German Journal of Natural Products Research
eISSN:3052-0886

Research article

Anthelmintic activities of diatomaceous earth in sheep

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Article History:
Received: 13-Dec-2024
Accepted: 20-Feb-2025
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Abstract

Internal parasites are the leading cause of mortality in sheep in resource-constrained countries, highlighting the need for affordable, non-conventional medicines. In the study, diatomaceous earth (DE), a relatively abundant and cheap non-conventional medicine, was assessed for its impact in controlling parasites and its effects on the behavior and growth performance of pasture-raised Wiltshire (WS) × Barbados Black Belly (BB) sheep. A total of 12 female weaner lambs with an average body weight of 21.37±2.28 kg were used in the study. The lambs were fed a basal diet and randomly assigned to one of three treatment groups. Sheep in group 1 (n=4) received a basal diet supplemented with 2% DE, while animals in group 2 (n=4) were administered subcutaneous injections of ivermectin (200 µg/kg body weight). Group 3 served as the control (n=4), with no treatment. Data collected over nine weeks included fecal egg counts, fecal consistency scores, time spent on different behavioral activities, and body weights. The results showed that Moniezia expansa egg counts were significantly lower in the ivermectin and DE-treated groups compared to the control group (P<0.05). *Haemonchus contortus* eggs were more predominant across all treatment groups. Fecal consistency was significantly improved (P<0.05) in the ivermectin group than in other groups. Sheep receiving DE and ivermectin treatment spent less time lying down compared to the control group with no anthelmintic (P<0.05). There were no differences in the average weight gain of sheep across all treatments. In conclusion, DE was more effective in reducing *M. expansa* than *H. contortus*, indicating its potential as a viable alternative treatment for internal parasites in sheep.

Keywords: Diatomaceous earth, Weaner sheep, Internal parasites, Feeding behaviour

Citation: Prasad, A. S., Zindove, T. J., Danmaigoro, A., and Bakare, A. G. 2025. Anthelmintic activities of diatomaceous earth in sheep. Ger. J. Nat. Prod. Res. 1 (1): 11–20. https://doi.org/10.51585/gjnpr.2025.1.0003

Introduction

Small ruminants are integral to farmers' livelihoods in developing countries due to their affordability, productivity, adaptability, and ability to coexist with large ruminants in rangelands (Jack et al., 2022). Rearing small ruminants can be advantageous because they can make optimal use of marginal and tiny plots of land, and the animals can convert low-quality forage to provide valuable protein sources such as meat and milk. In Fiji, sheep farming is popular among farmers (Ferew, 2008). The country's sheep population is estimated to be 37,435 heads (FAOSTAT, 2020). Common sheep breeds in the country include the Dorper, Barbados Black Belly, and Wiltshire (WS) × Barbados Black Belly (BB) crosses. The WS × BB cross was developed to best suit the Fijian production environment (Rokomatu et al., 2006). The WS × BB cross is reported to have high lambing and fast growth rates and sheds its wool as an adaptation feature to Fiji's hot and humid climate (Rokomatu et al., 2006). While most sheep have acclimatized to harsh environmental conditions, rearing sheep on the island nation has its challenges. Intestinal parasites are the primary cause of concern.

Internal parasites are the major cause of sheep mortalities on pasture-based systems and significantly reduce productivity in the event of persistent illnesses (Maqbool et al., 2017). Some of the most problematic internal parasites affecting sheep include *Haemonchus contortus*, *Telodorsagia circumcinta, Trichostrongylus* spp, *Nematodirus* spp, and *Cooperia* spp (Roeber et al., 2013). In Fiji, *H. contortus* and *T. colubriformis* have been reported to be the primary internal parasites in sheep (Cowley et al., 2019). The low productivity of sheep in Fiji despite the development of the WS × BB might be due to the high prevalence of internal parasites. Sheep exhibiting elevated intestinal parasite burdens have been documented to have suboptimal growth performance, anemia, diarrhea, diminished wool quality and quantity, and mortality (Taylor, 2010). In addition, high intestinal parasite loads also affect behavioral activities (Grant et al., 2020). Control of gastrointestinal nematodes is thus critical to intensifying sheep production under pastoral production systems.

