

# Soybean-Supplemented Nursing Ewes Showed Better Performance and Immune Response than Corn-Supplemented Ewes while Grazing in Woodlands

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#### ABSTRACT

Gastrointestinal (GI) parasites are a major cause of poor health and production loss in pasture-raised sheep in the Southern USA. Woodlands in this region typically have poor forage quality, and parasites are often a problem due to higher rainfall and warmer temperatures. Supplemental feeding could benefit lactating animals under such conditions, but the type of supplement with the most benefit is not known. This study hypothesizes that nursing ewes' performance and immune function would be better with soybean than with corn supplements. The objective of the study was to evaluate the immune response and performance of ewes supplemented with either corn or soybean while raised in woodlands. Eighteen Katahdin-St. Croix crossnursing ewes were divided into two groups, each grazing on separate sets of woodland plots (3 plots/group) and rotated in the respective plots with ad libitum hay from May to August 2022. One group received whole corn, and the other received whole soybean (0.5% of live weight). Animal performance data (live weight, FAMACHA© score, and body condition score (BCS)) were collected on day 1, fortnightly, and at the end of the study. Fecal and blood samples were collected on days 1, day 45, and day 80. Fecal samples were analyzed for species types and infection intensity of GI parasites, blood samples were analyzed for RBC, PCV, and WBC as well as immunoglobulins (IgA, IgG, and IgE). Data were analyzed in SAS 9.4. The soybean-fed ewes had higher live weight (6%, p<0.05), FAMACHA© score (13%, p<0.01), BCS (8%, p<0.001), and IgG level (p<0.0001) compared with corn fed ewes. Soybean supplementation to nursing ewes could enhance their performance and resiliency against GI parasites in woodlands.

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# 1. Introduction

Gastrointestinal (GI) parasites pose a major health problem to small ruminants in the southeast USA (Karki, 2013). Among GI nematodes, *Haemonchus contortus* (barber pole worm) is highly prevalent in the region and pathogenic to small ruminants due to its hematophagous nature (Burgunder et al., 2018). *Haemonchosis* causes gastroenteritis, anemia, weight loss, and even death of susceptible animals, resulting economic losses to small-ruminant producers (Karki, 2013; Torres-Acosta et al., 2004). Continuous use of anthelmintics has led to the development of anthelmintic resistance in parasites (Bosco et al., 2020; Sepúlveda-Vázquez et al., 2021). This situation reinforces the need for sustainable strategies to prevent and/or control GI parasites. One of the most promising parasite-preventive methods could be to enhance the host's immune response through nutritional supplementation (Cériac et al., 2019; Houdjik, 2012).

Small ruminants infested with GI parasites require more energy and protein to produce immune cells and mediators for immune functions, as well as to repair the tissues damaged by the invading parasites (Knox et al., 2006). This increased need is in addition to the maintenance requirements of healthy animals (Colditz, 2008). Several studies have confirmed the impacts of nutrients on the resilience and resistance of small ruminants to GI parasites (Coop & Kyriazakis, 1999; Hoste et al., 2016; Torres-Acosta et al., 2004). López-Leyva et al. (2020) demonstrated that a high-energy diet (3.1 Mcal/kg) lowered the number of parasite eggs per gram of animal feces (EPG) by 59% and increased the live weight by 17% in Pelibuey sheep infested with *H. contortus*, compared to sheep on a low-energy diet (2.6 Mcal/kg), when both groups received a similar amount of crude protein (CP) (12%). Similarly, Can-Celis et al. (2022) found lower *H. contortus* EPG (71%) in Pelibuey lambs when CP content in their diet was increased from 8.48% to 14.96% while keeping the energy level constant. In the same

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study, a negative correlation was found between IgG and EPG (r = -0.85) and IgG and the number of total adult *H. contortus* (r = -0.72). Since energy and protein both play crucial roles in enhancing animal performance and maintaining immunity against parasites (Houdijk, 2012), strategic nutritional supplementation is required or can be helpful.

