

The Effects of Phytochemical Tannin Containing Diets on Meat Goat Performance and Drug Resistant *Haemonchus contortus* Control

Research Article

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Abstract

The objective of this study was to quantify the effects of condensed tannins (CT) containing diets of *sericea lespedeza* pellets (SLP; *Lespedeza cuneata*; 13.6% CT), pine bark powder (PB; *Pinus*; 16.3% CT), and a combination of SLP and PB on combating of the drug resistant *Haemonchus contortus* UGA/2004 in meat goats. Twenty four Kiko cross intact male goat (*Capra hircus*; BW = 38.6 ± 2.7 kg) of one year old were randomly assigned to four experimental treatments (n = 6): 1) 30% bermuda grass (*Cynodondactylon*) hay and 70% grain mix, 2) 30% PB and 70% grain mix, 3) 30% SLP and 70% grain mix, and 4) 15% PB, 15% SLP, and 70% grain mix. Each treatment diet (30%) was mixed with the remainder of each diet containing 70% commercial sweet feed and 30% alfalfa pellets for a 42-day trial. The animals were experimentally infected with 5000 of 3rd stage larvae of the drug resistant *H. contortus* 6 weeks before the initiation of the experiment. Starting from the day zero the animals were fed once daily and their performance monitored for the following 42 days. Biweekly body weight, fecal egg counts (FEC), packed cell volume, and FAMACHA score. Blood samples were collected at day 42 and blood serum chemistry was detected. At the end of the experiment, the animals were sent to the Meat Technology Center, Fort Valley State University, GA for slaughtering, abomasum sampling and carcass evaluation. Samples of abomasum content were preserved then sent to Louisiana State University for adult worm counts. The results showed that FEC and adult worm counts in the mix diet were significantly reduced by day 42 compared to other treatment groups. FAMACHA data was significant low in the PB diet on day 21, while it was significantly reduced in both the SLP and mix diet on day 42. Furthermore, the blood parasitemia infection in meat goats, and it is possible that this effect is depends on the source of CT or the CT-containing diet.

Keywords: Condensed Tannins; Goats; *Haemonchus contortus*; Pine Bark; *Sericea Lespedeza*.

Introduction

Haemonchus contortus one of the most pathogenic widely distributed blood sucking nematode of sheep and goats [20, 22]. Adult *H. contortus* can suck about 0.05 mL of blood/ worm/day [27]. Several highly effective anthelmintic drugs were used for controlling of such worms, however these drugs were no longer be used due to rapid developing of the parasite drug resistance [26]. Developing of new control programs to *H. contortus* critical for goat enterprise expansion. One of these control programs is using of the

phytochemical bioactive compounds to overcome *H. contortus* problems. Phytochemical condensed tannins (CT) used efficiently as natural alternative anthelmintics to control gastrointestinal nematodes (GIN), as it safe, eco-friendly and easily applicable feed additives [31].

Using of the phytochemicals tannins may offer more efficacious control to *H. contortus* compared with other chemical anthelmintics [2]. The CT could interact with digestible nutrients forming complex, thus inhibits the nutrient availability for larval growth

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and decrease GIN metabolism through the inhibition of the oxidative phosphorylation [28], causing larval death [1]. Feeding of high level of CT had negative impacts upon the animal digestion that it inhabits the protein digestibility [5]. Fortunately, goats carry tannin-tolerant bacteria (*Streptococcus caprinus*; *diplococcoid bacterium*) which overcome the problem of protein indigestion via producing tannin-binding salivary protein during chewing and ruminating [8]. In concurrent work, the effect of condensed tannins of pine bark (PB) and sericea lespedeza (SLP) were studied on goats experimentally infected with drug resistance *H. contortus* to develop a new combating technique for *H. contortus* in goats.

Material and Method

The study was done in the Caprine Research Center, Tuskegee University, Tuskegee, AL from March to May, 2014. Twenty four intact Kiko crossbred male goat of one year old (BW = 38.6 ± 2.7 kg) were randomly enrolled into 4 experimental groups of 6 animals each. Goats were individually housed indoors and managed according to the ideal standards of the goats keeping. Experimental treatments included the control diet (30% bermudagrass hay and 70% commercial sweet feed and alfalfa pellets), PB diet (30% PB plus 70% commercial sweet feed and alfalfa pellets), SLP diet (30% SLP plus 70% commercial sweet feed and alfalfa pellets) and mixed diet (15% PB plus 15% SLP with 70% commercial sweet feed and alfalfa pellets). Feed samples were taken from each treatment and analyzed for dry matter (DM), crude protein (CP), CT and total digestible nutrients (TDN).

