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Interaction between copper oxide wire particles and *Duddingtonia flagrans* in lambs

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Abstract

An experiment was completed to determine if copper oxide wire particles (COWP) had any effect on the activity of the nematode-trapping fungus *Duddingtonia flagrans* in growing lambs. COWP has been used recently as a dewormer in small ruminants because of nematode resistance to anthelmintics. *D. flagrans* has been used to control free-living stages of parasitic nematodes in livestock. Katahdin and Dorper lambs, 4 months of age, were administered no or 4 g COWP (n = 24/dose) in early October 2003. *Haemonchus contortus* was the predominant gastrointestinal parasite during the trial, which was acquired naturally from pasture. Half the lambs from each COWP group were supplemented with corn/soybean meal with or without *D. flagrans* for 35 days. Fecal egg counts (FEC) and packed cell volume (PCV) were determined weekly between days 0 (day of COWP administration) and 35. Feces from lambs in each treatment group were pooled and three replicates per group were cultured for 14 days at room temperature. Larvae (L3) were identified and counted per gram of feces cultured. Treatment with COWP was effective in decreasing FEC, which remained low compared with FEC from lambs not treated with COWP. This led to an increase in PCV in these lambs (COWP × day, $P < 0.001$). Number of larvae was decreased in feces from lambs treated with COWP and *D. flagrans* between days 14 and 35 compared to the other groups of lambs (COWP × *D. flagrans* × day, $P < 0.003$). Percentage of larvae identified as *H. contortus* decreased in feces collected from lambs treated with COWP and *D. flagrans* between days 14 and 28 compared with other treatments (COWP × *D. flagrans* × day, $P < 0.05$). Other trichostrongyles were present and remained less than 7% in feces collected from control lambs. There was no adverse effect of COWP on the ability of *D. flagrans* to trap residual larvae after COWP treatment. With fewer eggs being excreted due to the effect of copper on *H. contortus*, and the additional larval reducing effect exerted by the nematode destroying fungus *D. flagrans*, the

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expected result would be a much lower larval challenge on pasture when these two tools are used together in a sustainable control strategy.

Keywords: Copper; *Duddingtonia flagrans*; *Haemonchus contortus*; Lambs

1. Introduction

Parasite resistance to anthelmintics (Miller and Craig, 1996; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003) threatens the viability of the small ruminant industry in many countries including the USA. This has stimulated a worldwide shift in parasite management strategies from an attempt to kill the worms with anthelmintic drugs to development of sustainable programs for individual farms to manage parasite problems, which involves an integration of control methods such as grazing management, dewormers, and animal selection (Miller and Waller, 2004).

One bolus of copper oxide wire particles (COWP) has been used to control *Haemonchus contortus* in sheep and goats (Bang et al., 1990; Chartier et al., 2000; Knox, 2002; Burke et al., 2004) for at least a 4-week period (Chartier et al., 2000; Knox, 2002). A biological control agent that has been used in small ruminants for parasite control is *Duddingtonia flagrans* (Githigia et al., 1997; Waller et al., 2001; Pena et al., 2002; Terrill et al., 2004). Resting spores (chlamydospores) of this nematode destroying fungus pass through the digestive tract of ruminant livestock and are deposited in the feces (Larsen et al., 1992, 1998; Larsen, 2000; Grønvold et al., 1993; Faedo et al., 1997). In feces, the fungus develops along with the larvae on pasture, trapping and killing the larvae, including *H. contortus* (Fontenot et al., 2003; Waghorn et al., 2003; Chandrawathani et al., 2004). Thus, pasture infectivity of *H. contortus* has been shown to be greatly reduced in sheep pastures with *D. flagrans* feeding (Fontenot et al., 2003; Chandrawathani et al., 2004).

Copper is known to be able to act as a fungicide, depending on concentration and pH in the environment. Therefore, if a COWP bolus is to be used in conjunction with fungal spore supplementation, there ought not to be any antagonistic effects on the growth and/or trapping activity of the biological control agent due to the concentration of the released copper in the faecal environment of faeces from sheep. The interaction between COWP and *D. flagrans* used for control of *H. contortus* in sheep has not been examined. The objective of this experiment was to determine if COWP had any effect on the ability of the nematode-trapping fungus *D. flagrans* to reduce larval development in feces from growing lambs.

2. Materials and methods

All experimental procedures were reviewed and approved by the Agricultural Research Service Animal Care and Use Committee in accordance with the NIH guide for the care and use of laboratory animals. Pain and stress to animals was minimized throughout the experimental period.

