

## Case Study: Sericea Lespedeza Leaf Meal Fed to Sheep and Goats Reduces Serum Concentrations of Trace Minerals

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### Summary

Sericea lespedeza (SL; *Lespedeza cuneata*) is an important plant in the southeastern U.S. that aids in the control of gastrointestinal nematodes (GIN) and coccidiosis, and to reduce methane production in ruminant livestock. Extended feeding of SL has led to slower growth in lambs and kids. It was hypothesized that this may be related to changes in trace minerals bioavailability. The objective was to determine effects of feeding SL leaf meal pellets on serum concentrations of trace minerals in sheep and goats which participated in studies on gastrointestinal nematode parasite control (which will not be presented here). Lambs or kids weaned between 86 and 108 days of age (day 0) were supplemented with up to 900 g of a control supplement (CO) or SL leaf meal pellets for 56 to 112 days while grazing grass pastures at the USDA, Agricultural Research Service (ARS) in Booneville, AR or Louisiana State University (LSU), Baton Rouge, LA in 2010 (ARS lambs only), 2011 (lambs only), 2012, and 2013 (kids only). Blood was collected for serum concentrations of trace minerals

between days 56 and 112. There were marked changes in trace minerals found in serum between diets among study locations. Molybdenum was reduced in SL compared with CO fed animals in both Arkansas and Louisiana. Manganese, zinc, and selenium were reduced in SL compared with CO fed animals in most studies. A reduction in trace mineral status could influence growth rate of lambs and kids, possibly leading to other metabolic issues. It may be important to restrict feeding of SL to less than eight-week periods to minimize trace mineral deficiencies or feed a high-quality mineral supplement.

**Key Words:** Condensed Tannins, Goats, Trace Minerals, Molybdenum, Sericea Lespedeza, Sheep

**Abbreviations:** CO, control; FEC, fecal egg count; GIN, gastrointestinal nematodes; PCV, blood packed cell volume; SL, sericea lespedeza. Mineral abbreviations: Co, cobalt; Cu, copper; Mn, manganese; Mo, molybdenum; Se, selenium; Zn, zinc.

## Introduction

*Sericea lespedeza* [SL, *Lespedeza cuneata* (Dum.-Cours. G. Don)] has been grazed by or fed to sheep and goats to aid in the control of gastrointestinal nematodes (GIN; Min et al., 2004, 2005; Burke et al., 2012) or prevent or control *Eimeria* spp. infection in lambs (Burke et al., 2013). Burke et al. (2012, 2014) reported a reduction in body weight gains in lambs and kids after grazing for 28 to 56 days. The latter study describes growth of lambs and kids utilized in the current experiment.

Other uses of condensed tannin rich plants such as SL fed to ruminants are to reduce methane emissions (Woodward et al., 2004; Puchala et al., 2005; Waghorn and Woodward, 2006) and the incidence of bloat (Jones et al., 1973). Legumes such as SL can be included in pastures to reduce the need for nitrogen fertilizer (Ball et al., 1996).

The condensed tannins can bind to proteins, polymers such as cellulose and hemicelluloses, as well as pectin and minerals in the rumen, inhibiting their digestion (reviewed by McSweeney et al., 2001). The condensed tannin bound protein disassociates in the small intestine and becomes available for digestion. Binding of condensed tannin to minerals derived from feed sources could be an issue to the animal if disassociation does not occur in the abomasum or small intestine reducing bioavailability to the animal. Thus, the objective of these experiments was to determine the effect of feeding SL pellets to sheep and goats on serum concentrations of trace minerals.

## Materials and Methods

The experiments were conducted at the USDA, Agricultural Research Service, Dale Bumpers Small Farms Research Station, Booneville, Arkansas, or at Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana. All animal-related procedures were approved by the Animal Care and Use Committee of each research institution (ASAS, 2020). All experiments were part of a larger study to evaluate the effect of SL pellets on gastrointestinal nematode and *Eimeria* (coccidia) parasite infections. Diets between treatments were not balanced for trace minerals and feed or pasture groups per treatment were not replicated within experiments. Thus, results and interpretations should be considered accordingly.

### ARS Lambs

Katahdin lambs were born from ewes fed control or SL supplements for GIN control (data unpublished). Initially, Katahdin ewes were randomized to treatments; offspring remained in treatment plots to examine effects of SL or control diets on GIN infection. In 2011 and 2012, lambs were weaned at  $108.9 \pm 1.4$  d of age in late May (spring), or  $90.1 \pm 0.9$  d of age, respectively, at ARS (number and sex listed in Table 1). Lambs were offered a control supplement (CO; 37% corn, 16% wheat middlings, 14% soybean meal, 13% cottonseed hulls, 10% alfalfa pellets, 4% molasses, 4% soybean hulls; 15% crude protein or CP), or SL leaf meal pellets (15% CP; Sims Bros. Inc., Union Springs, AL; mineral concentrations listed in Table 2); diets were isonitrogenous. Mineral analyses of CO and SL determined in 2012 are listed in Table 2. Supplemental

