Spring 5-9-2015

The Effects of Phytochemical Tannin-Containing Diets on Animal Performance and Internal Parasite Control in Meat Goats

Chassity Wright
Tuskegee University

THESIS APPROVED BY:

Byeng Min, PhD
Major Professor

Walter A. Hill, PhD
Dean of College

Cesar D. Fermin, PhD
Dean of Graduate Programs

FOR:

Chassity Wright
Student

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Title of Thesis

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By
Chassity Wright

A Thesis Submitted to the Graduate Faculty
of Tuskegee University
in Partial Fulfillment of the Requirements
of the Degree

MASTER OF SCIENCE IN ANIMAL AND POULTRY SCIENCES

Tuskegee University
Tuskegee, Alabama 36088
May 2015

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for giving me the strength to complete this journey. Without his blessings, none of this would have been possible. A sincere and special thanks is in order to Dr. Byeng Min, my major advisor, for his undivided attention, guidance and support. I would also like to thank my fellow graduate buddy Ms. Danielle Perkins for all of her help and efforts with assisting me with my study. Lastly, I would like to give thanks to Drs. Nar Gurung, Wendell McElhenney, Chukwuemeka Okere, and William Witola for serving as my committee members and giving their advice and support.

Most importantly, a great big thanks to the Sustainable Agriculture Research and Education (SARE) Programs funded by USDA-NIFA, the USDA/NIFA Evans-Allen Research Program, and the Tuskegee University George Washington Carver Agricultural Research Station for their support of this project.

ABSTRACT

The Effects of Phytochemical Tannin-Containing Diets on Animal Performance and Internal Parasite Control in Meat Goats

By
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Haemonchus contortus (H. contortus) resistance has been reported against almost all chemical anthelmintics available for its control. *H. contortus* has a remarkable ability to develop resistance and threatens the viability of the goat industry in many regions of the world. Consequently, there is an urgent need to understand the genetic mechanisms underlying anthelmintic resistance and to discover new alternative methods of chemical and non-chemical control. With chemical anthemintics failing, this has led to the evaluation of plants as a natural source of anthemintic. Researchers are evaluating the effectiveness of phytochemical compounds, called tannins. Condensed tannins (CT) and hydrolyzable tannins (HT) exist, but HT is metabolized to toxic by-products. Therefore, CT are more widely used for research. The objective of this study was to compare the effects of CT containing diets of *sericea lespedeza* pellets (SLP), pine bark powder (PB), and a combination of SLP and PB on animal growth, fecal egg counts (FEC), packed cell volume using FAMACHA score, adult worm numbers, carcass characteristics, and blood serum chemistry in meat goats experimentally infected with 5,000 drug resistant *H. contortus* L3 larvae 6 weeks before the initiation of the study.

Twenty-four Kiko-cross intact male goat yearlings (*Capra hircus;* BW = 38.6 ± 2.7 kg) were randomly assigned to four experimental treatments: 1) 30% bermudagrass hay (BG), 2) 30% PB, 3) 30% SLP, and 4) 15% SLP + 15% PB. Each treatment diet was completed with

70% commercial sweet feed and alfalfa pellets. Each of the ingredients for all experimental diets were individually measured out, mixed together by hand and distributed accordingly for 42 days. Starting on day zero of the study, animals were fed once a day and monitored for a 42-day performance period. Body weights, FAMACHA, and fecal samples were taken on day 0 and every 2 weeks until day 42. Blood samples were collected only on day 0 and day 42. At the end of the experiment the animals were sent to Fort Valley State University for carcass evaluation and adult worm counts from the abmomasum were conducted at Louisiana State University.

The results showed that CT containing diets have no significant effects on final BW (P = 0.16) and ADG (P = 0.19). FEC results indicated that there was no significance among the CT treatments (P = 0.64) up to day 28. However, FEC in the mix diet was significantly reduced by day 42 (P = 0.05) compared to other treatment groups. There was interaction significance shown with day (P = 0.00), but no significance with treatment interaction and treatment per day interaction (P = 0.08). Carcass data indicated no significant differences in fasting weight (P = 0.24), hot carcass weight (P = 0.35), and dressing percentage (P = 0.83). Adult worm counts was lowest in the mixed diet (P = 0.02) compared to all other treatments (P < 0.03 – 0.01). FAMACHA data was significant on day 21 (P = 0.05), lowest in the PB diet, and on day 42 (P = 0.05), lowest in both the SLP and mix diet. In the blood serum chemistry, all values were within the normal range for goats, suggesting that no liver damage occurred. This was confirmed by postmortem necropsy and dissection of the liver and kidney in this study (data not presented), which indicated no anatomical lesions on liver and kidney organs.

CHAPTER I

INTRODUCTION

Although the use of condensed tannins (CT) from tanniferous plants has been largely documented as an alternative to chemical anthelmintics to control gastrointestinal nematodes (GIN) in sheep, studies remain scarce in goats. *Haemonchus contortus* (*H. contortus)* is one of the most pathogenic and widely distributed blood sucking abomasal nematode of sheep and goats. Adult *H. contortus* can suck about 0.05 mL of blood/ worm/ day (Rowe et al. 1988). *H. contortus* infections occasionally depress feed intake and utilization, and can impair tissue and skeletal growth (Parkins and Holmes 1989). The customary mode of control of GIN has been based on the use of chemical anthelmintics. However, *H. contortus* drug resistance has become an important issue in small ruminant husbandry, especially when anthelmintics are applied at high levels and increasing frequency and inappropriate doses (Pandey et al. 2001). Thus, environment friendly sustainable novel strategies are required, which could reduce the exclusive reliance on chemical anthelmintic treatments. Use of phytochemicals like CT may offer more efficacious control than chemical anthelmintics to treat *H. contortus* (Chandrawathani et al. 2000; Chaichisemsari et al. 2011; Hassanpour et al. 2011). The CT could complex with nutrients and inhibit nutrient availability for larval growth or decrease GIN metabolism directly through inhibition of oxidative phosphorylation (Scalbert 1991), causing larval death (Athanasiadou et al. 2001). This raises the possibility that feeding locally available plant species containing CT may prove to be a successful and economical alternative method for controlling *H. contortus* infections. Therefore, potential source and optimum level of CT to be used in the diets to reduce *H.*

contortus warrants investigation, which may provide some advantage for reducing *H. contortus* parasites with the strategic use of tanniferous plants. Fortunately, browsing ruminant animals such as goats, deer, and antelopes carry tannin-tolerant bacteria (e.g., *Streptococcus caprinus*; diplococcoid bacterium) and produce a tannin-binding salivary protein during chewing and ruminating to overcome negative impacts on digestibility in the rumen, a mechanism lacking or poorly developed in other ruminant species such as sheep and cattle (McSweeney et al. 2001).

Sericea lespedeza (SL), a forage containing 4–15% CT, has been shown to reduce parasite loads in sheep and goats; however, the mechanism of action is unknown. *Sericea lespedeza* is already well established as a forage for hay and grazing in the U.S., as well as in other parts of the world, such as South Africa. With a sericea leaf meal pellet (SLP) now commercially available, the use of SL as a nutritional/anti-parasitic feed supplement by small and large ruminant producers is likely to continue growing.

