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SUPPLEMENT TYPE INFLUENCED THE PERFORMANCE AND RESILIENCY AGAINST GASTROINTESTINAL PARASITES OF NURSING LAMBS RAISED IN WOODLANDS

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Abstract

Nutrition supplementation can be one of the integrated approaches to reduce gastrointestinal parasite (GIP) infestation. The study objective was to evaluate the impact of supplement type on the performance and resiliency against GIP of nursing lambs raised in woodlands. Katahdin-St. Croix cross lambs (23) with their mothers (18) were divided into two groups; they were rotationally stocked in separate sets of woodland plots and provided with *ad libitum* hay. Group-1 animals were supplemented with corn, and Group-2 with soybean (0.5% of live weight). Live weight, FAMACHA score, and body condition score were measured on Day 1, fortnightly, and at the end of the study. Fecal samples were analyzed for the type and quantity of GIP on Days 1, 45, and 80. Lambs supplemented with soybeans performed better than corn-supplemented lambs ($p<0.05$), indicating soybeans as a better supplement than corn for nursing lambs stocked in woodlands.
Keywords: Corn, FAMACHA score, Soybean, Woodland grazing

Introduction

Gastrointestinal (GI) parasite infestation is one of the significant threats to the health and production of sheep and goats in the Southeast USA (Miller, 2018). Among the GI parasites, *Haemonchus contortus* is a commonly occurring and highly pathogenic blood-sucking helminth (Dey et al., 2020). Anemia, weight loss, reduced milk production, suppressed immunity, and death are some consequences of GI parasitism (Miller et al., 2012). The impact of GI parasitism on the pathophysiology of animals varies depending on the host's immune response, which is usually weak in nursing animals. Because of low immunity, the susceptibility to *H. contortus* and other GI parasites is high in young, nursing lambs (Hoste et al., 2016). Young animals would be exposed to GI parasites from grazing contaminated pastures beginning when they start grazing at an early age. Supplementation of nursing ewes and lambs may help boost their immunity and enhance their resistance and resiliency against GI parasites, as nutrients play a major role in immune function. Therefore, the identification and implementation of supplementation tactics that enhance immunity, as revealed through high resistance and/or resilience against GI parasites, can be significant for promoting animals' health.

Many studies conducted in indoor pens on lambs, kids, ewes, and dairy goats reported that metabolizable protein supplementation improved hosts' resistance to GI nematodes, as indicated by a reduced number of parasite eggs in animals' feces, decreased worm burdens in the GI tract, and improved immune function (Coop & Kyriazakis, 1999; Houdijk & Athanasiadou, 2003; Houdijk, 2012). Likewise, young kids raised in woodlands with corn supplementation had a lower prevalence of *H. contortus* and *Moniezia* spp. compared to kids with supplemental grazing in silvopasture plots (Shrestha et al., 2022). However, the impact of feed supplementation against GI

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parasitism in nursing lambs raised in woodlands is scant. The objective of this study was to evaluate the influence of supplement type on the performance and resiliency against GI parasites of nursing lambs raised in woodlands.

Literature Review

Understory vegetation in woodlands has revealed a promising potential for complementing small ruminants' feed requirements during the warm-season portion of the grazing season (Bhattrai et al., 2021; Karki, 2017; Khatri, 2016; Paneru et al., 2019). Khatri (2016) reported young Kiko wethers eating 23 plant species out of 37 major species available in woodland plots. Similarly, Bhattrai et al. (2021) reported mature Katahdin rams consuming all plant species that were eaten by Kiko wethers while grazing in woodlands and showing satisfactory performance (FAMACHA score - 1 to 2.7 and body condition score - 2.8 to 4.4). In addition to grazing/browsing benefits, woodland grazing should minimize gastrointestinal (GI) parasite infestation if major plant species in the system contain condensed tannins (CT), which have anthelmintic properties. Min et al. (2005) reported that consumption of condensed tannins effectively suppressed gastrointestinal nematodes (GIN) infection in goats. Karki (2017) reported numerous woodland plant species containing a good amount of condensed tannins, e.g., wild plum (*Prunus* spp. L.) (5.7% CT) and winged elm (*Ulmus alata* Michx.) (7.7% CT). Additionally, animals browsing on woodland shrubs and trees lowers the chance of engulfing parasite larvae as a high density of larvae remains within 2-3 inches from the ground surface (Karki, 2017). However, the nutritional requirements of animals, such as when in late pregnancy, lactation, or growing stage, may not be fulfilled from woodland grazing (Shrestha et al., 2022; Ellis et al., 2021; Khatri, 2016). Deficient nutrition increases the susceptibility of young animals to GI parasites (Coop and Holmes, 1996).