Conventional anthelmintic drugs are widely used to manage intestinal parasites in sheep. Anthelmintic resistance in gastrointestinal nematodes has been a massive drawback to using conventional anthelmintic drugs to control internal parasites in sheep worldwide (Dolinská et al., 2014). Growing efforts have been made to explore using non-conventional methods to complement anthelmintic drugs in controlling gastrointestinal nematodes in sheep. Diatomaceous earth (DE), an abundant naturally occurring compound, is used in traditional medicine as an insecticide (Ikusika et al., 2019). It is composed of amorphous silicates and possesses distinct physical and chemical properties that make it suitable for various applications (McLean et al., 2005; Ikusika et al., 2019). It is postulated that DE's sharp, blade-like surfaces can cut and penetrate the parasitic worm's membrane, leading to dehydration and death (Beltran et al., 2016). The siliceous material also damages the cuticles of invertebrates, increasing their permeability and eventually causing death by dehydration. Nonetheless, very little scientific research has investigated the anthelmintic properties of DE in sheep. Therefore, this study aimed to assess the growth performance, behavioral changes, and health of weaner ewes treated with DE as an anthelmintic.

Materials and methods

Study site and animal ethics

The study was conducted at the College of Agriculture, Fisheries, and Forestry (CAFF) livestock farm located in Koronivia, Fiji. The farm lies between latitudes 15° to 20° S and longitudes 175° to 182° E, with an elevation ranging from 6 to 23 meters above sea level. Data loggers were employed to record the average ambient temperature and relative humidity, which were found to be 28±1.48°C and 70±4.17%, respectively. All animal-use procedures were approved by the Fiji National University (FNU) Animal Ethics Committee (FNU-AREC-23-001). The study was carried out in compliance with the ARRIVE guidelines.

Sheep, study design, and diet

A total of 12 female weaner lambs with an average body weight of 21.37±2.28 kg were used in this experiment. The sheep were purchased from Horeb farm. They were allowed to adapt to the environment, pens, and grazing paddocks for two weeks before the experiment. All the sheep were tagged and randomly allocated to three treatment groups, with four sheep each. Sheep kept in group 1 (n=4) were given a basal diet containing 2% food-grade DE [Sundat, Singapore]. The active ingredients in DE were silicon dioxide (87.88% w/w) and inert ingredients (12.12% w/w). Sheep kept in group 2 (n=4) received subcutaneous injections of ivermectin (IVO) (Ivomec®, Boehringer Ingelheim, Germany). The injection was administered according to the product instructions. Sheep in group 3 (control, n=4) were not treated with either of the products.

The sheep grazed on the same pasture daily and were housed in the evening in group pens. Each pen had a separate water trough and feed trough. The feed trough allowed an equal feed allocation for each sheep in each treatment. All sheep were supplemented with a basal diet of chopped rice straw, copra, soya bean meal, wheat and molasses (Table 1), and freshwater *ad libitum.* The basal diet for sheep in treatment group one had DE at a 2% inclusion level. DE is an absorbent; hence, incorporating more than 2% might affect moisture levels in the digestive system, consequently affecting nutrient utilization. The sheep were supplemented with feed slightly below what they would voluntarily consume daily. This would allow them to consume all the provided feed soon after it was made accessible, prevent them from exhibiting selective feeding behaviors, and allow them to consume the treatment (DE) under investigation.

Table 1: Composition of ingredients in basal diet.

Ingredients	Composition (%)
Rice straw	60
Coconut meal	15
Soya bean meal	18
Wheat	2
Molasses	4
Sodium chloride	1
Nutrient composition	
Crude protein (%)	15
Metabolizable energy (MJ/kg)	5.4

Parasitic examination

Faecal samples were collected from the rectum of the sheep weekly, labelled accordingly, and stored in an ice-packed container to slow down the process of nematode egg development. Samples were examined grossly for consistency and screened qualitatively using the flotation technique to identify and quantify parasite eggs. Faecal consistency was scored on a 1-5 scale, whereby 1 indicated watery faeces and 5 indicated normal hard faeces as earlier described by Le Jambre et al. (2007). The quantitative method or egg per gram (EPG) examination was done using the modified McMaster technique (Ahmed et al., 2017). The method involved weighing 3 g of faecal sample and mixing it with 20 mL of saturated salt (Sodium chloride) solution. The saturated salt solution was prepared by diluting 400 g of common salt in 1 L of distilled water. The faecal pellets were ground using a pestle and mortar to distribute parasite eggs evenly. The ground pellets with salt solution were strained through a cheesecloth and transferred into a small beaker. A 2 mL aliquot was taken from the beaker and filled into the McMaster chamber using a plastic pipette. The viewing and counting were done 5 minutes after filling the chamber under the light microscope, as described by Ikurior et al. (2020). The parasites were identified, and counts were recorded. *H. contortus* egg is approximately 70–85 µm long by 44 µm wide and contains about 16 and 32 cells during the early cleavage stages (Boylu and Onder, 2020).

