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**Use of Diatomaceous Earth and Copper Oxide Wire Particles to Control
Gastrointestinal Nematodes in Lambs**

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Abstract

Anthelmintic resistance (AR) urges alternatives to control gastrointestinal nematodes (GIN). Copper oxide wire particles (COWP) are more efficacious when used with other dewormers and little is known on efficacy of diatomaceous earth (DE) to control gastrointestinal parasites. The objective was to examine the effects of DE and COWP on GIN control. Katahdin lambs ($n = 32$; ~ 150 d of age; 25.0 ± 1.8 kg) were randomly assigned to receive: 1) DE fed at an estimated 2% dry matter intake (with the assumption of moderate consumption of bermudagrass forage and provided supplement), 2) 1g COWP, 3) both 2% DE and 1g COWP, and 4) neither DE or COWP. Feces and blood were collected on d 0, 7, 14, 21 and 28 to determine fecal egg counts (FEC) and blood packed cell volume (PCV). Feces were cultured to determine GIN genera. Fecal egg counts were log transformed; data were analyzed as a 2 x 2 factorial arrangement using Proc Mixed of SAS with day as a repeated measurement. There was a mixed GIN population including 58% *Haemonchus contortus* and 30% *Trichostrongylus* spp. Fecal egg counts tended to be lower by d 28 in DE (175 eggs/g) than no DE (753 eggs/g) fed lambs ($P = 0.09$), but in general, were relatively low and no other differences among treatments detected. Packed cell volume tended to be higher in COWP than no COWP lambs by d 28 ($P = 0.10$), and no other differences detected. In conclusion, during a low GIN challenge, there may have been a small effect of DE on FEC, but no advantage observed in combination treatment.

Keywords: copper, diatomaceous earth, internal parasites, sheep

Introduction

There are multiple classes of anthelmintics, or “dewormers”, used in livestock to kill internal parasitic worms without harming the host. The improper management and use of them has brought about populations of nematodes, such as *Haemonchus contortus*, that show resistance to these dewormers in the USA (Kaplan, 2004; Howell et al., 2008; Crook et al., 2016). In addition to problems pertaining to resistance, consumers are becoming increasingly aware of the chemical contamination of meat and milk and are pushing for natural dewormers to be used instead (Ahmed et al., 2013). Due to these problems, many researchers are looking for alternative biological methods to rid gastrointestinal parasites from livestock.

Literature Review

Resistance and Its Consequences

There are many negative health consequences associated with high gastrointestinal parasite burdens. Gastrointestinal parasitism can lead to delayed growth, weight loss, reduced feed consumption, decreased milk production, low fertility and, in cases of massive infections, high mortality rates (Macedo et al., 2012). The principle parasitic offender in warm humid climates is *Haemonchus contortus*, which is responsible for major economic loss within goat and sheep production (Terrill et al., 2004). Three broad spectrum anthelmintics are commonly used to rid livestock of gastrointestinal parasites. One study found that amongst the farms tested, 82 percent had multiple resistance to at least two drug classes. In addition to this, 12 percent of the farms demonstrated total anthelmintic failure (Crook et al., 2016). *Haemonchus contortus* and *Trichostrongylus* spp. in Arkansas have developed resistance to all classes of anthelmintics (J.M.

Burke, personal communication). Coles et al. (2006) found that during anthelmintic treatment, burdens were greatly decreased, but a small number of the population survived due to adaptations to resist anthelmintic activity. The nematodes that survive are typically the most resistant and live to pass eggs carrying resistant genes through the host's feces. As the nematode life cycle continues, the pasture becomes increasingly contaminated with disease causing parasites that are largely resistant to anthelmintics.

Diatomaceous Earth

Some research shows that the ingestion of diatomaceous earth (DE), a natural siliceous substance that is formed from the skeletal remains of unicellular organisms, or diatoms, may offer a solution to rising anthelmintic resistance (Ahmed et al., 2013). This sedimentary deposit of low toxicity is known to be soft to the touch, but microscopically, the diatom particles that it is composed of have sharp edges. When diatomaceous earth is ingested by the nematodes' host, the microscopic shards are thought to use mechanical movements within the gut to cause injury to the cuticle of nematodes and ultimately lead to the dehydration then death of the adult parasites.

