Effects of Condensed Tannin-Rich Pine Bark Diet on Experimentally Infected With Haemonchus Contortus in Meat Goats

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Abstract

This study evaluated the effects of dietary supplementation of a novel condensed tannin-rich pine bark (PB) mixture diet on an in vitro gas production, in vivo animal performance, internal parasites, carcass production, and plasma metabolites in meat goats. Fifteen Kiko-cross meat goats (Capra hircus; body weight (BW) = 28.0 ± 1.0 kg) were randomly assigned to two experimental diets (control vs. PB supplementation): 1) control diet (70% grain mixture and 30% wheat straw (WS) and 2) 70% grain mixture with 30% PB. Animals were dewormed on day 10 and animals were artificially inoculated (day 20) with 5,000 infective stage (L3) Haemonchus contortus larvae. Feed intake and performance were monitored for 87 days. Blood samples were taken twice, once at the beginning and once at the end of the experiment. Abomasal worms were identified and counted. All analyses were conducted using a GLM or MIXED procedure of SAS, but in vitro gas analysis was conducted using non-linear procedure of SAS. Overall, there were no differences (P >0.10) in dry matter intake, body weight, and carcass traits between diets. H. contortus adult worm numbers were lower (P <0.05) for goats that were fed PB diet than for control. On day 84, control goats on control WS diet had greater (P = 0.01) FEC than PB diet group. It was concluded that feeding ground PB as a feed ingredient has the potential to decrease internal parasite infection without detrimental effects.

Keywords: Condensed Tannins; Animal Performance; Parasites; Pine Bark.

Introduction

Implications

Infestation with gastro-intestinal nematodes (GIN) in small ruminants can cause economic losses and endanger animal welfare. Although sheep and goats get numerous types of parasites, Haemonchus contortus is the most clinically significant nematode parasite and most important with respect to dewormer resistance. Unfortunately, the long term, heavy use of dewormers has led to parasite resistance to these products. That means that on some farms, there are no dewormers left that will effectively eliminate parasites. We have developed alternative natural dewormer that can control internal parasites infestation in goats. A noble approach of such practical strategy will help to make recommendation to improve the management of animal health and well being, especially organic livestock farmers.

Gastrointestinal (GI) parasites (especially Haemonchus contortus) present the greatest danger to the goat and sheep industry. Infected animals have lower growth rates, reduced reproductive performance, and have higher rates of illness and death. In the past, sheep and goat producers relied heavily on anti-parasite drugs. Unfortunately, GI parasites have become increasingly resistant to many of the anthelmintics. Alternative methods of GI parasite control for animals raised primarily on forages are vital for the sustainability and profitability of sheep and goat farms in the southeastern U.S. Consequently, alternative, sustainable, and affordable methods of parasitic control are required that are practical and realistic for introduction into farm production systems. Research has shown that legumes such as sericea lespedeza (Lespedeza cuneata) contain condensed tannin (CT) have anti-parasitic properties. The anti-parasitic properties of CT have been demonstrated to reduce GI parasitic infection in goats at Oklahoma [1] and with hay-feeding trials with goats at Georgia [2] and sheep at Louisiana, U.S.A [3]. Recently, researchers in Tuskegee University...
[4] found potential benefits of pine bark (PB) supplementation on anti-parasitic effects and improved feed efficiency. Pine bark is one of the abundant forest by-products in the southern U.S. and contains 11–13% condensed tannins (CT) on a dry matter basis [4].

However, relationships among CT levels in PB, experimentally infected worm burden, feed intake, animal performance, blood metabolites, and carcass characteristics have not been explored in meat goats. The aim of this study was to assess possible anthelmintic effects of PB powder supplementation against *H. contortus* in infected goats and the associated consequences on the animal production and health.

**Materials and Methods**

This experiment was conducted at Tuskegee University’s Small Ruminant Research and Education Unit over an 87-day period (16 weeks). This experiment was piloted in compliance with Tuskegee University Institutional Animal Care and Use Committee regulations.