Nutritional supplementation has been recommended to improve the performance of young lambs (Ellis et al., 2021), kids (Khatri, 2016), and lactating does (Shrestha, 2022) when stocked in woodlands. However, Bhattrai et al. (2022) and Paneru (2020) reported that mature rams and wethers raised in woodlands without any supplement showed satisfactory body condition and FAMACHA© scores. These studies showed the potential of woodlands to extend the grazing opportunity for small ruminants, with the provision of supplements for nursing and growing animals. However, information is limited about the role of supplement type in improving the performance and immune response of nursing ewes against GI parasites while grazing in woodlands. The study tested the hypothesis that the performance and immune response of nursing ewes to the natural challenge of GI parasites would be better with soybean than with corn supplements. The objective of the study was to evaluate the immune response and performance of ewes supplemented with either corn or soybean while raised in woodlands.

#### 2. Methods

All the procedures applied related to animal handling, care, and sample and data collection during the study were as per the protocol approved by the Animal Care and Use Committee of Tuskegee University (Protocol Request Number: R12-2018-19), approved on 12/10/2018.

#### 2.1. Study Site

The study was conducted from May to August 2022 at the Atkins Agroforestry Research and Demonstration site (Latitude 32°26'35.7" N, Longitude 85°43'56.5" W), Tuskegee University, Tuskegee, Alabama, USA. The study site had six woodland plots, each of 0.4 ha, consisting of mixed southern pines (longleaf (*Pinus palustris* Mill.) and loblolly (*Pinus taeda* L.), and numerous understory plant species. Water oak (*Quercus nigra* L., 29%), yaupon (*Ilex vomitoria* Aiton,12%), blackberry (*Rubus spp.* L.,12%), and sweet gum (*Liquidambar* L., 5%) were some of the major plant species (Khatri, 2016) in woodland plots. The study plots consisted of Uchee loamy sand (78.4 %) with 1 to 5 percent slopes and Cowarts loamy sand (21.6 %) with 5 to 15 percent slopes (USDA-NRCS, 2022). The weather data for the site and study period were used from the secondary source

https://www.wunderground.com/history/monthly/us/al/montgomery/KMG M/date/2022-5) that was recorded at the Montgomery regional airport station for Tuskegee Institute, Alabama which was approximately 72 kilometers west of the study site. The Temperature Humidity Index (THI) was calculated using the equation: THI = 0.8\*T + RH\*(T-14.4) + 46.4 (Pericoli, 2022). Each study plot was equipped with a mineral feeder, mobile shelters (2), and a water trough with water line.

### 2.2. Quality of Understory Vegetation and Supplements

Ten vegetation samples per plot were collected using a 1-m2 quadrat before bringing animals to the study plots and at each rotation throughout the study period. Vegetation grown to 10 cm and up to 1.8 m from the ground level was included in the sample. All herbaceous vegetation within the quadrat and the young twigs and leaves of woody plants present within the sampling volume were collected from each sampling location. The collected samples

were dried at 60°C for 72 hours in a hot air oven in the Agroforestry and Grazing Land Laboratory (AG Lab) at Tuskegee University. Dried vegetation samples were ground and analyzed for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) using the Fourier Transform Near-Infrared Spectroscopy (FT-NIR) in the AG Lab. Total digestible nutrient (TDN) was calculated from ADF values (TDN%=87.84- (0.7\*ADF%)) (CSU, 2022). To determine hay quality, hay samples from each square bale were taken, oven dried, ground, and analyzed for quality following the same method that was used for analyzing vegetation quality described above. The quality of the whole corn and whole soybean used as supplements during the study were assumed to be similar to that reported by NRC (2007) (Table 1).