A 2×2 factorial design of no or 4 g COWP (Copinox; Animax Veterinary Technology, UK; in a gelatin capsule administered per os on day 0) and no or 5×10^5 spores/kg body weight *D. flagrans* was implemented with randomly assigned lambs ($n = 12$/cell) in early October 2003. Naturally infected Katahdin and Dorper lambs, 4 months of age, were blocked by breed, sex, and fecal egg count (FEC), which was determined 3 days before treatments were initiated. Lambs grazed bermudagrass pastures heavily contaminated with parasitic nematode larvae before and throughout the trial. Because there were two feeding groups, lambs grazed on different pastures and were rotated between the two pastures every 7 days. Fecal culture, from feces collected weekly throughout the trial, determined that the predominant gastrointestinal parasite was *H. contortus*. Lambs were supplemented with 500 g of corn/soybean meal (15.0% crude protein, dry matter basis) with or without a top-dressing of *D. flagrans* for 35 days. Trace-mineralized salt containing no copper (Land O'Lakes Sheep and Goat Mineral, Shoreview, MN, USA) and water were provided ad libitum. Lambs were weighed on days 0 and 35.

Blood and feces were collected every 7 days for determination of blood packed cell volume (PCV) and FEC, as determined by a modified McMaster technique (Whitlock, 1948), between days 0 (day of COWP administration) and 35. Lambs were dewormed with moxidectin (Cydectin®; 0.2 mg/kg oral administration) if PCV was less than 16%, which occurred on day 21 in five control and three fungus-fed lambs that had not been administered COWP and on day 28 in two fungus-fed lambs receiving no COWP.

Feces also were collected for fecal culture of larval nematodes. Feces collected per rectum from lambs of each treatment group were pooled, mixed thoroughly and three replicates of approximately 28 g (weights ranged between 12 and 50 g) were mixed with water to moisten and then mixed with sterile peat moss of equal volume. The mixture was loosely covered with aluminum foil, and left at room temperature. The culture was moistened and stirred three times weekly. After 14 days the cultures were wrapped in cheese cloth, and the cheese cloth immersed in warm water (approximately 38 °C), and baermannized overnight in a funnel with an attached culture tube. L3 larvae were collected from the culture tube, identified and counted per gram of feces cultured according to Pena et al. (2002).

Data were analyzed using the mixed models procedure of SAS (1996). The mathematical model used for PCV and FEC included COWP treatment, dietary *D. flagrans*, breed, sex, date, the interactions, and a repeated statement for date of measurement (Littell et al., 1996). Contrasts were determined using the PDIFF option (all probability values for the hypothesis) in SAS. FEC and number of larvae hatched were log transformed: ln (FEC + 1). Statistical inferences were made on transformed data and untransformed LS means were presented. Body weight of lambs was analyzed by GLM with COWP and fungus treatment, the interaction, sex, and breed. LS means and standard errors of the mean of all response variables were presented. Once lambs were dewormed, they were removed from the study.

3. Results

The use of COWP bolus decreased FEC from more than a mean of 5000 to 250 eggs/g (epg) within 7 days and FEC remained lower than in lambs not treated with COWP (COWP × *D. flagrans* × day, $P < 0.05$; Fig. 1A). The FEC of lambs fed *D. flagrans* without COWP treatment were lower on days 7 and 14 than those not fed *D. flagrans*, but FEC were similar after 21 days in both groups of lambs not treated with COWP. The FEC of ewe lambs was greater than wether lambs ($3378 > 2251 \pm 480$; $P < 0.03$) and there were no breed differences. The PCV of lambs that did not receive COWP declined over the 35 days

