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Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs

J.M. Burke^{a,*}, J.E. Miller^{b,c,d}, D.D. Olcott^b,
B.M. Olcott^e, T.H. Terrill^f

^a Dale Bumpers Small Farms Research Center, USDA, ARS, Booneville, AR 72927, USA

^b Department of Pathobiological Sciences, School of Veterinary Medicine,
Louisiana State University, Baton Rouge, LA 70803, USA

^c Department of Animal Science, Louisiana State University, Baton Rouge, LA 70803, USA

^d Department of Veterinary Science, Louisiana State University, Baton Rouge, LA 70803, USA

^e Department of Veterinary Clinical Sciences, School of Veterinary Medicine,
Louisiana State University, Baton Rouge, LA 70803, USA

^f College of Agriculture, Home Economics and Allied Programs, Agricultural Research Station,
Fort Valley State University, Fort Valley, GA 31030, USA

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Abstract

The objective of the experiment was to determine the optimal dose of copper oxide wire particles (COWPs) to reduce infection of *Haemonchus contortus* in male lambs. Five to six-month-old hair breed lambs were housed on concrete and fed 450 (L; $n = 25$) or 675 g (H; $n = 25$) corn/soybean meal supplement and bermudagrass hay. In July, lambs were inoculated with 10,000 L_3 larvae (97% *H. contortus*; Day 0). Lambs were administered 0, 2, 4, or 6 g COWP on Day 28. Concentrations of copper in the liver were determined. There were no effects of supplement level on concentrations of copper in the liver and a linear relationship existed between COWP treatment and concentrations of copper in liver ($P < 0.001$). Least squares means of the 0, 2, 4, 6 g COWP treatments were 62.2, 135.7, 161.1, and 208.4 ppm ($P < 0.001$). Between Days 0 and 28, PCV declined and by Day 42, PCV of all COWP-treated lambs was markedly higher than control lambs and remained higher (COWP \times day, $P < 0.05$). By Day 21, PCV was greater in the H compared with the L group of lambs ($P < 0.001$). Within 14 days of COWP treatment FEC declined from more than 8000 eggs/g (epg) to less than 250 epg in all COWP-treated lambs (COWP \times day, $P < 0.001$). The numbers of

* Corresponding author. Tel.: +1 479 675 3834; fax: +1 479 675 2940.
E-mail address: jmburke@spa.ars.usda.gov (J.M. Burke).

H. contortus in the abomasum were greatly reduced in all COWP-treated groups of lambs and remaining nematodes were predominantly males. FEC were greater in L versus H supplemented lambs and values decreased to a greater extent in H lambs when treated with COWP (supplement \times COWP; $P < 0.02$). The 2 g COWP was effective in alleviating *H. contortus* infection and reducing number of egg-laying nematodes in the abomasum with the lowest concentration of copper in the liver of the COWP treatment groups. PCV values were more favorable for lambs fed the higher level of supplement, especially when FEC were greater than 8000 epg.

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1. Introduction

The small ruminant industry is threatened world-wide by parasitic nematode populations that have become increasingly resistant to anthelmintics. This has led to a search for alternate means of combating these parasites. USDA (2001) identified stomach or intestinal worms as the predominant disease condition present in 74% of the 1101 sheep operations surveyed. The most detrimental nematode accounting for health and economic losses is *Haemonchus contortus*, which is a blood feeder. In the southeastern US, this nematode represented 75–80% of the total fecal nematode egg output and worm burden (Mortensen et al., 2003; Miller et al., 1998). Nematode resistance to all three classes of anthelmintics has been reported (Hembry et al., 1986; Miller and Barras, 1994; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003).

Nutrition plays a role in tolerance to infection with *H. contortus* (Coop and Holmes, 1996; Coop and Kyriazakis, 1999). Immune response of lambs fed a high protein diet acted to decrease parasite infection (Strain and Stear, 2001). Lambs can be managed to some degree for internal parasites by feeding an increased level of supplement.

Copper oxide wire particles (COWPs) have been used to reduce *H. contortus* infection in sheep and goats. Worm burdens of *H. contortus* were reduced in growing lambs administered 2.5 or 5 g COWP (Bang et al., 1990; Knox, 2002). *H. contortus* worm burden and fecal egg counts (FEC) also were decreased in mature goats administered 4 g COWP (Chartier et al., 2000). The COWP pass from the rumen to the abomasum where it is retained for at least 32 days (Dewey, 1977). The COWP release free copper in the abomasum, increases concentrations of copper in the abomasal digesta and subsequently stores in the liver (Dewey, 1977; Bang et al., 1990). This concentration of soluble copper creates an environment that somehow affects the worms' ability to remain established and they are expelled (Chartier et al., 2000).

