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The Effect of *Sericea Lespedeza* (*Lespedeza Cuneata*) on *Eimeria* Spp. Infection in Broiler Chickens

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THE EFFECT OF SERICEA LESPEDEZA (*LESPDEZA CUNEATA*) ON
EIMERIA SPP. INFECTION IN BROILER CHICKENS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in the School of Animal Science

by
Long N. Trinh
B.S., Louisiana State University, 2013
May 2017

Dedicated to

My Family

&

Friends

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ABSTRACT

Parasites cause considerable economic losses in all fields of agriculture. In the poultry industry, coccidia infections are of major significance costing billions of dollars every year. Research on condensed tannins (CT) has been shown to be effective against various parasites such as gastrointestinal nematodes and *Eimeria* spp. in small ruminants. This study tested the effects of the CT containing forage, sericea lespedeza (SL), on broiler chickens infected with live *Eimeria* spp. oocysts. In Study 1, 300 Ross 708 broiler chicks were divided into 6 groups (negative control, positive control w/BioCox, 0% SL, 5% SL, 10% SL, 15% SL). In Study 2, 250 Ross 708 broiler chicks were divided into 5 groups (negative control, positive control w/BioCox, positive control w/Corrid, 0% SL, 10% SL). Chicks were grown for 18 days in battery cages. All groups, except the negative control, were infected with a high dose (approximately 100,000 oocysts per chick) of oocysts at day 4 via gavage. SL was fed to the appropriate groups from day 0 to day 18. Data analysis included fecal oocyst count (FOC), weight, feed conversion, lesion scoring and histopathology. Results from Study 1 indicated that 10% and 15% SL, significantly decreased FOC. Reduced body weight (BW) and feed conversion for all SL fed chicks were also noted. Lesion scoring was not of value. Histopathology suggested a potential mechanism of action of CT, in that only the SL fed chicks had developing stages in the intestinal mucosa. Therefore, CT may act to slow development allowing the immune response to mature. In Study 2, FOC was not affected by the 10% SL treatment. Reduced BW and feed conversion were also observed as in Study 1. Feeding broiler chicks, a diet containing SL may be beneficial for controlling *Eimeria* spp. infection, but production was negatively affected. Therefore, feeding SL should not be recommended at this time pending further research to address the production issues.

CHAPTER 1 INTRODUCTION

The poultry industry has been streamlined over the years to become an agricultural powerhouse in terms of production and technology. In just about 5 weeks, a single chicken house operation could have 50,000 broilers ready for market. As a multi-billion-dollar enterprise (\$48.3 billion in 2014), there are many obstacles that will have to be overcome in order to continue to meet the demand of consumers (USDA, 2014).

Chickens are hosts to a variety of parasites including nematodes, ticks, mites, lice and protozoa. Among the protozoa, coccidia (*Eimeria* spp.) are of most economic importance. This is due to modern production practices that rear a large number of chicks at high stocking densities. Broiler operations are confined geographically to areas that are ideal for parasite transmission (Chapman et al, 2013). Good husbandry plays an important role in reducing infection between flocks. However, in the United States, litter is frequently used to raise up to 6 flocks before being replaced each year. These unsanitary conditions are ideal for parasitic transmission, especially those with a fecal life cycle such as *Eimeria* spp. (Chapman et al. 2014).

Eimeria spp. infection costs the UK poultry industry in excess of \$54 million per annum as a result of reduced production efficiency and the costs of veterinary and prophylactic intervention (Shirley et al., 2007). Worldwide, the cost exceeds \$3 billion US dollars annually. These costs include low productivity, mortality, prophylaxis and treatment (Dalloul and Lillehoj, 2006). Coccidiosis is the most prevalent disease affecting the US broiler industry. An estimated \$90 million is spent in the US for coccidiosis prevention annually (Dinev, 2013).

Eimeria spp. are ubiquitous protozoan parasites that infect livestock in a host-specific manner. *Eimeria tenella*, *E. mivati*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. hagani*, *E. necatrix* and *E. praecox* infect chickens and no other hosts. In broiler chickens the most prevalent species are *E. acervulina*, *E. tenella* and *E. maxima* (Györke et al., 2013), of which *E. tenella* is highly pathogenic, causing hemorrhagic diarrhea, reduction of weight gain and mortality. *E. maxima* has moderate pathogenicity producing economical losses and mortality. *E. acervulina* is mildly pathogenic and is the most common species in chickens, causing poor feed conversion and mortality in heavy infections (McDougald and Fitz-Coy, 2008).

Development of the modern poultry production industry is largely dependent on anticoccidials and live vaccines to control coccidiosis. Anticoccidial drugs can be roughly generalized into two categories: ionophores (those produced by fermentation) and synthetic drugs (those produced by chemical synthesis). Using an ionophore in conjunction with a synthetic drug is a common practice in poultry production (Chapman, 2014).

A majority of available anticoccidial vaccines consist of live oocysts of attenuated or non-attenuated strains of *Eimeria* spp. (Shirley et al., 2007). The first vaccine to target *Eimeria* was introduced in 1952 (DM® Cecal Coccidiosis Vaccine; Dorn and Mitchell Inc., USA). It only contained live wild-type oocysts of *E. tenella*. Now vaccines cover a wide array of *Eimeria* spp. Some vaccines (e.g. Coccivac and Paracox) produced today contain species that were isolated before the introduction of most anticoccidial drugs. Their seed stocks have been maintained for years, free of exposure to medication. Therefore, the oocysts in these vaccines are thought to be inherently sensitive to most anticoccidials and their progeny will be drug-sensitive as well (Chapman, 2014).

Medicated feed is cheap, convenient and non-labor intensive factor that has allowed large commercial poultry operations the ability to rear large numbers of chicks in intensive conditions.

However, the frequent use of these drugs, especially in broiler production, has inevitably resulted in the development of resistance (Chapman, 1997). Continual use and misuse of anticoccidial drugs have also led to an increase of drug-resistant strains of *Eimeria* spp. The use of vaccines has alleviated some of the problems associated with drug-resistance, but not without its own negative effects. Live vaccines could potentially produce severe reactions, affecting the performance of chickens whereas attenuated vaccines are very expensive to produce. Vaccines may not be efficient in all geographical areas (Chapman, 2000; Abbas et al., 2012). Increasing regulations and bans on the use of anticoccidial drugs coupled with the associated costs for developing new drugs and live vaccines has stimulated the need for developing novel approaches and alternative control strategies for controlling coccidiosis (Dalloul et al., 2006).

Sericea lespedeza (SL, *Lespedeza cuneata*), also known as Chinese Bushclover, is a perennial upright legume of the plant Family Fabaceae that was introduced into the southeastern United States from eastern Asia for soil conversion, erosion control, forage and hay (Anon, 2002). SL grows in low fertility and acid soils and was widely planted to rebuild eroded and depleted soils. It is commonly used for planting on surface mine spoils, road banks, and other disturbed areas. SL is a high condensed tannin (CT) forage that has been shown to reduce gastrointestinal nematode (GIN) infection in sheep and goats (Burke et al. 2010). It is believed that the plant CT may affect GIN either directly or indirectly.

The mechanism of action is not yet known. It has been shown that CT could bind with feed nutrients. This could possibly prevent bacterial growth in the feces by limiting the feed available for larval growth and movement (Coffey, 2007). Some other plant products have been tested for efficacy against *Eimeria* spp. in the chicken. Pine bark extracts containing

35% CT were shown to significantly inhibit the sporulation of *E. acervulina*, *E. tenella*, and *E. maxima* oocytes in vitro (Molan et al., 2009). Artemisinin, a sesquiterpene lactone derived from *Artemisia annua*, were shown to have some effect as an alternative to control mixed *Eimeria* spp. infections (Popa et al., 2015). Grape seed and green tea extracts have also been shown to reduce lesions scores and inhibit sporulation of oocysts (Wang et al., 2008; Molan and Thomas, 2007). The aim of this study was to evaluate SL as a natural alternative for *Eimeria* spp. control in broiler chickens.

CHAPTER 2 LITERATURE REVIEW

2.1. *Eimeria*

Coccidia is a subclass of single-celled obligate intracellular protozoan parasites belonging to the Phylum Apicomplexa (cells with cluster of organelles known as apical complex). Coccidia affecting poultry, belong to the genus *Eimeria*, infecting various sites along the intestines. The infection is rapid (between 4–7 days) and is defined by replication (i.e. asexual reproduction) in host cells causing harm to the intestinal mucosa. Poultry *Eimeria* spp. are generally organ-specific, and the different species parasitize specific parts of the intestines (Yun et al., 2000).

The majority of the scientific literature recognizes 7 species of *Eimeria* which infect chickens: *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. praecox*, *E. necatrix* and *E. tenella*. For consistency with updated research, *E. mivati* and *E. hagani* will also be included in this thesis.

