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The Effect of Tannin-Containing Peanut Skin Supplementation on Drug-resistant *Haemonchus contortus* Control in Meat Goat

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BRM and AS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors WM and NG managed the analyses of the study. Author JWH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was to determine whether the phytochemical CT-containing peanut skin would have effects on animal performance and drug-resistant *Haemonchus contortus* control in meat goats or not. Twenty two Kiko-crossbreed intact male goats (*Capra hircus*) were randomly assigned to three treatments (n = 6): 1) control (no-peanut skin), 2) 25 % peanut skin and 3) 50% peanut skin (PS). The total mixed ration (TMR) associated with peanut skin was applied included soybean meal, alfalfa pellet, ground corn, vitamins and minerals mix. Animals were confined indoors for a period of 50 days. Animals were dewormed on day -10 and all the goats were artificially inoculated on day 0 with 5000 infective stage (L3) drug-resistant *H. contortus* larvae. Dry matter intake (DMI) and average daily gain (ADG) were monitored. Blood samples were taken at the end of the experiment. Fecal egg counts (FEC) and FAMACH scores were determined approximately every 2 week. All analyses were conducted using a GLM procedure of SAS with linear and quadratic effects. The

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results indicated that animals on 25% peanut skin tended to be grow 31% faster ADG (quadratic; P=0.10) when compared to control or 50% PS supplementation group. Average DMI were similar among treatments (P>0.05), but there were treatment by time of day interactions (P<0.01). Goats receiving PS supplementation had linearly decreased (P<0.05) FEC, with maximum responses in 25% PS supplementation. Blood plasma metabolites were not different among treatments, but Ca (*P*<0.01), Cl (*P*=0.08), urea-N (*P*=0.07), and Alkaline phosphatase (*P*=0.09) tended to be higher or were higher for the PS diet than for control. Results from the current study indicated that animals consuming PS supplementation has the potential to improve ADG and might have the ability to reduce FEC at the moderate level of CT-containing PS supplementation (up to 25%).

Keywords: Peanut skin; Haemonchus contortus; goats; condensed tannins.

1. INTRODUCTION

Most studies investigating the effects of bioactive plants against gastrointestinal nematodes have focused on grazing sheep and goats, including grazing on forage legumes (sainfoin (*Onobrychis viciifolia*), sulla (*Hedysarum coronarium*), birdsfoot trefoil (*Lotus corniculatus*), and sericea lespedeza (*Lespedeza cuneata*)), herbs (chicory; *Cichorium intybus*) and some fodder trees [1,2]. There appears to be no data on the drug resistant *Haemonchus contortus* parasites and on this subject in small ruminants fed condensed tannin-containing peanut skin on drug-resistant *H. contortus* in the literature. At present, most research on bioactive forages can still be considered to be in its early stages, as it is largely focused on the screening and identification of plants that have demonstrable activity against nematodes.

The most significant internal parasite to small ruminants in the Southern United States is the *H. contortus,* also known as the barber pole worm (round worm) [3]. *Haemonchus contortus* is a blood sucking parasite that can cause severe anemia, protein loss and death in goats and sheep, and thus is the most important to control [3]. Moreover, many parasitic helminthes of veterinary importance have genetic features that favor development of anthelmintic resistance, this becoming a major worldwide constrain in livestock production [3,4]. The development of anthelmintic resistance poses a large risk to future production and well-being of grazing sheep [5] and goats [3]. Development of variable degrees of resistance among different species of gastrointestinal nematodes has been reported for all the major groups of anthelmintic drugs [6].

The feeding value of peanut skin (PS) has been evaluated for dairy [7], beef cattle [8], and goats [9] as a supplementary feedstuff. Approximately 20,000 to 30,000 metric tons of PS are produced in the U.S. annually and most of it is used as animal feed [10]. The typical nutrient contents of PS are 17% protein, 25% fat, and 65% total digestible nutrients (TDN). Because of high levels of tannins (up to 21%), the proteins are poorly digested and its energy value is low despite high fat content. That is why its inclusion rates are low in cattle. However, goats have been shown to be especially adept at handling moderately high levels of tannins in their diet [11]. However, producers have become very conscious about animal health and well-being; hence, it is important to determine the anthelmintic potential when feeding PS before recommending them as a suitable supplemental feed for meat goats. Studies using goats relating to the use of tannin-containing PS on drug resistant *H. controtus* control are limited.