Researchers believe that the plant tannins may affect *H. contortus* either directly, in-directly, or both. Tannins may react directly with adult *H. contortus* worms by attaching to the skin, causing them distress, or indirectly by improving protein nutrition of the goat and boosting the immune system. In addition, tannins appear to reduce the hatching of fecal eggs and development of *H. contortus* larvae, perhaps by binding to the larvae (Min et al. 2003). According to Pathak et al. (2013), the tannins could also bind with feed nutrients and possibly prevent bacterial growth in the feces (larva feed on bacteria) and so limit the feed available for larval growth, or in some other way inhibit larvae growth and movement. Molan, Waghorn and McNabb (1999; 2002) showed that CT extracts can disrupt the life cycle of *H. contortus* by inhibition of egg hatching and larval development. Shaik et al. (2006) also reported lower percentage of *H. contortus* larval development in goats fed SL hay compared with Bermuda grass (BG) hay. As animals are fed SL for longer periods of time, research has shown a reduction in *H. contortus* mature worms as well (Min et al. 2003, Shaik et al. 2006, Lange et al. 2006). With SL and its many agronomic advantages, including tolerance of acidic soils and animal grazing tolerance, combined with benefits for animal health (anti-bloat, anti-parasitic, good nutritional quality) and the environment (reduced methane production in livestock) (Min et al. 2012), the future of SL in agriculture and research looks very bright indeed.

In the case of pine bark (PB), it is one of the abundant timber industry by-products and contains up to 16% CT on a DM basis. Pine bark has been studied in goats by Min et al. (2012) and results showed that there was no difference in initial body weight (BW), and total neutral detergent fiber (NDF) intake among treatments; however, final BW, ADG, and total dry matter intake (DMI) increased linearly as the PB supplementation increased in the diets. Min et al. (2012) study also showed average fecal egg count (FEC) was linearly reduced by 52 to 56% with 15 to 30% PB inclusion in the diet, respectively. Therefore, this study aimed to compare the individual effects of SL and PB as well as the combination effects of SL and PB. We hypothesized that not only should we observe an individual tannin effect, but also a combination effect.

The objective of this study was to compare the effects of CT in pine bark powder (PB), *sericea lespedeza* pellets (SLP), and the combination of SLP and PB on animal growth, fecal egg counts (FEC), FAMACHA score, adult *H. contortus* worm numbers, carcass characteristics, and blood serum chemistry in meat goat yearlings.

CHAPTER II

LITERATURE REVIEW

Small Ruminant History

There are approximately 150 species of ruminants, which include both domestic and wild species (Hackman and Spain 2010). Ruminating mammals include cattle, goats, sheep, giraffes, yaks, deer, camels, llamas, and antelope. Wild ruminants number at least 75 million and are native to all continents except Antarctica (Hackman and Spain 2010). Nearly 90% of all species are found in Eurasia and Africa (Hackman and Spain 2010). Ruminant species inhabit a wide range of climates from tropic to arctic and habitats from open plains to forests. The population of domestic ruminants is greater than 3.5 billion, with cattle, sheep, and goats accounting for about 95% of the total population (Hackman and Spain 2010). Goats were domesticated in the Near East around 8000 B.C (Hackman and Spain 2010). Most other species were domesticated by 2500 B.C., either in the Near East or southern Asia (Hackman and Spain 2010). Ruminants are mammals that are able to acquire nutrients from plant-based food by fermenting it in a specialized stomach prior to digestion, principally through bacterial actions. The process typically requires the fermented ingesta, known as cud, to be regurgitated and chewed again. The process of rechewing the cud to further break down plant matter and stimulate digestion is called rumination.

Ruminant production is a very significant component of livestock production throughout the world and more especially in the developing countries (Timon 1985). More specifically, small ruminants such as sheep and goats have adaptive capacities to survive and produce in difficult environments whether it be arid, high altitude or extreme cold. Generally small ruminants are efficient converters of forage feeds whether they are farmed in temperate, arid or semi-tropical conditions. Small ruminants have a great advantage relative to large ruminants in their low cost, small size, their suitability to small-holdings and in many of the developing countries, their triple purpose use for meat, milk and fiber. Noticeable in the world trends in livestock numbers over the past twenty years is the steady increase in sheep and goat numbers. According to FAOSTAT (2005), world goat population was almost 800 million goats, up 165% from 485 million in 1985.

Nutrient Requirements of Meat Goats

Feed is the single largest cost associated with raising small ruminants, typically accounting for 60% or more of total production costs. Small ruminants require energy, protein, vitamins, minerals, fiber, and water. Energy, or calories, is usually the most limiting nutrient, whereas protein is the most expensive. Deficiencies, excesses, and imbalances of vitamins and minerals can limit animal performance and lead to various health problems. Many factors affect the nutritional requirements of small ruminants: maintenance, growth, pregnancy, lactation, fiber production, activity, and environment. As a general rule of thumb, goats will consume 2 to 4 percent of their body weight on a dry matter basis in feed (Schoenian 2009). Fiber is necessary to maintain a healthy digestive environment and prevent digestive upsets. Water is the cheapest feed ingredient, yet often the most neglected. The exact percentage varies according to the size and/or weight of the animal as well as age, with younger animals needing a higher intake to maintain their growth and development (Schoenian 2009).

Pasture, forbs, and browse are usually the primary and most economical source of nutrients for goats. During the early part of the grazing season, browse (woody plants, vines and brush) and forbs (weeds) tend to be higher in protein and energy than ordinary pasture. Goats are natural browsers and have the unique ability to select plants when they are at their most nutritious state (Malechek and Provenza 1983). Legume hays such as alfalfa, clover, and lespedeza tend to be higher in protein, vitamins and minerals, especially calcium, than grass hays. Protein quantity is generally more important than protein quality (amino acid content) in ruminant livestock because the microorganisms in the rumen manufacture their own body protein (Hackman and Spain 2010). Livestock do not store excess protein; it is burned as energy or eliminated as nitrogen by the kidneys. By-product feeds, such as fat, soy hulls, wheat middling, and broiler litter may contain high levels of various nutrients and can be incorporated into small ruminant diets if they are cost effective.

Gastrointestinal Nematodes in Ruminants

Gastrointestinal nematodes (GIN) are widespread and appear in ruminants of all ages (Rossanigo and Gruner 1995). Infections can present a variety of clinical signs, that range from no clinical manifestation at all to diarrhea, anorexia and weight loss leading to impaired growth, decrease in productivity and, in severe cases, death. Subclinical infections represent the majority of cases and cause economical losses due to the suboptimal performance of the animals. As a consequence of GIN infestation and the immune response of the host, several nutritional parameters inside the infected host are malfunctioned, such as food intake and nutrient digestibility, nitrogen retention and protein loss, mineral and energy metabolism and utilization (Rossanigo and Gruner 1995) resulting in decreased performance of animal production and efficiency.

In a study conducted by Rossanigo et al. (1988), a reduction of 15% in feed intake was recorded in GIN infected calves with GIN burdens lower than 300 eggs per gram (epg) compared to non-GIN infected animals and of 18% in animals with more than 300 epg compared to controls. A reduction of food intake is believed to take place with burdens above 150 epg. In another study by Rossanigo, Avila and Sager (1992), a reduction of 8.36% in dry matter intake was observed in GIN infected animals compared to a control group. After 329 days the non-GIN infected animals had gained 26 kg more than the GIN infected ones. The appetite depression can vary between 15-20% according to Coop and Kyriazakis (1999), while other authors state that reductions of up to 30% (Van Hountert, Sykes 1996) or even 50% (Knox, Torres-Acosta and Aguilar-Caballero, 2006) can result.