diets were fed as single groups on pasture (though deworming events were on an individual basis). The CO and SL ration offered at weaning increased from no supplement (pre-weaning) incrementally to 450 g/lamb between 0 and 14 days, 675 g/lamb between 14 and 42 days, and 900 g/lamb between 42 and 126 days in 2011, or from no supplement (pre-weaning) incrementally to 900 g/lamb daily within 14 days in 2012. This resulted in feeding SL pellets at a mean of 1.5% (day 0) to 2.5% of body weight (day 42; highest percent body weight fed) in 2011, or a mean of 3.7% (day 0) to 2.7% (day 56) of body weight daily in 2012.

Lambs were offered free choice trace mineral mix (2011: Land O'Lakes Sheep and Goat Mineral, Shoreview, MN; a minimum of 28% salt, 15% calcium, 7% phosphorus, 0.3% potassium, 26.5 µg/g Se, 69,873 IU/kg vitamin A, 8,167 IU/kg vitamin D<sub>3</sub>, 136 IU/kg vitamin E; also contained an unspecified amount of magnesium, iron, iodine, Mn, Co, and Mo; 2012: Nutra Blend, LLC, Neosho, MO; 20% salt, 16% calcium, 8% phosphorus, 3% magnesium, 1.5% potassium, 2,700 µg/g Zn, 2,000 µg/g Mn, 320 µg/g iodine, 125 µg/g Cu, 20 µg/g Se, 63,520 IU/kg vitamin A, 15,880 IU/kg vitamin D<sub>3</sub>, 104 IU/kg vitamin E), water and grazed primarily bermudagrass (*Cynodon dactylon*) pastures or were offered bermudagrass hay when forage in pasture was limiting due to drought conditions.

On day 70 (2011) or 56 (2012), blood was collected from the jugular vein in trace element serum vacutainer tubes (Bectin Dickinson, Franklin Lakes, NJ). Serum was collected, placed on ice and submitted for trace mineral analysis using inductively coupled

**Table 1. Experiments conducted among Agricultural Research Service (Booneville, AR) and Louisiana State University (LSU) lambs and goat kids fed control (CO) or sericea lespedeza (SL) supplement.**

Trait	ARS 2011, Lambs		ARS 2012, Lambs		LSU 2011, Lambs		LSU 2012, Lambs		ARS 2012, Kids		ARS 2013, Kids	
	CO	SL	CO	SL	CO	SL	CO	SL	CO	SL	CO	SL
n	15	13	25	25	14	15	15	14	16	16	17	16
Females	15	13	17	13	2	7	8	5	--	--	--	--
Males	--	--	8	12	13	8	7	9	16	16	17	16
Age at start, days	108.9		90.1		85.5		80.0		80.0		86.5	
Supplement, g	900		900		900		450		450		450	
Day of blood collection	72		56		112		112		64		56	

**Table 2. Concentrations of micro-minerals ( $\mu\text{g/g}$ ) in control (CO) or sericea lespedeza (SL) supplements offered in 2012 to lambs and kids at the Agricultural Research Service (Booneville, AR) or Louisiana State University (LSU). Feed samples were ground and analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).**

Mineral	CO <sup>1</sup>	SL <sup>2</sup>	CO <sup>3</sup>	SL <sup>4</sup>	CO <sup>5</sup>	CO <sup>6</sup>
Co	0.12	0.27	0.54	0.48	1.0	0.17
Cu	7.6	7.5	8.5	7.2	26.3	6.8
Fe	157	245	883	166	178	382
Mn	55.9	85.2	75.4	62.7	113.7	59.8
Mo	2.29	0.32	2.68	0.80	0.70	1.45
Zn	38.2	26.3	26.7	18.1	122.5	27.5
Se	0.35	0.08	0.76	0.07	0.67	0.16

<sup>1</sup> CO supplement fed to ARS lambs in 2012.

<sup>2</sup> SL pellets fed to ARS lambs in 2012.

<sup>3</sup> CO supplement fed to LSU lambs in 2012.

<sup>4</sup> SL pellets fed to LSU lambs and ARS kids in 2012 and 2013.

<sup>5</sup> CO supplement fed to ARS kids in 2012.

<sup>6</sup> CO supplement (alfalfa meal pellets) fed to ARS kids in 2013.

plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI).