Diatomaceous earth is composed primarily of silicon dioxide and is safe to use as a feed additive for mammals. It is soft and crumbly to the touch and is textured much like flour. According to McLean et al. (2005), this fine dust has abrasive properties and can absorb lipids to about three times or more of a substance's particle mass. The effect of diatomaceous earth on intestinal parasites is unclear, but it has been suggested that the abrasive qualities of this powder act on invertebrates by mechanically piercing or scratching the outer cuticle of the organism, resulting in death by dehydration (McLean, et al., 2005).

The use of biological agents to control parasites has increased in recent decades. A study conducted by Ahmed et al. (2013) on the use of *Bacillus thuringiensis* and *Clonostachys Rosea*, and a commercial diatomaceous earth product examined the *in vivo* effect of each variable on fecal egg count (FEC) using Merino sheep. The subjects used were already naturally infected with *Haemonchus contortus*. For each group, one biological agent was added to the diet as a feed additive. One of the agents tested, diatomaceous earth, was added at a rate of two percent of the diet. Eggs per gram were not affected by any of the variables, including diatomaceous earth. Larvae per gram showed lower numbers of nematode larvae and larvae development was significantly different with fewer numbers of larvae developing. It was concluded that all of the biological agents studied have the potential to reduce counts of nematode larvae in sheep manure (Ahmed et al., 2013). Based on this study, diatomaceous earth appears to have little to no impact on nematode eggs but may potentially halt larval development and larvae per gram. If less larvae are excreted through feces, or if the larvae are unable to fully mature, there could potentially be fewer gastrointestinal parasites in pastures and therefore could lead to decreased fecal egg count long term. If research were to show that diatomaceous earth could be effective over time, there would still be the immediate threat of major parasite burdens and disease while waiting for the population of nematodes to decrease in numbers. If anthelmintics were used in addition to diatomaceous earth until nematodes are no longer a prevalent threat, the anthelmintics could potentially be pulled and diatomaceous earth could be used as a primary dewormer with the occasional dosage of anthelmintics to hinder resistance. While there is research available on the effectiveness of the biological agent, diatomaceous earth, on adult parasites, it has not been adequately published regarding its effects on gastrointestinal nematode infected small ruminants, and more studies need to be conducted.

While fecal egg count appeared to be unaffected by the dietary addition of diatomaceous earth in the previous study, results from McLean et al. (2005) suggested otherwise. McLean et al. (2005) carried out two studies—one utilizing yearling Welsh Black Heifers and the other utilizing single bearing pregnant ewes. Amongst the cattle there were three treatment groups receiving either an anthelmintic drench prior to turnout, addition of dietary diatomaceous earth, or no treatment (control). The same system was used to treat the pregnant ewes. Amongst the cattle, no significant differences were found in fecal egg count. Cattle in the untreated (control) group had significantly higher fecal egg counts ($P < 0.05$) at week seven compared to their counterparts in the anthelmintic drench group and cattle receiving diatomaceous earth had lower fecal egg counts by week seven than the control group, however significance was narrowly missed. In regard to the pregnant ewes that were observed throughout the study, it was concluded that diatomaceous earth may decrease gastrointestinal parasite burdens within grazing ruminants but needs to be studied over a more extensive time frame.

Copper Oxide Wire Particles

Another possible alternative for gastrointestinal parasite control, copper oxide wire particles (COWP), has been studied in the past, and has shown promising results, but requires further inquisition on interaction with other methods. Copper oxide needles are 476 – 548 μm in diameter and were developed to treat copper deficiency. When administered as a bolus, copper oxide needles are released from the capsule and lodge in the folds of the abomasum and are thought to act directly on the adult *Haemonchus contortus* but are not efficacious against intestinal genera. Due to blood-sucking nature of *Haemonchus contortus*, eliminating this species from the gut will increase packed cell volume (PCV) overall or reduce anemia.

A previous study by Bang et al. (1990b) mentions the bioavailability of copper in the GI tract associated with low pH levels. Lambs uninfected by gastrointestinal parasites have an abomasal pH of less than one. The copper oxide wire particles are insoluble in the abomasum at pH greater than 3.4. The same study suggests that it is possible that copper oxide wire particles cannot ease parasite burdens when they are extremely high due to the increased pH that occurs with infection. It is also important to note that when administering copper oxide wire particles in sheep with severe diarrhea, there is a possibility of the bolus passing through the animal without being retained within the abomasum (Burke & Miller, 2020). Despite these challenges, evidence using electron microscopy supported that copper oxide wire particles directly acts on *Haemonchus contortus* by fatally damaging its cuticle.