**Experimental Procedures**

Fifteen male 9 month old Kiko-cross meat goats (*Capra hircus*; BW = 28.0 ± 1.0 kg) were randomly assigned to two experimental diets (control vs. PB supplementation): 1) 0% pine bark (PB) and 30% wheat straw (WS) and 2) 30% ground PB and 0% WS (Table 1). The remainder of each diet was a mix of 85% grain and 15% bermuda grass hay. Experimental goats for this experiment were strategically dewormed on day 0 with 5 mL/100 lbsBW of Pyrantel (Pfizer Animal Health) under supervision of a veterinarian to sequentially determine using an ANKOM 200 Fiber Analyzer [4].

The ingredients composition of the concentrate mix and bermuda grass hay offered to goats is summarized in Table 1. The fresh PB was donated by a wood processing company (West Fraser Inc., British Columbia, Canada) and incorporated in the grain mix portion of the diet. The final concentration of CT in 30% PB diet was 3.2% DM. Mixtures containing ground PB and WS were commercially prepared at the local feed mill (Eclectic Feed Mill, Eclectic, AL). Experimental diets met all animals’ requirements for growth and gain according to NRC [5]. The mixtures of ground PB, and WS (n = 3) were dried for 48 h at 55°C in a convection oven (model 420, NAPCO, Pittsburgh, PA). Samples were ground to pass a 1-mm screen (Wiley Mill, Arthur Thomas Co., Philadelphia, PA) according to the method described by Min et al. [5]. Samples were obtained (about 100 mL) from 4 animals using a stomach tube and transported to the laboratory within 30 min of collection. The rumen fluid was immediately placed on 300 mL thermostatic bottle with that was capped and immediately returned to the Lab for determination of rumen gas production. Rumen gas production was determined by combining, in 18 x 150 mm crimp top tubes, 5 mL mixed rumen fluid with 5 mL anaerobic artificial saliva [6] containing 0, 5, 10 mg/mL of ground PB (to pass 2 mm sieves). These tubes were capped, attached with 60 mL syringe, and incubated at 39°C under H<sub>2</sub>:CO<sub>2</sub> (50:50 mix) atmosphere. *In vitro* gas production was measured as plunger displacement (mL) at 0, 1, 2, 3, 4, 5, 6, 8, and 12 h incubation periods [6]. *In vitro* incubation was under taken in duplicate. Total *in vitro* gas produced was corrected to blank incubations (i.e. no ruminal fluid). All gases were collected from the *in vitro* rumen incubation for total gas and methane gas production analyses [7].

**Performance Measurement**

Feed intake was calculated as difference between feed offered and refused. Feed intake and refusal was treated as daily measures for 87 days for growth performance and gain efficiency determinations. Average daily gain (ADG) was calculated by difference in initial and final BW divided by 87 days of growth performance. Gain-to-feed ratio was calculated as ADG (g/d) divided by total dry matter intake (DMI; g/d). The animals used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee of the Tuskegee University, Tuskegee, AL.

**Feed sample collection and analysis**

Diet samples were collected every 2-week. Composite samples for grain mixes and ingredient samples for Bermuda grass hay, PB, and WS (n = 3) were dried for 48 h at 55°C in a convection oven (model 420, NAPCO, Pittsburgh, PA). Samples were ground to pass a 1-mm screen (Wiley Mill, Arthur Thomas Co., Philadelphia, PA). Ground composite samples were analyzed for DM, lignin, ether extract, and minerals according to the methods described by AOAC [8]. Crude protein was determined using a Kjeldahl-N method [8] by multiplying N by 6.25. Dietary neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using a method described by Van Soest et al. [9]. Sample were obtained (about 100 mL) from 4 animals using a stomach tube and transported to the laboratory. Rumen fluid was assayed using the method described by Min et al. [5]. Samples were obtained (about 100 mL) from 4 animals using a stomach tube and transported to the laboratory within 30 min of collection. The rumen fluid was immediately placed on 300 mL thermostatic bottle with that was capped and immediately returned to the Lab for determination of rumen gas production. Rumen gas production was determined by combining, in 18 x 150 mm crimp top tubes, 5 mL mixed rumen fluid with 5 mL anaerobic artificial saliva [6] containing 0, 5, 10 mg/mL of ground PB (to pass 2 mm sieves). These tubes were capped, attached with 60 mL syringe, and incubated at 39°C under H<sub>2</sub>:CO<sub>2</sub> (50:50 mix) atmosphere. *In vitro* gas production was measured as plunger displacement (mL) at 0, 1, 2, 3, 4, 5, 6, 8, and 12 h incubation periods [6]. *In vitro* incubation was under taken in duplicate. Total *in vitro* gas produced was corrected to blank incubations (i.e. no ruminal fluid). All gases were collected from the *in vitro* rumen incubation for total gas and methane gas production analyses [7].

