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Research Article

Growth Performance and Nematode Infestation in Grazing Lambs: Impact of Diatomaceous Earth

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Abstract | Gastrointestinal parasites cause substantial economic losses worldwide, affecting the United Nations' food security plans due to their detrimental influences on livestock productivity. The study evaluated the effect of supplementing weaner lamb diets with diatomaceous earth (DME) on weight gain and gastrointestinal parasite load. Forty-eight Dohne merino weaner lambs weighing 31.4 ±0.55kg were randomly assigned to 1 of 4 dietary treatments: 0% DME (T1), 2% DME (T2), 5% DME (T3), 10% DME (T4) of dry matter(DM) and twelve animals were used per treatment. Animals were allowed to graze at a Kikuyu pasture and were supplemented with lamb diet at 1.5% of body weight. Bodyweight was measured weekly, while faecal samples were collected every fortnight. Faecal egg count was performed using the McMaster and modified Stoll methods, while body weight was measured using an electronic scale. Treatments had a significant difference (P<0.05) in the weight gain of lambs. Improvement in the average daily weight gain performance of lambs was also observed at 10% DME inclusion levels, followed by T1 (31.96±7.787 kg), T2 (31.86±7.787 kg), and T3 (31.5±7.787 kg), respectively. Coccidia and roundworms were the only species found in the collected faecal samples. Significant differences were observed in roundworms (P<0.05) among treatment groups, but DME did not affect coccidia parasites. Overall, diatomaceous earth improved growth performance and reduced parasitic infestation up to a 5% inclusion level.

Keywords | Weight gain, Nematodes, Lambs, Diatomaceous earth

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INTRODUCTION

The gastrointestinal parasite has been fingered as one of the significant impediments to efficient and profitable livestock production, especially small stock (Waller et al., 1996; Chiejina et al., 2000; Eysker and Ploeger, 2000; Baker et al., 2003; Graham et al., 2007; Bennett et al., 2011; Tariq, 2018). The direct and indirect effects of parasites resulted in reduced production through reduced feed intake, decreased live weight gain and wool production, and sometimes death (Liu et al., 2003).

Mavrot et al. (2015) reported that the clinical signs in an animal depend on the parasite fauna present and the intensity of infection. Global losses to sheep due to gastrointestinal parasites are estimated at 1 billion dollars per annum (Sackett and Holmes, 2006). There are various ways to control gastrointestinal parasites, but every method of endoparasite control has certain shortcomings (Fernandes et al., 2019). As early as the late 1950s, using anthelmintic drugs became a recognized strategy to prevent nematode infections and improve livestock performance (Kaplan, 2004). Administration of pastureland, suitable stocking rate, and correct rotational grazing were other strategies to control gastrointestinal parasite infections in livestock (Akhtar et al., 2000; Newland et al., 2001).

Diatomaceous earth (DME) inclusion in ruminants was beneficial in reducing external parasites (Iatrou, 2010). However, its influence on internal parasites has not been fully explored in small ruminants. Hitherto, chemical-based anthelmintics have been considered one of the best methods in the fight against helminths (Shalaby, 2013). In addition, gastrointestinal nematodes have developed resistance against various anthelmintic drugs (Molento et al., 2011). Also, these chemicals are costly, not readily available in rural areas, and some might be toxic (Sutherland et al., 2010; Wagil et al., 2014). Furthermore, an increase in consumer awareness of drug residues in the food chain has resulted in farmers searching for an alternative organic control strategy to mitigate these constraints (consumer concern and anthelmintic drug resistance), as Cornejo et al. (2018) reported. Bwalya (2013) and Ikusika et al. (2019) observed that no toxins in food-grade DME are recognized as feed additives. Diatomaceous earth is a million diatomes originating from sea and lake plant algae (Ikusika et al., 2019). Diatomaceous earth as anthelmintics in livestock production is widely reported (Maurer et al., 2009; Bernard et al., 2009; Sokerya, 2009). Studies have suggested that adding food-grade DME to the livestock diet may offer benefits in mitigating gastrointestinal parasites, thereby improving performance (McLean et al., 2005; Köster, 2010; Ikusika et al., 2019). Ikusika et al. (2019b) reported that the inclusion of DME into the diets of Dohne Merino sheep, up to 4% of DME, improves the Animal's growth performance and health status. In contrast, Mclean et al. (2005) recommended a 2% inclusion rate for heifer diets. However, a paucity of scientific evidence supports its efficacy, especially in growing lambs.