Table 1. Quality of whole corn and whole soybean used for supplementing nursing ewes during the study, May – August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

Supplements	CP %	ADF %	NDF %	TDN %
Whole corn	9	3	9	88
Whole soybean	40	11	15	93

Source: NRC (2007)

#### 2.3. Research Animals

Eighteen Katahdin × St. Croix cross-nursing ewes aged 44 to 45 months were randomly divided into two uniform groups based on the initial performance data (live weight, BCS, and FAMACHA© score). Each group consisted of nine nursing ewes. The ewes were allocated to separate sets of plots (three plots/group) and rotationally grazed in those plots throughout the study period with *ad libitum* hay. One group of ewes was supplemented with whole corn (corn group) and another group with whole soybean (soybean group) at the rate of 0.5% of their live weight. The supplement amount was adjusted fortnightly based on animal live weight taken every two weeks during the study. Ewes were monitored twice a day: morning and evening. Minerals (Purina loose mix), water, and hay were provided *ad libitum*.

# 2.4. Ewe Performance Data

Live weight, BCS, and FAMACHA© scores were measured on Day 1, fortnightly during the study, and at the end of the study (day 80). A digital weighing scale was used to measure the live weight of animals. The BCS was taken on a range of 1 to 5 - 1 indicating the lean and thin animals and 5 obese animals - by feeling the muscles and fat tissues over the backbone, ribs, and brisket bone (Griffiths, 2018). The FAMACHA© score of ewes was assessed by matching the color of the conjunctiva of the lower eyelids of both eyes to the color category (1–5: 1 = bright red color indicating nonanemic condition; 5 = pale or white color indicating the severely anemic condition) on the FAMACHA© card (Karki, 2017). Both FAMACHA© score and BCS were assessed by a single trained person throughout the study period.

# 2.5. Fecal Sample Collection and Analysis

Fecal samples were collected from each animal on Days 1, 45, and 80 of the study and analyzed for the type and quantity of GI parasites using the McMaster technique (Whitlock, 1948). In addition, fecal samples were collected from animals with FAMACHA© scores of 3 or greater and analyzed for GI parasites. The samples were assessed during the fortnightly performance measurements and, if needed, more frequently based on daily observations.

#### 2.6. Blood Sample Collection and Analysis

Two sets of blood samples were collected on day 1, day 45, and day 80 of the study from each animal via the jugular vein. One set of samples was collected in ethylenediaminetetraacetic acid (EDTA) treated vacutainer tubes (BD Vacutainer™ K2EDTA Tubes, Fisher Scientific) with a purple cap and another in untreated vacutainer tubes (BD Vacutainer™ Serum Tubes, Fisher Scientific) with a red cap. Blood samples with EDTA were gently mixed immediately after collection to prevent coagulation. Samples without EDTA were kept undisturbed in a tube holder. After collection, blood samples were taken to the Pathobiology Laboratory of Tuskegee University for analysis. Blood samples in purple-cap tubes were analyzed for hematological parameters using the ProCyte Dx analyzer. Samples from the red-cap tubes were centrifuged for 15 minutes at 1500 rpm to separate serum and stored in an Eppendorf tube at −20 °C for Enzyme-Linked Immunosorbent Assay (ELISA) for immunoglobulin analysis (IgA, IgG, and IgE).

A separate set of sheep ELISA kits was used from antibodies.com for each serum immunoglobulin. ELISA kit consisted of a 96-well ELISA plate coated with mouse monoclonal antibody. For analysis, frozen serum samples were thawed and kept at room temperature for about 30 minutes, then diluted for assay with the sample dilution solution. For IgG and IgA, samples were diluted at 1:100, and IgE at 1:2. Standards and samples were then pipetted into the wells and incubated for 90 minutes at 37°C to let immunoglobulins present in each sample bind to the monoclonal antibodies present in the wells. After incubation, the wells were washed, and biotin HRP-conjugated streptavidin was pipetted into the wells and incubated for 60 minutes at 37°C. After another round of washing, Tetramethylbenzidine (TMB) substrate solution was added to the wells and incubated for 15-20 minutes. As the TMB substrate reacted, a blue color developed in the wells in proportion to the amount of immunoglobulins bound with the monoclonal antibody in the wells. Then, a stop solution was added to the wells, changing the color from blue to yellow. The intensity of the color was measured at 450 nm in a microplate reader. The coefficient of variance (CV) for each sample was read, and samples with CVs greater than 20% were reanalyzed until the CV was found to be 20% or less for each sample.

