

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 ± 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 D₃, 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 D₃, 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.

	ARS 2 Lan	2011, nbs	ARS Lai	2012, mbs	LSU : Lai	2011, mbs	LSU Lar	2012, nbs	ARS 2 Ki	2012, ids	ARS Ki	2013, ids
Trait	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	3.9	90	.1	85	.5	80	0.0	80	.0	86	5.5
Supplement, g	90	00	90	00	90	00	44	50	45	0	44	50
Day of blood collection	7	2	5	6	11	2	11	12	6	4	5	6

Table 2. Concentrations of micro-minerals (μ 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	CO1	SL ²	CO ³	SL ⁴	CO ⁵	CO ⁶
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
Мо	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

 1 CO supplement fed to ARS lambs in 2012.

 2 SL pellets fed to ARS lambs in 2012.

 3 CO supplement fed to LSU lambs in 2012.

⁴ SL pellets fed to LSU lambs and ARS kids in 2012 and 2013.

⁵ CO supplement fed to ARS kids in 2012.

⁶ 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 × Suffolk crossbred lambs were weaned at 85.5 ± 1.5 and 84.9 ± 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 µg/g Zn, 2,850 µg/g Mn, 34 μg/g iodine, 25 μg/g Se, 10 μg/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 x Spanish (n = 21) or French Alpine x Kiko (n = 12). Kids (Table 1) were weaned at 83.0 ± 0.5 days of age, weighed 16.8 ± 0.6 kg in 2012 and 18.5 ± 0.6 kg in 2013. In 2012, kids were supplemented with 680 g of a control supplement (CO; NobleTM 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 µg/g Zn, 4,324 µg/g iron, 2,157 µg/g Mn, 1,267 µg/g Cu, 790 µg/g iodine, 25 µg/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 tallfescue 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 lambed 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 90fold 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 (Schweinzer 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).

							Diet
	20	11	20	12	Diet	Year	× Year
Mineral	СО	SL	СО	SL	P <	P <	P <
		AI	RS				
n	20	19	25	25			
Co	2.6 ± 0.21^{a}	1.6 ± 0.22^{b}	$0.84 \pm 0.19^{\circ}$	1.5 ± 0.19^{b}	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^{a}	1.8 ± 0.18^{c}	2.5 ± 0.16^{b}	$1.9 \pm 0.16^{\circ}$	0.001	0.01	0.001
Мо	9.8 ± 1.2^{b}	$0.87 \pm 1.2^{\circ}$	16.9 ± 1.1^{a}	$0.45 \pm 1.1^{\circ}$	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^{a}	98.9 ± 5.5^{a}	108.2 ± 4.9^{a}	82.1 ± 4.7^{b}	0.005	0.30	0.03
		LS	SU				
n	14	15	16	16			
Со	0.43 ± 0.08^{b}	0.37 ± 0.07^{b}	0.48 ± 0.07^{b}	1.1 ± 0.07^{a}	0.001	0.001	0.001
Cu	$0.45 \pm 0.04^{\rm bc}$	0.51 ± 0.03^{b}	0.64 ± 0.03^{a}	$0.37 \pm 0.03^{\circ}$	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
Мо	72.8 ± 7.6^{a}	$0.80 \pm 6.7^{\circ}$	23.2 ± 6.4^{b}	$2.1 \pm 6.7^{\circ}$	0.001	0.001	0.001
Zn	0.38 ± 0.02^{b}	0.38 ± 0.02^{b}	0.52 ± 0.02^{a}	0.41 ± 0.02^{b}	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 feed-stuffs (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).

