5-2014

Improving Lamb Performance with Sericea Lespedeza and Molybdenum

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A thesis submitted in partial fulfillment
of the requirement for the degree of
Master of Science in Animal Science

by

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Tribhuvan University
Bachelors in Veterinary Science and Animal Husbandry, 2010

May 2014
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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Abstract

The objective of this research was to find the effect of prolonged feeding of sericea lespedeza (**SL**) supplemented with sodium molybdate on changes in body weight, serum and liver concentration of trace minerals (**TM**), hematology, serum biochemistry, and gastrointestinal parasites. Thirty ram lambs (Katahdin and ¾ Katahdin × ¼ Romanov) were weaned; blood, liver, and fecal samples were collected and signs of internal parasitic infection on lambs were recorded. Lambs were supplemented with 900 g of alfalfa (n = 10; **CON**) or SL (n = 20) based supplement for 103 d. Within the SL group, half of the lambs were administered sodium molybdate (n = 10; **SL+MO**) to ameliorate a reduction of serum molybdenum concentrations observed previously. Body weight and body condition scores (**BCS**) were recorded every two wk. Supplementation of sodium molybdate ($P < 0.001$) increased serum and liver concentrations of molybdenum in SL+MO lambs similar to that of CON diet fed lambs; however body weight was similar ($P = 0.74$) between molybdenum supplemented and non-supplemented lambs. Serum and liver concentrations of TM, mainly molybdenum, zinc, copper, selenium, and cobalt were reduced in SL compared with CON fed lambs. Supplementation with SL reduced most of the hematological and serum biochemical values in SL lambs compared with CON fed lambs. Body weight, BCS, fecal egg counts and fecal oocyst counts were similar between the dietary treatments; however clinical signs associated with parasitic infection were reduced in SL compared to CON diet fed lambs.

Acknowledgements

I would like to express my deepest gratitude and sincere appreciation to my research advisor Dr. Joan M. Burke for her patience, encouragement, guidance and support for helping me to complete this project. She never waited a single minute and always showed prompt enthusiasm to cooperate for every approach I made in quest of learning. I can't imagine a scenario where I would not choose you as my advisor.

I would also like to thank Dr. Rosenkrans for his guidance and mentoring. I will never forget your kind words, smiling face and helping me to face every challenge. I am proud to say that 'I LOVE SCIENCE'.

My dear parents, Chhabilal Acharya and Sabitra Acharya, I could never achieved this without your love and guidance. Thank you for sending me to great institutions since I was child. Thanks to my brother and sister, Kamal Prasad Acharya and Ranjana Acharya for your guidance, support and love all these years.

In addition, I would like to acknowledge Dr. Kenneth Coffey, and Dr. Dan J. Donoghue for serving in my MS thesis committee and providing valuable suggestions.My achievement today woun not be possible without the help of USDA-ARS, Booneville, AR folks, Jackie Cherry, Erin Smyth, Chad Lee, Connie Cox, Samuel Tabler, Lucas Hotubbee, Kellee Williams, Hannah Toland and Karen Chapman for feeding experinmental animals and helping during sample collection. Graduate students at the University of Arkansas, Fayetteville, AR, Orr Casey, Smith Brandon, Tony Ryan, Saleh A. Alrashedi and Basanta Raj Wagle for their help during mixing feed. Dr. Kenneth Coffey for feed formulation and providing feed mill. Sonia Tsai and Shawn Curtis for blood analysis and mixing feed. Dr. Matt Welborn and his graduate students, Velvet Rich and Alex Thiels for sampling liver samples.

This study was funded by USDA, NIFA, Organic Research and Education Initiative (Project No. 2010-51300-21641), USDA NIFA Small Business Innovative Research program (Project No. 2011-33610-30836) and Southern Region SARE Grant (Project No. GS13-122). I would like to acknowledge NIFA and SARE for providing this fund. Appreciation is extended to B. Lambert, Tarleton University, Stephenville, for analyzing condensed tannins in sericea lespedeza and alfalfa pellets.

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Chapter I. Introduction

In the U.S., there is a growing preference for goat and sheep meat because of the increased ethnic population that consumes these products, high reproduction rate, and ability of goat to survive on noxious plants (Glimp, 1994). The Southern U.S. has an abundance of pasture for small ruminants, although internal parasites can be challenging. Gastrointestinal parasitism is one of the leading causes of loss of sheeps and goat because gastrointestinal nematodes (GIN) have become resistant to all available anthelminics (Howell et al., 2008). Because of this and a demand for certified organic meat there is a continued need for studies on non-chemical parasite control.

Forage plants like sericea lespedeza (SL; *Lespedeza cuneata*) may aid in controlling both GIN, especially *Haemonchus contortus* (Min et al., 2004; Shaik et al., 2006) and coccidia species (Burke et al., 2013). Sericea lespedeza can be grazed or can be fed in a dry form (hay or pellets). Sericea lespedeza leaf meal pellets were found to be more effective in the control of *H. contortus* compared to SL hay (Terrill et al., 2007). Weight gain in goats fed 75% and 95% SL leaf meal pellets was higher compared to those fed a commercial supplements when fed for 77 d (Gujja et al., 2013) or goats fed 75% SL hay compared with those fed berumdagrass (*Cyanodon dactylon*) hay for 98 d (Moore et al., 2008) .

However, prolonged grazing or feeding of SL leaf meal pellets (> 56 d) decreased weight gain in lambs and kids (Burke et al., 2012; Burke et al., 2014) and serum concentration of trace minerals mainly Mo, Mn, Ze and Se (J.M. Burke and J.E. Miller, unpublished data) compared with control diet fed animals. A marked reduction in Mo occurred in SL-fed sheep and goats compared with control animals, with as high as 90-fold reduction (J. M. Burke and J. E. Miller, unpublished observation).

Earlier studies also have reported a reduction of fecal egg count (FEC) and adult worms of *H. contortus* (Suttle., a, b), *Trichostrongylus vitrinus* (Suttle et al., 1992 b), and *T. colubriformis* (McClure et al., 1999) in lambs supplemented with Mo. Molybdenum is an essential component of enzyme complexes (de Renzo et al., 1953) that could be important to growth and other physiological functions. Sericea lespedeza contains condensed tannins (CT), a plant secondary metabolite. Toxic effect due to CT on lambs and kids fed SL is unknown. Because of the recent popularity of grazing or feeding SL hay or pellets, which are both commercially available, for an aid in the control of parasites, it is important to understand potential limitations to production of small ruminants. Therefore the objectives of this study are,

1. to determine changes in trace minerals in lambs fed SL and administrated sodium molybdate;
2. to determine hematological and serum chemical profile in lambs fed SL and
3. to determine FEC and weight gain in lambs fed SL and administrated sodium molybdate.

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Chapter II: Review of Literature

Importance of Small Ruminant Production in Southern U.S. and Effect of Internal Parasites on Small Ruminants Production.

Coccidia and gastrointestinal nematodes (GIN) are the most pathogenic gastrointestinal parasites in small ruminants, causing a significant loss of sheep and goat production world-wide. Pathogenic parasites in the gastrointestinal tract alters the host's feed intake, digestion, metabolism and various physiological processes (Dargie and Allonby, 1975; Dynes, 1990). The most common GIN in warm and humid climate is *Haemonchus contortus*, a voracious blood feeder that can lead to anemia and death. Other GIN can alter feed conversion ratio, decreas weight gain, delay maturity, decrease immunity to disease resistance, and lead to death (Chiejina and Sewell, 1974; Smith, 1985; Munn et al., 1987; Gatongi, et al., 1997). Direct estimation of economic losses due to parasitic diseases can be challenging because of the environmental interaction; however, cost analysis, by McLeod (1995) in the an Australian grazing industry have shown a loss of millions of dollars every year. This problem is similar to the recent report published by National Animal Health Monitoring System (NAHMS, 2011). In at report, internal parasitism was the second leading cause of non-predator loss of sheep in the U.S. About 65% of the respondents in the same survey ranked internal parasitism as one of the top three disease problems they encountered.

Distribution, Life Cycle, Pathogenesis and Clinical Symptoms of Some Commonly Available Gastrointestinal Nematodes.

Gastrointestinal nematodes are a serious problem for the sheep and goat production in the Southern U.S. Most pathogenic GIN in small ruminants belong to the order *Strongylida* and superfamily *Trichostrongylidea*. *Haemonchus* spp., *Teladorsagia* spp., and *Trychostrongylus*

spp., are the most common GIN belonging to the superfamily *Trichostrongylidae*. *Haemonchus contortus* is the predominant GIN, in the Southern U.S. (Craig, 1986). Other GIN present in the area are *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Nematodirus* spp., *Cooperia* spp., *Trichuris* spp., *Trichostrongylus axei* and *Oesophagostomum* spp. (Miller et al., 1998).

Abomasal Nematodes

Haemonchus contortus (Barber's pole worm/Wire worm/Stomach worm)

A female *H. contortus* deposits 5000-7000 eggs daily in the abomasum of the host (Coyne et al., 1991). Eggs pass through the feces, and hatch into first-stage larvae (L_1) under warm temperature and humid conditions. However, the optimal hatching takes place at 30°C, pH 6 and relative humidity of 80-90% (Khatun et al., 2013). First stage larvae molt into second-stage larvae (L_2) after shedding their protective cuticle. Both L_1 and L_2 survive by feeding on bacteria and organic matter in the manure. Second-stage larvae develop into third-stage larvae (L_3), but retain their cuticle. This stage of larvae feeds only on the stored nutrients, and survival is optimal if the outside temperature is warm and humid. Animals consume L_3 from the pasture and become infected. Third-stage larvae sheds their cuticle in the rumen when exposed to CO_2, enter the abomasum, and penetrates deep into the mucosa, and molt into fourth-stage larvae (L_4). Fourth-stage larvae molt into fifth stage larvae (L_5), or adult (Veglia, 1916). The pre-patent period is 3 weeks unless the L_4 undergo hypobiosis. Hypobiosis is the process of arrested development of L_4 stage larvae (Blitz and Gibbs, 1971a, b). Factors such as adverse environmental conditions, host immune response, and negative feedback of overcrowded adult worms are responsible for hypobiosis (Capitini et al., 1990; Adams, 1983). Growth, development and reproduction of L_4, L_5 and adult *H. contortus* takes place in the abomasal mucosa. They are voracious blood feeders

(Rowe et al., 1988). A single adult can ingest 0.05 ml of blood daily (Clark et al., 1962). In severely infected lambs, blood loss per day can be as high as 253 mL (Rowe et al., 1988). A reduction in packed blood cell volume (PCV) is an indirect estimation of haemonchosis (Barger and Dash, 1987).

Severe haemorrhage can be seen in the abomasal mucosa (Dargie and Allonby, 1975). There are various reports of a reduction in plasma albumin concentrations. This decreases plasma-colloid osmotic pressure in capillaries. Reduction in blood osmotic pressure results in outflow of fluid from blood which accumulates in the interstitial space. Fluid accumulated in the submandibular space is very prominent in sheep and goats and usually referred to as 'bottle jaw' (Abbott et al., 1986). 'Bottle Jaw' is another indirect indication of severe haemonchosis. Young sheep and goats are more susceptible to *H. contortus* than adult animals (Albers et al., 1990). The time immediately around lambing or kidding is called the peri-parturent period. Peri-parturent rise of *H. contortus*is is also common (Van Geldorp et al., 1976). Severely infected animals are unthrifty, emaciated, and weak. Diarrhea may or may not be seen (Abbott et al., 1988). Mortalities are reported during heavy infection (Abbott et al., 1986).

Teladorsagia circumcincta (Brown stomach worm)

The life cycle of *T. circumcincta* is similar to other trichostrongylid nematodes. Adult females lay eggs in the abomasum of the host which are shed in the feces. Eggs develop into L_1 stage within a day. First stage larvae then moult into L_2 and L_3 under favorable temperature and moisture conditions (Sommerville, 1954). The optimum temperature for survival at this stage is 18 – 26 °C; however, this nematode can tolerate much lower temperatures (Pandey, 1972), hence, these parasites are commonly found in temperate regions. Once ingested by the host, L_3

exsheath in the rumen and migrate to the abomasum. In the abomasum, L_3 molt into L_4 and finally into the adult stage.

Infection of *T. circumcincta* results in irritation and damage to parietal cells (Scott et al., 2000). Damage to parietal cell causes a decrease in hydrochloric acid (HCl) secretion. Hydrochloric acid is essential for the conversion of pepsinogen to pepsin needed for protein digestion. Hence, there will be decreased protein digestion. Feed intake decreases sharply due to inappetance (Downey et al., 1972). Major clinical signs are anorexia, diarrhea, dehydration and decreased weight gain (Jayawickrama and Gibbs, 1967; Sykes, 1977).

Small Intestinal Nematodes

Trichostrongylus colubriformis (Black scour worm)

The life cycle of *T. colubriformis* is similar to other GIN. Eggs pass though feces, which then hatch to give rise to L_1 and L_2 (Gibson and Everett, 1967). Third-stage larvae are resistant to adverse temperatures, due to the retained protective cuticle. This is the infective stage. These then shed their cuticle in rumen, move to the intestine, and penetrate deep forming tunnels (Barker, 1975a). There, L_3 molts into L_4 and finally to the adult in the abomasum. The life cycle will be completed in 3 weeks.

Larvae and adults of *T. colubriformis* penetrate into the intestinal mucosa underneath the epithelium, causing desquamation of cells (Barker, 1975b). This becomes extensive upon heavy infection (Barker, 1975b). *T. colubriformis* are not blood suckers. Anemia can be observed, which is due to shortening of the life cycle of red blood cells and impaired erythropoiesis. Edema and loss of plasma proteins occur in the intestinal lumen due to burrowing of tunnels. Decreased feed intake, slow weight gain, and soft feces are some of the clinical signs (Barker, 1973).

Nematodirus spp.

Nematodirus spp. has a unique life cycle. Infected lambs shed eggs in the pasture during spring, which will hatch into L_1 within the egg in the summer following spring. These larvae then remain dormant for several months, molt into L_2 and L_3 and infect the host (Thomas and Stevens, 1960). Hatching is stimulated by low temperature (Van Dijk and Morgan, 2008), so during the cold winter, hatching occurs. L_3 molts into L_4 in the abomasum, and moves towards the intestine. *Nematodirus* spp., then penetrate the host's intestinal wall and develops into the adult. In a heavy infection, there will be damage of intestinal villi, erosion of intestinal mucosa and infiltration of cells in the lamina propria (Coop et al., 1973). Diarrhea and loss of appetite are observed with heavy infection (Zurliiski, 1979).

Cooperia spp.

Cooperia spp. are not very common in sheep, but more common in cattle. These are mostly prevalent in winter and spring, and least during summer (Charles and Baker, 1988). They have a similar life cycle as other GIN. Adult parasites reside in the duodenum and anterior jejunum (Coop et al., 1979). Infection of *Cooperia* spp. causes slight intestinal lesions. There will be lymphocytosis and eosinophilia; however, most of the blood parameters will be in the normal range (Ahluwalia and Charleston, 1975).

Large Intestinal Nematodes

***Trichuris spp.* (Whip worm)**

Females lay small numbers of unembryonated eggs which pass out with the feces. Eggs are very resistant to adverse weather conditions; and therefore can survive up to six months. The L_1 stage develops inside the egg under optimum temperatures and moisture conditions. Hatching does not occur unless ingested by a suitable host. Once eggs are ingested, mucoid plugs are dissolved by the digestive enzymes in the duodenum, allowing larvae to escape. These larvae

than migrate to the cecum, where all three molts occur. In about 12 weeks, ingested infective eggs mature into the adult stage (Deo, 1960).

There are rare reports of disease due to naturally infected *Trichuris* spp. However, few studies have shown haemorrhagic necrosis, and edema of cecal mucosa, which later turn into nodules. In acute cases, profuse watery diarrhea, retarded growth, and death can be observed (Deo, 1960; Nath et al., 2011).

***Oesophagostomum spp.* (Nodule worm)**

Under warm and moist conditions, eggs in the feces hatch into L_1. The L_1 molt into the L_2 stage, and both are susceptible to desiccation. The L_3 develop inside the cuticle of L_2. This is the infective stage. After being ingested by the host, larvae cast their sheath and penetrate into the wall of the intestine. Third ecdysis takes place in the intestine, anywhere between the pylorus and rectum (Robert et al., 1963). In normal conditions these larvae return to the lumen, then pass to the colon and molt into L_4 and adult (Dash, 1973).

Marked inflammation will be seen around each larvae, while making their way inside the intestinal submucosa. Eosinophils and giant cells collect around these larvae which will be encapsulated by fibroblasts. Encapsulated larvae stay inside these nodules for a few months (Bawden, 1969). Some of these larvae may die inside the encapsulated fibroblast due to calcification. Other may come out through a narrow opening, and wander in between muscle fibers. Very few make their way into the cecum where they molt into an adult stage. A large number of nodules in the intestine interfere with absorption and digestion. Mucoid diarrhea and death can be seen during heavy infection (Shelton and Griffiths, 1967).

Coccidiosis

Introduction

Coccidiosis is one of the most common gastrointestinal parasitic diseases of young lambs (Salisbury et al., 1953). It is caused by the infection of a protozoan parasite, *Eimeria* spp. There are approximately 10 *Eimeria* spp. prevalent in sheep; however, *E. ovinoidalis*, *E. crandallis*, and *E. ahsata* are the most pathogenic species (Gregory, 1989). Loss of body weight, diarrhea and death are common signs of infected lambs (Smith et al., 1960; Catchpole and Gregory, 1985). This problem is prevalent worldwide including the U.S. and includes other livestock species, goats, cattle, poultry and pig (Chhabra and Pandey, 1991; Maingi and Munyua, 1994; Yakhchali and Rezaei, 2010; Burke et al., 2013).

Life Cycle

Ingestion of sporulated oocysts occurs via contaminated feed or water (Helle, 1970). Sporozoites are released from the oocyst and enter into epithelial cells in the intestine (Gregory et al., 1989). This stage of sporozoites are called tropozoites. Sporozoites grow larger, undergo nuclear division or schizogony, and form schizont. Schizonts produce the first generation of merozoites (Gregory et al., 1989). Each merozoite invades new cells and undergoes a second generation of schizogony. Schizogony may repeat many times causing great damage to intestinal epithelial cells (Gregory et al., 1989), or undergo sexual multiplication, in which merozoites develop into macrogametocytes or microgametocytes in the large intestine (Gregory and Catchpole, 1986; Gregory et al., 1989).

Macrogametocytes store food material to induce hypertrophy of both the cytoplasm and nucleus to form female sex cells (McLaren, 1969). Microgametocytes, on the other hand, undergo nuclear division, to form multinucleates with biflagellate, male sex cells (McLaren,

1969; Gregory et al., 1989). After the maturation, microgamonts leave their host cell, seek out macrogamonts and penetrate the cell having macrogamonts (Davis et al., 1957). Only a small fraction of microgamonts find and fertilize macrogamonts to form zygotes (Davis et al., 1957). The zygote lays a wall around itself to form an oocyst (Gregory et al., 1989). Oocysts break out of host cells into the intestinal lumen and are excreted with feces (Vieira et al., 1997). Sporulation outside the body can be completed within 2-3 days under favorable conditions (Vieira et al., 1997). The life cycle will be completed within 2-4 weeks, depending on the species of *Eimeria* (Vieira et al., 1997).

Pathogenesis

Pathological lesions are seen, both in the small and large intestine. Lesions in the small intestine are due to the release of the first generation of merozoites (Gregory and Catchpole, 1990). However, damage in the large intestine, is due to the second generation of merozoites and/or due to gametocytes (Gregory et al., 1987). Villus atrophy, hyperplasia, and atrophy of crypts can be seen on histological examinations. These are accompanied by losses of epithelial cells from the mucosal surface and from the crypts (Gregory et al., 1987). There will be massive shedding of infected cells. Loss of non-infected cells attached to infected cell also will occur (Gregory and Catchpole, 1986). Lesions created by cell loss are very prone to secondary bacterial infection (Gregory et al., 1987). Healing is slow in the large intestine. So diarrhea is seen even after a decrease in fecal oocyst count (Gregory and Catchpole, 1986).

Treatment and Control

Economic losses due to coccidiosis include production losses and losses for treatment costs. Severe coccidosis can lead to permanent damage of the intestinal lining and stunted growth. Coccidiostat drugs can be used for the control of coccidiosis in lambs (Platzer et al.,

2005; Le Sueur et al., 2009; Tauseef-ur-Rehman et al., 2011; Diaferia et al., 2013). However recent studies have reported coccidia resistant to anticoccidial drugs (Burke et al., 2013). Some consumers are concerned about drug treatment in animals, its residue and potential impact on human, and there are no pharmaceutical products that can be used in organic production and demand for organic meat is increasing day by day (Dimitri and Oberholtzer, 2009). This led to search for the organic method for the control of coccidia.

Organic methods for the control of coccidia have been tested recently. Lambs grazing on sericea lespedeza (SL; *Lespedeza cuneata*) pasture (Burke et al., 2013) and those fed with foliage of mastic tree (*Pistacia lentiscus*; Markovics et al., 2012) reduced the need for anticoccidial drugs. Feeding chinaberry (*Melia azedarach*) fruit was as effective as anticoccidial drugs for the reduction of fecal oocysts (Madibela and Kelemogile, 2008). In vitro studies with sainfoin (*Onobrychisviciifolia*) (Saratsis et al., 2012) and feeding water extract from monterey pine (*Pinus radiate* Molan et al., 2009) potentially decreased the need for anticoccidial drugs. Hence, these methods may be used for prevention and treatment of coccidia.

Diagnosis of Gastrointestinal Nematodes and Coccidiosis in Lambs

Fecal Egg Count (FEC).

The McMaster technique of egg counts is based on the principle of flotation in a salt solution and has been extensively used for the estimation of GIN burden in animals (Whitlock, 1948; Rossanigo and Gruner, 1991; Nicholls and Obendorf, 1994). In this technique, estimation of FEC is done by measuring eggs per gram of feces (EPG), calculated from the microscopic examination of a fraction of fecal suspension from the known volume of fecal sample (Nicholls et al., 1994). Counting of total eggs is done adding the number of eggs in two specialized McMaster chambers within a slide, where 1 egg represents 50, and number thus obtained give

FEC per gram in feces. Estimation of FEC by the McMaster technique is quick, but, it has some limitations. The GIN burden at an early stage of parasitism is hard to determine. Hypobiosis, host immunity, and variation in egg output by nematode species are some of the factors that make McMaster technique inexact (Gordon, 1967; Jasmer and McGuire, 1991). Uneven distribution of eggs in feces is another limiting factor. This technique also does not tell genera or their pathogenicity. To determine genera a fecal culture can be conducted to hatch eggs and grow to L_3 stage and identify microscopically (Peňa et al., 2002).

Nevertheless, measurement of FEC in sheep and goats by this method is relatively accurate when the predominant genera is *Haemonchus* (Tembely et al., 1997). There is a strong correlation between FEC and adult GIN in young lambs (McKenna, 1981). McMaster technique can be used to measure the changes in FEC over a period of time (Brunsdon, 1970). The eggs of some GIN genera such as strongyloides, and *Trichuris* spp., can be recognized morphologically by the McMaster technique (reviewed by Taylor, 2010). This also is a simple technique for the estimation of *Eimeria* spp. in feces. However, the McMaster technique may not be a proper diagnostic tool for most pathogenic coccidian species because clinical signs may be seen before oocysts appear in feces (reviewed by Taylor, 2010).

