7 is associated with the consumption of beef products, *Campylobacter jejuni* is associated with consumption of raw or undercooked poultry and poultry products and *Salmonella enterica* outbreaks commonly involve poultry and produce. Despite efforts to reduce risk factors implicated in foodborne outbreaks, the incidence of these illnesses is not decreasing.

Salmonella

Salmonella is the leading cause of hospitalizations and death due to foodborne pathogens. Salmonellosis causes 23,128 hospitalizations and 452 deaths each year in the United States

(Scallan et al., 2011). It is the most frequently reported foodborne pathogen with the highest incidence in children under 5 years old and adults over 60 years old. *Salmonella* causes gastroenteritis with abdominal pain, diarrhea, fever, nausea, vomiting and headache as common symptoms. Recently, *Salmonella* outbreaks reported by the CDC have involved fruits and vegetables, live poultry, and peanut butter, but it is widely known that poultry products are the main vehicle of human salmonellosis. *Salmonella* is found in the intestinal tract of birds but it is not part of the normal flora, it is acquired from feed and environment. Salmonellosis symptoms usually start from 12 to 72 hours after infection and the duration of the illness is typically 4 to 7 days. Most persons recover without treatment but in some cases hospitalization is needed due to a severe diarrhea (CDC, 2013a).

Campylobacter

Campylobacter is a primary concern for public health because it is one of the most common causes of foodborne illness worldwide. In the United States, there are over 1.3 million cases, 13,240 hospitalizations and 119 deaths each year related to campylobacteriosis (Scallan et al. 2011). *Campylobacter* is the most frequent cause of acute bacterial diarrhea in many developed countries and other common symptoms may include fever, abdominal pain, malaise and vomiting. In very few cases, *Campylobacter* infections cause sequelae including the Guillain-Barré syndrome and reactive arthritis (Altekruse et al., 1999; Humphrey et al., 2007). The highest incidence occurs in infants and adults between 20 and 30 years old. When campylobacteriosis occurs, antibiotic therapy is not recommended but fluid balance and bed rest are important, the typical duration of the illness is less than 10 days. Like *Salmonella*, poultry is a common vehicle for *Campylobacter* because they are reservoirs of the pathogen without causing harm or disease to the bird.

Poultry Production and Processing

Live poultry and poultry products are a frequent vehicle of *Salmonella enterica* or *Campylobacter jejuni* infection in humans. In recent years, the CDC has reported several outbreaks related to *Salmonella* in poultry and poultry products, including live poultry, chicken products, ground turkey and shell eggs (CDC, 2013b). Conversely, *Campylobacter* occurs sporadically and there are few reported outbreaks (Finch and Blake, 1995; Pearson et al., 2000; Allerberger et al., 2003).

From farm to fork, *Salmonella* and *Campylobacter* can contaminate poultry in a variety of ways. On the farm, the environment in close proximity to the rearing houses is the most likely source of contamination. The farm workers as well as vectors including birds, reptiles, insects and vermin, also serve as reservoirs of enteric pathogens and propitiate the contamination of poultry with *Salmonella* and *Campylobacter* (Kazwala et al., 1990). *Salmonella* is a common contaminant of feed but it also can survive in litter and soil for several weeks and *Campylobacter* can be found in the air, litter and drinking water containers contaminated with feed or fecal material (Bryan and Doyle, 1995). Intensive rearing is conducive to horizontal transmission of *Salmonella* and *Campylobacter* colonization. One infected bird can easily spread pathogens to many birds because pathogens are shed in feces and birds habitually peck at litter (White et al., 1997). *Salmonella* and *Campylobacter* mainly colonize the ceca of chickens. The mechanism of *Salmonella* colonization is not fully understood while *Campylobacter jejuni* is known to be drawn to mucin and L-fucose in the ceca and utilizes mucin as a source of nutrients (Hugdahl et al., 1988).

Processing is a very important step for the microbiological quality of poultry meat. If it is not done correctly, meat contamination may occur. The crucial steps include scalding, defeathering and evisceration, where transfer of microorganisms from the GI tract or contamination of the equipment, personnel or utensils to the poultry meat can occur. Bacteria adhere firmly to poultry carcasses and migrate from the skin to ridges and crevices where they become entrapped (Bryan and Doyle, 1995). Pathogens present in the carcasses increase the risk of outbreaks and people getting sick from foodborne illness. Food handlers and final consumers have important roles in preventing illness. Improper cooling and inadequate cooking or thermal processing of meat products and cross-contamination during food preparation can have a detrimental impact on the food quality and may cause foodborne illness or outbreaks. Governmental agencies and producers should not assume that the risk of getting a foodborne illness is eliminated with proper food handling; they need to eliminate the problem to reduce the risk of contamination.

Poultry Microbiota

Extensive research has been carried out to learn and understand chicken intestinal microbiota, its complex associations and dynamic relations. Intestinal microbiota is of much importance because it is related to health and well-being of the host. Microbial interactions influence the intestinal environment, affecting the development and responses of the host against pathogenic and non-pathogenic bacteria (Ricke et al., 1999). A wide variety of digestive flora are present in the gastrointestinal tract of birds, including bacteria, fungi and protozoans (Gabriel et al., 2006) and birds obtain the flora from the feed and the environment within a few hours after hatching. The microorganisms of the digestive flora are located in the gut lumen, buried in the mucus layer or adhering to the digestive mucosa.

These mucosal bacteria form a very important cell layer that plays an important role in the health and well-being of the host (Gong et al., 2002; Gabriel et al., 2006).

A wide number of studies have been carried out, culturing on a variety of selective and non-selective media, to characterize and understand the chicken digestive ecosystem (Barnes et al., 1979). In these studies most of the cultures obtained from ceca showed a high density and variability of Gram-positive bacteria, as compared with cultures from the small intestine that had a simpler bacterial community dominated by *Lactobacilli* (Gong et al., 2007). However, traditional methods of classical culturing of digestive microflora only identified 20 to 50% of bacteria present in the microbiota (Patterson and Burkholder, 2003). Therefore, molecular techniques have been developed using 16S ribosomal DNA gene sequencing analysis, which gives a more precise and complete image of the microbial density than culturing (Gabriel et al., 2006). Those studies using molecular methods showed a more detailed characterization of the microflora present in the gastrointestinal tract, mainly dominated by *Lactobacilli*. In a study conducted by Lu et al. (2003), 70% of the bacterial sequences in the ileum were related to those of *Lactobacillus*, 11% to *Clostridiaceae,* 6.5 % to *Streptococcus* and 6.5 % to *Enterococcus*, while in the ceca, 65% of the bacterial sequences were related to *Clostridiaceae*, 14% to *Fusobacterium, 8% to Lactobacillus* and 5% to *Bacteroides*; but these numbers vary considerably from bird to bird. *Lactobacilli* were also predominant in the small intestine, gizzard and crop (Gong et al., 2007; Zhu et al., 2002).

Factors related to each specific animal, like sex, age and immune system influence the microflora present in the gastrointestinal (GI) tract. It has been determined that each individual possess a specific bacterial community in the GI tract that can be modulated by

several factors related to the environment and rearing conditions. This profile is also a function of the diet as dietary ingredients are potential substrates for bacterial growth (Gabriel et al., 2006). Furthermore, all the biochemical processes occurring during digestion, modulate the microbiota present on the GI tract (Zhu et al., 2002).

The numbers of microbes can reach 10^{11} CFU/g and $l0^{9}$ CFU/g of caecal and ileal digesta, respectively, during the first three days post hatch and remain relatively stable for the following 30 days. This large amount of bacteria can use 10 to 20% of carbohydrates and amino acids that could be otherwise utilized by the host (Apajalahti et al., 2004). Although, there exists an internal competition between the host and microbiota for dietary nutriments, these microorganisms also have a positive effect on the host by releasing factors including vitamins and fatty acids that the host can absorb in the intestines and the ceca. Most non-digestible carbohydrates are fermented by the microflora in the ceca. Nitrogenous compounds which persist in the ceca are broken down by bacteria into short chain fatty acids (SCFA), which are later absorbed by the host (Gabriel et al., 2006). Schaedler (1973) concluded that and ideal flora allows optimum growth performance while an alteration could be deleterious to the host. Changes in dietary composition or nutrient availability can have dramatic effects on the intestinal microflora populations, which in turn can influence the ability of the animal to digest and absorb dietary nutrients (Lu et al., 2003; Apajalahti et al., 2004).

Pathogen Control

In general, intestinal bacteria may be divided into species that exert beneficial effects on the host (*Lactobacilli* and *Bifidobacterium*) or pathogenic bacteria to the bird or human (*E. coli*, *Campylobacter* and *Salmonella*) (Li et al., 2009). Intestinal microbiota plays an

important role in the health status of host animals and it is the first barrier against foodborne zoonotic pathogens (Zhu et al., 2002; Li et al., 2009). Proposed mechanisms of pathogen inhibition by the intestinal microbiota include competition for nutrients, production of toxic conditions and compounds (volatile fatty acids, low pH, and bacteroicins), competition for binding sites on the intestinal epithelium and stimulation of the immune system (Patterson and Burkholder, 2003). The intestinal epithelium, together with the mucus, provides the first line of defense against pathogens and antigens (Gaggia et al., 2010). The concept of a gastrointestinal probiotic or competitive exclusion culture is to prevent pre-harvest colonization of the gastrointestinal tract of food animals by foodborne zoonotic pathogens. Development and application of effective competitive exclusion cultures may prevent *Salmonella* colonization mainly based on the understanding of the progression and establishment of the intestinal microflora as the bird ages (Ricke et al., 1999). Furthermore, the intestinal microflora participates in the maintenance of an effective intestinal immune system. It influences the number, distribution and degree of activation of cell populations of the intestinal immune system by activating phagocytosis and cytokine synthesis by macrophages (Gabriel et al., 2006).