### LSU Lambs

The objective of the LSU experiment was the same as for ARS lambs using a second location with different environmental conditions. The experimental design was the same. In 2011 and 2012 at LSU, Louisiana Native  $\times$  Suffolk crossbred lambs were weaned at  $85.5 \pm 1.5$  and  $84.9 \pm 1.7$  days of age, respectively. Lambs were fed 900 g of CO (Purina Lamb Show Ration CTC 50; Purina Mills, LLC, St. Louis, MO; 16% CP; mineral analysis found in Table 2) or SL (number and sex of animals described in Table 1; mineral analyses found in Table 2; 12.3% CP) between 0 and 112 days. In 2012, the SL pellets (Sims Brothers, Inc.) fed were lower in CP than in 2011. The CO lambs had access to bermudagrass pasture (forage quality not measured) and the SL lambs had access to a mixed SL and grass pasture. Free choice water and trace mineral (Purina Wind and Rain Sheep All Season 7 Complete, Purina Mills, LLC, St. Louis, MO; a minimum of 37% salt, 7% calcium, 7% phosphorus, 1% potassium, 0.5% magnesium, 3,230  $\mu\text{g/g}$  Zn, 2,850  $\mu\text{g/g}$  Mn, 34  $\mu\text{g/g}$  iodine, 25  $\mu\text{g/g}$  Se, 10  $\mu\text{g/g}$  Co, 440,800 IU/kg vitamin A, 440,800 IU/kg vitamin D, 181 IU/kg vitamin E)

were available. Blood was collected on day 112 for serum analysis of trace minerals.

### ARS Goat Kids

The objective of this experiment was to determine the effects of feeding a control or SL supplement for 64 days on serum concentrations of trace minerals in goat kids. The experiment was a complete block design (blocked for breed) and was repeated over 2 years. Only two pastures were available and supplement group was the experimental unit. At ARS, buck kids used in 2012 were Spanish ( $n = 16$ ) or Kiko ( $n = 16$ ), and in 2013 were French Alpine  $\times$  Spanish ( $n = 21$ ) or French Alpine  $\times$  Kiko ( $n = 12$ ). Kids (Table 1) were weaned at  $83.0 \pm 0.5$  days of age, weighed  $16.8 \pm 0.6$  kg in 2012 and  $18.5 \pm 0.6$  kg in 2013. In 2012, kids were supplemented with 680 g of a control supplement (CO; Noble<sup>TM</sup> Goat Grower, Purina Mills, LLC, St. Louis, MO; 16% CP; mineral analyses in Table 2) or 450 g SL pellets (same as fed for 2012 LSU lambs;  $n = 16$ /supplement) plus 230 g of the CO supplement to balance CP while grazing grass pastures. In 2013, buck kids were randomly assigned to be supplemented with 225 g/day of alfalfa pellets (16% CP;  $n = 17$ ) or SL pellets (same as used in 2012;  $n = 16$ ; Table 1) for 28 days, then supplement increased to 450 g/d for an additional 28 days.

There was little forage on pastures in 2012 because of a severe drought; therefore, free choice bermudagrass hay was offered. In 2013, predominantly bermudagrass pasture was never limiting based on visual appraisal. In both years, free choice trace mineral mix (Nutra Blend LLC, Neosho, MO; 20% salt, 12% calcium, 8% phosphorus, 8% sodium, 3% magnesium, 1.4% potassium, 1% sulfur, 4,465  $\mu\text{g/g}$  Zn, 4,324  $\mu\text{g/g}$  iron, 2,157  $\mu\text{g/g}$  Mn, 1,267  $\mu\text{g/g}$  Cu, 790  $\mu\text{g/g}$  iodine, 25  $\mu\text{g/g}$  Se, 46 KU/kg vitamin A, 103 IU/kg vitamin E, 15 KU/kg vitamin D3) formulated for goats was offered.

All goats were administered copper oxide wire particles for control of GIN (Burke et al., 2007a) on day 14 and were selectively dewormed or individually administered with a combination of albendazole (15 mg/kg oral drench; Valbazen, Pfizer Animal Health, Exton, PA) and moxidectin (0.4 mg/kg oral drench; Cydectin, Fort Dodge Animal Health, Fort Dodge, IA) if PCV  $\leq 19\%$ .

### Peri-Parturient Ewes

The objective of this experiment was to determine the long-term effect of feeding a CO or SL supplement and pasture on serum concentrations of trace minerals of peri-parturient ewes. Experimental design was a randomized complete block, blocking for age of ewe, with a  $2 \times 2$  Latin square analyzing supplemental treatment (CO or SL) and two forages. Pasture was the experimental

unit (n = 1 per treatment × forage combination). Ewes were randomly assigned to treatments in 2010 to examine effects of long-term supplementation with SL on *H. contortus* (unpublished data), and replacements added in 2011 to account for necessary culling.