In a study conducted by Bang et al. (1990a), there was a 96 percent reduction in adult *Haemonchus contortus* as well as a 56 percent reduction in another abomasal species, *Teladorsagia circumcincta*, in lambs given copper oxide wire particles. Another study concluded that copper oxide wire particles treatment reduced fecal egg count, increased packed cell volume, and lowered abomasal gastrointestinal nematode population numbers in sheep and goats (Soli et al., 2010). For both species in this study, the copper oxide wire particle treatment was equally as effective at ridding the body of *Haemonchus contortus* with burdens of 67.2 percent and 85.8 percent lower amongst copper oxide wire particles treated lambs and kids, respectively. While these results are very promising, it is important to consider the risks associated with increasing the copper status in ruminants. Before administering copper in any form to lambs or goats, the natural levels of copper as well as any additive minerals that the small ruminants may have access should be considered. Copper oxide wire particles have reduced gastrointestinal parasites infections of *Haemonchus contortus*. Further studies should be conducted in which diatomaceous

earth and copper oxide wire particles are administered in combination to see if they decrease parasitic burdens or improve anemia when lambs receive both together similar to that that occurred when copper oxide wire particles was administered with albendazole (Burke et al., 2016). In that study, both products were less effective in reducing fecal egg count by themselves than when used in combination.

Materials and Methods

This experiment determined the effectiveness of diatomaceous earth and copper oxide wire particles on diminishing gastrointestinal parasite burdens in lambs. The objective of this study was to examine the effects of diatomaceous earth and copper oxide wire particles on gastrointestinal parasites infection in Katahdin sheep. Katahdin sheep (n=32) aged approximately 150 days and weighing 25 ± 1.8 kg were supplemented with 653 g corn/soybean meal and soyhull pellets per day with access to bermudagrass pastures. The lambs were randomly divided into 4 treatment groups (n = 8/treatment), but only 2 feeding groups, one with and one without diatomaceous earth. Thus, in each feeding group, half the lambs were administered 1 g copper oxide wire particles each and half untreated. The following treatments were administered. Feeding groups were rotated between two pastures to minimize and confounding effect of pasture. A 2 x 2 factorial design that considered the effects of administration of copper oxide wire particles on Day 7, and/or diatomaceous earth supplement on Days 0 through 14 with number of lambs used per cell can be found in Table 1. Every 7 days, a fecal sample was collected to determine fecal consistency/fecal score (FS; 1 = solid pellets and 5 = slurry) and fecal egg counts. A blood sample was collected from the jugular vein and spun in a centrifuge to determine blood packed cell volume. On days 0 and 21, body weight and condition (1 =

emaciated and 5 = fat) were recorded. A modified McMaster's method which uses a saturated salt solution with a sensitivity of 50 eggs for every 1 egg counted, was used to determine fecal egg counts (Whitlock et al., 1948). A pooled fecal sample from each group was used for coproculture to determine gastrointestinal parasite genera. Feces was cultured for 14 days at 26°C then baermannized to recover larvae (Pena et al., 2002). Larvae in water were cleaned of fecal debris by allowing larvae to settle, then removing top layer of water. Cleaned L3 stage larvae were shipped to Dr. James Miller at Louisiana State University for identification and proportion per 100 larvae.

Data Analyses

For this study, a 2 x 2 factorial design using Proc MIXED procedure of SAS (SAS Institute, Cary, N.C.) with day of study as a repeated measure was used. The model included copper oxide wire particles and diatomaceous earth treatments, day of study, and interactions. Fecal egg counts were log transformed [$\ln(\text{fecal egg counts} + 10)$], and untransformed least squares means presented.

