Effects of grazing birdsfoot trefoil–enriched pasture on managing *Haemonchus contortus* infection in Suffolk crossbred lambs¹

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ABSTRACT: High-tannin forages can be used to help mitigate the serious limitations associated with gastrointestinal nematode (GIN) infections on efficient small ruminant production. The objective of this experiment was to determine how grazing a GIN-free, established stand of a high-tannin cultivar of birdsfoot trefoil (*Lotus corniculatus* L.) influenced the prevention or treatment of *Haemonchus contortus* (*Hc*) infection in lambs. A birdsfoot trefoil–enriched pasture was established on an area that was previously row cropped and not grazed for at least 15 yr. Treatments included preventative (PREV) with parasite-naïve lambs transitioned onto pasture 1 wk prior to receiving an infection of 10,000 *Hc* larvae, therapy (THER) with parasite-naïve lambs infected with 10,000 *Hc* larvae 4 wk prior to the start of grazing, and control (CONT) with naïve, uninfected lambs to verify that natural infection did not occur on pasture. Each treatment group of 12 Suffolk crossbred lambs was divided into

3 replicates per treatment, and all were supplemented with a grain mix to provide 16% CP. Fecal egg count (FEC, eggs/g wet feces) in THER lambs peaked 1 wk after the start of grazing (9,404) and after 4 wk fell to 1,068, equivalent to a FEC reduction of 88.6%. Lambs in PREV had a peak FEC of 4872 at 4 wk after infection where peak FEC was 48% less in PREV than THER lambs. Lambs in CONT did not have measurable FEC for the duration of this study. Packed cell volume (PCV, %) reflected infection status of the lambs in each group, where CONT (32%) had the highest ($P < 0.05$) PCV followed by THER (29%) and PREV (26%). Total weight gained in CONT lambs was greatest at 5.51 kg ($P < 0.01$), whereas THER and PREV (2.68 and 2.97 kg, respectively) did not differ. Grazing birdsfoot trefoil–enriched pasture can have both therapeutic and preventative effects on *Hc* infection in lambs and can be used in a systems approach to control GIN parasites in grazing sheep.

Key words: birdsfoot trefoil, grazing, *Haemonchus*, sheep

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INTRODUCTION

Gastrointestinal nematodes (GIN), including *Haemonchus contortus* (*Hc*), are a serious impediment to efficient small ruminant production on a global scale (Roeber et al., 2013). Control strategies relied almost exclusively on chemotherapeutics which led to anthelmintic-resistant GIN populations. Declining anthelmintic effectiveness dictates

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need for innovative control methods that sustains livestock health, performance, and farm profitability. An integrated approach to control GIN infection could include pasture management practices that improve grazing animal nutrition and optimize the interaction of grazers and forages. One approach includes use of condensed tannin (CT)-rich legumes to help mitigate GIN infections in small ruminants (Hoste et al., 2015, 2016; Turner et al., 2017). Forages with high CT may counteract parasitism by direct action on GIN (Athanasiadou et al., 2005), improve host nutrition (Hoste et al., 2016), or influence specific aspects of host physiology and immune response (Hoste et al., 2015). Birdsfoot trefoil (*Lotus corniculatus*, L.) may deliver improved nutrition and provision of bioactive plant secondary metabolites, such as CT (Malaguti et al., 2014).

The influence of high CT forage on GIN-infected small ruminants was explored in numerous in vitro and in vivo experiments (Hoste et al., 2015, 2016). Results suggest that forages, including birdsfoot trefoil, can be components of parasite control strategies in small ruminants; however, grazing studies in temperate regions focusing on the effects of CT-rich legumes are rare or inconclusive. Defining the efficacy of forages under field conditions is difficult because CT concentrations vary among plant parts, with herbage mass, and with edaphic and environmental conditions (Lowther et al., 1987; Hoste et al., 2006). The objective of this experiment was to determine whether birdsfoot trefoil-enriched pasture could help prevent or reduce *Hc* infection in grazing lambs.

MATERIALS AND METHODS

The West Virginia University Institutional Animal Care and Use Committee reviewed and approved all animal procedures; protocol # 13-0801.

