**Examination of commercially available copper oxide wire particles in combination
with albendazole for control of gastrointestinal nematodes in lambs**

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Abstract

Control of gastrointestinal nematodes (GIN) remains a critical issue due to the prevalence of anthelmintic resistance. The objective of the experiment was to determine the efficacy of copper oxide wire particles (COWP) from three commercial sources and a combination of COWP and albendazole to control GIN and/or *Haemonchus contortus* in lambs. Naturally infected Katahdin lambs in early June 2014 and 2015 were randomly assigned to receive no COWP (CON; $n = 9$ and 12) or 2 g COWP in a gel capsule as Copasure[®] (COP; $n = 4$ and 17 ; Animax Ltd.), Copper Oxide – Wire Form (AUS; $n = 7$ in 2014 only; Pharmplex), Ultracruz[™] (ULT; $n = 8$ and 15 ; Santa Cruz Animal Health[™]), no COWP and albendazole (CON+alb; $n = 10$ in 2015 only; 15 mg/kg BW; Valbazen[®]; Zoetis Animal Health), or COWP+alb ($n = 7$ and 11 ; in 2014, lambs were administered alb on day 3). Lambs grazed grass pastures as a group and were supplemented with 227 g/lamb daily of a commercial grain mix (15% crude protein) and the same amount of alfalfa pellets. Feces were collected on days 0 (day of COWP treatment), 7, and 14 for determination of fecal egg counts (FEC). Pooled (2014) or pooled treatment group feces were cultured on days 0, 7, and 14 (2015 only) to determine GIN genera. Data were analyzed using repeated measures in a mixed model, and FEC were log transformed. The predominant GIN on day 0 was *H. contortus* (87%) in 2014, and there was a mixed population in 2015. The mean FEC was reduced by day 7 in AUS and ULT lambs (treatment $\times$ day, $P = 0.001$), and all of the COWP products were similar. By day 14, the AUS FEC were lower than the CON and COP groups. When examining the combination of COWP and synthetic anthelmintic, the FEC of COWP+alb were reduced to nearly 0 eggs/g (back-transformed) and lower than the other groups (treatment

× day, $P = 0.001$). The percentage of *H. contortus* in cultured feces was reduced to a greater extent in the COWP than CON or CON+alb groups of lambs. In a mixed GIN population, the COWP products appeared to be similar in efficacy and using a combination of COWP+alb increased the efficacy not only against *H. contortus*, but all GIN genera present, offering options in the face of resistance to benzimidazoles.

Keywords: Anthelmintic resistance, Copper oxide, Gastrointestinal nematodes, Sheep

1. Introduction

Gastrointestinal nematodes (GIN) are the greatest health and production challenge for small ruminants in warm, humid climates. *Haemonchus contortus* or barber pole worm is a blood feeder that can cause severe anemia and death if left untreated. A recent study conducted on U.S. sheep flocks found *H. contortus* in nearly every state in the U.S. examined (USDA, 2014a; R. Kaplan, personal communication). The exploration of alternatives to synthetic anthelmintics for the control of *H. contortus* in small ruminants remains critical because of the prevalence of anthelmintic resistance (Kaplan et al., 2005; Howell et al., 2008). Not only do organic farms need to worry about anthelmintic resistance, but also a means of control that is acceptable to the USDA National Organic Program (USDA, 2014b) is paramount. Some organic certifiers in the US allow the use of copper oxide wire particles (COWP) as an anti-parasitic agent. Our laboratory (Burke et al., 2004; Burke and Miller, 2006) and others (Bang et al., 1990; Knox, 2002) have examined the use of COWP to control *H. contortus*. We have used a product marketed to

alleviate copper deficiency in cattle (Copasure[®]; Animax Ltd., Suffolk, England). Recently, similar products have become available for small ruminants to treat copper deficiency. Producers may be using these products as an anthelmintic without any knowledge of their efficacy.

The objective of the experiment was to determine the efficacy of COWP from three commercial sources and a combination of COWP and albendazole to control a natural infection of *H. contortus* or mixed GIN in lambs.