Results

Cultured gastrointestinal parasites were 58% *Haemonchus contortus* and 30% *Trichostrongylus* spp. Mean fecal score was 3.0 ± 0.1 and was not influenced by treatment or time (Table 2). A fecal score greater than one may have been due to the presence of *Trichostrongylus* spp., *Strongyloides* and possibly *Coccidia* as well. Toward the end of the study, the mean weight increased for each treatment group. Lighter weights were recorded ($P = 0.087$) on day 0 for lambs receiving diatomaceous earth, which may be due to differences ($P = .0096$) in

initial weight of animals randomly assigned across treatments (Table 3). There was no diatomaceous earth x copper oxide wire particles interaction detected for fecal egg counts analyses ($P = 0.59$) so the main effects of diatomaceous earth and copper oxide wire particles were analyzed. Also, there was no effect ($P = 0.26$) of copper oxide wire particles or copper oxide wire particles x day ($P = 0.2$) on fecal egg counts, but there tended ($P = 0.09$) to be an interaction between diatomaceous earth by day.

Effect of Diatomaceous Earth and Copper Oxide Wire Particles on Fecal Egg Count

There tended to be a reduction in fecal egg count by days 21 and 28 in lambs fed diatomaceous earth. The mean fecal egg counts in the diatomaceous earth treatment on days 0, 7 and 14, were 567, 662 and 803 eggs/g, respectively. By day 21 of treatment, the mean fecal egg counts dropped to 537 eggs/g and continued to drop to a mean of 175 eggs/g by day 28. In contrast, mean fecal egg counts in the diatomaceous earth control group continued to increase throughout the study and reached a mean of 753 eggs/g by day 28. Therefore, there was a diatomaceous earth by day interaction ($P = 0.09$; Table 4; Figure 1). Lambs in treatment groups receiving copper oxide wire particles had a copper oxide wire particles bolus administered on day seven. The mean fecal egg counts in the copper oxide wire particles bolus treatment on days 0, 7 and 14, were 563, 707, and 365 eggs/g, respectively. By day 21 of treatment, the mean fecal egg counts were to 328 eggs/g and continued to drop to a mean of 140 eggs/g by day 28. In contrast, mean fecal egg counts in the copper oxide wire particles control group continued displayed higher numbers than the copper oxide wire particles treatment throughout the study and reached a mean of 787 eggs/g by day 28. No significant interaction was found between copper oxide wire particles treatment and sampling date, perhaps due to the mixed populations of

nematodes. The copper oxide wire particles likely acted on existing *Haemonchus contortus* present, but not other gastrointestinal parasites such as *Trichostrongylus* spp. Numerically, the mean fecal egg counts for lambs receiving copper wire particles was lower versus their control group lambs (140 versus 787), however, these differences were not significant ($P = 0.26$; Table 4; Figure 2).

Effect of Diatomaceous Earth and Copper Oxide Wire Particles on Packed Cell Volume

In lambs receiving diatomaceous earth, packed cell volume percentage increased over time ($P < 0.001$), from 24 at the initiation of the treatment, to 26 by day 28; however, this increase was not influenced ($P = 0.24$) by diatomaceous earth (Table 5; Figure 3). Packed cell volume tended to increase by days 21 and 28 in lambs treated with copper oxide wire particles ($P = 0.09$; Table 5; Figure 4). On day 0, packed cell volume was 25 percent as compared with 28 percent and 27 percent on days 21 and 28, respectively. This is possibly due to a targeting effect of copper oxide wire particles on *Haemonchus contortus*, which is a blood sucking nematode.

Discussion

Both diatomaceous earth and copper oxide wire particles have been researched in the past, but until now, have never been studied together to examine how the combined administration can affect parasitic burdens. For this study, we were interested in exploring the interaction of diatomaceous earth and copper oxide wire particles on gastrointestinal nematode infection in moderately infected Katahdin lambs.

The nematode species, *Haemonchus contortus*, is particularly prolific compared to other gastrointestinal nematode genera in Katahdin lambs. Untreated Katahdin lambs at weaning can

have fecal egg counts as high as 5,000 eggs/g or even considerably higher (Burke et al. 2010) At these higher levels, lambs may show signs of anemia if not tolerant or may not show signs of anemia if genetically tolerant. They may even self-cure. Thus, in this study with fecal egg counts of less than 1000 eggs/g, the PCV shows that these lambs are genetically tolerant. When PCV starts approaching 22 percent or less, it is an indication that the lamb is becoming anemic and needs medical intervention for gastrointestinal nematode infection. Throughout the course of this study, there were not any lambs with packed cell volumes below 24 percent. Thus, no intervening treatment was required to quickly correct problems pertaining to anemia as all subjects had healthy PCV percentages. Further research could be conducted on the effects of these treatments on varying species.