E. tenella infections are found only in the ceca. Its presence can be recognized by bloody diarrhea and build-up of blood in the ceca. *E. necatrix* produces lesions in the upper and middle areas of the small intestine. Small white and red spots of various sizes, can be seen on the serosal surface similar to “salt and pepper.” *E. acervulina* is the most common cause of infection with lesions characterized as whitish, oval patches in the upper portion of the small intestines. *E. brunetti* is found in the lower small intestine, rectum and ceca and is associated with pale mucosa and, in severe cases, sloughing of the mucosa. *E. maxima* establishes itself in the small intestine where it thickens the wall and causes hemorrhaging turning the mucous reddish/pink. *E. mitis* is pathogenic in the lower intestine and its lesions

are indistinct. *E. praecox* infects the upper small intestine and its lesions are also indistinct. Infection causes decrease rate of growth and watery intestinal contents. *E. hagani* and *E. mivati* develop in the upper small intestine. *E. hagani* are indistinct and difficult to characterize. *E. mivati* causes severe lesions similar to those of *E. acervulina* (Gerhold, 2014).

2.2. Life Cycle

The life cycle of a typical *Eimeria* spp. takes about 4 to 7 days to complete and involves three phases: sporogony, schizogony and gametogony (Figure 1). Sporogony or sporulation,

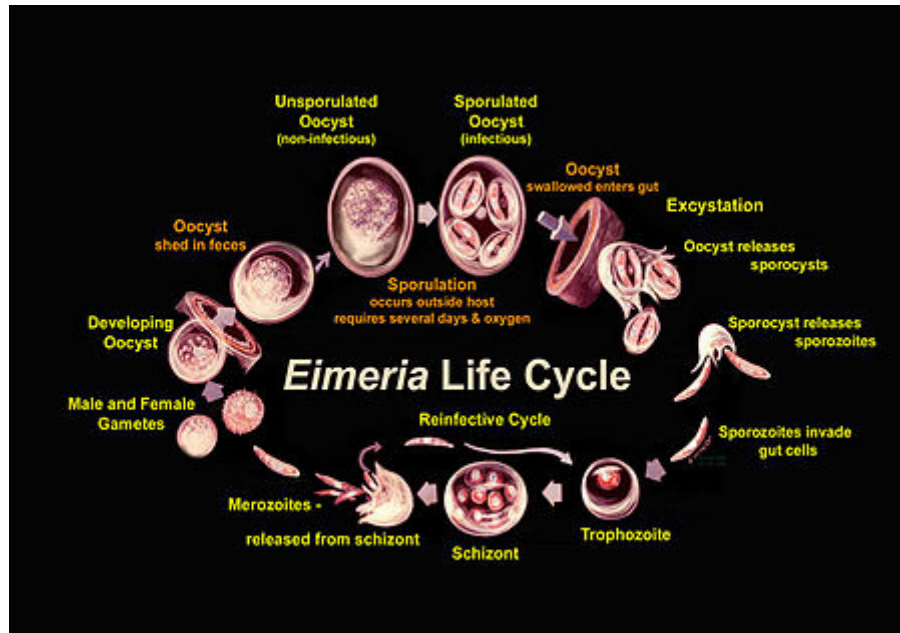


Figure 1: *Eimeria* spp. life cycle diagram USDA website. <<https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-agricultural-research-center/animal-parasitic-diseases-laboratory/docs/coccidiosis/>>

is the formation of the infective transmission stage known as the sporulated oocysts. This occurs outside the host once optimal conditions are met. Sporulation engages meiotic division and mitotic division consecutively, resulting in the development of 4 sporocysts. A mitotic division then occurs within each sporocyst to form 2 genetically identical haploid sporozoites, totaling to 8 sporozoites in each oocyst (Canning and Anwar, 1968; Canning and

Morgan, 1975). Oocysts have a tough outer wall that allows it to survive weeks to months in the environment (Farr and Wehr, 1949). The optimal temperature for sporulation is approximately 22°C. Sporulation also requires adequate oxygen and moisture to begin. The rate of sporulation is slowed by excess hot or cold temperatures. Oocysts are killed either by freezing or very high temperatures (Fanatico, 2006). The endogenous phases of the life cycle begin once sporulated oocysts are ingested. The oocyst wall is pulled apart by the crop or gizzard freeing the sporocysts. Excystation is the process of releasing sporozoites by digestive enzymes. This occurs as the sporocysts pass through the intestines. As the sporocysts pass through the digestive tract, pancreatic enzymes and trypsin activation dissolve the sporocyst plug. The sporozoites are released and invade the epithelial cells. Each *Eimeria* spp. has a specific location that it infects and develops (Blake and Tomley, 2013).

Inside the epithelial cell, sporozoites assemble into trophozoites before undergoing schizogony. Schizogony is a process of asexual reproduction, also known as merogony, which results in multiplication of parasite numbers in the intestine (Yun et al., 2000). The first generation of merozoites rupture and leave the host cell. These merozoites invade other epithelial cells to proceed with the formation of second generation merozoites. This process could repeat itself over and over depending on the species of *Eimeria* (Blake and Tomley, 2013).

Finally, gametogony occurs with production of male and female gametes which, following fertilization form a zygote and become the unsporulated oocyst. After final schizogony occurs, the last generation of merozoites differentiate into macrogametes (female) or microgametes (male). Mature microgametes penetrate neighboring cells and fertilize mature macrogametes to form zygotes (Yun et al., 2000). The fertilized macrogametes then

form the outer wall to become oocysts. The oocysts are excreted in feces and wait for ideal conditions to sporulate and become infective (Blake and Tomley, 2013).

2.3. Coccidiosis

Coccidiosis is usually a disease of young chicks, but older chickens can be infected at any time if never before exposed (Vermeulen et al., 2001). Coccidiosis is also correlated with intestinal diseases, because the damage done allows bacteria to enter and cause secondary infections (Dinev, 2013).

Manifestations of coccidiosis include decreased growth rate, visibly sick chicks, severe diarrhea, and increased mortality (Gilbert et al., 2011). Feed and water consumption are also depressed. Increased culls and decreased egg production may accompany outbreaks. Less severe infections may lead to secondary infection, particularly *Clostridium* spp. (Vermeulen et al., 2001). Survivors of severe infections usually recover in about 2 weeks but may not recover from the loss of performance (Gerhold, 2014).

An outbreak of coccidiosis eventually runs its course and most of the flock will survive. A coccidial infection differs from bacterial and viral infections in that coccidia are “self-limiting” and usually stop multiplying before killing the chick. The chicks that recover from coccidiosis gain immunity. If the infection is severe, the gut remains scarred and impaired (Fanatico, 2006).

During infection, both cellular and humoral immune responses are stimulated. Since the majority of the *Eimeria* spp. lifecycle occurs intracellularly, the most effective immune response is of a cellular nature, and not humoral (Lillehoj and Trout, 1996). The development of immunity is also influenced the severity of the parasitic infection. In a heavy infection, a short-term humoral immune response is produced. In a low infection, the immune system reacts with a cellular immune response offering longer term immunity (Brake et al., 1997).

2.4. Coccidia Control

2.4.1. Husbandry

Environmental management practices to control and prevent coccidiosis are crucial to big and small chicken operations. Sanitation of feed, water, equipment and litter reduces exposure to infective sporulated oocysts. The focus of litter management is to reduce moisture. Proper heating, ventilation and feed should be met (Fanatico, 2006). Biosecurity in larger poultry operations include: controlling farm access, restricting movement and implementing rodent/insect control.

2.4.2. Anticoccidials

Anticoccidial drugs are given in the feed or water to prevent disease and the economic loss is often associated with subclinical infection. Prophylactic (preventative) use is preferred, because drugs cannot thoroughly stop an outbreak and damage usually occurs before symptoms arise. Supplementing the feed with antibiotics and vitamins (A and K) improve rate of recovery and help to prevent secondary infections (Gerhold, 2014).

Anticoccidial drugs fall into two categories: ionophores and synthetic drugs. Ionophores (lasalocid, monensin, narasin, salinomycin, and semduramicin) are thought to disrupt ion gradients across the parasite cell membrane (Chapman, 1997). These drugs affect both extra- and intracellular stages of the parasite during the asexual phases of development (Gerhold, 2014). Synthetic drugs (decoquinate, clopidol, sulphonamides and amprolium) include a assorted range of compounds with varying modes of action. Decoquinate and clopidol inhibit the parasites mitochondrial respiration. Sulphonamides inhibit the folic acid pathway in the parasite. Amprolium inhibits thiamine uptake; rapidly dividing coccidia have a high requirement for thiamine (Chapman and Jeffers, 2014). The use of amprolium today is

for prevention before infection and during clinical outbreaks (Gerhold, 2014). The mode of action of some anticoccidials (e.g. diclazuril, halofuginone, nicarbazin, and robenidine) are still unknown (Chapman and Jeffers, 2014). Diclazuril is a feed additive and is used for prevention. Nicarbazin was the first to have a broad-spectrum activity and Robenidine prevents the formation of mature schizonts.

The effects of anticoccidial drugs fall into two categories: coccidiostatic or coccidiocidal. In short term use, anticoccidial drugs may be coccidiostatic, where developmental stages are stunted but may progress after drug withdrawal. In long term use, anticoccidial drugs may be coccidiocidal, where developmental stages are killed. Most anticoccidials currently used in poultry production are coccidiocidal (Chapman, 1997). To reduce cost and meet regulatory requirements, anticoccidials are usually withdrawn between 3 to 7 days before slaughter. A longer withdrawal period may result in higher risk of secondary coccidiosis outbreaks (Gerhold, 2014).

