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The Use of Organic Pinot Noir Grape Extract as a Natural Anthelmintic in Katahdin Lambs

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Summary

Gastrointestinal nematode parasitism is one of the greatest threats to economic sheep production in the United States. With increased incidences of anthelmintic resistance and constraints of organic production, there is heightened interest in alternative natural dewormers, such as plants containing condensed tannins. Therefore, the objective of this study was to evaluate effects of organic fermented Pinot Noir (PN) grape extract on parasite level and performance in Katahdin lambs. On October 14, 2014, Katahdin ewe and ram lambs (n = 45; 23.13 kg ± 0.60 BW) were stratified by fecal egg count, weight, sex, and were allocated randomly to one of three treatments: 1) an oral dose (10-mL per 4.5 kg of BW) of fermented PN at 7 d (D7) intervals, 2) the same dose of PN at 14 d (D14) intervals, or 3) control (C; 30-mL oral dose of water at 14 d intervals). Condensed tannins were extracted, purified, and standardized from organic PN and were found to have a concentration of 0.20 mg/mL. Lambs were maintained on tall fescue [*Lolium arundinaceaum* (Schreb.) Darbysh] pasture, with no additional feed for the duration of the 63-d study. Data were analyzed using PROC MIXED of SAS. Two contrast statements were used to compare the mean of C compared with D7 and D14 and the mean of D7 compared with D14. Average daily gain and total weight gain were greater ($P = 0.02$) from D7 and D14 compared with C. Start, end, and start to end change body condition scores and FAMACHA© scores did not differ ($P \geq 0.05$) across treatments. End of study and change from start to end fecal egg counts were greater ($P \leq 0.05$) from C compared with D7 and D14. Change in packed cell volume from start of study to end were greater ($P = 0.05$) from D7 compared with D14. End monocytes and white blood cell counts were less ($P = 0.05$ and $P = 0.03$, respectively) from D7 compared with D14. Other blood parameters were similar across treatments. Therefore, fermented grape extract may be an effective organic and sustainable strategy for controlling gastrointestinal nematodes and increasing performance in Katahdin lambs.

Key Words: Anthelmintic, Condensed Tannin, Lambs, Organic Grape Extract

Introduction

Gastrointestinal nematodes (GIN) can endanger animal health and welfare and cause severe economic damage in small ruminants (Miller and Horohov, 2006; Shaik et al., 2006). Since their introduction in the 1960s, broad-spectrum, synthetic anthelmintics have been the primary defense against GIN infection in small ruminants worldwide (Hoste, 2011). However, with evolution of resistant strains of parasites, there is a greater necessity for exploration of natural alternatives (Shaik et al., 2006; Rahmann and Seip, 2007; Terrill et al., 2009).

Research by Rahmann and Seip (2007) suggested that phytotherapy (the use of plants as a natural anthelmintic) should be evaluated. Found in nearly all families of plants, the most abundant phytochemicals are tannins. Tannins are plant secondary metabolites, which are closely associated with plant-defense mechanisms against insects (Githiori et al., 2006; Oksana et al., 2012) and are broken into two groups, condensed tannins (CT) and hydrolysable tannins (Anthanasiadou et al., 2001). Condensed tannins are compounds that may demonstrate biological activities in ruminants, such as binding to proteins and suppression of GIN infection (Naumann et al., 2013). Collectively, it has been reported (King and Young, 1999; Gu et al., 2004; Mattivi et al., 2009) that high concentrations of CT have been measured in fruits with dark red, blue, or black pigment skin, such as grapes; many dark orange or red-skin vegetables; some legume cereals and beans; tree nuts, such as almonds, pecans and hazelnuts; cocoa beans, coffee, tea, wine, and spices, such as cinnamon. Components of pH, astringency or dryness, and bitterness are indications of CT concentration (King and Young, 1999). As degree of polymerization and molecular weight increases, astringency may also increase (Naumann et al., 2013). Further, an increase in concentration of CT is observed comparing red grape juice to red wine (King and Young, 1999), suggesting that fermentation may influence CT accessibility to the ruminant animal (Githiori et al., 2006). Therefore, our objective was to evaluate effects of organic, fermented Pinot Noir grape extract on parasite level and performance in Katahdin lambs.