The eggs were cylindrical and had reddish appearances due to their blood-sucking habit. The egg of *M. expansa* is about 70 µm in diameter and irregular in shape. The egg contains a hexacanth larva surrounded by a pyriform apparatus, giving the appearance of a triangular cone (Roberts and Janovy, 2004).

Behavioral observation and growth performance

Since the direct observation method for determining behavioral activities was used in the study, two lambs from each treatment group were randomly selected for behavioral observation. Sheep are also known to be highly social animals, and their behaviors are affected by the presence of other sheep. It was, therefore, assumed that the behaviors exhibited by the two sheep selected in each group would be representative of the behaviors of the other sheep in the same group. Any variations observed among groups could be attributed to the worm burden, which might indicate the efficacy of the treatments administered to the sheep. The sheep were marked on both sides of the abdomen with spray paint of different colors for easy identification during behavioral observations. The feeding behavior of the sheep was recorded over three consecutive days within a week, from 08:00 to 16:00 hours of daylight. The same animals were used for behavioral observation throughout the experimental trial. Each sheep was observed for 10 minutes sequentially using stopwatches by well-trained observers. The chosen time frame allowed for numerous behavioral observations to be accurately documented, accurately estimating the time spent engaging in specific behaviors. Each observer was assigned two sheep per treatment and monitored the animals throughout the experimental trial. Time spent on the following activities was recorded: grazing, grazing and walking, standing, lying down, and ruminating (Table 2). These activities were observed from a distance of about 15 m away from the sheep so as not to disturb or influence normal behavioral activities. The growth performance of the sheep was measured by weighing the lamb every week. The weight gain was calculated by subtracting the initial live weight from the final live weight.

Table 2: Ethogram of behaviors recorded for the experimental sheep.

Behavior	Behavior description
Grazing	Time spent grazing with their head down or chewing with their head up
Grazing and walking	Time spent grazing with their head down or chewing with their head up and walking at the same time
Standing	Animals are idle and standing upright with the body equally supported by all four legs
Lying down	Resting and lying on grass supported by the belly
Drinking	Time spent inserting the mouth into the drinker and drinking water
Rumination	Re-chewing and re-swallowing food from the rumen

Statistical analysis

Data was first analyzed to check if it was normally distributed. The data that was not normally distributed (Fecal egg counts and time spent on behavioural activities) was transformed ($\log_{10}$ [x + 1]). The effects of the parasite control method, week, and possible interactions on parasitic fecal egg counts, fecal consistency, and weight gain scores were analyzed using the mixed model procedures for repeated measures (SAS, 2012). Only significant interactions were included in the results. The following statistical model was used:

$Y_{ijk} = \mu + C_i + W_j + C*W_{ij} + E_{ijk}$, where Yijk = the response variable (faecal egg counts, faecal consistency scores, weight gain). M=overall mean; C_i = effect of i^{th} parasite control method (i^{th} = Control [No anthelminthic], DE [Diatomaceous earth], IVO [Ivermectin]); W_j = effect of j^{th} week (j^{th} = 1,2,3,4,5,6,7,8,9); $C*W_{ij}$=interaction of parasite control method and week and E_{ijk}=random error.

The parasite control method was used in the statistical model as the only independent factor to analyze data on time spent on different behavioral activities. The BY statement in SAS (2012) was used to analyze data for each behavioral activity. Mean separation was performed using the PDIFF option of SAS (2012) at a 5% significance level.

Results

The fecal egg count of sheep subjected to different anthelmintic treatments is shown in Figures 1 and 2. No significant difference (P>0.05) was observed in the fecal egg count between sheep treated with DE and those treated with ivermectin. However, control animals had significantly higher (P<0.05) fecal egg counts compared to the treated groups. Control animals generally had significantly higher (P<0.05) egg counts from weeks 2 to 5. The fecal egg counts of sheep treated with DE and ivermectin remained similar throughout the study (P>0.05).