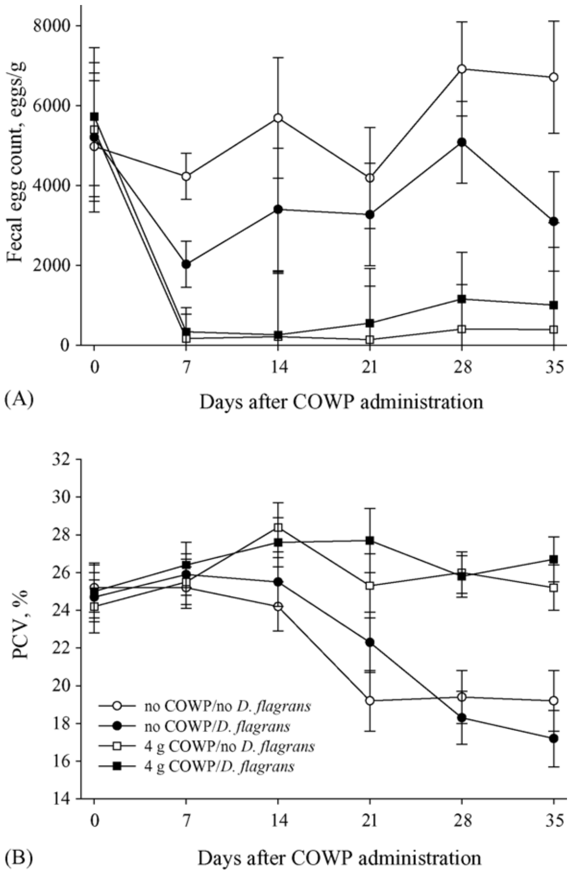

Fig. 1. Least squares means and standard errors of fecal egg counts (FEC; A) and packed cell volume (PCV; B) of lambs administered no (circles) or 4 g copper oxide wire particles (COWP; squares) and fed no fungus (open symbols) or *Duddingtonia flagrans* (closed symbols). COWP was administered on day 0. There was a three-way interaction between COWP, *D. flagrans* and day after COWP administration for the analysis of FEC ($P < 0.004$). Effect of COWP administration over time ($P < 0.001$) and *D. flagrans* over time ($P < 0.04$) was significant in the PCV analysis. Statistical inferences are presented for the log-transformed FEC and untransformed least squares means are plotted.

study compared with COWP-treated lambs (COWP × day, $P < 0.001$; Fig. 1B). The PCV of *D. flagrans*-fed lambs was greater on day 21 than control-fed lambs, but was otherwise similar (*D. flagrans* × day, $P < 0.007$). There were no differences in PCV between sexes or breeds.

Number of larvae developed was decreased in feces from lambs treated with COWP or *D. flagrans* between days 14 and 35 compared with lambs administered no COWP or *D. flagrans* (COWP × *D. flagrans* × day, $P < 0.003$; Fig. 2A). Larval development began to increase on day 21 in lambs not fed *D. flagrans*. Between days 7 and 35 number of larvae cultured was reduced in feces from *D. flagrans*-fed compared with feces from lambs not receiving *D. flagrans* and to a greater extent in COWP-treated lambs (no COWP/no *D. flagrans*, 1875; no COWP/*D. flagrans*, 308; COWP/no *D. flagrans*, 186; COWP/*D. flagrans*, 36 ± 131 larvae, $P < 0.001$).

Percentage of larvae identified as *H. contortus* decreased in feces collected from lambs treated with COWP and *D. flagrans* were similar to other treatment groups on days 0 and 7, but decreased between days 14 and 28 (COWP × *D. flagrans* × day, $P < 0.05$; Fig. 2B). There were no larvae present in feces cultured from the COWP/*D. flagrans* group collected on day 21. Percentage of *H. contortus* declined in COWP-treated lambs that did not receive *D. flagrans* after day 21. In lambs not treated with COWP percentage of *H. contortus* was nearly 100% with the exception of a decline in *D. flagrans*-fed lambs on day 21. Other trichostrongyles nematodes were present but remained less than 7% in feces collected from control lambs.

Weight gains were minimal during the 35 days trial and were similar among treatment groups (day 0, 26.7 kg; day 35, 27.5 kg). Wethers were heavier than ewe lambs ($P < 0.05$) and DO heavier than KA ($P < 0.05$).

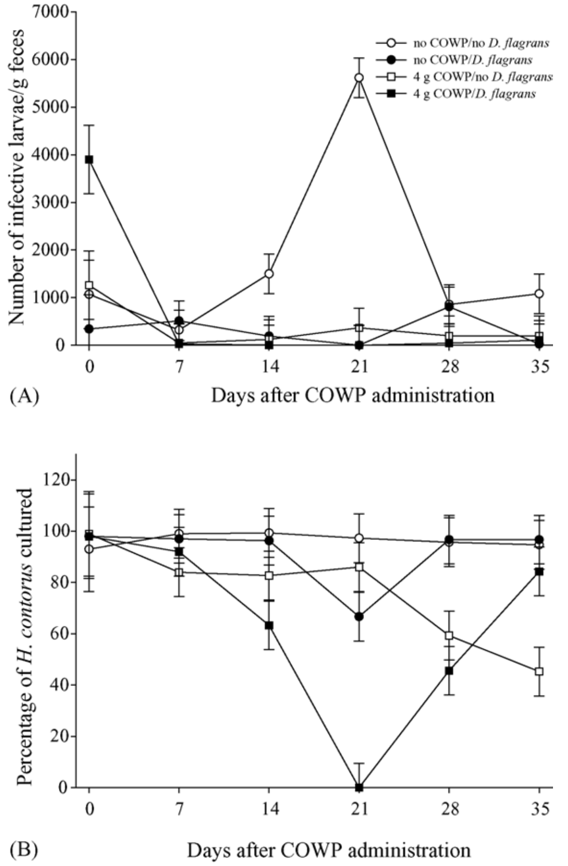

Fig. 2. Least squares means and standard errors of larval hatch from cultured feces (A) and percentage of *Haemonchus contortus* cultured from feces (B) from lambs administered no (circles) or 4 g copper oxide wire particle bolus (COWP; squares) and fed no fungus (open symbols) or *Duddingtonia flagrans* (closed symbols). COWP was administered on day 0. There was a three-way interaction between COWP administration, dietary *D. flagrans*, and days for change in larval hatch ($P < 0.03$) and percentage *H. contortus* cultured ($P < 0.05$). Statistical inferences are presented for the log-transformed larval hatch and untransformed least squares means are plotted.