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Blood Sample and Analysis

Blood samples were taken before (day 0) and after inoculated (day 60). Blood samples were collected using 20 gauge 1 inch needles via jugular vein, in EDTA (3 mL) and non-EDTA (10 mL) containing vacutainer tubes (BD, Franklin Lakes, NJ) and blood samples were placed on ice immediately following collection and relocated to Tuskegee University Clinical Pathology Laboratory for analysis. Samples were analyzed for complete blood counts using CELL-DYN 3700 Model System (Abott Diagnostic Division, Chicago, IL), packed cell volume (PCV), white blood cells (WBC) differential count, red blood cell count (RBC), hematocrit (HCT), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, and mean platelet volume (MPV) and blood serum metabolites [11] were analyzed at the Tuskegee University Diagnostic Laboratory, immediately after sampling. Total white blood cell numbers were determined by the method of Natt and Herrick [12]. Lymphocyte population was evaluated from stained blood smears with brilliant cresyl blue [13].

Harvest Measures

Final body weight (BW) was obtained after 87 days and animals were transported to Auburn University Lambert-Powell Meats Lab for harvesting according to the USDA approved guidelines [14]. All of the internal organs with the exception of the abomasum and the kidneys were disposed of after slaughter. The Post-mortem necropsy examination and dissecting the kidney and liver were conducted according to the method of Nietfeld [15].

Hot carcass weight (HCC) was determined on the day of slaughter, and carcasses were chilled at 4°C for 24 h. Then, cold carcass weight (CCW) and carcass shrink weight were measured. Carcass-water over the midpoint of longissimus muscle (LM) at the 12th rib, body wall fat (BWF) measured at lower point of the 12th rib, and fat depth over the midpoint of longissimus muscle (LM) at the 12th rib, body wall fat (BWF) measured at lower point of the 12th rib, kidney-pelvic fat (KPF), dressing percentage (DP; HCW/live wt.), and LM area, were determined by certified USDA grader 24 h postmortem.

Fecal Collection and Sampling

Fecal samples were taken every two weeks using fecal bags for collection. Goats for this experiment were strategically dewormed on day 10 as mentioned above to reduce or eliminate gastrointestinal parasites; however, most resistant worms and coccidian survived under controlled environment. Fecal samples for fecal egg counts (FEC) were obtained on d0 (in quarantine d0-21), 10, 24, 40, 49, 56, 72, and 84. Fecal egg counts were determined by a modification of the McMaster technique [16].

Parasite Identifications

After harvest, the rumen of each animal was obtained where the abomasum was sectioned off and secured individually into an air locked zip locked bag and labeled. The abomasum was frozen and placed into two Styrofoam containers to be kept frozen and shipped to the School of Veterinary Medicine on the campus of Louisiana State University (LSU) for parasite count and identification. Abomasum was thawed and washed by opening the entire length and emptying the contents into a 20 L bucket, and then adjusted by tap water up to 10 L (final volume). Collected digesta was washed 3-4 times with 10 L of fresh water each time, and mixed well; two 100 ml (1%) samples were taken from each bucket and mixed with 100 mL of 10% (v/v) formalin solution, then examined under a stereoscope at 30X magnification.