For all treatment methods, sheep had more *H. contortus* eggs than *M. expansa* eggs (P<0.05). Sheep in the control group had the same number of *H. contortus* eggs as those treated with DE (P>0.05). However, sheep treated with ivermectin had fewer *H. contortus* eggs than those treated with DE or control (P<0.05). The egg counts of *M. expansa* were significantly lower in sheep treated with ivermectin and DE compared to the control group (P<0.05). Figure 3 shows images of *H. contortus* and *M. expansa* observed during coprology.

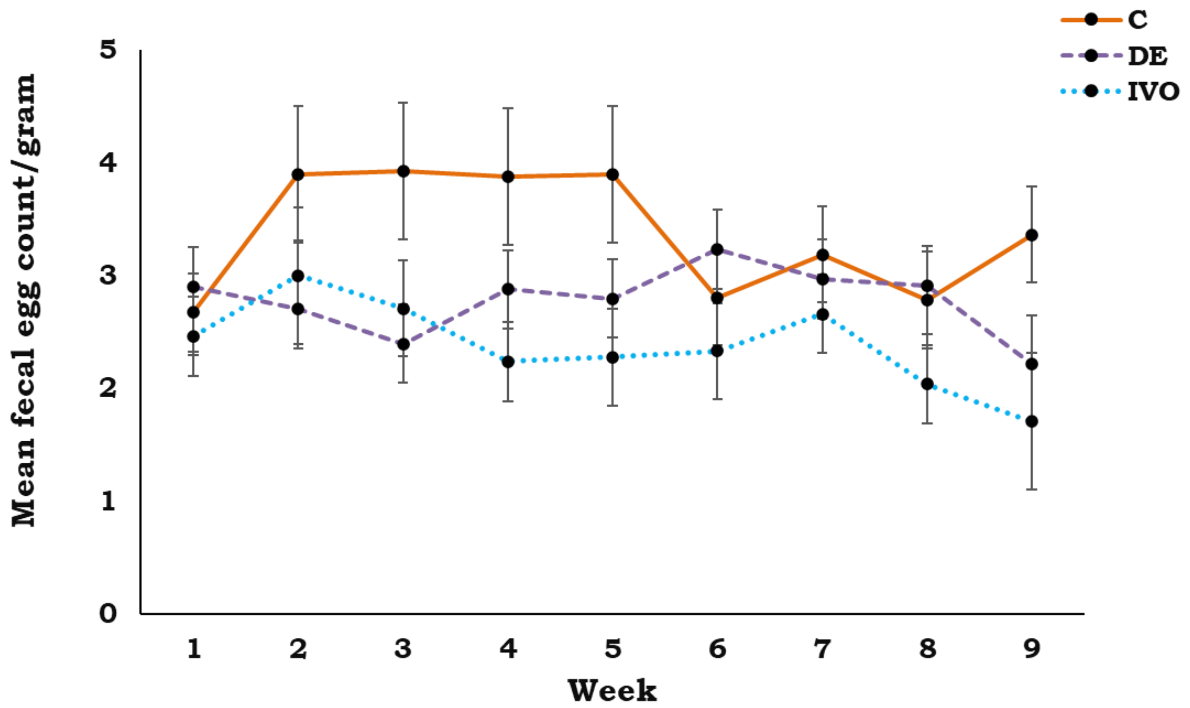

Figure 1: Effect of treatment × week on mean fecal egg count (per gram) shed by WS × BB lambs subjected to different anthelmintic treatment. The quantitative method or egg per gram (EPG) examination was done using the modified McMaster technique (Ahmed et al., 2017). C=Control (No anthelminthic); DE=Diatomaceous earth; IVO=Ivermectin.