### ARS Ewes

In 2012, ARS Katahdin ewes between 2 and 4 years of age grazed bermudagrass (*Cynodon dactylon*) overseeded with cereal rye (*Secale cereale*) and wheat (*Triticum aestivum*) pasture, or tall fescue (*Lolium arundinaceum* infected with the endophyte *Epichloë coenophiala*) overseeded with hairy vetch (*Vicia villosa*) at ARS, and were fed 450 g of a CO supplement (n = 14 on bermudagrass, n = 9 on tall fescue pastures; 50% soybean hulls, 14% soybean meal, 13.5% corn, 10% wheat middlings, 5% alfalfa pellets, 5% molasses; 15% CP) or SL pellets described for 2012 ARS lambs above (n = 11 on bermudagrass; n = 10 on tall fescue pastures) starting 35 days before lambing, which occurred over a 28 day period in February. Supplement was increased to 1 kg at lambing and reduced to 450 g 60 days post-lambing until weaning (131 days of supplement). For the last 30 days before weaning/blood collection, SL-fed ewes grazed SL pasture (AU Grazer; CP ranged between 11.0–13.2%). Forage was never limiting based on visual appraisal. On the day that diet was initiated, all ewes were administered 2 ml BoSe® (4.2 mg sodium selenite, 100 mg vitamin E; Merck Animal Health, Madison, NJ) i.m. as a selenium supplement. Trace mineral mix was offered free choice (Nutra Blend, LLC, Neosho, MO; 7.5% salt, 17% calcium, 8% phosphorus, 3% magnesium, 1.5% potassium, 2,708 µg/g Zn, 2,033 µg/g Mn, 318 µg/g iodine, 259 µg/g Cu, 20 µg/g Se, 63,520 IU/kg vitamin A, 15,880 IU/kg vitamin D3, 104 IU/kg vitamin E). All but one CO ewe had multiple lambs born, and all but 4 SL ewes had multiple lambs born. Blood was collected 131 days after dietary supplement began.

### LSU Ewes

A second replicate of ewes was used at LSU in 2012, under somewhat different conditions and a different breed using a completely randomized design.

Louisiana Native × Suffolk crossbred ewes were randomly assigned to be fed 1 kg of control (n = 15) or SL (n = 14) supplement 30 days before lambing began. The ewes were managed on pasture and given trace mineral mix similar to LSU lambs above. Ewes lambled between March 2 and April 2. Blood was collected after 120 days of feeding supplements for serum analysis of trace minerals.

### Analysis of Minerals in Serum and Feed

Serum was collected, placed on ice and shipped within 48 hours. Serum and feed samples were analyzed for macro (feed only) and trace minerals using inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University, Lansing, MI). Serum concentration of trace minerals were considered adequate or deficient based on reference ranges determined at Michigan State University Veterinary Diagnostic Laboratory in 2021 (Table 3). Values between the deficiency and adequate concentrations are considered marginal.

### Statistical Analyses

General linear models (SAS) in a completely randomized or complete block design were used for serum concentrations of minerals. All independent variables were considered fixed. The model used for lambs included dietary supplement, sex, year, and interactions. The model used for goat kids included dietary supplement, breed, year, and interactions. The model used for ARS ewes included dietary supplement, forage and the interaction; only supplement

was included in the LSU model. Outliers (more than two SD) of any value from individuals were removed from the data set. Concentrations of molybdenum were below the detectable level (0.5 ng/ml) of the assay for 12 of 17 SL (2012), and 12 of 16 SL (2013) supplemented goat kids; a value of 0.5 was assigned to these kids.

## Results and Discussion

### Manganese, Molybdenum, Zinc, and Selenium

There were changes in serum concentrations of trace minerals in lambs (Table 4), kids (Table 5), and mature ewes (Table 6) between CO and SL-fed animals. Manganese, Mo, Zn, and Se were almost always lower in SL compared with CO-fed animals among experiments with Mo having the greatest reduction (Tables 4, 5, 6). The exception was a greater concentration of Zn in SL-fed goats in 2013 (Table 5; diet × year,  $P < 0.001$ ). There was a 10 to 90-fold reduction of serum concentrations of Mo in lambs, a 3 to 8-fold reduction in goat kids, and a 3 to 4-fold reduction in ewes. Concentrations of trace minerals in lambs were generally within normal range (Mn, 1-6 ng/mL; Se, 60-200 ng/mL; Zn, 0.55-1.2 µg/mL), but concentrations of Se and Zn were often at the low end of the range, regardless of diet or experiment (Herdt and Hoff, 2011). Serum concentrations of zinc are not good indicators of zinc deficiency, except when low. Less is known about normal range of serum concentrations of trace minerals in goats, but in the current study, Mn and Zn were in reported range (Schweizer et al., 2017). Hamil-

**Table 3. Criteria for determination of adequate or deficient serum concentrations of trace minerals according to Michigan State University Veterinary Diagnostic Laboratory (2021) in ranges on adult sheep. Values between adequate and deficient are considered marginal.**

Mineral	Adequate	Deficient
Co, ng/ml	0.17 – 1.35	--
Cu, µg/ml	0.54 – 1.04	<0.5
Mn, ng/ml	0.8 – 4.4	--
Mo, ng/ml	1.1 – 79.8	--
Zn, µg/ml	0.48 – 0.94	<0.43
Se, ng/ml	95 - 190	<81