#### 2.7. Data Analyses

All data sets were analyzed in SAS 9.4 at a 95% confidence level. The quality data for vegetation and hay (CP, ADF, NDF, and TDN) were analyzed using the MEANS Procedure. The EPG data were analyzed using the Wilcoxon rank sum test. The prevalence and odd ratio of GI parasites were calculated in Microsoft Excel. Animal performance, blood parameters, and immunoglobulin data were analyzed using the GLM procedure with the multivariate analysis of variance (MANOVA) option. The general models used to analyze various data sets are presented below.  $Y_{(1-n)ii} = \mu + \alpha_i + (\alpha\beta)_{ii} + e_{ii}$ 

MANOVA h = Animal group and the interaction of animal group and observation date. Where,

 $Y_{(1-n)ij}$  = dependent variables from  $i^{th}$  group and  $j^{th}$  observation date,  $\mu$  = grand mean,  $\alpha_i$  = 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.  $Y_{(1-n)}$  for each data set is described below:

- 1. In animal performance data set,  $Y_{(1-3)ij}$  = Performance variables (Live weight, BCS, FAMACHA© score) of animals
- In hematological parameter data set, Y<sub>(1-9)ij</sub> = Hematological variables (RBC, Hematocrit, Mean Corpuscular Volume, total WBC, Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil)

3. In immunoglobulin data set,  $Y_{(1-3)ij} = Immunoglobulins$  (IgG, IgA, IgE)

#### 3. Results

#### 3.1. Weather Data

The weather conditions during the study were hot and humid, with an average temperature of 27°C and an average relative humidity of 73% (Fig. 1). The THI was found to be in between 66.2 to 79.2 during the study. The optimal temperatures for *H. contortus* larval development range from 25°C to 30°C, and 60–70% humidity levels support the survival of infective larvae on pasture. These conditions help nematode larvae remain active and migrate onto vegetation, increasing the likelihood of ingestion by grazing animals.

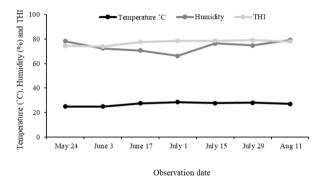


Figure 1. Weather condition of the study site during the study period, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (THI: temperature-humidity index).

#### 3.2. Vegetation and Hay Quality

Table 2. Quality of the woodland vegetation and hay used for supplementing ewes, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

	CP	ADF	NDF	TDN				
	-	% (LSMean $\pm$ SE)						
Woodland								
vegetation	12 ±0.2	$41\pm0.3$	$51\pm0.3$	$51 \pm 0.2$				
Hay	$12 \pm 0.1$	$32 \pm 0.2$	$71 \pm 0.2$	$65 \pm 0.2$				

CP = Crude protein; ADF = Acid detergent fiber; NDF = Neutral detergent fiber; TDN = Total digestible nutrients

Both woodland vegetation and coastal bermudagrass (*Cynodon dactylon* L.) hay used for supplementing ewes were found to be similar in nutrient content (Table 2). Likewise, the biomass of understory vegetation in plots allocated to the corn group (1048  $\pm$  117.72) and soybean group (1128.35  $\pm$  109.12) was similar.

### 3.3. Performance of Ewes

Overall, soybean-supplemented ewes had higher bodyweight (6%, p<0.05) (Fig. 2), BCS (10%, p<0.001) (Fig. 3) and FAMACHA© score (17%, p<0.01) (Fig. 4) in comparison to corn-supplemented ewes. The range of BCS for corn-supplemented ewes was between 3.0 and 3.1, while soybean-supplemented ewes were between 3.1 and 3.5. Additionally, the range of

FAMACHA© scores for corn-supplemented ewes was 1.7 to 2.0, while soybean-supplemented ewes had a range of 1.2 to 1.7.