The immune response that takes place against the infections requires amino acids, therefore they will not be available for other processes if they are used for immune purposes. The availability of amino acids for the body tissues can be reduced up to 30% because of the protein loss from GIN (Coop and Kyriazakis 1999). The hypoproteinemia and nitrogen loss affect the protein deposition in the growing animal, retarding the body development (Rossanigo et al. 1988). There is also abundant evidence concerning the importance of increasing the protein level. The increase in the metabolizable protein in sheep infected with *Ostertagia circumcincta* increased the milk production at the beginning while the reduction in GIN burden took place only at the end (Houdijk et al. 2000; Houdijk et al. 2006). In an experimental work conducted by Gennari et al. (1995), the group of calves that consumed a diet with high protein content showed a lower GIN burden, less clinical signs and less biochemical and hematological alterations than the group of animals maintained with a low protein level.

Haemonchus contortus

Infection with GINs can, and in some circumstances do, cause substantial losses to goat owners. These range from decreased utilization of feed in unthrifty animals to death. Eradication of GINs is impossible, but the simple presence of GINs in an animal does not indicate disease. An animal will show the symptoms of disease only when GIN loads become excessive or when an animal's natural immunity to disease becomes suppressed. A certain amount of understanding about the life cycle of these GINs is necessary to control them most effectively using anthelmintics or other means of parasite control. One of the most important GINs is roundworm *Haemonchus contortus* (*H. contortus*). *H. contortus* is the most damaging GIN for ruminants in tropical and subtropical regions, particularly for sheep and goats. Both the larvae and the adults feed on blood and cause considerable damage to the stomach tissues. While feeding they release anticoagulants to hinder blood clotting. All this causes numerous lesions in the stomach wall, which becomes irritated to cause gastritis (Junquera 2007). Other effects of chronic infections are edema, i.e. accumulation of liquid in the abdomen, thorax, and also in the submandibular tissue, which is known as "bottle jaw" and is characteristic of infections with *H. contortus* and other GINs (Junquera 2007). Severe infections can also cause liver damage, weight loss, unthriftiness, diarrhea, and dehydration. Fatalities are not infrequent and massive infection can be fatal in a few days without previous symptoms and without previous shedding of eggs in the feces. *H. contortus* resistance to anthelmintics is the number one problem with goat producers worldwide (Gilleard

2006). Resistance has been reported against almost all anthelmintics available for its control including benzimidazoles, macrocyclic lactones, levamisole, salicylanilides, tetrahydropyrimidines and organophosphates. Resistance to several chemical classes has also been reported in some countries such as Australia, Brazil, and USA. One striking feature of *H. contortus*, in common with related GIN species, is the extremely high level of genetic diversity that has been reported in both laboratory and field populations; this is thought to be predominantly a function of its large effective population size (Blouin et al. 1995). Genetic variation may lie both *H. contortus* remarkable ability to adapt to different climatic regions and host species (Brasil et al. 2012) and its alarming ability to develop drug resistance.

Current Methods to Control *H. contortus*

Anthelmintics such as closantel, nitroxinil and tetrahydropyrimidines (e.g. morantel, pyrantel) are effective against adult *H. contortus* but may not control larvae and other roundworm species that often infect livestock simultaneously with *H. contortus* (Junquera 2014). A few organophosphates such as trichlorfon are also available in some countries for the control of *H. contortus*, but must be used with utmost care because it has a very low safety margin. Numerous commercial products contain mixtures of two or even more active ingredients of different chemical classes. This is done to increase the chance that at least one active ingredient is effective against *H. contortus* that have become resistant, or to delay resistance development by those worms that are still susceptible (Junquera 2014). Depending on the country most of these anthelmintics are available for oral administration as drenches, feed additives and/or tablets. A few active ingredients are also available for livestock as pour-ons and slow-release boluses. Except slow-release boluses, most dewormers containing benzimidazoles (e.g. albendazole, febantel, fenbendazole, oxfendazole, etc.), levamisole, tetrapyrimidines (e.g. morantel, pyrantel) and other classic anthelmintics kill *H. contortus* shortly after treatment and are quickly metabolized and/or excreted within a few hours or days (Junquera 2014). This means that they have a short residual effect, or no residual effect at all. As a consequence treated animals are cured from worms but do not remain protected against new infections.

To ensure that they remain *H. contortus*-free or with low GIN numbers, the animals have to be dewormed periodically, depending on the local epidemiological, ecological and climatic conditions (Junquera 2014). An exception to this are macrocyclic lactones (e.g. abamectin, doramectin, eprinomectin, ivermectin, moxidectin), that offer several weeks protection against re-infestation, depending on the delivery form and the specific parasite. However, resistance to many of these lactones is a serious problem in many regions, particularly in sheep and goats, and the problem is increasing and spreading worldwide (Junquera 2014).

Natural Alternative Methods to Control *H. contortus*

Development of new drug classes for use in small ruminants is not likely in the foreseeable future because of the high costs associated with drug development and the relatively small market for these drugs for use with sheep and goats (Geary, Sangster and Thompson 1999). Use of anthelmintic drugs alone to control *H. contortus* infection in sheep and goats is not a sustainable strategy, and this approach has led to the current epidemic of anthelmintic resistance. A more sensible and sustainable approach is to take steps to preserve whatever efficacy is left in currently available drugs while incorporating effective non-chemical control technologies (SARE 2014).

The FAMACHA system of anemia detection for use in goats is now a useful tool for reducing overall use of anthelmintics by producers, while also preserving existing anthelmintic efficacy. With the FAMACHA system, the color of the lower eyelid is compared to colors on a laminated chart from red (healthy) to white (severely anemic) to determine animals most in need of treatment. Because approximately 20% of the animals will harbor 80% of the GINs (Terrill et al. 1989), identifying and treating only that 20% greatly saves on anthelmintic costs and also allows culling of the most susceptible GIN carriers, improving the genetics for GIN resistance in the herd.

Two non-chemical control methodologies that have been researched are the use of copper oxide wire particles (COWP) and forages high in condensed tannins (CT), particularly *Sericea lespedeza* (SL; *Lespedeza cuneata*). The COWP are given to goats as a bolus, whereas CT forages can be grazed or fed as hay in unground, ground, or pelleted form. In the latter case, each of these forms of SL has been shown to be effective against *H. contortus* in goats. Development of environmentally-safe biological agents for control of GIN that can be incorporated into sustainable, forage-based feeding systems and could greatly impact production systems for small ruminants throughout the world is the number one goal for researchers everywhere.

Apart from the obvious role of plants in herbivore nutrition, they are also a rich source of bioactive products that can operate either to the benefit or the detriment of grazing animals. In particular, tannin-rich plants have attracted most attention for their effect on *H. contortus* in ruminants (Junquera 2014). It is speculated that these plants could act through direct anti-parasitic activity but might also act indirectly by increasing host resistance (Junquera 2014). Assumingly, the effects may vary with the species of plant, GIN and host. More research is required to understand better the mechanisms of action, and therefore make more pertinent use of these bioactive plants in livestock systems (Hoste et al. 2006).