							Diet
	20	12	20	13	Diet	Year	× Year
Mineral	CO	SL	СО	SL	P <	P <	P <
Co	2.1 ± 0.15^{a}	1.4 ± 0.15^{b}	$0.50 \pm 0.14^{\circ}$	$0.87 \pm 0.15^{\circ}$	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^{a}	1.7 ± 0.13^{b}	1.7 ± 0.13^{b}	$1.9 \pm 0.13^{\rm b}$	0.009	0.003	0.001
Mo1	2.1 ± 0.26^{b}	$0.72 \pm 0.26^{\circ}$	3.8 ± 0.26^{a}	$0.53 \pm 0.26^{\circ}$	0.001	0.007	0.001
Zn	0.66 ± 0.02^{a}	$0.50 \pm 0.02^{\circ}$	$0.50 \pm 0.02^{\circ}$	0.58 ± 0.02^{b}	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).

¹ 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).

		ARS				LSU		
Mineral	СО		SL	P <	СО		SL	P <
n	23		21		15		14	
Day1		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
Мо	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, supp	lement ×	pasture			
		BG			-	TF		
n	14		11		9		10	
Cu	0.87 ± 0.05^{abc}		0.73 ± 0.06^{b}		0.72 ± 0.06^{b}		0.90 ± 0.06^{c}	0.008
Fe	133.5 ± 8.0^{a}		110.6 ± 9.1 ^{ab}		101.1 ± 10.0 ^b		120.4 ± 9.5 ^{ab}	0.03
Mn	2.1 ± 0.10^{a}		1.2 ± 0.12^{c}		1.6 ± 0.13^{b}		1.2 ± 0.12^{c}	0.03
Мо	10.7 ± 0.6^{a}		2.3 ± 0.7^{c}		4.6 ± 0.7^{b}		3.4 ± 0.7^{bc}	0.001
Zn	0.84 ± 0.02^{a}		$0.61 \pm 0.02^{\circ}$		0.70 ± 0.03^{b}		$0.58 \pm 0.03^{\circ}$	0.04

¹ Days after initiation of dietary treatment.

^{a-c} 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 nutrient 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 SLfed 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 \times year, P = 0.02; Table 5), increased in SL-fed lambs in 2012 at ARS and LSU (diet \times vear. 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 ± 0.04, male, 0.63 ± 0.06; SL: female, 0.63 ± 0.05, male, 0.69 ± 0.05 mg/dL).

Serum concentrations of Cu in lambs was often below the reported range $(0.75 - 1.7 \,\mu\text{g/mL}; \text{Herdt and Hoff},$ 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 further 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, endophyteinfected tall fescue produces ergot alkaloids that may have reduced concentrations of Se in blood in cattle (Burke et al., 2007b). Thus, the endophyteinfected 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

(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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Literature Cited