2. Materials and methods

All husbandry practices and experimental procedures were approved by the USDA, ARS Animal Care and Use Committee. The experiment was conducted at the Dale Bumpers Small Farms Research Center, Booneville, AR, in June (summer) of 2014 and 2015. This sheep flock is certified as Animal Welfare Approved (<http://animalwelfareapproved.org>).

Naturally infected Katahdin ewe (2014) and ewe and ram (2015) lambs that were 106 ± 1.3 days of age (born February; $n = 35$) and 21.6 ± 1.3 kg in 2014 and 149.4 ± 5.6 days of age (born December to January; $n = 65$) and 28.0 ± 1.4 kg in 2015 were randomly assigned to receive a gel capsule (NOW[®] Foods Single “0” VCAPS[™], Bloomington, IL) filled with soybean meal (CON; $n = 9$ and 12) or 2 g COWP as Copasure[®] (COP; $n = 4$ and 17; Animax Ltd.), Copper Oxide – Wire Form (AUS; $n = 7$ in 2014 only; Pharmplex), Ultracruz[™] (ULT; $n = 8$ and 15; Santa Cruz Animal Health[™]), no COWP and albendazole (CON+alb; $n = 10$ in 2015 only; 15 mg/kg BW; Valbazen[®], Zoetis

Animal Health, Kalamazoo, MI), or COWP+alb ($n = 7$ and 11). In 2014, the COWP+alb were 4 COP, 2 AUS, and 1 ULT lambs treated on day 3. In 2015, all COWP+alb were treated with COP as the COWP source. The intent of the albendazole treatment in 2014 was to eliminate *Trichostrongylus* spp. or other non-*H. contortus* GIN as lambs developed diarrhea (thus, these lambs were not randomly assigned). The *H. contortus* in this flock were determined to be resistant to benzimidazole according to Drenchrite (R. Kaplan, University of Georgia), which was thought to minimize the effect of deworming on *H. contortus*, the GIN of interest. Previous studies determined that COWP was primarily effective in reducing *H. contortus* (Bang et al., 1990; Knox, 2002).

When blood packed cell volume (PCV) fell below 19%, lambs were dewormed with levamisole (12 mg/kg BW, AgriLabs, St. Joseph, MO). This occurred on day 7 in 3 CON and 1 ULT in 2014 and 1 COP in 2015. The *H. contortus* in this flock was determined to have suspected resistance to levamisole according to the Drenchrite, but a recent fecal egg count (FEC) reduction test indicated a 98% reduction in FEC (Burke, unpublished results). Rather than penalize the lambs that were not dewormed by deleting the day 14 FEC of levamisole treated lambs, the day 7 value was also used for day 14 in these 4 lambs in 2014 and 1 lamb in 2015. This would be a conservative estimate because if left untreated, the FEC would most likely have increased by day 14. Because it is difficult to predict the PCV on day 14, these 5 observations were deleted for that analysis.

In both years, lambs grazed bermudagrass (*Cynodon dactylon*) pastures as a group and were supplemented with 227 g/lamb daily of a grain mix (soy hull pellets, wheat middlings pellets, corn gluten pellets, cracked corn, dried distillers grain; 15% CP; Farmer's Cooperative, Van Buren, AR) and the same amount of alfalfa pellets (16% CP;

Farmer's Cooperative, Van Buren, AR). On day of treatment, lambs were fed after COWP and/or albendazole was administered. Blood and feces were collected on days 0 (day of COWP treatment), 7, and 14 for determination of PCV and FEC (nematodes only), respectively, according to Whitlock et al. (1948), and pooled (2014) or pooled treatment group feces were cultured on days 0, 7, and 14 (2015 only) according to Peña et al. (2002) to determine GIN genera.

The COWP diameter was measured on 16 particles of COP, AUS, and ULT by standing particles on end and determining the average of the longest and shortest width. PROC GLM was used to determine differences among means.