Blood Packed Cell Volume (PCV)

Larval and adult stages of GIN in sheep and goats penetrate the gastrointestinal tract, damaging mucosal and submucosal layer, causing haemorrhage. Shortening the life cycle of red blood cells (RBC) and impaired erythropoiesis also is common. Among the GIN genera, *H. contortus* is notorious for sucking blood. Packed blood cell volume (PCV) is the volume percentage of red blood cells in blood. Blood packed cell volume is determined by centrifuging whole blood in a capillary tube which packs the red blood cell and the percentage of the blood

volume taken up by RBC is measured. High FEC and reduced PCV are could be correlated when the predominant GIN is *H. contortus* (Barger and Dash, 1987).

FAMACHA©

Hemoglobin is the part the RBC which gives red color in blood. Loss of blood or an anemic condition can be estimated by observing the color of the mucous membrane. Scientists in South Africa developed a card called FAMACHA©, to read the degree of redness (Malan et al., 1992; Bath et al., 1996). Fafa Malan was the lead scientist and the card was dubbed the Fafa Malan chart or FAMACHA©. In this method, the mucous membranes of the lower eyelid are examined and compared to a laminated color chart: 1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, mildly anemic; 4 = pink-white, anemic; and 5 = white, severely anemic. FAMACHA© score and PCV were correlated (Vatta et al., 2002; Kaplan et al., 2004). This method is effective to determine degree of *H. contortus* but not other GIN genera, because loss of blood is the primary symptom of haemonchosis. FAMACHA© also been validated in different parts of world, including the U.S. (Vatta et al., 2002; Keplan et al., 2004; Burke et al., 2007a; Molento et al., 2009).

Dag Score

Fecal soiling or accumulation of feces around the perineum in sheep and goats is caused by bacterial and viral pathogens, internal parasites, dietary composition, and mineral imbalance (Owen et al., 1958; Dohoo et al., 1985; Munoz et al., 1996; Larsen and Anderson, 2000). There is a higher incidence of diarrhea associated with coccidiosis, which occurs during times of stress (Janke et al., 1989). Some GIN genera cause fecal soiling in young lambs (Broughan and wall, 2007). Extent of fecal soiling or dag score is estimated by observing amount of fecal material adhering around perineum. Dag score is measured in a five point scale (1 = clean, and 5 =

extensively daggy; Larsen et al., 1994; French et al., 1996; Burke et al., 2013). Anthelmintic treatment, based on the dag score has been successively practiced in different field cases (Gogolewski et al., 1997; Broughan and Wall, 2007). However, a study has shown poor correlation between FEC and dag score when the predominant genera was either *Haemonchus* or *Trichostrongylus* (Karlsson et al., 2004).

Fecal Score

Fecal consistency in sheep or goats is defined as the degree of hardness of a fecal sample. Feces may vary from hard feces to watery. The degree of hardness of fecal pellets can be measured in a five point scale: 1 for solid pellets; 2 solid pellets, somewhat clomped; 3 solid mass of fecal matter; 4 consistency like pudding; and 5 consistency like pea soup (Cabaret, 2004; Burke et al., 2013). Diarrhea due to some genera of GIN and coccidiosis is common in young lambs (Broughan and Wall, 2007). Anthelmintic and anticoccidial drug treatment on the basis of fecal score has been successfully practiced in different parts of the world (Watts et al., 1978; Gogolewski et al., 1997; Broughan and Wall, 2007).

Body Condition Score (BCS)

Body condition score is an estimate of the degree of muscle and fat deposition over the spine, loin and ribs (Murray, 1919). Condition scoring for sheep can be done using a five point scale: 1 for emaciated, very thin; 2 for lean; 3 for moderate condition; 4 for fleshy, fat; and 5 for obese, very fat (Jefferies, 1961). Body condition score can be a useful technique to estimate the infection of non-hematophagous nematodes like *Teladorsagia* spp., *Trichostrongylus* spp., *Nematodirus* spp., and *Oesophagostomum* spp. (Gordon, 1981). Body condition score also may be reduced when coccidia, paratuberculosis, and sore mouth exists on the farm (Stafford et al., 1994; Ayaz, 1999; Kurade., 2004). If lambs of a similar age group, kept in a similar

environment, with similar nutrition and weather condition have different BCS then, internal parasite could be the cause for a reduction in BCS (Barger, 1985). Artificial infection of *H. contortus*, and *T. colubriformis* larvae in dairy goats led to a decrease in BCS in comparison to a control group (Hoste and Chartier, 1993). Researchers have found a negative correlation between BCS and FEC or number of adult worm (Idika et al., 2013). Selective anthelmintic treatment based on the BCS may be successfully practiced in sheep and goats (Gallidis et al., 2009)

Control of Gastrointestinal Nematodes

Grazing Management

Multispecies grazing can be done in which more than one livestock species (that rarely share common GIN) are grazed in a common pasture at the same time, or interchanged over time for the reduction GIN. Most GIN are species specific (Amarante et al., 1997). That is, cattle GIN affect cattle and not sheep and goats and vice-versa. Multispecies grazing can effectively reduce FEC in ruminants (Barger and Southcott, 1978). A study has shown decrease of sheep GIN, *H. contortus* and *T. colubriformis,* and cattle GIN, *H. similis, C. punctate*, and *O. radiatum* when cattle and sheep grazed alternatively in a pasture for three month intervals (Rocha et al., 2008). In another study, alternative grazing of horses with sheep decreased pasture larval and worm count of Cyathostomine and Strongylinae (Eysker et al., 1983). However, this method should be adopted with caution. Sometimes GIN, primarily of cattle origin, may infect sheep and cause clinical disease if they are not rotated for a long period (Bairden et al., 1995). Higher incidence of *H. contortus* in cattle was reported, when grazed alternately with sheep that were rotated in a weekly intervals (Mahieu and Aumont, 2009).

Stocking rate has a direct effect on parasite burden. Higher worm burden was observed in sheep when stocking rate was increased (Le Jambre, 1984). Feeding behavior also has a high

influence on GIN infection. Research done in France demonstrated that Saanen breed of goat browsed to greater extent than the Angora breed, leading to lower FEC (Hoste, 2001). Including any browsers on goats will reduce GIN uptake and, rotational grazing of a single species (sheep) compared with continuous grazing led to a reduced need for deworming (Burke et al., 2009).

Selection and Breeding

Heritability is defined as the degree of variation of a quantitative trait that can be passed from parents to offspring. Sheep resistant to GIN infection generally are less susceptible with GIN, which can be measured by FEC relative to other flock mates with more than one sire represented. Heritability estimate for parasite resistance and resilience can be predicted by repeated measurement of FEC and PCV over a period of time. Host resistance is defined as the host's ability to resist infection. Resistance to nematode infection is usually measured by a decrease in FEC after GIN infection. Resilience is defined as the host's ability to withstand infection. Here, FEC may be high, but PCV remains high during *H. contortus* infection, or body weight and BCS may be normal for GIN infection. Heritability estimate for parasite resistance in Merino, Perendale, and Katahdin sheep are 0.23, 0.22 and 0.45 respectively (Eady et al., 1996; Morris et al., 2005; Notter et al., 2007). Measurement of FEC after a natural infection gives estimation of heritability mainly due to genetic variation (Miller et al., 2006).

Genetic variation in parasite resistance is useful for the calculation of estimated breeding value (EBV) for parasite resistance. Estimated breeding values help us to predict a relative genetic merit of each member in a breeding population. Estimated breeding values for parasite resistance is available through the National Sheep Improvement Program (www.nsip.org). Selection in sheep, especially replacement rams is very useful for producing GIN resistant lambs (Notter et al., 2007).

Smart use of Anthelmintics

Refugia is defined as the proportion of nematode population that is not exposed to anthelmintics. This is a process of reducing the anthelmintic resistance in GIN by diluting genes that are resistant to anthelmintics. Veterinary parasitologists recommend treating all the animals at once without considering refugia, to maximize production led to the developement of GIN resistance of all classes (avermectins, benzimidazoles, imidothiazoles) of anthelmintics available. Prevalence of GIN resistance to available anthelmintics is widespread in most parts of the world (Uhlinger et al., 1992; Echevarria et al., 1996; Howell et al., 2008; Harfoush et al., 2010; Domke et al., 2012). After several years of research, a new class of anthelmintic drug, monepantel, was discovered (Kaminsky et al., 2008). However, nematodes developed resistance to monepantel after four years of discovery in goats (Dr. Stephen love unpublished data). Due to genetic diversity within GIN genera some develop resistance to anthelmintics and survive after the treatment. Repeated use of anthelmintics without regard to refugia will gradually kill all non-resistant worms leaving only resistant worms (Van Wyk et al., 2001). So, in order to overcome the problem of resistance, wise and limited use of anthelmintics should be practiced. Deworm and move to clean pasture for parasite control had been recommended in the past (Van Wyk, 1990). However this system has serious drawbacks. Only anthelmintic resistant GIN remain in the pasture, and over a period of time, the pasture will be totally contaminated with anthelmintic resistant GIN. In order to overcome this problem, a new concept of move and deworm came into practice. In this deworming system, in theory the pasture contains both anthelmintic resistant and susceptible worms. Hence, this management practice dilutes the resistant worms and reduces the chance of anthelmintic resistance (Van Wyk, 2001). Distribution of GIN is not equal in all the animals. One-third of animals harbor most of the nematodes. Selective treatment of only heavily

infected parasitic animals brings effective control of nematodes, and reduces chance of developing resistance (Hoste et al., 2002; Gallidis et al., 2009). Selective treatment using FAMACHA© is effective for only for the hematophagous nematodes (Van Wyk and bath, 2002). Selective treatment by BCS and/or body weight change over a short period of time is an effective way to selection of parasitized animals (reviewed by Van Wyk et al., 2006).

Oral anthelmintics also can be more effective if animals are off feed for more than 24 hours before drenching. Higher dissolution and absorption was experimentally documented in albendazole treated ruminants that were off feed for 48 hours before drenching (Sanchez et al., 1997). In a separate study, it was found that a combination of two or more classes of anthelmintics worked better than treating with a single class of anthelmintics (Echevarria et al., 1996).

Nutrition

A decrease in feed intake has been observed in parasitized lambs (Sykes and Coop, 1977). Poppi et al., (1986) reported an increase in the amount of ammonia and non-ammonia nitrogen at the terminal ileum in lambs a few weeks after being artificially infected with *T. colubriformis*. This occurred due to leakage of plasma from epithelial cells lining the intestinal wall (Poppi et al., 1986). A high amount of water also was found in the duodenum of *H. contortus* infected sheep (Bueno et al., 1982). In both of those experiments, increased lumen permeability of fluids was observed, which implies leakage of nutrients from the lumen of the parasitized lambs caused by damage in epithelial cells lining the intestine (Barker, 1975a). Loss of nutrients in parasitized lambs is confirmed by their slower growth in comparison to non-infected lambs (Sykes and Coop, 1977).

A protein rich diet increases the host's resilience to nematode infection (Coop et al., 1995; Donaldson et al., 1998; Valderrabano et al., Houdijk et al., 2000; Valderrabano et al., 2002; Louvandini et al., 2011). Supplementation of protein balances the endogenous loss and overcomes the problem of weight loss due to GIN infection (Abbott et al., 1988; Brown et al., 1991). Supplementation of some minerals decreased the total GIN burden in ruminants. Studies reported a decrease in FEC and adult worms of *H. contortus* (Suttle et al., 1992a), *T. vitrinus* (Suttle et al., 1992b), and *T. colubriformis* (McClure et al., 1999) in lambs by addition of molybdenum in the diet.

Copper Oxide Wire Particles (COWP)

Initially, copper oxide wire particles were investigated for the treatment of copper deficiency in sheep and goats. Commericially available copper oxide wire particles are generally less than 5 mm long and 1 mm long diameter. Studies showed that COWP reduced FEC of *H. contortus* (Bang et al., 1990a; Chariter et al., 2000; Knox, 2002; Burke et al., 2004; Burke et al., 2010), but was less effective in the control of *Trichostrongylus circumcincta* and *Trichostrongylus* spp. (Bang et al., 1990b; Chartier et al., 2000a). Administration of 0.5 - 1 g of COWP in young goats and lambs of less than a year of age and 2 g for older sheep and goats effectively reduced *H. contortus* (Burke and Miller 2006; Burke et al., 2007 b; Burke et al., 2010). Multiple low doses of 0.5 – 1 g of COWP also was administrated four times in six week intervals without any toxic effect in lambs (Burke and Miller, 2006). Multiple low doses of COWP in lambs was as effective as levamisole for the reduction of *H. contortus* (Burke and Miller, 2006). Excess copper in the diet can lead to copper toxicity, which can result in mortality. Copper oxide wire partilce administrated as a bolus moves from the rumen to abomasum, and remains in the abomasum for several weeks slowly releasing copper (Dewey, 1977). Copper

oxide is slowly absorbed in comparison to copper sulphate, and hence the chance for developing toxicity is minimized with low doses. Sheep are more susceptible to copper toxicity than goats; however toxicity symptoms were not observed in sheep when they were administered up to 10g of COWP but copper was increased in liver (Whitelaw et al., 1980; Hale et al., 2007). Nevertheless, precautions should be taken when sheep are raised on a farm containing high levels of copper in soil, feed or environment or when using high doses of COWP.

Vaccines

There are two classes of experimental vaccines used to control of GI nematodes. First is a natural antigen, recognized during natural infection, which has an excretory/secretory (E/S) or surface antigen. Second is a hidden gut antigen, derived from intestine of the parasite that is not exposed to the host's immune system during infection.

Sheep immunized with E/S antigen had lower FEC and abomasal worm burden in comparison to control groups (Arunkumar and Basith, 2013). Vaccines prepared from the gut membrane protein of adult *H. contortus* also reduced FEC, and re-occurring infection of *H. contortus* from pasture (LeJambre et al., 2008). A study was done to compare the efficacy of a whole worm homogenate antigen and E/S product antigen. Vaccination with whole worm homogenates showed a higher reduction of FEC in comparison to E/S antigen (Schallig, 1997). Efficacy of surface antigens can be increased by a adding a suitable adjuvant (Jacobs, 1999). After vaccination, antibody level reached a peak in 60 days and remained at that level for 6 months. This effect was seen when animals were vaccinated with gut antigen H11 followed by a challeng infection with *H. contortus* (Andrew et al., 1997). This antigen also in effective in the reduction of periparturient rise in FEC. Despite the success in the control of GIN, there are various hindrances for the production of the vaccine. Immune response produced by the immune

potentiators in the vaccine are not specific and often down regulates other critical immune responses (reviewed by Sher and Coffman, 1992). Vaccines failed to reach a balance between protecting from disease and maintaining productivity, are less adaptable to extensive farming systems, have a short protection period, and are hard to maintain stability (reviewed by Sonstegard and Gasbarre, 2001). Relying on the natural or hidden antigen would be impractical for mass production (reviewed by Sonstegard and Gasbarre, 2001) unless a recombined form can be developed.

Condensed Tannins Containing Plants; Sericea Lespedeza

Sericea lespedeza (SL) is a native plant of East Asia and Australia (Ohiwi, 1965; Stevens, 2002). Sericea lespedeza seeds were introduced to the U.S. from Japan in 1924. Initially SL was used for the control of soil erosion in dry and arid soil (reviewed by Ohlenbusch et al., 2001). From the 1950s, SL was used as hay and a grazing crop (Donnelly, 1954). High condensed tannins (CT) content in SL were thought to be less palatable and less digestible in comparison to those SL cultivar containing low CT (Wilkins et al., 1953; Donnelly and Anthony, 1970; Cope and Burns, 1971). However, that was found not to be true. After several years of breeding, the 'AU Grazer' variety of SL was developed and released in 1997 by Auburn University, Alabama Agriculture Experiment Station. This variety was developed after crossing between high CT-containing plant and low-CT containing plant. This variety contains pliable stems of smaller diameter, higher forage yield, abundant branching and greater survival under grazing in comparison to other varieties

Sericea lespedeza can be grazed by goats and sheep (Min et al., 2004; Burke et al., 2012a) or can be fed in dry form (hay or pellets; Shaik et al., 2006; Lange et al., 2006). Hay of SL was effective for the control of *H. contortus*; therefore, could replace chemical anthelmintics

where the predominant GIN is *H. contortus* (Shaik et al. 2006). Incorporating 50-75% SL hay in the diet was more effective for the reduction FEC compared with 25% of SL hay in the diet (Terrill et al., 2009). Pelleting SL adds an option for broader use of forage. A study showed a higher reduction of GIN in animals fed SL pellets compared to those fed SL hay (Terrill et al. 2007). Weight gain in goats fed 75% and 95% SL leaf meal pellets were higher compared to those fed commercial pellets without SL (Gujja et al., 2013). Sericea lespedeza aids in the control but does not kill adults. So, it may not be able to replace anthelmintics, but reduce their use.

Tannins: Hydrolysable and Condensed

Tannins are polyphenolic compounds having high molecular weight and complex structure (Foo and Porter, 1981). They are plant secondary compounds found mainly in vacuoles and surface wax of the cell (Parham and Kaustinen, 1977). Generally three classes of secondary metabolites are found in plants: nitrogen containing compounds, terpenoids, and phenolics (Gershenzon and Mabry, 1983). Tannins belong to the phenolic class. Tannins can broadly be subdivided into two groups: hydrolyzable and condensed tannins. Hydrolyzable tannins are those which can be hydrolyzed by water and tannases (Seikel et al., 1970). Gallotannins and ellagitannins are the hydrolyzable tannins. Tannins that are not hydrolyzed in water or by tannases are called the condensed tannins (William et al., 1986).

Hydrolyzable Tannins (HT)

Gallotannins and ellagitannins are the major classes of HT. The core structure of these tannins is occupied by carbohydrate (D-glucose). Gallotannins are formed by esterification of the hydroxyl group of the carbohydrate with gallic acid (Nishizawa et al., 1980). Ellagitannins on the other hand constitute: hexahydroxydiphenoyl units, galloyl units, and sanguisorboyl units (Vivas

et al., 1995). Hydrolyzable tannins are present in tree leaves and nut husks of *Ceratoniasiliqua*, *Climediahirta*, *Quercusrobur*, *Betula alba*, *Calluna vulgaris*, and *Vacciniummyrtillus* (Joslyn et al., 1968; Murdiati, 1990; González-Hernández et al., 2003; Salminen et al., 2004).

Hydrolyzable tannins are easily degradable. In a study where plants containing hydrolyzable and condensed tannins (CT) were fed to ruminants, HT were absent in feces; whereas, a large fraction of CT were present (Hagerman et al., 1992). Low concentrations of HT in the diet will not often produce any negative effects on health and production of ruminants. A study done with HT extract from chestnut (*Castanea sativa*) at the dose rate of 120 g per day in the diet of a dairy cow had no effect on milk production (Hagerman et al., 1992; Lavrencic et al, 2006). Quebracho contains more HT in comparison to CT. A study has shown incorportation of 1.5g of tannin extracts per kg live weight in feed of sheep without any negative effect on health or production (Hervas et al., 2003). Forages containing more than 4% hydrolyzable tannins on a DM basis, when measured as gallic acid equivalent, are considered a high level of HT. However, ruminal bacteria can degrade HT up to the level of 15% (wt/vol), of HT in their diet (Nelson et al., 1995). Prolonged feeding of HT results in a negative impact on digestion of feed in ruminants. Lambs fed pomegranate extracts containing 16.5% gallic acid equivalents decreased crude protein and fat digestion. Decrease in body weight was recorded in those lambs in comparison to a control diet (Oliveira et al., 2010). Toxic signs appeared if the concentration of HT exceeded 17% of gallic acid equivalents (Nelson et al., 1995). Gastroenteritis, hepatotoxicity, and nephrotoxicity are some of the pathological changes noticed in goats that were fed with soap bush (*Climedia hirta)* containing 19% of HT. However, the harmful effects can be ameliorated by calcium hydroxide [$Ca(OH)_2$] supplementation (Murdiati, 1990). Stressed animals cannot tolerate HT in their diet. Young bulls fed leaves of pyrenean oak (*Quercus pyrenaica)* after

several days of restricted feeding exhibited pathological signs like gastroenteritis, tubular necrosis, and renal failure (Pérez et al., 2011). Hydrolyzable tannins can play an important role in decreasing methane emission by ruminants. An in vitro study with rumen extract from different ruminants showed that 50 g/kg dry matter of hydrolyzable tannins decreased methane production without any harm on rumen microbes (Hassanat et al., 2013).

Condensed Tannins (CT)

Proanthocyanidins are the oligomers or polymers of flavonoid units linked by carbon-carbon bonds. These also are called condensed tannins because of their condensed structure. Cyanidin and delphinidin are the most common anthocyanidins (Hemingway et al., 1982). These may or may not dissolve in water. Condensed tannins are one of the most widely studied plant secondary metabolites. Some of the plants containing a high level of CT are birdsfoot trefoil (*Lotus corniculatus*), white leadtree (*Leucaena leucocephala*), sainfoin (*Onobrychis viciifolia*), sulla (*Hedysarum coronarium*), sericea lespedeza, and *Pinus* spp. (Tiarks et al., 1989; Terrill et al., 1990; Aerts et al., 1999; McNeill et al., 1998; Albrecht and Muck, 1991).

Interaction of Condensed Tannins with Protein, Carbohydrate, and Mineral

Interaction of Condensed Tannins with Proteins

The molar mass of CT varies from 400 to 13,000. There are various factors that affect the binding ability of proteins with CT. Small peptides have a lower affinity in comparison to large peptides. A greater number of proline-contanining proteins have a higher affinity for tannins in comparison to those containing a lower number of prolines. In CT, hydrogen bonds are the bonds that are formed between phenolic hydroxyl and peptide carbonyl. The greater the number of hydrogen bonds, the stronger the binding ability of CT with proteins. Compact bound globular proteins have a lower affinity in comparison to loosely bound globular proteins. The binding of

protein to CT also depends on the pH. Precipitation of proteins by tannins will be greater at pH 4.9 than pH below or above this value. The pH-dependent binding affinities of a protein with CT verify that the binding of the protein takes place in the rumen. Binding will not occur at pH more than 8.0 or below 3.0. A lower pH in the abomasum frees the bound protein from the CT. This leads to increased intestinal absorption of protein from CT-fed ruminants. Surprisingly, decreased nitrogen availability in the digestive tract was seen in sericea lespedeza-fed cattle (Jones and Mangan, 1977; Hagerman and Butler, 1978; Hagerman et al., 1981; Martin et al., 1983; Waghorn et al., 1987; Eckerle et al., 2011). Browsers, like goats and deer, secrete proline rich protein in their saliva, giving these animals a higher ability to tolerate CT in comparison to other grazing ruminants (Austin et al., 1989; Mole et al., 1990). However toxic signs related to CT have not yet been reported in other ruminants. Condensed tannins found in different plants also vary in chemical structures and their binding affinity for protein (Asquith and Butler, 1986). Studies also have shown decreased protein utilization in high-CTcontaining plants in comparison to low-CTcontaining plants (Donnelly et al., 1971). Forages that contain a high level of CT in the diet reduce the efficiency of degradation of plant protein by affecting rumen microorganisms (Aerts et al., 1999). The effect of tannins on rumen bacteria depends on the concentration of CT and the type of bacteria present. Condensed tannins purified from sainfoin (*O. vicifolia)* inhibit growth and protease activity of *Butyrivibrio fibrisolvens* and *Streptococcus bovis* when given at a concentration of 200 µl of CT/ml. However, this effect was not seen at a concentration of 100 µl of CT/ml. Some strains of bacteria even grow in numbers with 600 µg of CT/ml (Jones et al., 1994). Studies on interactions of rumen microbes with CT and its effect on protein digestion are rare.