- Acharya, M., J. M. Burke, K. P. Coffey, E. B. Kegley, J. E. Miller, G. R. Huff, E. Smyth, T. H. Terrill, and C. Rosenkrans Jr. 2015. Changes in hematology and serum biochemical profiles in lambs fed sericea lespedeza. J. Anim. Sci. 93:1952-1961. doi.org/10.2527/jas.2014-8584.
- Acharya, M., J. M. Burke, K. P. Coffey, E. B. Kegley, J. E. Miller, E. Smyth, M. G. Welborn, T. H. Terrill, and C. Rosenkrans Jr. 2016. Changes in concentrations of trace minerals in lambs fed sericea lespedeza leaf meal pellets with or without dietary sodium molybdate. J. Anim. Sci. 94:1592-1599. doi.org/10.2527/jas.2015-9910.
- Acharya, M., J. M. Burke, J. E. Miller, T. H. Terrill, E. L. Wood, and J. P. Muir. 2020. Quebracho tannins aid in the control of Eimeria spp. and gastrointestinal nematodes in lambs and goat kids. Vet. Parasitol. 288:109295. Doi.org/10.1016/ j.vetpar.2020.109295.
- ADSA-ASAS-PSA. 2020. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 4th Ed. Champaign, IL.
- Ball, D. M., C. S. Hoveland, and G. D. Lacefield. 1996. Southern Forages. 2nd ed. Potash and Phosphate Inst. and the Foundation for Agronomic Research, Norcross, GA.
- Burke, J. M. and J. E. Miller. 2006. Evaluation of multiple low doses of copper oxide wire particles compared with levamisole for control of *Haemonchus contortus* in lambs. Vet. Parasitol. 139:145-149. doi.org/10.1016/j.vetpar.2006. 02.030.
- Burke, J. M., J. E. Miller J. A. Mosjidis, and T. H. Terrill. 2012. Use of a mixed sericea lespedeza and grass pasture system for control of gastrointestinal nematodes in lambs and kids. Vet. Parasitol. 186:328-336. doi.org/10.1016/j.vetpar. 2011.11.074.
- Burke, J. M., J. E. Miller, D. D. Olcott, B. M. Olcott, and T. H. Terrill. 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs. Vet. Parasitol. 123:235-243. doi.org/ 10.1016/j.vetpar.2004.06.009.
- Burke, J. M., J. E. Miller, T. H. Terrill, and J. A. Mosjidis. 2014. The effects of supplemental sericea lespedeza pellets in lambs and kids on growth rate. Livest. Sci. 159:29-36. doi.org/10.1016/j.livsci.2013.10.030.
- Burke, J. M., J. E. Miller, T. H. Terrill, S. Orlik, M. Acharya, and J. A. Mosjidis. 2013. Sericea lespedeza as an aid in the control of *Eimeria* spp. in lambs. Vet. Parasitol. 193:39-46. doi.org/10.1016/j.vetpar.2012.11.046.
- Burke, J. M., T. H. Terrill, R. R. Kallu, and J. E. Miller. 2007a. Use of copper oxide wire particles to control gastrointestinal nematodes in goats. J. Anim. Sci. 85:2753-2761. doi.org/10.2527/jas.2006-784.
- Burke, N. C., G. Scaglia, K. E. Saker, D. J. Blodgett, and W. S. Swecker Jr. 2007b. Influence of endophyte consumption and heat stress on intravaginal temperatures, plasma lipid oxidation, blood selenium, and glutathione redox of mononuclear cells in heifers grazing tall fescue. J. Anim. Sci. 85:2932-2940. doi.org/10.2527/jas.2007-0303.

- Hamilton, T., T. Terrill, D. S. Kommuru, A. Rivers, J. Mosjidis, J. Miller, C. Drake, I. Mueller-Harvey, J. Burke. 2017. Effect of supplemental sericea lespedeza pellets on internal parasite infection and nutritional status of grazing goats. J. Agric. Sci. Tech. A 7:334-344. doi:10.17265/2161-6256/2017.05.005.
- Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. Food Anim. 27:255-283.
- Jones, W. T., L. B. Anderson, and M. D. Ross. 1973. Bloat in cattle. XXXIX. Detection of protein precipitants (flavolans) in legumes. N. Z. J. Agric. Res. 16:441-446. doi.org/10.1080/00288233.1973.10421128.
- McNabb, W. C., G. C. Waghorn, T. N. Barry, and I. D. Shelton. 1993. Effect of condensed tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cysteine and inorganic sulphur in sheep. Br. J. Nutr. 70:647-661. doi.org/10.1017/S0021859600078576.
- McSweeney, C. S., B. Palmer, D. M. McNeill, and D. O. Krause. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci. Tech. 91:83-93. doi.org/10.1016/S0377-8401(01)00232-2.
- Min, B. R., and S. P. Hart. 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81 (E. Suppl. 2):E102-E109. doi.org/10.2527/2003.8114_suppl_2E102x.
- Min, B. R., S. P. Hart, D. Miller, G. M. Tomita, E. Loetz, and T. Sahlu. 2005. The effect of grazing forage containing condensed tannins on gastro-intestinal parasite infection and milk composition in Angora does. Vet. Parasitol. 130:105-113. doi.org/10.1016/j.vetpar.2005.03.011.
- Min, B. R., W. E. Pomroy, S. P. Hart, and T. Sahlu. 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. Small Rum. Res. 51:279-283. doi.org/10.1016/S0921-4488(03)00204-9.
- National Research Council. 2007. Nutrient Requirements of Small Ruminants. Sheep, Goats, Cervids, and New World Camelids. National Academies Press. Washington, D.C.
- Naumann, H. D., L. O. Tedeschi, W. E. Zeller, and N. F. Huntley. 2017. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. R. Bras. Zootec. 46:929-949. http://dx.doi.org/ 10.1590/S1806-92902017001200009.
- Page, C. M., T. W. Murphy, M. L. Van Emon, J. G. P. Bowman, S. A. Wyffels, and W. C. Stewart. 2018. Blood serum mineral element concentrations of weaned Montana ram lambs and their relationship with water quality characteristics. Prof. Anim. Sci. 34:410-420. Doi.org/10.15232/ pas.2018-01747.
- Puchala, R., B. R. Min, A. L. Goetsch, and T. Sahlu. 2005. The effect of a condensed tannin-containing forage on methane emission by goats. J. Anim. Sci. 83:182-186. doi.org/10.2527/2005.831182x.
- Saratsis, A., I. Regos, N. Tzanidakis, N. Voutzourakis, A. Stefanakis, D. Treuter, D., A. Joachim, and S. Sotiraki. 2012. In vivo and in vitro efficacy of sainfoin (*Onobrychis viciifolia*) against Eimeria spp in lambs. Vet. Parasitol. 188:1-9. https://doi.org/10.1016/j.vetpar.2012.03.014.