An adjustment period of 6 weeks allowed goats to be dewormed, inoculated, acclimated to pen environment and routine feeding prior to the study initiation. During the 1st week of the adjustment period, the goats were dewormed (day -15) with fenbendazole (10 mg each/kg BW) and fed control diet without CT. The goats were orally inoculated (day 0) with 5000 3rd stage [14] drug resistant (benzimidazoles, levamisole, and ivermectin) *H. contortus* UGA/2004 larvae [32]. In this study, we used drug resistant *H. contortus* because gastrointestinal parasites in goats have become increasingly resistant to many of the anthelmintics.

Once the experiment began biweekly body weight and fecal samples were collected. The FAMACHA score were recorded at day 0, 21, and 42, while the blood samples were taken once at day 42. The fecal egg counts (FEC) were determined using a modified McMaster technique according to [33]. By the end of the experiment, goats were transported, weighted, kept for 24 hours, reweighted then humanely slaughtered at Meat Science Laboratory, Fort Valley State University, GA. The fasting weight loss was determined via subtracting the pre and post keeping period weights of each animal. Just after the slaughter, the animal hot carcass weight was detected and samples of the whole abomasal content were taken, preserved and rapidly send to Louisiana State University for counting and sexing of the adult *H. contortus* worms. Abomasum was opened, and then washed with 10 L of tap water into a 20 L bucket [11]. Collected digesta samples were washed three times (1 h interval each time) with fresh water each time to obtained clear digesta samples and mixed well; two 100 mL (1%) samples were taken from each bucket (final volume was 10 L each) and mixed with 100 mL of 10% (v/v) formalin solution, and then worms were counted under a stereoscope at 30

X magnification, and used the mean of the two sample counts.

Statistical Analysis

Data of body weight, fecal egg counts (FEC), packed cell volume, FAMACHA score, blood serum chemistry, adult worm counts were collected. The data were enrolled to further statistical analysis using ANOVA with Fishers protected LSD test, and the level of significance was set at $P < 0.05$.

Ethical approval

Goats which used in the experiment housed and kept under the ideal standards for keeping of the small ruminant species. The animals were treated according to standards of the bioethical committee of the Tuskegee University, USA.

Results

Feed Analysis

The results of CT analysis of crud PB and SLP Table 1 indicated that the pure PB contained (16.1% ± 0.5) CT, while SLP contained (13.5% ± 0.3). The forgoing results illustrated the high level of CT was attained in pure PB. Moreover, the results were confirmed by the results of the diet characteristics showed in Table 2, as the results indicated that the PB diet contained the highest level of CT at 4.88% followed by the mixed diet (4.5%) and the SLP diet (4.0%). However, the SLP diet had the highest level of crude protein level (14.5%), while the lowest value was recorded in the PB diet (11.2%). Furthermore, the mixed diet contained 13.2% crude protein, as this value became in-between the PB and SLP diets.

Growth Performance

Concerning to the effect of the ration supplemented with CT on the growth performance of meat goats Table 3, it was clearly that the different ration with different CT types possessed no significant difference on growth performance (initial, final BW, co-variated final BW, ADG and co-variated) of Kiko goats at ($P > 0.05$).

Fecal Egg Counts

Regarding to the effect of feeding time of CT on FEC of the experimentally infected Kiko goats with drug resistant *H. contortus* showed in Figure 1, it was evidenced that feeding of either control or CT diets didn't significantly affect the FEC till the day 28 from the start of feeding. While by the day 42 of the experiment the effect became to reach to the significant levels. The mixed diet decreased significantly the FEC ($P < 0.01$) followed with SLP and PB ($P < 0.05$) containing diet compared to control.

FAMACHA

FAMACHA score one of rapid assessment to anemic condition and general health status via eye mucus membrane color. Table 4 showed that there were significant differences among different treatment diets in the FAMACHA score values, as by the day 21 PB diet decreased significantly the FAMACHA score than other

Table 1. Means and standard errors of the pine bark and sericea lespedeza Condensed Tannins level (%).

