



## Evaluation of multiple low doses of copper oxide wire particles compared with levamisole for control of *Haemonchus contortus* in lambs

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### Abstract

High levels of anthelmintic resistance in gastrointestinal nematodes (GIN) of small ruminants have created the need for alternative approaches to parasite control. Copper oxide wire particles (COWP; 2 g) have proven effective in decreasing GIN infection in lambs. However, the risk of copper toxicity has limited the usefulness of this approach. Recently, smaller doses (0.5 and 1 g) have proven effective in GIN control, reducing the risk of toxicity. The objective of this study was to examine the effectiveness and risk of toxicity using multiple small doses of COWP for GIN control in lambs between weaning and market weight. Dorper crossbred ram lambs were orally administered levamisole (Levasol, 8.0 mg/kg;  $n = 8$ ), 0.5 g ( $n = 9$ ), or 1 g COWP ( $n = 9$ ) at weaning (Day 0;  $118 \pm 2$  days of age; late May 2005) and again at 6-week intervals for a total of four treatments. A pooled fecal culture determined that *Haemonchus contortus* was the predominant gastrointestinal parasite at weaning. Lambs grazed bermudagrass pastures and were supplemented with up to 500 g corn/soybean meal and free choice trace mineralized salt. Fecal egg counts (FEC), packed cell volume (PCV), and plasma aspartate aminotransferase (AST) activity were determined every 14 days and lambs weighed every 28 days. GIN infection reached a peak at Day 42 (high FEC, low PCV). COWP effectively reduced FEC on Days 0 and 42 compared with the previous week, but did not reduce FEC on Days 84 and 126 (treatment by time interaction,  $P < 0.005$ ). Plasma AST activity and weight gains were similar among treatment groups throughout the study period. Concentrations of copper in the liver on Day 155 were greater in COWP-treated lambs ( $P < 0.001$ ), but all concentrations were normal. Multiple doses of COWP were as effective as levamisole for control of *H. contortus* without risk of copper toxicity.

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## 1. Introduction

Anthelmintic resistance is highly prevalent throughout the southern U.S. (Miller and Craig, 1996; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003) and recently the first case of complete anthelmintic failure was reported (Kaplan et al., 2005). Consequently, the development of alternative means to control gastrointestinal nematodes (GIN) has become imperative. This problem is well recognized by sheep producers of whom 74% surveyed felt that the predominant disease condition present in flocks was stomach/intestinal worms (USDA, 2001). One particular class of animals that is highly susceptible to GIN includes growing lambs or kids. This places significant constraints on the productivity of young ruminants.

A single bolus of copper oxide wire particles (COWP) has been shown 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 dose of COWP as low as 2 g decreased fecal egg counts and worm burden in lambs (Burke et al., 2004). However, because of the accumulation of copper in the liver that may lead to toxicity, not more than one dose every 12 months was recommended (Langlands et al., 1983). Recently, smaller doses (0.5 and 1 g) have been found to be effective for GIN control (Miller et al., 2005). The objective of the current study was to determine the effectiveness and risk of toxicity of multiple small doses of COWP as GIN control in lambs from weaning until market age.

## 2. Materials and methods

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 were minimized throughout the experimental period.

In late May 2005, 26 crossbred (Katahdin, Dorper, St. Croix cross) ram lambs naturally infected with GIN were assigned randomly to three treatment groups. Lambs were administered levamisole (Levasol, 8.0 mg/kg;  $n = 8$ ; LEV), 0.5 or 1 g copper oxide wire