Animal and Grazing Management

This experiment was conducted at the West Virginia University Animal Sciences Farm, located in Morgantown, WV (39° 64' N, 79° 94' W; 287 m elevation) on the Appalachian High Plateau of the Eastern United States. The cultivated grassland used in this experiment was established on a site used for corn (*Zea mays*, L.) production for more than 15 yr and which had not been grazed by sheep or goats. Soil was classified as a Holly silt loam (Fine-loamy, mixed, active, nonacid, and mesic Fluvaquentic Endoaquepts). The site was prepared by broadcast applying Eraser, glyphosate

[N-(phosphono-methyl) glycine] at 2.5-kg/ha active ingredient, and ammonium sulfate (2.5 kg/ha) to control weeds and existing vegetation. The area was tilled and 15-kg/ha birdsfoot trefoil, cv. Bruce, was sown using a Brillion seeded into the prepared seedbed in spring of 2014. Once established, the plant stand was managed as a hay crop (cut twice) during 2015 prior to the start of the grazing experiment in 2016. An additional 10 kg/ha of Bruce birdsfoot trefoil was broadcast oversown in spring of 2016. The sward primarily consisted of birdsfoot trefoil, other volunteer legumes including white clover (*Trifolium repens* L.), red clover (*T. pratense* L.), and some common grasses and other forbs (Supplementary Material).

Thirty-six spring-born, weaned Suffolk-Hampshire crossbred lambs (*Ovis* spp.), (mean initial wt = 40.66 ± 5.84 kg) not exposed to GIN (naïve lambs) were maintained on an elevated-floor barn (see Jacobs et al. (2015)) prior to being randomly assigned to 3 treatments, with each treatment replicated 3 times. Treatments (Table 1) were as follows: 1) a preventative (PREV) with lambs first adapted to grazing then dosed with 10,000 *Hc* larvae; 2) a therapeutic (THER) with lambs dosed with 10,000 *Hc* larvae 4 wk before the start of grazing; and 3) a control (CONT) with naïve lambs not exposed to *Hc* and grazing birdsfoot trefoil-enriched pasture.

The site was mowed to a 10-cm residue height with a rotary mower 30 d prior to the start of grazing to stage pasture in terms of forage mass, sward height, and forage nutritive value. The area was divided into 36 paddocks each with an area of 0.03 ha, to accommodate 3 treatments, 3 replicates of each treatment, and 4 grazing intervals. The paddocks were grazed intensively at 27 lambs/ha, for 10 wk from June to August. Each paddock was grazed only once. Four grazing intervals were defined as the time lambs remained in each paddock. Grazing lambs were moved from paddocks after a 15-d period of stay, or earlier, based on mean residual herbage height of approximately 4 cm. Each paddock had a temporary shelter for shade and rain protection. In addition to pasture, lambs were given a grain supplement of corn and soybean (*Glycine max*, L.) meal providing 16% CP, at 0.75% body weight (BW) total, divided into 2 daily allotments, with a salt-trace mineral mix and water provided ad libitum.

GIN Dosing

Infective-stage *Hc* larvae, used to infect lambs while grazing, were obtained from monospecifically

Table 1. Grazing plot configuration for birdsfoot trefoil–enriched pasture, illustrating treatment (PREV, lambs infected after grazing began; THER, lambs infected 4 wk before grazing began; CONT, control naïve lambs) and replicate (I, II, III) positions for each grazing interval and the adaptation period

Grazing interval	Location of each treatment ¹ and replicate paddock ²		
Adaptation	CONT		
Start: 29 JUN 2016	THER		
End: 6 JUL 2016	PREV		
1st Interval	CONT III	PREV III	THER III
Start: 6 JUN 2016	THER II	CONT II	PREV II
End: 18 JUL 2016	PREV I	THER I	CONT I
PREV group infected 6 JUN			
2nd Interval	THER III	PREV III	CONT III
Start: 18 JUL 2016	PREV II	CONT II	THER II
End: 2 Aug 2016	CONT I	THER I	PREV I
3rd Interval	THER III	PREV III	CONT III
Start: 3 AUG 2016	PREV II	CONT II	THER II
End: 9 AUG 2016	CONT I	THER I	PREV I

¹PREV = lambs infected after grazing began; THER = lambs infected 4 wk before grazing began; CONT = control naïve lambs. Roman numerals I, II, III denote replicate lamb grazer groups.