- SAS. 1996. STAT® Software: Changes and Enhancements through Release 9.11. SAS Inst., Inc., Cary, NC.
- Schwarz, G., R. R. Mendel, and M. W. Ribbe. 2009. Molybdenum cofactors, enzymes and pathways. Nature 460/13:839-847. Doi:10.1038/nature08302.
- Schweinzer, V., M. Iwersen, M. Drillich, T. Wittek, A. Tichy, A. Mueller, and R. Krametter-Froetscher. 2017. Macromineral and trace element supply in sheep and goats in Austria. Vet. Medic. 62:62-73. doi: 10.17221/243/2015-VETMED.
- Stewart, W. C., J. D. Scasta, J. B. Taylor, T. W. Murphy, and A. A. M. Julian. Invited review: Mineral nutrition considerations for extensive sheep production system. Appl. Anim. Sci. 37:256-272. https://doi.org/10.15232/aas.2021-02143.

- Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutr. 11:121-140.
- Waghorn, G. C., and S. L. Woodward. 2006. Ruminant contributions to methane and global warming a New Zealand perspective. Pages 233-260 in Climate Change and Managed Ecosystems. J. S. Bhatti, R. Lal, M. J. Apps, M. A. Price, ed. Taylor and Francis, Boca Raton, FL.
- Woodward, S. L., G. C. Waghorn, and P. G. Laboyrie. 2004. Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduce methane emissions from dairy cows. Proc. N. Z. Soc. Anim. Prod. 64:160-164.



Technical Note: Impact of Temperature and Humidity on United States Fine-Wool Fiber Diameter Measurements

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Summary

Fiber diameter is one of the main factors determining wool value with small increases in fiber diameter leading to a loss in revenue. Understanding the factors that lead to inaccuracies in wool fiber diameter testing could help ensure fair pricing of wool for producers in different climates. To determine the effect of humidity and temperature on the diameter of United States fine-wool, 135 wool samples were placed in a temperature and humidity-controlled chamber and acclimated to a range of relative humidity and temperatures. Samples were then evaluated

on an Optical Fiber Diameter Analyzer 2000 to measure fiber diameter and other fiber quality metrics. Results show that increases in humidity increase the fiber diameter of wool (P < 0.05) but temperature and the interaction between the two had no effect. Producers testing wool fiber diameter in the field with the OFDA2000 may need to consider environmental humidity to increase the testing accuracy.