GI parasitism is one of the most frequently reported health issues by sheep and goat producers in Alabama and other states of the Southeast (Karki, 2013). The most prevalent GI parasites in young ruminants are GIN, coccidia (*Eimeria* spp.), and tapeworms. Some predominant GIN in the Southeast pastures are *Trichostrongylus* spp., *H. contortus*, *Teladorsagia circumcincta*, *Ostertagia* spp., and *Oesophagostomum* spp. (Edmondson, 2013). GI parasitism causes appetite loss and low absorption of digested nutrients, resulting in devastating economic losses due to anemia, lack of weight gain, poor body condition scores, reduced productivity, morbidity, and mortality. Among all, the most pathogenic parasite to sheep producers is *H. contortus* (barber pole worm), a blood-sucking parasite that can cause the acute death of young grazing lambs (Sanders et al., 2020). Adult *H. contortus* can consume one-fifth of the total circulating erythrocyte volume daily in lambs and one-tenth of erythrocyte volume in a day in adult sheep (Bowdridge et al., 2013) and cause anemia very quickly. The evidence of anemia is a pale or white ocular mucous membrane, and it is used as an "on-farm" diagnostic technique with a FAMACHA card. The FAMACHA system is used to detect the anemic condition caused by *H. contortus* in small ruminants (Karki, 2017).

A FAMACHA card has five colors ranging from bright red to pale or white. When examining animals with a FAMACHA card, the color categories on the card are matched with the color of an animal's conjunctiva on the lower eyelids (both eyes). A score is given to the animal based on the matching color on the card. The bright red color indicates non-anemic animals, 4 and 5 stand for the pale or whitish color that indicates severely anemic animals, and 3 is conditional, indicating the treatment of such animals would depend on various conditions. Treatment of conditional animals is required under these conditions: 5-10% or more of the herd are anemic, animals have poor body condition score, and overall health condition is concerning. FAMACHA score can be improved by enhancing anaimal nutrition through either supplementation or pasture improvement. Tiwari et al. (2021) reported a better FAMACHA score in ewes grazing legume-grass mixed pastures compared to ewes grazing sole-grass pastures. In addition to FAMACHA score, body condition score (BCS) and live weight are other important parameters for performance measurement in growing lambs. Both BCS and live weight of animals are affected by inadequate nutrition and GI parasitism (Hoste et al., 2016). Ramos et al. (2019) showed higher BCS (18%) and live weight (27%) in concentrate-supplemented lambs compared to lambs grazing native pastures. Suppressed immune responses were reported in GIN-infected sheep compared to non-infected Blackbelly sheep (González-Garduño et al., 2018). Torres Acosta et al. (2004) found higher daily weight gain (123%) in soybean-sorghum supplemented kids compared to non-supplemented kids when both groups were GIN-infected and raised in native vegetation.

The provision of adequate nutrients through supplementation can be one of the strategies for improving immunity and minimizing the impact of GI parasites in vulnerable animals. Animals suffering from GI parasites would have increased nutritional demands to improve immune response. At times when animals receive limited nutrients, there would be competition between the immune system and other bodily functions for the available nutrients with the resulting possibilities of unchecked establishment, fecundity, and survival of GIN within the host and low performance (Coop & Kyriazakis, 1999). Liveweight gain and the antibody titer against GIN antigens are negatively correlated, indicating the competition of immunity with liveweight gain for limited nutrients (Hoste et al., 2016). Previous studies have shown a positive impact of increased nutrients on the resiliency of young lambs and kids against GIN. A high-energy diet (1.4 Mcal/lb) fed to Pelibuey lambs infected with *H. contortus* decreased the parasite eggs per gram (EPG) of animal feces (59%) and increased liveweight (17%) as compared to lambs fed a low-energy diet (1.2 Mcal/lb) (López-Leyva et al., 2020). Likewise, Shrestha et al. (2022) reported a reduced prevalence of *H. contortus* and *Moniezia* spp. in nursing Kiko kids raised in woodlands with supplemental hay and corn compared to kids with supplemental grazing in silvopasture plots. However, the prevalence of GI parasites in nursing lambs raised in woodlands and the impacts of supplement type against GI parasitism in these lambs is limited.