2. MATERIALS AND METHODS

This experiment was conducted at Tuskegee University's Small Ruminant Research and Education Unit over 50-day period.

2.1 Experimental Design and Feed

Tuskegee University Animal Care and Use Committee approved animal care, handling and sampling procedures for this experiment. Twenty-two Kiko-crossbreed intact male goat yearlings (*Capra hircus*; average body weight (BW) =46.9±2.0 kg) were randomly assigned to 3 treatments (n = 6) to perform a study on condensed tannin (CT) containing peanut skin and its effects on drug-resistant *Haemonchus contortus* (*H. contortus*), fecal egg counts (FEC), packed cell volume (PCV), Faffa Malan Chart (FAMACHA) score, and body weight changes. Animals were randomly assigned to 3 treatments (n = 6): 1) control (no-peanut skin), 2) 25% peanut skin and 3) 50% peanut skin. Animals were confined indoors for a period of 50 days at the Caprine Research Unit located on the

Tuskegee University Research farm, Alabama State, USA during 2016. Each male goat was placed separately in pens.

Experimental goats for this experiment were strategically dewormed on day -10 with 5 mL/100 lbs BW of Cydectin® (Moxidectin, Fort Dodge Animal Health, Fort Dodge, IA; 5 mg/mL) followed by 3 mL/100 lbs BW of Valbazin (500 mL, Pfizer Animal Health) under supervision of a veterinarian to reduce or eliminate gastrointestinal parasites. All the goats were artificially inoculated with 5000 infective stage (L_3) drug-resistant *H. contortus* larvae. The drug resistant larvae were kindly donated from Dr. Kaplan Lab. at the Department of Infectious Disease, College of Veterinary Medicine, University of Georgia, Athens, GA. The goats were individually housed in 1.1 m x 1.2 m pens with plastic-coated expanded mesh floors to allow simple passage of feces and urine to minimize parasite re-infestation. Feed intake and performance were monitored for 50 days. Animal weight and fecal samples were collected on day 0 to determine the baseline weight and fecal egg count. A total mixed ration (TMR) associated with peanut skin (PS) and 200 g Bermuda grass hay supplementation a day was applied and included soybean meal, alfalfa pellet, ground corn, vitamins and minerals mix. Each male goat was fed once a day.

2.2 Sample Collection and Laboratory Analysis

Once the experiment began, samplings of fecal and weights were collected every 2 weeks on day 0, 14, 28, and 42. The FEC and FAMACHA status were collected on day 0 and 50, but Blood samples were collected at the end of trial. The fecal egg counts (FEC) were determined using a modified McMaster technique [11] using a McMaster slide with two chambers (McMaster Counting Slides – Green Grid by Chalex Corporation). Feed samples were composited for each treatment and analyzed for DM, crude protein (CP), fat in the completely mixed ration, and total digestible nutrients (TDN) according to the methods described by Dairy One Inc. (730 Warren Rd. Ithaca, NY, USA) in the Forage Testing Laboratory. Analytical DM concentration of dietary samples was determined by oven drying at 105°C for 3 h [12], and organic matter (OM) was determined by ashing at 550ºC for 5 h [12]. Ash, ether extract (crude fat), total digestible nutrient (TDN), and minerals were analyzed according to the methods described by AOAC [12]. Concentration of N was determined using an organic elemental analyzer (Flash 2000; CE Elantech Inc., Lakewood, NJ, USA; AOAC, [12]). Concentrations of NDF and ADF were sequentially determined using an ANKOM200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the methodology supplied by the company, which is based on the methods described by Van Soest and Robertson [13]. Sodium sulfite was used in the procedure for NDF determination and pre-treated with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO, USA). Ether extract was measured [12] using a fat analyzer (XT20, ANKOM Technology).

2.3 Data Processing

All analyses were conducted using a GLM procedure of SAS with linear and quadratic effects [14]. Linear and quadratic effects were determined utilizing polynominal orthogonal contrasts for equally space treatments. Animals were the experimental unit and treated as a random effect. Body measurements and dry matter intake (DMI) were analyzed as a repeated measure design with treatment, time of day, and treatment x time of day interactions. Mean separation was performed using least significant differences at probability level $P<0.05$, and trends were accepted if $0.05 < P < 0.10$.