Item	Condensed tannins level (%)
Pine bark	16.1% ± 0.5
Sericea lespedeza	13.5% ± 0.3

Table 2. Means and standard errors of selected diet characteristics by treatment group.

Item	Diet*			
	Control	PB	SLP	Mixed
Dry matter,%	90.3 ± 0.5	90.2 ± 0.15	90.3 ± 0.15	89.8 ± 0.7
Crude Protein,%	13.3 ± 0.35	11.2 ± 0.15	14.5 ± 0.2	13.2 ± 0.1
TDN,%	59.0 ± 1	51.5 ± 0.5	56.0 ± 1	53.5 ± 0.5
Condensed tannin,%	0.4 ± 0.055	4.88 ± 0.1	4.0 ± 0.045	4.5 ± 0.045

* Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis. Except Bermuda grass hay, all ingredients were incorporated in the grain mixes.

Table 3. Effects of condensed tannin supplementation on growth performance of Kiko goats.

Item	Diet*				SEM	P-value
	Control	PB	SLP	Mixed		
Initial BW, kg	39	33.8	36.9	44.7	2.73	0.06
Final BW, kg	45.2	36.5	41.8	47.9	3.38	0.12
Final BW co-variated, kg	44.9	42.2	43.9	41.8	1.02	0.16
ADG, g/d	148	65.1	81.8	78.6	26.54	0.15
ADG co-variated, g/d	147.4	75.5	85.7	83.5	24.95	0.19

^{abc} Means with different superscripts within the same row differ significantly at (P<0.05)

* Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis Initial BW was co-variated for final BW and ADG

Table 4. The effects of condensed tannin on FAMACHA.

Item	Diet*				SEM	P-value
	Control	PB	SLP	Mix		
Day 0	3.2	3.7	3.6	3.4	3.46	0.35
Day 21	3.5 ^a	2.7 ^b	3.7 ^a	3.2 ^{ab}	3.26	0.05
Day 42	3.8 ^a	2.0 ^b	1.8 ^b	1.8 ^b	2.36	0.05

^{abc} Means with different superscripts within the same row differ significantly at (P<0.05)

* Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis.

Table 5. Effects of condensed tannin on the means of adult male, female, and total *H. contortus* worm counts in meat goats (n =6).

Item	Diet*				SEM	P-value
	Control	PB	SLP	Mix		
Female Worms	15.75 ^a	18.20 ^a	5.17 ^a	1.58 ^b	4.45	0.03
Male Worms	16.25 ^a	12.80 ^a	6.00 ^a	2.33 ^b	3.86	0.01
Total Worms	31.92 ^a	31.00 ^a	11.17 ^a	3.67 ^b	8.19	0.02

^{abc} Means with different superscripts within the same row differ significantly at (P<0.05)

* Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis.

Table 6. The effects of condensed tannin on carcass characteristics.

Item	Diet*				SEM	P-value
	Control	PB	SLP	Mix		
Fasting wt, kg	41	33.3	37	37.3	2.44	0.24
Hot carcass wt, kg	17.4	13.6	15.9	16.2	1.4	0.35
Dressing %, kg	42.4	40.8	42.7	42.9	1.7	0.83

*Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis.

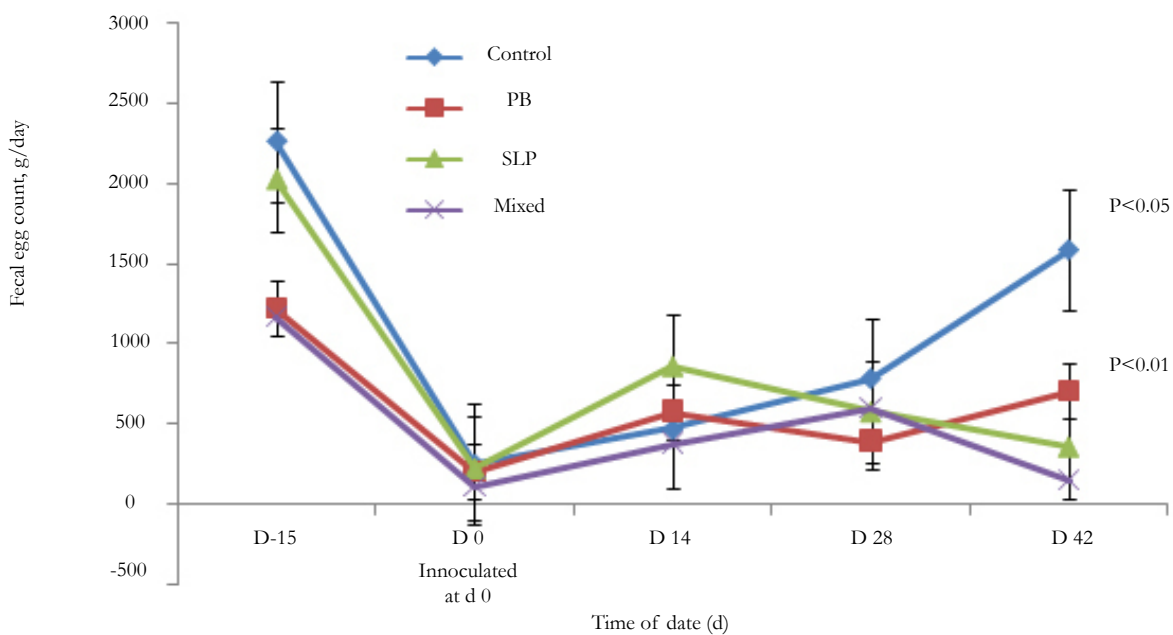
Table 7. The effects of condensed tannin on blood serum chemistry (mmol/L).