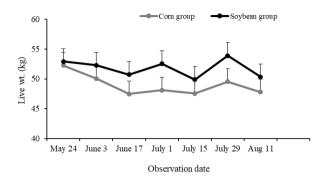


Figure 2. Live weight (LSMean ± SE) of nursing ewes with corn or soybean supplements when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

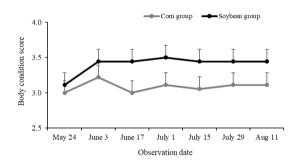


Figure 3. Body condition score (LSMean ± SE) of nursing ewes with corn or soybean supplement when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA

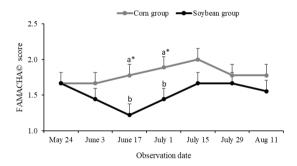


Figure 4. FAMACHA© score (LSMean ± SE) of nursing ewes with corn or soybean supplement when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (\*p<0.05).

# 3.4. Prevalence of GI Parasites in Ewes

There was no difference in the EPG or prevalence of nematodes between the groups. However, the prevalence of nematodes with EPG of 1000 or more was 11% in corn-supplemented nursing ewes, compared to none in soybean-supplemented ewes (Table 3).

Table 3. The severity of gastrointestinal nematode infection ( $EPG \ge 1000$ ) in nursing ewes with corn or soybean supplement when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

Obs. date	Corn group			Soybean group		
	Ewes	Prevalence	Total	Ewes	Ewes	Total
	having	of Ewes	obs.	having	having	obs.
	$\text{EPG} \geq$	having EPG	(N)	$EPG \ge$	$\text{EPG} \geq$	(N)
	1000	≥ 1000 (%)		1000	1000	
	(n)			(n)	(%)	
May 24	0	0	9	0	0	9
July 7	3	33	9	0	0	9
August 11	0	0	9	0	0	9
Total	3	11	27	0	0	27

#### 3.5. Hematological Parameters and Immunoglobulins

In both groups of nursing ewes, the concentration of hematological parameters was similar and in the normal range, except for an elevated level of eosinophils (Table 4). The concentration of serum IgG was higher in ewes supplemented with soybean for the entire study period (15%, p<0.0001) and on Day 80 (23.3%, p<0.001) in comparison to corn-supplemented ewes (Fig. 5). Similarly, serum IgA was higher in soybean group ewes on Day 1 (42.2%, p<0.0001), Day 45 (27.8%, p<0.001), and during the entire study period (23.15%, p<0.0001) (Fig. 6). However, serum IgE concentration was similar in both groups of ewes (Fig. 7).

Table 4. Eosinophil counts for ewes with corn or soybean supplement when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

Eosinophil (K/μL)	Corn	Soybean	P value	Normal
	group	group	_	value
May 24	$1.4 \pm 0.25$	$1.9 \pm 0.25$	0.16	0.05 - 0.95
July 7	$1.3\pm0.25$	$1.0\pm0.25$	0.43	
August 11	$2.1\pm0.24$	$1.8 \pm 0.24$	0.46	
Overall	$1.6\pm0.14$	$1.6 \pm 0.14$	0.95	

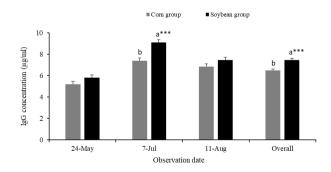


Figure 5. The concentration of serum IgG (LSMean  $\pm$  SE) in nursing ewes with corn or soybean supplement when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (\*\*\*p<0.001), \*\*\*\*p<0.0001).

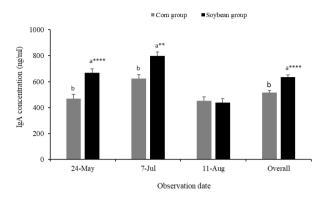


Figure 6. The concentration of serum IgA (LSMean ± SE) in nursing ewes with corn or soybean supplement when stocked in woodlands, May – August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA (\*\*\*p<0.001), \*\*\*\*p<0.0001).