²Each adaptation paddock was 21 by 63 m and each treatment paddock was 21 by 21 m. All groups were grazed simultaneously. Drawing not to scale.

infected wethers. Feces were collected from these wethers and mixed with sterile peat moss and activated carbon and then incubated at 30 °C for 7 d. Feces were then added to a Baermann apparatus and L3 larvae were separated and stored in PBS (pH 7.4) until use. Larvae were diluted to 1,000/mL in PBS and each lamb in PREV and THER groups were orally dosed with 10 mL of larval dilution (Jacobs et al., 2015).

Animal Sampling

Fecal egg count (FEC), blood-packed cell volume (PCV), and BW were measured weekly to evaluate influences of treatments on growing lambs on pasture. A uniform method for weighing livestock was utilized (Watson et al. 2013) and lambs were removed from paddocks at 0700 h and then FEC, PCV, and BW data collected at each date. Animals were returned to paddocks at 0830 h on the same day. Average daily gain (ADG) was calculated per period between sampling dates and total gain was calculated by difference between initial and final BW.

Parasitological Measures

To quantify FEC, feces were collected by rectal grab from each lamb and 2 g were added to 28 mL of a saturated salt solution (specific gravity 1.20) and the mixture filtered through a double layer of cheesecloth. Trichostrongylid eggs were enumerated using a McMaster counting chamber (Chalex Corporation, Wallowa, OR) at 100× magnification.

Total count was multiplied by 50 to determine eggs/g of wet feces (Whitlock, 1948). Data were log transformed [$\log_{10}(\text{EPG} + 25)$] for analyses and with raw means reported.

To measure PCV, whole blood was collected via jugular venipuncture into 5-mL EDTA-treated tubes, then loaded into heparinized microhematocrit tubes, and centrifuged for 2 min using a StatSpin hematocrit centrifuge (Westwood, MA) (Jacobs et al., 2015). Red blood cell percentage was determined by a digital hematocrit reader (StatSpin, Westwood, MA).

Plant Measurements

For herbage mass estimation, a plate meter technique was used (Rayburn and Rayburn (1998). The plate meter was calibrated using linear regression of 150 paired samples (plate height and pasture mass values). Samples were collected from paddocks during the interval June to August 2016. The quadrat was placed over the sample site after the plate height was measured and the plate meter removed. The forage was cut as close to ground level as possible. Sward height measurements were made before and after grazing, at 1-m intervals along 4 transects within each paddock. The difference between forage mass determined before grazing and residual mass after each grazing event was assumed to represent herbage disappearance; that is, herbage consumed by grazing animals as well as senesced, trampled, or fouled herbage. Sward height was converted to forage mass using paired samples collected from each of the paddocks. Forage was

clipped to soil surface within a 0.07 m² area, dried in a forced-air oven at 60 °C for 48 h, and weighed. Forage mass was computed as follows:

$$\text{Forage mass (kg / ha)} = 266.15 \bullet \text{sward height (cm)} + 265 \text{ } r^2 = 0.4336 \quad (1)$$

To determine botanical composition and nutritive value, prior to, during, and after grazing, 3 forage samples were collected at fixed intervals along transects in each paddock for botanical composition and nutritive value analyses, including CT concentrations. Herbage was collected from 0.07 m² quadrats along the same transects used to estimate forage mass. Samples collected were placed in paper bags and stored at 0 °C until processed. Samples were hand sorted by functional groups into birdsfoot trefoil, clover, other forbs and grasses, and dead. The separate groupings were placed in paper bags and dried at 60 °C in a forced-draft oven for 48 h. Air-dried weights were determined and percentage of each group as a component of the total was computed. Dry samples were weighed, recombined by paddock, and ground to pass a 1-mm screen using a stainless-steel Wiley mill (Model 4, A.H. Thomas, Philadelphia, PA). All nutritive value analyses were performed in duplicate. Dry matter (105 °C), ash, and total N were determined in pregrazing samples, using AOAC (2002) procedures. Crude protein was computed by multiplying total N (%) by 6.25. Pregrazing herbage samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991), using modifications outlined in the ANKOM fiber analysis procedure (ANKOM Technology, Macedon, NY). The ADF and NDF data obtained from the Van Soest fiber analysis were used to derive total digestible nutrients (TDN; NRC, 2007).

