Journal of Agricultural Science and Technology A 8 (2018) 377-387
doi: 10.17265/2161-6256/2018.06.005

Effect of Ensiling on Efficacy of Sericea Lespedeza against Gastrointestinal Nematodes and Coccidia in Goats

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Abstract: Effect of ensiled sericea lespedeza (SL, *Lespedeza cuneata*) on indicators of gastrointestinal parasitism was investigated in two trials using intact male Spanish goats (n = 36/trial). Naturally parasite-infected animals at 24.4 ± 3.7 kg body weight (BW) and nine months of age or 24.6 ± 0.57 kg BW at 4-6 months of age were used for 28 d or 21 d for Trials 1 and 2, respectively. For Trial 1, goats were fed SL silage (SLS), SL hay (SLH), or Bermuda grass (BG, *Cynodon dactylon*) hay at 70% of the diet. For Trial 2, goats were provided with SLH or SLS and orally drenched with distilled water (Hay or silage plus water, HW and SW, respectively) or polyethylene glycol (PEG; SLS only; SP) daily. Fecal and blood samples were collected weekly to determine gastrointestinal nematode fecal egg counts (FEC) and coccidia fecal oocyst counts (FOC) per gram of feces and blood packed cell volume (PCV). The SL diets significantly reduced ($p < 0.05$) FEC and FOC in both trials, with the SLH diet reducing FEC 7 d faster compared to SLS. In PEG-treated goats, FOC tended to increase ($p < 0.07$) at day 7 before decreasing. There was no treatment effect on PCV scores. Overall, SLH and SLS reduced fecal egg and oocyst counts in goats, but PEG results were inconclusive, so more research is needed.

Key words: Coccidia, gastrointestinal nematodes, goats, sericea lespedeza, silage.

1. Introduction

Sericea lespedeza (SL, *Lespedeza cuneata*) is a perennial, warm season legume that has been shown to be beneficial as a natural alternative for controlling gastrointestinal nematodes and coccidia in small ruminants [1, 2], thought to be due primarily to condensed tannins (CT) in the plant. Its efficacy was first reported in fresh grazed forage [3], and though processing (drying, pelleting or ensiling) of harvested plants may influence polyphenol composition and thus could impact plant bioactivity, research has indicated that effectiveness of tannin-containing plants can be maintained with processing in dried forms such as hay and pellets [1].

In the Southeastern U.S., or on small-scale farms, production of dried forages may not be feasible due to weather issues. Also, in drying SL, the leaves may over-dry and shatter, causing loss of leaves, the part of the plant with the highest levels of protein and tannin [4]. Processing of the forage into silage (fermented fresh forage) may solve some of these issues as well as provide an alternative sales product for SL growers. However, no known research has been conducted to determine if ensiling SL would alter its impact on gastrointestinal parasites or to determine if the action of SL might be mitigated by the use of polyethylene glycol (PEG) to bind CT during feeding. Therefore,

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the objective of the current research was to determine the effect of SL silage (SLS) on indicators of gastrointestinal nematode and coccidia infections in goats, and to compare anti-parasitic effects of SL with and without PEG in goats.

2. Materials and Methods

Two confinement feeding trials with goats were completed at the Fort Valley State University Agricultural Research Station in Fort Valley, GA, with Institutional Animal Care and Use Committee approval. For both studies, ‘AU Grazer’ SL was harvested from a well-established (nine-year) pasture at the University Agricultural Research Station in late summer (August). The forage was cut and chopped into approximately 1.27 cm lengths using a forage harvester (Troy-Bilt Chipper-Shredder CS 4323, Troy-Bilt, Valley City, OH). Half the chopped material was immediately packed into 132.3 L low-density black plastic bags and tied after manually removing as much air as possible. The bags were then placed inside a second bag, tied and stored in a barn to allow the bagged material to ensile. The bags were kept in storage for at least 12 weeks before opening for feeding. The other half of the chopped material was spread out on 30.5 m × 7.6 m plastic sheeting and sun-dried for 72 h, with daily turning to facilitate uniform drying. The dried material (85% DM) was then collected and placed in 207.9 L clear plastic bags for storage.

2.1 Animals and Treatments

2.1.1Trial 1

Thirty-six, naturally parasite-infected Spanish intact males at 24.4 ± 3.7 kg body weight and approximately nine months of age were used in a 28-day study. Animals were housed in stalls (3.66 m × 3.05 m) on packed dirt flooring with mulch bedding (three goats per stall; four stalls per diet). Treatment diets consisted of SLS, chopped SL hay (SLH), or ground Bermuda grass (*Cynodon dactylon*) hay (BGH) at 70% of the diet with 30% of the diet consisting of a grain mixture formulated to balance dietary protein and energy among diets according to National Research Council guidelines for small ruminants [5]. Rations were provided at 3.5% of body weight (DM basis) and water was provided *ad libitum*.