2.4.3. Vaccines

Live oocyst vaccines against *Eimeria* spp. in poultry have been successfully used by the industry since 1965. Vaccines have mostly been used by breeder flocks but, in recent years, have been used in other poultry (broilers, roasters and turkeys) operations. Coccidiosis vaccines stimulate a number of immunological responses (innate, specific and non-specific) and induce protective immunity by controlling re-infection during the first 4 to 5 weeks of a chick's life.

The 3 major vaccines available in the United States are Immucox®, Advent® and Coccivac®. Vaccines used in Europe are Paracox®, Livacox®, and Viracox®. The vaccines found in the United States typically fall under the non-precocious (non-attenuated) category.

In contrast, vaccines used in Europe are precocious (attenuated). The precocious types are altered, where the coccidia used are modified to mature quickly and have a shorter life cycle. These precocious vaccines are less pathogenic and cost more to produce than the non-attenuated vaccines (Fanatico, 2006).

Vaccines are usually administered at the hatchery or within the first week of life. Spray cabinets are used at hatcheries on day-old chicks. This method provides uniform application, resulting in 90 to 95 percent of chicks exposed to the vaccine (Chapman, 2000). Some vaccines, such as Immucox®, are given as edible gel. Brightly colored gel pucks are placed on the transport truck or flooring for the chicks to eat. Vaccines could also be applied via a feed sprayer (garden pressure-sprayer) over a 24-hr supply of feed. The final method of administering a vaccine is via drinking water. Oocysts are heavy and would normally fall to the bottom of drinkers. Mixing the oocysts with a suspension gel allows even distribution for chick access (Fanatico, 2006).

2.5. Resistance

Coccidiosis is mainly controlled using chemical coccidiostats administered in feed. The continual misuse of anticoccidial drugs (e.g. incorrect dosages) has led to the emergence of drug-resistant strains (Dauguschies et al., 1998; Long, 1982; Danforth et al., 1989). In the early 1980s, sulphaquinoxaline, nitrofurans and amprolium were commonly used to control coccidiosis, and with time, resistant *Eimeria* spp. field isolates in various countries were observed, China (Li et al, 2004), Pakistan (Abbas et al., 2008) India [Panda et al., 1973; Gill and Bajwa, 1979; Yadav and Gupta, 2001), and Brazil (Kawazoe and Difabio 1994).

Various programs are used in attempts to slow or stop selection of resistance. For instance, producers may use one anticoccidial continuously through succeeding flocks,

change to alternative anticoccidials every 4–6 mo, or change anticoccidials during a single growout (i.e., a shuttle program). “Shuttle programs,” in which one group of chick’s is treated sequentially with different drugs (usually a change between the starter and grower rations), are common practice and offer some benefit in slowing the emergence of resistance.

2.6. Alternatives

Coccidia resistance to drugs has led to interest in the development of alternative means of control such as the use of plant-based products and extracts. *Artemisia annua* (sweet wormwood) used in Chinese traditional medicine as an anti-parasitic agent to control skin bugs, itchy scabs, lice, and insects was demonstrated to have anticoccidial properties (Pirali-Kheirabadi et al., 2014; Popa et al., 2015). Other botanicals such as *Aloe excelsa* (Gadzirayi et al., 2005), *Azadirachta indica* (Tipu et al., 2002) and *Beta vulgaris* (Augustine et al., 1997) have reported anticoccidial effects. Therefore, in some countries plant based formulations, such as Apacox, Natustat and Zycox are used for the control of coccidiosis in chickens (Abbas et al., 2012). Most botanicals and plant-derived products are being extensively tested to establish the efficacy, mechanism of action and target parasite species (Athanasiadou et al., 2007).

2.7 Tannins

Tannin comes from an old German word for oak or fir tree. The tannins in the oak bark were used in the process of tanning; waterproofing and preservation of animal hides using plant extracts. The term tannin now extends to any large polyphenolic compound containing sufficient hydroxyls or carboxyls to form strong complexes with various macromolecules (proteins, bacterial cell membranes, carbohydrates and

polysaccharides). Some characteristics that make tannins unique to other secondary compounds found in plants are their molecular weight (500 – 20,000 Da), water solubility (except for some high molecular weight structures), ability to bind proteins and form tannin-protein complexes (soluble or insoluble). Tannins are also characterized as oligomeric compounds with various structure units with free phenolic groups. Based on their chemical structure, tannins are categorized as hydrolyzable or condensed tannins (proanthocyanidins) (Cannan, 2015).

Hydrolyzable tannins (HT) are molecules with a polyol (alcohol containing multiple hydroxyl groups) as a central foundation. The hydroxyl groups of HT are esterified (completely or partially) with phenolic groups like ellagic acid or gallic acid. These HT are usually found in low amounts in plants (Eastaugh et al., 2008).

Condensed tannins (CT) are polymeric flavonoids found in majority of tropical legumes. Some plants containing CT are birdsfoot trefoil (*Lotus corniculatus*), sainfoin (*Onobrychis viciifolia*), and sericea lespedeza (SL, *Lespedeza cuneata*) (MacAdam et al., 2013). CT might be associated with adverse effects as anti-nutritional factors, causing lower dry matter intake and reduced digestion of protein and fiber (Beelen et al., 2006). Lack of weight gain associated with SL has been observed in chickens and in lambs (Moyle et al., 2012; Burke et al., 2013). The effects depend on CT concentration in the plant and also other factors, such as type of CT, animal species, physiological status and diet composition (Schofield et al., 2001). In some cases, CT are reported to be beneficial to an animal's health. CT are relevant to ruminants in that they aid in bloat prevention and inhibit gastro-intestinal nematodes (GIN) (MacAdam et al., 2013). CT also allow more protein to be readily available for digestion due to its ability to attach to soluble proteins which bypass the rumen

(Kariuki and Norton, 2008). Lambs also benefit from CT by having lower fecal egg counts (FEC) when grazing on high CT forage (sulla (*Hedysarum coronarium*) and SL) as opposed to just alfalfa (*Medicago sativa*) (Niezen et al., 1995).

2.8 Sericea Lespedeza

SL is a legume that was introduced to the United States in the 1900s for erosion control. It is a warm season perennial and is native to eastern Asia. It is now found throughout the southern coast of the US and contains high amounts of CT. SL has also been used for forage/grazing. It has been declared a noxious weed in Kansas since 2001 (Anon, 2002) due primarily to its ability to take over pasture area and livestock refrain from eating it because of its bitter taste. As a nitrogen fixer, SL grows in poor soils and is tolerant of floods and droughts. It can survive in various levels of light, allowing it to thrive in a wide range of climates. SL grows also anywhere and stands 3 to 6 ft high creating dense stands at its base that inhibit the growth of surrounding plants (Powell et al., 2003). It has a hairy stem with club-shaped leaves.

SL has been investigated for controlling GIN in sheep and goats (Kommuru et al., 2015; Mechineni et al., 2014; Burke et al., 2013). Kommura et al. (2015) suggested that SL directly acts upon the cuticle of the nematodes when fed 75% SL leaf meal pellets. Mechineni et al. (2014) reported lower FEC in goats grazing on SL forage compared to goats grazing on bermudagrass. SL leaf meal pellets were also effective in preventing and treating coccidiosis in lambs (Burke et al., 2013). The use of pelleted SL reduced *Eimeria* spp. and GIN burdens in weaned goats (Kommuru et al., 2014). Moyle et al. (2012) reported that broiler chicks fed diets containing various concentrations of SL mixed with commercial feed

suggested that diets of more than 5% SL resulted in lower body weights than control chicks.

Palatability was not an issue and feed conversion was highest in their 20% SL diet.

CHAPTER 3 STUDY 1

3.1 Materials and Methods

3.1.1 Location

The study was conducted at the LSU AgCenter Poultry Research Lab, School of Animal Sciences, Louisiana State University, Baton Rouge, LA.

3.1.2 Animals

Day old male Ross 708 broiler chicks were obtained from Raeford Farms Hatchery located in Gibsland, Louisiana. Chicks were housed in starter battery cages and allowed *ad libitum* access to feed and water. Chicks were vaccinated for bursal disease, reovirus, Marek's disease, Newcastle disease, and bronchitis. Chicks were not vaccinated for coccidia. All experimental procedures for this study was approved by the LSU AgCenter Institutional Animal Care and Use Committee.

3.1.3 Infection

Chicks were infected at Day 4 by gavage with approximately 100,000 live sporulated oocysts (Coccivac-D2®, Merck Animal Health) in 0.5 ml of water using a 1 ml syringe. Uninfected chicks were given 0.5 ml distilled water. Coccivac-D2® contains *Eimeria tenella*, *E. mivati*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. hagani*, *E. necatrix*, and *E. praecox*.