While there is some research available such as Soli et al. (2010) which showed that copper oxide wire particles treatment was just as effective against *Haemonchus contortus* in sheep as it is goats and McLean et al. (2005) which examined the effects of diatomaceous earth in cattle as well as pregnant ewes, the combined treatment of diatomaceous earth and copper oxide wire particles could be tested in goats and other species as well. A previous study conducted by Ahmed et al. (2013) used multiple agents to explore the possibilities of ridding gastrointestinal parasites in lambs. This same study also examined diatomaceous earth as a possible solution to rising anthelmintic resistance. Its results suggested that diatomaceous earth, along with the other agents tested, had no impact on fecal egg counts. However, diatomaceous earth —along with the other agents tested in this study—appeared to reduce the number of nematode larvae (LPG) and found significantly fewer numbers of larvae developing. Contrary to the results of Ahmed et al. (2013), the results of the current study indicated that there was a reduction of fecal egg counts by d 21 and d 28 in lambs fed diatomaceous earth, whereas in the control, fecal egg counts tended

to increase each day. McLean et al. (2005) writes of similar findings regarding animals receiving diatomaceous earth. Amongst the fecal egg counts of cattle observed in this study, no significant differences were found when comparing the untreated (control) group to the diatomaceous earth group. However, the fecal egg counts of the cattle receiving diatomaceous earth appeared to be reduced at a level in which significance was just barely missed. At week seven, McLean et al. (2005) recorded average fecal egg counts of 137 eggs per gram, 172 eggs per gram, and 404 eggs per gram for the anthelmintic drench treatment, the diatomaceous earth treatment, and the untreated (control) group respectively.

Although these studies found that it is possible for diatomaceous earth to reduce gastrointestinal nematode infection in sheep, more can be examined in regard to larvae per gram and larvae development post diatomaceous administration. If the larvae are unable to fully mature, there could potentially be fewer gastrointestinal parasites in pastures and therefore could lead to decreased fecal egg counts long term. Previous research conducted by Bang et al. (1990b) shows that copper oxide wire particles seem to act particularly well against the adult *Haemonchus contortus*. The results of the current study suggest the same. Lambs that received copper oxide wire particles were more resilient against *Haemonchus contortus* as evidenced by a tendency for higher packed cell volume likely due to the aforementioned effect of the copper oxide wire particles bolus treatments on a species of nematode that consumes blood. Past research also shows that while copper oxide wire particles have a profound effect on that particular species, it does not work so well to diminish fecal egg counts of intestinal genera. The fecal egg counts of animals receiving the copper oxide wire particles remained unaffected by their treatment. One could hypothesize that this may be due to the presence of *Trichostrongylus* spp., which copper oxide wire particles does not seem to act against. Another potential study

could use research published by Bang et al. (1990b) to create a more current study that examines the effects of copper oxide wire particles on gastrointestinal nematode infected lambs with differing abomasal pH. This would be useful as Bang et al. (1990b) writes that lambs with higher abomasal pH due to heavy parasite burdens do not process copper oxide wire particles as effectively as lambs with lighter infections. As previously mentioned, this is because of copper oxide wire particles' insolubility in the abomasum with any pH greater than 3.4.

Summary and Implications

There was previously no research widely available regarding the combined administration of diatomaceous earth and copper oxide wire particles. For this reason, our study combined the two and examined the effects. There was no diatomaceous earth by copper oxide wire particles interaction for any measure. We did not see the typical reduction in fecal egg count in response to the administration of copper oxide wire particles, likely due to a high proportion of *Trichostrongylus* spp. which do not respond to copper oxide wire particles. However, packed cell volume did improve after treatment. Diatomaceous earth tended to have a favorable effect on fecal egg count, but not packed cell volume, perhaps targeting either *Trichostrongylus* spp. or *Haemonchus contortus*. Fecal egg counts were relatively low at the start of the study. These lambs were genetically resistant to parasites and had not needed to be dewormed like other lambs in their contemporary group before the study began. A higher fecal egg count at start of study would have been optimal to test this strategy. A longer treatment period would allow time to more accurately measure the effects of both agents. Further testing of more highly parasitized animals may be warranted.