The study hypothesized that the performance and resiliency against GI parasites of nursing lambs raised in woodlands would be the same, irrespective of the supplement type. The study objective was to evaluate the impact of supplement type on the performance and resiliency against GI parasites of nursing lambs raised in woodlands.

Methods

Study Site

The study site consisted of six woodland plots (1.1 acres/plot) located at Atkins Agroforestry Research and Demonstration site (Latitude 32°26’35.7” N, Longitude 85°43’56.5” W), Tuskegee University, Tuskegee, Alabama, USA. The Agroforestry site contained Uchee loamy sand (Loamy) (78.4 %) and Cowarts loamy sand (21.6 %) with a slope of 1 to 15 % (USDA-NRCS, 2022). Weather data (Table 1) were downloaded from https://www.wunderground.com/history/monthly/us/al/montgomery/KMGM/date/2022-5, recorded at the Montgomery regional airport station for Tuskegee Institute, Alabama. This station is located approximately 45 miles west of the study site.

The study site had a good perimeter fence, all plots were cross-fenced, and gates were installed in each plot to move animals in and out for rotational grazing. Additionally, sites were well equipped with grazing facilities for small ruminants (mobile shelters, water lines and troughs, and mineral feeders). Each woodland plot consisted of 17-year-old mixed southern pines (longleaf (*Pinus palustris* Mill.) and loblolly (*Pinus taeda* L.)) and various understory plant species.

Vegetation Quality

Ten random samples per plot, each within a 10.8 ft^2 quadrat up to a height of 6 ft., were collected before bringing animals into each plot. The subsequent set of samples were collected one day before each rotational stocking to each plot throughout the grazing period. Collected samples were dried for 72 hours at 140°F in a hot air oven in the Agroforestry and Grazing Land Laboratory (AG Lab), Tuskegee University. Dried samples were ground and analyzed for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) using Near-Infrared Spectroscopy (NIRS) in the AG Lab of Tuskegee University. Total digestible nutrient (TDN) was calculated from ADF values (TDN%= 82.38-(0.7515*ADF%)).

Supplement Quality

The quality of whole corn (TDN- 88%, CP- 9%) and whole soybean (TDN- 93%, CP- 40%) used for the study was assumed to be similar to that reported by the National Research Council [NRC] (2007). Samples of hay used to feed animals were collected from each square bale used during the study and followed the procedure as described for vegetation above to determine the quality.

Research Animals

Twenty-three Katahdin-St. Croix cross lambs (1-2 months) along with their mothers (18, 44-45 months) were used in the study. At the beginning of the study, animals were weighed and assessed for FAMACHA score and body condition score. Then, animals were divided into two identical groups with uniform performance values based on the initial data. Each group was allocated to separate sets of plots and rotationally grazed in those plots throughout the study. Animals were moved to new plots when 50% of foliage was consumed in the plots where they were stocked. Both groups were always provided ad libitum hay and loose mineral mix (Purina sheep mineral). Group-1 animals (9 nursing ewes and 12 lambs) were supplemented with whole corn (0.5% of live weight (LW), and Group-2 animals (9 nursing ewes and 11 lambs) were supplemented with whole soybean (0.5% of LW). Each plot had mobile shelters (2), mineral feeders, and watering systems. Animals were monitored and cared for properly twice a day, morning, and evening, and needed supplies added (minerals, water, hay) as per the Animal Care and Use Committee of Tuskegee University protocol.