3. RESULTS AND DISCUSSION

3.1 Diet Composition and Condensed Tannins

The peanut skin and diet chemical nutrient characteristics are shown in Tables 1 and 2, respectively. The peanut skin (PS) used in the current study (Table 1) was 22.7% crude protein (CP), 32.66% neutral detergent fiber (NDF), and 87.77% total digestible nutrient (TDN). The ether extract (EE) content (crude fat) of PS was 19.05%, which were lower values than the published values that ranged from 24% to 30% [8,15]. However, the CP value was 22.7%, which was higher than the values reported by other researchers. For example, an average value of 15.5% crude protein was reported by Utley et al. [7]. Similarly the TDN value was much higher 87% compared to an average of 65% reported by Hill [8]. The discrepancy in nutritional values reported by other researchers may have resulted due to different peanut blanching processes used by different processing plants and the different methods of TDN calculation by different labs. However, nutritional values are generally consistent within a particular blanching plant. Similarly, in the diet (Table 2), the CP content in the 0, 25, and 50% PS mixed TMR diets were 22.6, 5.7, and 11.3%, and TDN value was 87.6, 21.9, and 43.8%, respectively. Final CT concentration in the 0, 25, and 50% PS containing diet were 0.15, 3.9, and 7.8% CT/DM (Table 2). As expected, the different amounts of PS in the diets resulted in different concentrations of CP, TDN, and CT percent (calculated) in the diets. Total condensed tannins (CT) in the diet as % DM basis were 0.15, 3.9, and 7.8% CT DM for the 0, 25, and 50% PS diets, respectively.

3.2 Intake and Growth Performance

Growth performance and dry matter intake (DMI) data are summarized in Figure 1 and Table 3. As a result of Fig. 1 and Table 3, average DMI in control (0%), 25%, and 50% PS supplementation were similar among treatments (P > 0.05; 1.3, 1.6, and 1.3 kg/d DMI, respectively), but there were treatment by time of day interactions (P<0.01), with highest during days 29 and 30 for 25% PS treatment compared to control and 50% PS supplemented group. In the present study showed that daily DMI increased as tannin containing PS supplementation increased up to 25% PS in the diets. Similarly, Solaiman et al. [16] and Min et al. [11] reported that total DMI of growing goats increased when tannin-containing diets (sericea lespedeza or pine bark) replaced alfalfa meal in the grain mixes, and Turner et al. [17] reported that goats receiving the CT-containing sericea lespedeza (*Lespedeza cuneate*) hay (23.1 mg CT/g soluble protein) had greater DMI than those fed the alfalfa hay–based diet. Puchala et al. [18] also demonstrated increased DMI in Angora does fed CT-containing Sericea lespedeza (17% CT in DM) compared with a mixture of crabgrass (*Digitaria ischaemum*) and tall fescue (*Festuca arundinacea*). Some animal species have developed adaptive mechanisms to counteract the antinutritional effects of tannins. This may be attributed to the fact that goats naturally prefer browse that contains bioactive secondary compounds, including tannins and other alkaloids. In addition, animals previously exposed to plant secondary compounds eat much more feeds containing secondary compounds than inexperienced animals [19].

There was no difference in initial BW and final BW of goats among treatments (Table 3); however, ADG (quadratic; P = 0.08) and gain efficiency (gain: feed ratio; Linear and quadratic; P < 0.05) of goats were the highest for goats on the 25% PS diets than the control and 50% PS. The presence of optimum CT in the diet can reduce protein degradation in the rumen and improve bypass protein flow to the small intestine [1], thus enabling more hydrolysis of dietary protein in the lower tract [20]. Previous studies have shown that the supplementation of CT in ruminant animal diets has both beneficial and adverse effects on animal performance depending on the concentration and chemical structure of CT [1,21]. Min et al. [1] reported that beneficial effects of CT in the diet on sheep performance may occur in the range of 2% to 4% CT of diet DM. However, the wool growth response was negative when the CT concentration was above 5% CT of diet DM. Conversely, when CT concentration decreased below 2% of diet DM, the production response was variable. This may partially explain why the growth performance of goats in the present study was improved for those goats receiving diets containing 25% PS (3.9% CT DM; Table 3) compared with control diet (0.15% DM CT) or 50% PS diet (7.8% CT DM). It must be noted that higher gains in the present experiment for animals fed the 25% PS diet, similar to reported by Solaiman [15], were in part due to the higher dietary crude protein fed to goats and more optimum PS or CT (3.9%CT DM) levels in the diet when compared with 75% sericea lespedeza diet reported by Moore et al. [22] and Min et al. [1].