Condensed Tannins and Carbohydrate Interaction

Condensed tannins has a big impact on rumen microbial populations (Aerts et al., 1999). Sulla decreased the proportion of iso-butyrate and n-valerate in the rumen (Terrill et al., 1992). Like proteins, a decreased in carbohydrate digestion is due to the level of CT in the feed. Lower digestibility of carbohydrates was be seen in animals that are fed higher levels of CT in their diet. Decreased digestion of hemicelluloses and readily digestible carbohydrates in the rumen occured in sheep fed high-CT-containing *Lotus pedunculatus* (Barry and Manley, 1984). Higher dry matter and organic matter digestibility are reported in steers fed sericea lespedeza containing a lower level of CT in comparison to those containing a higher level of CT (Donnelly et al., 1971). Similar results were observed when sheep were fed *L. pedunculatus* containing a higher concentration of CT in the diet (Barry et al., 1986).

Condensed Tannins and Mineral Interaction

Studies have shown that a reduction of mineral absorption occurs in animals fed with a diet containing a high level of CT. Absorption of minerals decreases due to chelation of metal ions with CT. A study done with kernels of walnut, hazelnuts and almonds that are rich in CT showed that the affinity of chelation of metal ions with CT decreases in the order of Cu (II) > Fe (II) > Zn (II) (Karamac, 2009). A decrease in the absorption of calcium, but not magnesium was noticed in rabbits fed CT containing sorghum (Al-Mamary et al., 2001). The pH also plays an important role in the affinity of CT and absorption of certain metal ions. Metal salts of tannic acid have unique values for dissociation. Tannic acid with Mg dissociates at pH 7.0; Ca at 6.6; Zn at 5.2; Mn at 4.2; Co at 4.0; Cu at 3.7; Al at 3.2 and Fe^{3+} at 2.0. Most of the metal salt complexes could dissociate either in the rumen or abomasum. However iron-condensed tannin

complex can traverse the abomasum (pH 2.5 – 3.0) without dissociation. Hence iron could be less available in animals fed CT rich diet (Faithfull, 1984).

Interference in the absorption of minerals also depends upon the type of phenolic compounds. Iron ions form a complex with CT by forming Fe-galloyl and Fe-catechol complexes (Brune et al., 1991). Gallic acid forms stronger complexes with ferric ion in comparison to catechin (Brune et al., 1989).Tannic acid contains 8-10 molecules of gallotannins. These are widely studied in human and other monogastric animals for their role of reducing absorption of minerals (Marzo et al., 2002). Gallotannins found in plants have higher molecular weight than commercially available tannic acid. High molecular weight gallotannins can bind with protein and remain unhydrolyzed. However, low molecular weight tannins are hydrolyzed in the gut (reviewed by Hagerman et al., 1992). Lambs fed sainfoin showed a reduced absorption of Ca, P, Mg, and Na (Scharenberg, 2007). Similarly a three year study done at USDA, Agricultural Research Station (ARS) in Booneville, AR and Louisiana State University, Baton Rouge showed a reduction of trace minerals, mainly molybdenum, zinc, selenium and manganese in serum of lambs were fed sericea lespedeza (J. M. Burke and J. E. Miller, unpublished data). In this research, molybdenum was reduced as much as 90-fold in serum of sericea lespedeza-fed sheep in comparison to control fed sheep (J. M. Burke and J. E. Miller unpublished data). Decreased plasma concentrations of some mineral ions may also depends upon the competitive interaction of one metal ion with another.

1.8. Role of Some Trace Minerals Biological Function in the Body

Cobalt

Cobalt is an essential component of Vitamin B_{12} (Barker et al., 1958). Vitamin B_{12} is a cofactor for the enzymes methionine synthase and methylmalonyl Coenzyme A mutase

(Kennedy et al., 1990). Methionine synthase catalyzes the conversion of homocysteine to methionine. Methylmalonyl-CoA mutase converts methylmalonyl-CoA into succinyl-CoA and hence plays a crucial role in propionate metabolism (Kennedy et al., 1991; Marston et al., 1961). Cobalt deficiency in ruminants leads to rough hair coat, muscular wasting, weight loss and death (Grace and Sinclair, 1999; Judson et al., 1982).

Copper

Copper is an essential component for the synthesis of various enzymes in the body, including cytochrome-*c* oxidase, tyrosinase, ceruloplasmin, lysyl oxidase, superoxide dismutase, and dopamine β-monoxygenase (Reviewed by Bonham et al., 2002). Cytochrome-*c* oxidase plays an important role in the electron transport system (Reviewed by Brunori et al., 1987). Superoxide dimutase is required for the antioxidant defense in the cells exposed to oxygen (McCord et al., 1969).Tyrosinase catalyzes the production of melanin from tyrosine by oxidation (Reviewed by Brichard et al., 1993). Ceruloplasmin is a enzyme containing is a six copper atom. It helps in the oxidation of ferrous iron to ferric iron. Transferrin binds iron only in the ferric state, and finally oxygen is transported to the cells (Goldstein et al., 1979). Lysyl oxidase is essential for stabilization of the collagen fibrils and for integrity and elasticity of the mature elastin (Smith-Mungo and Kagan, 1998). Dopamine-beta-monooxygenase is an important enzyme for the hydroxylation of dopamine to nor-adrenaline (Goodall and Kirshner, 1957). Nor-adrenaline is an important neurotransmitter.Clinical signs related to copper deficiency are anemia, severe diarrhea, change in hair color, neonatal ataxia, infertility, heart failure, fragile bones and depressed growth (Reviewed by Gooneratne et al., 1989).

Iron

Iron is an essential component in hemoglobin (Stockman, 1893) and myoglobin (Dallman et al., 1965). It also is an essential component of the enzymes cytochrome (Dallman et al., 1965), catalase (Summer and Alexander, 1937) and peroxidase (reviewed by Poulos and Kraut, 1980). Haemoglobin is essential for the transport of oxygen in the blood whereas myoglobin is the oxygen-carrying pigment in the muscle tissue (Scholander, 1960). Cytochromes are membrane-bound proteins responsible for the generation of ATP via electron transport (Griffiths and Wharton, 1961). Catalase decomposes hydrogen peroxide to water and oxygen (Keilin and Hartree, 1938). This way it protects the cell from oxidative damage by reactive oxygen species. Peroxidases are thought to remove oxidizing agents from the body (reviewed by Poulos and Kraut, 1980). Deficiency of iron in ruminants leads to anemia, lowered feed intake, and chronic blood loss during parasitic infestation (Hibbs et al., 1963; Blaxter et al., 1957).

Manganese

The enzyme, pyruvate carboxylase requires manganese during conversion of pyruvate to oxaloacetate. This is an essential reaction for the metabolism of lipids and carbohydrates (Scrutton et al., 1966; Scrutton et al., 1972). Manganese is a cofactor of the enzyme superoxide dimutase (MnSOD). This enzyme together with the metalloprotein CuZnSOD helps in protecting cells from damage caused by reactive oxygen radicals (Okado-Matsumoto and Fridovich, 2001). Manganese is also essential for activation of the enzyme glycosyltransferases. This enzyme catalyzes the synthesis of glycosaminoglycans and glycoprotein (Reviewed by Lairson et al., 2008). Deficiency of manganese in ruminants leads to skeletal abnormalities, such as twisted legs, enlarged joint, weak bones. In mature ruminants manganese deficiency leads to irregular estrus, low conception rate, stil births and low birth weight (Reviewed by Hidiroglou, 1979).

Molybdenum

Molybdenum is an essential component of the enzyme xanthine oxidase (de Renzo et al., 1953). Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine. This enzyme is required for the catabolism of purine (Edwards et al., 1979). Molybdenum also is an essential cofactor for the enzyme sulfite oxidase which is required for the metabolism of sulfur-containing amino acids (Mudd et al., 1967). Little is known about the effects of low molybdenum in sheep and goats, except when withheld from the diet of goats over a long period of time; depressed growth, impaired reproduction, and eventual death occurred (Anke et al., 1978). Excess molybdenum can lead to a copper deficiency and too little molybdenum can lead to excess copper.

Zinc

The active sites of most carbonic anhydrases contain zinc (Todd et al., 1933). These enzymes are required for the interconversion of carbon dioxide to water and bicarbonate (Keilin and Mann, 1940). Zinc also is required for the regulation of genes that are required for signal transduction, response to stress, growth, and energy utilization (Cousins et al., 2003). Zinc plays an important role in the activation of the enzyme phospholipase A_2 (Lindahl and Tagesson, 1996). Phospholipase A_2 hydrolyses phosphatidylcholine, facilitating the absorption and the formation of chylomicrons that are necessary for the absorption of micelles (Noh and Koo, 2001). The enzyme superoxide dimutase contains zinc, which is necessary to protect cells from superoxide radicals (Zago and Oteiza, 2001). Deficiency of zinc leads to a reduction of testicular growth, stunted growth, lower reproductive performance, slow healing of wounds, hair loss and tissue lesions (reviewed by Miller, 1970).

Selenium

Selenium is a component of glutathione peroxidase which plays an important role in the metabolism of lipid hydroperoxides and hydrogen peroxide (Rotruck et al., 1973). The selenium containing enzyme deiodinase converts thyroxine to triiodothyronine. This is necessary to maintain basal metabolic rate (Behne et al., 1990). Thioredoxin reductase regulates transcription (Korhle et al., 2005), recylces vitamin C and vitamin E, and absorbs calcium (Moreno-Reyes et al., 2006). Deficiency of selenium leads to compromised immune function, unthriftiness, weight loss and diarrhea (reviewed by Wichtel et al., 1998).

1.9. Summary and Proposed Objective

Gastrointestinal parasites are a major contraint for the production of sheep and goats worldwide. Gastrointestinal nematodes developed resistance to all available anthelmintics, and due to the increasing demand for organic and 'natural' reduced chemical residue in meat, there is a continued need to study organic methods of parasite control. Forage plants like sericea lespedeza are very effective for the control of GIN and coccidia spp. However, recent studies have shown a decrease in weight gain by lambs kids fed SL, and a reduction of serum concentrations of molybdenum and other trace minerals. Molybdenum is an important co-factor in the integral enzyme complex that could be important in weight gain. It is important to know the relationship between molybdenum and weight gain in sericea lespedeza-fed lambs.

Therefore the major objectives of the studies presented in this thesis are: effect of incorporating 75% of alfalfa or sericea lespedeza leaf meal pellets on

1. changes in serum and liver concentrations of trace minerals;
2. hematological and serum biochemical parameters and

3. fecal egg count and weight gain in lambs fed sericea lespedeza and administrated molybdenum.

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Chapter III: Changes in Trace Minerals in Lambs Fed Sericea Lespedeza and Administered Sodium Molybdate

Abstract:

Prolonged feeding of sericea lespedeza (**SL;** *Lespedeza cuneata*) led to reduced serum concentrations of molybdenum, a co-factor in an enzyme complex that may contribute to weight gain. The objective of this study was to determine the effect of Mo supplementation on changes in body weight (**BW**), and serum, fecal, urine and liver concentrations of trace minerals (**TM**) in lambs fed SL. Lambs weaned in May (84 ±1.5 d of age; 27 ± 1.1 kg) were blocked by BW and parasite resistance, and assigned randomly to be fed 900 g of an alfalfa based (**CON**; $n = 10$) or **SL** based supplement ($n = 20$) for 103 d. Within the SL group, half of the lambs (**SL+MO**) were administered 163.3 mg of sodium molybdate per lamb three times/wk to ameliorate a reduction in serum molybdenum observed previously. Supplements were isonitrogenous, isocaloric, and similar in TM. Lambs were weighed and body condition scores (**BCS**) determined every 14 d. Serum was collected on d 28, 56, and 103, urine and feces on d 56, and a liver biopsy on d 104 to determine concentrations of TM (inductively coupled plasma-atomic emission spectroscopy). A mixed model was used for data analysis. Molybdenum (**Mo**) drench increased ($P < 0.001$) serum, liver, and urine Molybdenum (**Mo**) to that of CON lambs, while that of SL lambs without Mo was markedly reduced. Fecal Mo was greatest ($P = 0.02$) in SL+MO followed by SL then CON lambs. Serum concentrations of cobalt and zinc were reduced (**Co**; $P = 0.006$; **Zn**; $P < 0.001$) in SL compared with CON lambs, and Copper (**Cu**) was greatest ($P < 0.001$) in CON followed by SL then SL+MO lambs. Liver concentrations of Cu, Mo, selenium (**Se**), and Zn were reduced ($P < 0.05$) in SL compared with CON and SL+MO lambs. Fecal Cu, iron (**Fe**), Zn, and Co were reduced ($P = 0.01$) in SL compared with CON lambs, and manganese (**Mn**) was reduced ($P = 0.03$) in SL+MO compared with CON lambs. Urinary Co was reduced in SL compared with

CON lambs (P = 0.03). There tended to be a reduction (P = 0.06) in BW in SL compared with CON fed lambs, but BCS was similar (P = 0.83) between diets. Changes in TM of lambs, especially copper Cu, Mo, Se, and Zn could explain poor production reported in previous experiments.

Key words: sericea lespedeza, lambs, molybdenum, trace mineral

Introduction

Sericea lespedeza [**SL**, *Lespedeza cuneata* (Dum.-Cours. G. Don)] is a warm-season perennial legume containing a high concentration of condensed tannins (**CT**; Donnelly et al., 1971; Powell et al., 2003). Desirable characteristics of SL include adaptability to infertile soils, readily grazed by both sheep and goats (Min et al., 2004), and anti-bloat (Puchala et al., 2005) and anti-parasitic properties (Shaik et al., 2004; Min et al., 2004). Both hay and leaf meal pellets of the AU Grazer variety were reported to reduce gastrointestinal nematodes (**GIN**) in small ruminants, specifically *Haemonchus contortus* (Shaik et al., 2004; Lange et al., 2006; Terrill et al., 2007), and coccidia in lambs (Burke et al., 2013). However, prolonged grazing of SL or feeding SL leaf meal pellets of (> 56 d) decreased weight gain in lambs and kids (Burke et al., 2012; Burke et al., 2014).

The CT-protein complex bypasses the rumen and dissociates in the abomasum and intestine (Barry et al., 1986; Waghorn et al., 1987). Thus, greater N absorption occurs post-ruminally (Barry et al., 1986). The CT not only binds to protein, but also to carbohydrates and minerals (reviewed by McSweeney et al., 2001). Several authors have reported binding of commercial tannins and CT with minerals (Disler et al., 1975; Pritchard et al., 1992; Silverstein et al., 1996). Decreased mineral availability could be the cause for the decreased weight gain in SL-fed lambs if the CT-mineral complex does not dissociate in the abomasum.

Changes in macro-minerals in animals fed a CT rich diet were not consistent (Chang et al., 1994; Waghorn et al., 1994; Scharenberg, 2007), and in lambs and goats fed SL (J.M. Burke and J.E. Miller, unpublished data). A reduction in serum concentrations of trace minerals, mainly Mo, Mn, Zn, and Se, occurred in lambs fed CT-rich SL pellets. A marked reduction in Mo occurred in SL-fed sheep and goats compared with control animals, with as high as 90-fold

reduction (J. M. Burke and J. E. Miller, unpublished observation). Molybdenum is an essential component of enzyme complexes (de Renzo et al., 1953) that could be important to growth and other physiological functions.

Because of the popularity of grazing SL or feeding SL hay or pellets, which are both commercially available, for an aid in the control of parasites, it is important to understand potential limitation to production of small ruminants. Therefore, the objective of this study was to determine the effect of Mo supplementation on changes in body weight (**BW**) and serum and liver concentrations of trace minerals (TM) in lambs fed SL.

Materials and Methods

All animal procedures were approved and by the Institutional Animal Care and Use Committee of the Agricultural Research Service (protocol # USDA-ARS – 74-F-002).

Location

The trial was conducted at the USDA, Agriculture Research Service, Dale Bumpers Small Farms Research Center in Booneville, AR (35°N, 94°W) from May 8-August 21, 2013. Average rainfall per month during the trial was 113 mm. Average maximum and minimum temperature during the trial was 30ºC and 18ºC, respectively.

Animal Procedure

Thirty Katahdin (n = 14) or ¼ Romanov × ¾ Katahdin (n = 16) ram lambs weaned in May (84 ± 1.5 d of age; 27.2 ± 1.1 kg) were used. Lambs were blocked by breed, BW, and estimated breeding value for parasite resistance, and assigned randomly to be fed: 900 g of alfalfa based supplement (**CON**; n = 10), or a SL based supplement (n = 20). Supplements were balanced for energy, protein, minerals and vitamins (feed ingredients for CON and SL diets are listed in Table 1; mineral concentration of alfalfa or SL pellets used are listed in Table 2; mineral

concentrations of mixed supplements are listed in Table 3). Diets were balanced to meet moderate gain according to NRC (2007). Two lots of SL pellets (Sims Brothers, Inc., Union Spring, AL) were mixed and used for the first 5 wk, and another lot of SL pellets were used for the last 10 wk. Supplements were mixed at the University of Arkansas feed mill (126 kg per mixing, which occurred every 14 d). The first day of dietary treatment was considered d 0. Within the SL group, lambs were administered either water alone or 163.3 mg of sodium molybdate per lamb (mixed with 5 mL of water; Sodium Molybdate Dihydrate, North Metal & chemical Co, York, PA) by syringe on Monday, Wednesday, and Friday (n = 10/drench). The concentration of Mo in sodium molybdate was 39%; thus, the target dose of Mo was 27.3 mg/lamb daily. The dose was based on a previous study in which 13.6 mg Mo/d (van Ryssen, 1994) was administered to lambs daily to increase serum concentrations in SL fed lambs, but only a slight increase occurred (J. E. Miller unpublished data). Lambs grazed one of four 0.34 ha plots containing predominantly tall fescue (*Festuca arundinacea*; n = 2 replicate/diet) and rotated among plots every two wk to minimize plot effect. Water was always available and no trace mineral mix was offered as it was included in the supplement. Lambs were dewormed with a combination of albendazole (15 mg/kg BW; Valbazen, Pfizer Animal Health, Exton, PA) and moxidectin (0.4 mg/kg BW; Cydectin Fort Dodge Animal Health, Fort Dodge, IA) on d 56, which failed to reduce fecal egg counts (44.2% reduction). Hence lambs were dewormed again on d 70 with levamisole (12 mg/kg BW, AgriLabs, St. Joseph, MO) and fecal egg count reduction was 98.8%. Lambs were treated with sulfamethoxine (55 mg/kg BW; SulfaMed-G, Bimeda, Le Sueur, MN) if signs of coccidiosis were present (watery diarrhea), which occurred in 1 lamb each from SL and SL+MO group.

Mineral Analysis of Alfalfa and SL Pellets and Mixed Dietary Supplement

Random samples of pellets and mixed supplements were grabbed from bags, and ground to pass through a 1 mm screen in a Thomas-Wiley laboratory mill model 4 (Arthur H. Thomas Co. Philadelphia, PA). Samples were then shipped to the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI for mineral analysis. An Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc, Santa Clara, CA) was used for the analysis of TM in feed samples as described by Wahlen et al. (2005).

Forage and Feed Quality Forage Availability

Forage quality was analyzed every 2 wk between June and August (Table 4). Ten random grab samples per plot were combined. Alfalfa and SL pellets collected for mineral analysis also were used to determine feed quality. Forage samples were dried at 22°C for 24 to 72 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F). Dry matter was determined, and samples were sent to the University of Arkansas Agricultural Diagnostic Service Laboratory (Fayetteville, AR) for analysis. Both the forage and pellet samples were ground to pass through 1 mm screen in a Thomas-Wiley laboratory mill model 4 (Arthur H. Thomas Co., Philadelphia, PA), subsampled, and ground through a 0.5mm screen using Cyclotec mill (Tecator 1093, Hoganas, Sweden). Nitrogen content was determined by combustion method, using the Elementar Rapid NIII combustion N analyzer (Elementar Americas Inc.; Mount Laurel, NJ). Crude protein was determined from N content through multiplication by 6.25 and expressed as percentage of forage DM. Acid detergent fiber and NDF were measured with a fiber digester (Labconco, USA) following Goering and Van Soest (1970) procedures. Forage availability was measured using disc method. For this forage mass beneath the 0.218 m^2 disc was clipped,

collected, weighted and dried at 37.7°C for 24 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F). Dry matter was determined calculate kg/ha.

Condensed Tannins

Ground SL pellets and the CON supplement were analyzed for extractable, protein-bound, and fiber-bound CT content by the Terrill et al. (1992) method using purified SL tannins as the standard (courtesy of B. Lambert, Tarleton State University/Texas A&M AgriLife Research, Stephenville).

Serum Trace Mineral Analysis

Blood was collected every 28 d by jugular venipuncture into 6.0 mL trace element serum tubes (Bectin Dickson, Franklin Lakes, NJ). Blood was allowed to clot for 3 h at room temperature and then centrifuged (Beckman Coulter T J6 refrigerated centrifuge, Fullerton, CA) at 1000 ×*g* for 20 minutes. Serum was collected and stored at 2°C until shipped on ice-packs to the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI for TM analysis. An Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc., Santa Clara, CA) was used for the analysis of serum concentration of TM as described by Wahlen et al. (2005).

Liver Concentrations of Trace Minerals

Fifteen milligrams of liver was collected using a Tru Cut biopsy needle (LVWR Scientific Products Corp., Seattle, WA) inserted between the 11^{th} and 12^{th} rib diagonally between the tuber coxae and the olecranon process, after clipping hair and administering lidocaine (20 mg/mL; Lido-epi, Radix Labs, Eau Claire, WI). Tissue was placed in 6.0 ml trace element serum tubes (BD, Franklin Lakes, NJ). Samples were kept refrigerated until shipped on ice-packs to the

Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI for trace mineral analysis as described for serum.

Fecal and Urine Trace Mineral Analyses

Fecal and urine samples were collected on d 56. Fecal samples were collected directly from the rectum and dried at 22°C for 16 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F). Samples were ground and shipped to the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI. Urine was collected from the individual animal in 120 mL container. Ten milliliters of each sample then was transferred into TM free vector test tubes (BD, Franklin Lakes, NJ). Then 200 µL of 30% HCl was added. Urine samples were then freeze dried at -20°C and sent to Michigan State University. An Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc., Santa Clara, CA) was used for the analysis of fecal and urine concentrations of TM as described by Wahlen et al. (2005).

Body Weight and Condition

Body condition score (**BCS**) and BW were determined every two wk between d 0 and 112. The degree of fat covering in the lower lumber area was assessed and scored on a scale of 1 to 5 (1 = emaciated; 5 = obese; detail regarding the assessment of BCS is given in Table 5). Body weight was determined without fasting using a Gallaghar scale (130 West 23rd Av. North Kansas City, MO).