Individual parasite larvae or adults examine were morphologically confirmed according to the methods of Merck Veterinary Manual [17] and Zajac [18]. In the process of parasite identification, a100 mL sample was taken from the 10 L size of plastic bottle and was placed into a 1 L beaker. The sample was then washed using small amounts of water through a 200 mesh sieve. Remain samples were placed into the 1 L beaker and water was added until the original volume (100mL) was reached. A drop of small amounts of the sample were poured into a Petri dish (with grids) and de-colored with hypochlorite solution. Using a dissecting microscope, the contents were slowly scanned to search for adult and larval worms. Using a tuberculin syringe, worms were transferred from the solution to the slide with a drop of lactophenol. The worms were counted, sexed, and identified to determine worm burden and worm percent distribution within each section of the abomasum for each animal. Once the worms on the slides have been counted the differentiated (%) for a given section of GI tract in a given animal is calculated. For calculating total worm count, a dilution factor of 100 (10 L water in washtub, with 1 L sub-sample, and 100 mL sample that was analyzed) was applied to sample worm count.

Statistical Analysis

The study was conducted to two comparable treatments (control vs. PB supplementation) as the main effect using a completely randomized design. The two factors were dietary amendments (PB powder or wheat straw supplementations). Goats were assigned randomly to one of the two treatment combinations. All analyses were conducted using SAS (SAS Inst., Inc., Cary, NC). For characteristics measured only once on the experimental unit (goat), the GLM procedure was used. For characteristics measured more than one time on the experimental unit, the MIXED procedure using the repeated option was used [19]. To evaluate treatment differences in fecal egg counts and worm counts, the GLIMMIX procedure was used with a Poisson error distribution, the log link function and Satterthwaite’s approximation of the denominator degrees of freedom [19]. The level of significance was P<0.05.

An in vitro gas production rate was measured repeatedly and calculated using the exponential equation of Drskov and McDonald [20]: Y = a + b(1-e^-ct).

Where Y was defined as gas production in time t; a, b, and c being constants of the exponential equation where a = the gas production at time 0, b = the proportion of gas production during time t, and c = the rate of gas production of the ‘b’ fraction. The constants b and c for each treatment were calculated with the method described by Min et al. [6] using the Non-Linear Regression (NLIN) procedure from SAS Institute (SAS Inst. Inc., Cary, NC). Cumulative in vitro gas production in each time point was
analyzed using the MIXED procedure of SAS Institute. The F-test-protected least squares means procedure of SAS was used to separate treatment means.

**Results**

**Diet Composition**

Chemical composition of the experimental diets including PB and WS are presented in Table 1. The grain mixes were isonitrogenous. Total CT concentration in PB and WS on a DM basis was 11.0% and 0.03%, respectively. However, total CT concentration in these diets (0% PB and 30% PB) was 0.19 and 3.2% CT DM, respectively. According to Table 1, ADF, NDF, and lignin content were higher for PB than for control diet. Total digestible nutrient (TDN) was higher for WS than for PB-contained ration.

**In vitro rumen digestibility and gas production**

We were concerned about the rumen fermentation of ground PB due to a high level of CT as well as dry form of PB. Our in vitro data in Figure 1 shows that addition of ground pine bark up to 10 mg PB/mL with concentrate mixture, incubated with rumen fluid obtained from grazing goats, had significantly reduced $\text{P}<0.01$ for total gas and methane gas production (Figure 1), indicating that minimum levels of ground PB supplementation to impact rumen fermentation was started at >10 mg PB/mL.

**Intake and growth performance**

The summary of dry matter intake (DMI), growth performance and gain: feed (G: F) ratio were presented in Table 2. There were no differences between animals in diet groups in any of the variables shown in Table 2.

**Carcass characteristics**

As shown in Table 3, carcass characteristics of animals fed experimental diets are presented. Of variables fasting BW, HCW, CCW, carcass shrink, DP, 12th rib fat thickness and KPF, there was no significant difference in the characteristics of carcass between the dietary treatment groups.