Animal Performance

Animal performance data (live weight, FAMACHA score, and BCS) were taken on the first day of the study, fortnightly during the study, and on the last day of the study. Live weight was measured using a digital weighing scale installed at the handling facility located at the study site. The BCS was evaluated by feeling the muscles and fat tissues over the backbone, ribs, and brisket bone, and scores were provided ranging from 1 to 5. Scores 1 and 5 indicate, respectively, extremely thin animals and obese animals. The FAMACHA score was assessed by using the FAMACHA card and following the guidelines for the FAMACHA system. The color of animals’ conjunctiva on both eyes was compared with the color on the FAMACHA card and scored with the most matching color. Both FAMACHA score and BCS were assessed by a single trained person (the first author of this article) throughout the study period to avoid any potential bias that would have occurred if multiple people were involved in such evaluations.

Fecal Sample Collection and Analysis

Fecal samples were collected on Days 1 and 45, and at the end of the study. Fresh feces were collected directly from the rectum and kept individually in airtight ziploc bags. Fecal samples were kept in a refrigerator (37˚F - 41˚F) until analyzed on the same day of collection for the type and quantity of GI parasites by using the McMaster technique (Storey, 2013).

Animal Treatment

Lambs having 500 or more parasite eggs per gram of feces were treated with appropriate anthelmintic for the type of GI parasite (Albendazole, Moxidectin, Amprolium) (Pugh et al., 2021).

Data Analysis

All data sets were analyzed in SAS 9.4 with 95% confidence level for rejecting the null hypothesis ($\alpha = 0.05$). Quality analysis (CP, ADF, NDF, and TDN) of woodland vegetation and hay was done by MEANS Procedure. Animal performance data (live weight, FAMACHA score, and BCS) were analyzed by using the Generalized Linear Model (GLM) procedure with Multivariate Analysis of Variance (MANOVA) option. The model used for the analysis of animal performance is given below.

$$Y_{1ij}Y_{2ij}Y_{3ij} = \mu + \alpha_i + (\alpha\beta)_{ij} + e_{ij}$$

MANOVA h = Animal group and interaction between animal group and observation date
Repeated factor = Individual animal

Where $Y_{1ij}Y_{2ij}Y_{3ij}$ = performance variable of animals from i^{th} group and j^{th} observation date, μ = mean, α_i = group effect, $(\alpha\beta)_{ij}$ = interaction of i^{th} group and j^{th} observation date, e_{ij} = error associated with the i^{th} group and j^{th} observation date.

The EPG data were analyzed by using the Wilcoxon two-sample test because the data were not normally distributed. Prevalence and odd ratio of GI parasites were calculated in Microsoft Excel®.

Results and Discussion

Weather Data

The average humidity (73.27%) and temperature (81.19 ˚F) (1) showed the humid and subtropical weather for the study site. In Alabama, the climate is humid and subtropical (>64˚F) which means hot summers and mild winters (Climate-Alabama, n.d.).

Vegetation and Hay Quality

The nutrients in the woodland vegetation and hay are presented in Table 2. Both woodland vegetation and hay showed similar CP content of around 12%; however, hay had much higher (26%) TDN content compared to woodland vegetation. The quality of vegetation in the woodlands

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was similar to the results reported by Shrestha (2022). In the study, the author reported that woodland vegetation had 11% CP and 51% TDN.

Table 1. Weather data, May-August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

Weather parameters	Daily average	Minimum	Maximum
	LSMean ± SE		
Rainfall (inches)	0.14 ± 0.047	0	2.59
Temperature (˚F)	81.19 ± 0.391	61	102
Humidity (%)	73.27 ± 0.860	0	100
Wind (mph)	4.79 ± 0.199	0	43

Table 2. Quality of the Woodland Vegetation and Hay used for Supplementing Lambs, May-August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

	CP[†] (%)	ADF (%)	NDF (%)	TDN (%)
	LSMean ± SE			
Woodland vegetation	11.9 ± 0.19	41.3 ± 0.31	50.6 ± 0.27	51.3 ± 0.24
Hay	11.9 ± 0.09	32.3 ± 0.22	70.6 ± 0.24	64.8 ± 0.23

[†]CP- Crude protein; ADF- Acid detergent fiber; NDF- Neutral detergent fiber; TDN- Total digestible nutrient