2.1.2 Trial 2

Thirty-six naturally parasite-infected, intact male Spanish goats 4-6 months of age and 24.6 ± 0.57 kg body weight were used in a 21-day trial. Goats were housed in 3.66 m × 3.05 m stalls (three goats per stall; four stalls per diet) and administered treatments consisting of pre-weighed rations of SLH or SLS and daily oral drenching with 60 mL distilled water daily (hay or silage plus water, HW and SW, respectively), or daily drenching with 50 g of 6,000 MW PEG (to bind plant tannins) in 60 mL distilled water daily (SLS only; SP). Animals were provided with *ad libitum* water and loose goat minerals (Southern States Top Choice Goat Mineral, Southern States Coop., Inc., Richmond, VA). Orts (leftover feed) were measured weekly to calculate dry matter intake (DMI).

2.2 Fecal Samples

For the determination of gastrointestinal nematode fecal eggs counts (FEC) and coccidia fecal oocyst counts (FOC) per gram using the modified McMaster technique [6], fecal samples were collected on day 0 and weekly thereafter from the rectum of each animal during each trial. Fecal consistency was also scored (Trial 2) just prior to fecal egg counting using a system described by Burke *et al.* [7] in which a score of 1 = solid pellets, 2 = solid pellets that stuck together, 3 = a soft mass of feces, 4 = unformed, soft feces and 5 = slurry.

2.3 Packed Cell Volume (PCV)

Blood samples were collected via jugular venipuncture weekly to monitor anemia level during each trial. Blood was collected, placed into 3 mL vacutainer tubes containing K_2ETDA and stored on ice until analyzed. For percentage red blood PCV, microhematocrit capillary tubes were filled with blood, sealed and centrifuged at 10,000× g for 10 min (IEC Model Mb, Needham Heights, MA). A microhematocrit reader was then used to determine the PCV. For Trial 1, FAMACHA© eyelid color scores were recorded on day 0 and weekly thereafter as an additional measure of anemia [8].

2.4 Statistical Analysis

Data for gastrointestinal nematode FEC, coccidia FOC, blood PCV and FAMACHA© were analyzed by repeated measures analysis in a completely randomized design using a mixed model in SAS 9.4, TS1M3 [9]. Treatment, day (sampling date) and the treatment × day interaction were included in the model. Data were log transformed for FEC, FOC and PCV (ln(FEC + 25), ln(FOC + 25), ln(PCV+1)) prior to statistical analysis. These data are reported as least squared means, with statistical inferences based upon log-transformed data analysis.

3. Results

3.1 Trial 1

3.1.1 Fecal Egg and Oocyst Counts

Gastrointestinal nematode FEC were impacted by a treatment by day interaction ($p < 0.01$) in which FEC for goats fed SLH were lower ($p < 0.001$) than for those fed BGH on days 14, 21 and 28 while SLS was only lower ($p < 0.04$) than BGH on days 21 and 28 (Fig. 1). Coccidia FOC, which started on day 7 after treatment (no day 0 value available), were impacted by treatment and day ($p < 0.001$ for both). Compared to BGH, SLH and SLS both decreased FOC ($p < 0.001$) and FOC were higher at day 7 than for all other time points measured ($p < 0.005$; Fig. 2).

3.1.2 PCV and FAMACHA©

Percentage PCV was not impacted by treatment, averaging 20.1% ± 0.2%. Goat FAMACHA© eyelid color scores were not impacted by treatment, but were impacted by day ($p < 0.02$). Scores were highest for day 0 and day 7, which were similar, and lowest for