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Tables**Table 1**

Diatomaceous Earth (DE) and Copper Oxide Wire Particle (COWP) Treatments in a 2 by 2 Factorial Design.

Treatments*	No DE	DE
No COWP	8	8
COWP	8	8

Note. Administration of COWP on Day 7, and DE supplement on Days 0 through 14 with number of lambs used per cell.

Table 2

Effects of Diatomaceous Earth (DE) and Copper Oxide Wire Particles (COWP) on Fecal Score (FS).

Item	Con (No DE)	DE	Con (No COWP)	COWP
No. of Sheep	8	8	8	8
Day 0 FS	3	3	3	3
Day 7 FS	3	3	3	3
Day 14 FS	3	3	3	3
Day 21 FS	3	3	3	3
Day 28 FS	3	3	3	3

Table 3

Effects of Diatomaceous Earth (DE) and Copper Oxide Wire Particles (COWP) on Body Weight (Kg).

Item	Con (No DE)	DE	Con (No COWP)	COWP
No. of Sheep	8	8	8	8
Day 0 BW	26	24	25	25
Day 21 BW	30	27	29	28

Note. Standard error—D 0 Wt = 25.0 +/- 0.91 kg and 28.5 +/- 0.91 kg.

Table 4

Effects of Diatomaceous Earth (DE) and Copper Oxide Wire Particle (COWP) on Fecal Egg Counts (FEC; eggs/g

Item	Con (No DE)	DE	Con (No COWP)	COWP
No. of Sheep	8	8	8	8
Day 0 FEC	377	567	381	563
Day 7 FEC	500	662	456	707
Day 14 FEC	431	803	868	365
Day 21 FEC	525	537	734	328
Day 28 FEC	753	175	787	140

Note. FEC (eggs/g) across sampling dates by lambs given DE at 2% of the estimated daily dry matter intake D 0- 14, lambs not receiving DE (CONTROL) or 1 g COWP administered on D 7 or without COWP (CONTROL).

Table 5

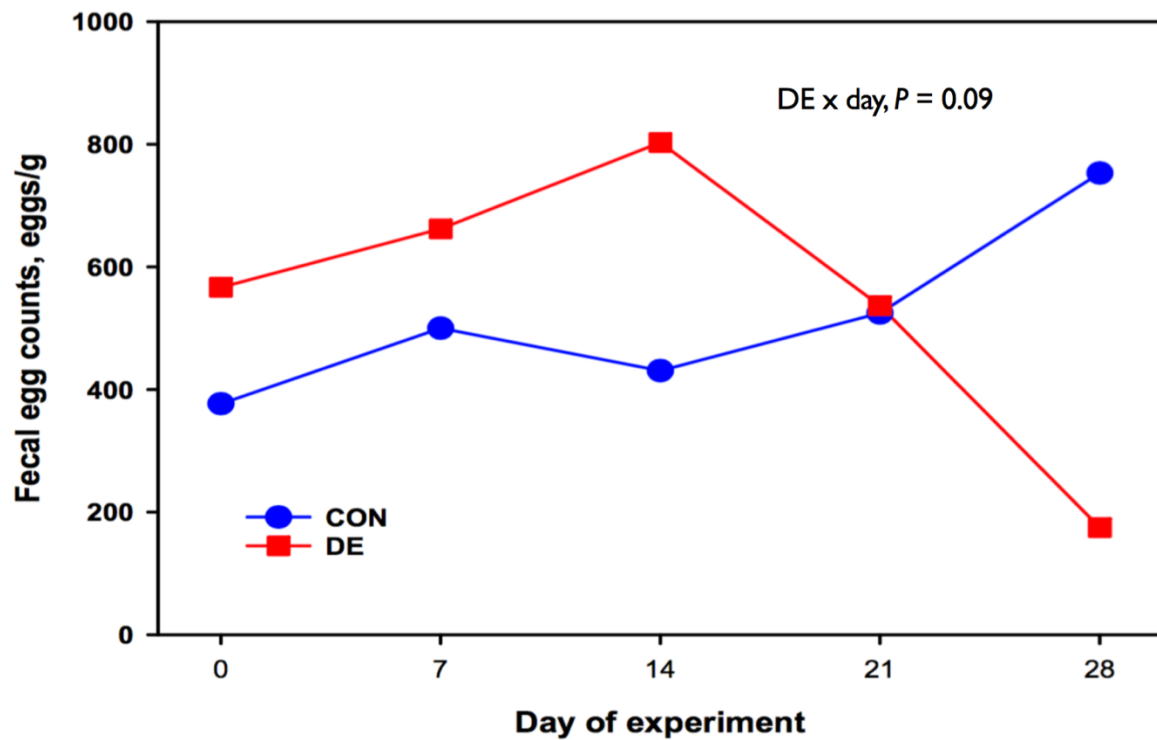
Effects of Diatomaceous Earth (DE) and Copper Oxide Wire Particle (COWP) on Packed Cell Volume (PCV) Over Time.