3.1.4 Source of Sericea Lespedeza

SL leaf-meal was obtained from Sims Brother Inc. Agricultural Seed Farm, Union Springs, Alabama. Complete amino acid profile, protein, calcium and phosphorus were conducted by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO (Table 1). Amino acids were added in the diet to meet the nutritive

requirements. CT level was measured (15%) by Dr. Irene Mueller Harvey, Reading University, UK.

Table 1: Nutrient composition of sericea lespedeza. Units are in grams per 100 grams of sample. Crude protein = %N*6.25.

Threonine	0.61
Serine	0.57
Glutamic Acid	1.38
Proline	0.69
Lanthionine	0.01
Glycine	0.69
Alanine	0.81
Cysteine	0.16
Valine	0.68
Methionine	0.22
Isoleucine	0.56
Leucine	1.16
Tyrosine	0.52
Phenylalanine	0.72
Hydroxylysine	0.18
Ornithine	0.01
Lysine	0.92
Histidine	0.29
Arginine	0.74
Tryptophan	<0.02
Total	12.44
Crude Protein	14.99
Calcium	0.84
Phosphorus	0.18

3.1.5 Experimental Design

The primary objectives of this study were to evaluate the efficacy of SL on reducing *Eimeria* spp. infection, and effect on weight gain and feed conversion. The secondary objectives were to determine any effect on change in *Eimeria* spp. population distribution, lesion scores or histopathology.

Three hundred male Ross 708 broiler chicks were randomly allocated to 6 treatment groups as follows:

1. Negative Control (no infection, no treatment)
2. Positive Control (infection, treatment with Biocox (0.05%) in feed)
3. 0% SL (infection, no treatment)
4. 5% SL (infection)
5. 10% SL (infection)
6. 15% SL (infection)

There were 10 replications of 5 broiler chicks per replicate. The experiment was conducted for 18 days (Aug. 27 to Sept. 14). Broiler chicks were weighed at the beginning, day 12 and termination of the study. Feed was weighed when broiler chicks were weighed and when feed was low. Fecal samples were collected on days 1, 4, 6, 7, 11, 13, 15 and 18. Fresh samples were collected from aluminum trays under each cage into airtight collection bags. Trays were cleaned after each collection. Feces were processed using a modified quantitative McMaster technique to determine *Eimeria* spp. fecal oocyst count (FOC) and was reported as oocysts per gram (OPG). If a FOC was zero, a qualitative double centrifugation sugar flotation was done. *Eimeria* spp. population distribution was determined on each collection day. At the end of the study, necropsies were done on all surviving chicks (252) for lesion scoring and histopathology.

3.2 Techniques

3.2.1. Feed Mixing

Diets of treatment groups were formulated to meet nutrient requirements (Table 2).

All diets were based on the Aviagen Broiler Nutrition Handbook 2014 for the Ross 708

Table 2: Composition of study diets (FeedMix program).

Treatments		1	2	3	4	5	6
Starter Feed		Neg. Ctrl	Pos. Ctrl	0% SL	5% SL	10% SL	15% SL
	Ingredient						
		%	%	%	%	%	%
	Corn Chick (Evonik)	51.05	50.96	51.05	44.71	38.37	32.02
	SBM Chick (Evonik)	42.08	42.09	42.08	41.04	39.99	38.94
	Sericea lespedeza	0.00	0.00	0.00	5.00	10.00	15.00
	Soy Oil	2.43	2.46	2.43	4.88	7.32	9.77
	Mono-cal PHOS 22/2	1.61	1.61	1.61	1.64	1.68	1.72
	Limestone 6x12	1.18	1.18	1.18	1.06	0.94	0.83
	Salt	0.50	0.50	0.50	0.50	0.50	0.50
	DL-Methionine	0.35	0.35	0.35	0.37	0.39	0.40
	Minerals	0.25	0.25	0.25	0.25	0.25	0.25
	Vitamins	0.25	0.25	0.25	0.25	0.25	0.25
	BioLys	0.20	0.20	0.20	0.20	0.20	0.20
	BioCox	0.00	0.05	0.00	0.00	0.00	0.00
	Choline Chloride LIQ/70	0.05	0.05	0.05	0.05	0.05	0.05
	L-Thr	0.05	0.05	0.05	0.06	0.06	0.07
	Total	100.0 0	100.0 0	100.0 0	100.0 0	100.0 0	100.0 0
	ME (kcal/kg)	3000	3000	3000	3000	3000	3000
	Calcium	0.96	0.96	0.96	0.96	0.96	0.96
	P	0.77	0.77	0.77	0.76	0.75	0.75
	aP	0.48	0.48	0.48	0.48	0.48	0.48
Total AA	Lysine	1.44	1.44	1.44	1.44	1.44	1.44
	Methionine	0.7	0.7	0.7	0.71	0.72	0.73
	M+C	1.08	1.08	1.08	1.08	1.08	1.08
	Threonine	0.97	0.97	0.97	0.97	0.97	0.97
	Tryptophan	0.3	0.3	0.3	0.29	0.28	0.27

breed. FeedMix program was used to input specification of SL amino acid inclusion. 45.5 kg of feed was formulated for each treatment group (272.7 kg total). All ingredients were weighed and mixed in a small feed mixer for 8 min. Feed was stored at room temperature (approximately 27 degrees C) in separate plastic 10 g containers until used. Feed samples

were sent to the Agricultural Experiment Station Chemical Laboratory, University of

Missouri, Columbia, MO for complete feed analysis (Table 3).

Table 3: Post-experiment feed analysis by the Agricultural Experiment Station Chemical Laboratories at the University of Missouri. Crude protein* = %N x 6.25. § Non-proteinogenic amino acids. Units are W/W% = grams per 100 grams of sample. Results are expressed on an “as is” basis.

	Neg. Ctrl	Pos. Ctrl	0%SL	5%SL	10%SL	15%SL
Taurine §	0.16	0.16	0.15	0.14	0.13	0.12
Hydroxyproline	0.10	0.07	0.06	0.09	0.08	0.10
Aspartic Acid	2.33	2.08	2.50	2.21	2.27	2.35
Threonine	0.89	0.81	0.95	0.87	0.90	0.94
Serine	0.99	0.84	1.00	0.97	0.96	1.05
Glutamic Acid	4.10	3.68	4.30	3.88	3.86	3.93
Proline	1.26	1.17	1.30	1.20	1.21	1.22
Lanthionine §	0.05	0.06	0.07	0.06	0.07	0.07
Glycine	0.96	0.84	1.00	0.93	0.94	0.97
Alanine	1.09	0.99	1.12	1.06	1.05	1.07
Cysteine	0.31	0.28	0.33	0.30	0.31	0.32
Valine	1.13	1.01	1.20	1.08	1.12	1.13
Methionine	0.57	0.48	0.69	0.62	0.66	0.61
Isoleucine	1.04	0.93	1.10	0.98	1.03	1.03
Leucine	1.92	1.78	2.01	1.83	1.86	1.86
Tyrosine	0.72	0.63	0.76	0.70	0.70	0.71
Phenylalanine	1.17	1.06	1.25	1.12	1.16	1.18
Hydroxylysine	0.04	0.04	0.05	0.05	0.06	0.06
Ornithine §	0.01	0.01	0.01	0.01	0.01	0.01
Lysine	1.46	1.31	1.53	1.38	1.51	1.45
Histidine	0.60	0.54	0.63	0.56	0.57	0.57
Arginine	1.49	1.33	1.63	1.46	1.47	1.48
Tryptophan	0.28	0.27	0.27	0.25	0.24	0.24
Total	22.67	20.37	23.91	21.75	22.17	22.47
Gross Energy (Cal/100g)	353	357	351	368	377	392
Salt	0.53	0.36	0.59	0.11	0.45	0.45
Crude protein*	23.13	21.74	24.49	22.75	23.36	23.13
Moisture	10.59	10.50	10.49	10.11	9.78	9.12
Crude Fat	4.19	4.31	4.19	6.27	8.32	10.73
Crude Fiber	3.24	2.98	2.84	3.42	4.02	5.19
Ash	6.49	5.70	7.08	5.64	6.36	6.33

3.2.2. Fecal Oocyst Count

Fecal samples were stored in a refrigerator (5 degrees C) until processed. FOC was determined using a modified McMasters technique. One g of feces was weighed for each sample and placed in a 125-ml plastic cup using a tongue depressor. Fifteen ml of saturated salt solution (737 g of iodized salt dissolved in 3000 ml of tap water) was added to the feces and mixed to a solution. An additional 15 ml was added, and thoroughly mixed using an electric mixer (Drinkmaster® Drink Mixer, Hamilton Beach Brands, Inc., Glen Allen, NC). A sample of the solution was extracted using a pipette, added to one chamber of a McMaster slide. The sample was remixed and an additional sample was pipetted and added to the other chamber. The oocysts were counted at 100x on a microscope. Total oocysts counted in both chambers were multiplied by 100 to get OPG. This multiplication factor of 100 is specific to the ratio of feces (1 g) to flotation solution (30 ml). Each oocyst observed represents 100 OPG, therefore, this procedure has a sensitivity of ≥ 100 OPG. The FOC was performed twice (replicate) for each sample.