Key Words: Wool, Humidity, Temperature, Fiber Diameter, Sheep

Introduction

In recent years wool production has been a secondary source of income for US sheep producers compared to other sheep producing countries. In 2021, the United States produced 12,332 tons of greasy wool, making up less than 1 percent of worldwide wool production (IWTO, 2021). Comparatively, countries like Australia and China have large shares of the global wool market, producing 18.3% and 17.1% of global wool production respectively (IWTO, 2021). Demand for wool has decreased in the United States due to increased competition with less expensive synthetic fibers (Ashton et al., 2000). Furthermore, traditional markets for wool, such as outer knitwear and woven suit attire, have shrunk due to increased casualization of the workforce, limited trans-seasonal clothing options, and increased discretionary spending during unfavorable economic conditions (Doyle et al., 2021).

Wool marketing has shifted toward new markets such as next-to-skin clothing (Rowe, 2010). Next-to-skin clothing requires wool to have a high comfort value, which is largely based upon the prickling factor of the fiber. The prickling factor of wool-based clothes is associated with increased buckling fibers that protrude from the fabric (Naebe et al., 2015). A fiber's buckling response (F) is determined by the equation

$$F = \frac{\pi^{3*}Ed^{4}}{31.36L^2},$$

where E is Young's modulus, d is the fiber diameter, and L is the length of the protruding fiber (Naebe et al., 2015). As fiber diameter increases, so does the buckling response, which then decreases the comfort level as more protruding fibers rub and irritate the wearer's skin. Thus, the importance of lower diameter fiber wool production has increased as the next-to-skin wool clothing markets expand. As lower fiber diameter wool is usable in a more extensive range of textiles, due to the higher comfort factor, it is priced higher on a weight basis, making wool prices inversely associated with fiber diameter (Cottle and Baxter, 2015). Therefore, to increase profits from wool, producers have a further incentive to breed animals that grow finer diameter wool.

To accurately determine the fiber diameter of wool, fiber analysis machines are used. Three common machines used to analyze wool for its diameter are the Fiber Lux Micron Meter, the Opticalbased Fiber Diameter Analyzer 2000 (OFDA 2000) and the Sirolan-LaserscanTM, The Sirolan-LaserscanTM machine works through laser-based fiber diameter analysis. First. the machine project lasers through the wool fibers within a measurement cell. The resulting beam is then split between two points a detector and a fiber optic discriminator which measures the beam and allows the processer to determine the width of the sampled wool (Botha and Hunter, 2010). The Fiber Lux uses light diffraction to determine fiber diameter (Walker et al., 2018). The OFDA 2000 works through optical diameter measurements. This machine is operated by first putting samples in a wire-framed slide where the frame is traversed by a low-powered microscope while being illuminated stroboscopically from below. The wool sample is traversed by the microscope at each increment of the staple and measures the fiber diameter at regular intervals (Baxter, 2001). These diameters are then averaged through the entire wool grab. The OFDA 2000 has been used to accurately sort lines of wool in-field in real-time for large producers (Kott et al., 2010). Accuracy in using these machines in the wool testing process is vital to fairly price wool and select sheep with lower fiber diameter wool. Differences in wool sample diameter between New Zealand and Montana State testing labs have been discovered that indicate there may be outside factors that are impacting the fiber diameter of samples.

As sheep producing regions differ in climates, changes in temperature and humidity may be affecting in-field fiber diameter measurements. Wool is water absorbent with an absorbance level of around 11% water weight per wool dry weight (Van Amber et al., 2015). Water molecules act in a diffusion-like manner with keratin structures in wool. Keratin chains "diffuse" into the space left vacant by the water molecules when water molecules evaporate and vice versa. The water molecules interact with the keratin proteins which replace the protein-protein interactions and form a keratin-water network instead (Feughelman and Robinson, 1967; Wortmann and De Jong, 1985). Wool appears to have a two-phase model for water absorption, where the first 10-15% of water absorbed is stored as monolayer at strongly reactive absorption sites along the fiber and when relative humidity is greater than 14% absorption occurs at weakly reactive sites (Wortmann and De Jong, 1985). However, this absorption rate is also controlled by the Hofmeister effect where differences in hydrophobic ions alter the presence of water in the wool fibers (Lo Nostro et al., 2002). In cases where these ions are present this could increase the water density in wool fibers making them test at a higher fiber diameter value. If the water from increased relative humidity is absorbed into wool and subsequently increases wool fiber diameter, fiber diameter analysis will be biased towards larger values.