Beneficial Bacteria

Competitive Exclusion

The term competitive exclusion was first introduced by Nurmi and Ratala (1973). They found that administering a suspension of adult cecal contents to baby chicks reduced *Salmonella* colonization. Today it is a common practice to administer cultures, mix of cultures or commercial probiotic products to day-of-hatch chicks to protect and reduce pathogens colonization. Due to the complete ban of antibiotics by the European Union in

2006, the poultry industry is increasing the research efforts in order to use probiotic, prebiotic and botanical feed supplementation as alternatives to improve host health, enhance feed intake, weight gain and control foodborne pathogens.

Probiotics

Probiotics are live microorganisms that promote host health and are associated with the concept of competitive exclusion. Many of the species of probiotics used are constituents of the normal gut microbiota of humans and animals. Probiotics can support the beneficial effects of commensal bacteria and protect from pathogen colonization through several modes of action. The most used probiotic bacteria in poultry production are *Lactobacillus*, *Bifidobacterium*, *Pedioccoccus*, *Enterococcus*, *Saccharomyces* and *Bacillus*. *Lactobacillus* is a lactic acid producing bacteria and a significant constituent of the gut microbiota of humans and animals, including chicken broilers (Zhu et al., 2002; Lu et al., 2003). *Bacillus* is a non-lactis, spore-forming Gram-positive microorganism normally found in the intestinal tract of animals. *Bifidobacterium* is one of the major bacteria found in the intestinal microbiota; it is associated with good health of the host maintaining the appropriate balance of the microbiota reducing the risk of pathogen colonization (Gaggia et al., 2010). When probiotics are delivered during early life of the host, the bacteria can modulate expression of genes in intestinal epithelial cells, thus creating a favorable habitat for themselves (Gaggia et al., 2010).

The beneficial effects of probiotics in the host have been widely studied, for example *Bifidobacteria* lowers cholesterol levels, acts as immunomodulator, produces vitamin B and folic acid, reduces blood ammonia levels and produces acetate and lactate which inhibit the growth of potential pathogens by acidifying the gut contents (Gibson and Roberfroid,

1995). Numerous studies *in vivo* have demonstrated the effectiveness of probiotics against pathogen colonization, including *Salmonella* Enteritidis (SE) and *Campylobacter jejuni*. For example, Higgins et al. (2007) recovered significantly less SE compared to the control when day-of-hatch chicks were treated with probiotics and subsequently challenged with *Salmonella* Enteritidis. A commercial product containing *Bacillus cereus* var. Toyoi was found to be effective at reducing *Salmonella* Enteritidis in broilers and Leghorn chickens (Vila et al., 2009). Santini et al (2010) did an in depth evaluation of 55 LAB and *Bifidobacteria* for desirable properties for potential probiotic strains and assessed the capability of the most promising strains to colonize the GI tract of poultry. They found that *Bifidobacterium longum* PCB 133 possessed the best probiotic properties *in vitro* and was able to colonize the gut and significantly reduced *Campylobacter jejuni* in live poultry.

Bird health and performance have also been increased with the use of probiotics. Vicente et al. (2007) significantly reduced mortality, improved body weight (BW) and reduced feed conversion ratio (FCR) treating broilers reared under commercial conditions with a *Lactobacillus* based probiotic product. They concluded that production costs decreased with this treatment due to the improvements caused by the probiotic. Supplementing chicken feed with a mix of twelve different *Lactobacillus* strains isolated from the chicken intestine, increased BW and decreased FCR, serum cholesterol levels and mortality for broiler chickens (Jin et al., 1998).

Prebiotics

Gibson and Roberfroid (1995) introduced the term prebiotic defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon”. To consider a feed additive as

a prebiotic, it must not be hydrolyzed or absorbed in the GI tract, it must be selective for a limited number of beneficial bacteria, it must beneficially alter the intestinal microbiota and their activities and fermentation of the substrate should induce beneficial effects within the host. (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003; Donalson et al., 2008; Gaggia et al., 2010).

Non-digestible oligosaccharides meet the definition of prebiotic and some of the most common prebiotics are fructooligosaccharides (FOS), galactooligosaccharides, transgalacto-oligosaccharidesand lactulose. Mannanoligosaccharides (MOS) have been uses as a prebiotic supplement but they do not selectively enrich for beneficial bacterial populations. MOS prevent bacteria from being excreted by promoting attachment due to mannose, which binds to the type 1 fimbriae used by many enteric bacteria to attach to the host cell (Gaggia et al., 2010; Kim et al., 2011).

Prebiotics are known to act as nutrients for colonic bacteria and produce SCFA, which modify bacterial ecosystems. SCFA in the GI tract inhibit the growth of pathogenic bacteria which increases performance in poultry due to better nutrient utilization. Kim et al. (2011) applied different FOS and MOS treatments in broilers and observed an increase in *Lactobacilli* population and a decrease of *Clostridium perfingens* and *E. coli*. Improvements in FCR have been obtained as well as enhanced growth of *Bifidobacterium* and *Lactobacillus* and decrease in *E. coli* with FOS supplementation (Xu et al., 2003). Sims et al. (2004) observed an improvement in BW and FCR and increase of *Lactobacilli* and *Bifidobacterium* counts when feeding an MOS treatment to turkeys.

Botanicals

General Information

Botanicals include plants and plant products. They can be solid, dried, ground, plant extract, oleoresin or EOs. They have a long history in human medicine and nutrition and they are commonly used for flavor, color and aroma or as preservatives in food and beverages systems. They have a great variety of phytochemical compounds which are responsible for the beneficial effect to the consumer (Windisch et al., 2008; Hippenstiel et al., 2011). One of the most economically important forms of botanicals are EOs which are odoriferous secondary metabolites obtained from plant materials including flowers, buds, seeds, leaves and fruits that contain most of the active substances. They play an important role in protection of plants acting as antibacterial, antiviral, antifungal and insecticide. They also may attract insects to help dispersion of pollen and seeds, or repel other undesirable insects (Bakkali et al., 2007; Applegate et al., 2010).

Antimicrobial Properties

Botanicals have gained researchers and industry attention for their effective use against foodborne pathogens. There are numerous *in vitro* studies evaluating the effects of a wide variety of botanicals, against bacteria but the most effective antimicrobial form is EOs (Burt, 2004; Bakkali et al., 2008). Mechanisms of actions of EOs involve cell wall deterioration, cell lysis, disintegration of the outer membrane but numerous authors conclude that the cell membrane is the main target of EOs (Burt, 2004). Ouwehand et al. (2010) evaluated the effect of several EOs on common pathogens and beneficial members of the microbiota. Carvacrol, cinnamaldehyde, citral and thymol were the most effective at reducing *S. enterica*, while *E. coli* strains were relatively sensitive to most EOs tested.

Owehand et al. (2010) and Si et al., (2006) have also reported that beneficial bacteria including *Bifidobacteria* and *Lactobacillus* are slightly more resistant to EOs than pathogens and some strains are actually growth stimulated by specific EOs, suggesting that EOs may be used to inhibit the growth of pathogens while stimulating the growth of beneficial bacteria.

Effects on Feed Intake and Passage Rates

Botanicals have been used as feed additives for poultry production because they increase performance and productivity due to their antioxidant activity, growth promoting effects and antimicrobial properties (Windisch et al., 2008). The use of botanical products including products from rosemary, thymol and carvacrol, as antioxidants in poultry production and processing, has been found to contribute to improvements in oxidative stability in chicken and turkey meat (Botsoglou, 2002a; 2003a; 2003b). In poultry production, EOs have been used to improve FCR and BW by beneficially altering the composition and activity of the gut microflora (Leusink et al., 2010), proving that an ideal flora promotes the optimum growth performance. The effect of botanicals on broiler performance and gut microbiota has been extensively evaluated and some authors conclude that EOs lower counts of pathogens including *Clostridium*, *E. coli* and *Salmonella*, while increasing counts of *Bifidobacteria* and *Lactobacillus*. The use of EOs also increases performance in terms of BW and FCR because the beneficial members of the microbiota are positively affected. Tiihonen et al. (2010) lowered *E. coli* and *Clostridium*, obtained higher counts of *Bifidobacteria* and *Lactobacillus* and increased BW measurements compared to the control when they fed a blend of EOs to broiler chickens. The use of EOs as feed additives is thought to be more effective than botanicals because EOs contain

polyphenols and other active compounds in a concentrated form. However, there is not enough information about which form is more effective at controlling pathogens and increasing performance. A study was conducted by Cross et al. (2007) to evaluate the effect of feeding herbs or its associated EOs on bird performance and intestinal microbiota. They reported that birds fed yarrow herb had greater BW than those fed yarrow oil, but the group fed thyme oil had the greatest BW. The authors concluded that herbs and EOs have different effects on broilers based on the terpene composition of the feed additives.

Polyphenols in Botanicals

Polyphenols are secondary metabolites present in EOs and botanicals. They occur in fruits, vegetables and byproducts including wine, tea and chocolate. They have several functions in plants including color of leaves, flowers and fruits, antimicrobial and antifungal, chelation of toxic heavy metals and antioxidants during photosynthesis (Gould and Lister, 2006). They are produced by plants as defense against herbivores, insects and pathogens to avoid predation (Khokhar and Apenten, 2003). The most abundant polyphenols in the human diet are flavonoids including quercitin and kaempferol and phenolic acids including caffeic and chlorogenic acids. Other important water-soluble polyphenols include tannins, which give astringency or bitterness to fruits. Tannins include proanthocyanidins and tannic acid (Han et al., 2007). Polyphenols have received a lot of attention because they are thought to be beneficial for human and animal health for their antioxidant capacity.