A double centrifugation sugar flotation technique was used on samples with a zero McMaster count. Two g of feces were weighed for each sample and placed in a 125 ml plastic cup using a tongue depressor. Fifteen ml of water was added to the sample and mixed thoroughly to make a solution. The solution was filtered through a tea strainer into a 15 ml plastic centrifuge tube. The tube was placed into a centrifuge and spun at 1500 rpm for 10 min. The supernatant was poured off and 10 ml of sugar solution was added. The precipitate was mixed with two applicator sticks until a solution was formed. The tube was placed into a centrifuge and sugar solution was added to a positive meniscus. A coverslip (Fisherbrand) was placed on top of the meniscus and the tube was spun at 1500 rpm for 10 min. The

coverslip was removed and placed on a microscope slide (Globe Scientific Inc). The number of oocysts were counted at 100x. and divided by 2 to get the OPG. The sensitivity is ≥ 1 OPG. Sugar flotation slides were also used to identify oocysts to species for population distribution.

3.2.3. Weight

Broiler chicks were weighed (g) using a digital scale at initiation, day 12 and termination of the study to determine average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency comparing gain to feed (G:F). Chick days (number of deceased chicks and the days they were not part of the study) were accounted for and applied to the feed efficiency on the days feed was weighed.

$ADG = \text{Weight gained (g)}/\text{Number of days fed (days)}$

$ADFI = \text{Total Feed (g)}/\text{Number of days}$

$FEED\ CONVERSION\ (G:F) = \text{Total Feed (g)}/\text{Weight gained (g)}$

3.2.4. Lesion Scoring

Necropsies were performed after euthanasia via Co2 asphyxiation at day 18. Lesion scoring (scale of 0-4) was recorded for each section of the intestine (upper, middle, lower, ceca) using visual aids (www.Immucox.com/Coccidiosis/Disease-Monitoring/Chicken-Lesion-Scores). A score of zero represented absence of gross lesions and 4 represented extensive hemorrhage or lesions. The lesion scores were then recorded as the average across the chicks (per group) for each segment. Total lesion score was calculated as the sum of lesion scores in the four intestinal segments.

3.2.5. Histopathology

Intestine samples were collected at necropsy from a random (arbitrarily selected from carcasses) group for each treatment. The samples consisted of duodenum, jejunum, ileum and ceca segments. The samples were cut, cleaned and placed into a 50 ml tube of 10% formalin solution. After 24 hr, the formalin was drained and replaced by a solution of 70% ethanol. Samples were sent to the LSU School of Veterinary Medicine Histology lab for hematoxylin and eosin staining. One chick was randomly selected per treatment group. Four sections of each sampled portion of the small and large intestine were embedded for microscopic evaluation. The microscopic lesion scoring system (MLS) based on methods described by Goodwin et al. (1998), was used in this study to identify development stages in the villi. For each intestinal segment, four fields (100x) of view were evaluated. The established MLS was determined as the sum of distribution and severity scores. The distribution score was based on the presence of any coccidial stage in the four fields examined. The scores were as follows: 0 = none of the fields contained coccidia; 1 = one field contained coccidia; 2 = two fields contained coccidia; 3 = three fields contained coccidia; and 4 = all fields contained coccidia. The severity score was based on the percentage of the villi in the four fields examined that were parasitized by coccidia. The scores were as follows: 0 = no villi were parasitized; 1 = < 25% of villi were parasitized; 2 = 25 to 75% of villi were parasitized; and 4 = > 75% of villi were parasitized.

3.2.6. *Eimeria* spp. Identification

Sugar flotation slides were read under 100x microscopic magnification. One hundred randomly viewed oocysts were identified to species for population distribution based on

descriptions (primarily size and shape) in “Coccidial Vaccines Manual” (MSD Animal Health).

3.3 Statistical Analysis

All data were analyzed using SAS with PROC GLM followed by Fishers Least Significant Difference. A $p \leq 0.05$ was significant.

3.4 Results

3.4.1 Fecal Oocyst Count

Fecal samples were collected and processed pre-infection to determine if any oocysts were present. No oocysts were observed pre-infection or on days 4 and 6. Treatment 1 maintained a low/zero FOC throughout the study (Figure 2).

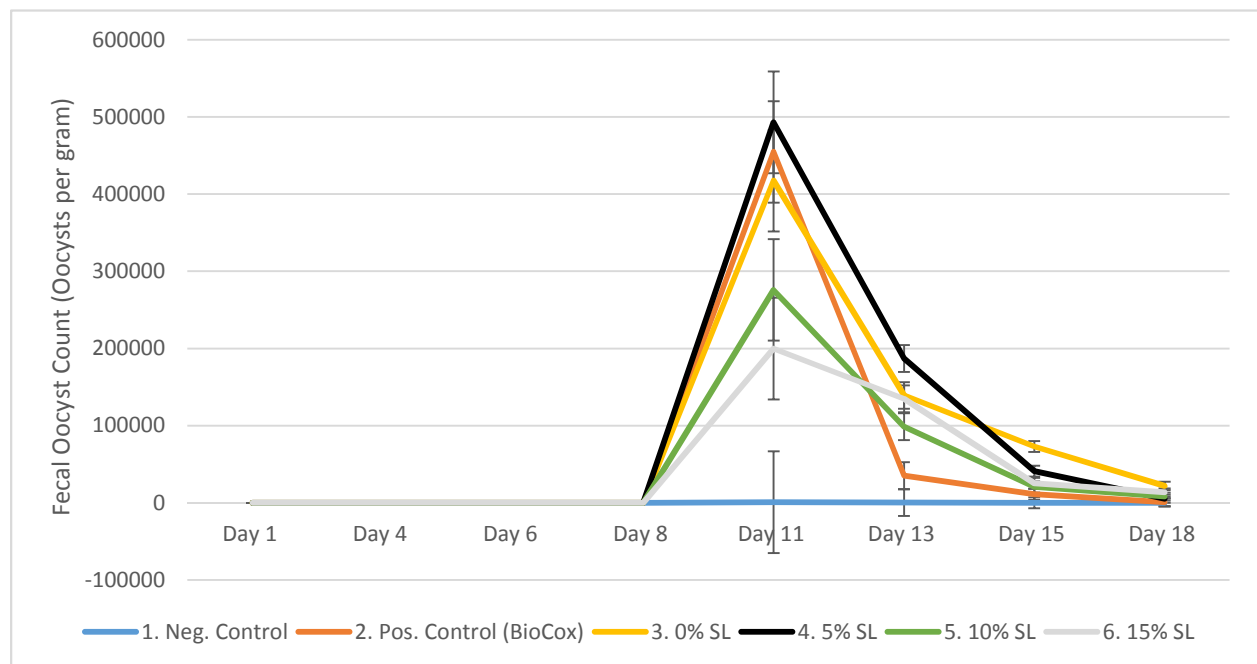


Figure 2: Mean (+/- SEM) fecal oocyst count (oocysts per gram of feces) of *Eimeria* spp. infected broiler chicks fed various levels of sericea lespedeza (SL). Infection was administered on day 4.

Oocyst production peaked on day 11 (7 dpi). Treatments 5 and 6 were significantly ($p < 0.05$) lower than treatments 2-4, which were not significantly ($p > 0.05$) different. Subsequent to day 11, treatments 2-6 decreased and remained relatively similar for the duration of the study.

3.4.2 Weight

Initial bodyweight (BW) for treatments 1-6 were 34.56 g, 34.66 g, 34.72 g, 34.64 g, 34.69 g and 34.69 g, respectively and were not different ($p = 0.69$). At day 12, BW for treatment 4-6 chicks were significantly ($p < 0.05$) lower than treatment 1-3 chicks with treatment 3 chicks significantly ($p < 0.05$) lower than treatment 1-2 chicks (Table 4).

Table 4: Mean bodyweight (BW) data of *Eimeria* spp. infected broiler chicks fed various levels of sericea lespedeza (SL). ADG: Average daily gain. ADFI: Average daily feed intake. G:F (Gain:Feed =ADG/ADFI, adjusted to accommodate mortality over the study). SEM: Standard error of the mean. Pr > F: p-value associated with the F statistic. Unlike superscripts within columns are significantly ($p < 0.05$) different.