Furthermore, conflicting results have been reported by Dolatabadi & Guardia (2011) in which the impact of DME products on gastrointestinal nematode infection did not always yield positive results in sheep, cattle, and goats. In light of the value placed by organic livestock producers in feeding DME and response to the need for alternatives to anthelmintics currently in use. The study evaluated the effect of DME on gastrointestinal parasitic load and the growth performance of lambs under a semi-intensive production system. Therefore, we hypothesized that supplementing varying levels of DME could reduce parasite load and increase growth performance in lambs.

MATERIALS AND METHODS

Institutional Animal Care and Use Committee Statement (IACUC)

Routine care and experimental protocols used in this study were approved by the Animal

Care and Use Committee of Dohne Research Institution, Stutterheim Eastern Cape, South Africa, and conformed to published guidelines for ethical conduct and animal science research reporting (Kilkenny et al., 2010).

Description of the study site

The feeding trial was conducted at the Campagna small stock Production section of Dohne Research Farm. The experimental farm lies on 32^0 29'S and $27^0$29'E, under Amahlathi Local Municipality, and the veld type is classified as Bhisho Thornveld of the Eastern Cape as described by Mucina and Rutherford (2006). The main grass species are *Eragrostis curvula, Eragrostis plana, Heteropogon contortus, Andropogon appendiculatus*. An area of eight hectares of Kikuyu (*Pennisetum clandestinum*) pastures, divided into four by two-hectare paddocks, was utilized for experimental grazing lambs. These pastures had been grazed by heifer that had previously tested positive for gastrointestinal parasites up to an average of 360g/faeces of eggs calculated from larva recovered in faeces cultures (Horak et al., 2004)

Experimental diets

A lamb meal diet was used in the current study, and the nutrient composition is shown in Table 1.

Table 1: The nutrient composition of the lamb meal diet before the addition of DME

Ingredient items	Percentage (%)
Crushed maize	17
Sorghum offal	52
Dried molasses	15
Cotton seed oil cake	13.4
Limestone	2.0
Sheep premix	0.25
Salt	0.35
Chemical composition	
DM%	89.92
CP%	13.49
CF%	23.51
Fat%	5.92
Me Mj/kg	8.87
NE Mcal/kg	0.78
NDF%	32.39

DM.: dry matter; CP: crude protein; NDF.: neutral detergent fibre; CF.: crude fibre; ME: metabolizable energy; NE: net energy

Analytical procedures

The Dry Matter (DM) content of the diets, orts and faecal samples was measured by drying samples in an air-forced oven at 135°C for 24 h (AOAC, 2002, method 930.15). Ash content was measured by placing samples into a muffle furnace at 550°C for 5h as described by AOAC (2005) method 938.08. Organic matter (OM) was measured as the difference between DM and ash (A) content. Nitrogen (N) was measured by the Kjeldahl method using Se as a catalyst, and crude protein (CP) was calculated as 6.25 × N. Gross energy (GE) was measured using a bomb calorimeter (C200, IKA Works Inc., Staufen, Germany). Ether extracts (EE) were measured by weight loss of the DM on extraction with diethyl ether in Soxhlet extraction apparatus for 8h as described by AOAC (2005) method 920.85. The crude fibre was determined by allowing the sample to boil with 1.25% dilute H_2SO_4, washed with water, and boiled with 1.25% dilute sodium hydroxide. After digestion, the remaining residue is crude fibre (method 978.10), as Thiex (2009) described.