3.3 Fecal Egg Count

Table 3 displays the FAMACHA score and mean number of FEC of drug resistant *H. contortus*. There was no significant among groups on FAMACHA score and FEC on day 0, but FEC was quadratic (P<0.05) decreased in goats fed 25% tannin containing PS diet compared to control and 50% PS supplementation. Average FEC (an indication of parasite load) was reduced (qudratic; P < 0.05) by 53% with 25% PS inclusion in the diet (Table 4). Results from the current study indicated that animals consuming CT containing PS might have the ability to reduce FEC or inhibit the fecundity of the resistant worm parasite such as *H. corntotus*. In more detail, these were resistant worms that

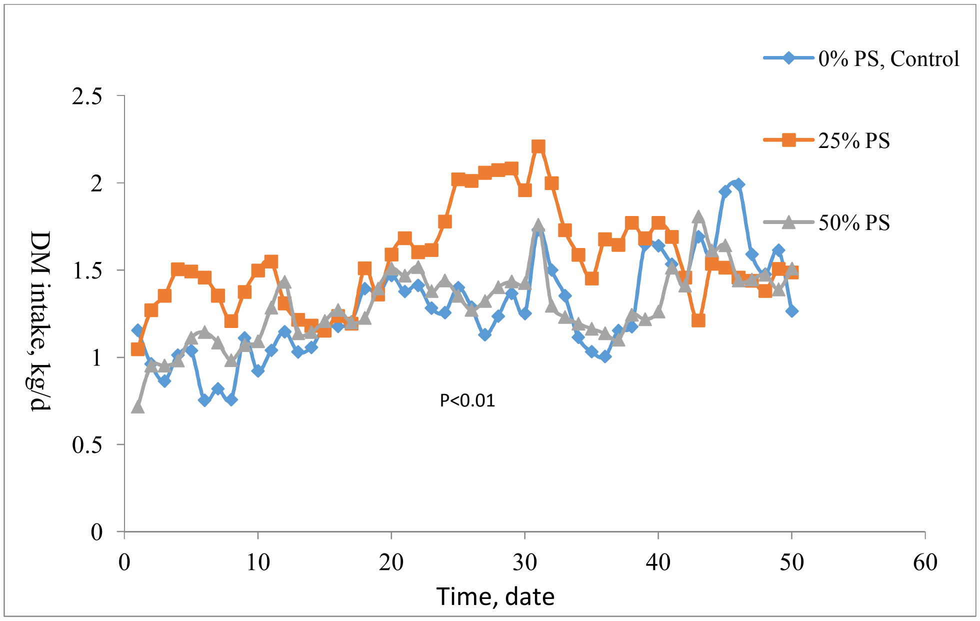

Fig. 1. The effect of peanut skin (PS) supplementation on daily dry matter (DM) intake (DMI) in meat goats

Table 1. Nutrient composition of peanut skins (PS) and Bermuda grass hay (BGH)

Item	Units	PS	BGH
DM	%	89.6	92.3
CP	%	20.0	10.1
ADF	%	21.8	36.8
NDF	%	39.7	68.2
NFC	%	12.5	15.1
TDN	%	91.0	58.0
Condensed tannins	%	15.6	0.2
Minerals			
Calcium	%	0.37	0.35
Phosphorus	%	0.14	0.21
Magnesium	%	0.17	0.15
Potassium	%	0.50	1.48
Sulfur	%	0.18	0.17
Sodium	%	0.025	0.24
Copper	Ppm	73	-
Manganese	Ppm	39	-
Zn	Ppm	51	-
Iron	Ppm	203	-

PS=peanut skin; DM=dry matter; CP=Crude Protein; ADF=Acid detergent fiber; NDF=Neutral detergent fiber; NFC=Non-fibrous carbohydrate; TDN=Total digestible nutrients.