Tannin standards for the spectrophotometric assay were isolated from samples of birdsfoot trefoil plant material based on procedures described by Hagerman (2011). Briefly, a suspension of 10% (wt/vol) finely ground plant material in 1% (vol/vol) acetic acid, 24% (vol/vol) water, and 75% (vol/vol) acetone was sonicated for 30 min with periodic mixing. Mixtures were centrifuged for 10 min at 3,000 × g and the supernatant filtered through a coarse fritted disk; plant material was extracted a total of 3 times and supernatants combined. Supernatant was mixed with an equal volume of ethyl ether and the aqueous layer was retained; the supernatant was extracted a total of 3 times with equal volumes of ethyl ether. The acetone and ethyl ether remaining in the aqueous solution were removed by rotary evaporation.

The aqueous solution was mixed with Sephadex LH 20 resin equilibrated in a 4:1 (vol/vol) ethyl alcohol:water solution, rinsed with 95% ethyl alcohol, and extracted with a 3:1 (vol/vol) acetone:water solution. Acetone was removed by rotary evaporation, and the aqueous solution was frozen and freeze-dried.

Herbage samples representing mid-June, and early- and late-July grazing intervals were analyzed for CT using the butanol-HCl-acetone method of Grabber et al. (2013). The CT assay solution contained 0.15% (wt/vol) ammonium iron (III) sulfate dodecahydrate, 3.3% (vol/vol) water, 5% (vol/vol) concentrated HCl, 41.7% (vol/vol) butyl alcohol, and 50% (vol/vol) acetone. Briefly, triplicates of dried ground plant tissue (0.030 g each) was suspended in 15 mL of tannin assay solution and heated for 2.5 h in a 70 °C water bath. Samples were mixed periodically during heating. Standard, blank solutions and check samples were included in each run. After tubes cooled, they were centrifuged at 5,000 × g for 10 min and the absorbance of the supernatant was determined at 554 nm.

Statistical Analysis

Paddock was the experimental unit for all measurements. Data were analyzed as a completely random design using the PROC-MIXED procedure of SAS with a repeated measures statement (Littell et al., 1996). The model for FEC and PCV included treatment, sampling date, and the interaction of treatment and sampling date. The model for ADG included treatment, grazing interval and the interaction of treatment and grazing interval. Least square means were calculated and then compared using residuals when the *F* test for means was significant (*P* < 0.05). For grazing management, botanical composition, and nutritive value parameters, the model included treatment, grazing interval, and the interaction of treatment and grazing interval. The influence of treatments on total weight gain was evaluated using one-way ANOVA and means. Significant (*P* < 0.05), *F*-test means were compared using the Bonferroni method. Analysis of initial weight, final weight, and ADG were conducted using a one-way ANOVA.

RESULTS

Parasitological and Gain Analysis

Fecal egg count. The THER lambs had highest FEC across all time points (Table 2) and peaked at 1 wk after grazing (Figure 1a). After which, FEC in

Table 2. The influence of grazing birdsfoot trefoil-enriched pasture on fecal egg count (FEC), packed cell volume (PCV), and total body weight (BW) gain of Suffolk lambs infected with *Haemonchus contortus* larvae after the start of grazing (PREV), or before grazing began (THER), and not infected, naïve controls (CONT)

Item	Treatment			SEM	P value
	PREV	THER	CONT		
FEC, eggs/g	224 ^b	1685 ^a	11 ^c	1.399	<0.01
PCV, %	29 ^b	26 ^c	32 ^a	0.755	<0.01
Total BW gain, kg	2.68 ^b	2.97 ^b	5.51 ^a	0.712	<0.01

Means in the same row followed by a different lowercase letter are different at $P \leq 0.05$.

Figure 1. (a) Fecal egg count (egg/g) and (b) packed cell volume (raw data) from Suffolk lambs grazing birdsfoot trefoil and infected with *Haemonchus contortus* larvae after the start of grazing (PREV) or 4 wk before grazing began (THER), and not infected, naïve controls (CONT).

THER was reduced by 4 wk after grazing resulting in a FEC reduction of 88.6%.

Fecal egg count was higher for ($P < 0.01$) PREV and THER, whereas the FEC for CONT lambs had a minimal number of trichostrongylid eggs and maintained the naïve status throughout the experiment (Table 2). Fecal egg count differed with sampling date ($P < 0.05$) and the interaction of treatment and sampling date ($P < 0.01$) (Figure 1a). Fecal egg count in PREV was about 5,000 eggs/g and about 4 times greater than FEC in THER and

CONT at 4 wk after infection. The FEC of lambs in THER decreased from 9,000 eggs/g during first week of grazing to about 1,000 eggs/g by week 4. The reduced FEC continued for THER until the end of the experiment. The FEC of lambs on CONT was negligible throughout the experiment.