**Table 4. Serum concentrations of micro-minerals in control (CO) or sericea lespedeza (SL) supplemented lambs at the Agricultural Research Service (ARS; Booneville, AR) or Louisiana State University (LSU). At ARS, samples were collected on day 72 (2011) or 56 (2012), and at LSU on day 112 after initiation of dietary treatment (day 0 = day of weaning). Serum was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).**

Mineral	2011		2012		Diet P <	Year P <	Diet × Year P <
	CO	SL	CO	SL			
ARS							
n	20	19	25	25			
Co	2.6 ± 0.21 <sup>a</sup>	1.6 ± 0.22 <sup>b</sup>	0.84 ± 0.19 <sup>c</sup>	1.5 ± 0.19 <sup>b</sup>	0.001	0.41	0.001
Cu	0.68 ± 0.04	0.62 ± 0.04	0.76 ± 0.03	0.66 ± 0.03	0.02	0.12	0.54
Mn	3.6 ± 0.18 <sup>a</sup>	1.8 ± 0.18 <sup>c</sup>	2.5 ± 0.16 <sup>b</sup>	1.9 ± 0.16 <sup>c</sup>	0.001	0.01	0.001
Mo	9.8 ± 1.2 <sup>b</sup>	0.87 ± 1.2 <sup>c</sup>	16.9 ± 1.1 <sup>a</sup>	0.45 ± 1.1 <sup>c</sup>	0.001	0.005	0.001
Zn	0.79 ± 0.03	0.70 ± 0.03	0.83 ± 0.03	0.70 ± 0.03	0.001	0.45	0.50
Se	102.1 ± 5.5 <sup>a</sup>	98.9 ± 5.5 <sup>a</sup>	108.2 ± 4.9 <sup>a</sup>	82.1 ± 4.7 <sup>b</sup>	0.005	0.30	0.03
LSU							
n	14	15	16	16			
Co	0.43 ± 0.08 <sup>b</sup>	0.37 ± 0.07 <sup>b</sup>	0.48 ± 0.07 <sup>b</sup>	1.1 ± 0.07 <sup>a</sup>	0.001	0.001	0.001
Cu	0.45 ± 0.04 <sup>bc</sup>	0.51 ± 0.03 <sup>b</sup>	0.64 ± 0.03 <sup>a</sup>	0.37 ± 0.03 <sup>c</sup>	0.001	0.50	0.001
Mn	1.8 ± 0.08	1.9 ± 0.07	1.4 ± 0.07	1.5 ± 0.07	0.068	0.001	0.85
Mo	72.8 ± 7.6 <sup>a</sup>	0.80 ± 6.7 <sup>c</sup>	23.2 ± 6.4 <sup>b</sup>	2.1 ± 6.7 <sup>c</sup>	0.001	0.001	0.001
Zn	0.38 ± 0.02 <sup>b</sup>	0.38 ± 0.02 <sup>b</sup>	0.52 ± 0.02 <sup>a</sup>	0.41 ± 0.02 <sup>b</sup>	0.005	0.001	0.01
Se	126.9 ± 4.2	97.0 ± 3.7	135.7 ± 3.6	105.7 ± 3.7	0.001	0.03	0.99

abc Means with unlike superscripts differ ( $P < 0.05$ ).

Mineral abbreviations (units): Cobalt = Co (ng/ml); Copper = Cu (µg/ml); Manganese = Mn (ng/ml); Molybdenum = Mo (ng/ml); Zinc = Zn (µg/ml); Selenium = Se (ng/ml).

ton et al. (2017) also noted a reduction in serum concentrations of Se and Zn in goats fed SL, but not Mo which was quite low in both the CO and SL-fed goats. Similarly, quebracho tannins

reduced serum Mo and Se in lambs compared with a control-fed diet (Acharya et al., 2020).

Less is known about the normal range of Mo in livestock, but at present

is considered between 1 – 50 ng/mL (Herdt and Hoff, 2011). Molybdenum is prevalent in forages and the requirement is considered to be met by common feedstuffs (Suttle, 1991). The trace mineral

**Table 5. Serum concentrations of minerals in control (CO) or sericea lespedeza (SL) supplemented kids (n = 16 or 17/diet in 2012 and 2013) at the Agricultural Research Service (Booneville, AR) collected after 64 (2012) or 56 (2013) days of dietary treatment. Serum was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).**