**Blood parameters and metabolites**

Hemogram and blood serum chemistry of goats consuming the separate diets of PB and WS are presented in Tables 4 and 5, respectively. In the current study, of the hemogram and serum chemistry showed no significant difference except for the mean corpuscular hemoglobin concentration ($\text{P}<0.02$), mean platelet volume ($\text{P}<0.03$), cholesterol ($\text{P}<0.03$), creatine kinase ($\text{P}<0.001$), and blood urea ($\text{P}<0.001$). Mean corpuscular hemoglobin concentration, cholesterol, triglyceride ($\text{P}<0.03$), and blood mineral P ($\text{P}<0.001$) were lower in PB diet, but mean platelet volume, creatine kinase, and blood urea N for PB diet was higher ($\text{P}<0.001$) than for those WS diet.

**Table 1. Chemical composition of experimental diets containing pine bark (PB) and wheat straw (WS).**

| Item                        | Mixedrations (% DM) | SEM Control (WS) | PB  |
|-----------------------------|---------------------|------------------|-----|
| Dry matter                  | 87.4                | 90.2             | 0.84|
| Crude protein               | 16.6                | 17.5             | 2.43|
| Acid detergent fiber        | 20.8                | 31.4             | 1.73|
| Neutral detergent fiber     | 34.5                | 37.7             | 3.97|
| NFC $^2$                    | 40.3                | 37.8             | 5.38|
| Lignin                      | 2.03                | 12.3             | 0.52|
| Crude Fat                   | 3.78                | 3.45             | 0.70|
| Total digestible nutrients  | 71.3                | 61.0             | 1.94|
| Ca                          | 0.78                | 0.44             | 0.001|
| P                           | 0.50                | 0.44             | 0.001|
| Mg                          | 0.28                | 0.20             | 0.001|
| K                           | 1.63                | 1.36             | 0.01|
| S                           | 0.31                | 0.29             | 0.001|
| Na                          | 0.19                | 0.37             | 0.001|
| Cu, ppm                     | 8.75                | 25.5             | 5.19|
| Mn, ppm                     | 161                 | 98.8             | 10.66|
| Zn, ppm                     | 126                 | 125              | 39.70|
| Fe, ppm                     | 375                 | 255              | 15.14|
| Condense tannins (% DM) $^3$| 0.19                | 3.20             | 0.01|

1 The total mixed ration (% DM) in control and pine bark (PB) contained diets consisted of ground PB (0, 30), ground wheat straw (30, 0), corn (20, 20), soybean meal (19, 21), soy hulls (4, 4), alfalfa meal (5, 3), molasses (6, 6), vitamins and mineral mix (0.5, 0.5), salt (0.5, 0.5), ammonium chloride (0.5, 0.5), and bermuda grass hay (15, 15) on an as-fed basis, respectively. Calcium (Ca); Phosphorous (P); Magnesium (Mg); Potassium (K); Sulfur (S); Sodium (Na); Copper (Cu); Manganese (Mn); Zinc (Zn); Iron (Fe).

2 Non fibrous carbohydrates.

3 Condensed tannins (CT) are relative to a purified Quebracho condensed tannins standard (on DM basis).
Fecal egg counts
Data presented in Figure 2, displays the effects of PB versus WS diets fed to inoculated animals on FEC. During the performance period, FEC fluctuated between diet treatments. On day 84, control group had greater \( (P<0.01) \) FEC than PB diet group.

Worm burdens
Experimentally infected GI parasites in PB and WS diets are presented in Table 6. GI infected parasites animals that received PB had lower total worm burdens \( (P<0.05) \) included \textit{H. Contortus} than WS mixed diet. Feeding PB diet reduced both male and female worm counts compared to WS diet.
Table 4. Effects of condensed tannin-containing pine bark (PB) supplementation on hemogram of Kiko-cross goat.

| Item                     | Mixed rations (% PB) | SEM  | P-value |
|--------------------------|----------------------|------|---------|
|                          | 0                    | 30   |         |
| No. of animals           | 7                    | 8    |         |
| Hemoglobin, g/dL         | 10.5                 | 9.98 | 0.36    | 0.12    |
| Hematocrit, %            | 13.8                 | 15.7 | 0.71    | 0.03    |
| Mean corpuscular volume, fl | 21.3               | 21.7 | 0.22    | 0.97    |
| Mean corpuscular hemoglobin, g/dL | 16.2           | 13.9 | 0.62    | 0.02    |
| Red cell distribution width, % | 32.8             | 31.5 | 0.97    | 0.82    |
| Mean platelet volume, fl | 11.0                 | 12.7 | 0.36    | 0.03    |
| Red blood cell, 10^6/µL  | 6.49                 | 7.26 | 0.28    | 0.34    |
| White blood cell, x 10^9/µL | 10.2              | 9.72 | 0.89    | 0.11    |