Individuals were removed from the data set if the FEC on day 0 was less than 400 eggs/g; numbers per treatment above reflect lambs that remained in the data set. Three lambs were removed in 2014 and 38 were removed in 2015. Data were analyzed as repeated measures (Littell et al., 1996) using mixed models (SAS 9.3, SAS Institute Inc., Cary, NC) and included the COWP treatment and year in the model. An autoregressive covariance structure was used and orthogonal contrasts (CON vs. COWP, COP vs. ULT, ULT vs. AUS). A second analysis was conducted to determine the effect of albendazole. For this, the COWP groups were pooled and orthogonal contrasts were CON vs. CON+alb, COWP vs. COWP+alb, and CON+alb vs. COWP+alb. In addition, means were separated using pre-planned pairwise comparisons using the t-test when treatment effect was $P < 0.05$. The FEC data were log transformed [$\ln(\text{FEC} + 10)$] and presented as back-transformed means.

A FEC reduction for each COWP treatment per year was calculated using arithmetic means (2014) as discussed in Miller et al. (2006). First, post-treatment was

considered on day 7 and day 14 using all eggs counted relative to the CON group of lambs: $[100 \times (1 - \text{mean post-treatment}/\text{mean post-CON})]$. Second, efficacy against *H. contortus* (using percentage *H. contortus* larvae counted on days 7 and 14) was considered on days 7 and 14 post-treatment: $1 - \{[\text{mean post-treatment} \times \% H. contortus \text{ treatment}/\text{mean pre-treatment} \times \% H. contortus \text{ CON}] \times [\text{mean pre-CON}/\text{mean post-CON}]\}$, whereas “treatment” is COP, AUS, ULT, CON+alb, and COWP+alb. For 2015 data, a mixed model with treatment as the variable was used to determine differences in FEC reduction (for all GIN and estimating only *H. contortus* based on the culture) between COP and ULT, and the COWP treatments and CON+alb and COWP+alb.

3. Results

The predominant GIN on day 0 in 2014 was *H. contortus* (87%), and in 2015 was a mix of *H. contortus*, *Trichostrongylus* spp., *Cooperia* spp., and *Oesophagostomum* spp. (Table 1). In addition, *Strongyloides* spp. were highly prevalent. The mean FEC was reduced by day 7 to the greatest extent in AUS and ULT lambs (treatment $\times$ day, $P = 0.001$; Figure 1). Means separation revealed that AUS ($P = 0.016$), and ULT ($P = 0.016$) groups were reduced compared with CON, and all of the COWP products were similar. By day 14, the AUS FEC were lower than the CON and COP groups. The FEC tended to be greater in 2014 than 2015 ($P = 0.07$; data not shown). There tended to be a higher PCV in the COWP (COP, $25.9 \pm 0.8\%$; AUS, $26.9 \pm 1.3\%$; ULT, $25.3 \pm 0.7\%$; $P = 0.07$) than CON lambs ($24.4 \pm 0.7\%$), and all values dropped between days 0 and 14 except

159 ULT treated lambs, which increased (treatment $\times$ day, $P = 0.03$). The PCV was higher in
 160 2015 than 2014 ($26.9 \pm 0.6\% > 24.3 \pm 0.6\%$; $P = 0.006$).

161 When examining the combination of COWP and synthetic anthelmintic, the FEC
 162 of COWP+alb were reduced to nearly 0 eggs/g (back-transformed, or 270 eggs/g using
 163 untransformed LS means) on days 7 and 14, which were lower than CON, CON+alb, or
 164 COWP alone (treatment $\times$ day, $P = 0.001$; Figure 2). On day 7, CON+alb FEC were
 165 lower than CON ($P = 0.001$), but this difference disappeared by day 14. By day 14, only
 166 the COWP+alb FEC were lower than the other treatment groups which were not different
 167 from each other. The PCV tended to be greater in COWP+alb treated lambs than COWP
 168 alone ($P = 0.08$) and overall treatment effect was $P = 0.09$ without any interaction
 169 detected.

170 In 2014, on day 7, the percentage of *H. contortus* in feces remained high in CON
 171 and was lowest in COP (Table 1). The FEC reduction of GIN relative to CON on days 7
 172 and 14 was relatively similar among COP, AUS, and ULT. The estimate of the FEC
 173 reduction considering only *H. contortus* on day 7 increased compared with all GIN
 174 (Table 1). In 2015, there was a $99.1 \pm 23.3\%$ reduction in FEC on day 7 and $90.2 \pm$
 175 24.9% on day 14 in COWP+alb compared with the other treatments ($P = 0.02$, $P = 0.05$,
 176 respectfully), which was still significant after estimating the reduction based only on *H.*
 177 *contortus* ($P = 0.001$, $P = 0.02$, respectfully), but only against CON+alb on day 14 (Table
 178 1). The FEC reduction of all GIN after 14 days was low for ULT and especially COP, but
 179 increased when estimating the reduction of only *H. contortus*.