Oxidative stress plays an important role in pathogenesis of aging and several degenerative diseases including atherosclerosis, cardiovascular diseases, type II diabetes and cancer (Gutteridge, 1993). Dietary polyphenols are excellent at reducing this oxidative stress which is why consumers believe polyphenols supplementation will be beneficial to their

health as natural antioxidants. Polyphenols reduce oxidative stress by scavenging free radicals inhibiting oxidant enzymes, impacting cell cycles and inducing endogenous antioxidant enzymes. They possess diverse biological properties including antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and cell proliferation activity (Han et al., 2007; Stevenson and Hurst, 2007). They may also help protect the GI tract against damage by reactive species present in food or generated within the stomach and intestines (Halliwell, 2007).

One of the many reasons why polyphenols are becoming very popular in human nutrition is because they are believed to play a role in inhibiting cancer development by modulating cell signaling pathway and inducing apoptosis in malignant cells (Stevenson and Hurst, 2007). A clear example of the potential beneficial effects of polyphenols is the polyphenol gossypol extracted from cotton oil, which has anti-viral activity in vitro, including human immunodeficiency virus (HIV). It has been proposed as a male anti-fertility agent (Polsky et al., 1989) but gossypol also possesses antinutritional properties.

Once polyphenols are consumed, they are extensively metabolized to simpler phenolic and non-phenolic compounds. They are absorbed through the gut barrier and metabolized in the tissues, and if not absorbed, they serve as substrates to the colonic flora, which metabolize them (Scalbert et al., 2002; Rechner et al., 2003). Unabsorbed dietary polyphenols and their metabolites may play a key role in the maintenance of intestinal health and can modulate gut microflora (Selma et al., 2009). Phenolic compounds including quercitin and caffeic acids have been reported to inhibit various pathogenic bacteria in vitro (Aziz, 1998). As an example of an application of polyphenols in animal production, Viveros et al. (2011) feed the botanical grape seed extract (GSE) to broiler chickens and concluded that polyphenols

found in GSE increased populations of beneficial bacteria in the ileum as well as increasing villus height:crypth depth ratio in the jejunum.

Polyphenols are complex molecules and have multiple potential actions other than antioxidant or antimicrobial. Given that they are a natural defense mechanism of plants against predators, they may have a negative effect in humans and animals when they are consumed, due to antinutritional factors. Makkar (1993) defined antinutrients as substances that interfere with food utilization and affect health and production of consumers. Plants are known to contain a wide variety of antinutritional substances that can be anti-vitamins or could affect protein, lipid or mineral utilization and digestion (Francis et al., 2001). Some examples of foods with important antinutritional factors are legumes, oil seeds and leaves rich in polyphenols.

Extensive research to include botanical feed supplements in animal production is being conducted. New plants and EOs are being evaluated to look for an ideal product that achieves an increase in animal performance and controls intestinal pathogens. But there is scare information and awareness that these botanical supplementations can include antinutrients which can cause a detrimental effects or reductions in performance of animals. Longstaff and McNab (1991) evaluated a tannin-rich diet in young chicks and found that tannins inhibit digestive enzymes including trypsin, lipase, α-amylase and α-glycosidas, which decrease digestibility of proteins, starches and lipids. Yuste et al. (1992) obtained similar findings in chickens and other authors concluded the same (Sarwar Gilani et al., 2005; Han et al.,2007).

Not only for animal production but in humans, polyphenols might have a negative impact on the health of the consumer. Fang et al (2007) concluded that the consumption of excessive amounts of polyphenols in dietary supplements may affect DNA methylation status, but its toxicity needs to be further demonstrated. Polyphenols can be toxic to the liver and kidney and cause stomach cancer in rats (Ferry et al., 1996; Galati et al., 2006; Stevenson and Hurst, 2007). Moreover, depending on the consumer or application, polyphenols can have either a beneficial or detrimental effect, as is the case of the polyphenol gossypol. Francis et al. (2001) summarized that feeding fish a cottonseed meal containing gossypol could cause growth depression, intestinal and internal organ abnormalities, liver and kidney damage and alterations in spermatic activity. There is still a long way to go and research to be conducted to prove if polyphenols are an effective feed additive in poultry production. It is certain that some polyphenols have evolved to be toxic to organisms that feed on them. Humans and animals are relatively resistant to them, but at definitively high doses, polyphenols could be harmful (Stevenson and Hurst, 2007).

Conclusions and future directions

Salmonella enterica and *Campylobacter jejuni* cause approximately 2.5 million cases of foodborne illness each year in the United States. The CDC reports poultry as a common vehicle to human infection for these two pathogens. The reason is that they easily colonize the chicken intestine during production and carcass contamination with GI contents during processing commonly occurs. To reduce the number of infections and outbreaks, pre-harvest intervention and exclusion strategies including the use of probiotics, prebiotics and botanicals were proposed.

Extensive research is being conducted to evaluate the use of probiotics, prebiotics and botanicals in chicken performance and microbiological quality. The main target is to find a feed additive that provides an ideal flora that allows optimum growth performance while inhibiting the colonization of pathogenic bacteria in the GI tract to reduce the number of foodborne illness related to poultry consumption. A new approach is the use of EOs as feed additive because they possess polyphenols and other active compounds in a concentrated form. Several bird trials have demonstrated that polyphenols can maintain a healthy gut microflora while inhibiting pathogen bacteria. Polyphenols possess antinutritional factors that could decrease protein or lipid digestibility or be toxic to the consumer. Future research should evaluate the use of feed additives with synergistic capacities to increases chicken performance, promote gut health and reduce pathogen colonization. However, antinutritional factors of polyphenols should be considered before evaluating new feed additives.

References

Altekruse, S.F., N.J. Stern, P.I. Fields and D.L. Swerdlow. 1999. Campylobacter jejuni—an emerging foodborne pathogen. Emerging Infectious Diseases. 5:28-35

Allerberger, F., N. Al-Jazrawi, P. Kreidl, M.P. Dierich, G. Feierl, I. Hein and M. Wagner. 2003. Barbecued chicken causing a multi-state outbreak of Campylobacter jejuni enteritis. Infection. 31:19-23

Apajalahti, J., A. Kettunen and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poultry Science Journal. 60:223-232

Applegate, T.J., V. Klose, T. Steiner, A. Ganner and G. Schatzmayr. 2010. Probiotics and phytogenics for poultry: Myth or reality?. J. Appl. Poult. Res. 19:194–210

Aziz, N.H., S.E. Farag, L.A.A. Mousa, and M.A. Abo-Zaid. 1998. Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios 93:43–54.

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar. 2008. Biological effects of essential oils – A review. Food and Chemical Toxicology. 46:446–475

Barnes, E.M. 1979. The intestinal microflora of poultry and game birds during life and after storage. Journal of Applied Bacteriology. 46:407-419

Botsogloua, N.A., D.J. Fletourisb, P. Florou-Paneria, E. Christakia, A.B. Spaisa. 2003a. Inhibition of lipid oxidation in long-term frozen stored chicken meat by dietary

oregano essential oil and a-tocopheryl acetate supplementation. Food Research International 36:207–213

Botsoglou, N.A., P. Florou-Paneri, E. Christaki, D.J. Fletouris and A.B. Spais. 2002a. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. British Poultry Science. 43:223–230

Botsogloua, N.A., S. H. Grigoropouloub, E. Botsogloub, A. Govarisc and G. Papageorgiou. 2003b. The effects of dietary oregano essential oil and a-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage.

Bryan, F.L. and M.P. Doyle. 1995. Health risks and consequences of Salmonella and Campylobacter jejuni in raw poultry. 58:326-344

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology.94:223–253

Centers for Disease Control and Prevention (CDC). 2011. 2011 Estimates of foodborne illness in the United States. http://www.cdc.gov/features/dsfoodborneestimates/. January 2014

Centers for Disease Control and Prevention (CDC). 2013a. Salmonella. http://www.cdc.gov/salmonella/. January 2014

Centers for Disease Control and Prevention (CDC). 2013b. Salmonella. Reports of Selected *Salmonella* Outbreak Investigations. http://www.cdc.gov/salmonella/ outbreaks .html. January 2014

Cross, D.E., R.M. McDevitt, K. Hillman and T. Acamovic. 2007. The effect of herbs and their associated essential oil on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poult. Sci. 48:496–506

Donalson, L.M., J.L. McReynolds, W.K. Kim, V.I. Chalova, C.L. Woodward, L.F. Kubena, D.J. Nisbet and S.C. Ricke. 2008. The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract fermentation, Salmonella Enteritidis infection, and intestinal shedding in laying hens. Poult. Sci. 87:1253–1262

Doyle, M.P. and M.C. Erickson. 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. Poultry Science. 85:960–973

Fang, M.,D. Chen and C.S. Yang. 2007. Dietary Polyphenols May Affect DNA Methylation. Journal of Nutrition. 137:223-228

Ferry, D.R., A. Smith, J. Malkhandi, D.W. Fyfe, P.G. deTakats, D. Anderson, J. Baker, and D.J. Kerr. 1996. Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. Clin. Cancer Res. 2:659-668.

Finch, M. and P. Blake. 1995. Foodborne outbreaks of campylobacteriosis: the United States experience, 1980-1982. American Journal of Epidemiology. 122:262-268

Francis, G., P.S.H. Makkar and K. Becker. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199:197–227

Gabriel, I., M. Lessire, S. Mallat and J.F. Guillot. 2006. Microbiota of the digestive tract: critical factors and consequences for poultry. World's Poultry Science Journal. 62:499-511

Gaggia, F., P. Mattarelli and B. Biavati. 2010. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology. 141:S15-S28

Galati, G., A. Lin, A.M. Sultan, and P.J. O_Brien. 2006 Cellular and in vivo hepatotoxicity caused by green tea phenolic acids and catechins. Free Radic.Biol. Med. 40:570-580

Gibson, G.R. and M.B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition. 125:1401-1412

Gould, K. and Lister, C. 2006. Flavonoid functions in plants. In: Andersen, O. M. and K. R. Markham, (Eds.), Flavonoid: Chemistry, Biochemistry and Applications. Taylor and Francis, Boca Raton, FL, pp. 397–441.