White blood cell (Diff. – absolute count/µL; % total)

|                |       |     |       |
|----------------|-------|-----|-------|
| Lymphocyte     | 4.06  | 3.20| 0.62  | 0.42  |
| Neutrophil     | 5.60  | 6.20| 0.61  | 0.89  |
| Monocyte       | 0.43  | 0.58| 0.07  | 0.55  |
| Eosinophil     | 0.08  | 0.12| 0.04  | 0.36  |
| Basophil       | 0.05  | 0.13| 0.04  | 0.22  |

Table 5. Effects of condensed tannin-containing pine bark (PB) supplementation on blood serum chemistry in Kiko-cross goat.

| Item                         | Mixed rations (% PB) | SEM  | P-value |
|------------------------------|----------------------|------|---------|
|                              | 0                    | 30   |         |
| No. of animals               | 7                    | 8    |         |
| Cholesterol, mg/dL           | 64.2                 | 59.1 | 5.12    | 0.03    |
| Enzymes, U/L                 | 191                  | 251  | 20.1    | 0.001   |
| Creatine kinase (CK)         | 19.8                 | 23.6 | 1.56    | 0.48    |
| Alanine transaminase (ALT)   | 36.2                 | 44.6 | 16.9    | 0.19    |
| Amylase                      | 219                  | 241  | 27.2    | 0.63    |
| Alkaline phosphatase(ALP)    | 47.1                 | 47.8 | 2.69    | 0.28    |
| Gamma-glutamyl transpeptidase(GGT) | 72.5       | 69.9 | 4.28    | 0.99    |
| Blood serum protein, g/dL    | 6.89                 | 7.16 | 0.20    | 0.74    |
| Blood serum protein, g/dL    | 2.79                 | 2.91 | 0.07    | 0.8     |
| Blood serum metabolites      |                      |      |         |
| Bilirubin, mg/dL             | 0.19                 | 0.24 | 0.02    | 0.81    |
| Bilirubin, total; mg/dL      | 0.19                 | 0.34 | 0.05    | 0.23    |
| Glucose, g/dL                | 70.9                 | 73.3 | 1.98    | 0.97    |
| Blood urea nitrogen, mg/dL   | 23.4                 | 34.4 | 1.78    | 0.001   |
| Creatinine, mg/dL            | 0.81                 | 0.88 | 0.03    | 0.44    |
| Triglyceride, mg/dL          | 30.9                 | 25.7 | 2.82    | 0.03    |
| Blood serum minerals, mg/dL  |                      |      |         |
| Ca                           | 9.84                 | 9.61 | 0.21    | 0.84    |
| P                            | 8.79                 | 7.01 | 0.44    | 0.001   |
| Blood serum electrolytes, mmol/L |                   |      |         |
| Na                           | 150                  | 150  | 1.45    | 0.88    |
| K                            | 5.63                 | 5.83 | 0.15    | 0.76    |
| Cl                           | 113                  | 114  | 1.18    | 0.57    |
| CO₂-LC, mM/L                 | 23.1                 | 21.9 | 0.68    | 0.81    |
Figure 2. Fecal egg counts of goats fed different diets throughout the experimental period. **Means with different superscripts differ (P<0.01). Experimental goats were dewormed on day 10 and inoculated on day 20.

Table 6. Effects of condensed tannin-containing pine bark (PB) supplementation on total adult worm count.

| Item               | Mixed rations (% PB) | SEM  | P-value |
|--------------------|----------------------|------|---------|
| Haemonchus contortus | 0                    | 30   | 0.01    |
| Female             | 13.9                 | 4.17 | 0.14    |
| Male               | 13.6                 | 4.67 | 0.11    |
| Total              | 27.5                 | 8.84 | 1.23    |

Discussion

The most significant findings of this study were that experimentally parasite infected animals reduced H. contortus load in the presence of PB diet with no changes in DMI and animal performance. The increase efficacy of anti-parasites activity was due to decreases fecundity at both fecal egg shedding and adult worm burden.