There is a growing data base on the effectiveness of alternate technologies/strategies which could constitute major components in a sustainable worm control program and COWP is one that can be immediately applicable. The objective of the current experiment was to determine the effectiveness of 2, 4, and 6 g COWP dosages to reduce infection of *H. contortus* in male lambs fed at a low and high level of grain supplement.

2. Materials and methods

2.1. Experimental protocol

Fifty (15 Katahdin, 16 Dorper, and 19 Dorper crossbred) ram and wether lambs were weaned between 60 and 90 days of age, dewormed with moxidectin (Cydectin[®]; 0.2 mg/kg oral administration) and housed on concrete for 83 days before starting the COWP treatment and during study period. During that time, lambs were fed 225 (first 41 days) to 450 g (last 42 days; L; $n = 25$) or 450 (first 41 days) to 675 g (last 42 days; H; $n = 25$) grain supplement (15.0% crude protein, dry matter basis) with free choice access to bermudagrass hay, trace mineral and water. Lambs had been randomly assigned to dietary treatments, blocked by breed and sex. On Day 0 lambs were inoculated with 10,000 L₃ (97% *H. contortus*). Lambs continued on the L and H level of supplement. On Day 28, lambs from each supplement group were blocked by FEC determined the previous week (separated into high, medium, and low FEC relative to the group) and randomly assigned to receive 0, 2, 4, or 6 g COWP (Copinox; Animax Veterinary Technology, UK; $n = 6$ per 0, 4, and 6 g COWP treatment and $n = 7$ for the 2 g COWP treatment for each supplement group) in a gelatin capsule administered per os. Blood and feces were collected every 7 days for determination of packed cell volume (PCV) and FEC, as determined by a modified McMaster technique (Whitlock, 1948), between Day 0 and day of slaughter on Day 54 ($n = 25$) or 63 ($n = 24$). A control lamb fed the L supplement died on Day 34 likely from haemonchosis (PCV from Day 28 was 21). Nematodes were recovered from the abomasum and small intestine at slaughter and counted and identified as described by Miller et al. (1987). The recovery procedure was modified in that organ contents were brought to a volume of 5 L and a 500 ml aliquot was taken and preserved with formalin. A liver sample was collected at slaughter and the wet weight concentration of copper was determined (Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, LA). Wet and dry weights of liver in 6–9-month-old lambs were 1.65 and 0.48%, respectively (van Ryssen, 1980). Therefore, copper concentration of dry weight is approximately 3.44-fold greater than wet weight. The pH of the contents of abomasal digesta was determined within 30 min after slaughter. Body weight was determined on Days 7, 28, and 49.

All experimental procedures were reviewed and accepted 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.

2.2. Statistical analysis

Data were analyzed using the mixed models procedure of SAS (1996). The mathematical model used for PCV, FEC, and body weight included COWP treatment, diet, breed, sex (ram or wether), the interactions and a repeated statement for date of measurement (Littell et al., 1996). Contrasts were determined using the PDIF option (all probability values for the hypothesis) in SAS. FEC and worm count data were log transformed: $\ln(\text{FEC} + 1)$. Statistical inferences were made on transformed data and untransformed LS means were

presented. Least squares means and standard errors of the mean of all response variables were presented.

3. Results

The PCV of lambs consuming the L level of supplement decreased to a greater extent in response to the parasite challenge compared with lambs consuming the H level of supplement (diet \times date, $P < 0.001$; Fig. 1A). FEC tended to be greater in the L compared with the H group of lambs (2923 ± 300 versus 2259 ± 307 epg; $P < 0.07$; Fig. 1B). PCV declined and was similar among COWP treatments between Days 0 and 35 (Fig. 2A). On Day 42, 14 days after COWP treatment, PCV remained low in the control group of lambs and increased in the 2, 4, and 6 g COWP-treated lambs (COWP \times date, $P < 0.03$). FEC were similar among all COWP treatments between Day 0 and 28 and increased in response to the parasite challenge by Day 21 (Fig. 2B). Seven days after lambs received COWP treatment, FEC was reduced in the 2, 4, 6 g group of lambs compared with the 0 g group of lambs ($P < 0.001$). FEC were lower in the 4 and 6 g COWP-treated lambs on Day 35 compared with the 2 g treatment ($P < 0.02$) and lower in the 4 g compared with the 2 g treated lambs on Day 42 ($P < 0.02$). FEC were similar among the COWP-treated groups of lambs on Days 49 and 56.