remained after repeated deworming that were eliminated by feeding PS diets. Alternative parasite control strategies have recently been suggested on the basis of using CT-containing forages [2,23]. Shaik et al. [23] reported that there was a direct effect of tannin-containing S. lespedeza hay (3.6% DM extractable CT) on adult worms, with significantly fewer numbers of both abomasal (*Haemonchus contortus*, *Teladorsagia circumcincta*) and small-intestinal (*Trichostrongylus colubriformis*) nematodes compared with goats fed Bermuda grass hay (0% CT). The present study strongly supports this view, showing that goats consuming a CT-containing PS diet had a reduced FEC of the resistant worm compared with those receiving

CT-free diets, which may have impacted the gain efficiency and ADG of our growing goats at the range of 25% PS supplementation.

3.4 Blood Parameters

Blood parameters and mean pack cell volume is shown in Table 5. In the blood mean packed cell volume (PCV), cholesterol, CK, amylase, TP, glucose, P, Na, K, triglyceride, GGT, and AST were not significance. However, Albumin and DBIL (quadratic; $P < 0.05$), creatinine (CA; linear; $P < 0.01$) were significantly different compared to control. ATP (P=0.09), BUN (P=0.07), and CL ($P = 0.08$) tended to increased (Linear) with increasing tannin-containing PS diets; however, all values were within the normal range for goats, suggesting that no liver damage occurred. Serum levels of AST, gamma glutamyl transpeptidase (GGT), ALP and cholesterol are those conventionally used for diagnosing human and domestic animal hepatic damage [24]. The

Table 2. Chemical composition of experimental diets containing peanut skin (PS)

Items	Treatment group (% as-feed basis)[1]			SEM
	0	25	50	
DM	89.0	89.1	89.1	1.55
CP	20.0	23.4	23.0	0.35
ADF	15.8	17.9	18.2	0.28
NDF	26.1	23.1	22.9	0.33
TDN	79.0	90.0	80.0	0.55
CT	0.15	3.9	7.8	0.045

[1]Control 0%, 25%, and 50% peanut skin (PS), Mixed on an as-fed basis. Ingredients were incorporated in the corn, soybean meal, alfalfa pellet, ground corn, vitamins and minerals mixed.
DM= dry matter; CP=Crude Protein; ADF=Acid detergent fiber; NDF=Neutral detergent fiber; TDN=Total digestible nutrients; CT=condensed tannins; SEM= standard errors of the means.

Table 3. The effect of peanut skin (PS) supplementation on animal performance in meat goats

Items	Treatment group (% as-fed basis)			SEM	Linear	Quadratic[2]
	0	25	50			
BW, kg						
D 0	24.3	20.9	19.1	3.93	0.36	0.88
D 25	25.3	26.6	24.2	3.57	0.81	0.67
D 50	29.1	27.9	24.6	3.80	0.44	0.82
ADG, g/d	90.0	140.0	110	3.67	0.42	0.08
DMI, kg/d	2.1	2.6	2.2	0.12	0.96	0.34
Gain:feed	0.75	0.89	0.85	0.009	0.04	0.05

BW= body weight; d= day; ADG = average daily gain; DMI= average dry matter intake; d= day
[2]Polynomial contrasts for equally spaced treatments.

Table 4. The effect of peanut skin (PS) supplementation on gastrointestinal fecal egg count (FEC) and FAMACHA score in meat goats

Items	Treatment group			SEM	Linear	Qudratic
	0	25	50			
FEC						
D 0	460.9	170.1	330.0	120.45	0.50	0.45
D 50	1970.5	920.7	1570.7	360.75	0.44	0.05
FAMACHA						
D 0	3.3	2.6	2.6	0.416	0.23	0.26
D 50	1.6	2.0	2.1	0.287	0.21	0.68

FEC=fecal egg counts.
[2]Polynomial contrasts for equally spaced treatments.