Gong, J., R.J. Forster, H. Yu, J.R. Chambers, R. Wheatcroft, P.M. Sabour and S. Chen. 2002. Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. FEMS Microbiol. Ecol. 41:171-179

Gong, J., W. Si, R.J. Forster, R. Huang, H. Yu, Y. Yin, C. Yang and Y. Han. 2007. 16S rRNAgene-based analysis of mucosa-associated bacterial communityand phylogeny in the chicken gastrointestinal tracts: from crops to ceca. FEMS Microbiol. Ecol. 59:147-157

Gutteridge, J.M. 1993. Free radicals in diseases processes: a compilation of cause and consequence. Free Radic. Res. Commun. 19:141-158

Halliwell, B. 2007. Review. Dietary polyphenols: Good, bad, or indifferent for your health?. Cardiovascular Research. 73:341–347

Han, X., T. Shen and H. Lou. 2007. Dietary polyphenols and their biological significance. Int. J. Mol. Sci. 8:950-988

Higgins, J.P., S.E. Higgins, J.L. Vicente, A.D. Wolfenden, G. Tellez, and B.M. Hargis. 2007. Temporal effects of lactic acid bacteria probiotic culture on Salmonella in neonatal broilers. Poult. Sci. 86:1662–1666

Hippenstiel1, F., A.A.A. Abdel-Wareth, S. Kehraus and K.H. Sudekum. 2011. Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers – a review. Arch. Geflugelk. 75(4):226–234

Hugdahl, M.B., J.T. Beery and M.P. Doyle. 1988. Chemotactic behavior of Campylobacter jejuni. Infect. Immun. 56(6):1560-1566

Humphrey, T., S. O'Brien and M. Madsen. 2007. Campylobacters as zoonotic pathogens: A food production perspective. 117:237-257

Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin. 1998. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poult. Sci. 77:1259–1265

Kazwala, R.R., J.D. Collins, J. Hannan, R.A. Crinion and H. O'Mahony. 1990. Factors responsible for the introduction and spread of Campylobacter jejuni infection incommercial poultry production. Veterinary Record. 126:305-306

Khokhar, S., and R.K. O. Apenten. 2003. Antinutritional factors in food legumes and effects of processing. In Squires, R.V. (Ed.), The Role of food, Agriculture, Forestry and Fisheries in Human Nutrition. Encyclopedia of Life Support Systems (EOLSS). Publishers Co Ltd., Oxford, UK, pp. 82–116. Kim, G. B., Y. M. Seo, C. H. Kim and I. K. Paik. 2011. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poult. Sci.90:75–82

Leusink, G., H. Rempel, B. Skura, M. Berkyto, W. White, Y. Yang, J.Y. Rhee, S.Y. Xuan, S. Chiu, F. Silversides, S. Fitzpatrick and M.S. Diarra. 2010. Growth performance, meat quality, and gut microflora of broiler chickens fed with cranberry extract. Poult. Sci. 89:1514–1523

Li, S.P., X.J. Zhao and J.Y. Wang. 2009. Synergy of Astragalus polysaccharides and probiotics (Lactobacillus and Bacillus cereus) on immunity and intestinal microbiota in chicks. Poult. Sci. 88:519–525

Longstaff, M. and J.M. McNab. 1991. The inhibitory effects of hull polysaccharides and tannins of field beans (Vicia faba L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. British Journal of Nutrition. 65:199-216

Lu, J., U. Idris, B. Harmon, C. Hofacre, J.J. Maurer and M.D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Applied and Environmental Microbiology. 69:6816-6824

Makkar, H.P.S. 1993. Antinutritional Factors in Foods for Livestock. In: Gill M, Owen E, Pollott GE and Lawrence TLJ (eds) Animal Production in Developing Countries, Occassional Publication. British Society of Animal Production. No. 16, pp 69–85.

Nurmi, E. and M. Rantala. 1973. New aspects of Salmonella infection in broiler production. Nature. 241:210-211

Ouwehand, A.C., K. Tiihonen, H. Kettunen, S. Peuranen, H. Schulze and N. Rautonen. 2010. In vitro effects of essential oils on potential pathogens and beneficial members of the normal microbiota. Veterinarni Medicina. 55(2):71–78

Patterson, J.A. and K.M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627–631

Pearson, A.D., M.H. Greenwood, J. Donalson, T.D. Healing, D.M. Jones, M. Shahamat, R.K.A. Feltham and R.R. Colwell. 2000. Continuous source outbreak of campylobacteriosis traced to chicken. Journal of Food Protection. 63:309-314

Polsky, B., S.J. Segal, P.A. Baron, J.W.M. Gold, H. Ueno, D. Armstrong. 1989. Inactivation of human immunodeficiency virus in vitro by gossypol. Contraception. 36(6): 579–587

Rechner, A.R., M.A. Smith, G. Kuhnle, G.T. Gibson, E.S. Debnam, S.K.S. Srai, K.P. Moore and C.A. Rice-Evans. 2004. Colonic metabolism of the dietary polyphenols:

influence of structure on microbial fermentation products. Free Radical Biology Medicine.36(2):212–225

Ricke, S.C. and S.D. Pillai. 1999. Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. Critical Reviews in Microbiology. 25(1):19-38

Santini, C., L. Baffoni, F. Gaggia, M. Granata, R. Gasbarri, D. Di Gioia and B. Biavati. 2010. Characterization of probiotic strains: An application as feed additives in poultry against Campylobacter jejuni. International Journal of Food Microbiology. 141:S98-S108

Sargar Gilani, G., K.A. Cockell and E. Sepehr. 2005. Effects of antinutritional factors on protein digestibility and amino acid availability in foods. Journal of AOAC International. 88(3): 967-987

Scalbert, A., C. Morand, C. Manach, C. Remesy. 2002. Absorption and metabolism of polyphenols in the gut and impact on health. Biomed. Pharmacother. 56:276–282

Scallan, E., R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M. Widdowson, S.L. Roy, J.L. Jones, and P.M. Griffin. 2011. Foodborne Illness Acquired in the United States—Major Pathogens. Emerg. Infect. Dis. 17:7-15

Schaedler, R.W. 1973. The relationship between the host and its intestinal microflora. Proc. Nutr. Soc. 32:41-47

Scharff, R.L. 2010. Health-related cost from foodborne illness in the United States. Produce Safety Project, Georgetown University, Washington, DC.

Selma, M.V., J.C. Espin, and F.A. Tomas-Barberan. 2009. Interaction between phenolics and gut microbiota: Role in human health. J. Agric. Food Chem. 57:6485–6501.

Si, W., J. Gong, R. Tsao, T. Zhou, H. Yu, C. Poppe, R. Johnson and Z. Du. 2006. Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. Journal of Applied Microbiology. 100:296-305

Sims, M.D., K.A. Dawson, K.E. Newman, P. Spring and D.M. Hooge. 2004. Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poult. Sci. 83:1148–1154

Stevenson, D.E. and R.D. Hurst. 2007. Review. Polyphenolic phytochemicals – just antioxidants or much more?. Cell. Mol. Life Sci. 64:2900–2916

Tauxe, R. 2002. Emerging foodborne pathogens. International Journal of Food Microbiology. 78:31–41

Tiihonen, K., H. Kettunen, M.H.L. Bento, M. Saarinen, S. Lahtinen, A.C. Ouwehand, H. Schulze and N. Rautonen. The effect of feeding essential oils on broiler performance and gut microbiota. British Poultry Science. 51(3):381-392.

Vicente, J.L., L. Aviña, A. Torres-Rodriguez, B. Hargis and G. Tellez. 2007. Effect of a Lactobacillus Spp-based probiotic culture product on broiler chicks performance under commercial conditions. International Journal of Poultry Science. 6(3):154-156

Vila, B., A. Fontgibell, I. Badiola, E. Esteve-Garcia, G. Jimenez, M. Castillo and J. Brufau. 2009. Reduction of Salmonella enterica var. Enteritidis colonization and invasion by Bacillus cereus var. toyoi inclusion in poultry feeds. Poult. Sci. 88:975–979

Viveros, A., S. Chamorro, M. Pizarro, I. Arija, C. Centeno and A. Brenes. 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult. Sci. 90:566–578

Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 2008. 86:E140–E148

White, P.L., A.R. Baker and W.O. James. 1997. Strategies to control Salmonella and Campylobacter in raw poultry products. Rev. sci. tech. int. Epiz. 16(2):525-541

Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang. 2003. Effects of Dietary Fructooligosaccharide on Digestive Enzyme Activities, Intestinal Microflora and Morphology of Male Broilers. Poult. Sci. 82:1030–1036

Yuste, P., M. Longstaff, and C. McCorquodale. 1992. The effect of proanthocyanidin-rich hulls and proanthocyanidin extracts from bean (Vicia faba L.) hulls on nutrient digestibility and digestive enzyme activities in young chicks. British Journal of Nutrition. 67:57-65

Zhu, X.Y., T. Zhong, Y. Pandya and R.D. Joerger. 2002. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Applied and Environmental Microbiology. 68:124-137

CHAPTER II:

YERBA MATE ENHANCES PROBIOTIC BACTERIA GROWTH *IN VITRO* BUT AS A FEED ADDITIVE DOES NOT REDUCE *SALMONELLA* ENTERITIDIS COLONIZATION *IN VIVO*

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Abstract

Yerba mate (*Ilex paraguariensis*) is a tea known to have beneficial effects on human health and antimicrobial activity against some foodborne pathogens. Thus, the application of yerba mate as a feed additive for broiler chickens to reduce *Salmonella* colonization was evaluated. First *in vitro* evaluation was conducted by suspending *Salmonella* Enteritidis (SE) and lactic acid bacteria (LAB) in yerba mate extract. The *in vivo* evaluations were conducted using preventative and horizontal transmission experiments. In all experiments, day-of-hatch chicks were treated with one of the following 1) no treatment (control); 2) ground yerba mate in feed; 3) probiotic treatment (*Lactobacillus acidophilus* and *Pediococcus;* 9:1 administered once on day of hatch by gavage) or 4) both yerba mate and probiotic treatments. At day 3, all chicks were challenged with SE (preventative experiment) or 5 of 20 chicks (horizontal transmission experiment). At day 10, all birds were euthanized, weighed, and cecal contents enumerated for *Salmonella*. For the *in vitro* evaluation, antimicrobial activity was observed against *Salmonella* while the same treatment enhanced growth of LAB. For *in vivo* evaluations, none of the yerba mate treatments significantly reduced SE colonization, while the probiotic treatment significantly reduced *Salmonella* colonization in the horizontal transmission experiment. Yerba mate decreased chicken body weight and decreased the performance of the probiotic treatment when used in combination. In conclusion, yerba mate had antimicrobial activity against foodborne pathogens and enhanced the growth of LAB *in vitro*, but *in vivo* yerba mate did not decrease SE colonization.