Temperature and humidities within and between fine wool producing regions can vary widely depending on location and time of day. Great Falls, Montana has an annual average relative humidity of 45% and annual average temperature of 5.3 °C compared to Houston, Texas that has an annual average relative humidity of 59% and annual average temperature of 19.2 °C (NCEI, 2018; NCEI, 2023). There is variation present within each region as well with Kalispell, Montana having an annual average relative humidity of 54%, 9% higher than Billings, Montana (NCEI, 2018). Therefore, there may be inaccuracies in testing for fiber diameter even when comparing flocks within regions if it is affected by the humidity conditions in which it is stored. Humidity and temperature also changes based upon the time of day. Between morning and afternoon, the average humidity for Billings, Montana differs from 66% to 48% (NCEI, 2018). Therefore, fiber diameter tested by an OFDA in the field may be biased if these variables change through the day or wool brought to a pool for testing has been stored in different conditions. Understanding if and how the environmental differences of temperature and humidity is impacting fine wool analysis when testing in the field is crucial to ensuring the accuracy of wool fiber diameter testing.

One of the issues the Montana Wool Lab faces when helping small and

medium sized growers is the inability to be at every farm and ranch during shearing with wool handling equipment and many shearing crews cannot economically pay a classer for small flocks. Due to this, the OFDA2000 is used at wool pool deliveries to test samples of baled or packaged wool as it is delivered to determine the appropriate market line. There is no published research on testing packaged wool in-field that has been stored under different environmental conditions and may vary in moisture content while testing. The objective of this study was to evaluate how different relative humidity and temperatures may influence wool fiber diameter measurements when using the OFDA 2000 for possible adjustments when in-field testing of United States fine wool.

Materials and Methods

One hundred thirty-five 7.5 to 12.5 cm staple length wool clips were collected over five different shearing dates. Wool samples were shipped to the Montana Wool Lab where all fiber measurements were taken. Wool samples were washed through a sonicating bath of hexanes for 10 minutes and allowed to dry for 30 minutes. A controlled humidity and temperature chamber (The-SausageMaker, Model# 19100, Buffalo, NY) was used to subject the wool samples to the temperature: relative humidity combinations listed in table 1. Each individual sample was subjected to every temperature: relative humidity combination (Table 1). Samples were acclimated to each temperature and relative humidity level for at least 12 hours prior to measurement. Samples were removed and analyzed in ten sample batches to avoid loss of acclimation before measurement. The samples were measured for fiber micron diameter with the OFDA 2000.

Using R, a repeated measurements ANOVA was performed to evaluate the effects of the independent variables (Temperature and Relative Humidity) on the dependent variable of mean fiber diameter. An interaction between Temperature and Relative Humidity was included in the model.

Results and Discussion:

Average sample fiber diameter

Treatment Combination RH¹ Temperature (°C) 1 4.4 35 2 10.0 35 3 15.5 35 4 40 4.4 5 40 10.0 6 15.5 40 7 45 4.4 45 8 10.0 9 45 15.5 10 50 4.4 50 11 10.0 12 50 15.5 13 55 4.4 55 14 10.0 15 55 15.5 16 4.4 60 17 10.0 60 18 15.5 60 19 65 4.4 20 10.0 65 21 15.5 65 ¹ Relative Humidity Percentage

ranged from 15.4 to 24.47 microns across all treatment combinations. Average fiber diameter for each temperature and relative humidity combination can be found in Table 2. No significant interaction effect of temperature and relative humidity was detected (P > 0.05). Wool fiber diameter was found not to be associated with temperature (P > 0.05) but associated with relative humidity (P < 0.05). As humidity increases, so does the mean wool fiber diameter, as shown in Figure 1. However, the effect size of humidity is small, with an increase of approximately 0.005 microns in diameter for every one RH percent increase within the range tested (Figure 1).