Item	Diet*				SEM	P-value
	Control	PB	SLP	Mix		
Alkaline phosphatase	198.2	285.2	205.3	463.3	288	0.15
Ace carbon dioxide reagent	20.5	18	19.9	20.4	19.7	0.1
Aspartate aminotransferase	70	72	62.3	57.6	65.47	0.08
Triglycerides	26.7 ^a	19.0 ^b	25.3 ^a	21.8 ^{ab}	23.2	0.04
Sodium	144.3 ^{ab}	146.9 ^a	144.3 ^{ab}	141.4 ^b	144.22	0.03
Cholesterol	71.0 ^a	45.0 ^b	64.3 ^a	55.2 ^{ab}	58.87	0.02
Alanine transaminase	12.3 ^a	3.5 ^b	8.0 ^{ab}	8.0 ^{ab}	7.95	0.02
Chlorine	108.6 ^b	112.9 ^a	109.0 ^b	106.2 ^b	109.17	0.001

^{abc}Means with different superscripts within the same row differ significantly at (P<0.05)

*Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis.

Figure.1. Effects of condensed tannin on fecal egg count (FEC) in Kiko crossbred male goat yearlings. Means with different superscripts within the same row differ significantly at (P<0.05). Control 0%, 30% pine bark (PB), 30% Sericea lespedeza pellets (SLP), and 15% PB + 15% SLP (Mixed) on an as-fed basis.



treatment groups at ($P < 0.05$), however the later groups were not significantly differed from each other at ($P > 0.05$). While, by the day 42 from the onset of the experiment PB, SLP, and mixed diets decreased significantly the FAMACHA score (2.0; 1.8; 1.8, respectively) compare with the control one (3.8) at ($P < 0.05$).

Adult Worm Count

These results were confirmed throughout adult worm counts Table 5.

Goats that supplemented both SLP and mixed diets had lower numbers of female ($P < 0.03$), male ($P < 0.01$), and total ($P < 0.02$) of *H. contortus* in the abomasum than those in the control treatment group. The PB diet possessed no significant differences in the adult worm counts, but mixed tannins diets with PB and SLP decrease significantly female, male and adult worm counts compare with other tannins containing diets at ($P < 0.02$).

Carcass Characteristics

Generally, Carcass characteristics taken after 42 day from the beginning of the experiment expressed in Table 6 possessed no significant differences among different treatment diets at ($P > 0.05$).

Blood Parameters

Blood serum biochemistry was significantly affected with tannins containing diets in goat Table 7, as the experimentally infected Kiko goats with *H. contortus* fed on PB containing diet showed significantly high serum aspartate aminotransferase (72.0), sodium (146.9), chlorine levels (112.9) and low cholesterol (45.0), alanine transaminase (3.5), and triglyceride levels (19.0) compared with goats fed on other treatment diets at ($P < 0.05$).