Mineral	2012		2013		Diet P <	Year P <	Diet × Year P <
	CO	SL	CO	SL			
Co	2.1 ± 0.15 <sup>a</sup>	1.4 ± 0.15 <sup>b</sup>	0.50 ± 0.14 <sup>c</sup>	0.87 ± 0.15 <sup>c</sup>	0.27	0.001	0.001
Cu	0.91 ± 0.03	0.83 ± 0.03	0.75 ± 0.03	0.69 ± 0.03	0.04	0.001	0.78
Mn	2.7 ± 0.13 <sup>a</sup>	1.7 ± 0.13 <sup>b</sup>	1.7 ± 0.13 <sup>b</sup>	1.9 ± 0.13 <sup>b</sup>	0.009	0.003	0.001
Mo <sup>1</sup>	2.1 ± 0.26 <sup>b</sup>	0.72 ± 0.26 <sup>c</sup>	3.8 ± 0.26 <sup>a</sup>	0.53 ± 0.26 <sup>c</sup>	0.001	0.007	0.001
Zn	0.66 ± 0.02 <sup>a</sup>	0.50 ± 0.02 <sup>c</sup>	0.50 ± 0.02 <sup>c</sup>	0.58 ± 0.02 <sup>b</sup>	0.07	0.03	0.001
Se	97.6 ± 3.5	81.9 ± 3.5	93.1 ± 3.5	71.3 ± 3.5	0.001	0.04	0.39

abc Means with unlike superscripts differ ( $P < 0.05$ ).

<sup>1</sup> Concentrations of Mo were below the detectable level (0.5 ng/ml) of the assay for 12 of 17 SL (2012), and 12 of 16 SL (2013) supplemented goat kids; a value of 0.5 was assigned to these kids.

Mineral abbreviations (units): Cobalt = Co (ng/ml); Copper = Cu (µg/ml); Manganese = Mn (ng/ml); Molybdenum = Mo (ng/ml); Zinc = Zn (µg/ml); Selenium = Se (ng/ml).

**Table 6.** Serum concentrations of trace minerals in control (CO) or sericea lespedeza (SL) supplemented ewes at the Agricultural Research Service (ARS; Booneville, AR) or Louisiana State University (LSU). There was a supplement × pasture effect for Cu, Fe, Mn, Mo, and Zn, presented in last five rows [the CO and SL supplements were offered to ewes grazing primarily bermudagrass (BG) and tall fescue (TF) pastures]. Serum was analyzed by inductively coupled plasma/mass spectroscopy (Diagnostic Center for Population and Animal Health at Michigan State University).

Mineral	ARS			LSU		
	CO	SL	P <	CO	SL	P <
n	23	21		15	14	
Day <sup>1</sup>	131			120		
Co	0.61 ± 0.07	0.68 ± 0.07	0.45	0.60 ± 0.09	0.87 ± 0.09	0.04
Cu	0.80 ± 0.04	0.81 ± 0.04	0.80	0.70 ± 0.02	0.62 ± 0.02	0.03
Mn	1.8 ± 0.08	1.2 ± 0.08	0.001	1.3 ± 0.09	1.4 ± 0.10	0.50
Mo	7.7 ± 0.5	2.9 ± 0.5	0.001	39.9 ± 2.9	9.2 ± 3.0	0.001
Zn	0.78 ± 0.02	0.59 ± 0.02	0.001	0.83 ± 0.03	0.77 ± 0.03	0.13
Se	105.0 ± 4.3	108.8 ± 4.5	0.54	165.3 ± 3.4	138.4 ± 3.6	0.001
ARS, supplement × pasture						
	BG			TF		
n	14	11		9	10	
Cu	0.87 ± 0.05 <sup>abc</sup>	0.73 ± 0.06 <sup>b</sup>		0.72 ± 0.06 <sup>b</sup>	0.90 ± 0.06 <sup>c</sup>	0.008
Fe	133.5 ± 8.0 <sup>a</sup>	110.6 ± 9.1 <sup>ab</sup>		101.1 ± 10.0 <sup>b</sup>	120.4 ± 9.5 <sup>ab</sup>	0.03
Mn	2.1 ± 0.10 <sup>a</sup>	1.2 ± 0.12 <sup>c</sup>		1.6 ± 0.13 <sup>b</sup>	1.2 ± 0.12 <sup>c</sup>	0.03
Mo	10.7 ± 0.6 <sup>a</sup>	2.3 ± 0.7 <sup>c</sup>		4.6 ± 0.7 <sup>b</sup>	3.4 ± 0.7 <sup>bc</sup>	0.001
Zn	0.84 ± 0.02 <sup>a</sup>	0.61 ± 0.02 <sup>c</sup>		0.70 ± 0.03 <sup>b</sup>	0.58 ± 0.03 <sup>c</sup>	0.04

<sup>1</sup> Days after initiation of dietary treatment.

<sup>a-c</sup> Means within a row lacking a common superscript differ ( $P < 0.05$ ).