Performance of Lambs

Lambs supplemented with soybeans were heavier (22%) (Figure 1) ($p<0.0001$) and had better BCS (13%) (Figure 3) ($p<0.0001$) and FAMACHA score (8%) (Figure 2) ($p<0.05$) compared to corn-supplemented lambs. Soybean-supplemented lambs had a gain rate of 5.8 oz./day vs. 5.1 oz./day for corn-supplemented lambs. The better performance of lambs with soybean supplements than those with corn supplements could be due to a higher crude protein content in soybeans (40% CP) than in corn (9% CP). Because of these results, the study hypothesis that lambs stocked in woodlands would perform equally well, irrespective of supplement type, was rejected. The greater live weight, daily gain rate, and BCS of soybean-supplemented lambs agree with the study by Ramos et al. (2019). These authors found greater body weight gain (5%) and BCS (18%) in castrated Merino Dohne-Corriedale-cross lambs on a high-protein (20% CP) vs. low-protein (12% CP) diet. Similarly, a better FAMACHA score in lambs supplemented with soybeans when stocked in woodlands in the current study is found to be consistent with the findings of Tiwari et al. (2021), who reported a better FAMACHA score for Kiko does grazing legume-grass (33% higher CP) vs. does grazing sole-grass pastures. Moreover, young lambs performed better when supplemented with soybean meal (20% CP) than lambs fed a diet containing 10% CP with equivalent energy provided to both groups (Singh et al., 2015).

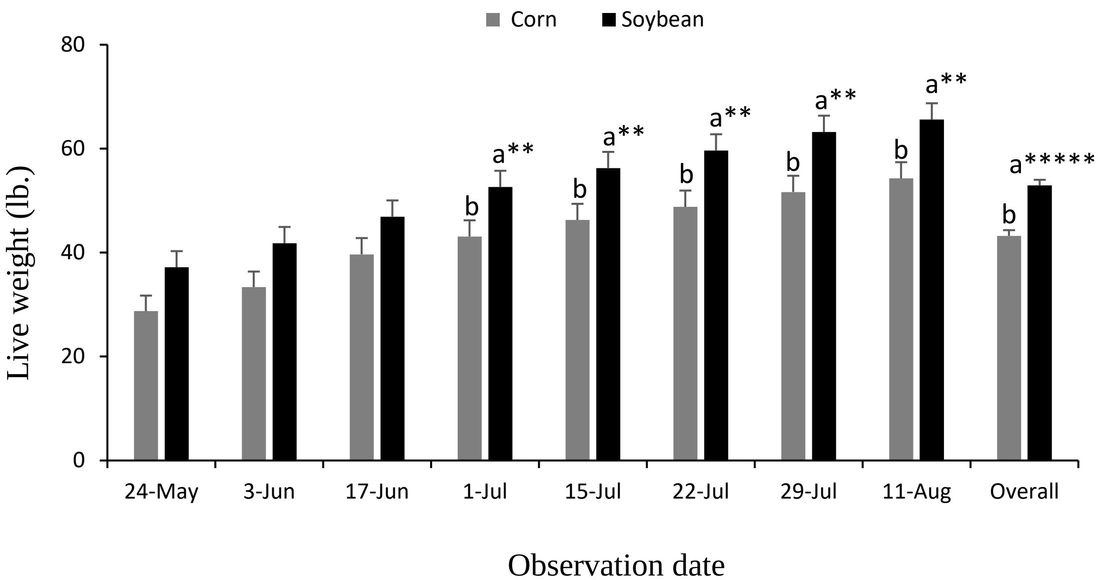

Figure 1. Live Weight of Katahdin-St. Croix Cross Lambs Supplemented with Corn or Soybean in Woodlands, May-August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (**p<0.05, *****p<0.0001).