Statistical Analyses

Serum concentrations of TM, BW, and BCS were analyzed as repeated measures (Littell et al., 1996) using mixed model with compound symmetry covariance structures (SAS Inst. Inc., Cary, NC). General linear model (SAS) in a completely randomized design were used to

determine differences in liver, fecal, and urine concentrations of TM. Two models were used. Variables in the first model included diet (CON, SL), MO administration (yes or no), breed type, day (repeated measures), and interactions. If *P* value of the interaction was > 0.10, it was dropped from the model. Variables in the second model included treatment (CON, SL, SL+MO), breed type, day (for repeated measures), and interactions. A covariate using the initial BW ($P < 0.001$) and BCS ($P < 0.001$) was used. Outliers (more than two SD) of TM values from individual lambs were removed from the data set. If an effect of MO administration was absent ($P > 0.10$), results from the first model (including diet, CON or SL) will be presented; otherwise the results from the second model (including treatment, CON, SL, SL+MO) will be presented for all analyses.

Results

Analysis of Condesned Tannins and Forage Availability

Extractable, protein-bound, fiber-bound, and total CT in the SL pellet fed to the lambs were 2.4, 1.6, 0.027, and 4.0 %, respectively. There was no detectable (< 0.05 %) CT in the CON supplement. This resulted in total CT consumption in SL supplemented lambs of 27 g/d. Forage availability in plot 1, 2, 3, and 4 at the end of the trial was 4563, 3585, 3121, 2174 kg DM per ha respectively.

Serum Concentration of Trace Minerals

Changes in serum concentrations of TM occurred between the dietary or treatment groups (Table 6). There was no effect of Mo administration ($P = 0.59$) on Co, but there was a diet × day ($P < 0.001$) effect in that Co was greater in CON than SL lambs on d 56 but were similar on d 0 and 112 (Figure 1). No other diet × time or treatment × time interaction were detected. Molybdenum administration reduced ($P = 0.001$), serum concentration of Cu increased ($P <$

0.001), Mo tended to increase (P = 0.08), Se but did not influence Fe, Mn, or Zn (Table 6). Molybdenum concentrations were greatest in SL+MO, intermediate in CON and lowest in SL. Serum concentration of Cu were greatest (P = 0.001) in CON lambs, lowest in SL+MO (P < 0.001) and intermediate in SL. Serum concentration of Se were similar between CON and SL+MO which were greater (P = 0.03) than SL lambs. Serum concentration of Zn were reduced (P < 0.001), in SL compared with CON fed lambs whereas concentrations of Fe tended to increase (P = 0.07) in SL compared with CON lambs, and concentrations of Mn were similar (P = 0.64; Table 6) between diets. There was no effect of breed type on serum concentration of TM except Se which was lower (P = 0.013), in crossbred lambs.

Liver Concentrations of Trace Minerals

Results in liver concentration of TM are presented in a similar way as mentioned for serum concentrations of TM. Changes in liver concentrations of TM occurred between the dietary or treatment group (Table 7). Molybdenum administration reduced (P = 0.07) liver concentration of Cu but increased (P < 0.001) Mo. There was no effect (P > 0.39) of Mo administration on Co, Fe, Mn, and Zn. Liver concentration of Cu were similar between SL and SL+MO which were lower than CON lambs. Molybdenum concentrations were similar between CON and SL+MO and were greater (P < 0.001) than SL lambs. Liver concentrations of Se and Zn were reduced (Se, P = 0.012; Se, P = 0.005) in SL compared with CON fed lambs. There was no effect (P > 0.12) of diet on liver concentrations of Co, Fe, and Mn. A breed effect was observed for Fe and Zn. Katahdin lambs tended (P = 0.09), to have lower Fe and higher Zn concentrations compared with crossbred lambs.

Fecal Concentrations of Trace Minerals

Changes in fecal concentrations of TM occurred between the dietary or treatments groups. Molybdenum administration increased ($P = 0.003$) fecal concentration of Mo, but did not influence ($P > 0.30$) Co, Cu, Fe, Mn, or Zn. Fecal Mo concentrations were greatest ($P = 0.02$) in CON, lowest in SL+MO and intermediate in SL lambs. Fecal concentrations of Co, Cu, Fe, Mn, and Zn were increased ($P < 0.001$) in CON compared with SL fed lambs. Katahdin lambs tended to have higher ($P < 0.01$) fecal concentrations of Co than crossbred lambs. Otherwise, no other breed effects were detected.

Urine Concentrations of Trace Minerals

Changes in urine concentrations of TM occurred between dietary or treatment groups. Administration of Mo increased ($P < 0.001$) urine concentrations of Mo but not ($P > 0.53$) Co, Mn, Zn or Se. Urine Mo concentrations were similar between CON and SL+MO, which were greater ($P < 0.001$) than SL lambs. Urine concentrations of Cu and Fe were lower than the detectable level. Urine concentrations of Co, Zn, and Se were greater (Co, $P = 0.01$; Zn, $P < 0.08$; Se, $P = 0.07$) in CON lambs compared with SL fed lambs. Katahdin lambs tended to have higher ($P = 0.03$) urine concentration of Se, but lower ($P < 0.09$) Zn concentration compared with crossbred lambs. No other breed effects were detected.

Body Weight and Body Condition Score

Administration of Mo did not influence ($P > 0.57$) BW or BCS. There tended to be an interaction between diet and time in that weight gain of SL lambs lowered ($P = 0.054$; Figure 2) in SL lambs compared to CON diet fed lambs. The BW was similar between breed type ($P = 0.98$), and BCS was similar between diets (2.8 ± 0.06; $P = 0.99$) and breed type ($P = 0.88$).

Discussion

This is the first experiment in which the concentrations of minerals in the diet were similar between control and SL fed lambs. Changes in TM described in the current experiment reflect an effect of an alfalfa compared with a SL diet, most likely associated with its CT. Additional Mo provided to the SL lambs increased serum and liver concentrations close to that of CON lambs, while that of SL lambs without supplemental Mo remained low. Reduced Mo was discovered in previous research in SL fed sheep and goats (J. M. Burke and J. E. Miller unpublished data). Little is known about the effects of low Mo in sheep and goats, except when withheld from the diet of goats over a long period of time; depressed growth, impaired reproduction, and eventual death occurred (Anke et al., 1978). Serum and liver concentrations of Mo were within the normal range as depicted by Herdt and Hoff (2011).

Molybdenum is essential in the enzyme complexes xanthine oxidase and sulphite oxidase (Schwarz et al., 2009). Molybdenum forms a complex as a metal cofactor or an enzyme complex to catalyze a redox reaction. Xanthine oxidase is important in purine degradation, catalyzing the oxidation of hypoxanthine via xanthine to uric acid, essentially detoxifying cells (Schwarz et al., 2009). Excess uric acid forms and urate is reduced in serum in humans who lack xanthine oxidase (Duran et al., 1978). Sulfite oxidase is a dimeric enzyme with a Mo domain and heme cofactors, and is important in cysteine metabolism (Schwarz et al., 2009).

Molybdenum is also important in Cu and sulfur interactions. There is an antagonistic relationship between Mo and Cu, whereby an excess of one leads to a deficiency of the other (reviewed by Suttle, 1991). This antagonism was displayed in Mo supplemented lambs in the current study as Cu was reduced in serum and liver compared with both the CON and SL lambs

without Mo. Interestingly, the low Mo in the SL fed lambs without Mo in the current study did not lead to an increase in Cu.

In previous research at ARS and LSU, Cu was reduced in some groups of SL fed lambs, but not consistently (Burke and Miller, unpublished). Serum concentrations of Cu in SL fed lambs in the current experiment, and often in ARS lambs (Burke et al., 2004; Burke and Miller, 2006), would be considered marginal or deficient (Herdt and Hoff, 2011). In the CON group in the current experiment, Cu would be considered adequate, likely because of the added copper sulfate in the supplement provided and lack of CT effect.

Manganese, Zn, and Se, all of which can complex as metalloprotein enzymes, were often reduced in SL fed sheep and goats (Burke and Miller, unpublished). In the current experiment, serum and liver concentrations of Zn and Se also were reduced in SL fed lambs, but not Mn. Selenium is an essential component of more than 12 enzymes and plays an important role in reproduction, immune function, and growth (NRC, 2007; Herdt and Hoff, 2011). Zinc is important in cell division, regulation of appetite and growth, and immune function. The homeostasis of both Mn and Zn is tightly regulated. Thus, the reduction of liver concentrations of Zn could be significant to the physiology of the SL fed animal.

Cobalt in serum was not consistently reduced in SL fed animals in previous research at ARS and LSU, which may have reflected different concentrations between control and SL supplements used. In the current experiment, serum concentrations of Co were above the normal range (Herdt and Hoff, 2011) on d 28 in both groups and on d 56 in the CON group. The lower Co in both dietary groups on d 112 could have been due to the amount of free or dietary source of the small cobalt carbonate added to the supplement. This suggests that changes in serum Co in the animal associated with SL diets are sensitive to concentrations of Co in the diet. However,

liver concentrations of Co were similar between diets, and liver is a better indicator of Co than serum (Herdt and Hoff, 2011).

Iron was similar between dietary groups in the current study and in the previous study at ARS and LSU. Similarly, Fe was similar between Zebu cattle fed a control or high CT forage (Yisehak et al., 2012).

It was of interest to understand the fate of dietary TM that were reduced in the serum or liver of SL fed relative to CON lambs. Mineral excretion by urine and feces may be correlated with mineral status of the animal, depending on the mineral (Suttle, 2010). For most TM (Co, Mn, Mo, Zn), results of urine were similar to serum concentrations, regarding diets or Mo supplementation. Numerical trends of urine concentrations of Se were similar to serum. Results of fecal concentrations of TM were similar to that of serum for Co and Zn. However, fecal Cu concentrations were similar between lambs supplemented with Mo or not, perhaps because of the timing of Mo administration to fecal collection. The most interesting relationship occurred between serum or liver concentrations of Mo and that of fecal or urinary Mo. While serum concentrations of Mo was markedly reduced in SL compared with CON lambs as previously described in ARS sheep and goats, as well as liver concentrations of Mo, very low concentrations were found in urine and there was greater fecal output by SL lambs. The low urine concentrations in SL lambs could indicate that lambs are utilizing much of the dietary sources of Mo, but the greater fecal Mo relative to CON lambs may indicate that Mo is still bound to CT without dissociation in the small intestine and much is being eliminated. Supplemental Mo in SL+MO lambs resulted in similar concentrations of Mo in serum, liver, and urine, but a greater concentration of Mo in feces for similar reasons as just described for the SL lambs.

It is not clear why serum and urine concentrations of Se would be lower in crossbred lambs, although liver concentrations were similar. The Romanov breed is more flighty than the Katahdin breed and each originates from different geographical areas of the world (Russia vs. the U.S. with a tropical influence of the St. Croix breed, respectively). It was observed that even the ¼ Romanov lambs retained similar behavioral traits as straightbred Romanov sheep.

Trends in lower BW gain after prolonged feeding (42 d in the current experiment) were consistent with earlier studies (Burke et al., 2012; 2014). The BCS, a subjective measurement that was considered good for this lamb class, was similar between dietary groups and not influenced by Mo supplementation.

In general administration of anthelmintics decreases fecal egg counts (FEC) and increases weight gain in lambs. Anthelmintic was administrated twice as first anthelmintic administration (mixture of albendazole and moxidectin) failed to reduce FEC (44.2 % reduction). Levamisole was administrated after a week interval which reduced 98.8% of the FEC. However, weight gain in both CON lambs and SL diet fed lambs slowed down after the administration of anthelmintics. Reasons underlining slow weight gain after anthelmintic treatment in the current study is unknown.

Conclusions

Diets balanced for CP, energy, and TM between control and SL groups of lambs allowed us to determine true changes in concentrations of minerals in the animal associated with the CT rich legume product. There was a clear reduction in Cu, Mo, Se, and Zn in SL compared with CON fed lambs. Supplemental Mo did increase serum and liver concentrations of Mo similar to that of CON lambs, but the additional Mo in SL lambs did not influence BW gain. The reduction of Mo in the liver of SL lambs was not as dramatic as that of serum, indicating that the lambs

may have met Mo requirements for Mo enzyme functions, at least for the duration of this experiment. Further studies could focus on the reduction of Cu that occurred, especially because it became deficient in the liver of SL fed lambs after 104 d of feeding.

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Table 1. Feed ingredient (expressed on % of DM basis; unless stated) used for experimental diet per batch.

	Diets			
Ingredients	SL[1]	SL[2]	CON[1]	CON[2]
Corn	1.76	1.78		
Soybean Hulls			16.4	16.4
Dehydrated alfalfa			76.3	76.3
Sericea lespedeza	75.5	75.0		
Soybean oil	1.5	1.5		
Soybean meal	9.0	9.0		
Cobalt(II) carbonate[3]			41	6
Molasses	5.0	4.9	5.0	5.0
Salt	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.4	0.6	0.6
Limestone	2.2	2.9		
Selenium premix	81	96	67	79
Potassium chloride	2.36	2.31		
Copper sulphate [4]	0.1	0.3	0.6	0.6
Manganese (II) sulphate [4]			4.5	6.7
Calcium Iodide [4]	0.1		0.1	0.1
Zinc sulphate [4]	2.2	2.4		
Vitamin ADE premix	0.20	0.18		
Vitamin E premix	1.05	1.05	0.80	0.80
Sodium molybdate [4]	0.22	0.32		
Total	100%	100%	100%	100%

[1]SL = Sericea lespedeza (75% SL; Sims Bros. Inc., Union Springs), used before wk 5.

[2]SL = 75% SL and 25% mixed ingredients, used after wk 5.

[1]CON= Control (75% alfalfa; Manzanola feeds; Manzanola, CO) used before wk 5.

[2]CON = 75% alfalfa and 25% mixed ingredients used after wk 5.

[3]Values expressed in milligram, used per batch, [4]values expressed in grams, used per batch.

Table 2. Concentration of macro-minerals (percent DM) and micro-minerals (µg/g) in diets.

Mineral[4]	SL[1]	SL[2]	SL[3]	CON
Ca	1.45	0.88	0.81	0.87
P	0.16	0.22	0.19	0.22
Mg	0.21	0.23	0.23	0.23
K	0.78	1.21	1.02	1.21
Na	0.05	0.03	0.05	0.03
S	0.37	0.29	0.32	0.29
Co	0.48	0.27	0.26	0.27
Cu	7.2	7.4	6.7	7.4
Fe	166	245	216	245
Mn	62.7	85.2	87.3	85.2
Mo	0.80	0.32	0.19	0.32
Zn	18.1	26.3	21.0	26.3
Se	0.07	0.08	0.08	0.08

[1, 2]SL pellets mixed in equal proportion to formulate SL feed supplement before wk 5. Alfalfa pellets as a control (CON) or sericea lespedeza (SL) leaf meal pellets used as ingredients in the diets. Pellets were ground and analyzed by inductively coupled plasma/mass spectrometry (Diagnostic Center for Population and Animal Health at Michigan State University).

[3]SL pellets used after wk 5.

[4]Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Co = Cobalt, Cu = Copper, Fe = Iron, Mn = Manganese, Mo = Molybdenum, Zn = Zinc, Se = Selenium.

Table 3. Mineral concentration of mixed supplement. Concentrations of macro-minerals (% DM) and micro-minerals (µg/g) in diet.

Mineral[1]	SL mixed	CON mixed
Ca	1.6	2.0
P	0.24	0.25
Mg	0.23	0.26
K	1.76	2.52
Na	0.25	0.36
S	0.35	0.34
Co	0.31	0.24
Cu	8.6	8.5
Fe	278	411
Mn	84.4	48.6
Mo	0.98	1.81
Zn	24	24
Se	0.10	0.12

[1]Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Co = Cobalt, Cu = Copper, Fe = Iron, Mn = Manganese, Mo = Molybdenum, Zn = Zinc, Se = Selenium. Control (CON) or sericea lespedeza (SL) supplements offered to lambs. Feed samples were ground and analyzed by inductively coupled plasma/mass spectrometry (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 4. Analyses of feed (alfalfa or sericea lespedeza pellets) or forage on DM basis for percentage crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF).

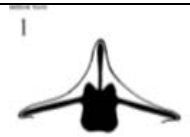
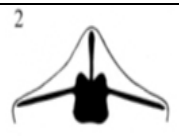
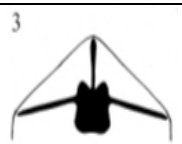
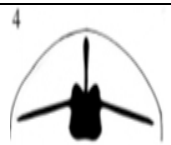
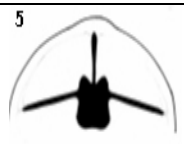
Date	Forage or pellets	CP	ADF	NDF
Forage				
June 12	Plot 1	11.00	35.97	57.47
	Plot 2	14.48	32.20	56.18
	Plot 3	14.19	32.68	5.3.07
	Plot 4	13.03	33.43	56.39
June 24	Plot 1	10.26	37.47	62.00
	Plot 2	12.56	35.42	57.63
	Plot 3	11.63	34.77	57.00
	Plot 4	10.07	37.66	60.43
July 18	Plot 1	16.59	28.79	59.08
	Plot 2	12.18	36.93	58.79
	Plot 3	10.44	38.57	64.05
	Plot 4	10.07	37.66	60.43
July 31	Plot 1	18.64	28.09	58.24
	Plot 2	13.49	33.37	54.49
	Plot 3	16.21	33.88	55.36
	Plot 4	14.24	36.36	62.51
August 14	Plot 1	14.68	32.74	59.58
	Plot 2	13.39	31.79	50.26
	Plot 3	12.27	33.74	56.20
	Plot 4	10.36	36.36	62.51
Pellets				
April 2013	Alfalfa	16.4	27.6	34.0
July 2012	SL[1]	14.3	23.8	32.7
July 2012	SL[2]	14.4	24.2	27.6
June 2013	SL[3]	15.3	25.8	30.2

[1,2]SL = sericea lespedeza mixed in equal proportion during the first 5 wk.

[3]SL = sericea lespedeza was used after wk 5 till the end of the trial.

Samples were ground and analyzed at the University of Arkansas, Agricultural Diagnostic Laboratory, Fayetteville, AR.

Table 5. Assessment of body condition score (BCS), was done on the basis of table below.

Body condition scores					
					
Spines	Sharp and form narrow ridge	Spines forms narrow ridge but points are rounded	Slightly elevated Vertebrae	Vertebrae could be felt only on pressure	Spines can be felt by pressing down firmly between fats
Transverse processes	Finger easily pass under	Smooth rounded, finger go under pressure	Smooth rounded. Fingers need hard pressure to find ends	Transverse process cannot be felt	Transverse process cannot be felt
Muscle	Very little	Medium depth	Muscle full	Muscle full	Muscle very full
Fat	No fat cover	Thin fat cover	Moderate fat cover	Fat cover thick	Fat cover dense

(Based on the information Available online http://www.lifetimewool.com.au/conditionscore.aspx retrived on 5th April, 2014)

Table 6. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), Mo administration (no or yes), and breed type (Katahdin or K; ¼ Romanov × ¾ Katahdin or R) on serum trace minerals concentrations.

Variables[1]	*n*	Co, ng/mL	Cu, µg/mL	Fe,µg/mL	Mn, ng/mL	Mo, ng/mL	Zn, µg/mL	Se, ng/mL
CON	10	2.3[a]	0.92[a]	138	3.6	11.2[b]	0.69[a]	156
SL	10	1.4[b]	0.74[b]	164	3.3	2.2 [c]	0.59[b]	143
SL+MO	10	1.6[b]	0.593[c]	158	2.5	15.8[a]	0.59[b]	152
SE		0.17	0.03	10	0.50	1.2	0.02	4.0
P =		0.001[2]	0.001	0.15	0.22	0.001	0.001	0.10
CON	10	2.37	0.85	135	3.18	18.07	0.69	162
SL	20	1.49	0.66	160	2.89	9.01	0.59	147
SE		0.15	0.03	9	0.39	1.1	0.02	3.5
P =		< 0.001[2]	< 0.001	0.074	0.64	< 0.001	< 0.001	0.009
No	20	1.86	0.83	150	3.42	6.69	0.64	149
Yes	10	1.99	0.68	145	2.65	20.37	0.63	159
SE		0.15	0.03	9	0.39	1.1	0.02	3.5
P =		0.59	0.001	0.69	0.22	< 0.001	0.79	0.08
K	14	1.95	0.76	148	2.18	13.79	0.63	160
R	16	1.91	0.75	147	1.61	13.27	0.64	148
SE		0.15	0.03	9	0.40	1.1	0.02	3.6
P =		0.83	0.66	0.88	0.62	0.72	0.40	0.013

[a-c]Means within column with different superscripts differ ($P < 0.05$). [2]Diet × day, $P < 0.05$.

[1]Co = cobalt, Cu = cupper, Fe = iron, Mn = manganese, Mo = molybdenum, Zn = zinc, and Se = selenium,

Values were expressed on least square mean, in lambs from samples collected on d 28, 56, 84, and 112. Serum was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 7. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), Mo administration (no or yes), and breed type (Katahdin or K; 1/4 Romanov × 3/4 Katahdin or R) on liver trace mineral concentrations.

Variables[1]	*n*	Co, ng/g	Cu, ng/g	Fe, ng/g	Mn, ng/g	Mo, ng/g	Zn, ng/g	Se, ng/g
CON	10	0.23	199[a]	358	12.8	5.4[a]	175[a]	9.2[a]
SL	10	0.22	83[b]	432	14.8	3.8[b]	120[b]	5.6[b]
SL+MO	10	0.24	44[b]	461	13.8	5.4[a]	127[b]	5.4[b]
SE		0.02	15	41	0.85	0.28	15	0.9
P=		0.61	< 0.001	0.21	0.26	< 0.001	0.02	0.003
CON	10	0.23	184	355	12.3	6.3	184	8.6
SL	20	0.22	63	446	14.3	4.6	123	4.9
SE		0.015	9	39	0.85	0.25	14	0.85
P=		0.7	< 0.001	0.12	0.14	< 0.001	0.005	0.012
No	20	0.22	140	394	13.8	4.6	147	7.3
Yes	10	0.24	106	407	13.2	6.3	160	6.1
SE		0.01	9	39	0.65	0.25	13.5	0.85
P =		0.39	0.07	0.8	0.4	< 0.001	0.52	0.37
K	14	0.22	136	359	13.4	5.6	166	7.3
R	16	0.23	111	442	13.2	5.3	139	6.5
SE		0.01	13	37	0.75	0.2	13	0.85
P =		0.7	0.16	0.09	0.8	0.4	0.08	0.66

[a-c]Means within column with different superscripts differ ($P < 0.05$)

[1]Co = cobalt, Cu = cupper, Fe = iron, Mn = manganese, Mo = molybdenum, Zn = zinc, and Se = selenium

Trace minerals expressed on least square means, in lambs from samples collected on d 104. Liver tissue was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 8. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), Mo administration (no or yes), and breed type (Katahdin or K; ¼ Romanov × ¾ Katahdin or R) on fecal trace mineral concentrations.