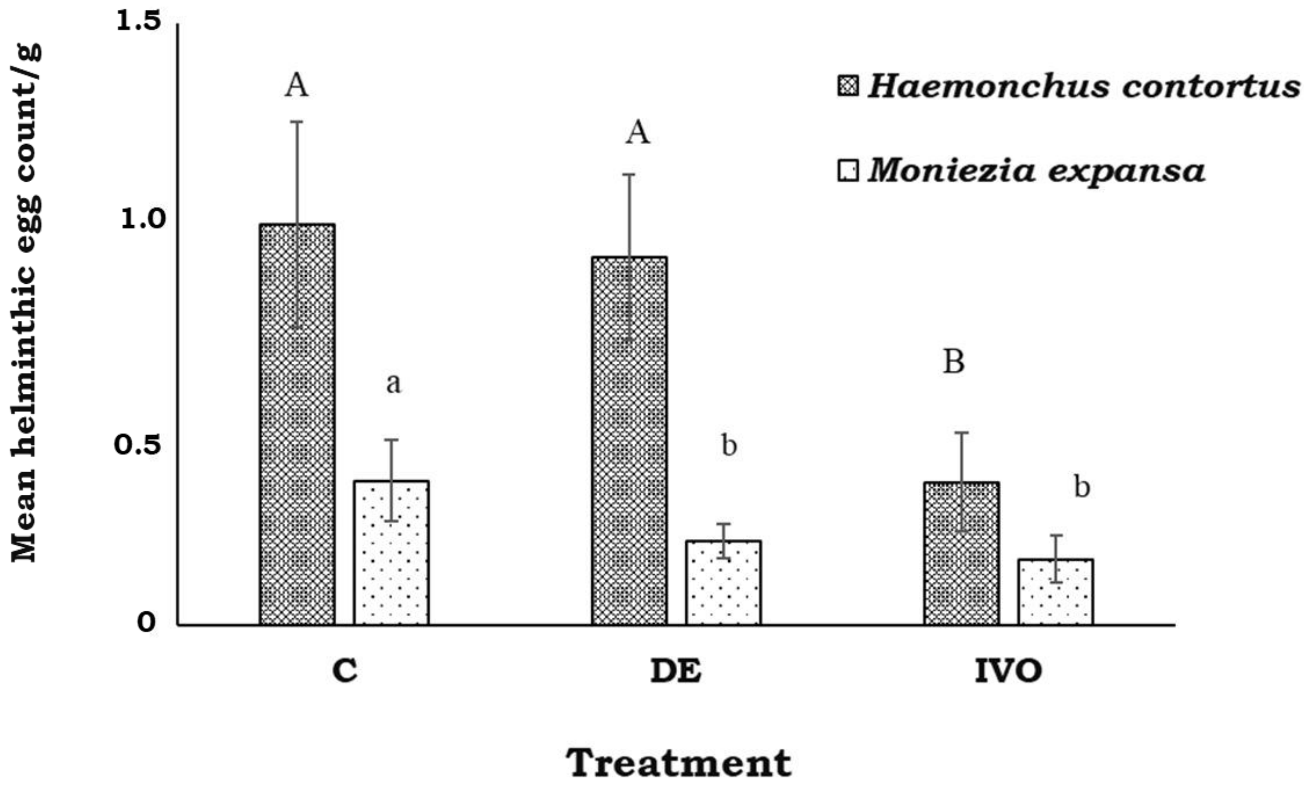

Figure 2: Mean helminthic egg count (per gram) in fecal matter shed by WS × BB lambs subjected to different anthelmintic treatment methods. Different uppercase letters represent differences in means for *H. contortus*, while different lowercase letters indicate differences in means for M. expansa. H. contortus eggs are identified according to Boylu and Onder (2020), while the eggs of *M. expansa* were identified according to Roberts and Janovy (2004). C=Control, no anthelminthic; DE=Diatomaceous earth; IVO=Ivermectin.