Tannins are a plant secondary compound of high molecular weight phenolic compounds with the capacity to form reversible and irreversible complexes with proteins and other nutrients (McLeod 1974). Tannins are mainly classified chemically into two groups: hydrolyzable and condensed. Hydrolyzable tannins (HT) can be further metabolized to toxic compounds, which are potentially toxic to ruminants (Dollahite, Shaver and Camp 1962). Condensed tannins (CT), which are less toxic to ruminants, are the most common type of tannin and are found in forage legumes, trees, and shrubs (Barry and McNabb 1999). CT in birdsfoot trefoil (*Lotus corniculatus*) have been reported to bind to protein in the rumen, reducing protein solubility and increasing the flow of essential amino acids to the small intestines, increasing their availability for growth (Wang et al. 1996). CT may enhance resistance of *H. contortus* infection through increases in protein supply, which are prioritized for tissue repair and immune response (Niezen et al. 2002). The CT could complex with nutrients and inhibit nutrient availability for larval growth or decrease *H. contortus* metabolism directly through inhibition of oxidative phosphorylation (Scalbert 1991), causing larval death (Athanasiadou et al. 2001).

Sericea lespedeza is a widely adapted, non-bloating warm season perennial legume that can be used for grazing, hay, or as a conservation plant. It is a deep-rooted plant that although it does best on deep, well-drained upland soils, it can be grown on a wide range of soil types and sites (Lange et al. 2006). It is particularly well-adapted to acid, infertile soils commonly found in the southeast of the USA.

The level of *H. contortus* reduction that has been reported for SL compared with non-tannin diets, up to 98% for fecal egg counts (FEC) (Lange et al. 2006), and 94% for adult *H. contortus* (Min et al. 2003), is often 2-3 times higher than that reported for other anti-parasitic plants. The reason for this is not clear, but may possibly be due to interacting directly or indirectly with *H. contortus* in the abomasum. Evidence of this was recently provided by scanning electron micrographs of female *H. contortus* recovered from the abomasum of goats fed pelleted diets containing SL leaf meal or non-SL commercial pellets. The *H. contortus* from the goats fed SL had a shrunken, shriveled appearance, while the control *H. contortus* looked smooth (Kommuru et al. 2012).

The anti-parasitic properties of SL were first documented in grazing trials with goats in Oklahoma (Min et al. 2003; 2004) and with hay-feeding trials with goats in Georgia (Shaik et al. 2004; 2006) and sheep in Louisiana (Lange et al. 2006). In studies with sheep (Lange et al. 2006) and goats (Shaik et al. 2004 2006) lower FEC and *H. contortus* burdens were reported when feeding SL compared with bermudagrass (BG; *Cynodon dactylon* (L.) Pers.). Shaik et al. (2006) reported an 80% drop in FEC in SL-fed goats relative to BG-fed goats 1 week after initial hay feeding. Negative effects observed included reduced intake and reduced digestibility, leading to a decline in animal productivity. According to Min et al. (2003), low concentrations of CT (20-45 g CT/kg dry matter (DM)) are helpful to animals, while high forage CT concentrations (>55 g CT/kg DM) may have negative effects. However, results vary according to CT concentration and structure and the animal that is grazing the forage, Min et al. (2003) showed that *H. contortus* were controlled when Angora does were grazed (81 d) on SL (52 g of CT/kg of DM) in spring and summer, but not when goats were grazed on control forages (crabgrass/tall fescue; 2.0 g of CT/kg of DM). Tracer goats that grazed SL had a 76% reduction in total *H. contortus* burdens compared with the control. The SL diet was also associated with a reduction in the numbers of *H. contortus* (94%) and *Teladorsagia* spp. (100%) in the abomasum and *Trichostrogylus* (45%) in the small intestine. The effects of CT in SL (46 g of CT/kg of DM) on FEC in goats have also been studied using a crossover design with ryegrass/crabgrass (0.6 g of CT/kg of DM) (Min et al. 2002). Goats that consumed SL had significantly lower FEC (1,162 eggs/g) than goats that grazed on non CT-containing control forage (2,722 eggs/g). This research (Min et al. 2002) showed a 57% reduction in FEC and a 61% reduction in total fecal egg output in goats that consumed forage SL (66 × 104 eggs/d) compared with control forage (168 × 104 eggs/d).

Pine bark (PB; *Pinus taeda L.*) is one of the abundant timber industry by-products that contains up to 16% CT on a DM basis (Min et al. 2012) and is also under current investigation for use in controlling *H. contortus*. Research demonstrates that goats fed on a 30% PB-containing diet have up to 30 percent fewer *H. contortus* in a total mixed ration (TMR), as well as lower instances of FEC and fecal coccidian oocyst count. Feeding PB diet (30% PB powder mixed with TMR diet) reduced both male (64%) and female (59%) *H. contortus* worm counts compared with the control (without PB) diet. In a study conducted by Min et. al. (2012), experimental diets provided a total of 1.9, 16.3, and 32 g CT/kg DM of freshly ground and air-dried PB. The published data highlighted that PB has the potential to increase average daily gain (ADG), body weight (BW) gain efficiency, and carcass traits, with no adverse effects on animal health. The data also showed improved animal performance by altering rumen fermentation (volatile fatty acid and ammonia production) as well as reducing *H. contortus* infection. It has been suggested by Min et al. (2012) that PB may have affected rumen fermentation by altering rumen microorganisms, and therefore warrants further investigation for its effects on rumen microbial composition as well as its anti-parasitic properties.

Secondary Plant Compounds

Tannins are a complex group of polyphenolic compounds found in a wide range of plant species commonly consumed by ruminants. Although for a long time tannins were thought to be detrimental to ruminants, their effect have been found to be either beneficial or harmful depending on the type of tannin consumed, its chemical structure and molecular weight, the amount ingested, and the animal species involved (Frutos et al. 2004). High concentrations of tannins reduce voluntary feed intake and nutrient digestibility, whereas low to moderate concentrations may improve the digestive utilization of feed mainly due to a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine (Frutos et al. 2004). In general, tannins are more abundant in the parts of the plant that are most valuable to it such as new leaves and flowers which are more likely to be eaten by herbivores (Terril et al. 1992). Typically, grasses do not contain much tannin, although sorghum (*Sorghum bicolor*) has significant tannin content. Tannins are often found in higher concentrations in broadleaf plants adapted to warm climates. Tannins bind salivary proteins and produce an astringent or pucker sensation when ingested. Two general traits of tannins relevant to grazing ruminants are the prevention of bloat (Lees 1992) and the suppression of GINs

(Hoste et al. 2006). The suppression of *H. contortus* by tannins has been documented for sainfoin and birdsfoot trefoil, and for purified tannins from woody plant species used as dietary supplements such as quebracho and chestnut. The effect of tannins on GINs depends on the tannin concentration and chemical structure as well as the species of GIN. The effectiveness of tannins also differs by the stage of growth of the GIN, and the location in the gastrointestinal tract where the tannin is active. In tannin-containing diets, proteins that become bound to tannins leave the rumen without being digested. In these cases the protein digestion is limited and they release these proteins in the abomasum in response to low pH. This allows the protein to be digested and absorbed in the small intestine (Waghorn et al. 1987) and results in high productivity in both sheep (Douglas et al. 1995) and cattle (Wen et al. 2002). In Utah, season- long average daily gains of 2.87 to 3.35 lbs. per day have been achieved on birdsfoot trefoil pastures (MacAdam et al. 2011).