Total number of *H. contortus* in the abomasum was reduced in COWP-treated lambs (0 g: 1415 ± 175 ; 2 g: 144 ± 153 ; 4 g: 90 ± 168 ; 6 g: 92 ± 174 ; $P < 0.001$). Both male ($P < 0.003$) and female ($P < 0.001$) nematodes were reduced in COWP-treated lambs, but percentage of females was reduced to a greater extent (0 g: 42.2 ± 8.1 ; 2 g: 13.0 ± 8.4 ; 4 g: 10.4 ± 9.4 ; 6 g: 11.0 ± 8.6 ; $P < 0.008$). The stage of *H. contortus* was primarily mature (only two 0 g and two 2 g COWP lambs found with immature nematodes). Only one lamb (2 g COWP, L feed supplement) harbored *Trichostrongylus axei* in the abomasum. Abomasal worm counts were similar between dietary treatments. Small numbers of *Trichostrongylus* and *Nematodirus* were found in the small intestine, but there were no differences between treatment groups.

There was a linear effect between dose of COWP and concentration of copper in the liver ($y = 74.6 + 22.8x$, where y = copper concentration in the liver, mg/kg wet weight and x = dose of COWP; $P < 0.001$; $R^2 = 0.54$). As dose of COWP increased, concentrations of copper in the liver increased ($P < 0.001$; Table 1) and there was no effect of feed supplement.

Table 1

Concentration of copper measured from the liver collected at slaughter in lambs treated with 0 ($n = 11$), 2 ($n = 14$), 4 ($n = 12$), or 6 ($n = 12$) g copper oxide wire particles (COWP)

COWP dose (g)	Liver concentration of copper (mg/kg wet weight)				
	LS mean	S.E.	Minimum	Maximum	Dry weight ^a
0	62.2	14.7	31.7	116.9	217
2	135.7	13.0	53.7	194.2	467
4	161.1	14.0	87.1	309.3	554
6	208.4	14.0	131.4	298.2	717

^a Conversion of LS mean of wet weight to dry weight ($\times 3.44$).