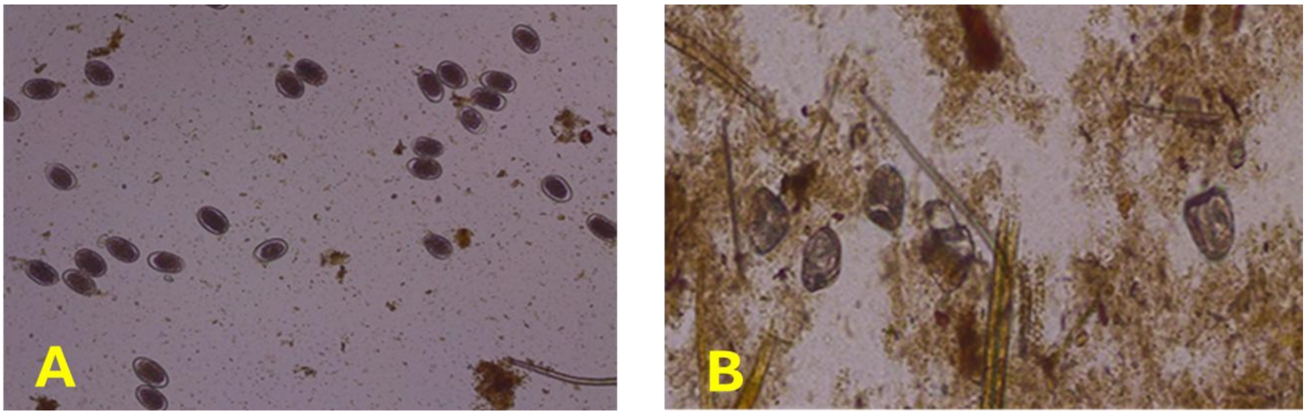

Figure 3: Morphology of *Haemonchus contortus* (A) and *Moniezia expansa* eggs (B). *H. contortus* eggs are cylindrical and have a reddish appearance. While the eggs of *M. expansa* are irregular in shape. It also contains hexacanth larvae surrounded by a pyriform apparatus, giving the appearance of a triangular cone.

There was no difference in fecal consistency of sheep subjected to the control and the DE as anthelminthic. The fecal consistency fluctuated throughout the experimental period ($P<0.05$; Figure 4). Sheep subjected to all anthelmintic treatment methods had higher ($P<0.05$) fecal consistency from week 1 to 5 and low from week 7 to 9. However, sheep treated with ivermectin showed a higher fecal consistency score compared to those in the control group and those given DE during weeks 3, 5, and 8. Sheep subjected to all anthelmintic treatment methods showed a higher ($P<0.05$) fecal consistency score from week 1 to week 5 but lower scores from week 7 to week 9.

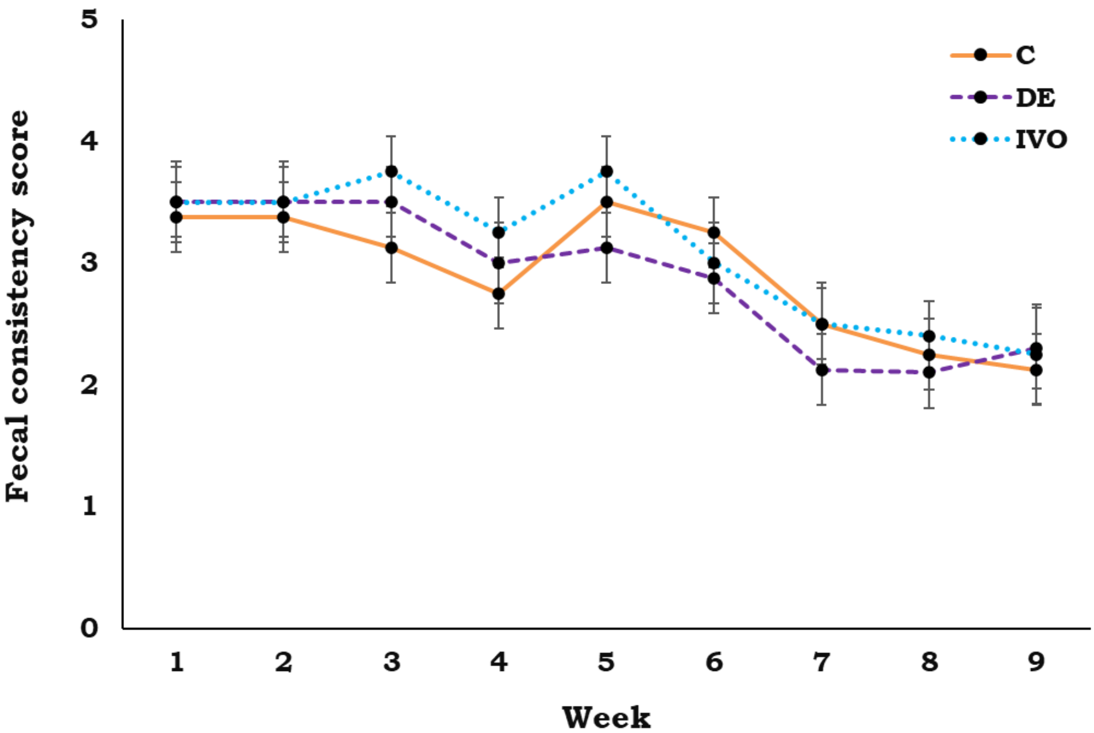

Figure 4: Effect of different treatments on the fecal consistency score of WS × BB lambs. Fecal consistency was scored on a 1-5 scale, whereby 1 indicated watery faeces and 5 indicated normal hard faeces as earlier described by Le Jambre et al. (2007). C=control; DE=Diatomaceous earth; IVO=Ivermectin.