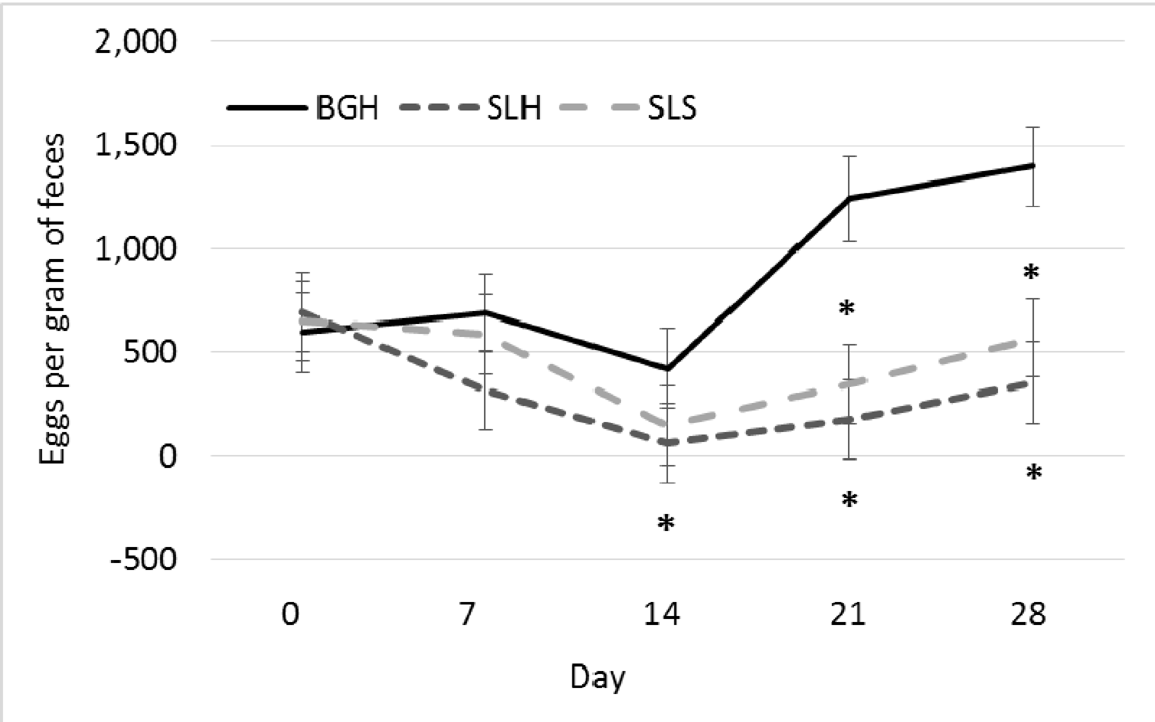
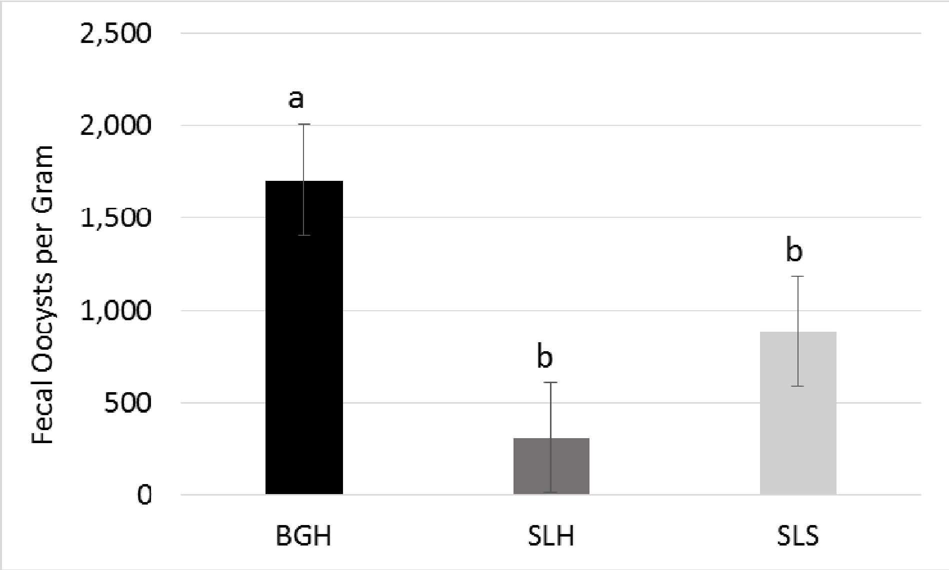


Fig. 1 Fecal egg counts (eggs per gram) for goats fed Bermuda grass hay (BGH) or sericea lespdeza hay (SLH) or silage (SLS) for 28 d.

Treatment by day interaction, $p < 0.008$; points with a * are different from BGH at $p < 0.04$.