Materials and Methods

Animals and experimental design

This project was conducted at the Lincoln University Allen T. Busby Farm in Jefferson City, Mo. and was approved by the Animal Care and Use Committee (14-4). Certified organic Katahdin mixed-sex lambs (n = 45; 23.13 kg ± 0.60 BW) were weaned and grazed tall fescue [*Lolium arundinaceaum* (Schreb.) Darbysh] pastures for 81 d. Lambs were then weighed, fecal egg counts (FEC) were determined, and body condition scores (BCS) and FAMACHA© scores were assigned by an experienced evaluator. Starting October 14, 2014, lambs were then stratified by FEC, weight, and sex, and allocated randomly to one of three treatments: 1) drenched with organic PN every 7 d (D7) at a rate of 10-mL per 4.5 kg of BW; 2) drenched with organic PN every 14 d (D14) at a rate of 10-mL per 4.5 kg of BW; and 3) drenched with 30-mL of water every 14 d (C). In accordance with established farm protocols, animals were removed from the study if they met three out of the following four criteria: 1) FEC of ≥ 4,000; 2) FAMACHA© score of ≥ 4; 3) packed cell volume (PCV) of ≤ 21 percent; or 4) a BCS ≤ 2. For the duration of the 63 d trial, lambs rotationally grazed six tall fescue [*Lolium arundinaceaum* (Schreb.) Darbysh] pastures, had *ad libitum* access to water and organic approved mineral (Redmond Naturals, Redmond, Utah), with no additional supplementation to the diet. Throughout the study, lambs were maintained in a single group with ear tag numbers as the primary identification method.

Chemical analysis and quantification of condensed tannins

Condensed tannins were extracted and purified from organic PN by the CT isolation method using Sephadex LH-20 gel filtration (GE Healthcare Bio-Sciences Corp, Piscataway, N.J.; Strumeyer and Malin, 1975) then quantified by the Protein-Precipitable Phenolic method (Hagerman and Butler, 1978, which uses iron phenolate to detect tannins by UV Spectrophotometer (Beckman Coulter Inc., Model DU730, Fullerton, Calif.).

Condensed tannins were purified using Sephadex LH-20 for subsequent use as a standard from PN extract according to Naumann et al. (2013). The aqueous portion containing the CT was retained. The extract, along with enough 1:1 (v/v) methanol:water to form a slurry, was mixed with Sephadex LH-20, and the slurry was repeatedly washed with 1:1 methanol:water until cast off was near clear. Condensed tannins bound to the Sephadex were released by washing with 7:3 (v/v) acetone:water followed by evaporation of residual acetone by air stream/vacuum. The aqueous phase containing CT was frozen at -80°C and lyophilized (Strumeyer and Malin, 1975; Cooper et al., 2014).

To determine Protein-Precipitable Phenolic, 50 µL of supernatant from PN extracts were combined with 250 µL buffer A (0.20 M acetic acid, 0.17 sodium chloride, pH 4.9), 50 µL bovine serum albumin, and 50 µL 1:1 (v/v) methanol:water and incubated at room temperature for 30 min prior to centrifuging for 5 min. Supernatant was removed by vacuum aspiration and the protein-phenolic pellet was washed with 250 µL buffer A before re-centrifuging and aspirating. The protein-phenolic pellet was dissolved in 800 µL of SDS/TEA (sodium dodecyl sulfate [1 percent w/v]-triethanolamine [5 percent v/v] before adding 200 µL FeCl3 (0.01 M FeCl3 in 0.01 M HCl). Absorbance was read at 510 nm after 30 min and quantified via external standards (Hagerman and Butler, 1978).