Variables	*n*	Co, ng/mL	Cu, µg/mL	Fe, µg/mL	Mn, ng/mL	Mo, ng/mL	Zn, µg/mL
CON	10	1045[a]	22.1[a]	1033[a]	217080[a]	2125[a]	145.9[a]
SL	10	552[b]	14.6[b]	735[b]	197220[ab]	3288[b]	58.6[b]
SL+MO	10	543[b]	13.8[b]	689[b]	185940[b]	4690[c]	55.7[b]
SE		38	0.8	76	7651	344	8.7
P =		0.001	0.001	0.007	0.02	0.001	0.001
CON	10	1061	22.01	1029	212842	2939	144
SL	20	547	14.24	712	191580	3974	57
SE		33	0.35	74	7533	313	9
P =		< 0.001	< 0.001	0.007	0.06	0.03	< 0.001
No	20	798	18.38	884	207150	2706	102
Yes	10	810	17.87	857	197272	4207	99
SE		33	0.7	74	7533	313	9
P =		0.80	0.60	0.80	0.3	0.003	0.8
K	14	858	18.7	918	205718	3776	100
R	16	751	17.7	823	198704	3138	101
SE		32	0.65	70	7130	297	9
P =		0.01	0.19	0.29	0.44	0.10	0.9

[a-c]Means within column with different superscripts differ ($P < 0.05$)

[1]Co = cobalt, Cu = cupper, Fe = iron, Mn = manganese, Mo = molybdenum, and Zn = zinc

Trace minerals were expressed on least square mean, in lambs from samples collected on d 56. Fecal was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 9. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), Mo administration (no or yes), and breed type (Katahdin or K; ¼ Romanov × ¾ Katahdin or R) on urine trace mineral concentrations.

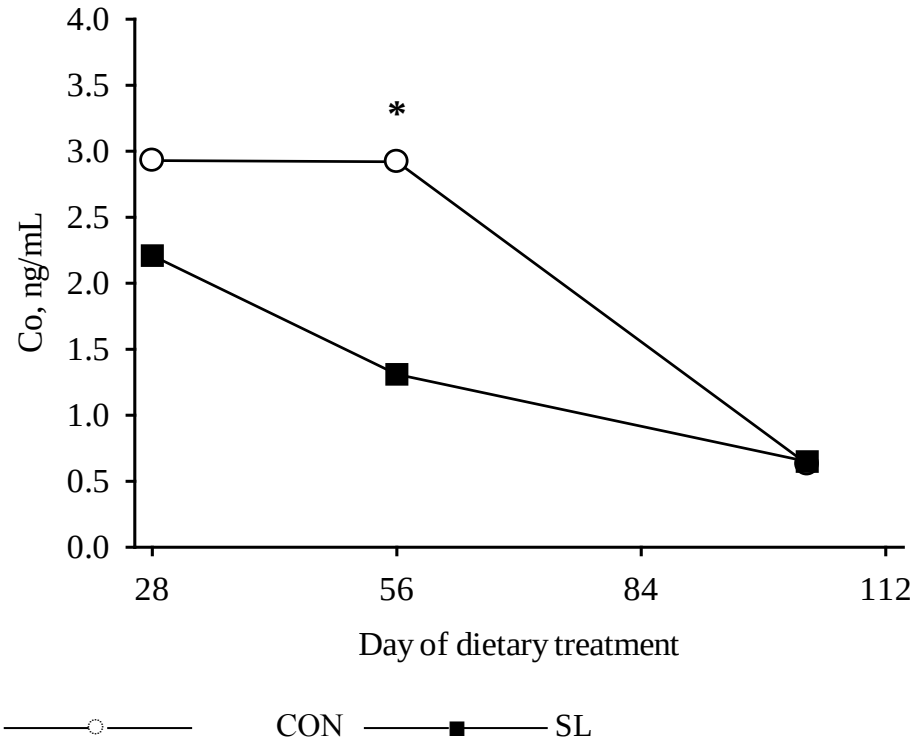
Variables	*n*	Co, ng/mL	Mn, ng/mL	Mo, ng/mL	Zn, µg/mL	Se, ng/mL
CON	10	7.2[a]	17.5	80.4[a]	0.43	1044
SL	10	2.4[b]	13.9	5.1[b]	0.16	728
SL+MO	10	2.4[b]	15.4	83.4[a]	0.11	730
SE		1.4	2.5	15	0.1	158
P =		0.03	0.60	< 0.001	0.06	0.28
CON	10	7.4	17.3	144	0.37	1121
SL	20	2.4	14.6	44	0.13	729
SE		1.3	2.4	14	0.1	145
P =		0.01	0.44	< 0.001	0.08	0.07
No	20	4.8	15.7	42	0.29	886
Yes	10	5.1	16.3	124	0.21	964
SE		1.3	2.4	14	0.1	145
P =		8.9	0.86	< 0.001	0.53	0.71
K	14	5.5	13.8	90	0.15	1116
R	16	4.3	18.2	76	0.35	734
SE		1.2	2.25	14	0.1	137
P =		0.45	0.14	0.44	0.09	0.03

[a-b]Means within column with different superscripts differ ($P < 0.05$).

[1]Co = cobalt, Cu = cupper, Fe = iron, Mn = manganese, Mo = molybdenum, Zn = zinc, Se = selenium.

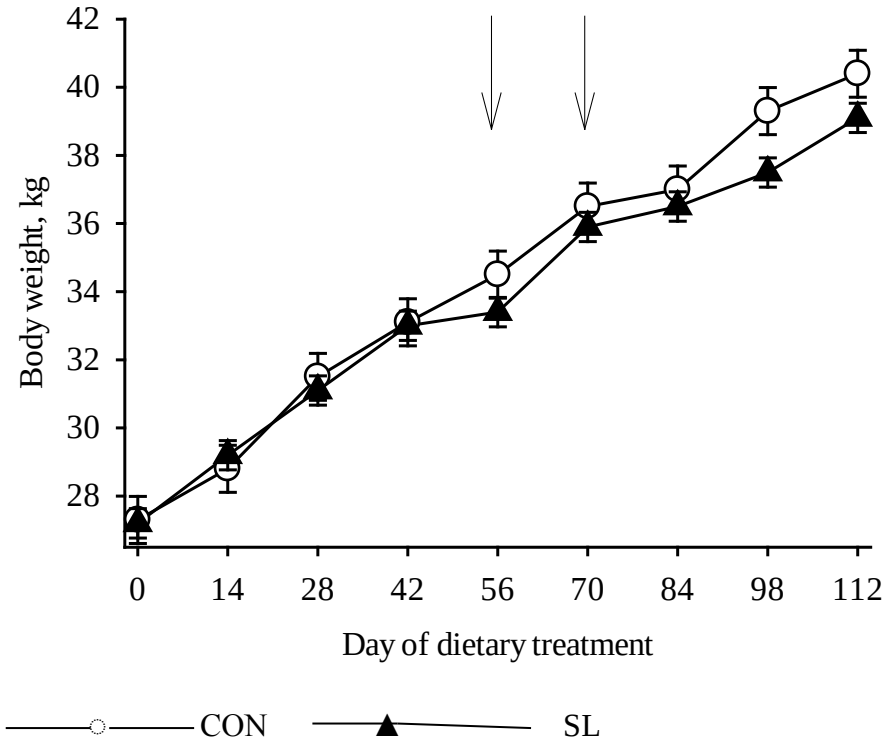
Trace minerals expressed on least square mean, in lambs from samples collected on d 56. Urine was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).

Figure 1.



Effect of diet (Control or CON, $n = 10$; sericea lespedeza or SL, $n = 20$) on serum concentration of cobalt (Co). Diet × day for serum concentration of Co, $P < 0.001$.

Figure 2.



Effect of diet (Control or CON, *n* = 10; sericea lespedeza or SL, *n* = 20). Arrows represent day of deworming, the first date failed to reduce fecal egg counts of lambs. Diet × day of on BW, *P* = 0.054.

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From: Sam Tabler, Chairman of USDA-ARS-Booneville IACUC

RE: Mohan Acharya Thesis Pre-Check

Dr. Charles Rosenkrans, Mohan Acharya, Dr. Joan Burke,

I write this letter in an effort to clear up any confusion concerning the USDA-ARS -IACUC approval for the graduate research project of Mohan Acharya.

The title of the project was "Improving lamb performance with serica lespedeza and molybdenum". The ARS – Booneville IACUC number for the project was USDA-ARS-74-F-0023. The ARS – Booneville IACUC met in April of 2013 to review and discuss the protocol of the study including the liver biopsy component. The committee consisted of the attending veterinarian, two other USDA-ARS employees, an unaffiliated member, and me. All five members approved the protocol. The research project timeframe was May 8, 2013 – August 20, 2013. All procedures and protocols that had been presented to the Booneville-IACUC were followed completely.

I hope this clears up any questions concerning Mohan Acharya's graduate project involving IACUC approval.

Sincerely,

Sam Tabler, Chairman USDA-ARS-Booneville IACUC

[redacted]

Chapter IV: Hematological and serum chemical profiles in lambs fed sericea lespedeza

Abstract:

Sericea lespedeza (**SL**; *Lespedeza cuneata*) is a plant grazed or fed to small ruminants for parasite control. Condensed tannins (**CT**) in SL may lead to unintended consequences such as changes in production. The objective of this study was to determine the effect of SL with or without molybdenum supplementation on changes in body weight (**BW**), hematology, and serum chemistry in lambs. Thirty ram lambs weaned in May (84 ±1.5 d of age; 27 ± 1.1kg) were blocked by BW, breed, and estimated breeding value of parasite resistance, and randomly assigned to be fed 900 g of alfalfa based supplement (**CON**; n = 10) or SL based supplement (n = 20) for 103 d. Supplements were isonitrogenous, isocaloric, and similar in trace mineral concentrations. Within the SL group, half of the lambs were administered 163.3 mg sodium molybdate per lamb of sodium molybdate three times a wk to ameliorate a reduction in serum molybdenum (**SL+MO**) observed previously. Lambs were weighed and body condition scores (BCS) determined every 14 d. Blood was collected every 14 d to determine hematological and serum chemical profiles. Data were analyzed using a mixed model with repeated measures. Blood packed cell volume (**PCV)**, and red blood cell count (**RBC)**, increased (P = 0.043) or tended to increase (P = 0.09) with Mo administration, and was greater (PCV, P = 0.09; RBC, P = 0.03) in CON and SL+MO than SL lambs. Mean corpuscular hemoglobin (**MCH**), and total protein (**TP**) decreased (MCH, P = 0.05; TP, P < 0.001) with molybdenum supplementation. Mean corpuscular hemoglobin was higher (P = 0.01) in CON and SL+MO than SL lambs. Total protein was higher (P < 0.001) in SL than CON and SL+MO lambs. White blood cell count (**WBC**; P = 0.05), percentage of neutrophils (P < 0.001), albumin (P = 0.006), blood urea nitrogen (**BUN**; P < 0.001), and creatinine (P = 0.004) were greater in CON than SL lambs.

Activity of aspartate aminotransferase (**AST)** was greater ($P < 0.001$) n SL than CON lambs. Mean corpuscular volume (**MCV)**, platelets, red cell distribution width (**RDW)**, percentage of lymphocytes and basophil, and serum concentrations of uric acid were similar ($P > 0.16$) among the diets. There was a treatment × time interaction for blood urea nitrogen (**BUN**) in that values were greater ($P < 0.001$) in CON lambs between d 14 and d 42 than SL fed lambs, and otherwise similar. Body weight tended to be reduced ($P = 0.06$) in SL compared with CON fed lambs, but BCS was similar ($P = 0.83$) between diets. Based on hematological and serum biochemical values, means of both groups appeared to be normal, though there were subtle but significant differences between dietary groups that may or may not be associated with reduced performance of SL lambs.

Keywords: hematology, serum chemistry, molybdenum, sericea lespedeza

Introduction

Sericea lespedeza [**SL**, *Lespedeza cuneata* (Dum.-Cours. G. Don)] is a warm-season perennial legume containing a large amount amount of condensed tannins (Donnelly et al., 1971; Powell et al., 2003). Sericea lespedeza can adapt to acidic soils with low fertility, and hence has potential for being a very useful low-input forage (Puchala et al., 2005). Other desirable characteristics of SL include readily grazed by both sheep and goats (Min et al., 2004) and has anti-bloat and anti-parasitic properties (Shaik et al., 2004; Min et al., 2004; Puchala et al., 2005). Both hay and leaf meal pellets of the AU grazer variety were reported to reduce gastrointestinal nematodes (**GIN**), especially *Haemonchus contortus*, and coccidia in lambs (Lange et al., 2006; Terrill et al., 2007; Burke et al., 2012).

Tannins are the polyphenolic compounds having high molecular weight and complex structure (Foo and Porter, 1981). They are plant secondary compounds found mainly in vacuoles and surface wax of the cell (Parham and Kaustinen, 1977).Tannins can broadly be subdivided into two groups: hydrolyzable and condensed tannins. Hydrolyzable tannins (**HT**) are those which can be hydrolyzed by water and tannases (Seikel et al., 1970). Gallotannins and ellagitannins are primary HT. Tannins which are not hydrolyzed in water or tannases are called condensed tannins (**CT;** William et al., 1986).

Hydrolyzable tannins are degraded by either acids or enzymes in the rumen (Nelson et al., 1995), and are absorbed through the gastrointestinal tract. There are several reports of toxic effects of feeding HT rich forage in ruminants (Nelson et al., 1995; Oliveira et al., 2010; Pérez et al., 2011). Toxicity of HT mainly depends upon percentage of HT in the diet, animal physiology, and continuous grazing on HT rich forages (Hervas et al., 2003; Lavrencic et al, 2006; Oliveira et al., 2010; Pérez et al., 2011). Condensed tannins, on the other hand, have high molecular size,

are less susceptible to hydrolysis, and are not readily absorbed. Studies regarding the systemic toxic effect of CT related forage in ruminant production are less clear, therefore knowing the toxic effect in ruminants consuming CT-rich diet is important. The objective of this study is to determine change in body weight (**BW**), hematology, and serum chemistry in lambs fed CT-rich SL pellets.

Materials and Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Agricultural Research Service (protocol # USDA-ARS – 74-F-002).

Location

Trial was conducted at the USDA-ARS, Dale Bumpers Small Farms Research Center in Booneville, AR (35^0N, 94^0W); from May 8-August 21, 2013. Average rainfall per month during the trial was 113 mm. Average maximum and minimum temperature during the trial was 30ºC and 18ºC, respectively.

Animal Procedure

Thirty Katahdin (*n* = 14) or ¼ Romanov × ¾ Katahdin (*n* = 16) ram lambs weaned in May (84 ± 1.5 d of age; 27.2 ± 1.1 kg) were used. Lambs were blocked by body condition score (**BCS**), BW, and estimated breeding value for parasite resistance, and assigned randomly to be fed: 900 g/d of alfalfa based supplement (**CON**; *n* = 10), or a SL based supplement (*n* = 20). Supplements were balanced for energy, proteins, minerals and vitamins (feed ingredients for CON and SL diets are listed in Table 1; mineral concentrations of alfalfa or SL pellets used are listed in Table 2; mineral concentrations of mixed supplements are listed in Table 3). Diets were balanced to meet moderate gains according to NRC (2007). Two lots of SL pellets (Sims Brothers, Inc., Union Spring, AL) were mixed and used for the first 5 wk, and another lot of SL

pellets were used for the last 10 wk. Supplements were mixed at the University of Arkansas feed mill (126 kg per mixing which occurred every 14 d). The first day of dietary treatment was considered d 0. Within the SL group, lambs were administered either water alone or 163.3 mg of sodium molybdate per lamb (mixed with 5 mL of water; sodium molybdate dihydrate, North Metal & chemical Co., York, PA) by syringe on Monday, Wednesday, and Friday (n = 10/drench) and remaining lambs with an equal volume of water. The concentration of molybdenum (**Mo**) in sodium molybdate is 39%; thus, the target dose of Mo was 27.3 mg/lamb daily. The dose was based on a previous study in which 13.6 mg Mo/d (van Ryssen, 1994) was administered to lambs daily to increase serum concentrations in SL fed lambs, but only a slight increase occurred (J. E. Miller unpublished data). Lambs grazed one of four 0.34 ha plots containing predominantly tall fescue (*Festuca arundinacea*; n = 2 replicate/diet) and rotated among plots every two wk to minimize plot effect. Water was always available and no trace mineral mix was offered as it was included in the supplement. Lambs were dewormed with a combination of albendazole (15 mg/kg BW; Valbazen, Pfizer Animal Health, Exton, PA) and moxidectin (0.4 mg/kg BW; Cydectin Fort Dodge Animal Health, Fort Dodge, IA) on d 56, which failed to reduce fecal egg counts (44.2% reduction). Hence, lambs were dewormed again on d 70 with levamisole (12 mg/kg BW, AgriLabs, St. Joseph, MO) and fecal egg count reduction was 98.8%. Lambs were treated with sulfamethoxine (55 mg/kg BW; SulfaMed-G, Bimeda, Le Sueur, MN) if signs of coccidiosis were present (watery diarrhea), which occurred in 1 lamb each from SL and SL+MO group.

Mineral Analysis of Alfalfa and Sericea Lespedeza Pellets and Mixed Dietary Supplement

Random samples of each pellet or mixed supplement were grabbed from bags, and ground to pass through a 1 mm screen in a Thomas-Wiley laboratory mill model 4 (Arthur H.

Thomas Co. Philadelphia, PA). Samples were then shipped to the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI for mineral analysis. An Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc, Santa Clara, CA) was used for the analysis of trace minerals (**TM**) in feed samples as described by Wahlen et al. (2005).

Forage and Feed Quality and Forage Availability

Forage quality was analyzed every two wk between June and August (Table 4). Ten random grab samples per plot were combined. Alfalfa and SL pellets collected for mineral analysis were also used to determine feed quality. Forage samples were dried at 22°C for 24 to 72 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F). Dry matter was determined, and samples were sent to the University of Arkansas, Agricultural Diagnostic Service Laboratory (Fayetteville, AR) for further analysis. Both the forage and pellet samples were ground to pass through 1 mm screen in a Thomas-Wiley laboratory mill model 4 (Arthur H. Thomas Co., Philadelphia, PA), subsampled, and ground through a 0.5mm screen using Cyclotec mill (Tecator 1093, Hoganas, Sweden). Nitrogen content was determined by combustion method, using the Elementar Rapid NIII combustion N analyzer (Elementar Americas Inc.; Mount Laurel, NJ). Crude protein was determined from N content through multiplication by 6.25 and expressed as percentage of forage DM. Acid detergent fiber and NDF were measured with a fiber digester (Labconco, USA) following Goering and Van Soest (1970) procedures. Forage availability as DM/ha was measured using a disc method. Forage mass beneath a 0.19 m^2 disc was clipped, collected, weighed and dried at 37.7°C for 24 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F).

Condensed Tannins

Ground SL pellets and the CON supplement were analyzed for extractable, protein-bound, and fiber-bound CT content by the Terrill et al. (1992) method using purified SL tannins as the standard (courtesy of B. Lambert, Tarleton State University/Texas A&M AgriLife Research, Stephenville).

Hematology

Blood samples were obtained by jugular venipuncture in 4 mL vacutainer tubes (Bectin Dickensen; Franklin Lakes, NJ, USA) containing an anticoagulant (K_2EDTA 7.2 mg). Samples were shipped in an ice-packed cooler to the USDA-ARS lab in Fayetteville, AR. Whole blood was analyzed within 6 h of collection for white blood cells (**WBC**), neutrophils, lymphocytes, monocytes, basophils, eosinophils, red blood cells (**RBC**), blood packed cell volume (**PCV**), hemoglobin (**Hb**), mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), mean corpuscular hemoglobin concentration (**MCHC**), red cell distribution width (**RCD**), and platelets, using hematological analyzer (CELL DYN 3500SL, System; Abbott, Abbot Park, I11U).

Serum Chemistry

Blood was collected every two wk for serum chemistry by jugular venipuncture. Corvac integrated serum separator tubes (TM Monoject Scientific) of 12.5 ml size, without anticoagulants, were used for blood collection. Blood samples were held in refrigerator for 3 h. Then tubes were centrifuged in a swinging bucket rotor (Beckman Coulter, Inc. Model TJ-6, South Kremer Boulevard Brea) at 1000 × *g* for 20 min. Serum was transferred to eppendorf tubes, and frozen at -20˚C. Samples were shipped on ice to the USDA-ARS lab, Fayetteville, AR, USA. An autoanalyzer (Express Plus; Ciba Corning Diagnostics Corp., Medfield, MA) with

appropriate reagent kits was used for the analyses of aspartate aminotransferase activity (**AST**), or concentrations of blood urea nitrogen (**BUN**; Bayer Heath care, Tarrytown, NY), albumin, uric acid (**UA**; Ciba Corning Diagnostic Corp, Oberlin, OH, USA), creatinine (Chiron Diagnostics Corporation, East Walpole, MA), and total protein (**TP**; Siemens Medical Solutions, Tarrytown, NY).

Body Weight and Condition

Body condition score and BW were determined every two wk. The degree of fat covering in the lower lumber area was assessed and scored on a scale 1 to 5 (1 = emaciated; 5 = obese; detail regarding the assessment of BCS is given in table 5). Body weight was determined without fasting using a Gallaghar scale (130 West 23rd Av. North Kansas City, MO).

Statistical Analyses

Blood leukocytes (WBC, neutrophil, lymphocyte, monocyte, basophil, and eosinophil), red blood cell parameters (RBC, PCV, Hb, MCV, MCH, MCHC, RCD, and platelets), and serum chemistry (albumin, AST, creatinine, TP, UA, and BUN), BW, and BCS were analyzed as repeated measures (Littell et al., 1996) using mixed models with an compound symmetry covariance structure (SAS Inst. Inc., Cary, NC). Log transformations were necessary to normalize data for neutrophil [ln(neutrophil)], lymphocyte [ln(lymphocyte)], monocyte [ln(monocyte × 10)], basophil [ln(basophil × 100)], MCV [ln(MCV)], MCH [ln(MCH)], platelets [ln(platelets)], AST [ln(AST)] and UA [ln(UA)]. Square-root transformations were necessary for eosinophil [sqrt(eosinophil × 100)], and RBC [sqrt(RBC)]. For transformed data, LS means were presented as back-transformed. Two models were usedfor all analysis. Variables in the first model included diet (CON, SL), Mo administration (yes or no), breed type, day (repeated measures), and interactions. If *P* value of the interaction was > 0.10 it was dropped from the

model. Variables in the second model included treatment (CON, SL, SL+MO), breed type, day (for repeated measures), and the interaction. A covariate using the initial BW ($P < 0.001$) and BCS ($P < 0.001$) was used. Outliers (more than two SD) of any hematology and biochemical value from individual lambs were removed from the data set. If the effect of Mo administration was absent ($P > 0.10$), results from the first model (including diet, CON or SL) will be presented; otherwise the results from the second model (including treatment, CON, SL, SL+MO) will be presented.

Results

Analysis of Condensed Tannins and Forage Availability

Extractable, protein-bound, fiber-bound, and total CT in the SL pellet fed to the lambs were 2.4, 1.6, 0.027, and 4.0 %, respectively. There was no detectable (< 0.05%) CT in the CON supplement. This resulted in total CT consumption in SL supplemented lambs of 27 g/d. Forage availability in plot 1, 2, 3, and 4 was 4563, 3585, 3121, 2174 kg DM per ha respectively.

Leukocyte Counts.