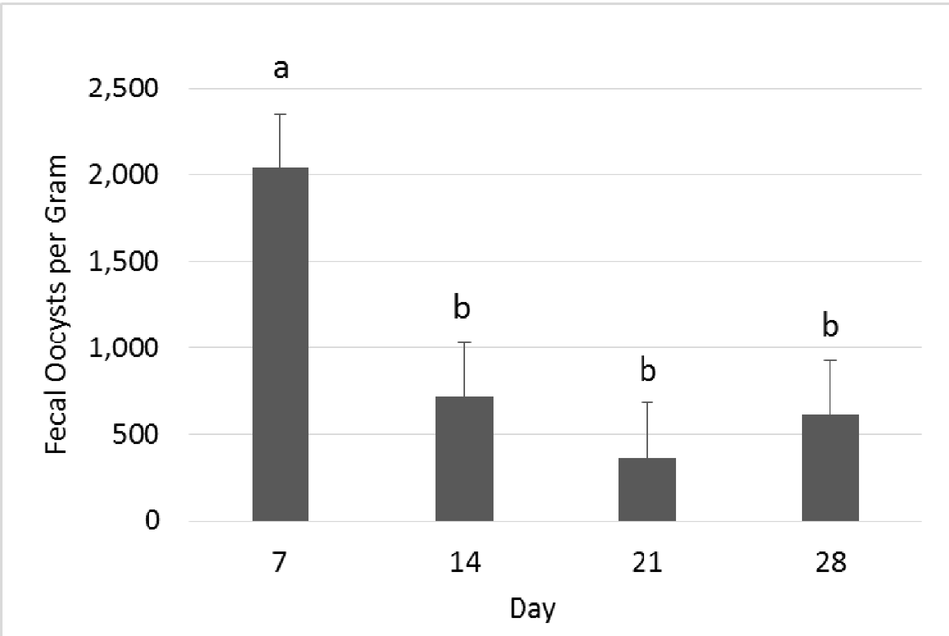


Fig. 2 Fecal oocysts counts (per gram of feces) for goats fed BGH or SLH or SLS for 28 d.
Treatment affect, $p < 0.001$ (top panel); oocysts counts (bottom panel) were also impacted by day, $p < 0.001$; bars with different superscripts differ $p < 0.01$ for both panels.

day 28 while day 14 and day 21 were intermediate ($p < 0.004$; Fig. 3).

3.1.3 Body Weight and Average Daily Gain

Goat body weights were impacted by a treatment by day interaction ($p < 0.001$; Fig. 4). Initial body weights were not different between the treatment groups and increased ($p < 0.001$) over time for all goats, regardless of treatment, but was greater for BGH than for SLH on day 21 and day 28 but only different for SLS on day 28 ($p < 0.04$). Average daily gain was impacted by treatment ($p < 0.01$), with goats fed BGH (207 ± 15.6 g/d) gaining more than SLH (153 ± 15.6 g/d) and SLS (122 ± 15.6 g/d) fed goats ($p < 0.03$).

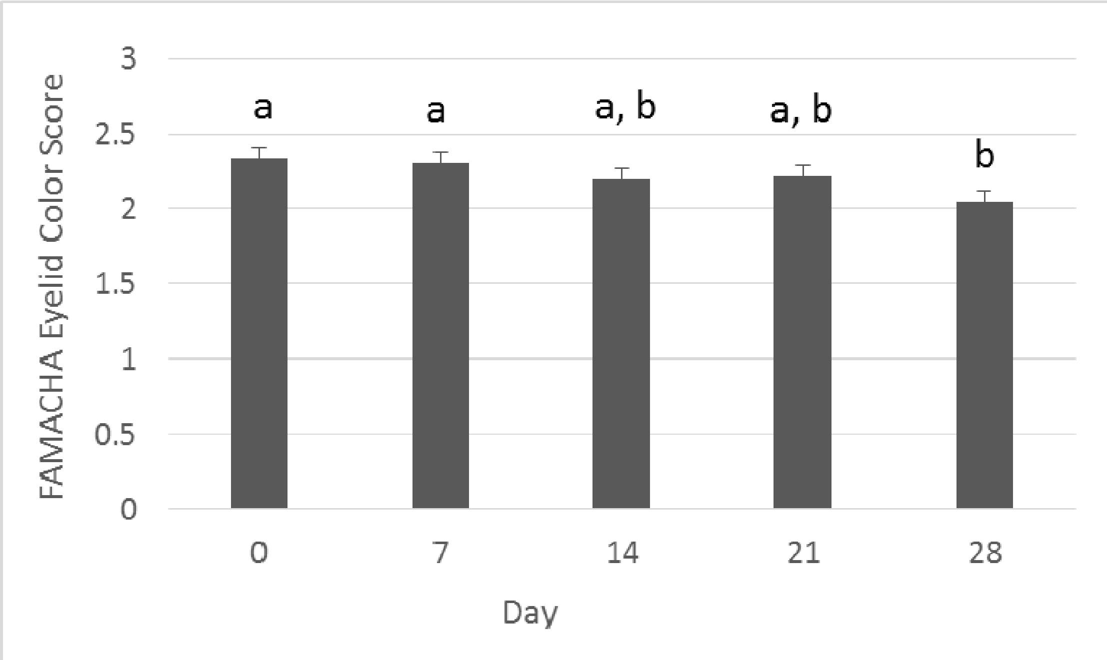


Fig. 3 FAMACHA© eyelid color scores for goats fed BGH or SLH or SLS for 28 d.
Day effect $p < 0.02$; bars with different superscripts differ, $p < 0.004$.