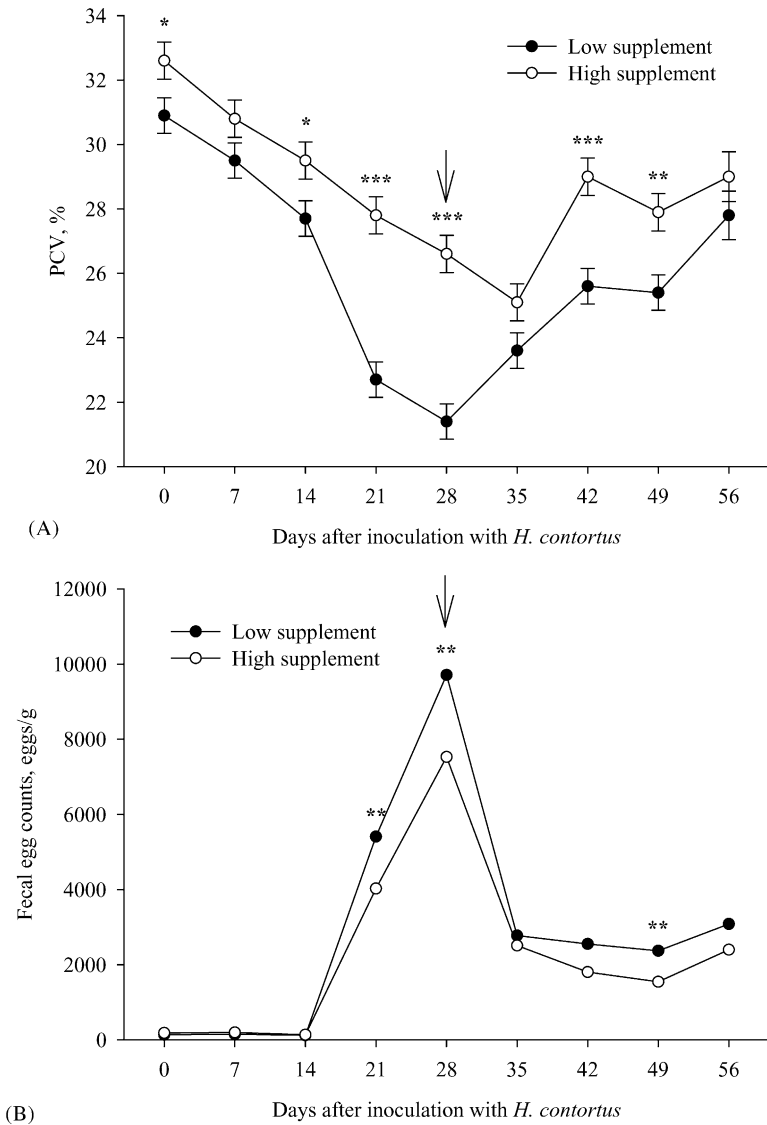


Fig. 1. Least squares means and standard errors of packed cell volume (PCV; A) and fecal egg counts (FEC; B) of lambs fed low supplement (450 g corn/soybean meal supplement per day; closed circles) or high supplement (675 g supplement per day; open circles). Arrow represents day of copper oxide wire supplement (0, 2, 4, or 6 g). Lambs were inoculated with 10,000 L₃ (97% *Haemonchus contortus*) on Day 0. Effect of dietary treatment over time was significant on PCV ($P < 0.001$) and tended to be significant on FEC ($P < 0.07$). Difference between dietary treatments for a particular time point represented by a single ($P < 0.05$), double ($P < 0.01$) or triple asterisk ($P < 0.001$).

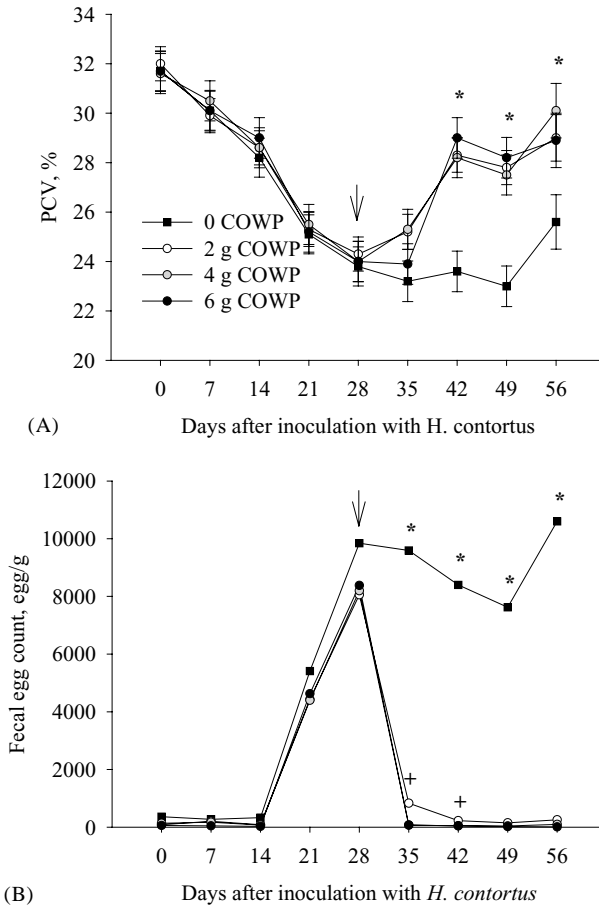


Fig. 2. Least squares means and standard errors of packed cell volume (PCV; A) and fecal egg counts (B) of lambs treated with 0 (closed squares), 2 (open circles), 4 (gray circles), and 6 (closed circles) g copper oxide wire particles (COWP) on Day 28, indicated by arrow. Lambs were inoculated with 10,000 L₃ (97% *Haemonchus contortus*) on Day 0. Arrow represents day of copper oxide wire supplement (0, 2, 4, or 6 g). There was a significant effect of COWP treatment over time ($P < 0.001$). Difference between lambs treated with 0 g compared with 2, 4, or 6 g COWP represented by an asterisk ($P < 0.001$) and difference between lambs treated with 2 g compared with 4 and 6 g COWP represented by a plus sign ($P < 0.05$).

The range of copper concentrations of the liver are reported in Table 1. The pH of abomasal digesta was similar among COWP-treated lambs and between dietary treatments (2.94 ± 0.22).

Body weight was greater in the H compared with L fed lambs on Day 7 ($42.3 \pm 1.9 > 32.8 \pm 1.6$ kg), 28 ($42.2 \pm 1.8 > 33.5 \pm 1.5$ kg), and 49 ($46.1 \pm 1.8 > 37.6 \pm 1.6$ kg; diet, $P < 0.002$). There was no effect of COWP treatment on body weight ($P = 0.75$).