4. Discussion

Heavy parasite loads and moderate forage quality likely contributed to minimal weight gains during the trial. A marked FEC reduction in COWP-treated lambs was similar to a previous study in this laboratory (Burke et al., 2004). COWP-treated lambs resisted further parasite infection for the duration of this trial, consistent with other reports in which animals were exposed to a constant challenge (Chartier et al., 2000; Knox, 2002). A reduction in percentage of *H. contortus* cultured in feces from COWP-treated lambs reflects a decreased proportion of *H. contortus* to other trichostrongyles species, demonstrating the targeted effect of COWP on abomasal rather than intestinal nematodes (Bang et al., 1990; Knox, 2002). The marked decrease in percentage of *H. contortus* on day 21 is a reflection of a reduced number of nematode eggs and no surviving larvae in lambs receiving the combination treatment. A reduction in percentage of *H. contortus* in feces from COWP-treated lambs not receiving *D. flagrans* on days 28 and 35 is not clear.

In lambs not receiving COWP, there may have been some initial advantage (day 7) in FEC reduction in *D. flagrans*-treated compared with lambs fed no fungus. However, between days 14 and 28 FEC were similar between these two groups of lambs. There was a numerical decrease in FEC in no COWP/*D. flagrans*-treated lambs on day 35 because of the deletion of two lambs that had been dewormed on day 28 that had high FEC on day of deworming (8050 and 13,750 epg). There appeared to be some advantage to dietary *D. flagrans* in PCV level on day 21, although PCV was similar between *D. flagrans* groups otherwise.

There was an 83% reduction in number of infective larvae in feces from *D. flagrans*-treated lambs compared with those not fed the fungus, consistent with other reports (Fontenot et al., 2003; Waghorn et al., 2003; Chandrawathani et al., 2004). There were very few eggs to culture in feces from COWP-treated lambs, though there appeared to be no adverse effect of COWP on the ability of *D. flagrans* to trap residual larvae after COWP treatment. As the effectiveness of the COWP declined by day 21, larval development increased in lambs not fed *D. flagrans*.

An immediate and delayed effect of reduced FEC after COWP administration, combined with the ability of *D. flagrans* to kill hatching larvae, will lead to very low pasture infectivity. Pasture infectivity was reduced after 18 weeks of feeding *D. flagrans* to mature ewes in Louisiana (Fontenot et al., 2003). For highly infective pastures it may take many months to break the cycle of *H. contortus* to a point in which a reduction in necessary animal treatment may be observed.

The small ruminant industry will become reliant on combination management strategies for *H. contortus* in the southeastern U.S. and other worldwide locations. Use of COWP and *D. flagrans* will greatly reduce infective larvae on pastures over time. Further combining these control methods with FAMACHA (Kaplan et al., 2004) and rotational grazing (Chandrawathani et al., 2004) may lead to minimal reliance on chemical dewormers. Because COWP was so efficient at reducing the egg counts and improving the PCV in lambs in this study and a previous study (Burke et al., 2004) it may not be necessary to combine use of both COWP and *D. flagrans* concurrently. However, to reduce the pasture infectivity to an absolute minimum for the initial and subsequent seasons, a logical approach would be to initially administer COWP followed by *D. flagrans* 4 weeks later.

COWP administration should not occur more than once every 12 months or longer because of the accumulation of copper in the liver (Langlands et al., 1983). In geographical areas where copper is prevalent in the environment or feed or mineral is contaminated with copper, use of COWP may lead to copper toxicity. Signs of copper toxicity include diarrhea, anorexia, dehydration, and hemolytic crisis (Aiello, 1998). Copper metabolism may differ between wool breeds and hair breeds, such as those used in the present study.

5. Conclusion

There was no adverse effect of COWP on the ability of *D. flagrans* to inhibit residual larval development after COWP treatment. There was a beneficial effect of treating lambs with both COWP and *D. flagrans*. With fewer eggs being excreted due to the effect of copper on *H. contortus*, and the additional larval reducing effect exerted by the nematode destroying fungus *D. flagrans*, the result would be an anticipated much lower larval challenge on pasture when these two technologies are used in a sustainable control strategy.

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