The diameter of COP, AUS, and ULT was 476 ± 11.4 , 560 ± 11.4 , and 548 ± 11.4 μm , respectively ($P < 0.001$), whereas AUS and ULT were similar in diameter and greater than that of COP ($P < 0.001$).

4. Discussion

The use of COWP in small ruminants as an aid in the control of *H. contortus* is important because of limited alternatives in flocks with multiple anthelmintic resistance. In those flocks, farmers should be using management strategies that minimize GIN infection, such as excellent nutrition to maintain good body condition allowing greater tolerance to GIN, grazing practices to minimize GIN larval development on pasture, and culling practices to remove animals that repeatedly become infected (reviewed by Waller, 2003; Houdijk et al., 2012; Terrill et al., 2012). However, even the most judicious farmers will need to treat animals that succumb to GIN infection.

In the current study, it was necessary to use a natural infection due to the presence of multi-drug resistance. Thus, while *H. contortus* was the predominant GIN in 2014, it was not in 2015. Copper oxide wire particles target *H. contortus* rather than the other genera that were present, *Trichostrongylus* spp., *Cooperia* spp., or *Oesophagostomum* spp. (Bang et al., 1991; Knox, 2000). The diameter of COP was less than that of AUS and ULT, which did not appear to influence efficacy. Both the AUS and ULT products reduced FEC within 7 days compared with the CON group of lambs and the AUS remained lower. The AUS was only used in 2014 when *H. contortus* was the predominant genera. The COP did not significantly reduce FEC compared with CON, which was most

likely influenced by only having 36% *H. contortus* in 2015 when most of the observations occurred.

The combination of COWP and albendazole used in the seven lambs that required intervention for diarrhea in 2014 suggested a greater FEC reduction in these animals. Because the flock is managed using high animal welfare criteria, intervention for minor health problems is necessary even when animals are on experiment. Even though animals were not randomly assigned to the COWP+alb treatment in 2014, the FEC reduction on individual animals was striking and prompted further study. The experiment was replicated in 2015 to increase the observations in all treatments (except AUS in order to accommodate the additional combination treatment) and to adequately examine the effect of the combination treatment. A potential confounding effect remains in that albendazole was administered on day 3 in 2014 and on day 0 in 2015. Because the particles remain in the abomasum for more than 3 days and the reduction in FEC was clearly greater than the other treatments, we believe this to be a significant finding. Miller et al. (2011) observed a similar phenomenon in lambs with a multi-drug resistant population of *H. contortus* treated with COWP alone (Copasure[®]) compared with COWP combined with albendazole and levamisole at the recommended doses. In that study, FEC were lower in the combination treatment than the group of lambs treated with COWP alone on day 7, but similar by day 14. The combination treatment may be important in animals with multi-drug resistant *H. contortus* with haemonchosis requiring salvage treatment. It is intriguing that the FEC reduction of the COWP+alb was greater than 90% even with the mixed GIN population. This may increase options for farms with anthelmintic resistance, especially to benzimidazoles.

Because of the risk of copper toxicity, caution must be employed if COWP is used as an anthelmintic. The lowest effective dose should be used, especially for sheep. A dose of 0.5 g COP was effective in reducing FEC in lambs (Burke and Miller, 2006) and a dose of 1 g was effective in ewes (Burke et al., 2007).

Farmers should be able to rely on the COWP products examined as an aid in the control of *H. contortus*, being mindful that only adults are targeted. It appears promising that COWP in combination with albendazole increased efficacy with a greater reduction in FEC compared with COWP alone. Further research is needed on combining COWP and other synthetic anthelmintics.