particles (COWP; Copasure; Animax Veterinary Technology, UK;  $n = 9$ /dose) in a gelatin capsule at weaning (Day 0;  $118 \pm 2$  days of age) and every 6 weeks until mid-October, for a total of four deworming treatments. All treatments were administered per os. No rejected boluses were found following treatment. Lambs grazed the same bermudagrass pastures at USDA, Agricultural Research Service, Dale Bumpers Small Farms Research Center in Booneville, AR, which had been grazed previously by sheep at a stocking rate of 15 lambs/ha and were supplemented with 500 g corn/soybean meal between Days 0 and 28 (typical of post-weaning management) and Days 56 and 98 (supplemented the latter period because of poor gains) and free choice trace mineralized salt (Land O'Lakes Sheep and Goat Mineral, Shoreview, MN) and water throughout. Feces were collected directly from the rectum. Fecal egg counts (FEC; modified McMaster technique with a sensitivity of 50 eggs/g; Whitlock, 1948), blood packed cell volume (PCV), and activity of the liver enzyme, aspartate aminotransferase in plasma (AST; Booneville Community Hospital, Booneville, AR) were determined every 14 days between Days 0 and 154. Plasma AST activity is a measurement of liver copper status (Buckley and Tait, 1981). A liver sample was collected at slaughter on Day 155 and the wet and dry matter concentration of copper was determined (Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, LA). Body weight was determined every 28 days on a calibrated scale.

Feces were collected from all lambs on Days 0 and 154 for fecal culture of larval nematodes. A pooled fecal sample from all lambs was used on Day 0. On Day 154, feces were pooled for each treatment group, gently mixed, but pellets were not broken, and misted with water every 2–3 days, and loosely covered with aluminum foil. After 14 days, the cultures were wrapped in cheesecloth, and the cheesecloth immersed in warm water (approximately 38 °C). The fecal cultures were subjected to the Baermann method overnight in a funnel with an attached culture tube. L3 larvae were collected from the culture tube and identified.

Data were analyzed using the mixed models procedure of SAS (1996). The mathematical model used for PCV, FEC, AST, and body weight included treatment, day, treatment by day, and a repeated statement for day of measurement (Littell et al., 1996).

Contrasts were determined using the PDIF option (all probability values for the hypothesis) in SAS when probability was less than 0.05%. FEC data were log transformed:  $\ln(\text{FEC} + 1)$ . Statistical inferences were made on transformed data and untransformed LS means were presented.

### 3. Results

*H. contortus* was the predominant (97%) gastrointestinal parasite on Day 0. Administration of LEV, 0.5 or 1 g COWP on Days 0 and 42 led to a reduction in FEC (Fig. 1A). There was a decrease in FEC after treatment with LEV on Days 84 and 126 but not after COWP treatment (treatment by time,  $P < 0.005$ ). FEC were similar between Days 56 and 126 among all groups of lambs. FEC of LEV-treated lambs was lower than that of COWP-treated lambs on Days 140 ( $P < 0.001$ ) and 154 ( $P < 0.003$ ). There was a decrease in PCV for all groups of lambs between Days 0 and 42 (Fig. 1B). All treatments on Days 42 and 84 were associated with an increase in PCV ( $P < 0.001$ ). The final treatment on Day 126 was associated with an increase in PCV in lambs administered LEV, no change in 1 g COWP-treated lambs, and a decrease in 0.5 g COWP-treated lambs (treatment by time,  $P < 0.001$ ). The increase in PCV on Day 56 was greater for LEV than COWP-treated lambs ( $P < 0.05$ ). By the final treatment on Day 126, PCV was lower in LEV than 0.5 g COWP-treated lambs ( $P < 0.04$ ). A greater proportion of *H. contortus* were recovered from Day 154 fecal cultures in the LEV-treated group compared with COWP-treated lambs (Table 1).

Within 14 days after each treatment, AST activity increased similarly in all groups (time,  $P < 0.001$ ) and was similar among treatment groups (Fig. 2). On day of slaughter (Day 155), concentrations of copper in the liver were greater in COWP-treated than LEV-treated lambs ( $P < 0.001$ ; Table 2).

Weight gains were similar among all groups of lambs throughout the trial, slowed during hotter months (Days 28–84), and increased in response to supplementation (data not shown). Weights on Day 0 were  $34.1 \pm 1.8$ ,  $32.1 \pm 1.1.7$ , and  $34.3 \pm 1.7$  kg and Day 154 were  $50.7 \pm 1.8$ ,  $49.8 \pm 1.7$ , and  $51.0 \pm 1.7$  kg for LEV-, 0.5 and 1 g COWP-treated lambs, respectively.