The concentration of protein bound by CT was determined as described by Naumann et al. (2014). The procedure was carried out as described above, but the protein-phenolic pellet was analyzed for N to quantify precipitated protein. Rather than dissolving the protein-phenolic pellet in SDS/TEA, the pellet was dissolved in 500 µL of buffer A, and the solution was transferred into foil cups and allowed to dry. A Elementar Vario Macro Cube C-N Analyzer (Donaustraße 7, Hanau, Germany) was used to analyze the dried protein-phenolic residue for percent N, which was multiplied by 6.25 to calculate the amount of protein bound CT. To determine total phenolics, 50 µL of supernatant from the crude plant extract was combined with 850 µL of SDS/TEA before adding 200 µL of FeCl3. Absorbance at 510 nm was read after 30 min and quantified via external standards as described for the Protein-Precipitable Phenolic assay.

The procyanidin:prodelphinidin ratio of CT from organic PN grape extract was measured by High Performance Liquid Chromatography (Li et al., 2010) using a Thermo Fisher Dionex Ultimate 3000 UHPLC (Thermo Scientific, Indianapolis, Ind.).

Feedstuff analysis

Carbon, N, CP, and ratios were analyzed for fescue pasture, by a C/N analyzer (Elementar Vario Macro Cube; Donaustraße 7, Hanau, Germany). Organic PN was analyzed for CP by the same method. Neutral detergent fiber, ADF, and DM were determined on grab samples, harvested at a 2.54 cm stubble height, which were taken from pastures pre-, mid-, and post-grazing of the trial. Samples were freeze dried with a FreezeZone12 (Labconco Corp., Kansas City, Mo.), ground to pass through a 1 mm screen using a Wiley Mill (Arthur H. Thomas, Penn., USA), and analyzed using the Van Soest (1991) method without α-amylase, using an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, N.Y.).

Parasitological procedure and measures

During the 63-d trial, individual fecal samples were taken from the rectum of each animal every 7 d. Fecal egg count was determined within 24 h by the modified McMaster procedure (Whitlock, 1948; Mines, 1977) and quantified by using 2-g subsamples of fresh feces from each lamb. Oocytes were counted under a microscope, but not identified by species; however, based on previous work in our lab, *Haemonchus Contortus* is the primary GIN at this locale. Every 7 d, individual blood samples were taken by jugular venipuncture into hematocrit tubes and PCV was determined using a HemataSTAT II Centrifuge (Separation Technology, Inc., Sanford, Fla.) within 6 h of blood collection. Additionally, weights, FAMACHA© scores (Hepworth et al., 2006) and BCS (Russell, 1991) were taken every 7 d by the same experienced evaluator throughout the entirety of the study.

Analysis of complete blood cell counts

Blood samples for complete blood cell (CBC) counts were taken by jugular venipuncture every 14 d into BD Vacutaine K3 EDTA 12-mg blood collection tubes (Fisher Scientific, Pittsburgh, Penn.). Samples were shipped to University of Arkansas (Fayetteville, Ark.) in cold storage to maintain sample integrity, and within 24 h of collection CBC counts were analyzed by an Abbott Cell-Dyn 3700SL Automate Hematology Analyzer (GMI Inc., Ramsey, Minn.).

Table 1. Effects of organic Pinot Noir on performance in Katahdin lambs.

	Treatment[a]				
Item	C	D7	D14	SEM[b]	Contrast[c]
Start BW, kg	23.8	22.7	23.4	1.06	ns
End BW, kg	28.0	28.2	28.9	1.05	ns
ADG, kg	0.07	0.09	0.08	0.01	W
Gain, kg	4.2	5.4	5.2	0.39	W
Start BCS[d]	2.9	2.9	2.7	0.14	ns
End BCS[d]	2.5	2.6	2.5	0.11	ns
BCSd change[e]	-0.3	-0.3	-0.3	0.13	ns

[a] C = Control drenched with 30 mL of water every 14 d, D7 = drenched with Pinot Noir every 7 d at a rate of 10-mL per 4.5 kg of BW, and D14 = drenched with Pinot Noir every 14 d at a rate of 10-mL per 4.5 kg of BW.
[b] SEM = Pooled standard error of means.
[c] Contrast statements: W = mean of C compared with the mean of D7 and D14 ($P \leq 0.05$); ns = no significant difference ($P > 0.10$).
[d] BCS = Body condition score, based on 5-point scale, with 1 being thin and 5 being obese.
[e] BCS change = Change of start body condition score compared with end body condition score.