	BW, g	ADG, g	ADFI, g	G:F
Day 0 to 12	Day 12			
1. Neg. Control	289.98 ^a	21.28 ^a	23.87 ^a	0.89 ^{ab}
2. Pos. Control (BioCox)	273.55 ^a	19.9 ^a	21.73 ^{ab}	0.92 ^a
3. 0% SL	240.1 ^b	17.125 ^b	20.33 ^b	0.83 ^b
4. 5% SL	161 ^c	10.53 ^c	16.02 ^c	0.65 ^c
5. 10% SL	150.99 ^c	9.69 ^c	15.09 ^c	0.64 ^c
6. 15% SL	152.49 ^c	9.76 ^c	15.15 ^c	0.61 ^c
SEM	9.89	0.82	0.95	0.02
Pr>F	<0.0001	<.0001	<.0001	<.0001
Day 12 to 18	Day 18			
1. Neg. Control	588.45 ^a	49.74 ^a	65.29 ^a	0.76 ^b
2. Pos. Control (BioCox)	537.31 ^{ab}	43.96 ^b	53.43 ^b	0.84 ^{ab}
3. 0% SL	487.22 ^b	41.18 ^b	57.6 ^{ab}	0.72 ^b
4. 5% SL	266.35 ^c	17.55 ^d	35.52 ^c	0.49 ^c
5. 10% SL	309.66 ^c	26.16 ^c	29.83 ^c	0.99 ^a
6. 15% SL	298.73 ^c	25.4 ^c	34.76 ^c	0.83 ^{ab}
SEM	19.95	1.72	3.41	0.06
Pr>F	<0.0001	<.0001	<.0001	0.0002

ADG,ADFI and G:F for treatment 4-6 chicks were significantly ($p < 0.05$) lower than treatment 1-3 chicks. At day 18, BW for treatment 4-6 chicks was significantly ($p < 0.05$) lower than treatment 1-3 chicks with treatment 3 chicks similar ($p > 0.05$) to treatment 2

chicks. ADG and ADFI for treatment 4-6 chicks were significantly ($p<0.05$) lower than treatment 1-3 chicks. From days 0-12, G:F was significantly ($p<0.05$) lower for SL diets compared to non-SL diets (Table 4). From days 12-18, G:F was highest in the 10% and 15% SL diets compared to the other groups.

3.4.3 Lesion Scores

There was no difference ($p>0.05$) between groups for duodenum, cecum or total lesion scores (Table 5). Treatment 2 had significantly ($P<0.05$) higher jejunum and ileum lesion scores than treatment 4 and treatment 5, respectively.

Table 5: Lesion scores of necropsied broiler chicks (n=252). Values represent the mean across the birds (per group) in each segment. Total lesion score was calculated as the mean of the 4 intestinal segments (duodenum, jejunum, ileum and cecum). *Significant difference ($p<0.05$)

Lesion Scores	Duodenum	Jejunum	Ileum	Cecum	Total
1. Neg. Control	0.09	0.05	0.03	0.28	0.11
2. Pos. Control (BioCox)	0	0.16*	0.16*	0.25	0.15
3. 0% Sericea	0.06	0.05	0.05	0.17	0.08
4. 5% Sericea	0	0*	0.06	0.15	0.05
5. 10% Sericea	0	0.05	0*	0.31	0.09
6. 15% Sericea	0	0.09	0.14	0.18	0.1

3.4.4 Histopathology

No developing stages were observed in any of the sections of the intestine from treatments 1-3 (Table 6). Treatments 4-5 had developing stages present in the duodenum, jejunum and ceca. Treatment 6 had developing stages in the duodenum and jejunum. The ileum lacked developing stages across all treatments. Treatment 5 had the highest number of developing stages in the ceca, accompanied by distortion of the mucosal architecture as well as marked mixed inflammation and fibrosis that multifocally extended into the submucosa. In addition, variable heterophilic infiltrate was evident in all treatments.

Table 6: Histopathology scoring of intestinal histopathology for development stages of *Eimeria* spp. based on the microscopic lesion scoring system.

Distribution Score				
	Duodenum	Jejunum	Ileum	Ceca
TRT1 ^a	0	0	0	0
TRT2 ^b	0	0	0	0
TRT3 ^c	0	0	0	0
TRT4 ^d	1	1	0	1
TRT5 ^e	4	4	0	4
TRT6 ^f	4	4	0	0
Severity Score				
	Duodenum	Jejunum	Ileum	Ceca
TRT1 ^a	0	0	0	0
TRT2 ^b	0	0	0	0
TRT3 ^c	0	0	0	0
TRT4 ^d	1	1	0	1
TRT5 ^e	2	1	0	3
TRT6 ^f	1	1	0	0
Microscopic Lesion Scoring (MLS)				
	Duodenum	Jejunum	Ileum	Ceca
TRT1 ^a	0	0	0	0
TRT2 ^b	0	0	0	0
TRT3 ^c	0	0	0	0
TRT4 ^d	2	2	0	2
TRT5 ^e	6	5	0	7
TRT6 ^f	5	5	0	0

Superscripts ^{a-f} represent groups in the following treatment (TRT1-6) order: (a: Negative control; b: Positive control; c: 0%SL; d: 5%SL; e: 10%SL; f: 15%SL).

3.4.5 *Eimeria* spp. Identification

No *Eimeria* spp. oocysts were found in Treatment 1 feces (Table 7). No *E. hagani* oocysts were found for any treatment group. All other *Eimeria* spp. were present. *Eimeria tenella* was the predominant species followed by *E. praecox* throughout the study. *Eimeria miavati* was the least predominant species throughout the study. *Eimeria acervulina*,

Table 7: Population distribution (percent) of *Eimeria spp.* oocysts found in feces of sericea lespedeza (SL) fed and non-SL fed broiler chicks.

Day 11								
	<i>E. tenella</i>	<i>E. miavati</i>	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. brunetti</i>	<i>E. hagani</i>	<i>E. necatrix</i>	<i>E. praecox</i>
TRT1 ^a	0	0	0	0	0	0	0	0
TRT2 ^b	32	6	24	1	2	0	16	19
TRT3 ^c	35	8	19	4	8	0	13	13
TRT4 ^d	17	7	28	1	1	0	22	24
TRT5 ^e	18	3	20	20	13	0	16	10
TRT6 ^f	19	6	10	26	16	0	11	12
Day 13								
	<i>E. tenella</i>	<i>E. miavati</i>	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. brunetti</i>	<i>E. hagani</i>	<i>E. necatrix</i>	<i>E. praecox</i>
TRT1 ^a	0	0	0	0	0	0	0	0
TRT2 ^b	41	2	11	1	10	0	15	20
TRT3 ^c	37	1	10	4	11	0	18	19
TRT4 ^d	32	2	12	1	18	0	17	18
TRT5 ^e	31	1	8	6	26	0	14	14
TRT6 ^f	34	1	9	4	26	0	8	18
Day 15								
	<i>E. tenella</i>	<i>E. miavati</i>	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. brunetti</i>	<i>E. hagani</i>	<i>E. necatrix</i>	<i>E. praecox</i>
TRT1 ^a	0	0	0	0	0	0	0	0
TRT2 ^b	39	1	15	1	4	0	13	27
TRT3 ^c	28	5	14	8	3	0	16	26
TRT4 ^d	36	3	19	5	4	0	13	20
TRT5 ^e	24	1	4	36	18	0	6	11
TRT6 ^f	25	0	5	44	13	0	6	7
Day 18								
	<i>E. tenella</i>	<i>E. miavati</i>	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. brunetti</i>	<i>E. hagani</i>	<i>E. necatrix</i>	<i>E. praecox</i>
TRT1 ^a	0	0	0	0	0	0	0	0
TRT2 ^b	43	2	6	5	7	0	15	22
TRT3 ^c	23	1	10	8	21	0	11	26
TRT4 ^d	32	1	6	5	31	0	12	13
TRT5 ^e	9	1	3	55	19	0	7	6
TRT6 ^f	13	0	3	39	35	0	4	6

Superscripts ^{a-f} represent groups in the following treatment (TRT1-6) order: (a:Negative control; b:Positive control; c:0%SL; d:5%SL; e:10%SL; f:15%SL).

E. maxima, *E. brunetti* and *E. necatrix* were all present in relatively equivalent numbers across treatments with the exception of high numbers of *E. maxima* in treatments 5 and 6 on days 15 and 18.

3.5 Discussion

Lambs and kids fed SL has been shown to control of *Eimeria* spp. based on a reduction in FOC (Burke et al., 2013). *Eimeria* spp. are also pathogenic in chickens and SL might also be effective in controlling infection. The only study evaluating such an effect demonstrated that feeding broiler chicks SL up to 4% of the diet, did not control infection and the conclusion was that SL should not be used for *Eimeria* spp. control (Rathinam et al., 2014). It was also shown that they had reduced weight gain. The SL used in that study was the same as that used in another study that showed no effect on reducing *Eimeria* spp. infection in lambs (J.M. Burke, personal communication). It was suspected that the SL product used had been subjected to something that could have adversely affected the activity of CT. The CT level was 5% in that study. The objective of this study was to evaluate higher levels of SL for effect on infection. Results suggested that feeding 10-15%SL in the feed reduced infection by approximately 50% on Day 11 which was the peak of infection. Treatment 4 had no effect on infection which is in accordance with Rathinam et al. (2014). This study used fresh SL that contained 15% CL. As expected, there was no effect of 0 and 5% SL on reducing infection, but it was unexpected that the positive control (Biocox) also had no effect. This suggested that resistance had developed to the coccidiostat.

Reduction in weight gain has been observed with feeding broiler chicks SL up to 20% of the diet (Moyle et al. 2012). Results of this study supported reduced weight gain in that all SL diets did not gain as well as non-SL diets. Although the diets were formulated to meet the nutritive requirements of the Ross 708 breed, substituting a large portion with SL was not

beneficial to growth. A decrease in feed consumption was also observed with the SL diets which was not observed in the Moyle et al. (2012) study. It appears there was a palatability issue with the SL diets of this study which was not the case in the Moyle et al. (2012) study. There was no apparent reason for this difference.