Table 3 presents a summary of the behavioral observations of sheep subjected to various anthelmintic treatment methods. Sheep treated with ivermectin treatment spent less time grazing than those treated with DE and the control group with no anthelmintic ($P<0.05$). No differences were observed for time spent drinking and grazing/walking. Sheep receiving DE and ivermectin treatment spent less time lying down compared to the control group with no anthelmintic (P<0.05). Additionally, sheep in the control group spent more time ruminating and standing compared to DE and ivermectin-treated sheep (P<0.05). There were no differences in the average weight gain of sheep across all treatments. The average weekly weights of all sheep under all treatments fluctuated throughout the experiment.

Table 3: Average time spent (minutes/day) on different behavioral activities by sheep subjected to anthelmintic treatment methods.

Behavioral activity (min/day)	**Treatment**			**SE**
	C	DE	IVO	
Grazing	2.310[ab]	2.345[a]	2.300[b]	0.0140
Grazing/walking	1.713[a]	1.691[a]	1.770[a]	0.0339
Drinking	0.090[a]	0.039[a]	0.079[a]	0.0238
Lying down	0.319[a]	0.087[b]	0.121[b]	0.0544
Standing	0.592[a]	0.299[b]	0.434[ab]	0.0597
Ruminating	1.306[a]	0.809[b]	1.477[a]	0.0794

[abc] Means with different superscripts indicate significant differences at (P<0.05). C=control; DE=Diatomaceous earth; IVO=Ivermectin; SE=standard error.

Discussion

In this study, sheep behavior was observed in one flock to mimic Fiji's production system. Also, most farmers release their sheep to graze the lush undergrowth during the day and return them to the sheds in the evening; hence, observations in the study were recorded from 8 am to 5 pm. The health status of the sheep subjected to all treatments was assessed daily throughout the experimental trial with a veterinarian. All the sheep were healthy, with no mortalities recorded for all the sheep subjected to the treatments. One notable limitation of our study is the relatively small number of animals used, which may influence our findings' statistical power and overall robustness (Ahmed et al., 2013; Mthi et al., 2024). However, the insights gained into the study of feeding behavior and control parasites are valuable and can guide future research, contributing to a more comprehensive understanding of the subject areas.

The observation that sheep subjected to control had a higher fecal egg count than those in other treatments was expected. This is mainly because the sheep were not treated with any anthelmintic, making them more susceptible to internal parasite infestations from ingesting infective larvae on natural pastures. Overall, fluctuations in fecal consistency and fecal egg count over time may be attributed to sheep grazing in the same paddock for the duration of the experiment, leading to the re-infestation of parasites. Consequently, the nematode egg cycle continued in the sheep. The same paddock was used in this study to mimic small-scale farm settings in the Pacific Island nation of Fiji, where most farmers do not have enough land for rotational grazing. Our findings highlight the need for alternative methods to reduce the internal parasite load in sheep. Continuous or excessive use of conventional drugs, such as ivermectin, may lead to the development of resistance in the parasites.

In this study, *H. contortus* and *M. expansa* were found in weaner sheep, and *H. contortus* was the predominant gastrointestinal parasite. Kour et al. (2025) found that *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., and *Strongyloides* spp. were the common internal parasite species in sheep in various regions of the Pacific Island nation of Fiji. Surprisingly, we found no other internal parasite species in our study. We assert that the adaptability and prevalence of infective larvae vary with different geographical locations. In the experiment, sheep in the control group had higher *M. expansa* egg counts than those given DE. This suggests DE was effective in reducing the parasite. However, this was not the case with *H. contortus*. More studies are required to investigate whether the sharp edges of diatom shells of DE can penetrate all cell membranes of the parasite eggs.