The high affinity of tannins for proteins lies in their great number of phenolic groups which provide many points at which bonding may occur with the carbonyl groups of peptides (McLeod 1974; Hagerman and Butler 1991). The formation of such complexes is specific, both in terms of the tannin and protein involved, and the degree of affinity between the participating molecules residing in the chemical characteristics of each (Zucker 1983; Mangan 1988). With respect to tannins, the factors promoting the formation of complexes include their relatively high molecular weight and their great structural flexibility (Mueller-Harvey and McAllan 1992). The proteins that show the most affinity for tannins are relatively large and hydrophobic with an open, flexible structure and are rich in proline (Kumar and Singh 1984). The bonds uniting tannins and proteins continually break and re-form. Kumar and Singh (1984) suggested that complexes could come about through four types of bonds: 1) hydrogen bonds (reversible and dependent on pH) between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups in the peptide bonds of proteins, 2) by hydrophobic interactions (reversible and dependent of pH) between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein, 3) by ionic bonds (reversible) between the phenolate ion and the cationic site of the protein (exclusive to hydrolysable tannin), and 4) by covalent bonding (irreversible) through the oxidation of polyphenols to quinones and their subsequent condensation with nucleophilic groups of the protein. For a long time it was believed that the formation of tannin-protein complexes was owed mainly to hydrogen bonds. However, it is now known that hydrophobic interactions are important.

Structure of Plant Tannins

On the basis of their structural characteristics, tannins are mainly classified into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins.

(1) Gallotannins are all those tannins in which galloyl units or their metadepsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.

(2) Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.

(3) Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin. One of

the striking properties of the monomeric catechins is that they have no tanning properties and their ability to be converted into oligomers and polymers that do have tanning properties, by the action of acids or enzymes (Haslam 1989).

Non-hydrolysable oligomeric and polymeric proanthocyanidins were classified as CT (Haslam 1989). Therefore, the term HT includes both the gallotannins and the ellagitannins (Haslam 1989). It should also be mentioned here that there are ellagitannins that are not hydrolysable, because of a further C–C coupling of their polyphenolic residue with the polyol unit, but are nevertheless for historical reasons classified as HT. In 1985 the first tannins that were described contained in addition to the hexahydroxydiphenoyl (HHDP) units (the characteristic structural element of the monomeric ellagitannins), also contained C-glycosidic catechin units.

These tannins were originally classified as ‘non-classified tannins’, because they are only partially hydrolysable due to the C–C coupling of their catechin unit with the glycosidic part. To properly place these ‘non-classified tannins’ in some scheme, the terms ‘complex tannins’ and flavanoellagitannins were established over the following years. These examples clearly show that the division of the tannins into two groups, HT and non-hydrolysable or CT, cannot do justice to the structural diversity of the tannins. The terms ‘flavanotannins’ or ‘condensed flavanoid tanning substances’ that are occasionally found in the literature denote tannins consisting of catechin units. The polymeric flavanotannins, constructed from coupled flavan-3-ol (catechin) units, belong to the CT group (oligomeric and polymeric proanthocyanidins). Oligomers and polymers consisting of two to ten catechin units are also known as flavolans.

The coupling pattern of the catechin units in CT can vary considerably. For example, many CT are known where the coupling of the single units is by way of position C-4 of the first unit linked with C-8 (or C-6) of the second unit, which may have a different substitution pattern (Ferreira and Bekker 1996). The tannins found in red wine (and to a lesser extent in white wine) are this type of CT. The properties of these tannins depend on their specific reaction with proteins, which in turn is directly related to their degree of polymerization.

Tannin Mode of Action

Two main, non-exclusive, hypotheses (direct vs. indirect) have been evoked to explain the effect of tannin rich forages against *H. contortus* in ruminants. (i.) Tannins might directly affect the biology of *H. contortus* by attaching to the outer skin and consequently causing dysfunction and death. (ii.) Alternatively, tannins may act indirectly through the improvement of the host immune system response against the worms. Because of tannin-protein binding ability, tannins protect proteins from ruminal degradations. It is known that any increase in the metabolizable proteins favors the two components of the host immune response of resistance and resilience to *H. contortus* (Coop and Kyriazakis 2001). The direct hypothesis has been supported by repeated and consistent results acquired through in-vitro assays and experimental in-vivo studies (Hoste et al. 2006). In contrast, only a few studies have addressed the indirect hypothesis, by comparing the local cellular changes related to host immunity in the digestive mucosae of animals receiving or not receiving tannin rich forages (Hoste et al. 2006). Overall, the results of the few studies performed in either sheep or goats did not show main and consistent changes in the number of mucosal mast cells, eosinophils or globule leucocytes. They remain largely inconclusive (Paolini et al. 2003).

Based on the hypothesis that tannins might affect *H. contortus* directly, some recent insights have been obtained on the interactions between these polyphenolic compounds and the infective larvae, using sainfoin as a model of tannin rich forage. Both in-vitro and in-vivo data indicate that the presence of tannins disturb the two early steps of *H. contortus* establishment, first the larval exsheathment (Brunet et al. 2007) and second, the penetration of the exsheated larvae within the digestive mucosae (Brunet et al. 2007).

These functional modifications have been associated with major changes in the larval ultrastructure. Similarly, observations in Transmission and Scanning electron microscopy on adult *H. contortus*, after in-vitro and/or in-vivo contact with tannin rich plants have shown major modifications to the cuticle, the digestive tract and the female reproductive tract which can explain the negative consequences on *H. contortus* populations, and particularly affecting the egg excretion. However, the exact mechanisms of action remain obscure and could differ depending on the parasite, its stage of development and possibly the biochemical characteristics of forage species. Despite the recent progresses, further studies remain necessary to better understand how tannins and other flavonoids affect the different stages of *H. contortus*.

From the in-vivo results, the consumption of tannin rich forages by sheep or goats has been associated with a modulation of *H. contortus* biology explained by impacts on different key stages of the life cycle.

Metabolic Fate of Tannins

Although it has been suggested that CT may increase intestinal digestibility (McSweeney, Kennedy and John 1988), Driedger and Hatfield (1972) report that tannins exert a negative effect on nutrient absorption from the small intestine which could be due to the persistence in the intestine of tannin-protein complexes which failed to dissociate in the abomasum, to the formation of tannin-digestive enzyme complexes or new tannin-dietary protein complexes, or to changes in intestinal absorption due to the interaction of tannins with intestinal mucosa. Though tannin-protein complexes dissociate at pH < 3.5 (the pH of the abomasum), McNabb et al. (1998) indicate that the pH at the beginning of the intestine (≈5.5) might allow tannin-protein complexes to reform, and therefore impede digestion. Kumar and Singh (1984) suggest that tannins might also be able to inhibit the digestive enzymes because of their ability to bind to them to form insoluble complexes (or soluble but inactive complexes). Silanikove, Nitsan and Perevolotsky (1994) mention the inhibition of the activity of some digestive enzymes (trypsin and amylase) because of CT. Mehansho (1987) uphold that tannins have the opportunity to form complexes with a wide variety of dietary proteins long before coming into contact with the digestive enzymes.