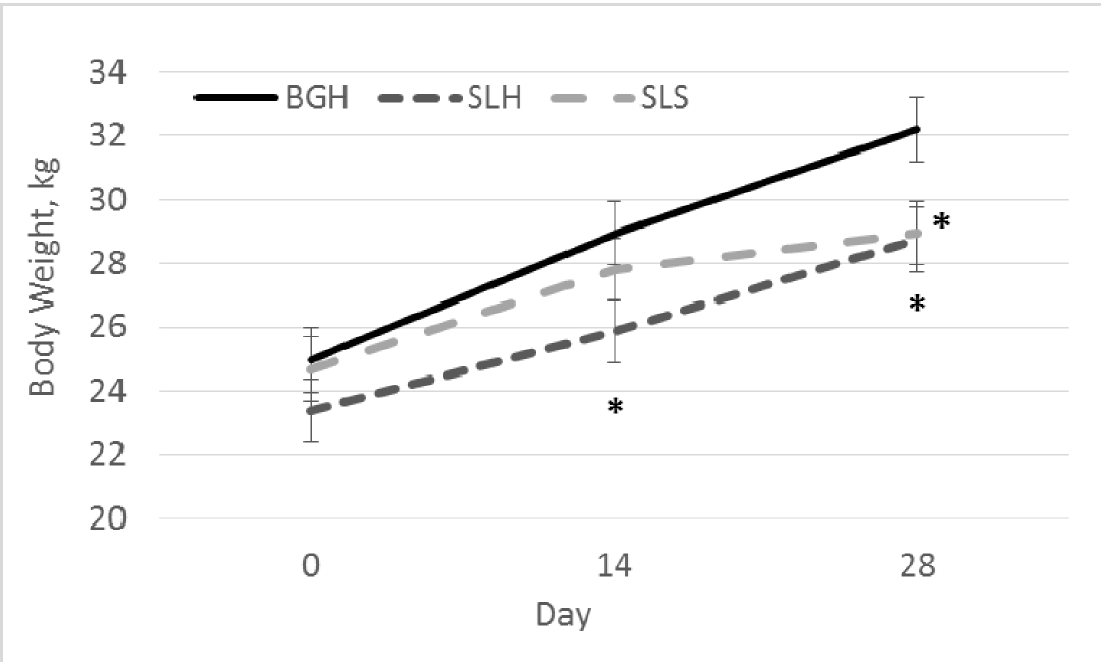


Fig. 4 Body weights (kg) for goats fed BGH or SLH or SLS for 28 d.
Treatment by day interaction, $p < 0.001$; points with different * differ from BGH, $p < 0.03$.

3.2 Trial 2

3.2.1 Fecal Egg and Oocyst Counts

Gastrointestinal nematode FEC were impacted by a treatment by day interaction, $p < 0.01$ (Fig. 5). The FEC for goats fed silage and administered PEG (SP) and those fed silage and administered water (SW) were similar except for day 14 ($p < 0.04$) in which SP resulted in higher FEC than for SW (Fig. 5). Coccidia FOC were also impacted by a treatment by day interaction ($p < 0.01$; Fig. 6). Compared to day 0,