Table 2. Effects of organic Pinot Noir on parasite parameters in Katahdin lambs.

	Treatment[a]				
Item	C	D7	D14	SEM[b]	Contrast[c]
Start FEC, eggs/g[d]	43.0	39.6	48.7	8.11	ns
End FEC, eggs/g[d]	50.6	28.1	24.7	9.57	W
FECd change, eggs/g[e]	10.5	-13.1	-18.5	10.82	W
Start FAMACHA©[f]	1.6	1.4	1.8	0.60	ns
End FAMACHA©[f]	1.5	1.5	1.5	0.12	ns
FAMACHA©[f] change[g]	-0.2	-0.1	0.0	0.20	ns
Start PCV, %[h]	34.2	31.4	33.4	1.31	ns
End PCV, %[h]	36.3	37.0	36.8	1.05	ns
PCVh change, %[i]	2.2	5.6	2.2	1.19	X

[a] C = Control drenched with 30 mL of water every 14 d, D7 = drenched with Pinot Noir every 7 d at a rate of 10-mL per 4.5 kg of BW, and D14 = drenched with Pinot Noir every 14 d at a rate of 10-mL per 4.5 kg of BW.
[b] SEM = Pooled standard error of means.
[c] Contrast statements: W = mean of control compared with the mean of D7 and D14 ($P \leq 0.05$); X = mean of D7 compared with the mean of D14 ($P \leq 0.05$); ns = no significant difference ($P > 0.10$).
[d] FEC = Fecal egg count.
[e] FEC change = Change of start fecal egg count compared with end fecal egg count.
[f] FAMACHA© score = 1 - not anemic to 5 - severely anemic.
[g] FAMACHA© change = Change of start FAMACHA© compared with end FAMACHA©.
[h] PCV = Packed cell volume.
[i] PCV change = Change of start packed cell volume compared with end packed cell volume.

Statistical analyses

Data were analyzed using PROC MIXED of SAS 9.3 (SAS Inst. Inc., Cary, N.C.). Animal was considered the experimental unit. Treatment means are reported as least squares means with the contrast statements of the mean of control compared with D7 and D14 and the mean of D7 compared with D14. Differences were considered significant at $P \leq 0.05$.

Results

Pasture averages for all sample dates included: CP = 14.2 percent; NDF = 56.7 percent; ADF = 30.1 percent; DM = 91 percent. Organic PN grape extract was found to have a concentration of 0.20 mg/mL of CT. Crude protein was 1.6 mg/mL by sample. The concentration of PBCT was determined and found to bind 12.7 mg/mL of protein with a 32.8 percent binding capability. The level of combined procyanidins and prodelphinidin was 0.0007 mg/mL with 15.5 percent Galloylated tannin.

As shown in Table 1, ADG and total weight gain were greater ($P = 0.02$) from D7 (5.4 kg) and D14 (5.2 kg) compared with C (4.2 kg) lambs. Start, end, and change BCS averaged 2.5 and did not differ ($P \geq 0.50$) across treatments.

Natural GIN infection was apparent in all lambs with an average FEC of 43.8 ± 8.11 eggs per g of feces. Two lambs were removed from D14 because they met three of four health threshold criteria. As displayed in Table 2, end of study ($P = 0.05$) and change from beginning to end ($P = 0.04$) FEC were greater from C compared with D7 and D14. Change in PCV from start of study to end differed ($P = 0.05$) from D7 and D14. Overall, FAMACHA© scores were not different ($P \geq 0.50$) across all treatments.