The relative value of lesion scoring might be considered minimal in that scoring was done at the end of the study when FOC was low and the course of infection had passed. Some differences in the jejunum and ileum were observed, but overall nothing significant. Gross lesions (spots, blood clots) were not apparent on the outer and inner intestinal walls. Some discoloration was observed but nothing that matched the signs of *Eimeria* spp. infection. Therefore, lesion scoring was not of value in this study.

Histopathology showed that only SL fed broiler chicks had developing stages of *Eimeria* spp. in the mucosa in contrast to no developing stages in non-SL broiler chicks. This suggested that SL might act to curtail development whereas development was completed without SL. This might also suggest that the reduced FOC was due to lack of complete development in the mucosa. In high infections, asexual reproduction results in damage to intestinal mucosal cells and compromised function. By inhibiting asexual reproduction, SL-fed chick intestinal function may have been compromised which in turn may have been the cause of the reduced weight gain. However, since feed conversion was also reduced in SL-fed chicks, that may be the cause of reduced weight gain, or a combination of both. On the other hand, SL may simply have had a negative impact on the development and growth of the chicks intestinal tissue during the course of feeding.

Eimeria spp. population distribution was done to evaluate any change in species composition between treatments. There was no apparent difference in species composition,

therefore, all species were affected to the same extent, with *E. tenella* being the most predominant and recognized as the most pathogenic.

In conclusion, under the conditions of this study, diets including 10 and 15% SL significantly reduced the peak of *Eimeria* spp. infection, but weight gain was compromised. In addition, the coccidiostat Biocox was not effective probably due to development of resistance. Since weight gain is a major factor in broiler chick production, using SL for *Eimeria* spp. control may not be warranted.

CHAPTER 4 STUDY 2

4.1 Materials and Methods

4.1.1 Location

The study was conducted at the LSU AgCenter Poultry Research Lab, School of Animal Sciences, Louisiana State University, Baton Rouge, LA.

4.1.2 Animals

Day old male Ross 708 broiler chicks were obtained from Raeford Farms Hatchery located in Gibsland, Louisiana. Chicks were housed in starter battery cages and allowed *ad libitum* access to feed and water. Chicks were vaccinated for bursal disease, reovirus, Marek's disease, Newcastle disease, and bronchitis. Chicks were not vaccinated for coccidia.

4.1.3 Infection

Chicks were infected at Day 4 by gavage with approximately 100,000 live sporulated oocysts (Coccivac-D2®, Merck Animal Health) in 1ml of water using a 1ml syringe. Uninfected chickens were given 1ml distilled water. Coccivac-D2® contains *Eimeria tenella*, *E. mivati*, *E. acervulina*, *E. maxima*, *E. brunetti* and *E. necatrix*. For the second trial, *E. hagani* and *E. praecox* were not present.

4.1.4 Source of Sericea Lespedeza

SL leaf-meal used in this study was the same batch as study 1 but had been stored for approximately one year.

4.1.5 Experimental Design

The primary objectives of this study were to determine if the results from Study 1 could be repeated using feed with only 10% SL and to evaluate another coccidiostat

treatment (Corid, positive control) against *Eimeria* spp. Two-hundred fifty male Ross 708 broiler chicks were randomly allocated to 5 treatment groups as follows:

1. Negative Control (no infection, no treatment)
2. Postive Control (infection, treatment with Biocox (0.05%) in feed)
3. Postive Control (infection, treatment with Corid (2.5 mL/g) in water)
4. 0% SL (infection, no treatment)
5. 10% SL (infection)

There were 10 replications of 5 broiler chicks per replicate. The experiment was conducted for 18 days (Aug. 22 to Sept. 9). Broiler chicks were weighed at the beginning, day 12 and termination of the study. Feed was weighed when chicks were weighed and when feed was low. Fecal samples were collected on days 1, 4, 6, 7, 11, 13, 15 and 18. Fresh samples were collected from aluminum trays under each cage into airtight collection bags. Trays were cleaned after each collection. Feces were processed using a modified quantitative McMaster technique to determine *Eimeria* spp. FOC and was reported as OPG. If a FOC was zero, a qualitative double centrifugation sugar flotation was done.

4.2 Techniques

Techniques discussed in chapter 3 were used for this study. Histopathology, lesion scoring and oocyst identification for population distribution were not done.

4.3 Statistical Analysis

All data were analyzed using SAS with PROC GLM followed by Fishers Least Significant Difference. A $p \leq 0.05$ was significant.

4.4 Results

4.4.1 Fecal Oocyst Count

Fecal samples were collected and processed pre-infection to determine if any oocysts were present. No oocysts were observed pre-infection or on days 4 and 6 (Figure 3).

Treatment 1 maintained a low/zero OPG throughout the study. Oocyst production peaked on day 11 (7 dpi). Treatment 3 was significantly ($p < 0.05$) lower than treatments 2, 4 and 5 which were not significantly ($p > 0.05$) different. Subsequent to day 11, treatments 2-5 decreased and remained relatively similar for the duration of the study. On day 15 all treatments, except 1 and 2, slightly increased from day 13.

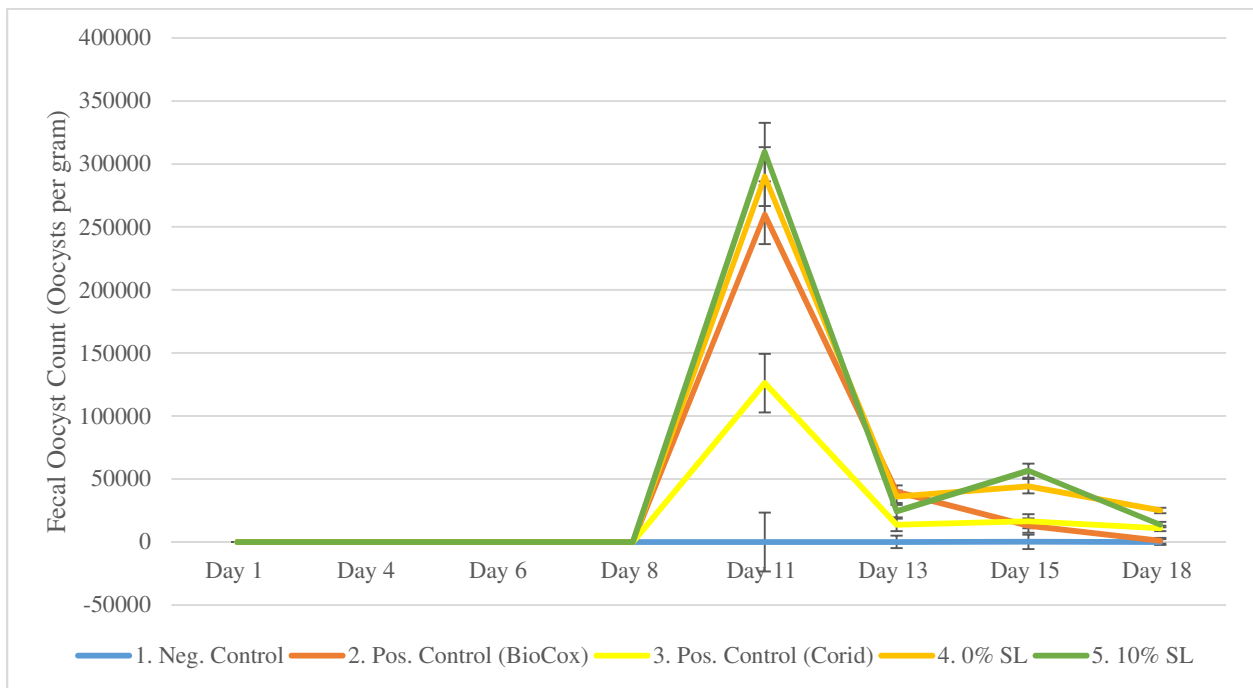


Figure 3: Mean (+/- SEM) fecal oocyst count (oocysts per gram of feces) of *Eimeria* spp. infected broiler chicks fed various levels of sericea lespedeza (SL). Infection was administered on day 4.

4.4.2 Weight

Initial BW for treatments 1-5 were 36.34 g, 36.38 g, 36.36 g, 36.36g, and 36.34 g, respectively and were not different ($p = 0.99$). At day 12, BW and ADG for treatment 2-5

chicks were significantly ($p < 0.05$) lower than treatment 1 chicks with treatments 4-5 chicks significantly ($p < 0.05$) lower than treatment 2-3 chicks (Table 8). ADFI for treatments 4-5

Table 8: Mean bodyweight (BW) for *Eimeria* spp. infected broiler chicks fed various levels of sericea lespedeza (SL). ADG: Average daily gain. ADFI: Average daily feed intake. G:F(Gain:Feed) = ADG/ADFI (adjusted to accommodate mortality over the study)SEM: Standard error of the mean. Pr > F: p-value associated with the F statistic. Unlike superscripts within columns are significantly ($p < 0.05$) different.