Mineral abbreviations (units): Cobalt = Co (ng/ml); Copper = Cu (µg/ml); Manganese = Mn (ng/ml); Molybdenum = Mo (ng/ml); Zinc = Zn (µg/ml); Selenium = Se (ng/ml).

mix offered to LSU sheep and ARS lambs in 2010 and 2011 and ARS ewes contained sodium molybdate; the mineral mix offered to ARS lambs and goats in 2012 did not. Molybdenum is essential in the enzyme complexes xanthine oxidase and sulphite oxidase (Schwarz et al., 2009). Our laboratory was first to report a reduction in Mo associated with feeding SL (Acharya et al., 2015, 2016). However, in that study, when drenching lambs with sodium molybdate, body weight gains were still reduced similar to that of SL without supplemental Mo, compared with a control diet (Acharya et al., 2015).

A reduction in serum concentrations of Se occurred in all SL compared with CO-fed lambs and kids except ARS lambs in 2011. Animals were provided Se as sodium selenite in the trace mineral mix offered. 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). Selenium appears to be absorbed from

the gut based on dietary availability (Herdt and Hoff, 2011). If Se binds to condensed tannin in the rumen, likely as a metalloprotein, and is able to dissociate in the small intestine, it would be absorbed and be transported to the liver (Herdt and Hoff, 2011). Serum represents a transport pool for Se and is a good indicator of dietary intake. Thus, the reduction in serum concentrations of Se in SL animals could have been associated with the lower concentration of Se in the SL compared with CO supplement (Table 2). Concentration of Se in liver is a more useful indicator of Se status in the animal. Indeed, liver concentrations, but not serum, were reduced in SL compared with CO-fed lambs in a study in which diets were balanced for trace minerals (Acharya et al., 2016).

Manganese and Zn can be found in forages and were provided in the trace mineral mix as manganous oxide and manganese sulfate, and zinc sulfate and zinc oxide. These trace minerals also complex with enzymes (Herdt and Hoff, 2011). Manganese is essential for nutri-

ent use, bone development and maintenance. Zinc is important in cell division, regulation of appetite and growth, and immune function. The homeostasis of both Mn and Zn is tightly regulated. When we examined these minerals in liver of CO or SL-fed lambs in a more controlled study, Mn was similar between dietary treatments, but concentrations of Zn were still reduced in SL-fed lambs (Acharya et al., 2016).

Like Se and Mo, Mn and Zn can complex with metalloproteins. Minerals and digestive enzymes can become bound to condensed tannins in the rumen and will bypass the rumen (reviewed by McSweeney et al., 2001 and Min and Hart, 2003; Naumann et al., 2017). Trace minerals complexed as metalloproteins should dissociate in the lower pH of the abomasum, but can form indigestible compounds with other feed components reducing their availability for absorption in the small intestine, the primary site of absorption (Gressley, 2009).

## Cobalt

Serum concentrations of Co were lower in SL compared with CO-fed lambs at ARS in 2011 (diet × year,  $P = 0.025$ ; Table 4) and goats in 2011 (diet × year,  $P = 0.02$ ; Table 5), increased in SL-fed lambs in 2012 at ARS and LSU (diet × year,  $P < 0.001$ ), and LSU ewes ( $P < 0.04$ ; Table 6), and goats in 2013, and similar between dietary lamb groups at LSU in 2011. Serum concentrations of Co were higher than reported range (0.18- 2.0 ng/mL) in the CO group of ARS lambs in 2011, but otherwise, most values were within range (Herdt and Hoff, 2011). Changes in Co could be related to differences in the concentration found in the diet, which was only measured in 2012. However, Co in the LSU CO supplement was slightly higher than the SL supplement. It was reported that quebracho tannin supplementation to lambs reduced serum concentrations of Co (Acharya et al., 2020).

## Copper

Copper was lower in SL compared with CO-fed ARS lambs ( $P = 0.02$ ; Table 4), was lower in LSU lambs in 2012 but not 2011 (diet × year,  $P < 0.001$ ; Table 4) and LSU ewes ( $P < 0.03$ ; Table 6), but otherwise similar between dietary groups in other experiments. In the 2012 lambs at ARS, CO-fed ewe lambs had higher serum Cu than ram lambs or SL-fed lambs (diet by sex,  $P = 0.014$ ; CO: female,  $0.83 \pm 0.04$ , male,  $0.63 \pm 0.06$ ; SL: female,  $0.63 \pm 0.05$ , male,  $0.69 \pm 0.05$  mg/dL).

Serum concentrations of Cu in lambs was often below the reported range (0.75 – 1.7 µg/mL; Herdt and Hoff, 2011), which could indicate a deficiency. Even though serum copper is a relatively insensitive marker, it is reasonably specific as an indicator of deficiency (Herdt and Hoff, 2011). The serum copper of goats in current study was in a similar range as reported by Schweinzer et al. (2017). In previous studies at ARS, concentrations of Cu in the liver were marginal (Burke et al., 2004; Burke and Miller, 2006). In a subsequent experiment in our laboratory in which CO and SL diets were balanced for nutrients and fed to lambs, both serum and liver concentrations of Cu were reduced in SL-fed lambs (fed for 103 days), and even fur-

ther when sodium molybdate was administered to balance molybdenum in serum of lambs (Acharya et al., 2016).