Packed cell volume. Differences in PCV (Figure 1b) occurred with treatment ($P < 0.01$) and sampling date ($P < 0.01$). Mean PCV for CONT was about 32% through the experiment (Table 2). The PCV

values for PREV lambs tended to decrease during the experiment. The THER lambs had consistently lower PCV relative to PREV and CONT lambs during most of the grazing season, but PCV values began to increase by week 4. The greatest PCV was 33% in CONT lambs and the least was 22% in THER lambs (Figure 1b). There was an interaction ($P < 0.01$) between treatment and sampling date.

BW gain. Lambs in CONT were significantly heavier ($P < 0.01$) at the end of the experiment than those in either PREV or THER (Table 2). Liveweight gains from all treatments were modest, being more consistent among the weighing periods in the naïve lamb group, averaging around 150 g/d. Parasitized groups (PREV and THER) were losing weight starting with the sampling made 15 July and were very low, regardless of treatment by the end of the grazing interval (Figure 2).

Pasture Characteristics

Forage mass. Mean forage mass determined prior to each grazing interval did not differ between treatments and averaged 3114 kg/ha during the

experiment; residual herbage mass averaged 1864 kg/ha, with a resultant herbage disappearance of 1250 kg/ha (Table 3). In addition, forage mass and residual herbage mass differed ($P < 0.01$ and $P < 0.01$, respectively) with grazing intervals, which is predictable because the experiment lasted from very early to late summer where changes in precipitation and air temperature influenced herbage growth and increased warm-season grasses and forbs as a proportion of the entire sward. Evaporative demand may exceed precipitation, and temperatures are usually greatest during this time of year at this location (Figure 3). Herbage disappearance was not influenced by treatment or grazing interval nor was the interaction of treatment and grazing interval significant (Table 3).

Botanical composition. Birdsfoot trefoil was the predominant component of sward composition during all grazing intervals. In the first grazing interval, a relatively wet and cool period (Figure 3), the proportion of birdsfoot trefoil fluctuated between 50% and 80% (Table 4). As summer progressed, trefoil proportion declined slowly and by the last grazing interval, the warmest period during

Figure 2. (a) Average daily gain (ADG), and (b) body weight (BW, kg) of Suffolk lambs grazing birdsfoot trefoil and infected with *Haemonchus contortus* larvae after the start of grazing (PREV) or 4 wk before grazing began (THER) and not infected naïve controls (CONT).

Table 3. Pregrazing, postgrazing, and herbage mass disappearance (pre- minus post-grazing) of birds-foot trefoil-enriched pasture as a function of treatment (T) and grazing interval (GI)

T ¹	GI	Pregrazing	Postgrazing	Disappearance
		kg/ha		
PREV	1	3173	1616	1557
	2	2335	1493	842
	3	3409	2182	1227
	4	2919	1917	1002
THER	1	3073	1782	1291
	2	2403	1650	754
	3	3717	2097	1620
	4	3474	2251	1223
CONT	1	2986	1543	1444
	2	2418	1445	973
	3	3825	2273	1515
	4	3633	2117	1515
Source of variation		P value		
T ¹		0.78	0.49	0.82
GI		0.01	0.01	0.27
T by GI		0.99	0.77	0.99

¹PREV = lambs infected after grazing began; THER = lambs infected before grazing began; CONT = control naïve lambs.

the experiment, the proportion of the sward attributable to birdsfoot trefoil averaged 32% (Table 4). The distribution of sward components comprising pregraze forage was similar across treatments. Proportion of birdsfoot trefoil and dead were different in postgrazing measurements (Table 4). The relatively greater amount of dead herbage in postgraze samples, regardless of treatments or grazing interval, indicates the obvious avoidance of senesced material by grazing lambs. The proportion of birdsfoot trefoil in pregraze swards was not different among treatments, but did differ with grazing interval. Differences associated with grazing interval in pregraze swards reflect competition among sward components, especially increases in the grass and other forb components. Pre- and post-grazing trefoil contribution declined with grazing period and did so most noticeably in PREV and THER paddocks suggesting selective removal by GIN-parasitized lambs reflected in postgraze birdsfoot trefoil proportions. All of the botanical components determined were influenced by grazing interval, except clover in both pre- and post-grazing sampling and “other forbs and grasses” in the post-graze. Clover, both red and white, made a considerable contribution to the legume available for grazing when the relative proportion of birdsfoot trefoil dropped. Legume proportion including both birdsfoot trefoil and clovers averaged 65% across