After their dissociation from proteins in the abomasum, the tannins might once again bind to dietary proteins in the intestine (Mole, Waterman 1987; Blytt, Guscar and Butler 1988). Changes in the permeability of the intestinal wall caused by the reaction between tannins and the membrane proteins of the intestinal mucosal cells, and the resulting reduction in intestinal absorption, may also lie behind reduced intestinal digestibility (McLeod 1974; Silanikove, Perevolotsky and Provenza 2001). However, it is important to bear in mind that the majority of studies affirming that tannins negatively affect intestinal digestibility have been performed in-vitro. Blytt, Guscar and Butler (1988) indicated that these tests do not take into account factors such as the presence of bile salts (Blytt, Guscar and Butler 1988), which could act as detergents and prevent the binding of tannins to digestive enzymes. Ruminants can benefit from dietary CT when the increases in protein flow from the rumen exceed the reduction in the absorption of amino acids from the intestine (Waghorn et al. 1994). Numerous articles exist on the ability of tannins to reduce the digestibility of the diet because tannins mainly exert this effect on proteins, but they also affect other feed components to different degrees (Kumar and Singh 1984). Evidently, the modifications of the digestibility caused by tannin ingestion are mainly associated with changes in the ruminal fermentation pattern, along with changes in intestinal digestibility. The reduction of ruminal protein degradation may be the most significant and well-known effect of tannins (McLeod 1974; Hagerman et al. 1992). The affinity of tannins for proteins is high, and the pH of the rumen medium favors the formation of tannin-protein complexes. In general, this reduction in protein degradation is associated with a lower production of ammonia nitrogen and a greater non-ammonia nitrogen flow to the duodenum (Waghorn et al. 1994). Though tannins mainly exert their effects on proteins, they also have effects on carbohydrates, particularly hemicellulose, cellulose, starch and pectins (Schofield, Mbugua and Pell 2001). For a long time, the effect of tannins on the degradation of fiber was seen as a secondary anti-nutritional effect. However, several studies have shown that fiber degradation in the rumen can be drastically reduced in animals that consume tannin-rich feeds (McSweeney et al. 2001).

The mechanisms by which tannins reduce ruminal degradation of different dietary components are not clear. Tannins may prevent or at least interfere with the attachment of rumen microorganisms to plant cell walls, and it is well known that such attachment is essential for degradation to occur (Chiquette et al. 1988; McAllister et al. 1994). Further, the formation of complexes with proteins and carbohydrates renders these nutrients inaccessible to microorganisms (Mangan 1988; Mueller-Harvey and McAllan 1992). Tannins are also chelating agents, and this could reduce the availability of certain metallic ions necessary for the metabolism of rumen microorganisms (Scalbert 1991).

With respect to enzyme inhibition, tannins can react with microbial (both bacterial and fungal) enzymes, inhibiting their activity (Makkar, Singh and Dawra 1988; McSweeney et al. 2001). Several authors (Leinmüller et al. 1991; O'Donovan et al. 2001) indicate that tannins alter the activity of proteolytic, cellulolytic and other enzyme activities, but it is important to point out that the binding of tannins to enzymes whether bacterial or endogenous does not necessarily imply their inhibition (Makkar et al. 1988). With respect to fibrolytic enzymes, CT more easily inhibits the activity of hemicellulases than cellulases (Waghorn 1996). This is possibly due to the fact that the latter are associated with bacterial cell walls while the hemicellulases are extracellular and therefore more sensitive (Van Soest 1994). This would explain why the majority of researchers report a greater reduction in the degradability of hemicellulose in the presence of tannins (Barry et al. 1984; Waghorn et al. 1994). However, this can vary depending on the tannin in question (McAllister et al. 1994).

Finally, tannins might have a direct effect on rumen microorganisms, such as altering the permeability of their membranes (Leinmüller, Steingass and Menke 1991; Scalbert 1991). Nonetheless, some rumen microorganisms can tolerate tannins (Nelson et al. 1998; O'Donovan and Brooker 2001). The degree of tolerance is specific to the microorganism in question, explaining the different susceptibility of bacterial strains. It also depends on the tannin, and the differences between HT and CT in this respect are notorious. Though few tolerant rumen microorganisms have been described, it is very likely that their true diversity is much greater than currently known (McSweeney et al. 2001). Several species of the ruminal microbiota respond to the presence of tannins by changing their morphology (McAllister et al. 1994). Chiquette et al. (1988) observed a thick glycocalyx on rumen bacterial walls in response to high levels of CT from *Lotus corniculatus*, which did not occur when the concentration of the same compounds was lower. This phenomenon is similar to the secretion of glycoproteins in the saliva (Scalbert 1991) for neutralizing the action of tannins. With respect to the inhibition of enzyme activity, apart from different sensitivities at different concentrations (Jones et al. 1994), O'Donovan and Brooker (2001) indicate that proteolytic bacteria, which are initially sensitive to tannins, can, after a short period of adaptation, respond by modifying their metabolism. This is only one example of how rumen bacteria with proteolytic and cellulolytic activity can continue to function when tannin levels are not too high (Jones et al. 1994).

CHAPTER III

MATERIALS AND METHODS

<u>Experimental Design and Protocol</u>

Twenty-four intact Kiko-crossbred male goat yearlings (*Capra hircus;* BW = 38.6 ± 2.7 kg) were randomly assigned to one of 4 experimental treatment groups (n = 6). Goats were individually housed indoors in pens of approximately 1.2 m^2 (Figure 1) with slotted floors and self-serve water-spout to determine the effects of feeding finely ground pine bark (PB; *Pinus taeda L.*), *sericea lespedeza* pellets (SLP; *Lespedeza cuneata*), and a mixture of SLP and PB on drug-resistant *Haemonchus contortus* (*H. contortus*). The fresh PB mix was donated by a wood-processing company (West Fraser Timber Co. Ltd., Opelika, Alabama) and air-dried under an outdoor shed before processing. Freshly dried PB was ground (Hammer Mill Model 1250; Lorenz MFG Co., Benson, Minnesota) to approximately 3 mm particle.

Figure 1. Experimental animals housed in the Tuskegee University Caprine Research and Education Unit.

Experimental treatments included the control diet [30% bermudagrass hay; *Cynodon dactylon* (BG) and 70% commercial sweet feed and alfalfa pellets], mixed diet [15% PB plus 15% SLP with 70% commercial sweet feed and alfalfa pellets], PB diet

[30% PB plus 70% commercial sweet feed and alfalfa pellets], and SLP diet [30% SLP plus 70% commercial sweet feed and alfalfa pellets]. Each of the diet ingredients were individually weighed out and mixed together by hand (Figure 2). The percentage of CT in the total mixed ration of PB plus grain mix was 4.88%, SLP plus grain mix was 4.05%, control was 0.44%, and the mixed diet contained 4.51%, respectively.

Figure 2. Each of the diet ingredients for each treatment was individually weighed out on a scale.

Animals were fed once a day and monitored for a 42-day performance period. An adjustment period of 6 weeks allowed goats to be dewormed, inoculated, acclimated to pen living and routine feeding, and to allot time for proper diet adjustment prior to study initiation. In week 1, all animals were fed a diet without CT (control diet) and dewormed with albendazole plus fenbendazole (4 mL each/100 kg BW). Starting on week 2, the animals were inoculated with 5000 3rd stage drug resistant *H. contortus* larvae, kindly donated by Dr. Kepline at the University of Georgia. From weeks 3 to 6, the animals were continually fed the control diet to allow for the maturation and acclimation of abomasal larvae.