Item	Con (No DE)	DE	Con (No COWP)	COWP
No. of Sheep	8	8	8	8
Day 0 PCV	25	24	24	25
Day 7 PCV	25	24	24	25
Day 14 PCV	25	25	25	25
Day 21 PCV	27	26	26	28
Day 28 PCV	27	26	26	27

Note. Packed cell volume (%) across sampling dates by lambs given DE at 2% of the estimated daily dry matter intake D 0- 14, lambs not receiving DE (CONTROL) or 1 g COWP administered on D 7 or without COWP (CONTROL).

Figures**Figure 1**

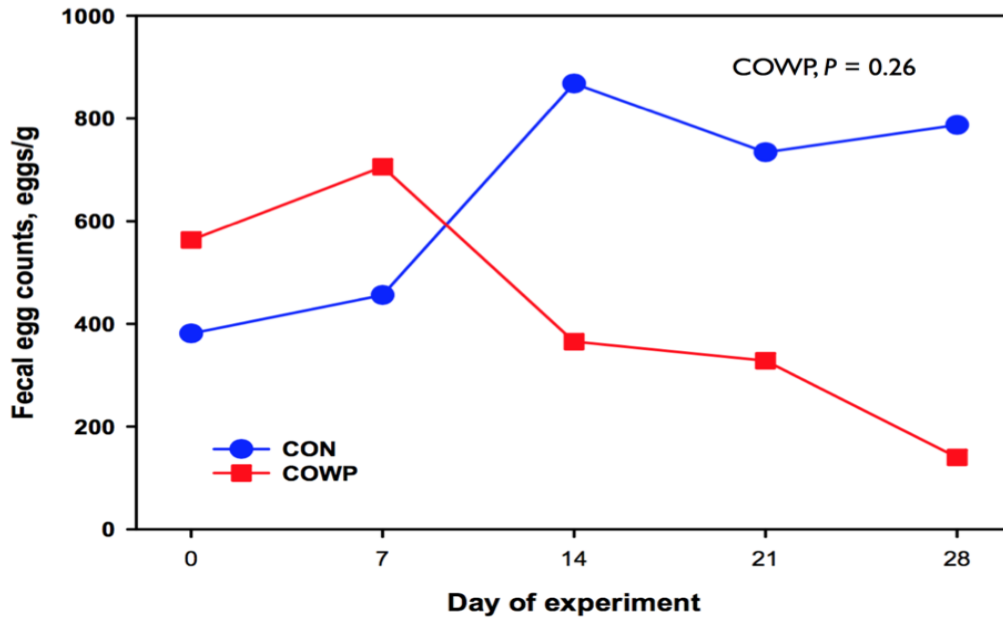
Effects of Diatomaceous Earth (DE) on Fecal Egg Counts.



Note. Effect on FEC across sampling dates by lambs offered diets with diatomaceous earth (DE) at 2% of the estimated daily dry matter intake or a diet without DE (CON)