White blood cell (WBC) counts and monocytes (MONO) were higher ($P = 0.03$ and $P = 0.05$, respectively) at end of study from D7 compared with D14. A significant ($P = 0.02$) change was found in basophil (BASO) concentrations from D7 compared with D14. A tendency ($P = 0.07$, $P = 0.09$, and $P = 0.09$, respectively) was found for change in WBC, hemoglobin (HGB), and mean corpuscular hemoglobin concentrations (MCHC percent) from D7 compared with D14, with D7 concentrations being less than D14 concentrations. Other blood parameter concentrations were similar ($P \geq 0.10$) across treatments (Table 3).

Discussion

The main purpose of chemical anthelmintics is to achieve >90 percent reduction of adult and larval parasites in the host animal (Ketzis et al., 2006). However, remaining refugia create the opportunity for resistant parasitic infections to occur (Ketzis et al., 2006). Consequently, the purpose of novel anthelmintics establishes a different approach towards the control of GIN in ruminants. Novel control methods do not always have a direct effect on the parasite, but instead use the animal's own ability to recover and assist in maintaining parasite infections below the economic threshold of the physical capabilities of the animal (Ketzis et al., 2006). This not only relates to the efficacy of the control method used, but also to the epidemiology of the parasites, climate, animal management program, and the ease of integration as a sustainable program (Ketzis et al., 2006). The precise mechanism by which CT acts as a natural anthelmintic needs to be better understood, and a concerted effort on isolation, development, and validation of the effects needs to be undertaken before they are more widely accepted (Githiori et al., 2006). High CT content of red grape products (Mattivi et al., 2009; Yang et al., 2009) and world-wide availability, make it a potential source of natural anthelmintics (Kammerer et al., 2004).

All lambs grazed the same forages with nutritional constituents averaged for all samples. These included CP = 14.2 percent; NDF = 56.7 percent; ADF = 30.1 percent; DM = 91 percent. Organic PN grape extract used in this study had a CT concentration of 0.20 mg/mL and demonstrated a natural bioactive anthelmintic effect by reducing nematode FEC of pasture-grazed Katahdin lambs. Reduction in FEC may have been due to temporary reduction of nematode numbers, reductions in female worm fecundity, reduced nematode excretion (measured by FEC) and/or egg output (Heckendorn et al., 2007; Hoste et al., 2006). It is also possible that CT may alter the L3 larval ex-sheathment process, thereby reducing persistence to the host animal (Alonso-Diaz et al., 2011). In research conducted by LeShure (2014), grape pomace extract resulted in 100 percent inhibition of egg hatching into third-stage larvae. Results indicated that grape pomace had efficacy in decreasing hatchability of helminth eggs, as well as decreasing parasite viability in an in vitro setting (LeShure, 2014). Research by Iqball et al. (2007) found when sheep were divided into three treatment groups of low CT, high CT, and a control, that high CT facilitated protection of protein from degradation by rumen microbes, which minimized the effects of internal parasites. This was due in part to CT binding with plant protein following mastication. The result was the formation of larger CT-protein complexes while in the rumen, which protected the protein from rumen microbes. It remained as a complex until reaching the abomasum where pH changes and protein was released (Hoste et al., 2006). The CT directly affect overall GIN numbers and increase animal performance by influencing the physiological function and environment available to the parasite (Githiori et al., 2006). At this point, free CT may interact with the parasite and proteins are released to be absorbed in the lower gastrointestinal tract (Min and Hart, 2003). Parasite level may also be indirectly influenced through improved availability of protein allowing the animal to launch a counter attack by increased immunity (Hoste and Torres-Acosta, 2011).