Discussion

The interesting results of the current work are the results of the worm count, FEC and FAMACHA score. Concerning to the adult worm counts results, it was clearly that the mixed diet with PB and SLP attained the lowest total, male and female worm counts. This indicated that the combination of the PB and SLP condensed tannins exaggerated their effects in reduction of the drug resistant *H. contortus* worms in the gut. These results may be due to the direct disrupting effect of the two different types of CT on the *H. contortus* cuticle and/or indirect effect throughout stimulation of the host immune resistance and resilience against gastrointestinal nematodes [3, 7]. The CT could form a complex with nutrients causing nutrients sacristy for larval growth also it may decrease GINs metabolism directly through inhibition of oxidative phosphorylation [28], also it caused larval death [1]. Shaik et al. (2006) reported that there was a direct effect of tannin-containing sericea lespedeza hayon adult worm counts, as it decreased significantly the numbers of *H. contortus*. These results go in parallel with those of Singh et al. (2015).

The FEC results showed no differences among different experimental groups till the day 42 of the experiment. This might be explained by the reduction of the adult worm counts by this

time. Also, the results may confirm that the effect of the CT containing diets may need prolonged time to amplify their effects on the drug resistant *H. contortus*. Mixed tannins diet decreased significantly the FEC by the day 42 of the experiment this may be indicated that the combination of the two types of the CT enhanced their effects against drug resistant *H. contortus*. Min et al. (2015) revealed that PB CT was mostly procyanidins (PC): total CT consisted of 87.6% PC and 12.4% prodelphinidins (PD) with mDP-values (mean degree of polymerization) of 10.5. However, SLP was mostly PD with mDP values of 30.0. The reduction of the FEC might be due to direct effect of the CT by reducing of the worm fecundity, killing of adult worms, and indirectly by stimulating of the immune resistance against GIN via enhancement of the tissue protein supply [16]. Pathak et al. (2013) have reported that CT extracts from various tree leaves can disrupt the life cycle of *H. contortus* by preventing their eggs from hatching and by preventing larval development to the infective stage. The results were in agreement with those of [22, 25, 29], who reported that CT supplementation may be used as an alternative parasite management strategy.

Min et al. (2003) reported that, CT had ability to bind with the proteins of *H. contortus* cuticle and change their physical and chemical properties. This ability could be explained the electron microscopical changes which occurred to the *H. contortus* cuticle after contact with CT [7]. Also, massive lesions were reported in the worm digestive and reproductive tracts after exposure to CT [15]. The FAMACHA results showed improvement towards CT containing diets, that the CT containing diet lowered the FAMACHA score this could be due to the effect of the CT on the *H. contortus* (results of worm counts) as well as this result may be due to indirect effect of the CT via enhancing of the immune functions.

Regarding to the results of the carcass quality measures, the current work possess no significant differences among different diet treatments. These results were in disagreement with [12], as they reported differences in cold carcass, breast, sirloin, trim trait, liver, and hide weight which tended to increase linearly in goats fed 15% and 30% PB.

Serum concentrations of alanine transaminase, aspartate aminotransferase, alkaline phosphatase, and cholesterol are conventionally used for diagnosing human and domestic animal hepatic damage, while alkaline phosphatase and cholesterol are also used to detect bile obstruction, mild and progressive liver damage [30]. The normal ranges for ALT, AST and ALP are 7-24 IU/L, 43-132 IU/L and 7-30 IU/L [4]. The blood metabolites results in this paper fall within the normal ranges of goats thus evidenced that no liver damage occurred when the goats fed on tannin rich diets. These results agreed with those of [17]. These results may be due to goats had high concentration of the salivary proline secreted from their parotid salivary gland [6]. This salivary proline have had high binding ability to tannins thus inhibits their bad effects upon protein digestibility [9]. This could be explained the reason for the lack of tannin systemic toxicity in goats when fed on tannins rich diets [17]. However, pine bark feeding diet could be improved health condition in goats infected with *H. contortus*, this may evident by decreasing of the cholesterol and triglyceride values in the serum of such cases.

Conclusion

Condensed tannins of the PB, SLP and/or their combination might be used as socioeconomic and eco-friendly anthelmintic against drug resistant *H. contortus* UGA/2004 in meat goats. Supplementation of meat goat diets with 30% CT of PB, SLP could be used as an effective combating tool against drug-resistant *H. contortus* with no adverse health hazards. Rations containing CT could reduce FAMACHA scores, adult worm counts, and FEC over the time in *H. contortus* infected goats. Furthermore, the combination between PB and SLP condensed tannins enhanced their actions on FAMACHA scores and adult worm counts. Further researches are needed to understand the combination effects.

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