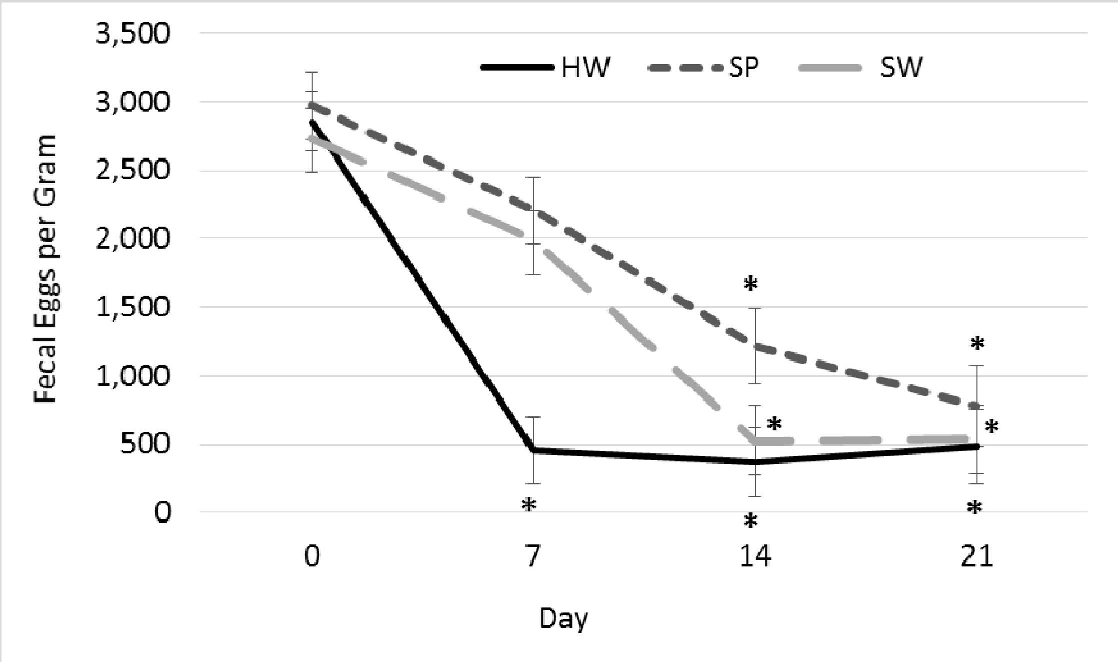


Fig. 5 Fecal egg counts (eggs per gram) for goats fed SL hay with water (HW) or SL silage with water (SW) or polyethylene glycol (SP) daily for 21 d.

Treatment × day interaction, $p < 0.01$; points with a * are different from day 0 within treatment, $p < 0.02$.

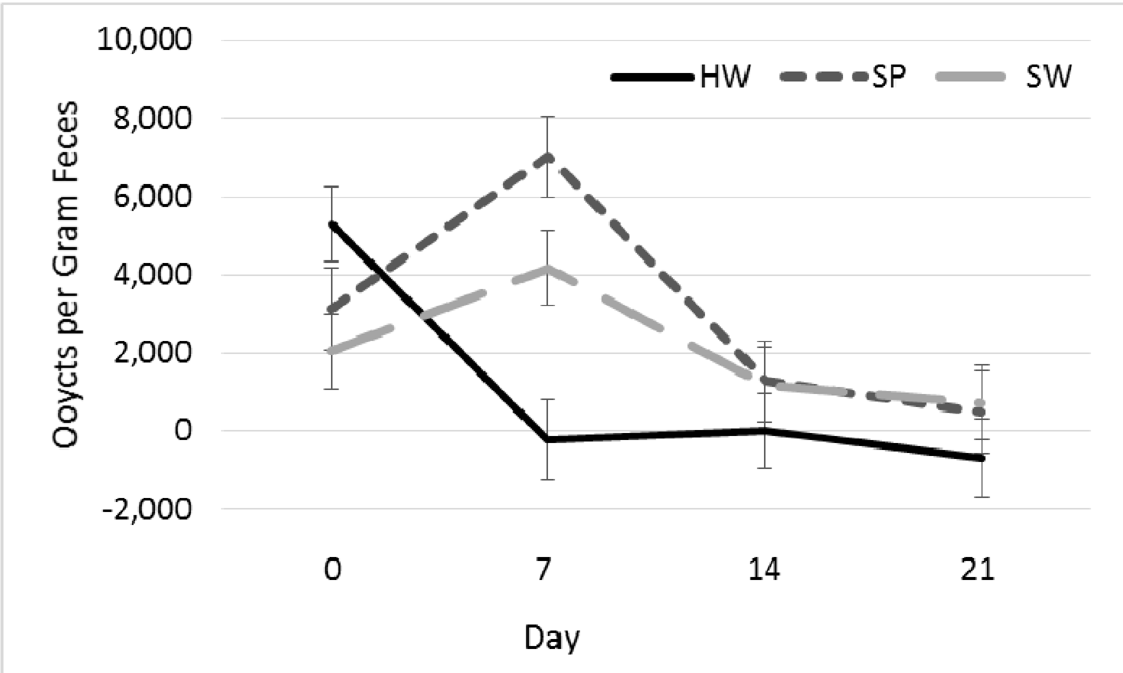


Fig. 6 Fecal oocyst egg counts (oocysts per gram) for goats fed SL hay with water (HW) or SL silage with water (SW) or polyethylene glycol (SP) for 21 d.

Treatment by day interaction, $p < 0.01$; points with a * are different from day 0 within treatment, $p < 0.05$, except for SP on day 7, $p = 0.0682$.