Sample Collection and Laboratory Analysis

Once the experiment began, samplings of fecal and weights were collected every 2 weeks on day 0, 14, 28, and 42. Blood samples and FAMACHA status were collected on day 0, 21, and 42. Feed samples were composited for each treatment and analyzed for DM, crude protein (CP), CT in the completely mixed ration, and total digestible nutrients (TDN) according to the methods described by Dairy One Inc. in the Forage Testing Laboratory. The fecal egg counts (FEC) were determined using a modified McMaster technique using a McMaster slide with two chambers (McMaster Counting Slides – Green Grid by Chalex Corporation). Two grams of feces were broken up in 28 mL of fecasol solution using a mesh filter and metal spoon. The sample solution was then thoroughly mixed immediately after filtering. A sample of the solution was extracted using a pipette and placed into one chamber of the McMaster slide. This was repeated to fill the other chamber. The number of eggs was counted using a compound microscope to look through both chambers on the slide. Lastly, the final number of eggs was multiplied by 50 to estimate the total number of eggs in the fecal sample. This was repeated every two weeks for all twenty-four goats.

Carcass Characteristics

Goats were transported to Fort Valley State University Meat Science Laboratory and kept over-night. On the next morning before slaughter, goats were weighed after twenty-four hours without feed. Fasting weight loss was measured by weighing animals before and after transport. Hot carcass weight was determined on the day of slaughter

(Figure 3). After the animals were slaughtered, the abomasum of each goat was sent to Louisiana State University for an adult male and female worm count.

Figure 3. Slaughter day at Fort Valley State University Meat Science Laboratory.

<u>Data Processing and Statistical Analysis</u>

Data was analyzed using Fishers protected lsd which provided and separated mean differences. Significant effects of the treatment were declared *P* > 0.05, and trends were accepted if 0.05 < *P* < 0.10. The FEC was log transformed prior to analysis.

CHAPTER IV

RESULTS

Feed Analysis

The diet characteristics are shown in Table 1. The CT analysis of 100% PB and SLP without addition into the diets was 16.1% ± 0.5 for PB and 13.5% ± 0.3 for SLP. Therefore, final CT concentration in the PB diet contained the highest level of CT at 4.88%, compared to the mixed diet at 4.5% and the SLP diet of 4.0%. The SLP diet had the highest level of crude protein at 14.5%, compared to the mixed diet with 13.2% and the PB diet with 11.2%.

Table 1. Means and standard errors of selected diet characteristics by treatment group.

Item	Diet[1]			
	Control	PB	SLP	Mixed
Diet characteristics,	------------------	(%)	------------------	------------------
Dry matter	90.3 ± 0.5	90.2 ± 0.15	90.3 ± 0.15	89.8 ± 0.7
Crude Protein	13.3 ± 0.35	11.2 ± 0.15	14.5 ± 0.2	13.2 ± 0.1
TDN	59.0 ± 1	51.5 ± 0.5	56.0 ± 1	53.5 ± 0.5
Condensed tannin	0.4 ± 0.055	4.88 ± 0.1	4.0 ± 0.045	4.5 ± 0.045

[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis. Except Bermuda grass hay, all ingredients were incorporated in the grain mixes.

Growth Performance

Growth performance data are summarized in Table 2. The weights of the goats at the beginning of the trial differed due to the goats being randomly assigned to each treatment so initial weight was used as a covariate with final BW and ADG. The data indicated that CT containing diets have no significant effects on final BW (P = 0.16) and ADG (P = 0.19).

Table 2. Effects of condensed tannin supplementation on growth performance of Kiko crossbred male goat yearlings.

	Diet[1]					P-value
Item	Control	PB	SLP	Mixed	SEM	
Initial BW, kg	39.0	33.8	36.9	44.7	2.73	0.06
Final BW, kg	45.2	36.5	41.8	47.9	3.38	0.12
co-variated Final BW, kg	44.9	42.2	43.9	41.8	1.02	0.16
ADG, g/d	148.0	65.1	81.8	78.6	26.54	0.15
co-variated ADG, g/d	147.4	75.5	85.7	83.5	24.95	0.19

[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis
Initial BW was co-variated for final BW and ADG

Fecal Egg Counts

FEC data is presented in Table 3 and Figure 4. Results indicate that there was no significance among the tannin treatments (P = 0.64) up to day 28. However, FEC in the mix diet significantly reduced by day 42 (P = 0.05) compared to other treatment groups. There was significance shown with day (P = 0.00), but no significance with treatment and treatment per day (P = 0.08).

Table 3. Effects of condensed tannin on log transformed fecal egg count (FEC) in Kiko crossbred male goat yearlings.

	Diet[1]			
	Control	PB	SLP	Mix
Day 0	6.4^{a}	6.6^{a}	7.3^{a}	6.8^{a}
Day 14	5.8^{a}	5.9^{a}	6.2^{a}	5.5^{a}
Day 28	6.3^{a}	5.9^{a}	6.1^{a}	6.2^{a}
Day 42	7.0^{a}	5.9^{ab}	4.8^{bc}	4.2^{c}
Diet	6.4^{a}	6.1^{a}	6.1^{a}	5.7^{a}

[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis
[abc]Means with different superscripts within a row differ significantly

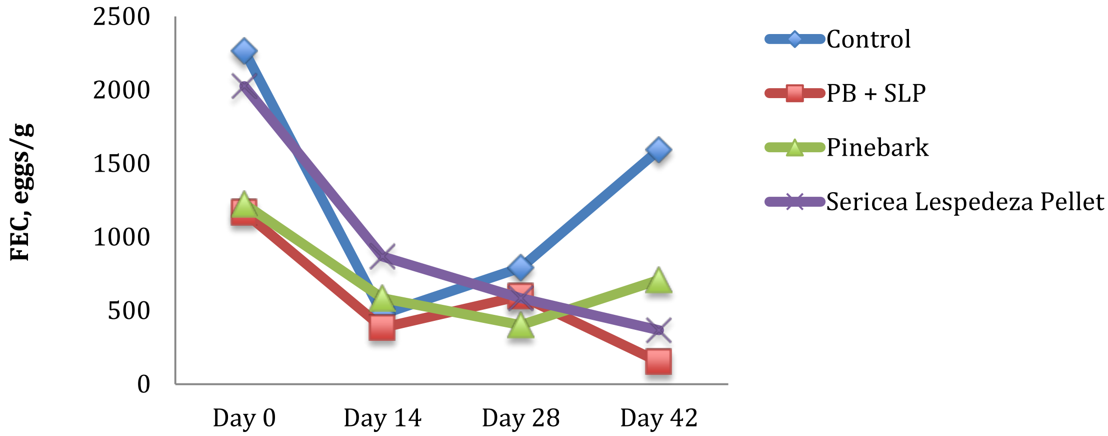

Figure 4. The effects of pine bark (PB), sericea lespedeza pellets (SLP), and a combination of SLP and PB on fecal egg counts.

Adult Worm Count

Female and male worm count data is shown in Table 4. In all of the treatments, the mixed diet showed the lowest numbers of total worm counts ($P = 0.02$) compared to all other treatments. Amongst the female and male worms, mixed diet has lower ($P = 0.01$) adult worm count than other treatments ($P < 0.01 – 0.03$).

Table 4. Effects of condensed tannin on total adult male and female *H. contortus* worms.

	Diets[1]					P-values
Item	Control	PB	SLP	Mixed	SEM	
Female Worms	15.75[a]	18.20[a]	5.17[a]	1.58[b]	4.45	0.03
Male Worms	16.25[a]	12.80[a]	6.00[a]	2.33[b]	3.86	0.01
Total Worms	31.92[a]	31.00[a]	11.17[a]	3.67[b]	8.19	0.02

[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis

Carcass Characteristics

Carcass characteristics are presented in Table 5. Carcass measurements taken after day 42 indicate no significant differences in fasting weight (*P* = 0.24), hot carcass weight (*P* = 0.35), or dressing percentage (*P* = 0.83).