Changes in the WBC count occurred between the dietary or treatment groups (Table 6). There was no effect of Mo administration on any of WBC differential count. White blood cell counts ($P = 0.03$), percentage of neutrophils ($P < 0.001$), and eosinophils ($P = 0.08$) were reduced or tended to reduce in SL compared with CON fed lambs. There was no diet effect ($P > 0.33$), on percentage of lymphocytes, monocyte, or basophils. A breed effect was detected for percentage of lymphocytes and neutrophils in that Katahdins had a higher ($P < 0.001$) percentage of lymphocytes and lower neutrophils than crossbred lambs (Table 6).

Erythrocyte Parameters

Changes in RBC parameters occurred between the dietary or treatment groups (Table 7). Molybdenum administration increased or tended to increase ($P = 0.09$) RBC, plasma concentrations of Hb ($P = 0.09$), and PCV ($P = 0.04$), reduced ($P = 0.05$) MCH, but did not influence ($P > 0.19$) MCV, platelets, RCD, or MCHC. Red blood cell, and PCV, were similar between CON and SL+MO, which were greater (RBC, $P = 0.03$; PCV, $P = 0.09$) than SL lambs. MCH was higher ($P < 0.01$) in SL compared to CON and SL+MO. Hemoglobin and MCHC tended to be reduced (Hb, $P = 0.06$; $P = 0.09$) in SL compared to CON fed lambs. Red blood cells, Hb, PCV and MCV were greater ($P < 0.05$), in Katahdin lambs compared to crossbred lambs, whereas MCHC, and platelets were higher in ($P < 0.001$) crossbred lambs compared to Katahdin lambs.

Serum Biochemistry

Changes in serum biochemical parameters occurred between the dietary or treatment groups (Table 8). Administration of Mo reduced ($P < 0.001$) serum concentrations of TP but not other biochemical parameters. Total protein was similar between CON and SL lambs, which was greater ($P < 0.001$) than SL+MO lambs. Serum concentrations of albumin and creatinine were reduced (albumin, $P = 0.006$; $P = 0.004$) in SL compared with CON fed lambs, whereas AST activity increased ($P < 0.001$) in SL compared to CON lambs. Uric acid was similar ($P = 0.23$) between the diets. There was an interaction of diet and time for BUN, in that concentrations were initially reduced in SL compared with CON fed lambs, but similar after d 56 (Figure 1). Katahdin lambs had higher ($P < 0.05$), serum concentration of albumin, BUN, creatinine, TP, and UA compared with crossbred lambs. Activity of AST was higher ($P < 0.001$) in crossbred than Katahdin lambs.

Body Weight and Condition

Administration of MO did not influence (BW, $P = 0.74$; BCS, $P = 0.57$) BW or BCS. There tended to be an interaction between diet and time in that weight gain of SL lambs slowed ($P = 0.054$; Figure 2) than CON diet fed lambs. The BW was similar ($P = 0.98$), between breed type and BCS was similar ($P > 0.88$) between diets and breed type.

Discussion

Changes in hematological and serum biochemical parameters in the current experiment reflects the effects of an CON compared with SL diet, most likely associated with its CT. Concentrations of CT in the diet could impact changes in hematological values. In the current study and several others, increases or decreases in most hematological and serum biochemical parameters occurred in lambs and kids fed a CT-rich diet compared with those fed a CT-free diet (Woodward and Reed, 1997; Turner et al., 2005; Azuhnwi et al., 2013). Condensed tannins tended to decrease or decreased Hb level in the current study and in kids fed a CT rich shrub forage *Prosopis cineraria* (Bhatta et al., 2002). However, Solaiman et al. (2010) reported increased Hb in kids fed SL hay. This discrepancy in results could be due to a difference in the concentration of CT. Condensed tannin concentrations in SL in the current study was 4% on a DM basis, or more in the study reported by Bhatta et al. (2005); whereas, Solaiman et al. (2010) reported feeding 2.2%.

Red blood cells contain Hb that binds oxygen, transporting it from the lungs to body tissues for various metabolic functions. In the current study, supplementation of SL pellets decreased RBC and Hb; however the mechanism by which CT decreases RBC count is unknown. Further investigation is needed to determine the suppressive effect of a CT-rich diet on haematopoietic stem cells or on the process of maturation of red cells.

The MCV, MCH, and MCHC are related to individual red blood cells and are important for the diagnosis of anemia. Mean corpuscular volume is the average volume of red blood cells. Increased MCV may be seen in anemic conditions or when there are a large number or immature RBC. In the current study, an increase in MCV occurred in SL lambs compared with CON fed lambs. It may be that CT in SL degraded mature RBC or delayed the maturation of RBC resulting in increased percentage of MCV in the blood. Increased MCV in SL lambs in the current study is consistent with results reported by Solaiman et al. (2010).

An increase in MCH in the current study is reasonable in SL fed lambs which could have resulted from a greater degeneration of RBC. However earlier studies have reported decreased MCH in kids and goats fed SL and other CT rich forages (Solaiman et al., 2010; Olafadehan, 2011).

Human studies suggested that a copper (**Cu**) deficiency diminishes erythropoiesis (Dunlap et al., 1974). A negative interaction occurs between Cu and Mo (Humphries et al., 1987). Supplementation of Mo in the current study decreased serum and liver Cu concentrations compared with both CON and SL lambs without Mo. However, blood parameters, mainly RBC, and Hb were higher in Mo supplemented compared to non-supplemented lambs. It could be possible that Cu deficient lambs were not severely hypocupremic, so, reduced erythropoiesis was not detected.

In the current study, a reduction of WBC occurred in SL compared with CON fed lambs, which was attributed to a reduction in neutrophils and maybe eosinophils. A similar reduction in WBC in animals fed a CT rich diet were reported in earlier studies (Solaiman et al. 2010; Olafadehan, 2011). A reduced WBC count could be related to a suppressive effect of CT in the bone marrow. Eosinophils increase during inflammation and allergic reactions for combating

parasites and allergens respectively. Decrease in percentage of eosinophils in the current study in SL compared to CON fed lambs is similar to that reported by Thomas et al. (1985) they reported increased anti-inflammatory response in rats injected with CT extracts.
However, the mechanism by which such an effect on lymphoid tissue and an inflammatory response was not uncovered. Further investigation is needed to determine the effect of dietary CT on the inflammatory response in growing lambs.

Supplementation of Mo in the current study had no effect on the WBC count. Similar results were reported by Arthington et al. (1996) in Mo-supplemented heifers. In the current study lymphocytes, esoinophils, neutrophils, monocyte, and basophils were similar between Mo supplemented and non-supplemented lambs. No change of monocytes in Mo supplemented lambs the current study is in contrast with results reported by Randhawa et al. 2001. Neutrophils, macrophage, and monocytes play important role in immune response. No change in immune cells in the current study indicates supplementation of SL did not have negative effects on immune response in lambs.

Blood albumin is an indicator of protein and carbohydrate status in the body (Sykes and Field, 1972). In the current study, serum concentrations of albumin were reduced in SL compared with CON fed lambs. In contrast, Solaiman et al. (2010) and Olafadhan (2011) reported increased albumin concentrations in animals fed a CT rich diet. Condensed tannins bind with protein and carbohydrate in the rumen reducing their availability (Donnelly et al., 1971). However, the CT-protein complex dissociates in the abomasum. Increased postruminal absorption of nitrogen in animals fed a CT-rich diet was reported by Barry and Manley (1984). A discrepancy in blood albumin concentrations in reported studies could be due to differences in

structure and protein binding affinity of CT found among different plants (Asquith and Butler, 1986).

Blood urea nitrogen is a good indicator of protein utilization in the body. In the current study, BUN decreased between d 14 and d 42 in SL compared with CON fed lambs indicating that more protein reached the intestine for absorption. It is not clear why BUN would increase over time in SL lambs, but it coincides when weight gain slowed. A similar reduction was reported by Turner et al. (2005) in kids, and Moore et al. (2008) in kids fed SL hay. Decreased BUN in SL compared with CON fed lambs could be due to a greater deamination of amino acids and less utilization in the body. In the current study, serum TP were similar between the diets, which is similar to that reported by Turner et al. (2005).

Creatinine is an indicator of kidney function. Increased level of creatinine reflects poor kidney function. Feeding SL in the current study reduced creatinine compared with CON fed lambs. This shows that 4% CT on a DM basis can be incorporated in a SL diet without hampering kidney function.

Serum activity of AST is used for the diagnosis of hepatic injury; but in ruminants this also could occur during skeletal muscle necrosis or injury (Braun et al., 1992). In the current study, AST activity increased in SL compared with CON fed lambs, but this may not have any physiological significance because the difference was so small. Similar results were reported by Zhu et al. (1992) in tannic acid intoxicated mice and sheep. The level of AST activity of lambs in the current study would not be indicative of any particular physiological stress (Burke and Miller, 2006).

Molybdenum is essential in the enzyme complexes xanthine oxidase and sulphite oxidase (Schwarz et al., 2009). Molybdenum forms a complex as a metal cofactor or an enzyme

complex to catalyze a redox reaction. Xanthine oxidase is important in purine degradation, catalyzing the oxidation of hypoxanthine via xanthine to uric acid, essentially detoxifying cells (Schwarz et al., 2009). In normal conditions uric acid formed is either recycled in the body or excrected through urine. In the current study, uric acid concentrations were similar between the dietary treatments indicating that Mo was not deficient even though there was a reduction in molybdenum concentration in serum of these lambs and in earlier ARS experiments.

Changes in hematological and serum biochemical parameters occurred between Katahdin and Romanov breeds. Such differences could be due to their origin (Russia vs. the U.S. with a tropical influence of the St. Croix breed, respectively). It was observed that even the ¼ Romanov lambs retained similar behavioral trait as straightbred Romanov sheep, which are flighty breed. A lower PCV likely is associated with a greater susceptibility to gastrointestinal nematodes. Protein digestion could also be more efficient in the crossbred lambs (lower albumin, BUN, TP)

In previous studies at two sites (ARS and Louisiana State University), goat kids, and mature ewes had reduced weight gain when fed SL for a prolonged period of time (Burke et al., 2014). A marked reduction in Mo, as much as 90-fold (J. M. Burke and J. E. Miller, unpublished data) was observed in the SL-fed animals compared with control. In the current study, diets were isonitrogenous, isocaloric, and similar in minerals and vitamins. Supplementation of Mo in SL+MO lambs increased serum and liver concentrations of Mo and were similar to CON fed lambs. Molybdenum concentrations in SL were lower than CON and SL+MO lambs.

Administration of effective anthelmintics decreased fecal egg counts (FEC) and increased weight gain in lambs (Akanda et al., 2012). An anthelmintic was administrated twice, as first anthelmintic administration (combination of albendazole and moxidectin) failed to reduce FEC (44.2 % reduction). Levamisole was administrated after 14 d which reduced FEC by 98.8%.

However, weight gain in both CON lambs and SL diet fed lambs slowed down after the administration of anthelmintics. Reasons underlying slower weight gain is unknown. Changes in hematological and serum biochemical values were within the reference range Wilhelmi et al. (2012). Any change in animal health or production associated with CT in SL observed in previous studies may not be related to those variables examined in the study.

Conclusion

Supplementation of a balanced diet containing 75% SL pellets tended to slow weight gain compared with alfalfa pellets. Based on hematological and serum biochemical values, means of both groups appeared to be normal, though there were subtle but significant differences between dietary groups that may or may not be associated with reduced performance of SL lambs.

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Table 1. Feed ingredient (expressed on % of DM basis; unless stated) used for experimental diet per batch.

	Diets			
Ingredients	SL[1]	SL[2]	CON[1]	CON[2]
Corn	1.76	1.78		
Soybean Hulls			16.4	16.4
Dehydrated alfalfa			76.3	76.3
Sericea lespedeza	75.5	75.0		
Soybean oil	1.5	1.5		
Soybean meal	9.0	9.0		
Cobalt(II) carbonate [3]			41	6
Molasses	5.0	4.9	5.0	5.0
Salt	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.4	0.6	0.6
Limestone	2.2	2.9		
Selenium premix	81	96	67	79
Potassium chloride	2.36	2.31		
Copper sulphate [4]	0.1	0.3	0.6	0.6
Manganese (II) sulphate [4]			4.5	6.7
Calcium Iodide [4]	0.1		0.1	0.1
Zinc sulphate [4]	2.2	2.4		
Vitamin ADE premix	0.20	0.18		
Vitamin E premix	1.05	1.05	0.80	0.80
Sodium molybdate [4]	0.22	0.32		
Total	100%	100%	100%	100%

[1]SL = Sericea lespedeza (75% SL; Sims Bros. Inc., Union Springs), used before wk 5.

[2]SL = 75% SL and 25% mixed ingredients, used after wk 5.

[1]CON= Control (75% alfalfa; Manzanola feeds; Manzanola, CO) used before wk 5.

[2]CON = 75% alfalfa and 25% mixed ingredients used after wk 5.

[3]Values expressed in milligram, used per batch, 4values expressed in gram, used per batch

Table 2. Concentration of macro-minerals (percent DM) and micro-minerals (µg/g) in the diet.

Mineral[1]	SL[2]	SL[3]	SL[4]	CON
Ca	1.45	0.88	0.81	0.87
P	0.16	0.22	0.19	0.22
Mg	0.21	0.23	0.23	0.23
K	0.78	1.21	1.02	1.21
Na	0.05	0.03	0.05	0.03
S	0.37	0.29	0.32	0.29
Co	0.48	0.27	0.26	0.27
Cu	7.2	7.4	6.7	7.4
Fe	166	245	216	245
Mn	62.7	85.2	87.3	85.2
Mo	0.80	0.32	0.19	0.32
Zn	18.1	26.3	21.0	26.3
Se	0.07	0.08	0.08	0.08

[1]Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Co = Cobalt, Cu = Copper, Fe = Iron, Mn = Manganese, Mo = Molybdenum, Zn = Zinc, Se = Selenium

[2, 3]SL pellets mixed in equal proportion to formulate SL feed supplement before wk 5.

[4]SL pellets used after wk 5.

Alfalfa pellets as a control (CON) or sericea lespedeza (SL) leaf meal pellets used as ingredients in the diets. Pellets were ground and analyzed by inductively coupled plasma/mass spectrometry (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 3. Mineral concentration of mixed supplement. Concentration of macro-minerals (% DM) and micro-minerals (µg/g).

Mineral[1]	SL mixed	Control mixed
Ca	1.6	2.0
P	0.24	0.25
Mg	0.23	0.26
K	1.76	2.52
Na	0.25	0.36
S	0.35	0.34
Co	0.31	0.24
Cu	8.6	8.5
Fe	278	411
Mn	84.4	48.6
Mo	0.98	1.81
Zn	24	24
Se	0.10	0.12

[1]Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Co = Cobalt, Cu = Copper, Fe = Iron, Mn = Manganese, Mo = Molybdenum, Zn = Zinc, Se = Selenium

Control (CON) or sericea lespedeza (SL) mixed supplements offered to lambs. Feed samples were ground and analyzed by inductively coupled plasma/mass spectrometry (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 4. Analyses of feed (alfalfa or sericea lespedeza pellets) or forage on DM basis for percentage crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF).

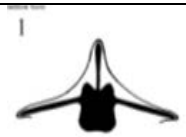
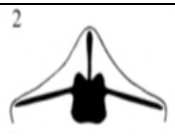
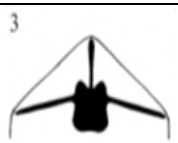
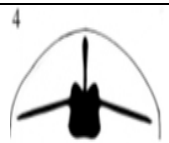
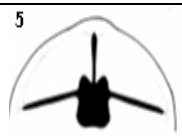
Date	Forage or pellets	CP	ADF	NDF
Forage				
June 12	Plot 1	11.00	35.97	57.47
	Plot 2	14.48	32.20	56.18
	Plot 3	14.19	32.68	5.3.07
	Plot 4	13.03	33.43	56.39
June 24	Plot 1	10.26	37.47	62.00
	Plot 2	12.56	35.42	57.63
	Plot 3	11.63	34.77	57.00
	Plot 4	10.07	37.66	60.43
July 18	Plot 1	16.59	28.79	59.08
	Plot 2	12.18	36.93	58.79
	Plot 3	10.44	38.57	64.05
	Plot 4	10.07	37.66	60.43
July 31	Plot 1	18.64	28.09	58.24
	Plot 2	13.49	33.37	54.49
	Plot 3	16.21	33.88	55.36
	Plot 4	14.24	36.36	62.51
August 14	Plot 1	14.68	32.74	59.58
	Plot 2	13.39	31.79	50.26
	Plot 3	12.27	33.74	56.20
	Plot 4	10.36	36.36	62.51
Pellets				
April 2013	Alfalfa	16.4	27.6	34.0
July 2012	SL[1]	14.3	23.8	32.7
July 2012	SL[2]	14.4	24.2	27.6
June 2013	SL[3]	15.3	25.8	30.2

[1,2]SL = sericea lespedeza mixed in equal proportion during the first 5 wk.

[3]SL = sericea lespedeza was used after wk 5 till the end of the trial.

Samples were ground and analyzed at the University of Arkansas, Agriculture Diagnostic Laboratory, Fayetteville, AR.

Table 5. Assessment of body condition score (BCS), was done on the basis of table below.

Body condition scores					
					
Spines	Sharp and form narrow ridge	Spines forms narrow ridge but points are rounded	Slightly elevated vertebrae	Vertebrae could be felt only on pressure	Spines can be felt by pressing down firmly between fats
Transverse processes	Finger easily pass under	Smooth rounded, finger go under pressure	Smooth rounded. Fingers need hard pressure to find ends	Transverse process cannot be felt	Transverse process cannot be felt
Muscle	Very little	Medium depth	Muscle full	Muscle full	Muscle very full
Fat	No fat cover	Thin fat cover	Moderate fat cover	Fat cover thick	Fat cover dense

(Based on the information Available online http://www.lifetimewool.com.au/conditionscore.aspx retrived on 5th April, 2014)

Table 6. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), MO administration (no or yes), and breed type (Katahdin or K; ¼ Romanov × ¾ Katahdin or R) on white blood cell parameters.

Variable	*n*	WBC[1], $\times 10^3/\mu L$	Lymphocyte[2] $\times 10^3/\mu L$	Neutrophils[2] $\times 10^3/\mu L$	Eosinophils[3] (%)	Monocytes[2] (%)	Basophils[3] $\times 10^3/\mu L$
CON	10	9.47	2.8	3.7[a]	7.1	13.5	0.22
SL	10	8.68	3.09	2.9[b]	6.8	13.5	0.22
SL+MO	10	8.75	2.94	2.9[b]	6.50	14.9	0.24
SE		0.25					
P =		0.05	0.39	< 0.001	0.18	0.29	0.57
CON	10	9.51	2.77	3.66	7.0	15.7	0.23
SL	20	8.71	3.01	2.97	6.7	14.5	0.23
SE		0.24					
P =		0.03	0.16	< 0.001	0.08	0.33	0.92
No	20	9.07	2.94	3.32	6.8	14.2	0.39
Yes	10	9.15	2.82	3.32	7.1	15.9	0.23
SE		0.24					
P =		0.83	0.42	0.99	0.12	0.12	0.39
K	14	9.35	3.25	3.09	6.7	15.6	0.23
R	16	8.87	2.45	3.56	7.5	14.6	0.23
SE		0.23					
P =		0.83	< 0.001	0.006	0.25	0.37	0.88

[a-c]Means with different superscripts differ ($P < 0.05$). [1]WBC = White blood cell, [2]Back transformed log data, [3]Back transformed square root data. WBC differential count (% of WBC; expressed in least square means), in lambs from the samples collected every two wk till the end of the trial. White blood cell was analyzed by CELL DYN 3500, hematological analyzer (USDA-ARS lab at University of Arkansas, Fayetteville, AR).

Table 7. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), MO administration (no or yes), and breed type (Katahdin or K; 1/4 Romanov x 3/4 Katahdin or R) on red blood cell characteristics.

Variables	*n*	RBC[8], $\times 10^6$/µL	Hb[2], g/dl	PCV[3], dl/dl	MCV[4,9,] fl	MCH[5,9,] pg	Platelets[9,] $^{\times}10^3$/µL	RCD[6], (%)	MCHC[7], pg
CON	10	21.3[a]	9.7	29.8	3.4	10.3[b]	626	25.5	32.1
SL	10	19.2[b]	9.2	28.5	3.9	10.8[a]	595	29.9	31.8
SL+MO	20	20.4[a]	9.6	29.1	3.5	10.4[b]	555	24.5	31.8
SE			0.16	0.4				2.89	0.138
P =		0.03	0.13	0.09	0.23	0.01	0.26	0.38	0.14
CON	10	21.9	9.9	31.1	29.96	10.1	601	22.8	32.1
SL	20	19.8	9.4	30.0	29.96	10.6	601	27.2	31.8
SE			0.15	0.4				2.83	0.12
P =		0.008	0.06	0.02	0.35	0.005	0.53	0.29	0.09
No	20	20.2	9.5	29.2	29.96	10.5	607	27.7	32.0
Yes	10	21.5	9.8	29.7	29.96	10.2	569	22.3	31.9
SE			0.15	0.4				2.83	0.12
P =		0.09	0.09	0.043	0.44	0.05	0.32	0.19	0.91
K	14	21.9	10.1	30.7	33.11	10.3	512	22.2	31.8
R	16	19.8	9.3	28.1	29.96	10.4	671	22.3	32.1
SE			0.18	0.36				2.68	0.12
P =		< 0.001	< 0.001	< 0.001	0.03	0.26	< 0.001	0.19	0.03

[a-c]Means with different superscripts differ ($P < 0.05$).[9]Back transformed log data, [8]Back transformed square root data.

Red blood cells (RBC), in lambs form the samples collected every two wk until the end of the trial. Red blood cells were analyzed by CELL DYN 3500, hematological analyzer (USDA-ARS, at University of Arkansas, Fayetteville, AR)

[2]Hb = Hemoglobin, [3]PCV = packed cell volume, [4]MCV = mean corpuscular volume, [5]MCH = mean corpuscular hemoglobin,

[6]RCD = red cell distribution width, [7]MCHC = mean corpuscular hemoglobin concentration.

Table 8. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), MO administration (no or yes), and breed type (Katahdin or K; ¼ Romanov × ¾ Katahdin or R).