Experimental animals and study design

Forty-eight (24 males and 24 females) weaned lambs with an average weight of 31.4 kg±0.55 between 11 - 12 months old were obtained from the Campagna production system, Dohne Research Farm in Stutterheim. The experiment had four treatments with twelve animals per treatment. Basal diets were supplemented with DE as follows: Treatment 1 (Control) = 0.0%, Treatment 2 = 2%, Treatment 3 = 5%, and Treatment 4 = 10% on a dry matter basis. All experimental lambs had access to clean water *ad libitum.* The selected animals would graze (Kikuyu pasture) from 0800 hours until 1500 hours, after which they were penned and offered supplementary diets (concentrate with DME) in a feeding trough. According to research conducted by Correa et al. (2018), Kikuyu pasture has 11.3% CP, 35.2% CF., 41.1% ADF and 70.1% NDF. Group feeding was employed in this research (animals in the same treatment form a group). The experimental animals were allowed two weeks of acclimatization before the commencement of the experiment. Seven days before the start of the investigation, the animals were treated against gastrointestinal parasites using Lexicon (an active ingredient is Levamisole HCl) at 3ml/10kg body weight. During this period, the anaemic status of animals was determined using the FAMACHA technique as described by Mpendulo et al. (2020). Faecal samples were collected fortnightly from the rectum using the middle finger into plastic bags. Samples were kept in a cooler box with ice at 4^0C and dispatched to Eastern Cape Veterinary Laboratory, Grahamstown, South Africa, and were analysed using the McMaster and modified Stoll methods as described by Crook et al. (2016). Body weights were taken and recorded weekly fortnightly using a RUUDWEIGH, KM-2E electronic weighing system (RUUDSCALE, Durbanville, South Africa)

Statistical analysis

Data on growth performance and faecal egg count were analyzed using PROC MIXED of SAS 9.4 (SAS Institute 2012). The Least Significant Difference was used to compare the means with the level of significance accepted at $p<0.05$. Pearson's *chi-squared* test was also used to determine the statistically significant difference.

$Y_{ijk} = + T_i + W_j + TW_{ij} + e_{ijk}$

Where T_i is the treatment effect (i= 1,2,3,4)

W_j is the effect of time on measurement (j = 7, 14,21, 28,35, 42, 49)

W_{ij} is the interaction between treatments and time

E_{ijk} is the error term

RESULTS

The growth rate of sheep was determined over the study period, and the results showed that lambs fed with the treatment 3 diet had the highest weight gain value during the feeding trial, while the control (T1) had the lowest weight gain value, as shown in Figure 1. Treatments significantly influenced the daily weight gain of lambs, with supplemented treatment having higher values than the control ($P < 0.05$).

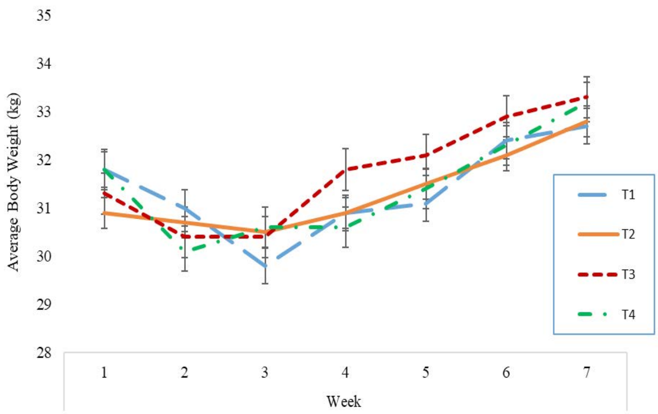

Figure 1: Weight changes in lambs supplemented with different inclusion levels of DME (means ± standard errors).

Table 2 shows the overall effects of treatments on TG, ADG, and the number of coccidial and roundworm eggs from lambs. Treatment three showed higher TG and ADG, followed by T3 and T4, while the control had the lowest TG and ADG values ($P<0.05$).

Table 2: Effects of varying levels of DME on growth performance of weaner lambs

Parameters	DME inclusion level				
	T1	**T2**	**T3**	**T4**	***P*-value**
Initial BW, kg	31.96±7.79	31.86±7.79	31.5±7.79	33.14±7.79	0.678
Final BW, kg	32.9[c]±0.908	33.8[ab]± 0.422	33.5[b]±0.964	34.6[a]±0.928	0.001
Total gain, kg	0.94[c]±0.281	1.94[a]±0.476	2.00[a]±0.303	1.46[b]±0.365	0.001
ADG, g/day	19.18[c]±3.150	39.59[a]±1.462	40.82[a]±3.340	29.80[b]±3.215	0.001

[ab]means with different superscripts within a row differ significantly (P<0.05); ADG: average daily gain; BW: Body Weight. T1 = 0%DME, T2 =2%DME, T3= 5%DME, T4= 10%DME.

Table 3: The anaemic status of lambs (%) fed various levels of DME.