In the current study, an increase in total weight gain and ADG in D7 and D14 lambs could suggest an added benefit of CT ability to bind protein, causing a by-pass protein effect. Research conducted by Dawson et al. (2011) found there were no difference in weaning weights or weight gain in lambs offered low protein diets compared with high protein diets. When a high protein diet was offered without CT tannins, lambs gained less weight as compared to lambs fed a low-protein diet with 80 g Quebracho CT tannin extract/kg BW. Therefore, diets with CT added may be more effective than diets concerned with protein content exclusively. The widely accepted explanation for positive effects of CT on protein digestion and metabo-

Table 3. Effects of organic Pinot Noir on complete blood cell counts in Katahdin lambs.

	Treatment[a]				
Item[b]	C	D7	D14	SEM[c]	Contrast[d]
Start WBC, K/µL	10.20	9.95	9.56	0.737	ns
End WBC, K/µL	9.59	10.26	7.97	0.728	X
WBC change, K/µL[e]	0.61	-0.31	1.95	0.845	x
Start NEU, K/µL	3.67	3.12	3.22	0.397	ns
End NEU, K/µL	3.67	3.49	2.95	0.456	ns
NEU change, K/µL[e]	0.00	-0.37	0.50	0.426	ns
Start LYM, K/µL	3.16	3.56	3.26	0.329	ns
End LYM, K/µL	3.24	3.35	2.52	0.362	ns
LYM change, K/µL[e]	-0.08	0.21	0.76	0.427	ns
Start MONO, K/µL	2.64	2.57	2.34	0.310	ns
End MONO, K/µL	2.10	2.59	1.89	0.246	X
MONO change, K/µL[e]	0.54	-0.02	0.53	0.319	ns
Start EOS, K/µL	0.15	0.29	0.67	0.057	ns
End EOS, K/µL	0.26	0.42	0.27	0.077	ns
EOS change, K/µL[e]	-0.11	-0.13	-0.09	0.065	ns
Start BASO, K/µL	0.57	0.40	0.56	0.064	ns
End BASO, K/µL	0.32	0.41	0.33	0.056	ns
BASO change, K/µL[e]	0.25	-0.01	0.23	0.079	X
Start RBC, K/µL	10.35	9.53	10.16	0.541	ns
End RBC, K/µL	10.19	9.76	9.89	0.669	ns
RBC change, K/µL[e]	0.16	-0.23	0.66	0.778	ns
Start HGB, g/dL	10.19	9.42	9.97	0.554	ns
End HGB, g/dL	10.28	10.29	9.68	0.522	ns
HGB change, g/dL[e]	-0.09	-0.87	0.69	0.641	x
Start HCT, %	33.20	33.34	32.57	1.180	ns
End HCT, %	33.07	33.89	33.87	1.044	ns
HCT change, %[e]	0.13	-0.22	0.31	0.973	ns
Start MCV, fL	32.03	32.71	32.20	0.412	ns
End MCV, fL	32.45	32.66	31.83	0.522	ns
MCV change, fL[e]	-0.42	0.17	0.08	0.324	ns
Start MCH, pg	9.79	9.89	9.81	0.109	ns
End MCH, pg	10.08	10.11	9.77	0.154	ns
MCH change, pg[e]	-0.29	-0.19	0.02	0.094	ns
Start MCHC%, g/dL	30.59	30.24	30.53	0.261	ns
End MCHC%, g/dL	31.05	31.01	30.75	0.223	ns
MCHC% change, g/dL[e]	-0.46	-0.77	0.02	0.311	X
Start RDW, %	26.39	25.35	25.85	0.536	ns
End RDW, %	31.65	29.39	29.30	1.403	ns
RDW change, %[e]	-5.27	-4.16	-2.75	1.340	ns
Start PLT, K/µL	745.60	750.43	747.80	85.924	ns
End PLT, K/µL	582.60	514.47	541.34	96.560	ns
PLT change, K/µL[e]	163.00	235.96	191.97	108.570	ns

[a] C = Control drenched with 30 mL of water every 14 d, D7 = drenched with Pinot Noir every 7 d at a rate of 10-mL per 4.5 kg of BW, and D14 = drenched with Pinot Noir every 14 d at a rate of 10-mL per 4.5 kg of BW.