Figure 3. Mean monthly air temperatures, °C (a), mean monthly precipitation, mm (b), and 30-yr mean values (dashed lines) for each at Morgantown, WV (39° 64' N, 79° 94' W; 287 m elevation).

treatments. As the summer advanced, plants representing “other forbs and grasses” increased gradually from 10% initially to 30% by the last grazing interval. There was a treatment by grazing interval interaction ($P < 0.01$) for dead material. The plant species identified in paddocks across treatments and through all grazing intervals are summarized in [Supplementary Material](#). Common pokeweed (*Phytolacca americana* L.) and annual sow thistle (*Sonchus oleraceus* L.) were present during all grazing intervals, Canadian thistle (*Cirsium arvense* L., Scop.), smartweed (*Polygonum pennsylvanicum* L.), and foxtail (*Setaria* spp.) increased in swards during later grazing intervals.

Forage nutritive value. Forage nutritive value by treatment during the 4 grazing intervals is presented in Table 5. No differences were identified by treatment for any forage nutritive component, during the experiment, although CP ranged from 13.3% to 17.4%. Concentration of all components differed ($P < 0.01$) with grazing interval and no interaction of treatment with grazing interval (Table 5).

The CT values in residual birdsfoot trefoil herbage were 19-g CT per kg, 30-g CT per kg, and 102-g CT per kg at 30, 45, and 60 d, respectively.

DISCUSSION

Bioactive plants, including grazed forages, offer a promising means to help control GIN in

Table 4. Pregrazing and postgrazing proportion of birdsfoot trefoil, clover, dead, and grasses and other forbs by treatment (T) and grazing interval (GI)

T ¹	GI	Sampling time	Birdsfoot trefoil	Clover	Dead	Grasses and forbs
			%			
PREV	1	Pregrazing	78.85	1.71	14.14	5.30
	2		70.77	2.23	8.11	18.89
	3		56.86	6.27	10.76	26.11
	4		38.70	2.94	34.79	23.57
THER	1		86.00	3.45	7.99	2.57
	2		53.38	19.58	9.61	17.44
	3		52.64	6.72	15.23	25.41
	4		15.71	17.02	31.84	35.44
CONT	1		48.24	24.93	6.65	20.18
	2		60.14	0.81	23.77	15.28
	3		58.10	11.62	8.73	21.55
	4		41.59	12.67	13.84	31.90
<i>P</i> value						
T			0.4314	0.1701	0.4597	0.8283
GI		Pregrazing	0.0013	0.9313	<0.0001	0.0416
T by GI			0.3552	0.2998	0.0013	0.8206
PREV	1	Postgrazing	36.92	2.61	30.49	29.97
	2		33.99	10.59	25.53	29.89
	3		31.97	0	39.50	28.54
	4		24.22	9.14	39.98	26.66
THER	1		75.99	6.71	7.96	9.35
	2		24.10	16.06	28.40	31.44
	3		50.40	10.52	15.18	23.90
	4		34.13	8.87	29.21	27.80
CONT	1		51.25	0.54	23.32	24.89
	2		42.78	7.41	35.72	14.09
	3		48.58	7.04	10.14	34.25
	4		30.13	1.84	33.34	34.69
<i>P</i> value						
T			0.0399	0.1581	0.0005	0.5249
GI		Postgrazing	0.0046	0.2368	0.0010	0.5341
T by GI			0.1437	0.7704	0.0011	0.1939

¹PREV = lambs infected after grazing began; THER = lambs infected before grazing began; CONT = control naïve lambs.

small ruminants (Niezen et al. 1998; Marley et al., 2003; Hoste et al., 2015); however, studies that define effects and modes of action of CT-rich forages growing in pasture on helminths are scarce. Bioactivity of high CT forages is attributed to polyphenolic constituents, specifically an array of condensed and hydrolyzable tannins (Hoste et al., 2012) that vary with trefoil species (Min et al., 2003) and cultivar (Wen et al., 2003). The CT-rich forages influence ruminant nutrition by modifying protein degradability (McMahon et al., 2000), grazing behavior (Villalba et al., 2014), and forage palatability (Häring et al., 2008). Possible effects of high CT forages on GIN include the following: 1) reduced establishment of infective L3; 2) reduced egg production by adult worms; and

3) impaired development of eggs into L3 (Hoste et al., 2012; Shea-Donohue et al., 2017).