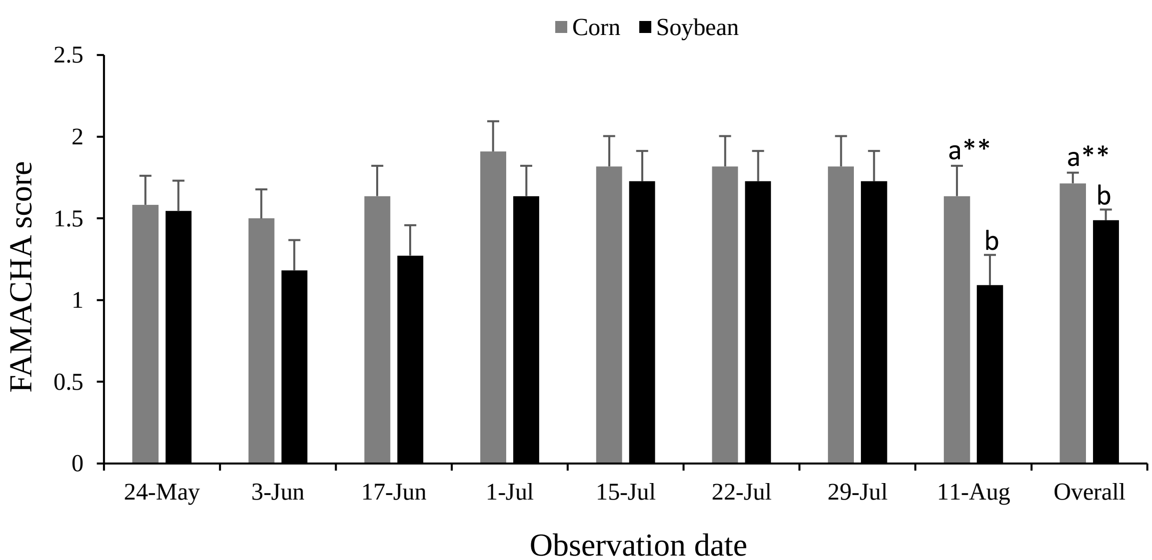

Figure 2. FAMACHA Score of Katahdin-St. Croix Cross Lambs Supplemented with Corn or Soybean in Woodlands, May-August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (**p<0.05).

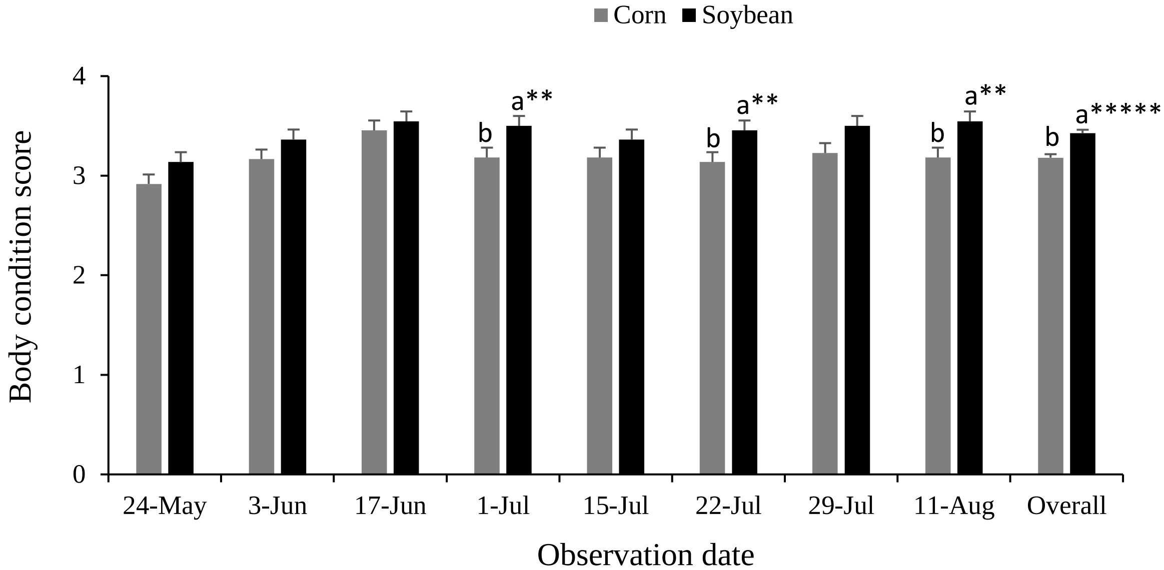

Figure 3. Body Condition Score of Katahdin-St. Croix Cross Lambs Supplemented with Corn or Soybean in Woodlands, May-August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (**p<0.05, *****p<0.0001).