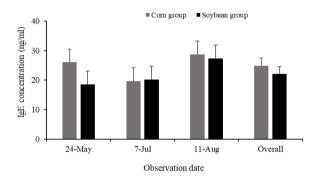


Figure 7. The concentration of serum IgE (LSMean ± SE) in nursing ewes with corn or soybean supplement when stocked in woodlands, May–August 2022, Atkins Agroforestry Research and Demonstration Site, Tuskegee University, Tuskegee, Alabama, USA.

### 4. Discussion

# 4.1. Performance of Nursing Ewes

The hypothesis that nursing ewes would perform better with soybean vs. corn supplement was accepted since the soybean-supplemented ewes performed better than ewes on the corn supplement. In the current study, the higher CP (40%) and TDN (93%) in soybeans than in corn (9% CP and 88% TDN) might be the reason for the performance difference between the animal groups. Wang et al. (2022) reported that increased dietary protein enhanced the nutrients available for utilization in lactating Hu sheep, due to increased production of volatile fatty acids and the activation of acetic acid. The higher live weight of the soybean group in the current study is consistent with Obeidat et al. (2019), who reported greater weight gain in the soybean-supplemented Awassi lambs (29%) than in lambs fed a forage mix diet (65% wheat straw and 35% alfalfa hay). Moreover, Ramos et al. (2019) observed greater body weight gain (5%) and BCS (18%) in castrated Merino Dohne-Corriedale-cross lambs fed a high-protein diet (20% CP) compared to those fed a low-protein diet (12% CP). This result aligns with the current study's findings, showing better BCS (10%) of soybeansupplemented ewes than corn-supplemented ewes. Tiwari et al. (2021) reported similar findings with Kiko does grazing legume-grass pastures (with 33% higher CP) exhibiting 12.5 % better FAMACHA© scores compared to those of grazing sole-grass pastures. In the current study, the BCS and FAMACHA© scores for nursing ewes with both supplement types fell within the desirable range. The desirable BCS for ewes has been reported to be 3 to 4 (Greiner, 2012), and the desirable FAMACHA© score is 1 to 2 (Karki, 2017), respectively.

## 4.2. Prevalence of GI Parasites in Nursing Ewes

Eleven percent of the ewes in the corn-supplemented group had nematodes with EPG 1000 or more, while none of the soybean-supplemented ewes had counts greater than or equal to 1000 EPG. This disparity could be due to the different nutritional profiles of the two supplements, with soybeans being rich in protein (CP, 40%) while corn containing low protein concentration (CP, 9%; National Research Council [NRC], 2007). These nutritional differences likely influenced the immune response of ewes, resulting in improved resistance and resilience against nematodes. H. contortus is the most prevalent nematode found in the southeast USA in the summer (Avery et al., 2024). Alba-Hurtado and Muñoz-Guzmán (2013) found IgG levels associated with sheep's resistance to GI parasites. Amarante et al. (2005) reported a negative correlation between the EPG of H. contortus and both mast cells (r = -0.49, p<0.05) and eosinophils (r =-0.714, p<0.01) in Santa Ines. Moreover, Wallace et al. (1996) reported no difference in EPG of H. contortus between infected Scottish blackface lambs that were provided a supplemented diet with soybean meal, containing 85% more CP per kg dry matter than lambs receiving only the basal diet (93 g CP per kg DM). However, the supplemented lambs had a higher live weight gain of 28% compared to the non-supplemented lambs. This finding aligns with the results of the current study, which showed a higher live weight of soybean-supplemented ewes vs. corn-supplemented ewes, but the average EPG of GI nematodes was not different between the groups.