According to Williams and Palmer (2012), there is a positive correlation between fecal consistency score and parasite load. Diarrheic feces may be an indication of high parasitic load compared to normal solid feces. In the study, a fecal consistency score of 5 was normal hard fecal pellets, while a score of 1 was watery/diarrhea. The sheep subjected to ivermectin and DE treatment had high fecal consistency scores, indicating that the level of nematode infection was low compared to the other sheep under the control treatment with low fecal consistency scores. The fecal consistency score was high in the early phases of the experiment and started to decrease with time for all the sheep under different treatments. It is not clear what caused the decrease in fecal consistency with time. We postulate that other factors, such as environmental temperatures and the state of gut physiology of digestion, might have caused the drop in fecal consistency scores (Roeber et al., 2013).

The observation that sheep subjected to DE treatment spent more time grazing compared to sheep subjected to control and ivermectin treatments was expected. This agrees with Slamova et al. (2011), who fed sheep diets with different inclusion levels of fossil shell flour (also known as DE) and reported an increase in feed intake. It is assumed that the DE might have prevented the sheep from utilizing nutrients. Therefore, sheep were not getting enough nutrients to meet their daily requirements, resulting in increased time spent grazing to compensate for the nutrient deficit in their bodies. Grazing/walking in the study resulted from sheep searching for palatable feed in the paddock. The observation that no differences were found in time spent grazing/walking among sheep subjected to different anthelmintic treatment methods was expected. According to Dwyer (2021), sheep are known to be highly social animals, and their behaviors are affected by the presence of other sheep. This could explain our findings as sheep movements were observed to be in a single group without splitting into subgroups or individual animals.

More time was spent standing and lying down for sheep in the control group compared to sheep subjected to DE and ivermectin treatment, which may result from a high level of parasite infestation. According to Grant et al. (2020), sheep with high parasite load have low energy, causing less active movements like standing and lying down, as observed in the study. The observed time spent standing for animals in the study can also be ascribed to changes in weather patterns, such as rainfall and high temperatures (De et al., 2017). Certain days of the experimental trial had rainfall followed by higher temperatures than other days, which forced the sheep to seek shade under a few trees in the paddock. After rainfall, the ground would be wet, so the sheep preferred not to lie down on the ground; therefore, more time was spent standing. We also postulate that time spent lying down or standing might be a result of rumen fill. We expected time spent ruminating to correlate to time spent grazing. However, this was not the case in our study. The inconsistent patterns in ruminating time may be attributed to individual variations among animals.

The findings on the average weight gain and weekly weights of sheep subjected to different anthelminthic treatments were not expected. However, our results align with a study conducted by Zhong et al. (2017), who treated ewes with an anthelmintic drug and reported no significant difference in body weight. Bernard et al. (2009) also found supplementing DE to have no effect on body weight in the goats. Since we used the same paddock for the duration of the experiment, we assume the sheep were likely re-infected with parasites present on pastures in the paddock, which may have affected their performance. Further research is required to investigate the extent of parasite infestation in the paddocks.

Conclusion

The study showed that diatomaceous earth influenced behavioral changes and the health of Wiltshire × Barbados Black Belly crossbred sheep. Internal parasite egg counts and fecal consistency varied across treatments. Diatomaceous earth was effective in reducing *M. expansa*. Time spent grazing, lying down, standing, and ruminating varied across treatments. Using diatomaceous earth would reduce the high cost and reliance on conventional anthelmintics. Future research should aim to include a more significant number of animals to validate and expand upon these findings. Additionally, it is crucial to implement rotational grazing and assess the level of parasite infestation in the paddocks.

Article Information

Funding. This work received no funding
Conflict of interest. The author declared no conflict of interest.
Authors contribution. A.S.P.: Data collection and manuscript writing; T.J.Z, A.D., and A.G.B.: Supervise and thoroughly review the manuscript.
Acknowledgments. We thank the Fiji National University for allowing us to conduct a research trial at the livestock farm. The assistance received in field data collection and laboratory work from laboratory technicians in the Department of Veterinary and Animal Science was appreciated.
Data availability. Not available
Publisher's Note. The claims and data contained in this manuscript are solely those of the author(s) and do not represent those of the GMPC publisher, editors, or reviewers. GMPC publisher and the editors disclaim the responsibility for any injury to people or property resulting from the contents of this article.

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