Introduction

Yerba mate is an herbal tea beverage made with dried leaves of *Ilex paraguariensis.* It is widely consumed in South America and gaining popularity worldwide because of its beneficial effects. Green mate leaves are blanched, dried, milled and aged before commercialization, and later consumed as infusion in hot water (Heck and de Mejia, 2007). Extensive analysis has been done to determine total phenol content, antioxidant activity and essential oil composition (Bastos et al., 2006).

Yerba mate contains a wide variety of polyphenols, xanthines, caffeoyl derivatives, saponins, and minerals (Anesini et al., 2006; Bastos et al., 2006; Heck and de Mejia, 2007). It has several beneficial pharmacologic effects on human health, including hypocholesteroliemic, hepatoprotective, central nervous system stimulant, diuretic capacity (Heck and de Mejia, 2007) and antifungal properties (Filip et al., 2009). Yerba mate has a high polyphenol content which acts as an antioxidant and chemoprotective agent to eliminate hydrogen peroxidase (Anesini et al., 2006). Popular medicine recommends the use of yerba mate for arthritis, headache, constipation, fatigue and hypertension (Bastos et al. 2006). Antimicrobial activity of yerba mate extracts against *Escherichia coli* O157:H7 and *Staphylococcus aureus* has been reported *in vitro* (Burris et al. 2011). Yerba mate has been used as a food additive in chicken meat to improve lipid stability (Racanicci et al., 2011).

The use of feed additives, including prebiotics, and probiotics in poultry have been investigated as means to improve gut health, decrease *Salmonella* Enteritidis (SE) colonization and increase the overall health of the flock (Donalson et al., 2008a,b). Given the effectiveness of yerba mate extracts against other foodborne pathogens, the aim of this

study was to assess, *in vitro*, the biocidal activity of lyophilized yerba mate extracts on SE and lactic acid bacteria (LAB) and, in *vivo,* assess the application of yerba mate as a feed additive treatment and compare it with a probiotic treatment with known efficacy as a method to decrease horizontal transmission and SE colonization.

Materials and Methods

Bacterial Cultures and Preparation

Salmonella Enteritidis 13A (13A), *Lactobacillus acidophilus* and *Pediococcus* (LP) were obtained from Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas. *Salmonella* Typhimurium DT104 (ST) and *Salmonella* Senftenburg (SS) were obtained from the culture collection at the Department of Food Science and Technology at the University of Tennessee, Knoxville. For the *in vitro* evaluation, 13A, ST and SS were cultured in Tryptic Soy Agar (TSA; BD Difco, Sparks, MD) and LP were cultured in *Lactobacilli* MRS agar (BD Difco, Sparks, MD), incubated for 24 h at 37 °C and diluted to 10^4-10^5 CFU/mL for the antimicrobial activity experiment. For the *in vivo* evaluation, 13A was cultured following Higgins et al. (2011) and LP was cultured in *Lactobacilli* MRS broth (BD Difco, Sparks, MD) for 24 h at 37°C and diluted to 10^7-10^6 CFU/mL. A mix of LP at a ratio of 9:1 respectively, was made and used for both *in vitro* and *in vivo* evaluations.

Yerba Mate Extraction for In Vitro Evaluation

Dried leaves of yerba mate brand Taragui (100% leaves, Taragui, Argentina) were purchased from a local international supermarket. Leaves were finely ground with a

blender. For yerba mate extractions, tea bags with 5 g of tea were made with miracloth (EMB Bioscience, San Diego, CA), placed in a plastic container and sterile deionized water was added at a ratio of 3.75 mL to 1g of ground tea. Suspensions were allowed to stand at 4°C for 24 h with occasional stirring. After 24 h, tea bags were removed from the container and extracts were centrifuged at 8000 x g for 10 min to remove sediments. The extracts were filter-sterilized with a 0.20-µm Fast PES Filter Unit (Nalgene, Rochester, NY) and frozen at -20 °C. Frozen extracts were lyophilized using the VirTis AdVantage Plus BenchTop freeze dryer (SP Industries, Gardiner, NY). Lyophilized yerba mate extracts were stored at -20 °C until used.

In Vitro Bactericidal Activity of Evaluation of Yerba Mate

Lyophilized yerba mate extracts were rehydrated with sterile deionized water to a final concentration of 500 mg/mL. To evaluate bactericidal activity of yerba mate, extracts (0-100 mg/mL) were mixed with 2 mL of bacterial suspensions harvested at late logarithmic phase and diluted to approximately 10^4-10^5 CFU/mL in phosphate buffered saline (PBS; FisherBiotech, Fair Lawn, NJ). Bacteria and extracts were placed in the incubator at 37 °C, at specific time points (0, 2, 4, 6 and 24 h) pH was measured using a pH meter (Denver Instrument, Bohemia, NY) and 100 µL of suspensions collected, serially diluted in PBS and plated. All *Salmonella* suspensions were plated on TSA and LP suspensions on MRS agar, incubated at 37°C for 24 h, and CFUs were enumerated. All experiments were duplicated and average values reported.

In Vivo Evaluations of Yerba Mate as a Feed Additive and Probiotic Treatments

Three trials were conducted for the *in vivo* evaluations. Experiments 1 and 2 were preventative experiments and experiment 3 was a horizontal transmission experiment to assess the application of yerba mate as a feed additive treatment to decrease SE colonization and horizontal transmission. In all trials, unsexed day-of-hatch broiler chicks were obtained from a local hatchery (Hubbard Co., Pikeville, TN) and were cared for using procedures approved by the University of Tennessee Institutional Animal Care and Use Committee. Chicks were randomly placed in conventional floor pens measuring approximately 5 ft^2 with paper bedding. The temperature was maintained at 35.5°C for the first 3 days and 26.6°C for the remainder of the experiment. Water and a feed starter formula were provided *ad libitum* for the entire experiment (Saleh et al. 1997). For all trials, at day 10, all birds were euthanized and weighed, ceca were collected and contents were serially diluted in PBS and plated on Brilliant Green Agar (BGA; BD Difco, Sparks, MD) containing novobiocin (25 μg/mL) and nalidixic acid (20 μg/mL). All plates were incubated for 24 h at 37 °C and *Salmonella* CFUs enumerated and data statistically analyzed.

For experiments 1 and 2 (preventative experiments), 120 chicks per bird trial were divided into four groups (n=30), each group was treated with one of the following 1) no treatment (control), 2) feed additive treatment (ground yerba mate leaves; 0.55% inclusion rate in feed), 3) probiotic treatment (*Lactobacillus acidophilus* and *Pediococcus*; 9:1 administered once on day of hatch by gavage; 10^7 CFU) or 4) both yerba mate feed additive-probiotic treatments. All chicks were challenged at day 3 with 13A (10^7 CFU). For experiment 3 (horizontal transmission experiment), higher concentrations of yerba mate (1% inclusion

rate) and lower SE concentrations (10^6 CFU) at challenge were evaluated. A total of 80 chicks were divided into four groups (n=20), each group was treated with one of the following 1) no treatment (control), 2) feed additive treatment (ground yerba mate leaves; 1% inclusion rate in feed), 3) probiotic treatment (*Lactobacillus acidophilus* and *Pediococcus;* 9:1 administered once on day of hatch by gavage; 10^7 CFU) or 4) both yerba mate as a feed additive and the probiotic treatments. At day 3, 5 chicks (seeders) from each group were challenged with 13A (10^6 CFU).

Statistical Analysis

Data were analyzed using the ANOVA procedure of SAS (SAS Institute Inc., 2002). A probability of $P<0.05$ was prerequisite for statistical significance. When ANOVA indicated differences, Tukey tests were conducted to evaluate any differences. All *Salmonella* data were transformed to logarithmic scale prior to analysis. Each group within a bird trial was considered one experimental unit. Data from each bird trial were analyzed separately.

Results

In this research, we found that *Salmonella* was very sensitive to the yerba mate extracts (MIC 7.4 mg/mL) while even high concentrations were not inhibitory but in fact enhanced the growth of the probiotic bacteria (83.33-100 mg/mL; Figure 1). Due to the promising results of the *in vitro* experiments, the ability of yerba mate to inhibit *Salmonella* colonization and promote probiotic colonization was evaluated *in vivo* using a broiler chick model. Two types of experiments were conducted, a horizontal transmission experiment and preventative experiments. In the preventative experiments, no statistically significant reductions in *Salmonella* were achieved in either trial 1 or 2 (Figure 2A). However, a

numerical reduction in *Salmonella* (approximately 1 log CFU g^{-1} cecal content) was observed in trial 1 for the yerba mate group. In both preventative experiments, body weight was highest for the probiotic group (Figure 2B).