Table 1 Dry matter intake (DMI) in grams per animal per day for goats fed SL hay with water (HW) or SL silage with water (SW) or polyethylene glycol (SP) for 21 d.

Treatment	Intake g/d	Letter	Day	Intake g/d	Letter
HW	350 ± 17.7	a	7	200 ± 18.6	A
SP	234 ± 17.7	b	14	277 ± 17.7	B
SW	224 ± 18.6	b	21	331 ± 17.7	C

Treatment and day effects, $p < 0.01$; different letters within treatment or day differ, $p < 0.05$.

coccidia FOC tended ($p < 0.07$) to increase for SP goats at day 7, but then decreased ($p < 0.03$) by day 14 and remained lower at day 21 (Fig. 6). For SLH fed goats administered water (HW), FOC were lowered ($p < 0.05$) by day 7 and at every other time point measured compared to day 0. For SLS fed goats with no PEG treatment, FOC were lowered by day 14 ($p < 0.05$) compared to day 0 and remained lower at day 21 (Fig. 6).

3.2.2 PCV and Fecal Consistency Scores

There was no effect of treatment or day on PCV, which averaged 22.4% ± 1.14%, 18.9% ± 0.87% and 20.3% ± 0.80% for HW, SP and SW, respectively. Fecal consistency scores were also not impacted by treatment or day and averaged 1.32 ± 0.15, 1.30 ± 0.12 and 1.27 ± 0.13 for HW, SP and SW, respectively.

3.2.3 Dry Matter Intake (DMI)

Average daily DMI in grams per animal per day was impacted treatment and day ($p < 0.001$ for both; Table 1). The DMI was lower for all SLS fed goats compared to that for goats fed SLH ($p < 0.001$), though DMI did increase over time for all goats ($p < 0.05$; Table 1).

4. Discussion

The primary question regarding processing and use of SL as silage was that biological activity might be impaired during the ensiling process. Minnee *et al.*[10] found that although total CT were not different between fresh and ensiled sulla and birdsfoot trefoil (both tannin-containing plants), there was a significant reduction of the proportion of free CT during the ensiling process. The authors suggested that during ensiling, cells rupture releasing CT, which then bind with other molecules such as proteins. If this was the case, the CT may then be unavailable for binding to protein on the parasites within the animal (one suggested mode of action for CT). However, in the present study, SLS was effective in reducing gastrointestinal nematode FEC and coccidia FOC in both trials, though the hay seemed to work faster (Trial 1).

For sheep fed the tannin-containing plant, sainfoin, gastrointestinal nematode FEC were also reduced for both hay (58% reduction in *Haemon chuscontortus*) and silage (48% reduction in *H. contortus*) [11]. Similarly, compared to ryegrass/clover silage, sainfoin silage was shown to reduce FEC by 60% at 10 d of feeding to peripaturient ewes during an on-farm study [12]. For goats on farms in other countries, FEC were lower for ensiled cassava (which contains tannins) compared to sun dried [13], lower for silage, hay and fresh cassava compared to control diets [14] and lower for cassava silage compared to grass and grass/soybean meal supplemented goats [15]. More recently, in cattle, chicory silage reduced worm burdens and/or FEC in two studies [16]. These studies, combined with results of the present study, indicates that ensiling does not significantly alter the ability of tannin-containing plants to negatively influence FEC.

The faster efficacy of SLH related to fecal egg count reduction in the current study could have been a consequence of lower DMI for the silage diet as noted in Trial 2. Similarly, in Friesian steers fed perennial ryegrass as hay or silage *ad libitum*, DMI of silage was significantly lower than that of steers fed hay (4.53 kg vs 5.16 kg) [17]. In contrast, DMI of sainfoin hay and silage in sheep were found to be similar (0.98 ± 0.02 kg/d vs 1.15 ± 0.01 kg/d) [11].

The current study is the first known report of a reduction in coccidial oocyst counts by tannin-containing forage silage. However, the use of SL and other tannin-containing feedstuff has been found to help control this gastrointestinal parasite in other studies, so the reduction found in the current study is not unexpected. For example, Burke *et al.* [7] conducted two trials and reported fecal oocysts reduction of 80% in one trial and 98% in the other study for lambs fed SL pellets compared to those fed alfalfa pellets. In addition, Kommuru *et al.* [2] conducted two confinement feeding studies and reported a 92.2% reduction in the first and 91.2% reduction in the second for fecal oocysts in goats fed SL pellets compared to those in the control group. For other tannin-containing feedstuffs, Madibela and Kelemogile [18] reported an 81% reduction in fecal oocysts for goats fed *Melia azedarach* and Markovics *et al.* [19] reported a 75.4% oocyst reduction in goats fed lentisk (*Pistacia lentiscus* L.) forage. Hur *et al.* [20] fed pine (*Pinus densifora*) needles and oak (*Quercus acutissima*) leaves, both which contain tannin, as well as lucerne chaff (control) to Korean native goats naturally infected with mixed species of *Eimeria*. For the goats fed pine needles, oocysts counts were reduced by 93% and those fed dried oak leaves were reduced by 85% compared to the control forage.