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Table 1. The effect of administration of a gel capsule filled with soybean meal (CON), or 2 g copper oxide wire particles (COWP) in a gel capsule as Copasure® (COP; Animax Ltd.), Copper Oxide – Wire Form (AUS; Pharmplex), or Ultracruz™ (ULT; Santa Cruz Animal Health™) on days 0 (day of COWP treatment), 7, and 14 on percentage of *Haemonchus contortus* (Hc) cultured from feces of lambs in 2014 and 2015. Other gastrointestinal nematode genera (GIN) cultured but not presented include *Trichostrongylus* spp., *Cooperia* spp., and *Oesophagostomum* spp. The fecal egg count reduction (FECR) of GIN and of Hc relative to CON 7 and 14 days post-treatment is presented.

	2014 ¹				2015				
	CON	COP	AUS	ULT	CON ²	COP	ULT	CON+alb	COWP+alb
<i>n</i>	9	4	7	8	12	17	15	10	11
Culture									
Day 0, Hc	87	-	-	-	-	36	61	35	23
Day 7, Hc	89	8	62	69	48	12	22	89	4
Day 14, Hc	-	-	-	-	47	12	24	100	13
Day 7, FECR _{GIN}	-	87.2	69.6	75.8	-	11.7 ± 18.4 ^a	58.0 ± 19.6 ^a	20.1 ± 25.7 ^a	99.1 ± 23.3 ^b
Day 14, FECR _{GIN}	-	80.7	59.5	51.8	-	1.3 ± 20.7 ^a	41.7 ± 22.1 ^{ab}	46.0 ± 26.2 ^{ab}	90.2 ± 24.9 ^b
Day 7, FECR _{Hc}	-	97.2	71.5	81.7	-	80.2 ± 3.1 ^b	85.1 ± 3.3 ^b	43.7 ± 9.1 ^a	99.9 ± 8.2 ^c
Day 14, FECR _{Hc}	-	-	-	-	-	76.9 ± 4.4 ^b	77.9 ± 4.7 ^b	22.3 ± 16.9 ^a	86.8 ± 16.0 ^b

¹Day 0 in 2014 was a bulk culture on all lambs. Feces were not cultured on day 14 samples. FECR were not analyzed statistically.

There were an additional 4 COP, 2 AUS, and 1 ULT lambs that received alb on day 3.

²Very few larvae were detected in Day 0 culture from CON lambs (2015), which may have inadvertently dried out.

^{abc}Least squares means within a row lacking a common superscript differ ($P < 0.05$; 2015 FECred).

Figure 1. Effect of administration of a gel capsule filled with soybean meal (CON; $n = 21$; white bars), or 2 g copper oxide wire particles (COWP) in a gel capsule as Copasure[®] (COP; $n = 21$; Animax Ltd.; solid orange bars), Copper Oxide – Wire Form (AUS; $n = 7$; Pharmplex; fine diagonal green bars), or Ultracruz[™] (ULT; $n = 23$; Santa Cruz Animal Health[™]; checked blue bars) to lambs on days 0 (day of COWP treatment), 7, and 14. Statistical analyses of fecal egg counts were performed on log transformed values (treatment $\times$ day, $P = 0.001$) and back transformed least squares means are presented (Panel A). Within day of treatment, individual means with different letters differ ($P < 0.05$). The back transformed standard error is 0.14. Untransformed least squares means are presented in Panel B as some readers are more accustomed to these.

Figure 2. Effect of administration of a gel capsule filled with soybean meal (CON; $n = 21$; white bars), CON and albendazole (CON+alb; Valbazen[®]; $n = 10$; white checked bars), 2 g copper oxide wire particles (COWP) in a gel capsule as Copasure[®], Copper Oxide – Wire Form, or Ultracruz[™] (Santa Cruz Animal Health[™]; $n = 51$; solid blue bars), or 2 g COWP+alb ($n = 18$; checked blue bars) to lambs on days 0 (day of COWP treatment), 7, and 14. Statistical analyses of fecal egg counts were performed on log transformed values (treatment $\times$ day, $P = 0.001$) and back transformed least squares means are presented (Panel A). Within day of treatment, individual means with different letters differ ($P < 0.05$). The back transformed standard error is 0.14. Untransformed least squares means are presented in Panel B.

Figure 1

Figure 2