In the horizontal transmission experiment, the yerba mate treatment (1% inclusion rate) was not effective at reducing transmission (Table 1). The probiotic treatment was the most effective at reducing transmission (4/15 positive birds) compared to the control (11/15 positive birds). The probiotic treatment significantly decreased SE concentrations in the ceca ($P<0.05$) while the yerba mate-probiotic treatment had higher counts than the probiotic treatment but less that the control (Figure 2A). The yerba mate treatment reduced body weight significantly compared to the other treatments ($P<0.05$; Figure 2B).

Discussion

It is not completely understood which compounds found in yerba mate are responsible for the antimicrobial activity, or whether they may have synergistic effects (Burris et al. 2011). Polyphenols found in yerba mate extracts may contribute to the antimicrobial activity, such as, caffeic and chlorogenic acids which are antimicrobial against Gram-negative bacteria (Herald and Davidson, 1983), and kaempferol and quercetin which inhibit the growth of *S. aureus* (Rauha et al. 2000). Some mechanisms of action are being investigated, including cell membrane damage, coagulation of cytoplasm and damage of lipids and proteins (Bakkali et al. 2008). Essential oils (concentrated plant extracts) have hydrophobic properties, for example carvacrol, an essential oil of oregano, dissolves the phospholipid bilayer of the cell membranes by pushing apart fatty acid chains of the phospholipids causing cell death. (Burt, 2004; Dorman and Deans, 2000; Ultee, 2000).

It is not surprising that yerba mate extracts enhance the growth of LAB *in vitro* because similar effects have been reported. Ouwehand et al. (2010) found that essential oils including eugenol, carvacrol, cinnamaldehyde and thymol stimulate the growth of LAB but are biostatic or biocidal to pathogenic bacteria. What is more interesting is that LP may be using compounds present in the yerba mate extracts as a nutrient source (Figure 1F). The reason why plant extracts and essential oils inhibit some bacteria while enhance the growth of others, is not very clear. Some studies agree that Gram-negative organisms are less susceptible to the action of biocidals, while Burt (2004) found no evidence for a difference in sensitivity. Ouattara et al. (1997) concluded that the variability of resistance depends on bacterial species.

In this work, we utilized a probiotic with known efficacy against SE colonization (Higgins et al. 2011; Vicente et al., 2007) against which to compare any yerba mate efficacy. Our evaluations showed that the probiotic treatment consistently improved body weight, however, decrease in SE colonization was not observed in the preventative experiments. This may be due to a very high challenge concentration (10^7 CFU) being used, which was chosen to ensure colonization while also ensuring a measurable reduction in colonization counts. Conversely, in the horizontal transmission experiment, the probiotic treatment resulted in significantly lower SE cecal concentrations and a significant reduction in horizontal transmission. Higgins et al. (2007) and Menconi et al. (2011) documented that LAB isolates were very effective at reducing SE when administrated therapeutically 1h after SE challenge while in our experiments, a prophylactic LP treatment three days before SE challenge were administrated to the chicks.

Yerba mate extracts were found to be biocidal against SE *in vitro*, however *in vivo* evaluations showed that supplementation of feed with raw yerba mate was not effective at reducing SE colonization in the ceca. The lack of effectiveness *in vitro* may have been for several reasons including: 1) reduced feed intake or change in feed passage rate; 2) an impact on host metabolic function by anti-nutritional chemicals possibly present in the plant; 3) form of supplementation of yerba mate used; or 4) impacting beneficial bacterial populations present in the gastrointestinal tract. In all trials, a reduced body weight was observed for the groups receiving the yerba mate, which may indicate that feed intake decreased or feed passage rate changed due to the yerba mate supplementation. Santa Cruz et al. (2003) reported that yerba mate had negative sensory attributes and consumers described the flavor as bitter, acidic or toasted. Because the flavor of yerba mate is somewhat strong, the birds may have refused the feed resulting in a reduced body weight. A reduced feed intake would also partially explain the lack of efficacy of yerba mate against SE colonization because it is know that higher feed intake will stimulate the gastric functions, hydrochloric acid secretion in the proventriculus and grinding process in the gizzard, resulting in a decrease in pH, making it more difficult for *Salmonella* to cross the foregut barrier (Bjerrum et al., 2004; Huang et al., 2006). Kallanoor-Johny et al. (2012) demonstrated that feed additives including eugenol and trans-cinnamaldehyde are effective at reducing SE colonization in broiler chickens however Cross et al. (2007) suggested that the form of supplementation (essential oil or herb) had an impact on bioactivity and antimicrobial activity. This suggests that the *in vitro* evaluations were effective because concentrated yerba mate extracts were used while ineffective *in vivo* because raw tea was used. Unfortunately, only raw tea was available for the *in vivo* evaluations because yerba

mate essential oil is not available and extracts were not able to be used due to the limited quantities obtained from the extraction process.

The literature agrees that some feed additive supplementation can be beneficial to the birds, by providing antimicrobial benefits and body weight gain (Cross et al. 2007; Erdogan et al., 2010; Hanning et al. 2012). Despite the beneficial effects of feed additive supplementation other possible effects may occur due to inappropriate inclusion levels. Negative impacts on body weight gain were observed by Cross et al. 2003 when supplementing 5 g/Kg of thyme essential oil into the feed. It would be expected that the intake of feed additives affect the gastrointestinal microflora, but "non-ideal" alteration of the indigenous flora by the feed additives can be deleterious to the host (Hippenstiel et al. 2011). Feed additives such as prebiotics can stimulate the production of digestive enzymes, including lipase, amylase or carbohydrates which may affect nutrient utilization and morphological changes in villus height and crypt depth can also occur (Applegate et al. 2010). Moreover, the effectiveness of the feed additives depends on factors such as environmental and vassal diet. If birds are housed under clean and healthy condition or diets are highly digestible, it is possible that the feed additives will have no impact on bird health (Hippenstiel et al. 2011).

Ouwehand et al. (2010) suggested designing diets using feed additives such as prebiotics in combination with probiotic treatments to inhibit the growth of potential pathogens while promoting the beneficial members of the intestinal microbiota. For this reason, the prebiotic-probiotic combination could theoretically work synergistically to strengthen resistance against pathogen colonization. Because yerba mate extracts were antimicrobial against SE and enhanced the growth of LP *in vitro*, synergist effects between yerba mate and LP to decrease SE colonization while stimulating LP growth were hopeful.

Unfortunately, the combination of treatments did not show any improvement in terms of SE colonization in the ceca and the combination of treatments actually decreased the beneficial effects of the probiotic treatment both in terms of weight gain and SE colonization resistance. This was not surprising given the poor results of the yerba mate treatment alone.

More prebiotic-probiotic treatment combination research is being conducted to investigate beneficial impacts on bird health and performance. For example, Li et al. (2009) reported administration of *Astragalus* polysaccharides and probiotic bacteria together improved cellular and humoral immunity and also increased *lactobacilli* and *bifidobacteria* intestinal concentrations. Bozkurt et al. (2009) and Falaki et al. (2010) obtained a synergistic effect in chicken body weight and feed conversion ratio using a prebiotic-probiotic treatment. In these experiments, treatments were optimized for digestion to convert feed to body mass more effectively.

Conclusions

Although no reduction in *Salmonella* was observed with our yerba mate treatment as a feed additive, other possible effects on bird health are possible and currently being investigated, including shifts in fatty acid profiles in the ceca and immune system responses. In conclusion, yerba mate has excellent antioxidant activity (Bastos et al., 2006), is antimicrobial against some foodborne pathogens (Burris et al. 2011), including *Salmonella*, and enhances the growth of beneficial bacteria. Yerba mate could have many applications for the food industry, but more evaluations need to be conducted to determine its application in food systems.

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References

Anesini, C., G. Ferraro and R. Filip. 2006. Peroxidase-like activity of Ilex paraguariensis. Food Chem. 97:459–464

Applegate, T. J., V. Klose, T. Steiner, A. Ganner and G. Schatzmayr. 2010. Probiotics and phytogenics for poultry: Myth or reality?. J. Appl. Poult. Res. 19:194–210

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar. 2008. Biological effects of essential oils – A review. Food Chem. Tox. 46:446–475

Bastos D.H.M., E.Y. Ishimoto, M.O.M. Marques, A.F. Ferri, E.A.F.S. Torres. 2006. Essential oil and antioxidant activity of green mate and mate tea (Ilex paraguariensis) infusions. J. Food Compost. Anal. 19:538–543.

Bjerrum, L., K. Pedersen and R.M. Engberg. 2004. The influence of whole wheat feeding on Salmonella infection and gut flora composition in broilers. Avian Dis. 49:9–15

Bozkurt M., K. Küçükyılmaz, A.U. Çatl and M. Çınar. 2009. The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. S.A. J. Food Sci. 39:197–205

Burris, K. P., P.M. Davidson, C.N. Stewart, Jr., and F.M. Harte. 2011. Antimicrobial activity of yerba mate (Ilex paraguariensis) aqueous extracts against Escherichia coli O157:H7 and Staphylococcus aureus. J. Food Sci. 76:456–462.

Burris, K.P., P.M. Davidson, C.N. Jr. Stewart, S. Zivanovic, F.M. Harte. 2012. Aqueous extracts of yerba mate (Ilex paraguariensis) as a natural antimicrobial against

Escherichia coli O157:H7 in a microbiological medium and pH 6.0 apple juice. J. Food Prot. 75:753–757

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Itl. J. Food Micr. 94:223–253

Cross, D. E., K. Svoboda, R.M. McDevitt, T. Acamovic. 2003. The performance of chicks feed diets with or without thyme oil and enzymes. Brit. Poult. Sci. 44:18–19

Cross, D.E., R.M. McDevitt, K. Hillman and T. Acamovic. 2007. The effect of herbs and their associated essential oil on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poult. Sci. 48:496–506

Donalson, L.M., J.L. McReynolds, W.K. Kim, V.I. Chalova, C.L. Woodward, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2008a. The influence of a fructooligosacharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract fermentation, Salmonella Enteritidis infection and intestinal shedding in laying hens. Poult. Sci. 87:1253–1262.