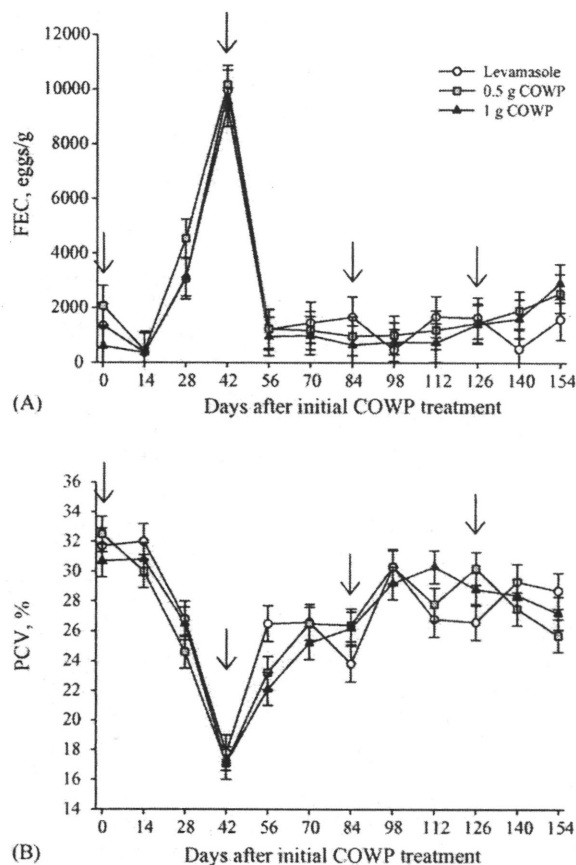


Fig. 1. Least squares means and standard errors of fecal egg counts (FEC; A) and packed cell volume (PCV; B) of lambs treated with levamisole ( $n = 8$ ; open circle), 0.5 g copper oxide wire particles (COWP;  $n = 9$ ; shaded square), or 1 g COWP ( $n = 9$ ; closed triangle) on Day 0. Arrows represent days on which treatment was administered.

### 4. Discussion

Administration of low doses of COWP on Days 0 and 42 was effective in reducing GIN infection similar to that reported in lambs with larger doses (Bang et al.,

Table 1  
Percentage of L3 larvae cultured from feces collected on Day 154 from lambs treated with levamisole (LEV;  $n = 8$ ), 0.5 or 1 g of copper oxide wire particles (COWP;  $n = 9$  per dose)

Treatment	<i>H. contortus</i>	<i>Trichostrongylus</i>	<i>Oesophagostomum</i>
LEV	98	1	1
0.5 g COWP	48	52	0
1 g COWP	65	34	1

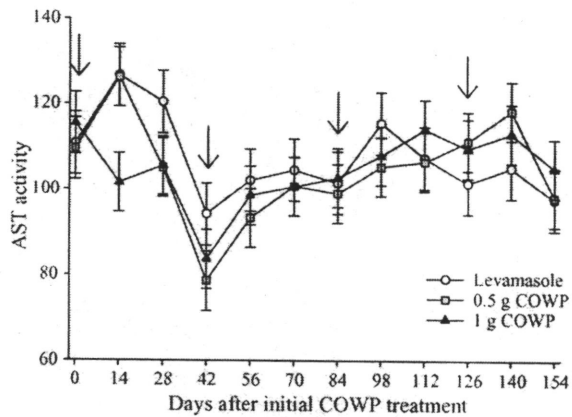


Fig. 2. Least squares means and standard errors of plasma aspartate aminotransferase (AST) activity (U/l) of lambs treated with levamisole ( $n=8$ ; open circle), 0.5 g copper oxide wire particles (COWP;  $n=9$ ; shaded square), or 1 g COWP ( $n=9$ ; closed triangle) on Day 0. Arrows represent days on which treatment was administered.