Molybdenum is 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). Interestingly, the low Mo in the SL-fed animals in the current study did not lead to an increase in Cu, although serum is not the best indicator of Cu status (Herdt and Hoff, 2011). Even in the subsequent experiment in which CO and SL diets were balanced for trace minerals, liver concentrations of Cu were reduced by 250% in SL-fed lambs (Acharya et al., 2016). Sulfur was not measured in the current experiments. However, McNabb et al. (1993) determined that condensed tannins from *Lotus pedunculatus* reduced degradation of sulfur amino acids to inorganic sulfide in the rumen, leading to greater absorption of methionine and increased utilization of cysteine. This suggests that sulfur from plant proteins binds to condensed tannins and dissociates in the small intestine and is available for absorption. This may also interfere with Cu absorption in SL-fed animals.

## Iron

Iron was similar between dietary groups in all experiments (data not shown).

## Forage Influence

Serum concentrations of Mo were reduced in peri-parturient ewes supplemented with SL compared with a CO at ARS and LSU farms (Table 6). At ARS, serum concentrations of Mo were reduced in SL compared with CO supplemented ewes on bermudagrass, but not tall fescue pasture, as the CO value was already lower. Serum concentrations of Mn and Zn were reduced in SL compared with CO-fed ewes on both forage types, but the magnitude of the reduction was different (Table 6). There was an increase in concentrations of Cu in SL-fed ewes on tall fescue compared with bermudagrass forage.

The lower concentrations of trace minerals in ewes that grazed tall fescue demonstrates the importance of forage type or quality when examining relationships among trace minerals in the blood

similar to that reported in rangeland plants reviewed by Stewart et al. (2021). There were some forage quality measurements collected, but not on or near the day of blood collection. Two weeks before blood collection, forage quality of bermudagrass and tall fescue pastures were comparable. However, endophyte-infected tall fescue produces ergot alkaloids that may have reduced concentrations of Se in blood in cattle (Burke et al., 2007b). Thus, the endophyte-infected tall fescue may have been associated with changes in trace minerals in serum in ARS ewes in the current study.

## Recommendations and Conclusions

The CO and SL supplements among studies were balanced for CP with the exception of LSU lambs and 2012 goat kids either due to feed mill mixing errors or lower quality feeds available which could have influenced serum trace mineral concentrations. Further, it cannot be ruled out that the presence of dietary condensed tannins may alter the satiety for salt or the mineral mix so that animals within one treatment group consumes more or less trace mineral mix. Animals fed CT feeds typically have drier feces (Saratsis et al., 2012; Burke et al., 2013), likely due to an inhibitory action on intestinal chloride channels (Tradtrantip et al., 2010), demonstrating a complexity of interactions in mineral metabolism in SL-fed animals. Though diets were not balanced for trace minerals, there were consistent trends in differences in serum concentrations of trace minerals among species, age groups, and the Arkansas and Louisiana locations, and in a subsequent study in which trace minerals were balanced between treatments (Acharya et al., 2016).

Growth of lambs and kids in these experiments (Burke et al., 2014) and others reported in the literature may have been impaired by consumption of a condensed tannin rich forage. Microminerals, especially Mo, Se, Mn, and Zn were nearly always reduced in SL-fed lambs, ewes, and kids. A reduction in these minerals could influence growth of lambs and kids, possibly leading to other metabolic issues. Selenium and Zn are often limiting minerals for animal grazing forages or extensive rangelands

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(Herdt and Hoff, 2011; Page et al., 2018). There is not enough known on whether offering trace minerals in excess will alleviate the deficiencies associated with feeding condensed tannin feeds, though when a diet was formulated by a nutritionist to meet needs for energy, protein, vitamins, and minerals according to NRC (2007) growth was similar between lambs fed SL or control diet (Acharya et al., 2015). High quality minerals, particularly focusing on trace minerals, should be offered before and during feeding of CT forages. If unsure of mineral status of animals, a blood or tissue sample can be submitted to a veterinary diagnostic lab to determine serum concentrations of trace minerals. Also, consider regional soil and forage tests for mineral deficiencies or consult with cooperative extension specialists or advisors to understand limitations such

as copper or selenium deficiencies or excesses. Although not presented here, a small number of ARS lambs fed SL in 2012 were sampled for serum concentrations of trace minerals 34 days after SL withdrawal and more closely aligned with CO-fed lambs. In other words, any potential trace mineral deficiency had disappeared after discontinuing condensed tannin feeding. It is suggested to supplement or graze SL for no more than six to eight weeks, which may occur during the greatest parasite challenges (around the time of weaning or during stress) or as needed for other mitigation strategies, or monitor serum trace mineral status on a portion of the animals being fed.

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