In vitro rumen fermentation

Plant tannins may have substantial effects on all phases of in vitro rumen fermentation and metabolism. In vitro ruminal fermentation incubated with PB supplementation reduced as evidenced by decreasing the rate of gas and methane gas concentration. The results indicate that the effect of CT-containing PB in this expression is a dose dependent. These findings agree with the data of Bento et al. [21] reported that the reduced gas production from mimosa tannin extracts during in vitro incubation reflects inhibition of cell walls, and its components, by mimosa tannins. As a result, mimosa tannins reduced microbial degradation of carbohydrates, and subsequently gas production [22, 23]. It has been shown that plant tannins or commercial tannin extracts modified microbial population in the rumen, reduced microbial numbers and/or enzyme production from the rumen microorganisms available to ferment substrates [24-26]. A decrease in rate of gas and methane gas production by PB tannins addition was consistent with the result of other in vitro studies [27, 1, 6].

Feed intake and growth performance

In the present study, there were no changes observed in DMI, ADG and carcass measurements. However, it has been reported that DMI was increased in the diets in goats fed PB (3.2% CT in DM) along with that of grain intake [4]. Similarly, Solaiman et al. [34] reported that total DMI of growing goats increased when sericea lespedeza (Lespedeza cuneata) ground hay (6.5% CT in DM) replaced alfalfa meal in the grain mixes, and Turner et al. (2005) reported that goats receiving the CT-containing sericea lespedeza hay (2.31 % CT/g soluble protein) had higher DMI than those fed the alfalfa hay based diet. Although this study was similar to the present experiment, variables such as inoculated animals may be the reason for DMI and animal performance difference. Other assumptions have been made such as, the feed intake by animals is usually reduced for diets with tannins in high concentrations due to a reduction of palatability, decreased rate of digestion in the rumen and the development of conditioned aversion [28-30].

Carcass traits

Previous research reported that animals grazing on CT-containing forage sulla (Hedysarum coronarium; 5-7 % CT DM) and birds foot trefoil (Lotus corniculatus, 3.4% DM CT) had greater animal performance and carcass production compared to those grazing alfalfa (Medicago sativa) [31, 32]. In the present study shown that there was no difference in HCW, CCW, transport shrink, DP, 12th rib fat thickness, and KPF, except LM area. According to the experiment conducted by Priolo et al. [33], twenty-four male Comisana lambs were assigned to one of three treatments; control, CT-containing sulla and polyethylene glycol drench group to eliminate condensed tannin effects. The carcass yield in the animals given sulla without polyethylene glycol was decreased. Solaiman et al. [34] reported similar results in carcass characteristics. There was no difference in HCW, CCW, shrink or dressing % of goats fed different dietary treatments of sericea lespedeza 0%, 10%, 20% and 30% for 63 days. Carcass characteristics are known to respond gradually to changes in nutrition. However, it is not fully understood how CT affects carcass traits, and so this area needs further investigation.