1990; Knox, 2002; Burke et al., 2004). Because FEC were relatively low on Day 84 there was no further reduction in COWP-treated lambs, although PCV increased in all lambs between Days 84 and 98. By Day 126 lambs no longer appeared to respond to COWP administration as a dewormer though FEC decreased in LEV-treated lambs. However, there were a lower proportion of *H. contortus* larvae in COWP-treated lambs on Day 154 with one-third to one-half of L3 being *Trichostrongylus*. COWP is reported to be more effective in reducing abomasal than intestinal nematodes (Bang et al., 1990; Knox, 2002) which could explain the difference in changes in FEC in response to COWP seen in this study after Day 84.

An alternate explanation may be that as lambs age there may be a change in the physiological response to supplemental copper oxide. There are several minerals (molybdenum, iron, or sulfur) that are antagonistic and can bind with copper in the GI tract. Exposure and/or accumulation of these minerals in the feed,

water, and the environment could lead to differences in reduction of abomasal GIN infection observed in COWP-treated sheep. Alternatively, a higher passage rate of feed could lead to a reduction in the retention of COWP in the abomasum and reduce efficacy as a dewormer.

According to Puls (1988), marginal concentrations of copper in the liver are 5–20 mg/kg wet weight, adequate levels are 25–100 mg/kg wet weight, and high levels are 100–500 mg/kg wet weight and toxic levels are greater than 250 mg/kg wet weight. In the current study, at least one lamb from each treatment group had marginal concentrations of copper in the liver on Day 155, 6 COWP-treated lambs were considered just above adequate (100–120 mg/kg wet weight), and all were below the toxic level. By Day 155, liver concentrations of copper in the 1 g COWP-treated lambs, which received a total of 4 g COWP, were below that reported for lambs administered a single 4 g dose where copper was measured 4 weeks later (Burke et al., 2004). In addition, AST activity was similar among treatment groups in the current study. Therefore, no risk of copper toxicity was detected in these lambs.

Changes in PCV observed were often associated with changes in FEC. But the increase observed between Days 84 and 98 may have also been attributed to supplemental feeding at that time. A decrease after Day 112 could have been attributed to a lower nutritional plane in addition to GIN infection. Also, low activity of AST on Day 42 may have been associated with reduced PCV at that time.

Using PCV cutoff values of less than 19% to determine which lambs are in need of deworming, treatment was unnecessary except on Day 42 (>57% were below PCV of 19%), and for one COWP-treated lamb on Day 0 and one LEV-treated lamb on Days 84 and 126. These data suggest that multiple low-dose COWP administered selectively using the FAMACHA system (Kaplan et al., 2004) could provide an

Table 2

Least squares means and standard errors of concentrations of copper measured from the liver collected at slaughter from lambs treated with levamisole (LEV;  $n=8$ ), 0.5 or 1 g of copper oxide wire particles (COWP;  $n=9$  per dose)

Treatment	Wet weight (mg/kg)	Dry weight (mg/kg)	Minimum dry weight (mg/kg)	Maximum dry weight (mg/kg)
LEV	29.5 ± 9.2	86.4 ± 26.2	31.5	195.5
0.5 g COWP	78.0 ± 8.7	223.8 ± 24.7	53.7	333.2
1 g COWP	89.7 ± 8.7	247.6 ± 24.7	60.6	336.8

alternative to chemical control. In addition, adequate nutrition should be provided, which aids in control of GIN (Coop and Holmes, 1996; Coop and Kyriazakis, 1999).

## 5. Conclusion

Low doses of COWP were effective for control of GIN early post-weaning, but was less effective as the lambs matured. This could be attributed in part to a reduction in the proportion of *H. contortus* relative to other GIN in the gut after initial administration of COWP. AST activity and concentrations of copper in the liver indicated minimal risk of copper toxicity in these lambs. However, only 18 lambs were tested in this study. Also, some breeds of sheep are more sensitive to copper toxicity and some flocks may have more accessibility to copper. Therefore, COWP should continue to be used with caution and copper levels should be monitored, particularly when there is a history of copper toxicity on a farm.

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