	Treatments				
Anaemic status	**T1**	**T2**	**T3**	**T4**	**Total**
Non- anaemic (score 1 & 2	27.4%	33.3%	26.2%	36.9%	31.0%
Moderate (score 3)	53.6%	47.6%	57.1%	48.8%	51.8%
Anaemic (score 4 & 5)	19.0%	19.0%	16.7%	14.3%	17.3%

Chi-sqaure value = 3.778; p value = 0.707, T1 = 0%DME, T2 =2%DME, T3= 5%DME, T4= 10%DME.

Table 4: Weekly anaemic status of lambs (%) fed various levels of DME.

	Time (weeks)							
Anaemic status	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Total**
Non- anaemic	25.0%	41.7%	47.9%	37.5%	14.6%	29.2%	20.8%	31.0%
Moderate	47.9%	39.6%	41.7%	50.0%	60.4%	56.3%	66.7%	51.8%
Anaemic	27.1%	18.8%	10.4%	12.5%	25.0%	14.6%	12.5%	17.3%

Chi-square value = 25.848; p-value = 0.011; Figures in parenthesis are percentages. T1 = 0%DME, T2 =2%DME, T3= 5%DME, T4= 10%DME

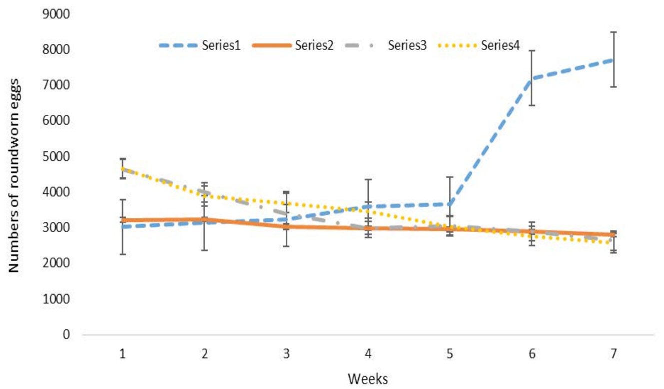

Figure 2: Graphical representation of the numbers of roundworms in lambs fed varying levels of DME for Seven weeks (means ± standard errors).

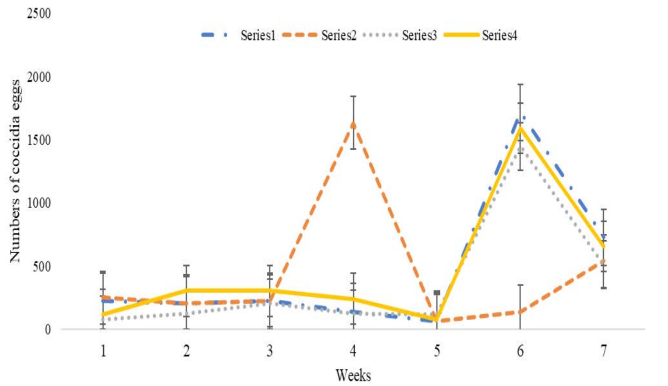

Figure 3: Graphical representation of the numbers of coccidia in lambs fed varying levels of DME for seven weeks (means ± standard errors).

Figures 2 and 3 showed the graphical pictures of the effect of different inclusion levels of DME on roundworms and coccidia. It showed that DME supplementation did not reduce the coccidia load but influenced the roundworm load (P<0.05). There was a significant difference in the mean count of roundworms among treatments (P<0.05). Lower roundworm counts were observed in treatments supplemented with DME compared to the control. However, the effect of DME supplementation was highest in T4 among the supplemented treatments. There was an interaction between treatments and week for roundworm faecal egg counts (P< 0.05). There was a linear relationship between the weeks and the faecal egg counts. As the weeks of the feeding of DME advance, the number of nematodes in the faeces decreases. Coccidia counts decreased (P<0.05) for T1 and T2 from weeks one to four, while they increased for T3 and T4. All treatments showed the least coccidia counts in week five except for T3 (P < 0.05), while the highest counts were observed in week six across all treatments. Overall, coccidia counts were higher for T1 compared to the supplemented treatments.