There was one lamb on the 2 g COWP treatment that may have received either a partial or no dose at the time of treatment. FEC was somewhat reduced after treatment (from 7400 to

1600 epg 14 days later) and PCV increased (from 23 to 33% 7 and 14 days after treatment). But, concentration of copper in the liver was similar to control lambs (53.7 mg/kg) and abomasal numbers and percent female of *H. contortus* were relatively similar to control lambs (900 and 25%, respectively).

4. Discussion

The data clearly show that infection with *H. contortus* was reduced by treatment with all doses of COWP examined. An additional 7 or more days was required for the 2 g COWP compared with the 4 or 6 g COWP to reduce FEC from more than 8000 epg to less than 250 epg. This reduction in FEC was consistent with Knox (2002), where lambs were exposed to a trickle infection with *H. contortus* and treated with 2.5 g COWP when FEC were low. FEC of untreated lambs then increased to approximately 25,000 epg and treated lambs remained below 2500 epg.

Abomasal worm numbers in the current study were consistent with a reduced level of infection with *H. contortus*. The impact of the reduced percentage of female *H. contortus* in COWP-treated lambs would translate to even further reduction of pasture contamination. There were no differences in worm counts among the doses of COWP and no effect of COWP on number of nematodes in the small intestine. Abomasal and small intestinal worm counts were similar between dietary treatments as were FEC on Day 56.

No signs of copper toxicity were apparent in any of the lambs treated with COWP, although concentrations of copper in the liver in lambs treated with 4 and 6 g COWP were above the normal range of 25–500 mg/kg dry matter (Whitelaw et al., 1982). It is well established that COWP can be used to treat copper deficiency in sheep (Dewey, 1977; Whitelaw et al., 1980; Suttle, 1981, 1987). Ewes given doses of COWP as high as 10 g did not show signs of copper toxicity although liver concentrations of copper were elevated (Suttle, 1987). Bang et al. (1990) used a dose of 5 g COWP in 10-week-old lambs without clinical signs of copper toxicity.

It is imperative to understand the copper status of the flock before recommendations can be made to producers on the use of COWP. Factors that influence copper status include dietary sources of copper (water, forage, feeds, etc.), antagonistic interactions of dietary minerals (molybdenum, sulphur, iron, zinc in feed, mineral, or water), and other environmental sources. Use of COWP should not exceed one dose per 12 months because of the accumulation of copper in the liver. Langlands et al. (1983) reported elevated hepatic copper concentrations for more than 8 months in mature ewes administered COWP. Breed differences may also influence flock susceptibility to copper toxicity. Liver concentrations of copper in the North Ronaldsay breed of sheep were increased in response to COWP treatment more so than the Cheviot breed (Suttle, 1987) and Scottish Blackface were more susceptible to copper toxicity than Finnish Landrace sheep (Suttle, 1977).

The reduction in nematode infection (greater PCV, lower FEC) in lambs on the H supplement during heavy infestation is consistent with previous studies. Roberts and Adams (1990) demonstrated reduced FEC in lambs receiving a trickle infection of *H. contortus* on a higher level of supplement. Similarly, researchers reported decreased FEC (Datta et al., 1999) and increased immune response to *H. contortus* (Strain and Stear, 2001) in lambs

consuming higher protein diets. The COWP supplement had no further effect on level of infection in the H supplemented lambs.

It cannot be expected that all animals will receive the full dose effect of COWP. The one lamb (2 g group) that had a decrease in FEC and increase in PCV appeared to be responsive to treatment, but *H. contortus* count, percent females and liver copper concentration were more in line with the control group. This suggested that the amount of COWP that reached the abomasum was not sufficient to substantially affect worm burden or liver accumulation.

A lack of effect on body weight gains in association with the COWP treatment and reduced nematode infection likely reflects environmental stressors that reduced rate of gain during the study which took place during the hottest part of summer. The higher body weights of the H compared with L lambs on days examined was associated with extended feeding period before and during the current trial.

5. Conclusion

The use of 2 g COWP can be used safely to reduce infection of *H. contortus* in 6-month-old hair breed lambs. No signs of copper toxicity were observed in lambs treated with 4 and 6 g COWP, but concentrations of copper were elevated to levels that could predispose lambs to the disease. Over time, copper toxicity may occur. The higher level of supplement decreased the anemia caused by haemonchosis and recovery was quicker after the treatment with COWP.

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