# 4.3. Hematological Parameters and Serum Immunoglobulins of Nursing Ewes

The similar RBC concentration in both groups of nursing ewes in the current study could be due to the similar overall prevalence and EPG of GI nematodes in both groups. *H. contortus* decreases RBC count due to its blood-sucking nature, causing anemia (Soulsby, 1982). However, in the current study, RBC and PCV were in the normal range, possibly due to fewer adult *H. contortus* than the number required to cause anemia. Alam et al. (2020) reported that when sheep were moderately infested with the *H. contortus* (400-1500 adult worms/animal), they had lower RBC (30%) and PCV (34%) compared to non-infested sheep.

Comparable to the findings for red blood cell (RBC) count and packed cell volume (PCV), leucocyte counts in both groups remained within the normal range, except for elevated eosinophil levels, which could reflect the ewes innate immune response to nematode infestation. Eosinophils provide a cellular innate immune response against parasite infestation (Tizard, 2009). Consistent with the current study, Alam et al. (2020), found a higher eosinophil count (58%) in the *H. contortus*-infested sheep in comparison with non-infested sheep. Ortolani et al. (2013) also recorded elevated eosinophil counts in sheep infested with *H. contortus*. Eosinophil is the main leukocyte to initiate the immune response during parasitism (Feldman et al., 2000). Increased production of eosinophil is due to the Th2 response against the *H. contortus* (Alba-Hurtado & Muñoz-Guzmán, 2013; Tizard, 2009). Eosinophil degranulation on the surface of the *H. contortus* and damages the cuticle resulting in the expulsion of parasites (Reinhardt et al., 2011). Previous research by Bowdridge et al. (2013) found a negative

correlation between eosinophil count and the number of *H. contortus* present in the abomasum of sheep.

The study hypothesis that the immune response would be enhanced in soybean-supplemented ewes compared to those supplemented with corn was partially accepted. Serum IgG concentration was found to be higher (15%) in ewes receiving soybean supplementation than in those receiving corn supplementation. The greater level of serum IgG in ewes on the soybean supplement might be because of more protein in soybean (40%) vs. corn (9%). IgG production by B cells requires adequate protein supplementation. Jin Ha et al. (2021) concluded that when mice were supplemented with dietary whey protein, they had increased IgG production, affecting Helper T cell population and B cells in response to influenza antigen exposure. IgG and FEC were negatively correlated when exposed to H. contortus on pasture in Santa Ines ewes and their crosses with Dorper, Ile de France, Suffolk, and Texel (Amarante et al., 2009). Strain and Stear (2001) stated that when the H. contortus-infested Hampshire Down lambs were supplemented with soybean meal, providing 76% more metabolizable protein compared to non-supplemented lambs, their parasite-specific IgA was increased (optical density: 1.4 in supplemented lambs vs. 0.8 in non-supplemented lambs). In the current study, higher IgA was observed in the soybean-supplemented ewes on Day 1. However, the difference was likely unrelated to the supplements, and the result on Day 45 may have been a carryover effect from the first observation. By the final day of the study, the differences had subsided for reasons that remain unclear. Since the ewes in this study were naturally infected with H. contortus, further investigation is needed to clarify these findings. Furthermore, serum IgE concentrations for both ewe groups were not different, which could be due to the transient nature of IgE response to parasitic infestation (Erb, 2007) and the short half-life (2-3 days) of serum IgE (Lawrence et al., 2017). Therefore, capturing an accurate snapshot of the IgE concentration at a single point-in-time in naturally infested animals is difficult.

# 5. Conclusions

Soybean-supplemented ewes lost less body weight (43%, p<0.05), and had better body condition scores (10%, p<0.001) and FAMACHA© scores (17%, p<0.01) compared to ewes supplemented with corn. Additionally, soybean-supplemented ewes demonstrated a better immune response regarding serum IgG (15%, p<0.0001) against gastrointestinal parasites than corn-supplemented ewes. Moreover, 1000 or more nematode EPG were found in the corn-supplemented ewes, but not observed in the soybean-supplemented ewes. This study suggests that soybean supplementation is more effective than corn in enhancing the performance and immune response of nursing ewes raised in woodlands.

#### **Declaration of Conflict of Interest**

None.

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