Table 5. The effects of condensed tannin on carcass characteristics.

	Diet[1]					P-value
Item	Control	PB	SLP	Mix	SEM	
Fasting wt, kg	41.0	33.3	37.0	37.3	2.44	0.24
Hot carcass wt, kg	17.4	13.6	15.9	16.2	1.40	0.35
Dressing %, kg	42.4	40.8	42.7	42.9	1.70	0.83

[abc]Means with different superscripts within a row differ significantly
[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis

FAMACHA

FAMACHA data is presented in Table 6. Significance was shown on day 21 (*P* = 0.05) with the lowest in the PB diet. There was also significance shown on day 42 (*P* = 0.05) with the lowest in both the SLP and mix diet.

Table 6. The effects of condensed tannin on FAMACHA.

	Diet[1]					P-value
	Control	PB	SLP	Mix	SEM	
Day 0	3.2^{a}	3.7^{a}	3.6^{a}	3.4^{a}	3.46	0.35
Day 21	3.5^{a}	2.7^{b}	3.7^{a}	3.2^{ab}	3.26	0.05
Day 42	3.8^{a}	2.0^{b}	1.8^{b}	1.8^{b}	2.36	0.05

[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis
[abc]Means with different superscripts within a row differ significantly

Blood Parameters

Blood serum data are shown in Table 7. In the blood serum chemistry, cholesterol, alanine transaminase, and triglyceride contents were lower ($P < 0.02 – 0.04$) for PB diet than for other diets. However, aspartate aminotransferase, sodium, and chlorine concentrations were higher for PB diet than for other treatment diets.

Table 7. The effects of condensed tannin on blood serum chemistry.

	Diet[1]					P-value
Item	Control	PB	SLP	Mix	SEM	
Alkaline phosphatase	198.2[a]	285.2[a]	205.3[a]	463.3[a]	288.00	0.15
Ace carbon dioxide reagent	20.5[a]	18.0[a]	19.9[a]	20.4[a]	19.70	0.10
Aspartate aminotransferase	70.0[a]	72.0[a]	62.3[a]	57.6[a]	65.47	0.08
Triglycerides	26.7[a]	19.0[b]	25.3[a]	21.8[ab]	23.20	0.04
Sodium	144.3[ab]	146.9[a]	144.3[ab]	141.4[b]	144.22	0.03
Cholesterol, mg/dL	71.0[a]	45.0[b]	64.3[a]	55.2[ab]	58.87	0.02
Alanine transaminase	12.3[a]	3.5[b]	8.0[ab]	8.0[ab]	7.95	0.02
Chlorine	108.6[b]	112.9[a]	109.0[b]	106.2[b]	109.17	0.00

[abc]Means with different superscripts within a row differ significantly

[1]Control 0%, 30% pine bark (PB), 30% sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis

CHAPTER V

DISCUSSION

The most significant results in this studyed show that FAMACHA, adult worm count, and FEC were reduced indicating that CT containing diets may have the potential to control drug-resistant *H. contortus* in meat goats. Based on the data in FEC, tannins seem to have a favorable outcome over a span of time, with the current study showing a maximum decrease on day 42. These results fall in line with previously conducted studies of goats grazing on high- CT-containing forage SL, 15.5% to 22.4% CT/ DM, (Min et al. 2005; Shaik et al. 2006) had decreased FEC. The ability of CT to bind to proteins and change their physical and chemical properties (Min et al. 2003) has to be considered, especially because *H. contortus* cuticle is known to be a proline and hydroxyproline-rich structure (Thompson and Geary 1995). This ability could explain the cuticular changes observed on *H. contortus* by scanning electron microscopy after contact with CT (Hoste et al. 2006). Previous studies have shown that massive lesions have been found in the digestive and reproductive tracts of worms after contact with ellagic tannins (Mori et al. 2000). With the FAMACHA data being shown to be favorable towards CT containing diets, it can be suggested that immune functions are enhanced.

As for the adult worm counts, the mixed diet had the lowest worm numbers for total, male, and female worm counts. This study clearly showed that diets mixed with two different types of CT, such as PB and SLP, had significant combination effects on adult *H. contortus* control, indicating that the actions of CT can increase when mixed with two types of CT. Shaik et al. (2006) reported that there was a direct effect of tannin-containing *sericea lespedeza* hay (3.6% DM extractable CT) on adult worms, with significantly fewer numbers of both abomasal (*Haemonchus contortus*, *Teladorsagia circumcincta*) and small-intestinal (*Trichostrongylus colubriformis*) nematodes compared with goats fed Bermuda grass hay (0% CT). The present study strongly supports this view, in regards to the observed effects resulting from a mixed CT containing diet.

Although the present study showed no significant differences in carcass quality, the overall data showed the highest impact for all carcass traits in the SLP and mixed treatments. In a similar study Min et al. (2012) showed differences in cold carcass weight (P = 0.06), which tended to increase linearly in goats fed 15% and 30% PB; breast, sirloin, trim trait, liver, and hide weight also increased (linear; $P < 0.01$) with addition of PB. For future implications, the enhancement of carcass traits using CT-containing diets would be desirable if it means more money for a meat goat producer from a high yielding carcass.

With no significant differences recorded in the growth characteristics, there was still growth amongst all of the tannin treatments nonetheless. Observed average final weights were higher than average initial weights. Although this study didn't significantly illustrate growth, previous studies containing PB and SL have illustrated significant results. Solaiman et al. (2010) reported that total dry matter intake (DMI) of growing goats increased when SL ground hay (6.5% CT in DM) replaced alfalfa meal in the grain mixes, and Turner et al. (2005) reported that goats receiving the CT-containing SL hay (23.1 mg CT/g soluble protein) had greater DMI than those fed the alfalfa hay–based diet. Puchala et al. (2005) also demonstrated increased DMI in Angora does fed CT containing SL (17% CT in DM) compared with a mixture of crabgrass (*Digitaria ischaemum*) and tall fescue (*Festuca arundinacea*).

Serum concentrations of alanine transaminase, aspartate aminotransferase, alkaline phosphatase, and cholesterol are conventionally used for diagnosing human and domestic animal hepatic damage (Silanikove, Tiomkin 1992), while alkaline phosphatase and cholesterol are also used to detect bile obstruction and mild liver damage (Silanikove, Tiomkin 1992). The present study showed significance in cholesterol (P = 0.02), alanine transaminase (P = 0.02) and triglycerides (P = 0.04). However, values were within the normal range for goats, suggesting that no liver damage occurred. This was confirmed by postmortem necropsy and dissection of the liver and kidney in this study (data not presented), which indicated no anatomical lesions on liver and kidney organs.

CHAPTER VI

SUMMARY

Results indicate that CT reduces FAMACHA scores, adult worm counts, and FEC reductions, over time. Furthermore combination effects with the mix diet out-performing the other treatments in FAMACHA and adult worm count. The mixed diet showed the most impact, statistically and/or nonstatistically, in all observations tested. According to all of the observations tested, PB had the lowest impact but the greatest amount of CT compared to SLP. Ultimately, this study highlights that diets containing up to 30% CT and a mixture of up to 30% CT has the potential to have an impact on drug-resistant *H. contortus* with no adverse effects on animal health. Mechanisms need exploring as an aid to understand the combination effects.

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