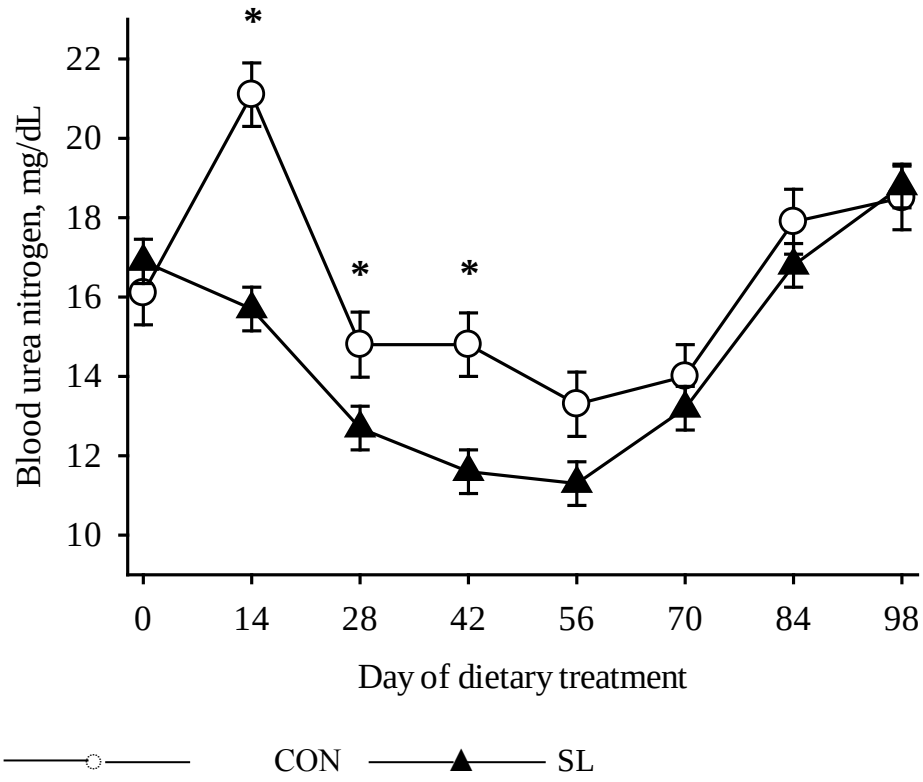
Treatment	*n*	Albumin, g/dL	AST[1, 5], IU/L	BUN[2], mg/dl	Creatinine, mg/dL	TP[3], g/dL	UA[4, 5], mg/dL
CON	10	3.21	76	16.52	0.60	5.7[a]	44.7
SL	10	3.07	85	14.85	0.56	5.6[a]	40.8
SL+MO	10	3.01	84	14.50	0.56	5.3[b]	40.1
SE		0.03		0.28	0.009	0.04	
P =		< 0.001	0.01	< 0.001	0.01	< 0.001	0.25
CON	10	3.18	75.18	16.34	0.61	5.56	44.25
SL	20	3.04	85.62	14.68	0.57	5.48	40.69
SE		0.03		0.25	0.008	0.04	
P =		0.01	< 0.001	< 0.001	0.004	0.17	0.23
No	20	3.14	81.45	15.68	0.58	5.66	42.98
Yes	10	3.08	79.04	15.33	0.60	5.38	42.09
SE		0.03		0.25	0.008	0.04	
P =		0.20	0.37	0.38	0.14	< 0.001	0.77
K	14	3.20	74.44	15.96	0.60	5.61	48.42
R	16	3.02	86.48	15.06	0.58	5.43	36.96
SE		0.03		0.25	0.007	0.04	
P =		< 0.001	< 0.001	0.006	0.04	< 0.001	< 0.001

[a-c]Means with different superscripts differ ($P < 0.05$). [5]Back transformed log data.

[1]AST = aspartate aminotransferase, [2]BUN = blood urea nitrogen, [3]TP = total protein, [4]UA = uric acid.

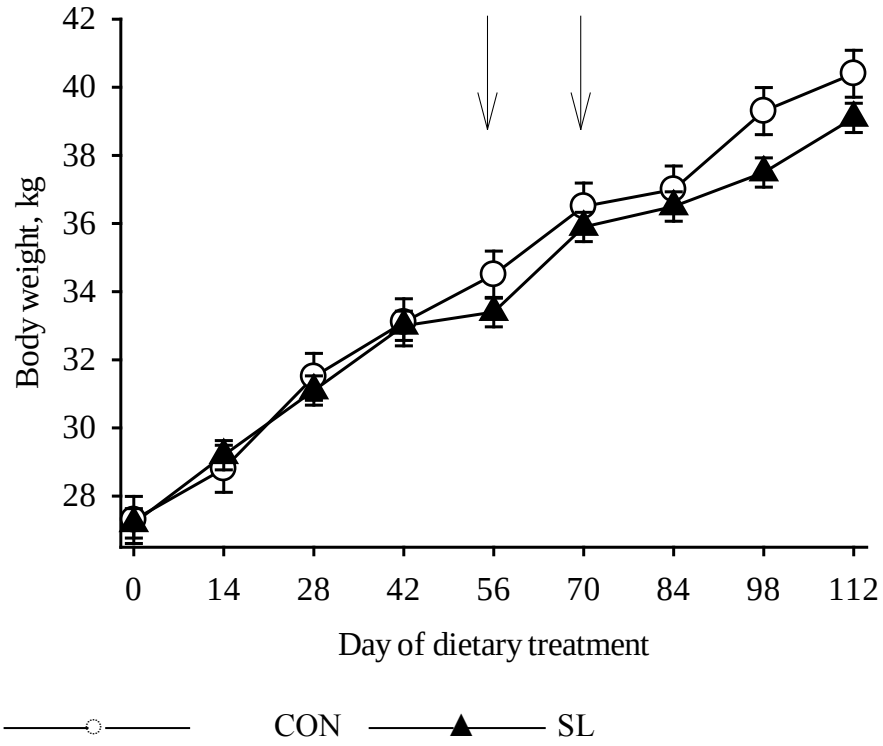
Serum chemistry, in lambs form the samples collected every two wk till the end of the trial. Serum chemistry was analyzed by an auto-analyzer at USDA-ARS lab at University of Arkansas, Fayetteville, AR.

Figure 1.



Effect of diet (Control or CON, n = 10; sericea lespedeza or SL, n = 20) on least square means and SE of lambs, on blood urea nitrogen (BUN) of lambs. Treatment × time for BUN, P< 0.001.

Figure 2.



Effect of diet (Control or CON, *n* = 10; sericea lespedeza or SL, *n* = 20 on SL least square means and SE on body weight). Arrows represent day of deworming the first date failed to reduce fecal egg counts of lambs). Diet × day, *P* = 0.054.

From: Sam Tabler, Chairman of USDA-ARS-Booneville IACUC

RE: Mohan Acharya Thesis Pre-Check

Dr. Charles Rosenkrans, Mohan Acharya, Dr. Joan Burke,

I write this letter in an effort to clear up any confusion concerning the USDA-ARS -IACUC approval for the graduate research project of Mohan Acharya.

The title of the project was "Improving lamb performance with serica lespedeza and molybdenum". The ARS – Booneville IACUC number for the project was USDA-ARS-74-F-0023. The ARS – Booneville IACUC met in April of 2013 to review and discuss the protocol of the study including the liver biopsy component. The committee consisted of the attending veterinarian, two other USDA-ARS employees, an unaffiliated member, and me. All five members approved the protocol. The research project timeframe was May 8, 2013 – August 20, 2013. All procedures and protocols that had been presented to the Booneville-IACUC were followed completely.

I hope this clears up any questions concerning Mohan Acharya's graduate project involving IACUC approval.

Sincerely,

Sam Tabler, Chairman USDA-ARS-Booneville IACUC

[redacted]

Chapter V: Fecal egg count and weight gain in lamb fed sericea lespedeza and administered sodium molybdate

Abstract:

Sericea lespedeza (**SL**; *Lespedeza cuneata*) is a plant grazed or fed to small ruminants for parasite control. Prolonged feeding of SL containing high condensed tannins (**CT**) may lead to unintended consequences such as changes in production. The objective of this experiment was to determine the effect of SL with or without molybdenum (**Mo**) supplementation on changes in gastrointestinal nematodes (**GIN**) and body weight (**BW**) in lambs. Thirty ram lambs weaned in May (84 ±1.5 d of age; 27 ± 1.1kg) were blocked by BW, breed, and estimated breeding value of parasite resistance, and randomly assigned to be fed 900 g of alfalfa based supplement (**CON**; *n* = 10) or SL based supplement (*n* = 20) for 103 d. Supplements were isonitrogenous, isocaloric, and similar in trace mineral concentrations. Within the SL group, half of the lambs were administered 163.3 mg of sodium molybdate three times/wk/lamb to ameliorate a reduction in serum molybdenum (**SL+MO**) observed previously. Lamb BW and body condition scores (**BCS**) were obtained every two wk. Blood and fecal samples were collected every 7 d during the first 5 wk and every 14 d thereafter to determine fecal egg count (**FEC**), fecal oocyst count (**FOC**), and blood packed cell volume (**PCV**). FAMACHA©, dag scores, and fecal scores were obtained during the sample collection. Data were analyzed using a mixed model with repeated measures. There was no reduction ($P > 0.80$) in FEC or FOC associated with the SL diet. Molybdenum administration tended to reduce ($P = 0.07$), FAMACHA© and dag score, but did not influence ($P > 0.38$) fecal score, PCV, FOC, and FEC. FAMACHA© scores were similar ($P = 0.05$) between CON and SL+MO lambs which were lower ($P = 0.05$) than SL lambs. Blood packed cell volume decreased ($P = 0.02$) in SL lambs compared to CON diet fed lambs. There was treatment × day interaction in dag score and fecal score in which both declined (dag score, *P*

= 0.008; *P* = 0.01) more rapidly in SL lambs compared to CON diet fed lambs. There tended to be a reduction (*P* = 0.06) in BW in SL lambs compared with CON diet fed lambs, but BCS (*P* = 0.83) was similar between the diet. A lack of control in GIN and possibly *Eimeria* spp. by SL was not observed in any of our previous experiments in which SL was fed to small ruminants.

Key words: - sericea lespedeza, condensed tannins, fecal egg count, molybdenum

Introduction

The southern U.S., holds a potential place for sheep and goat production because of increasing ethnic demand, high reproduction rate and ability of goats to survive on consumption of noxious plants (Glimp, 1994). Major constraints for small ruminant production in the region is infection with gastrointestinal nematodes (**GIN**), specifically *Haemonchus contotrus*; a predominant, blood sucking GIN. Prevalence to anthelmintic resistance by *H. contortus* is high (Howell et al., 2008), and consumers demand for organic and 'natural' meat has increased (USDA, 2010). Alternatives for parasite control are critically needed.

Sericea lespedeza [(SL, *Lespedeza cuneata* (Dum. –Cours. G. Don)], a plant high in condensed tannins, reduced GIN, especially *H. contortus*, and signs of coccidiosis in small ruminants (Min et al., 2004; Shaik et al., 2006, Lange et al., 2006; Terrill et al., 2007; Burke et al., 2013). Sericea lespedeza can be grazed or fed in a dry form (hay or pellets). Hay of SL was effective as an aid in the control of *H. contortus;* therefore it may minimized the need for chemical anthelmintics where the predominant GIN is *H. contortus* (Shaik et al. 2006). Incorporating 50-75% SL hay in the diet was more effective in the reduction of fecal egg count (**FEC**) compared with 25% of SL hay in the diet (Terrill et al., 2009). Pelleting SL adds an option for broader use of the forage geographically. The SL pellets may be more effective in *H. contortus* control in goats than SL hay (Terrill et al. 2007). Weight gain in goats fed 75% and 95% SL leaf meal pellets was higher compared to those fed a commercial supplement when fed for 77 d (Gujja et al., 2013) or goats fed 75% SL hay compared with those fed berumdagrass (*Cyanodon dactylon*) hay for 98 d (Moore et al., 2008) . However, prolonged grazing or feeding of SL leaf meal pellets (> 56 d) decreased weight gain in lambs and kids (Burke et al., 2012; Burke et al., 2014) compared with control fed animals.

The CT-protein complex bypasses the rumen and dissociates in the abomasum and intestine (Barry et al., 1986; Waghorn et al., 1987), which likely occurred in the Moore et al. (2008) and Gujja et al. (2013) experiments over a short time period. Thus, greater N absorption occured post-ruminally (Barry et al., 1986). The CT not only binds to protein, but also to carbohydrates and minerals (reviewed by McSweeney et al., 2001). Several authors have reported binding of CT with minerals (Disler et al., 1975; Pritchard et al., 1992; Silverstein et al., 1996). Decreased mineral availability could be associated with decreased weight gain.

Changes in macro-minerals in animals fed a CT rich diet were not consistent (Chang et al., 1994; Waghorn et al., 1994; Scharenberg, 2007), and in lambs and goats fed SL (J. M. Burke and J. E. Miller, unpublished data). A consistent reduction in serum concentrations of trace minerals, mainly Mo, Mn, Zn, and Se, occurred in sheep and goats fed SL (J. Burke and J. E. Miller unpublished observations). As much as a 90-fold reduction in Mo was found in SL-fed sheep and goats compared with control animals. Molybdenum is an essential component of enzme complexes (de Renzo et al., 1953) that could be important to growth and other physiological functions.

Because of the popularity of grazing SL or feeding commercially available SL hay or pellets for an aid in the control of parasites, it is important to understand potential limitations in the production of small ruminants. Therefore, the objective of this study was to determine the effect of Mo supplementation on reduction of gastrointestinal parasites and weight gain in lambs fed SL pellets.

Materials and Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee at the Agricultural Research Service (protocol # USDA-ARS-74-F-002).

Location

The trial was conducted at the USDA-ARS, Dale Bumpers Small Farms Research Center in Booneville, AR (35^0N, 94^0W); from May 8-August 21, 2013. Average rainfall per month during the trial was 113 mm. Average maximum and minimum temperature during the trial was 30ºC and 18ºC, respectively.

Animal Procedures

Thirty, Katahdin (*n* = 14) or ¼ Romanov × ¾ Katahdin (*n* = 16) ram lambs weaned in May (84 ± 1.5 d of age; 27.2 ± 1.1 kg) were used. Lambs were blocked by BCS, BW and estimated breeding value for parasite resistance, and assigned randomly to be fed: 900 g/d of alfalfa based supplement (**CON**; *n* = 10), or a SL based supplement (*n* = 20). Supplements were balanced for energy, protein, mineral and vitamin (feed ingredients for CON and SL diets are listed in Table 1; mineral concentration of feeds used are listed in Table 2; mineral concentrations of mixed supplements are listed in Table 3). Diets were balanced to meet moderate gains according to NRC (2007). Two lots of SL pellets (Sims Brothers, Inc., Union Spring, AL) were mixed and used for the first 5 wk, and another lot of SL pellets were used for the last 10 wk. Supplements were mixed at the University of Arkansas feed mill (126 kg per mixing which occurred every 14 d). The first day of dietary treatment was considered d 0. Within the SL group, lambs were administered either water alone or 163.3 mg of sodium molybdate per lamb of sodium molybdate (mixed with 5 mL of water; sodium molybdate dihydrate, North Metal & Chemical Co, York, PA) by syringe on Monday, Wednesday, and Friday (*n* = 10/drench). The concentration of Mo in sodium molybdate is 39%; thus, the target dose of Mo was 27.3 mg/lamb daily. The dose was based on a previous study (J. E. Miller unpublished data) in which 13.6 mg Mo/d (van Ryssen, 1994) was administered to lambs daily to increase serum

concentrations in SL fed lambs, but only a slight increase occurred (J. E. Miller unpublished data). Thus, twice that dose was used in the current experiment. Lambs grazed one of four 0.34 ha plots containing predominantly tall fescue (*Festuca arundinacea*; n = 2 replicates/diet), rotated among plots every two week to minimize plot effect. Water was always available and no trace mineral mix was offered as it was included in the supplement. Lambs were dewormed with a combination of albendazole (15 mg/kg BW; Valbazen, Pfizer Animal Health, Exton, PA) and moxidectin (0.4 mg/kg BW; Cydectin Fort Dodge Animal Health, Fort Dodge, IA) on d 56, which failed to reduce fecal egg counts (44.2% reduction). Hence lambs were dewormed again on d 70 with levamisole (12 mg/kg BW, AgriLabs, St. Joseph, MO) and fecal egg count reduction was 98.8%. Lambs were treated with sulfamethoxine (55 mg/kg BW; SulfaMed-G, Bimeda, Le Sueur, MN) if signs of coccidiosis were present (watery diarrhea), which occurred in one lamb each from SL and SL+MO group.

Mineral Analysis of Alfalfa and Sericea Lespedeza Pellets and Mixed Dietary Supplement

Random samples of each pellet or mixed supplement were grabbed from bags, and ground to pass through a 1 mm screen in a Thomas-Wiley laboratory mill model 4 (Arthur H. Thomas Co. Philadelphia, PA). Samples were then shipped to the Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI for mineral analysis. An Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP/MS; Agilent Technologies Inc, Santa Clara, CA) was used for the analysis of TM in feed samples as described by Wahlen et al. (2005).

Forage and Feed Quality and Forage Availability

Forage quality was analyzed every two wk between June and August (Table 4). Ten random grab samples per plot were combined. Alfalfa and SL pellets collected for mineral

analysis were also used to determine feed quality. Forage samples were dried at 22°C for 24 to 72 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F). Dry matter was determined, and samples were sent to the University of Arkansas, Agricultural Diagnostic Service Laboratory (Fayetteville, AR) for analysis. Both the forage and pellet samples were ground to pass through 1 mm screen in a Thomas-Wiley laboratory mill model 4 (Arthur H. Thomas Co., Philadelphia, PA), subsampled, and ground through a 0.5mm screen using Cyclotecmill (Tecator 1093, Hoganas, Sweden). Nitrogen content was determined by combustion method, using the Elementar Rapid NIII combustion N analyzer (Elementar Americas Inc.; Mount Laurel, NJ). Crude protein was determined from N content through multiplication by 6.25 and expressed as percentage of forage DM. Acid detergent fiber and NDF were measured with a fiber digester (Labconco, USA) following Goering and Van Soest (1970) procedures. Forage availability as DM/ha was measured using a disc method. Forage mass beneath a 0.19 m^2 disc was clipped, collected, weighted and dried at 37.7°C for 24 h (Fisher, Pittsburg, PA, Isotemp oven 300 series, model 338 F).

Fecal Culture

Feces collected directly from the rectum of each lamb were pooled according to the treatment groups every 14 d. Pooled fecal samples, weighing between 10 – 50 g were mixed thoroughly with water to make a soft mass in a glass beaker. The beaker was then covered with aluminium foil containing several holes and incubated at 25°C (Fisher Isotemp Incubator; Fisher Scientific, Pittsburgh, PA) for 12 – 14 d. During that period cultures were stirred, to aerate, and enough water was added to maintain the soft mass three times weekly. The fecal mass was then wrapped in cheese cloth, immersed in lukewarm water, and baermannized for 6 h or overnight in a funnel with a collection tube to recover third stage larvae (**L_3**). The clear supernatant was

suctioned off leaving a volume of approximately 1 mL. If larvae appeared to be mixed with particulant matter, they were washed with water, allowed to settle and clear supernatent removed again to a volume of 1 mL. To preserve larvae, 100 µL of 10% formalin was added (Pena et al., 2002). The larvae samples were sent to Louisiana State University (J. E. Miller) for identification of genera. The proportion of each genera was determined (Miller et al., 1987).

Fecal Egg and Oocyst Counts

Fecal samples were taken directly from the rectum of individual lambs and stored separately in a plastic bag in a refrigerator until processed. Fecal egg and oocyst counts were done by the modified McMaster technique (Whitlock, 1948). For this 2 g of feces were weighed and dissolved completely in 28 mL of saturated NaCl solution. The solution was then stirred, pipetted, and placed in both chambers of a McMaster slide (Chalex Corporation, Issaquah, WA). The number of nematode eggs or oocysts on both chambers of the slide was counted by a binocular microscope at 100 × power (Standard 20, Fisher Scientific, Raleigh, NC), and the result was multiplied by 50 to obtain eggs per gram (epg) of feces.

Blood Packed Cell Volume and FAMACHA©

Blood samples were collected every 2 wk by jugular venipuncture into 4 mL tubes containing 7.2 mg K_2EDTA (BD Vacutainer Whole Blood Tubes, Becton, Dickinson, & Co., Franklin Lakes, NJ). Blood packed cell volume was determined on the same day of collection. Micro-hematocrit capillary tubes were filled with whole blood, sealed, and centrifuged for 10 min at 10,000 rpm in a microcentrifuge (Legend Micro 17 centrifuge, Thermo Company, USA). Blood packed cell volume was determined by using a micro-hematocrit tube reader (Critocap, Stafford Mfg. Co. Inc., Brooklyn, NY, USA).

A FAMACHA© score was assigned that estimated the degree of anemia based on the

hematophagous GIN infection as described by Van Wyk and Bath (2002). Here, the color of the lower eyelid of each animal was compared to the FAMACHA© chart under natural light and scored on a scale of 1-5 (1 = red, non-anaemic to 5 = white, severely anaemic; provided in Table 8). During this trial FAMACHA©, was performed by the same trained person.

Indirect Measures of Coccidiosis

A dag score estimated the extent of fecal soiling around the hindquarters. Dag score was measured on a scale of 1 - 5 as described by Larsen et al. (1994). Score 1 represents no evidence of fecal soiling, and 5 represents severe fecal soiling on breech area and on the hind legs extending below the level of hocks. Details regarding the assessment of dag score in lambs with tails is given in Figure 3 (Burke et al., 2013). This procedure was conducted by a single trained person during the entire trial.

Fecal consistency score estimated the consistency of fecal samples collected every 14 d as an indirect measurement of coccidiosis. Fecal scoring was done on a five point scale (1 = solid pellets; 5 = slurry). Details regarding the assessment of fecal score in lambs are given in Figure 4 (Burke et al., 2013).

Body Weight and Condition

Body condition score and BW was determined every 14 d. The degree of fat covering the lower lumbar area was assessed and scored on a scale of 1 to 5 (1 = emaciated; 5 = obese; details regarding the assessment of BCS is provided in Table 5; Thompson and Mayer, 1994). Body weight was determined without fasting using a Gallaghar scale (130 West 23rd Av. North Kansas City, MO).

Condensed Tannins Analyses

Sericea lespedeza pellets were analyzed for extractable, protein-bound, and fiber-bound

CT content by the Terrill et al (1992) method using purified SL tannin as the standard.

Statistical Analyses

Fecal egg count, FOC, PCV, fecal score, dag score,FAMACHA©, BW, and BCS were analyzed as repeated measures (Littell et al., 1996) using mixed models with compound symmetry covariance structure (SAS Inst. Inc., Cary, NC). Log transformations were necessary for FEC [ln(FEC + 100)] and FOC [ln(FOC + 100)], and means were presented as back transformed data. Two models were used. Variables in the first model included diet (CON, SL), Mo administration (yes or no), breed type, day (repeated measures), and interactions. If *P* value of the interaction was > 0.10, it was dropped from the model. Variables in the second model included treatment (CON, SL, SL+MO), breed type, day (for repeated measures), and interaction. A covariate using the initial BW ($P < 0.001$) and BCS ($P < 0.001$) was used. If the effect of Mo administration was absent ($P > 0.10$), results from the first model (including diet, CON or SL) will be presented; otherwise the results from the second model (including treatment, CON, SL, SL+MO) will be presented for all analyses.

Results

Genera of GIN were mixed. There was 68% *H. contortus*, 24% *Trichostrongylus* spp., 5 *Cooperia* spp., and 3% *Oesophagostomum* spp., in cultured feces.

Extractable, protein-bound, fiber-bound, and total CT in the SL pellet fed to the lambs were 2.4, 1.6, 0.027, and 4.0 %, respectively. There was no detectable (< 0.05%) CT in the CON supplement. This resulted in total CT consumption in SL supplemented lambs of 27 g/d. Forage availability in plot 1, 2, 3, and 4 was 4563, 3585, 3121, 2174 kg DM per ha respectively.