Though both fecal egg and oocyst counts were negatively impacted by silage and hay treatment, percentage PCV was not impacted in either of these relatively short studies. This could be because animals were naturally infected, likely with multiple worm species. Similarly, animals fed a pine bark diet had lower FEC but no change in hematological parameters when compared to those fed a control diet [21]. *H. contortus* is the primary gastrointestinal nematode resulting in anemia (indicated by lower PCV), but other gastrointestinal nematode species augment fecal egg counts. SL has been found to suppress *H. contortus* more effectively than any other gastrointestinal nematode [22] and has been shown to increase (improve) PCV in other studies. Terrill *et al.* [23] reported a higher PCV in goats fed 75% SLH compared to the control group fed BGH. Lambs grazing SL also had higher PCV compared to those grazing tall fescue [24]. However, Terrill *et al.* [25] conducted a study in which goats were fed BGH, SLH and SL pellets with no overall treatment effect on PCV until the final sampling taken at slaughter. In addition, no change in PCV was noted for goats fed 50% and 25% SLH/BGH [25] or 75% SLH or 75% BGH [26].

Because PEG treated, silage-fed animals had an initial increase in FOC (Trial 2), it is possible that PEG was initially able to bind tannin and block its effects on this gastrointestinal nematode infection indicator. However, over time, as DMI increased, FOC were reduced by SLS with both water and PEG treatments, perhaps as an indication that the amount of tannin ingested overcame the effects of PEG. Other studies suggest that perhaps PEG added fresh directly to diets daily or different method of administration [27] (steers fed SL or control hay and grain supplementation with or without PEG mixed in daily), or a different molecular weight PEG [28] may have produced different results. The FEC were not impacted by PEG. The differences between FOC and FEC results with PEG may be due to a suggested greater sensitivity of FOC to tannins in SL [2, 7].

Although not a drastic difference, body weights and average daily gain were both reduced with time in SLH and SLS fed animals compared to BGH fed animals (Trial 1). Barry and Duncan [29] determined that in lambs, reduced intake, metabolizable energy, and hemicellulose and cellulose digestion was caused by the CT in *Lotus pedunculatus*. Although not measured in Trial 1, lower body weight for silage may be related to possible decrease in intake. Tannins bind to the proteins in saliva, causing astringency and bitterness, reducing palatability. However, Burke *et al.* [30] has also noted a reduction in weight gain and weight loss in sheep eating SL pellets with no difference in intake. The authors attributed the body weight differences to possible interactions of nutrients with CT in the ruminants' digestive tract. It was also a possibility the CT containing plant was of poor quality due to drought, so did not meet animal nutritional requirements. Because diets were balanced to meet nutritional requirements, unless intake were different (visually no difference, but not measured; Trial 1), this should not be the case for the present study. Waghorn [31] conducted an experiment in which examined diets including CT forages that did not meet crude protein requirements resulted in lower animal performance, but again, this was not likely in the present study.

Fecal consistency scores visually seemed to improve with treatment, however, no statistical difference was found in the present research (Trial 2). Waghorn and McNabb [32] reported that tannin-containing feeds generally tend to generate firmer feces. In addition, in goat kids fed old and new SL pellets compared to those fed a commercial goat diet pellets, fecal scores were improved by day 28 of treatment [2]. Hoste *et al.* [33] also reported lower fecal consistency scores in goats fed sainfoin hay compared to those fed lucerne hay. Similarly, weaned lambs fed SL pellets compared to alfalfa pellets had more solid fecal scores at weaning which continued to get firmer in the SL fed lambs compared to the lambs fed alfalfa pellets, whose scores stayed higher throughout the study [6].

5. Conclusions

In summary, SLS was effective in reducing gastrointestinal nematode FEC and coccidia FOC in growing goat kids. This could offer a possible alternative forage storage method for farmers in the south and additional product sales opportunities for agricultural businesses. However, the use of PEG to confirm effects related to tannins in the silage product was inconclusive and questions remain as to possible effects of SL on growth performance in small ruminants, so more research is needed.

Acknowledgments

This study was funded by a National Institute of Food and Agriculture (NIFA) Evans-Allen Research Program grant (Project No. GEOX-3220/Accession No. 1002851).

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