Our results suggest that relative humidity levels impact the fiber diameter measured in samples with more humid treated wool staples having higher micron values. These results are consistent with Naebe (2013) which indicates that the wool Comfort-MeterTM values, which are inversely related to fiber diameter, were reduced at

Table 2: Average fiber diameter (± standard deviation), in mi of 135 wool samples by relative humidity and temperature treatment.

RH ¹		Temperature °C	
	4.4	10	15.5
35	19.62 ±1.58	19.57 ±1.56	19.62 ±1.57
40	19.54 ±1.55	19.58 ±1.56	19.65 ±1.54
45	19.58 ±1.56	19.62 ±1.55	19.65 ±1.54
50	19.59 ±1.54	19.65 ±1.59	19.64 ±1.57
55	19.65 ±1.55	19.66 ±1.54	19.73 ±1.56
60	19.67 ±1.55	19.67 ±1.53	19.79 ±1.56
65	19.75 ±1.56	19.77 ±1.55	19.76 ±1.55

Table 1: Temperature and Relative Humidity Percentage treatment combinations applied to each individual wool sample.



Figure 1: Mean Fiber Diameter (with standard error bars) plotted against Relative Humidity Level.

greater levels of humidity (Naebe et al., 2013). The effect of humidity on wool fiber diameter appears to begin at around 50% relative humidity. Climate data indicates that relative humidities in areas with fine wool production often exceeds 50% humidity in parts of or throughout the whole day (NCEI, 2018). Therefore, the expanding effect relative humidity has on wool is relevant to in-field testing of wool produced and stored in these regions.

The impacts of humidity on fiber diameter may therefore be essential to control in order to increase in the field wool testing accuracy. In the field, wool testing may vary depending upon the humidity which is largely driven by time of day. Time of day variations of humidity levels can be as great as 30% between morning and night in places such as Bloomington, Texas (NCEI, 2018). Wool exposed to these higher humidity levels in the morning may have increased fiber diameter than if it were shorn and analyzed in the evening when it may be less humid. As many shearing operations start in the morning and shear all day there may be a difference in fiber diameter found between morning and afternoon shearing. Furthermore, wool fiber diameter may be artificially increased in regions of the United States with higher humidity such as the Northeast and Southeast as opposed to the Southwest and Intermountain West.

Differences in humidity levels between regions can be great as 30% on average (NCEI, 2018). Therefore, field testing in regions with higher humidity may assign wool samples higher fiber diameters than testing in less humid climates.

In the field testing of individual samples could lead to sheep being undervalued for their wool performance in high humidity areas and some sheep being overvalued in low humidity areas. From this misvaluing of breeding stock, selection towards smaller fiber diameter wool in flocks could be altered and progress potentially slowed if samples from different flocks are being tested at different in-field environmental conditions that are not accounted for. Differences in storage practices and processing of wool bales may result in differences in humidity within the bale, thereby affecting the in-field measurement of samples, and potentially changing the marketing line that wool is assigned to. Furthermore, while this study did not increase RH past 65% some wool producing areas may have relative humidities as great as 90% on any given day (NCEI, 2018). Using the results from this study, wool fiber diameter measurements on a 90% RH day would be 0.2 microns thicker than a 50% RH day. This line shift could cause an approximately \$0.20/pound grease weight reduction that producers receive for their wool, based on historical averages for the Eastern Consolidated Wool Pool (unpublished data).

While this study shows that fiber diameter is increased in higher relative humidity, further work is needed to determine if other factors in field may play a part in altering fiber diameter measurements. The study design does allow for interpretation of the effect humidity and temperature has on fiber diameter in a controlled setting. However, large in field data may be needed for more accurate information to what the actual effects of humidity and temperature are in field. Furthermore, interactions between the original fiber diameter and relative humidity might be limiting the effect relative humidity we found. Further studies may select for similar micron size in samples before application of treatments to control for beginning fiber diameter and relative humidity interactive effects better.