Table 5. The effect of peanut skin (PS) supplementation (% as-fed basis) on plasma chemical nutrients content in meat goats

Items	Treatment group[1]			SEM	Linear	Qudratic[2]
	0	25	50			
Cholesterol	79.3	84.3	79.2	8.55	0.99	0.69
CK	218.8	208.3	194.4	21.27	0.41	0.79
Amylase	55.8	60.8	53	10.49	0.75	0.61
ATP	142.5	214	660.3	215.7	0.09	0.54
TP	7.3	7.6	7.6	0.29	0.53	0.73
GLU	54.3	57.1	59.3	3.79	0.37	0.62
PHOS	6.7	6.8	5.3	0.65	0.16	0.86
TBILI	0.27	0.35	0.25	0.03	0.64	0.144
BUN	25.1	28	30.1	1.94	0.07	0.87
CREAT	0.79	0.73	0.76	0.056	0.89	0.45
CO2	25.1	24.9	23	0.98	0.12	0.50
NA	149.3	164.8	167.6	7.75	0.10	0.17
K	6.79	7.54	7.42	0.47	0.384	0.45
Cl	114.3	128.6	131.3	6.68	0.08	0.45
Ca	9.3	9.9	10.3	0.29	0.01	0.78
ALB	2.6	3.1	2.9	0.12	0.15	0.04
TRG	16.3	20.1	19.1	1.79	0.14	0.68
GGT	33	27.5	36.7	4.94	0.19	0.45
AST	75.8	78.7	68.7	5.46	0.35	0.19
DBILI	0.21	0.34	0.24	0.05	0.74	0.008
PCV	35.2	36.1	35.2	1.96	0.98	0.71

CK=creatinine kinase (IU/L), ATP=adenosine triphosphate, TP=total phosphate, GLU=glucose, PHOS=phosphate, TBILLI=total bilirubin (mg/dl), BUN=blood urea nitrogen, CA=creatinine, ALB=albumin, TRG=triglyceride, GGT=gamma-glutamyl transferase, AST=aspartate aminotransferase, DBILI= Bilirubin, PCV=packed cell volume, Ace carbon dioxide (CO_2-LC) reagent. SEM=standard errors of the means.
[1]Control (0% PS), 25% PB, and 50% PS on as-fed basis. Number of animals was 6, 6 and 6 in control, 25% PS, and 50% PS diets, respectively.
[2]Polynomial contrasts for equally spaced treatments.

plasma data obtained from the present study showed that the up to 25% PS supplementation is safe and beneficial, and not detrimental, because tannins at moderate levels (3.9% CT DM) are beneficial as they impact some qualities of rumen non-degradable protein, thus improving protein availability and utilization.

Albumin is one of the major proteins synthesized in the liver and is the most abundant protein in the plasma of healthy individuals. Plasma albumin performs important metabolic functions by regulating colloid osmotic pressure and transporting free fatty acids, bilirubin and many drugs [25,26]. Plasma albumin is one of the most common parameters used in evaluating nutritional status. In particular, the protein content and quality of a meal influence the rate of albumin synthesis [27,28]. To the best of our knowledge, the present results reported evidence that the plasma albumin changes in meat goats caused by moderate level of (25% PS) CT-containing PS diet. This is similar to other study [29] has been showed that goats received diet containing CT-containing diet tended to increased plasma albumin content. However, the mechanism by which such an effect of PS on animal reproductive per performance might occur and its potential side effects were not investigated in the present study.

4. CONCLUSION

This study has highlighted that CT-containing PS has the potential to increase ADG, daily DMI and FEC, with no adverse effect on animal health. Optimum level of peanut skin supplementation (25% DM) also reduced internal parasite infections and therefore warrants further investigation for its effects. Present results indicate that CT containing PS has potential as an energy supplement in feed for meat goats and may provide benefits as well as reducing drug resistant parasites infection. When compared with other CT plants used as feed PS contains a high content of energy (crude fat content; 27%

DM), CP (22.7), and TDN (87.7% DM). Ultimately, PS supplemented with other dietary ingredients (e.g. soybean meal, alfalfa meal etc.) showed an excellent results on meat goat and has the potential to reduce FEC and increase animal performance, especially during summer months where summer forages are not adequate for farmers seeking to have a viable product from meat goat production. However, small ruminants have been shown to be especially adept at handling moderately high levels of tannins in their diet. Thus, PS needs to be further researched as a potential low-cost feedstuff for ruminants.

DISCLAIMER

This manuscript (abstract only) partially published at the J. Anim. Sci. Proceedings [30].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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