The anaemic status of lambs was also evaluated, and the results are presented in Table 3. The more significant (51%) proportion of lambs fell within the moderate anaemic group (score 3). There was a significant effect of treatments on the anaemic status of lambs (P < 0.05), and time affected the anaemic status of lambs, as shown in Table 4. There is a general increase with the time of animals requiring management decisions over deworming or not (51.8%), while the proportion of animals showing anaemic symptoms remained low (17.3%) over time.

DISCUSSION

Lambs-fed DME gained more weight than the control group in the current study. Similar results have been reported by Ikusika et al. (2019) and Koster (2013). Contrarily, Osweiler and Carson (1997) and Milton and Klopfenstein (2000) reported a slight to no significant difference in growth rates in finishing beef cattle. In addition, Urbano (2012) affirmed that DME inclusion in rabbit diets did not affect live weight gain. This study concluded that the DME contained adequate nutrients and minerals for optimum growth (Urbano, 2012).

Furthermore, DME inclusion levels of up to 1.5% in broiler diets did not affect broiler production parameters or carcass quality characteristics (Motolwana, 2016). This could be due to the lower DME inclusion used in the broiler experiment than in this current study. Different feed ingredients and animals used in the two experiments could also be the reason for contradictory results.

The reduction in nematode infestation through the inclusion of DME observed in the current study has earlier been confirmed by Osweiler and Carson (1997) and Bwalya (2013). However, reports by, Murphy (2010), Ahmed (2013), Reigate et al. (2021) and Paswan (2016) show that no significant differences were observed in faecal egg counts or average daily weight gain in different ruminant species. Nonetheless, the effects of DME on internal parasites have sparked great attention from animal specialists, and results are sometimes inconclusive. However, there is evidence of a positive impact, as shown in the current study. Not much work on sheep has been done (Murphy et al., 2010). The mode of action of DME is also still not cleared. It has been envisaged that the physical nature of DME results in the destruction of the parasite or abrasion after ingesting the microscopically fine and sharp edges of the diatoms physically destroys the internal parasite. Nevertheless, Murphy et al. (2010) concluded that this might be untrue, proposing that DME absorbs water, thereby absorbing its effects by absorbing liquid from the waxy layer of the exoskeleton, which is not practical with internal parasites not posing this layer. Despite all this, our current study confirms that DME positively affects daily weight gain and reduces nematode load in sheep.

In the current study, DME did not affect coccidian parasites. The observed trend in the increase of parasites with time could follow this parasite's natural cycle, which requires approximately 21 days (Reigate et al., 2021). Internal parasites are known to suppress appetite, thereby reducing daily feed intake and daily weight gain. Similarly, its impaired mineral retention reduces protein metabolism, reducing animal muscle growth and carcass quality. Diatomaceous earth largely depended on the selected product as it has been proven that the chemical composition of DME varies widely (Crook et al., 2016). The same study proposed that the lack of reliable data depends on the choice of diatomaceous earth. The current study also affirms this observation for growth and antihelminthic properties, particularly against roundworms. Surprisingly, the present study's threshold levels of coccidia acceptable in sheep were exceeded in some treatments, but the animals did not show any clinical symptoms. Taylor et al. (2016) reported that most sheep were infected with coccidia, but the parasites caused little or no damage. Our results also confirm this position and that the disease only occurs when animals are subjected to heavy infections (Hashemnia et al., 2014).

The experimental animals showed no signs of anaemia and body weight loss, although the parasitic load for some treatments was above the recommended thresholds. The barber pole worm causes anaemia, thereby reducing sheep and goats' economic value during the grazing season (Villalba et al., 2011). The worm, which feeds on blood, penetrates and lacerates the Animal's stomach (abomasum).

CONCLUSIONS

This study showed that diatomaceous earth improves sheep's weight gain and reduces parasitic load. Further studies are warranted to investigate the DME effects using molecular and other advance diagnostic tools.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

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DATA AVAILABILITY STATEMENT.

Data would be available on reasonable request from anyone.

NOVELTY STATEMENT

This study is unique and novel in its ability to provide natural substance as an alternative to chemical-based anthelmintics in animal production.

AUTHORS CONTRIBUTION

Mthi Siza and Nowers carried out conceptualization, investigation, writing, data collection and methodology; Ikusika O. and Thando Mpendulo did validation, final draft, editing and supervision, while Washaya did the statistical analysis of the research work.

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