Figure 2

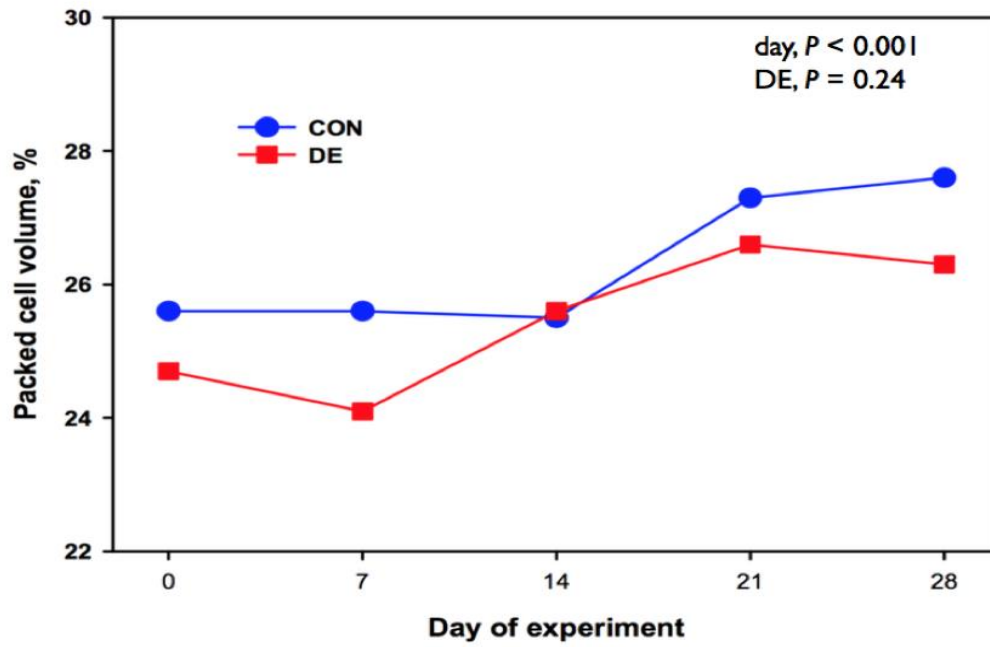
Effects of Copper Oxide Wire Particles (COWP) on Fecal Egg Counts.



Note. Effect of COWP across sampling dates by lambs given 1 g COWP administered on D 7 or without COWP (CON).

Figure 3

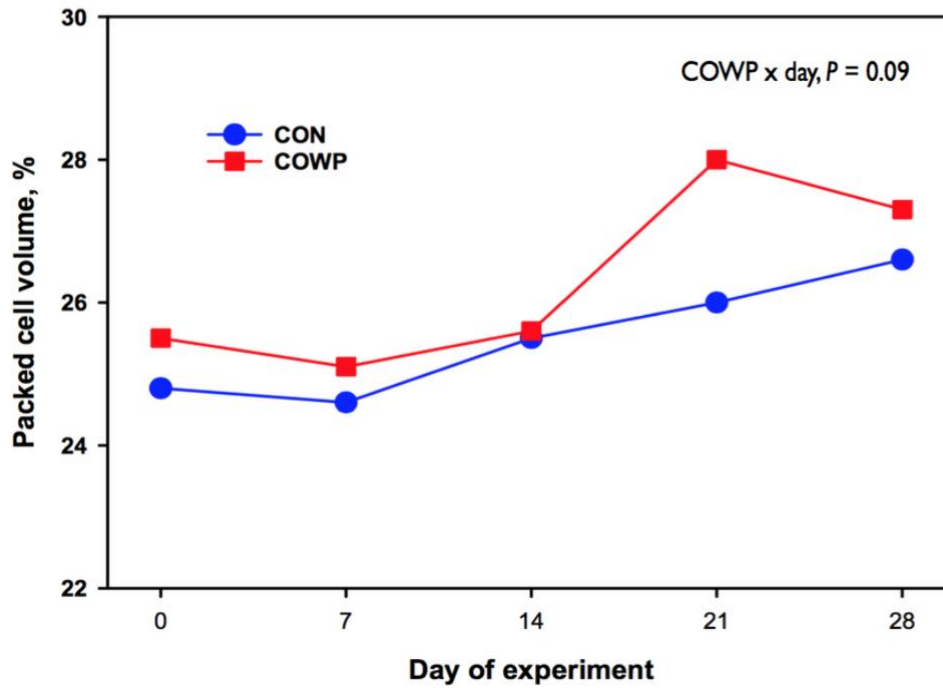
Effects of Diatomaceous Earth (DE) on Packed Cell Volume (PCV).



Note. PCV (%) across sampling dates by lambs offered diets with DE at 2% of the estimated daily dry matter intake or a diet without DE (CON)

Figure 4

Effects of Copper Oxide Wire Particles (COWP) on Packed Cell Volume (PCV).



Note. PCV (%) across sampling dates by lambs given 1 g COWP administered on D 7 or without COWP (CON).