[b] WBC = White blood cells; NEU = Neutrophils; LYM = Lymphocytes; MONO = Monocytes; EOS = Eosinophils; BASO = Basophils; RBC = Red blood cells; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC% = Mean corpuscular hemoglobin concentration percent; RDW = Red cell distribution width; PLT = Platelets.

[c] SEM = Pooled standard error of means.

[d] Contrast statements: W = mean of C compared with the mean of D7 and D14 ($P \leq 0.05$); X = mean of D7 compared with the mean of D14 ($P \leq 0.05$); lowercase letters represent statistical tendencies ($P \leq 0.10$); ns = no significant difference ($P > 0.10$).

[e] Change = Change of start complete blood cell parameters compared with end complete blood cell parameters.

lism is that CT-protein complexes escape ruminal degradation resulting in greater protein availability in the abomasum (Reed, 1995). In compiled research, Min and Hart (2003) found that moderate concentrations of 20 g to 40 g CT/kg of DM bind to protein by hydrogen bonding at near neutral pH (pH 6.0-7.0) in the rumen to form CT-complexes, but dissociate and release bound protein at pH less than 3.5 in the abomasum. Therefore, protecting dietary protein against degradation in the rumen subsequently increases amino acid supply to the abomasum and small intestine, resulting in improved nutritional status of the animal and possible improved production (Min and Hart, 2003; Hoste et al., 2006). The protein binding activity of CT could be affected by procyanidin:prodelphinidin and percent galloylation of CT. Condensed tannins with greater proportions of prodelphinidin and galloyl groups may have greater bioactivity (Naumann et al., 2015) and subsequent increased ability to bind protein over other types of tannins. It could also be suggested that organic PN is more easily absorbed in the abomasum of the ruminant animal due to solubility of tannin in fluid. Concentration and structure of CT present in different plant species seem to be the major factors modulating efficacy against nematodes (Oksana et al., 2012). However, there is some indication that molecular weight of CT also plays a role and that smaller molecular weight CT is more bioactive against GIN than those of larger molecular weight (Naumann et al., 2014).

Laboratory examination of the ruminant CBC can be an important addition to the physical examination (Jones and Allison, 2007). Consulting a CBC can often show an immune response to infection or virus before symptoms are presented in the animal. In this research, some changes were found in CBC results, including a significant increase in both MONO and WBC from D7 compared with D14 at the end of the study. A significant change in BASO concentrations were found, as well as tendencies for change in WBC, HGB, and MCHC percent from D7 compared with D14, with D7 concentrations tending to be less. An increase in PCV, MONO, and WBC concentrations may indicate that internal parasite load

was depressed. Further exploration is needed to determine the anthelmintic properties and the biological processes by which CT enhances the response of the host to the nematode. It can be hypothesized that the improved ability of the host to tolerate the negative effects of the parasite, or host resilience, and to respond to the parasite by resistance, might result from feed supplementation (Miller and Horohov, 2006; Hoste and Torres-Acosta, 2011). As such, it is possible that an enhanced immune response or resistance could be mediated by improved protein availability (Heckendorn et al., 2007). The ability of CT to bind proteins and increase an animals nutritional plane, could thereby increase the animals ability to fight GIN (Hoste et al., 2006). Key characteristics of CT that are most important to antiparasitic activity need to be elucidated.

Conclusion

Pinot Noir may be an effective organic and sustainable strategy for controlling nematodes and increasing performance in lambs. Use of Pinot Noir may be desirable because it is a sustainable resource, it is an easily accessible source of condensed tannins, and as a liquid it is simple to administer. The use of Pinot Noir could be applied by organic producers, thereby improving profitability of organic, small-ruminant production. However, additional research is needed to determine the extent of immunological response that may be observed by using phytochemicals in ruminant animals

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