A logistical challenge that contributes to the scarcity of pasture-based work is the need for GIN-free land area on which to conduct evaluations. Once a pasture is grazed by GIN-infected animals, the control or clean status is compromised, thus limiting the possibility to repeat an experiment in time on the same site and with the same suite of conditions. The effectiveness of birdsfoot trefoil-enriched pasture to either prevent or remediate GIN infection depended upon when lambs were exposed to *Hc* larvae relative to when lambs began grazing trefoil. Lambs adapted to grazing birdsfoot trefoil-enriched pasture before being exposed to *Hc* L3 had low FEC, and greater PCV and ADG,

Table 5. Mean pregrazing crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) of composite herbage collected from birdsfoot trefoil-enriched pasture as a function of treatment (T) and grazing interval (GI)

T ¹	GI	CP	ADF	NDF	TDN
			g/kg		%
PREV	1	171	287	468	69.8
	2	174	419	478	54.8
	3	140	325	388	65.5
	4	133	391	449	58.0
THER	1	169	302	422	68.2
	2	179	417	498	55.0
	3	152	314	408	66.8
	4	139	362	402	61.3
CONT	1	168	288	406	69.7
	2	172	413	481	55.5
	3	151	382	420	59.0
	4	136	321	386	56.0
Source of variation		P value			
T		0.0745	0.7012	0.2282	0.7005
GI		<0.0001	<0.0001	<0.0001	<0.0001
T by GI		0.3397	0.6847	0.2477	0.6855

¹PREV = lambs infected after grazing began; THER = lambs infected 4 wk before grazing began; CONT = control naïve lambs.

comparable to control naïve lambs, as long as trefoil was growing actively and present as a major component of the sward. The initially strong then gradual weakening responses in FEC, PCV, and ADG during the grazing season might be explained in part by changes in sward composition and associated changes in herbage composition, specifically CP. Minimal supplementation was provided; thus, these results need to be evaluated in context of lambs receiving some level of supplementation.

Sward changes included a decrease in birdsfoot trefoil as a fraction of available herbage during summer accompanied by an increase in grasses of secondary importance, forbs, and dead or senesced plant material. The change in sward composition was exacerbated by selective consumption of trefoil by grazing lambs. Changes in the contribution of birdsfoot trefoil to the sward reflected the preferential grazing by lambs in the PREV and THER groups and supported observations reported that GIN-infected animals sought to increase intake of tanniniferous herbage (Villalba et al., 2010). The CONT lambs remained free of *Hc* throughout the experiment, confirming that the only source of parasites in our experiment was via oral infection (dosing).

Birdsfoot trefoil pasture seems to be somewhat effective as a therapeutic treatment for lambs already infected with *Hc* before being offered the trefoil-enriched pasture. Infected lambs showed a decrease in FEC and an increase in PCV during the

early-grazing periods when consuming trefoil-enriched pasture. A contributing factor in the benefit of trefoil-enriched pasture to help reduce FEC was possibly associated with the use of the birdsfoot trefoil cultivar Bruce, considered to be a high CT content cultivar. An infected positive control group grazing common pastures was not used which may lead to uncertainty of birdsfoot trefoil leading to FEC reduction. Nonetheless, CTs influence intake and forage digestibility, as well as possess anthelmintic properties. Data presented here are consistent with data from other studies grazing high-tannin forages to reduce the impact of GIN parasitism in small ruminants (Min et al., 1997; Paolini et al., 2003, 2005; Athanasiadou et al., 2005; Mendez-Ortiz et al., 2012)

We observed an increase in CT concentration in birdsfoot trefoil as summer advanced. At the same time, birdsfoot trefoil growth was slowing which could contribute to the relative increased CT concentrations in herbage. Consequently, grazers may consume less birdsfoot trefoil, since not only is there less trefoil present in the sward, but greater herbage CT concentrations could deter selection of birdsfoot trefoil. The CT values observed were similar to concentrations of 12-g CT per kg, when A2 dimer standard was used and 40.9 g/kg when the Cranberry proanthocyanidin standard was used (unpublished data). However, our observations contradict Roberts et al. (1993) who noted that CT concentrations decreased during the summer

months, especially in accessions with high CT concentrations.