Prevalence of GI Parasites in Lambs

The major GI parasites found in corn- and soybean-supplemented lambs were *H. contortus*, coccidia and *Moniezia* spp. when raised in woodlands. The odds ratio for the prevalence of coccidia with egg per gram of feces (EPG) load ≥ 500 in corn-supplemented lambs was 2.5, and confidence interval (CI) was (3.02 - 1.98) as compared to the soybean-supplemented lambs (Table 3). This indicates that the corn-supplemented lambs were 2.5 times more likely to have coccidia EPG ≥ 500 than soybean-supplemented lambs. However, the odds ratios and CI for *H. contortus* and *Moniezia* spp. were 1.2 (2.06 - 0.34) and 1.5 (2.65 - 0.35), respectively, indicating a similar prevalence of these parasites in both groups. The higher coccidian EPG in corn-supplemented lambs vs. soybean-supplemented lambs could be due to the difference in protein contents in these supplements. Soybean contained much higher protein level (40% CP) in comparison to corn (9% CP) regardless of almost the same level of the total digestible nutrient (TDN) (corn - 88% and soybean - 93%) (NRC, 2007). Andrews (2022) suggested that nutritional supplementation is required along with the coccidiosis treatment because coccidia infect the

Table 3. Odds Ratio of Gastrointestinal-Parasite Load in Katahdin-St. Croix Cross Nursing Lambs Supplemented with Corn or Soybean in Woodlands, May – August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

Group comparison	Odds ratio for parasite load (EPG ≥ 500)		
	Haemonchus contortus	Coccidia	*Moniezia* spp.
	Odds ratio (95% confidence interval)		
Corn vs. Soybean	1.2 (2.06 - 0.34)	2.5 (3.02 - 1.98)	
Soybean vs. Corn			1.5 (2.65 - 0.35)

intestinal region leading to poor nutrient absorption and severe hemorrhages. Abo-Shehada and Muwalla (1989) demonstrated that Awassi yearling sheep supplemented with a higher level of concentrate (1.5 lb.) showed a lower infection with *Eimeria granulosa* than those supplemented with less concentrate (0.5 lb.).

Similar prevalence of *H. contortus* and *Moniezia* spp. between soybean- and corn-supplemented lambs observed in the current study agrees with the findings of Torres-Acosta et al. (2004), who reported no difference in EPG of GI nematodes between the soybean-sorghum supplemented Criollo kids and non-supplemented kids raised in native vegetation. However, the same study showed a greater weight gain in supplemented lambs. The current study showed a better performance of soybean-supplemented lambs vs. corn-supplemented lambs, although there was no difference in the EPG and prevalence of *H. contortus* and *Moniezia* spp. between groups. The better performance of soybean-supplemented lambs could be due to a higher crude protein in soybean vs. corn. Cériac et al. (2019) found a higher weight gain (15%) in *H. contortus*-infected Creole kids supplemented with high-protein (23% CP) diet compared to hay-supplemented kids (8% CP); however, no difference in EPG was observed. Supplementation with a high protein diet (12.5 % CP) (López-Leyva et al., 2020) in H. contortus-infected Pelibuey lambs was found to boost immunity, decrease parasite proliferation and maintain growth (9.5%) compared to lambs supplemented with low protein diet (8.5% CP). Animals supplemented with quality nutrition have sound immune systems, keeping the infections minimal, and are better equipped to resist the potential negative physiological effects caused by the GI parasites (Pugh et al., 2021).

Conclusion

The supplement type used in the study significantly affected the live weight, FAMACHA score, and body condition score of Katahdin-St. Croix cross lambs raised in woodlands. Soybean-supplemented nursing lambs grew heavier (22%; $p<0.0001$) and showed better body condition score (13%; $p<0.0001$) and FAMACHA score (8%; $p<0.05$) compared to corn-supplemented lambs. However, the supplement type did not affect the prevalence of H. contortus and Moniezia spp. in nursing lambs. Nevertheless, the odds of having a greater coccidia load (EPG≥500) was 2.5 in corn-supplemented lambs vs. soybean-supplemented lambs. The study's findings indicate that soybean supplement is a better option than corn to enhance the resiliency against the natural challenge of gastrointestinal parasites of nursing lambs raised in woodlands.

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