Hemogram and serum chemistry

In this study, visual inspection according to USDA regulations [14] standards indicated no anatomical lesions on liver and kidney organs. In correspondence with the data of Silanikove et al. [35] who reported using CT-containing diets that ranged from 5.0% - 7.0% CT, and Solaiman et al. [34] who reported that goats receiving the CT-containing sericea lespedeza hay (2.31 % CT/g soluble protein) had higher DMI than those fed the alfalfa hay based diet. Although this study was similar to the present experiment, variables such as inoculated animals may be the reason for DMI and animal performance difference. Other assumptions have been made such as, the feed intake by animals is usually reduced for diets with tannins in high concentrations due to a reduction of palatability, decreased rate of digestion in the rumen and the development of conditioned aversion [28-30].
CT (DM basis) in carob (*Ceratonia siliqua*) to 9.5% in oak (*Quercus-calyx*) and 20.5% in pistacia (*Pistacia lentiscus*) did not exhibit toxic symptoms in goats. The concentration of CT fed in current study was 0.19, and 3.2% CT DM in 0 and 30% PB diets, which was lower than the concentration used in the study of Silanikove et al. [35]. It is apparent that experimental animals were able to tolerate the amount equaling or less than 30% of CT content in diets fed. In the current study, of the hemogram and serum chemistry showed no significant difference except for mean corpuscular hemoglobin concentration, mean platelet volume, blood urea nitrogen, triglycerides, phosphorus, cholesterol, and creatine kinase; however, all values fell within the normal range for goats, suggesting that no damage in the liver occurred. This has been confirmed by post-mortem necropsy and dissecting the liver and kidney in this study that indicated no anatomical lesions on liver and kidney organs. These parameters were used as a diagnostic tool for screening animal health problems and abnormality.

Creatine kinase helps convert creatine into phosphocreatine by converting ATP into ADP in muscles. This is a reversible reaction where phosphocreatine acts as an energy reservoir used for rapid regeneration of ATP in muscles [36]. Previous study has been showed that goats received diet containing 15 and 30% PB inclusion had no change in blood creatine kinase enzyme. In the present study, however, creatine kinase level in PB diet was higher than control diet, indicating that animals received PB diet may associated with red blood cells increment that contribute to the enzymatic reaction for creatine kinase, artificially increasing values [37].

Blood urea nitrogen is waste from the liver, processed by the kidneys. According to Solaiman et al. [34], blood urea nitrogen level is not correlated with kidney dysfunction. However, blood urea nitrogen level increases in the present study may be due to the changes of protein metabolism in gastrointestinal tract as affected by CT containing PB or gastrointestinal parasites infection. Chaisemsaariat et al. [38] reported that parasitized sheep had a higher plasma blood urea nitrogen concentration than non-parasitized animal which attributed to the rate of irreversible loss and the rate of urinary excretion of urea.

**Fecal egg count and worm burdens**

Alternative parasites control strategies have recently been suggested based on using CT-containing forages [39, 40]. Min and Hart [39] reported that there was a direct effect of tannin-containing forages on adult worms, with significantly lower numbers of both abomasal (*H. contortus, Teladorsagia circumcincta*) and small intestinal (*Trichostrongylus*) nematodes compared with goats fed Bermuda grass hay (0% CT) [1, 6]. The present study strongly supports this view, showing that goats consuming CT-containing PB diet reduced both male and female worm burdens and fecundity as measured by FEC compared to those receiving CT-free diets. Of the common protein binding and precipitate characteristic of tannin, binding to parasitic worms are directly related [41]. These parasitic worms, *H. contortus*, are composed of protein, allowing for a binding and catabolism of the structure of the parasite’s mouth after the initial attachment to the intestinal wall. To compare and agree with an experiment conducted by Hur et al. [42], the effects of feeding pine (*Pinus densiflora* and *Picea abies*) leaves and lucerne chaff on coccidia egg yield were studied for a period of 10 days post-feeding. The results indicated that feeding fresh pine needles (40 g CT DM/day/goat) and oak leaves (40 g CT DM/day/goat) in blend with lucerne chaff had rapid anticoccidial activities in goats. This may begin to explain the results seen in this study although more research should be conducted for certain justifications. Regardless of the CT-parasite interactions, CT-containing diets may hold promise as a practical means of reducing contamination of pastures with parasites eggs and of decreasing exclusive reliance on commercial anthelmintics.

**Conclusion**

Pine bark powder supplementation up to 30% reduced fecal egg counts when fed for proper length of time. Feeding ground pine bark supplement reduced both male and female worm counts compared to wheat straw mixed diet. The results indicate that ground pine bark as a feed ingredient has potential to maintain animal performance while decreasing internal parasites infection. Thus, developing plant-based alternatives such as pine bark and other natural resources for gastrointestinal parasites control would be expected to have a greater impact on the goat and sheep industries. This will also development of best management practices to prevent or treat gastrointestinal parasites in ruminant livestock.

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