Donalson, L.M., W.K. Kim, V.I. Chalova, P. Herrera, J.L. McReynolds, V.G. Gotcheva, D. Vidanovic, C.L. Woodward, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2008b. In vitro fermentation response of laying hen cecal bacteria to combinations of fructooligosaccharide (FOS) prebiotic with alfalfa or layer ration. Poult. Sci. 87:1263–1275.

Dorman, H.J.D. and S.G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Micr. 88:208–316

Erdogan, Z., S. Erdogan, O. Aslantas and S. Celik. 2010. Effects of dietary supplementation of synbiotics and phytobiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broilers. J. Anim. Phys. Anim. Nut. 94:30–48

Falaki, M., M.S. Shargh, and B. Dastar. 2010. Effects of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. J. Anim. Vet. Adv. 9:2390–2395

Filip R, R. Davicino, C. Anesini. 2010. Antifungal activity of the aqueous extract of Ilex paraguariensis against Malassezia furfur. Phytother. Res. 24:715–724.

Hanning, I., A. Clement, C. Owens, S.H. Park, S. Pendleton, E.E. Scott, G. Almeida, F. Gonzalez Gil, and S.C. Ricke. 2012. Assessment of production performance in two breeds of broilers fed prebiotics as feed additives. Poult. Sci. 91:3295–3299

Heck, C.I., and E.G. de Mejia. 2007. Yerba Mate Tea (Ilex paraguariensis): a comprehensive review on chemistry, health implications, and technological considerations. J. Food Sci. 72:138–151.

Herald P.J., P.M. Davidson. 1983. Anti-bacterial activity of selected hydroxycinnamic acids. J. Food Sci. 48:1378–1387

Higgins, S.E., A.D. Wolfenden, G. Tellez, B.M. Hargis, and T.E. Porter. 2011. Transcriptional profiling of cecal gene expression in probiotic and Salmonella-challenged neonatal chicks. Poult. Sci. 90:901–913

Hippenstiel, F., A.A.A. Abdel-Wareth, S. Kehraus and K.H. Sudekum. 2011. Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers – a review. Arch. Geflugelk. 75: 226–234

Huang, D.S., D.F. Li, J.J. Xing, Y.X. Ma, Z.J. Li, and S.Q. Lv. 2006. Effects of feed particle size and feed form on survival of Salmonella typhimurium in the alimentary tract and cecal S. typhimurium reduction in growing broilers. Poult. Sci. 85:831–836

Li, S.P., X.J. Zhao and J.Y. Wang. 2009. Synergy of Astragalus polysaccharides and probiotics (Lactobacillus and Bacillus cereus) on immunity and intestinal microbiota in chicks. Poult. Sci. 88:519–525

Menconi, A., A.D. Wolfenden, S. Shivaramaiah, J.C. Terraes, T. Urbano, J. Kuttel, C. Kremer, B.M. Hargis, and G. Tellez. 2011. Effect of lactic acid bacteria probiotic culture for the treatment of Salmonella enterica serovar Heidelberg in neonatal broiler chickens and turkey poults. Poult. Sci. 90 :561–565

Ouattara, B., R.E. Simard, R.A. Holley and G.J.P. Piettea and A. Bégin. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Itl. J. Food Microb. 37:155–162

Ouwehand, A.C., K. Tiihonen, H. Kettunen, S. Peuranen, H. Schulze and N. Rautonen. 2010. In vitro effects of essential oils on potential pathogens and beneficial members of the normal microbiota. Vet. Med. 2:71–78

Rauha J.P., S. Remes, M. Heinonen, A. Hopia, M. Kahkonen, T. Kujala, K. Pihlaja, H. Vuorela, P. Vuorela. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Intl. J. Food Micr. 56:3–12

Racanicci A.M.C., B.H. Allesen-Holm, L.H. Skibsted. 2009. Sensory evaluation of precooked chicken meat with mate (Ilex paraguariensis) added as antioxidant. Eur. Food Res. Tech. 229:277–80.

Saleh, E.A, S.E. Watkins, and P.W. Waldroup. 1997. Changing time of feeding starter, grower and finisher diets for broilers 3. Birds grown to 3.3 KG. J. Appl. Poult. Res. 6:290–297

Santa Cruz, M.J., L.V. Garitta and G. Hough. 2003. Note: Relationships of consumer acceptability and sensory attributes of yerba mate (Ilex paraguariensis St. Hilarie) using preference mapping. Food Sci. Tech. Intl. 9: 347–352

Ultee, A., E.P.W. Kets, M. Alberda, F.A. Hoekstra, E.J. Smid. 2000. Adaptation of the food-borne pathogen*Bacillus cereus* to carvacrol. Arch. Micr. 174 :233–238

Vicente, J.L., L. Aviña, A. Torres-Rodriguez, B. Hargis and G. Tellez. 2007. Effect of a Lactobacillus spp-based probiotic culture product on broiler chicks performance under commercial conditions. J. Poult. Sci. 6:154–156.

CONCLUSIONS

Salmonella enterica and *Campylobacter jejuni* cause approximately 2.5 million cases of foodborne illness each year in the United States. The CDC reports poultry as a common vehicle to human infection for these two pathogens. The reason is that they easily colonize the chicken intestine during production and carcass contamination with GI contents during processing commonly occurs. To reduce the number of infections and outbreaks, pre-harvest intervention and exclusion strategies including the use of probiotics, prebiotics and botanicals were proposed. The use of each feed additive should be evaluated because each botanical could have beneficial or detrimental consequences to the bird depending on its properties. Future research should evaluate the use of feed additives with synergistic capacities to increases chicken performance, promote gut health and reduce pathogen colonization. However, antinutritional factors of polyphenols should be considered before evaluating new feed additives.

APPENDIX

Table 1. **Effect of the treatments on horizontal transmission, number of bird colonized by *Salmonella* and percentage reduction.**

Treatment	SE-positive/total	% Reduction
Control	11/15 (73%)[a1]	-
Yerba Mate	14/15 (93%)[a]	-27
Probiotic	4/15 (27%)[b]	64
Yerba Mate -Probiotic	9/15 (60%)[a]	18

[1]Values with different letters indicate statistically significant differences ($P<0.05$)

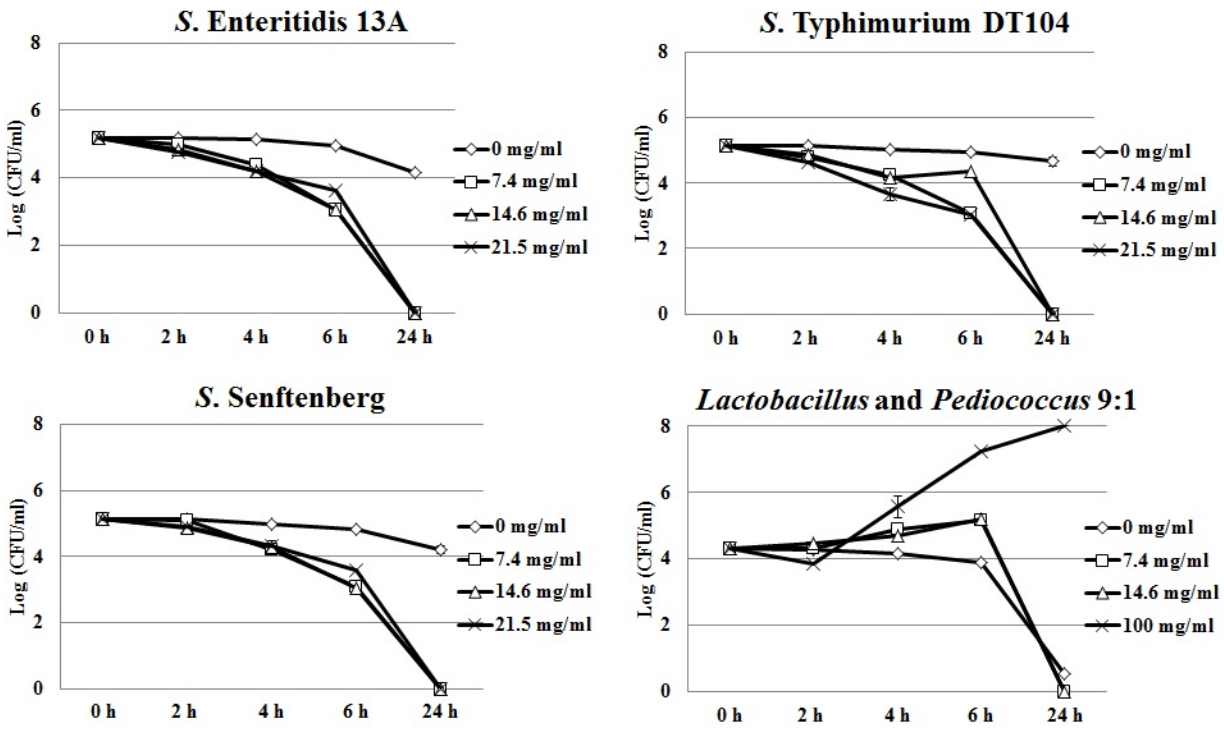

Figure 1. Effect of yerba mate extracts on the growth of *S.* Enteritidis 13A, *S.* Senftenberg, *S.* Typhimurium DT104 and *Lactobacillus acidophilus* and *Pediococcus* 9:1 in PBS, over time. Different concentrations of yerba mate extracts: 0-100mg/mL.