A desirable concentration of CT in ruminant diets should represent a balance between beneficial (e.g., anthelmintic; dietary protein protection) and detrimental (e.g., organoleptic) influences of tannins (Gebrehiwot et al., 2002). A number of other reports note that tannin-rich plants such as quebracho (*Schinopsis* spp.) or sainfoin (*Onobrychis vicifolia*), and others such as chicory (*Cichorium intybus* L.) that are rich in bioactive constituents, did not influence development of L3 of *Hc* and other strongylids in either in vivo or in vitro conditions (Paolini et al., 2003, 2005; Foster et al., 2011).

In addition to the beneficial nutritional aspects of lambs grazing tanniniferous legumes, host-nematode interactions also occur and influence the observed trends in response variables of FEC, PCV, and ADG (reviewed by Hoste et al., 2016). In the review, Hoste et al., (2016) addressed the interaction of dietary protein and susceptibility to GIN, noting that published literature supports the premise that initial establishment of GIN and early acquisition of resistance in young sheep were not affected by supplemental dietary protein. Supplementation, especially as rumen undegraded protein, appears to influence the degree of expression of immunity in sheep, which can manifest as reduced establishment or arrested development of incoming larvae and reduced survival and fecundity of established GIN.

The trends of PCV values for the treatment groups were reflective of *Hc* infection level as measured by FEC. The THER lambs compared with the other 2 groups maintained a lower PCV until the fourth week, then PCV values for that group increased and those for PREV decreased. This change is correlated with the change in FEC in lambs of those groups. An increase of PCV in the THER group and associated decrease in FEC may be explained by the influence of birdsfoot trefoil causing a reduction in FEC. Lambs in the PREV group never reached the level of fecal egg output as those in the THER group, yet PCV did decrease as a function of increasing FEC. It is important to keep in mind that lambs in the CONT group maintained higher PCV than either treatment group consistent with little to no strongylid egg output of these lambs. Burke et al. (2009) measured PCV in grazing Katahdin lambs and proposed that a PCV of 20% or less was an indicator that deworming was needed in a breed that is more resistant to GIN than is the Suffolk breed. Data from this study indicated that FEC of lambs in either PREV or THER did not require deworming based on PCV; therefore,

grazing birdsfoot trefoil may ameliorate pathological effects of *Hc* infection.

Previous research found that balance in herbage quality and quantity was needed to guarantee the appropriate nutritional level as a means to mitigate parasite burdens and obtain desirable gains (Clarke, 1959; Marley et al., 2003; Shea-Donohue et al., 2017). Although the final ADG did not differ among treatments when lambs grazed trefoil-enriched pasture, the ADG did differ among grazing intervals reflecting shifts in sward composition and changing herbage nutritive value. The CONT lambs tended to have greater ADG throughout the experiment. However, during the last grazing interval when herbage CP and herbage mass dropped, ADG of CONT lambs was similar to that of lambs on the other treatments. Precipitation and temperature patterns during the experiment, along with changes in sward botanical composition, and declining nutritive value probably contributed to these responses.

Results from this experiment suggest that strategic protein supplementation might be required in late summer to sustain weight gain and the beneficial influences of CT-rich forage on mitigating GIN infection. These findings should be taken in context with available energy in forages or further energy supplementation. Data from cattle studies indicate that when TDN:CP ratio is less than 5, then energy supplementation is needed relative to excess crude protein (Moore et al., 1999). Since this interaction has not been well-studied in sheep and TDN:CP ratios of this study were found to 4 and less, it is reasonable to conclude that both protein and energy supplementation are needed. Collectively, animal genetics, diet protein source and supplementation, and sward management including the use of CT-rich forages should be considered in a multifaceted approach to control GIN infection. The beneficial effects of birdsfoot trefoil as an anthelmintic preventative or therapy are noteworthy and contribute to an integrated approach to GIN infection control in growing lambs on pasture.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

Conflict of interest statement. None declared.

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