Molybdenum administration tended to reduce ($P = 0.05$) FAMACHA©, and dag score ($P= 0.05$) but did not influence ($P > 0.38$) fecal score, PCV, FOC, and FEC (Table 7). FAMACHA© scores were similar between CON and SL+MO lambs which were lower ($P <$

0.07) than SL lambs. Dag score was highest ($P < 0.001$) in CON, lowest in SL+MO and intermediate in SL. There was a treatment × day in dag score and fecal score, in that both declined ($P = 0.05$) over time. No other treatment × time or diet × time interactions were detected. Fecal score, and PCV was lower (fecal score, $P < 0.001$; PCV, $P = 0.02$) in SL lambs compared with CON fed lambs, whereas FOC and FEC were similar ($P > 0.69$) between the diets. There was treatment × day effect for fecal score and diet × time effect for (FEC), in which values both decreased ($P = 0.01$) with the progress of time. FAMACHA© and FEC were lower ($P < 0.001$), in Katahdin lambs compared to crossbred lambs.

Administration of MO did not influence ($P > 0.57$) BW or BCS. There tended to be an interaction ($P = 0.054$) between diet and time in that weight gain of SL lambs decreased compared to CON diet fed lambs. The BW was similar ($P = 0.88$), between breed type and BCS

Discussion

This is the first experiment in which a SL diet offered to lambs was isonitrogenous, isocaloric, and similar in minerals and vitamins compared with a control diet. Reduction in FEC was not observed in SL lambs compared to CON fed lambs. Lack of reduction of FEC or FOC in SL lambs compared with CON lambs in the current study contrasts with the previous reports (Shaik et al., 2004; 2006; 2006; Lange et al., 2006; Terrill et al., 2007, Burke et al., 2013). Condensed tannins in SL play an important role in reduction of FEC. It may be possible that CT in SL used in the current study were degraded due to various factors.

Fungal species *Ceriporiopsis subvermispora* and *Cyathus stercoreus* degrades CT in SL (Gebrehiwot et al. 2002). In their study, fungal treatments of CT in SL increased rumen biodegradability by rumen microorganisms. The first batch of SL pellets used in the current

study was stored for a long period of time (over winter). It may be possible that CT in SL pellets were degraded by fungal infection, reducing their effect in SL.

Several factors interfere with the bioavailability of CT, such as season during the harvest, drying method after harvest, temperature and moisture during storage, and duration of storage. Seasonal variation in CT level in SL was reported by Eckerle et al. (2010) and in *Lotus* spp. by Gebrehiwot et al. (2002). Field drying decreased CT concentrations in SL. Terrill et al. (1989) reported lower CT concentrations after freeze drying or sun cured preservation in a high-CT variety of SL compared to a low-CT containing variety of SL. Drying method before storage effects CT concentrations in plants in that extractable CT was lower in oven dried compared with freeze dried SL samples (Wolfe et al., 2008). Condensed tannins are affected by storage temperature, time of storage and percentage of moisture during the storage. Higher detannification occured at 37°C compared to 50°C or at room temp (20°C; Makkar and Singh, 1993). Increase in storage time, pH, and moisture enhanced the inactivation of tannins (Makkar and Singh, 1993) in oak (*Quercus incana*) leaves. Eunice et al. (1999) reported drought stress decreased CT in leaves and roots of *Lotus corniculatus*. Sericea lespedeza leaf meal in the second lot used in the current study was harvested in 2012. During that time there was drought in Alabama.

Suttle et al. (1992 a, b), and McClure et al. (1992) reported reduction of FEC and adult worm of *H. contortus*, *Trichostrongylus vitrinus* (Suttle et al., 1992b), and *T. colubriformis* (McClure et al., 1999) in lambs supplemented with dietary Mo. Reduced serum concentrations of Mo was discovered in previous (J. E. Burke and J. E. Miller personal communication) and current research in SL compared with CON-fed lambs. Supplementation of Mo in the current study increased serum concentrations of Mo in SL+MO lambs to that of CON lambs, while that

of SL lambs without supplemental Mo remained low (Chapter III). A reduction in FEC was not observed in Mo supplemented lambs compared to Mo non-supplemented lambs perhaps because Mo supplementation did not results in increased Mo in serum or liver. Suttle et al. (1992 a) suggested decrease in FEC in Mo supplemented lambs could be due to increased inflammatory response. In the current study inflammatory cells were similar between Mo supplemented and non-supplemented lambs (Chapter IV).

Sericea lespedeza pellets increased PCV in goats fed SL compared to those fed a control diet (Shaik et al., 2006; Lange et al. 2006; Terrill et al., 2007). A reduction in FEC and an increase in PCV in SL fed goats occurred only when the predominant GIN was *H. contortus* (Shaik et al., 2006). In the current study, the predominant GIN was *H. Contortus*. However, we did not observed greater PCV in SL compared with CON fed lambs. Instead, PCV was reduced and FAMACHA© scores were higher in SL compared with CON fed lambs. But in general, most lambs did have signs of anemia (PCV < 20%) except 8 lambs distributed among groups at the time of deworming and 4 lambs 28 d later (three of these were anemic a second time).

A CT-rich diet reduced FOC in sheep and goats (Madibela and Kelemogile, 2008; Saratsis et al., 2012; Markovics et al., 2012; Burke et al., 2013). No FOC reduction was observed in the current experiment as discussed, but signs of coccidiosis (dag score and fecal score) were minimized in SL compared with CON fed lambs. Dag and fecal scores were lower in SL than CON lambs during the first few weeks of the study.

In previous studies at two sites (ARS and Louisiana State University) and over more than a two year period on lambs and goat kids there was reduction in weight gain in lambs fed SL for prolonged period of time compared with control fed animals (Burke et al., 2012; 2014). There was a marked reduction in Mo in sheep and goats fed SL compared with control diet (J. M.

Burke and J. E. Miller, unpublished observations). In the current study, diets were isonitrogenous, isocaloric, and similar in minerals and vitamins. Even though Mo concentrations of serum and liver in SL+MO lambs were similar to that of CON lambs, a similar trend in slower weight gains were still observed similar to previous studies (Burke et al, 2012, 2014).

Administration of effective anthelmintics decreases fecal egg counts (FEC) and increases weight gain in lambs (Akanda et al., 2012). Anthelmintic was administrated twice, as first anthelmintic administration (combination of albendazole and moxidectin) failed to reduce FEC (44.2 % reduction). Levamisole was administrated after a 14 d interval which reduced FEC by 98.8%. However, weight gain in both CON lambs and SL diet fed lambs slowed down after the administration of anthelmintics. Reasons underlying slower weight gain are unknown.

Lower FEC, higher PCV, and lower FAMACHA® scores were observed in Katahdin lambs compared wih ¼ Romanov breeds. Such differences were likely due to a difference in susceptibility of the Romanov breed type to GIN. The Katahdin flock at ARS has been selected for GIN resistance for more than 12 yr.

Conclusions

It is not known why control of *H. contortus* or *Eimeria* spp. did not occur in SL fed lambs. Nevertheless, a similar GIN infection among lambs allowed us to examine BW under stress factors, which was still influenced by CT as weight gain of SL (with or without Mo supplementation) lambs still slowed after prolonged feeding relative to the control group.

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Table 1. Feed ingredient (expressed on % of DM basis; unless otherwise stated) used for experimental diet per batch.

	Diets			
Ingredients	SL[1]	SL[2]	CON[1]	CON[2]
Corn	1.76	1.78		
Soybean Hulls			16.4	16.4
Dehydrated alfalfa			76.3	76.3
Sericea lespedeza	75.5	75.0		
Soybean oil	1.5	1.5		
Soybean meal	9.0	9.0		
Cobalt(II) carbonate[3]			41	6
Molasses	5.0	4.9	5.0	5.0
Salt	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.4	0.6	0.6
Limestone	2.2	2.9		
Selenium premix	81	96	67	79
Potassium chloride	2.36	2.31		
Copper sulphate[4]	0.1	0.3	0.6	0.6
Manganese (II) sulphate[4]			4.5	6.7
Calcium Iodide[4]	0.1		0.1	0.1
Zinc sulphate[4]	2.2	2.4		
Vitamin ADE premix	0.20	0.18		
Vitamin E premix	1.05	1.05	0.80	0.80
Sodium molybdate[4]	0.22	0.32		
Total	100%	100%	100%	100%

[1]SL = Sericea lespedeza (75% SL; Sims Bros. Inc., Union Springs), used before wk5.

[2]SL = 75% SL and 25% mixed ingredients, used after wk 5.

[1]CON= Control (75% alfalfa; Manzanola feeds; Manzanola, CO) used before wk 5.

[2]CON = 75% alfalfa and 25% mixed ingredients used after wk 5.

[3]Valuesexpressed in mg/batch, [4]values expressed in g/batch.

Table 2. Concentration of macro-minerals (percent DM) and micro-minerals (µg/g) in alfalfa Pellets.

Mineral[1]	SL[2]	SL[3]	SL[4]	CON
Ca	1.45	0.88	0.81	0.87
P	0.16	0.22	0.19	0.22
Mg	0.21	0.23	0.23	0.23
K	0.78	1.21	1.02	1.21
Na	0.05	0.03	0.05	0.03
S	0.37	0.29	0.32	0.29
Co	0.48	0.27	0.26	0.27
Cu	7.2	7.4	6.7	7.4
Fe	166	245	216	245
Mn	62.7	85.2	87.3	85.2
Mo	0.80	0.32	0.19	0.32
Zn	18.1	26.3	21.0	26.3
Se	0.07	0.08	0.08	0.08

[1]Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Co = Cobalt, Cu = Copper, Fe = Iron, Mn = Manganese, Mo = Molybdenum, Zn = Zinc, Se = Selenium.

[2, 3]SL pellets mixed in equal proportion to formulate SL feed supplement before wk 5.

[4]SL pellets used after wk 5.

Control (CON) or sericea lespedeza (SL) leaf meal pellets used as ingredients in the diets. Pellets were ground and analyzed by inductively coupled plasma/mass spectrometry (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 3. Mineral concentrations of mixed supplement. Concentration of macro-minerals (% DM) and micro-minerals (µg/g) in control (CON) or sericea lespedeza (SL) supplements offered.

Mineral[1]	SL mixed	Control mixed
Ca	1.6	2.0
P	0.24	0.25
Mg	0.23	0.26
K	1.76	2.52
Na	0.25	0.36
S	0.35	0.34
Co	0.31	0.24
Cu	8.6	8.5
Fe	278	411
Mn	84.4	48.6
Mo	0.98	1.81
Zn	24	24
Se	0.10	0.12

[1]Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, Co = Cobalt, Cu = Copper, Fe = Iron, Mn = Manganese, Mo = Molybdenum, Zn = Zinc, Se = Selenium. Feed samples were ground and analyzed by inductively coupled plasma/mass spectrometry (Diagnostic Center for Population and Animal Health at Michigan State University).

Table 4. Analyses of feed (alfalfa or sericea lespedeza pellets) or forage on DM basis for percentage crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF).

Date	Forage or pellets	CP	ADF	NDF
Forage				
June 12	Plot 1	11.00	35.97	57.47
	Plot 2	14.48	32.20	56.18
	Plot 3	14.19	32.68	5.3.07
	Plot 4	13.03	33.43	56.39
June 24	Plot 1	10.26	37.47	62.00
	Plot 2	12.56	35.42	57.63
	Plot 3	11.63	34.77	57.00
	Plot 4	10.07	37.66	60.43
July 18	Plot 1	16.59	28.79	59.08
	Plot 2	12.18	36.93	58.79
	Plot 3	10.44	38.57	64.05
	Plot 4	10.07	37.66	60.43
July 31	Plot 1	18.64	28.09	58.24
	Plot 2	13.49	33.37	54.49
	Plot 3	16.21	33.88	55.36
	Plot 4	14.24	36.36	62.51
August 14	Plot 1	14.68	32.74	59.58
	Plot 2	13.39	31.79	50.26
	Plot 3	12.27	33.74	56.20
	Plot 4	10.36	36.36	62.51
Pellets				
April 2013	Alfalfa	16.4	27.6	34.0
July 2012	SL[1]	14.3	23.8	32.7
July 2012	SL[2]	14.4	24.2	27.6
June 2013	SL[3]	15.3	25.8	30.2

[1,2]SL = sericea lespedeza mixed in equal proportion during the first 5 wk.

[3]SL = sericea lespedeza was used after wk 5 until the end of the trial.

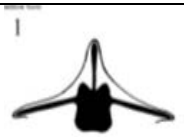
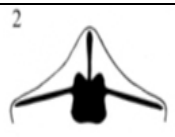
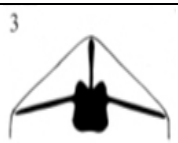
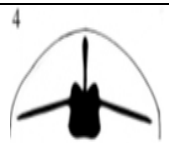
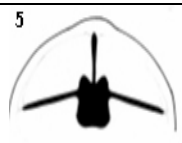
Samples were ground and analyzed at the University of Arkansas, Agriculture Diagnostic Laboratory, Fayetteville, AR.

Table 5. Genera of gastrointestinal nematodes in cultured feces in percentage of total larvae.

Date	Group	*n*/group	*Haemonchus contortus*	*Trichostrongylus* spp.	*Cooperia* Spp.	*Oesophagostomum* Spp.
5/15	Control 1[1]	5	67	33	0	0
	Control 2[2]	5	78	22	0	0
	SL 1[3]	10	…	…	…	…
	SL 2[4]	10	75	25	0	0
5/22	Control 1[1]	5	26	70	3	1
	Control 2[2]	5	85	11	3	2
	SL 1[3]	10	80	20	0	0
	SL 2[4]	10	77	15	8	0
5/29	Control 1[1]	5	60	40	0	0
	Control 2[2]	5	88	8	4	0
	SL 1[3]	10	88	10	0	2
	SL 2[4]	10	78	15	7	0
6/5	Control 1[1]	5	56	41	1	2
	Control 2[2]	5	69	29	1	1
	SL 1[3]	10	84	13	0	3
	SL 2[4]	10	73	14	9	4
6/19	Control 1[1]	5	79	17	0	4
	Control 2[2]	5	60	0	40	0
	SL 1[3]	10	76	11	0	13
	SL 2[4]	10	55	28	4	13
7/1	Control 1[1]	5	84	16	0	0
	Control 2[2]	5	46	31	15	8
	SL 1[3]	10	73	21	1	5
	SL 2[4]	10	75	25	0	0
7/17	Control 1[1]	5	100	0	0	0
	Control 2[2]	5	55	20	9	15
	SL 1[3]	10	100	0	0	0
	SL 2[4]	10	100	0	0	0
8/14	Control 1[1]	5	18	64	9	9
	Control 2[2]	5	40	53	7	0
	SL 1[3]	10	50	39	6	5
	SL 2[4]	10	17	67	17	0
			68%	24%	5%	3%

[1]Control lambs replicate 1; [2]Control lambs, replicate 2; [3]SL lambs, replicate 3, including Mo supplemented/non-supplemented; [4]SL lambs replicate 4, including Mo supplemented/non-supplemented.

Table 6. Assessment of body condition score (BCS), was done on the basis of table below.

Body condition scores					
					
Spines	Sharp and form narrow ridge	Spines forms narrow ridge but points are rounded	Slightly elevated vertebrae	Vertebrae could be felt only on pressure	Spines can be felt by pressing down firmly between fats
Transverse processes	Finger easily pass under	Smooth rounded, finger go under pressure	Smooth rounded. Fingers need hard pressure to find ends	Transverse process cannot be felt	Transverse process cannot be felt
Muscle	Very little	Medium depth	Muscle full	Muscle full	Muscle very full
Fat	No fat cover	Thin fat cover	Moderate fat cover	Fat cover thick	Fat cover dense

(Based on the information Available online http://www.lifetimewool.com.au/conditionscore.aspx retrived on 5th April, 2014)

Table 7. Effect of treatment (control or CON; sericea lespedeza or SL; SL and sodium molybdate administration or SL+MO), diet (CON or SL), Mo administration (no or yes), and breed type (Katahdin or K; ¼ Romanov × ¾ Katahdin or R) on following variables.

Variables	*n*	FAMACHA©	Dag score	Fecal score	PCV[1]	FOC[2]	FEC[3]
CON	10	1.48[b]	2.11[a]	2.63	29.83	2440	361
SL	10	1.77[a]	1.37[b]	1.69	28.95	2230	361
SL+MO	10	1.57[b]	1.15[c]	1.59	29.08	1939	333
SE		0.07	0.08	0.1			
P =		0.05	< 0.001	< 0.001	0.09	0.81	0.9
CON	10	1.39	2.00	2.58	30.08	2298	343
SL	20	1.67	1.26	1.64	28.84	2079	347
SE		0.07	0.07	0.8			
P =		0.008	< 0.001	< 0.001	0.02	0.80	0.96
No	20	1.63	1.74	2.16	29.21	2368	361
Yes	10	1.43	1.52	2.05	29.71	2018	330
SE		0.07	0.06	0.8			
P =		0.06	0.05	0.48	0.38	0.69	0.67
K	14	1.35	1.62	2.13	30.79	1998	239
R	16	1.71	1.65	2.09	28.13	2368	502
SE		0.06	0.07	0.09			
P =		< 0.001	0.7	0.77	< 0.001	0.60	< 0.001

[a-c]Means with different superscripts differ ($P < 0.05$).

[1]PCV = Blood packed cell volume

[2]FOC = Fecal oocyst count. Values are expressed in back transferred log data.

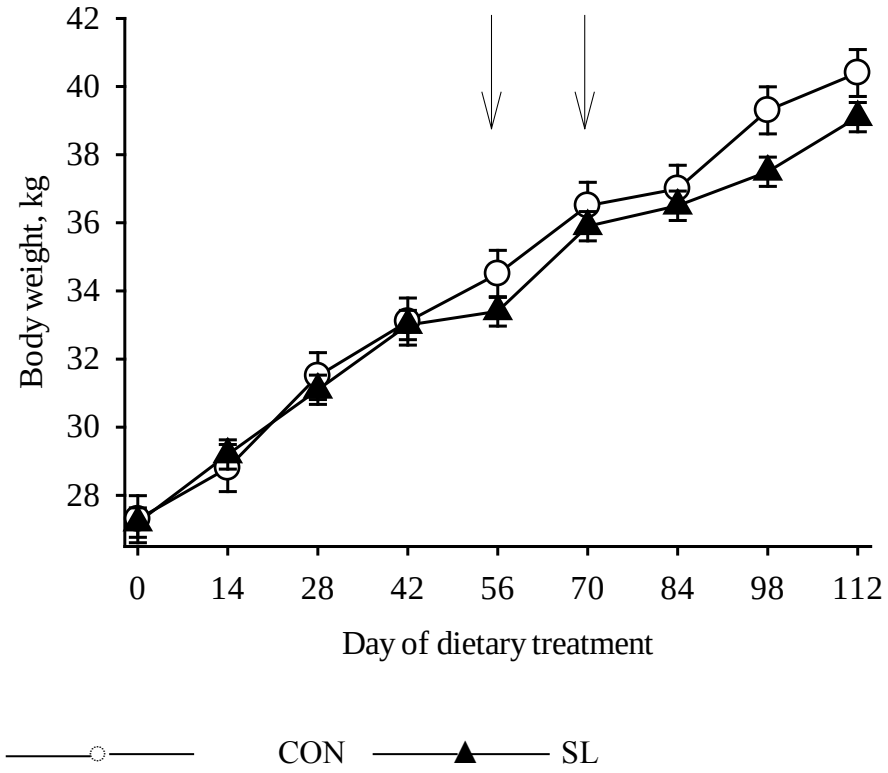
[3]FEC = Fecal egg count. Values are expressed in back transferred log data.

FAMACHA©, dag score, fecal score, PCV, FOC, and FEC (expressed as LS means, unless otherwise stated) in lambs form samples collected every two wk.

Table 8. FAMACHA© scoring to access the anemic condition in lamb.

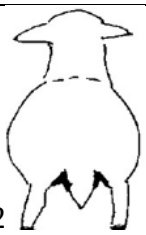
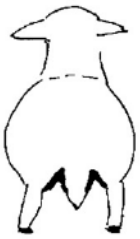
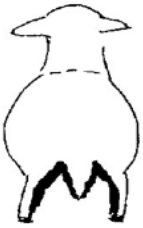
Clinical category	Color of ocular conjunctiva	Treatment recommendation
1	Red (non-anaemic)	Optimal
2	Red-Pink (non-anaemic)	Acceptable
3	Pink (mildly anaemic)	Borderline
4	Pink-White (anaemic)	Dangerous
5	White (severely anaemic)	Fatal

Figure 1.



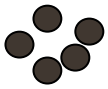
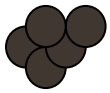
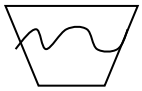
Effect of diet (Control or CON, *n* = 10; sericea lespedeza or SL, *n* = 20) on body weight of lambs. Arrows represent day of deworming. Diet × day, on BW; *P* = 0.054.

Figure 2. Dag scoring

Dag score	1 	2 	3 	4 	5 
Description	No soiling	Slight soiling	Moderate soiling, may be wet or dry.	Heavy soiling, will be wet with some sign of scouring	Heavy soiling, will be wet with sign of watery, diarrhea.
Treatment recommendation	No indication for treatment	No treatment	Consider treatment	Treatment, crutching needed	Treatment and crutching essential

Based on scoring system described by Burke et al., 2013

Figure 3. Fecal scoring

Fecal score	1	2	3	4	5
					
Description	Solid pellets	Solid pellets, but stuck together	Solid mass of fecal matter	Consistency like pudding	Consistency like soup
Treatment recommendation	No treatment	No treatment	Consider treatment	Treatment, recommended	Treatment highly recommended

Based on scoring system described by Burke et al., 2013.

From: Sam Tabler, Chairman of USDA-ARS-Booneville IACUC

RE: Mohan Acharya Thesis Pre-Check

Dr. Charles Rosenkrans, Mohan Acharya, Dr. Joan Burke,

I write this letter in an effort to clear up any confusion concerning the USDA-ARS -IACUC approval for the graduate research project of Mohan Acharya.

The title of the project was "Improving lamb performance with serica lespedeza and molybdenum". The ARS – Booneville IACUC number for the project was USDA-ARS-74-F-0023. The ARS – Booneville IACUC met in April of 2013 to review and discuss the protocol of the study including the liver biopsy component. The committee consisted of the attending veterinarian, two other USDA-ARS employees, an unaffiliated member, and me. All five members approved the protocol. The research project timeframe was May 8, 2013 – August 20, 2013. All procedures and protocols that had been presented to the Booneville-IACUC were followed completely.

I hope this clears up any questions concerning Mohan Acharya's graduate project involving IACUC approval.

Sincerely,

Sam Tabler, Chairman USDA-ARS-Booneville IACUC

[redacted]

Chapter VI: Conclusions

1. Diets used in current study were isonitrogenous, isocaloric and similar in minerals and vitamins. This helped us to determine the trace mineral status in the animals associated with feeding a condensed tannin (CT) rich legume plant. Copper, Mo, Se, and Zn were reduced in lambs fed CT rich sericea lespedeza (SL). Supplementation of Mo increased liver and serum concentrations of Mo in SL lambs similar to control fed lambs, but did not influence in weight gain. Further studies need to focus on reduced Cu and Se that occurred in SL fed lambs.
2. Supplementation of a balanced diet containing 75% SL pellets tended to slow weight gain, compared to alfalfa pellets. Based on hematological and serum biochemical values, means of both groups appeared to be normal, though there were subtle but significant differences between dietary groups that may or may not be associated with reduced performance of SL lambs.
3. Similar GIN infection among lambs fed SL or alfalfa fed lambs helped us to examine body weight under stress factors, which was slowed in SL (with or without Mo supplementation) compared with alfalfa fed lambs.