	BW, g	ADG, g	ADFI, g	G:F
Day 0 to 12	Day 12			
1. Neg. Control	292.18 ^a	21.32 ^a	25.05 ^a	0.85 ^a
2. Pos. Control (BioCox)	267.11 ^b	19.24 ^b	23.86 ^{ab}	0.8 ^b
3. Pos. Control (Corid)	247.7 ^b	17.61 ^b	23.08 ^{ab}	0.76 ^c
4. 0% SL	206.84 ^c	14.21 ^c	20.36 ^c	0.69 ^d
5. 10% SL	202.48 ^c	13.84 ^c	22.21 ^{bc}	0.62 ^e
SEM	7.83	0.65	0.7	0.01
Pr>F	<0.0001	<0.0001	0.0004	<.0001
Day 12 to 18	Day 18			
1. Neg. Control	553.84 ^a	43.61 ^a	59.74 ^a	0.72 ^a
2. Pos. Control (BioCox)	522.4 ^{ab}	42.54 ^{ab}	55.45 ^{ab}	0.77 ^a
3. Pos. Control (Corid)	494.82 ^b	41.18 ^{ab}	54.84 ^{ab}	0.75 ^a
4. 0% SL	432.18 ^c	37.55 ^{bc}	50.53 ^b	0.74 ^a
5. 10% SL	413.72 ^c	34.79 ^c	57.63 ^a	0.61 ^b
SEM	15.78	1.77	1.97	0.02
Pr>F	<0.0001	0.005	0.0259	0.0014

chicks was significantly ($p < 0.05$) lower than treatment 1-3 chicks with treatment 3 chicks similar ($p > 0.05$) to treatments 2 and 3 chicks. G:F was significantly different ($p < 0.05$) between all treatments, decreasing in value from treatment 1-5 respectfully. At day 18, BW for treatment 4-5 chicks was significantly ($p < 0.05$) lower than treatment 1-3 chicks with treatment 2 chicks similar ($p > 0.05$) to treatment 1 chicks. ADG for treatment 4-5 chicks were significantly ($p < 0.05$) lower than treatment 1-3 chicks with treatment 4 similar ($p > 0.05$) to treatment 2 and 3. ADFI for treatment 2-4 chicks were significantly ($p < 0.05$) lower than

treatment 1 and 5 chicks with treatment 4 chicks similar ($p>0.05$) to treatment 2-3 chicks. G:F for treatment 5 chicks were significantly ($p>0.05$) lower than treatment 1-4 chicks.

4.5 Discussion

The objective of this study was to determine if the 10% level of SL in the feed could repeat control of *Eimeria* spp. as observed in Study 1. FOC did not reflect the results observed in Study 1 in that the 10% SL group had the highest FOC of any group on day 11. One explanation for this lack of efficacy could be that the SL used was the same batch as used in Study 1 and had been stored for approximately a year. Storage may have adversely affected the bioactivity of the CT component. BioCox, once again, did not prevent infection, thus, verifying that resistance was present. However, Corid did effectively reduce FOC by approximately 50% which indicated that it might be of value for control.

Weight gain from days 0-12 and 12-18 for the broiler chicks fed 10% SL was again significantly lower than the control groups, but gain was substantially greater than that observed in Study 1. This result remains consistent with the results of Study 1 and in the Moyle et al. (2012) study. ADG was also significantly reduced, but in contrast to Study 1, ADFI was similar to the other diets. If storage affected the bioactivity, that may have resulted in increased palatability, which could have accounted for the increased ADFI. However, that did not result in better feed efficiency.

In conclusion, feeding a 10% SL feed to growing broiler chicks may not be of value for controlling *Eimeria* spp. infection which was in contrast to Study 1. The reason for this could be loss of bioactivity during the relatively long storage period. In addition, Corid was better at controlling infection than Biocox under the conditions of this study.

CHAPTER 5 DISCUSSION AND CONCLUSION

Eimeria spp. are a major threat to broiler chick production. Infection early in the growing (usually the first 18 days) phase destroys intestinal mucosal cells and compromises function. As a result, chicks do not grow well and the profit margin is adversely affected. Control has traditionally been accomplished by using coccidiostats either in the feed or in the water. These drugs are no longer as effective as they used to be as resistance has become an issue (Abbas et al., 2008; Blake and Tomley, 2013; Chapman, 2014). In light of this resistance, new methods for control need to be investigated. In addition, organic production has become popular and drug use is prohibited. By discovering and evaluating new non-drug alternatives, dependence on anticoccidial drugs is reduced both for traditional and organic production. Using alternatives that prove to be beneficial can prolong the efficacy of those drugs that still work which would improve the quality of life for chickens on small and large operations.

Sericea lespedeza is a legume forage that has demonstrated anti-coccidia properties when fed to lambs and kids at greater than 25% of the diet (Burke, 2013; Burke, 2010; Kommuru, 2014). Two previous studies attempted to evaluate feeding SL to broiler chicks. The first study showed that feeding SL at more than 5% (to 20%) of the diet compromised growth in that chicks did not grow as well as chicks fed a normal feed (Moyle et al., 2012). The second study used SL fed at 4% of the diet to evaluate effect on *Eimeria* spp. infection with minimal effect on growth (Rathinam, 2014). Results of that study showed no effect on controlling *Eimeria* spp. and it was concluded that feeding SL for control was not practical. Since higher levels of SL were needed to control *Eimeria* spp. in lambs and kids, higher

levels may also be necessary in chicks.

The objective of Study 1 was to evaluate the effect of 0, 5, 10 and 15% SL on *Eimeria* spp. infection to determine if the higher levels might be needed for control. Results indicated that at the higher levels (10% and 15%), SL reduced the FOC compared to other infected treatments at the peak of infection. And, in accordance with Moyle et al. (2012), weight gain was reduced more as the level of SL increased. This could have been a palatability issue in that SL fed chicks consumed less feed than the other treatments. What was unexpected was that the feed coccidiostat (Biocox) had no effect on infection. This suggested that resistance had developed to treatment. Since necropsy was done at the end of the study after the infection subsided, it was not surprising that there was no effect on intestinal lesion scores. However, histopathology on intestine samples revealed that there were residual developing stages of *Eimeria* spp. in the mucosa of SL fed broiler chicks only. This might suggest that SL had some effect on delaying development (i.e., asexual reproduction) and thus reduced FOC. It might be argued that this delay provided the opportunity for the host to develop the immunity needed to further control infection. In addition, the reduction in FOC would result in less environmental contamination and subsequent reinfection. Identification of oocysts over time did not reveal any apparent changes in population distribution between treatments. Therefore, SL did not appear to affect any species more than another.

The objectives of Study 2 were to determine if the observed effect of the 10% level of SL and apparent resistance to Biocox in study 1 could be repeated, and if a water administered coccidiostat (Corid) would provide adequate control. In contrast to Study 1, there was no reduction in FOC at the peak of infection. One logical explanation for this failure was that the SL used was the same as that used in Study 1 and the time in storage

(approximately a year) could have affected the bioactivity of the CT. If palatability was an issue in Study 1, it was not apparent in Study 2 as SL fed broiler chicks consumed almost as much as non-SL fed chicks. Weight gain was also much better in Study 2 but still less than that of non-SL fed broiler chicks. This might be expected if the bioactivity of the SL was affected by storage, thus making it more palatable (i.e., less bitter). As in Study 1, there was no reduction in FOC for the Biocox treatment, thus resistance to Biocox was repeated which indicated that this coccidiostat should be evaluated in production systems before relying on it for control. On the other hand, treatment with Corid did reduce FOC by approximately 50%. Therefore, Corid was somewhat effective and might be valuable for controlling infection.

Intestinal lesions scoring for Study 1 occurred at necropsy, day 18 of study and 14 days after infection with *Eimeria* spp. There were very few lesions observed and no apparent differences in lesion scoring between treatments were noted. Therefore, lesion scoring was not meaningful at the time scores were evaluated. It might have been better to necropsy chicks at the times FOC were done, thus evaluating lesions during the active infection period.

Results of these studies indicate that SL may still be useful for controlling *Eimeria* spp. infection, but further studies are required in view of not using fresh SL for Study 2 as was done for Study 1. It would also have been nice to include additional treatment groups in Study 1 (SL fed at 5, 10 and 15% without infection) just to see the effect of SL alone on weight gain. It should be noted that the normal growing period of broiler chicks is upwards of 40-45 days and not 18 days as done in these studies. The 18-day period was used to evaluate the effect of SL on infection and weight gain during the expected primary infection period. It

would have been interesting to have extended the studies beyond 18 days out to 40-45 days, the time at which broilers are normally harvested. This would have allowed the SL fed chicks an opportunity to display any compensatory gain from 18 days on after returning to their normal growing diet. That question needs to be answered if SL is to be considered for *Eimeria* spp. control.

In conclusion, there appears to be a benefit for feeding SL at 10% of the diet to control *Eimeria* spp. infection, but weight gain is negatively affected, at least during the first 18 days of the growing period when SL would be fed to control infection. In addition, there was an indication that SL delayed development of *Eimeria* spp. in the intestinal mucosa, which may elucidate a potential mechanism of action of the CT active component of SL. Finally, coccidiostats should not be used blindly as resistance is common. They should be evaluated before use to determine efficacy.

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