Figure 2. *In vivo* evaluation of prebiotic and probiotic treatments. Effect of the treatments on (A) *Salmonella* Enteritidis colonization in ceca samples and (B) chicken body weights, from 10 day old broiler chicks. For the preventive assays, all chicks were challenged with 10^7 CFU of *S*. Enteritidis 13A at day 3 and a 0.55% inclusion rate in feed for prebiotic treatments. For the horizontal transmission assay, 5 chicks (seeders) were challenged with 10^6 CFU of 13A at day 3 and 1% inclusion rate in feed for prebiotic treatment. Values with different letter (a,b) differed significantly within a bird trial ($P<0.05$)

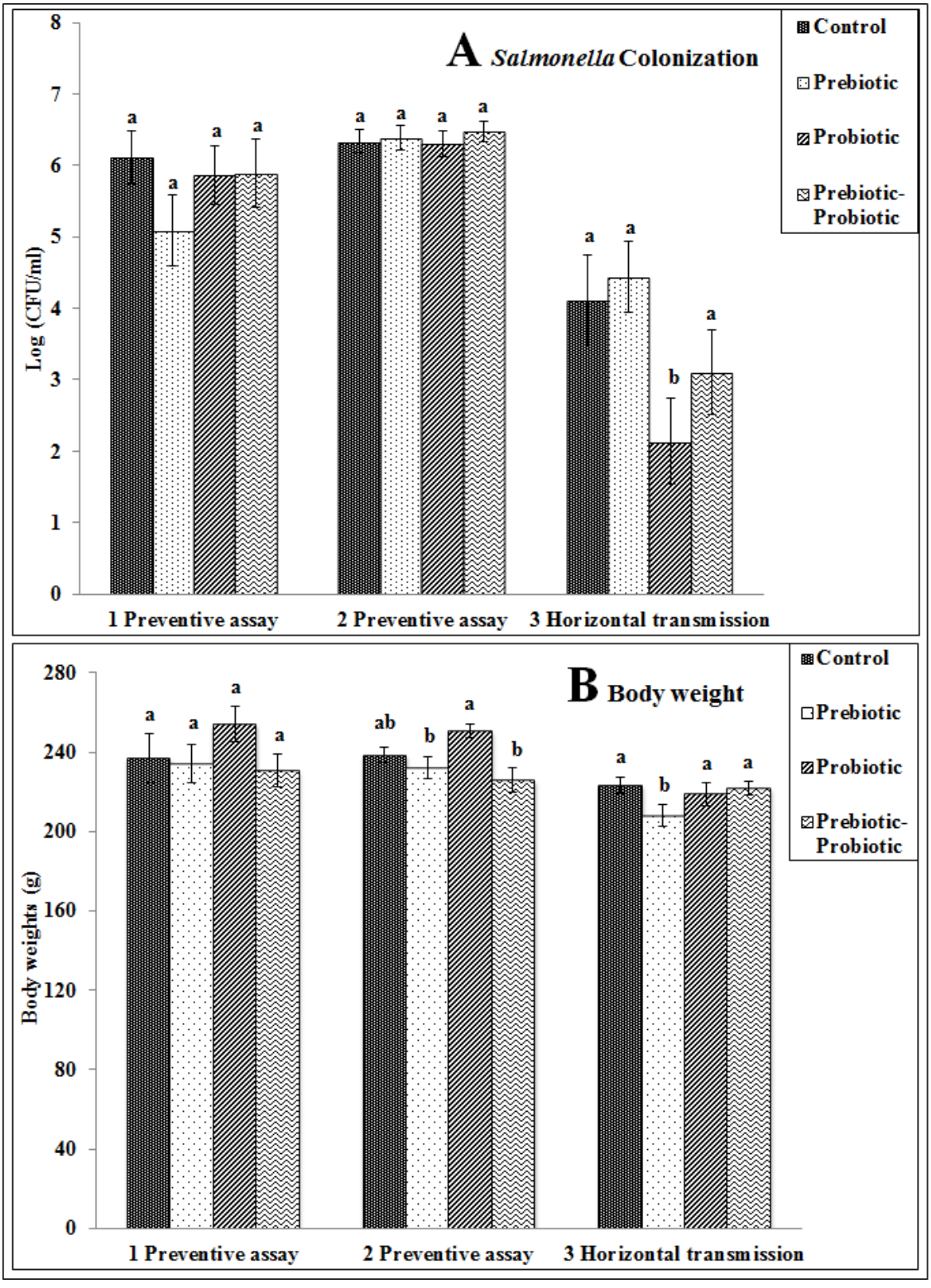

Figure 2 continued

Preparation of *Salmonella* Culture for Chicken Gavage

1. Obtain *Salmonella* Enteritidis 13A (SE) aliquot from ultra-low freezer.
2. Obtain 3 tubes with 10ml TSB
    a. Label tubes (1,2,3).
    b. Add 100µl SE to tube 1.
    c. Place all 3 tubes into the incubator (37C).
    d. Pass 100µl SE from tube 1 to tube 2 after 8 hours.
    e. Pass 100µl SE from tube 2 to tube 3 after 8 hours.
    f. After 8 hours remove culture from incubator.
3. Centrifuge culture from tube 3 at 8000 rpm for 5 min @ 4C and use other tube to balance bucket.
    a. Pour off supernatant.
    b. Wash culture pellet with PBS.
    c. Resuspend to original volume with PBS.
    d. Do at least 2 more washes.
4. Resuspend to 5ml with PBS.
5. Measure turbidity with the spectrophotometer at 630 nm.
    a. Add culture until the spectrophotometer reads 0.149
    b. This will be 10^8 CFU/ml.
6. Dilute culture with PBS to appropriate concentration for gavage.
7. Place diluted culture on ice until used for gavage.
8. Gavage chick with 0.25ml of 10^7 CFU culture.

Protocol for Isolation and Quantification of *Salmonella* from Ceca

1. Necropsy chicks at selected time point.
    a. Usually 10 days for *Salmonella.*
    b. Use alcohol and fire to sterilize tools between birds.
    c. Extract both ceca using sterile scissors and forceps and place them inside a necropsy bag properly labeled.
    d. Place necropsy bag in a cooler until processing in the lab.
2. Use sterile scissors and forceps to squeeze ceca content into a tube.
    a. Add approximately around 0.2-1 g of ceca into the tube (g ceca added).
3. Add PBS to the tube.
    a. ml of PBS to be added = (g ceca added)(9)
    b. Vortex
4. Serially dilute sample with PBS
5. Plate serial dilutions in Brilliant Green Agar containing 25 µg/ml of novobiocin and 20 µg/ml nalidixic acid. BGA NO/NA.
    a. Plate from 10^{-2} – 10^{-6}
6. Incubate plates at 37C for 24h.
7. Enumerate *Salmonella*.
    a. *Salmonella* appears as round pink colony.

In Vitro Evaluation of Yerba Mate

Yerba mate extraction

1. Ground yerba mate leaves with a blender until a fine powder is obtained.
2. Make a tea bag of ground yerba mate using miracloth.
    a. Add 5 g to each bag.
3. Place several tea bags with yerba mate in a sterile plastic container and add sterile deionized water.
    a. Add water at a ratio of 3.75 mL to 1g of ground tea.
    b. Let it stand at 4C for 24 h with occasional stirring.
4. Carefully remove used tea bags from container.
    a. Squeeze bag to extract all remaining the liquid into the plastic container.
5. Place extract into several plastic tubes and centrifuge.
    a. Centrifuge at 8000 rpm for 10 min to remove sediments.
6. Filter sterilize the extract with a 0.20-µm Fast PES Filter Unit.
7. Freeze at -20 °C overnight.
8. Lyophilize frozen extracts using the VirTis AdVantage Plus BenchTop freeze dryer.
9. Store lyophilized yerba mate extracts in a Ziploc bag at -20C until used.

Bacterial preparation

1. Obtain *Salmonella* and lactic acid bacteria (LAB) cultures from ultra-low freezer.
2. Plate *Salmonella* on TSA and LAB on MRS agar.
    a. Incubate overnight at 37C.
    b. Transfer one CFU into fresh plate and incubate 24h at 37C.
3. Mix several CFU with 10 ml of PBS in a plastic tube.
4. Measure turbidity with the spectrophotometer at 630 nm.
    a. Add culture until the spectrophotometer reads 0.149
    b. This will be 10^8 CFU/ml.
5. Dilute culture to appropriate concentration.
    a. Dilute to 10^4-10^5 CFU/mL in PBS
6. Use cultures for the *in vitro* bactericidal evaluation

In Vitro Bactericidal Evaluation of Yerba Mate

1. Rehydrate lyophilized yerba mate extracts with sterile deionized water.
    a. Final concentration 500 mg/ml.
2. Mix 2 mL of bacterial suspensions with desired concentration of yerba mate extract in a 12 well plate.
3. Incubate samples at 37C.
4. Measure pH
    a. Time points: 0, 2, 4, 6, 24h
5. Collect a 100ul of sample and serially dilute it in PBS.
    a. Time points: 0, 2, 4, 6, 24h
6. Plate samples and incubate at 37C for 24h
    a. Plate *Salmonella* on TSA and LAB on MRS agar.

7. Enumerate plates.
8. Duplicate experiment.

VITA

Francisco was born in Zamora, Mexico on March 7, 1989. His parents are Francisco Gonzalez and Leticia Gil. He is the second child of four kids. He earned a Bachelor's of Science in Food Engineering at the Monterrey Institute of Technology and Higher Education (ITESM) Campus Queretaro, Mexico in May 2012. After graduation he moved to Knoxville, TN and joined graduate school. He obtained a Master's of Science degree in Food Science and Technology in May 2014.