The overall differences in fiber diameter measured in environments with different humidity supports an equalizing process where wool samples are treated to the same humidity before analyzing for fiber diameter, which occurs in wool labs but is not practical when in field testing. However, as the effect size of humidity on fiber diameter is small, there may not be an economic incentive to push for such practices. Further study on the economics of this fiber diameter difference is needed to determine whether such a practice is economically beneficial.

Conclusion:

Here we show that humidity increases the diameter of wool fibers while temperature does not. The economic impact of the inaccuracy of testing due to humidity, however, is likely minimal but may be important for wools tested near cut-offs for specific lines when collecting at wool pools and downstream usages. Further research into other factors affecting fiber diameter may be needed to fully understand differences observed between fiber analysis equipment used in the field.

Literature Cited

- Ashton, D., S. Brittle, and T. Sheales. 2000. Demand for wool in a changing world. *Australian Commodities: Forecasts and Issues* 7(3):494-502.
- Baxter, B. 2001. Precision of Measurement of Diameter and Diameter-length Profile of Greasy Wool Staples On-farm using the OFDA2000 Instrument. *Wool Technology and Sheep Breeding* 49(1):42-52.
- Botha, A. F., and L. Hunter. 2010. The measurement of wool fibre properties and their effect on worsted processing performance and product quality. Part 1: The objective measurement of wool fibre properties. *Textile Progress* 42(4):227-339.
- Cottle, D., and B. P. Baxter. 2015. Wool metrology research and development to date. *Textile Progress* 47(3):163-315.
- Doyle, E. K., J. W. Preston, B. A. McGregor, and P. I. Hynd. 2021. The science behind the wool industry. The importance and value of wool production from sheep. *Animal Frontiers* 11(2):15-23.
- Feughelman, M., and M. Robinson. 1967. The relationship between some mechanical properties of single wool fibers and relative humidity. *Textile Research Journal* 37(6):441-446.
- IWTO. 2021. World Sheep Numbers and Wool Production No.17. International Wool Textile Organization.
- Kimble, C. A. 2010. Extreme Temperature Ranges. National Oceanic and Atmospheric Administration.
- Kott, R., B. Roeder, and L. Surber. 2010. Sorting Lines of Wool with the OFDA2000. International Journal of Sheep and Wool Science 58(1)

- Lo Nostro, P., L. Fratoni, B. W. Ninham, and P. Baglioni. 2002. Water absorbency by wool fibers: Hofmeister effect. *Biomacromolecules* 3(6):1217-1224.
- Naebe, M., B. A. McGregor, P. Swan, and D. Tester. 2015. Associations between the physiological basis of fabricevoked prickle, fiber and yarn characteristics and the Wool ComfortMeter value. *Textile Research Journal* 85(11):1122-1130.
- Naebe, M., Y. Yu, B. A. McGregor, D. Tester, and X. Wang. 2013. The effect of humidity and temperature on Wool ComfortMeter assessment of single jersey wool fabrics. *Textile Research Journal* 83(1):83-89.
- NCEI. 2018. Comparative Climatic Data.
- NCEI. 2023. Climate at a Glance Divisional Mapping.
- Rowe, J. B. 2010. The Australian sheep industry–undergoing transformation. *Animal Production Science* 50(12):991-997.
- Van Amber, R. R., C. A. Wilson, R. M. Laing, B. J. Lowe, and B. E. Niven. 2015. Thermal and moisture transfer properties of sock fabrics differing in fiber type, yarn, and fabric structure. *Textile Research Journal* 85(12):1269-1280.
- Walker, J., W. Stewart, R. Pope, S. Spear, M. Ebert, and T. Murphy. 2018. Evaluation of mean fiber diameter measurements by FibreLux micron meter and OFDA2000 in Texas and Intermountain west wool. *Small Ruminant Res* 159:31-37.
- Wortmann, F.-J., and S. De Jong. 1985. Analysis of the humidity-time superposition for wool fibers. *Textile Research Journal* 55(12):750-756.