Copper oxide wire particles effective against gastrointestinal nematodes in adult alpacas during a randomized clinical trial

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OBJECTIVE
To determine whether copper oxide wire particles (COWP) administered as an oral bolus effectively decrease fecal gastrointestinal nematode (GIN) egg counts in adult alpacas.

ANIMALS
57 adult alpacas.

PROCEDURES
A double-blind clinical trial was performed during the months of April through August 2019. Adult alpacas enrolled in the trial were administered 2 g of COWP or a placebo control capsule PO on day 0 and day 45. On day 0 and at 15-day intervals, fecal samples were collected, and Modified McMaster fecal egg counts (FEC) were performed as well as physical examinations, including FAMACHA scoring and body condition scoring (BCS). Hematocrits were measured in 30-day intervals (days 0, 30, 60, and 90). Results were compared across groups.

RESULTS
A significant decrease in mean FEC was observed in the COWP-treated group over time, compared with the placebo group. Mean BCS, FAMACHA score, and Hct did not differ significantly between groups. No clinical evidence of copper toxicosis was observed.

CLINICAL RELEVANCE
Oral administration of COWP was determined to be a safe and effective method of reducing GIN FEC in adult alpacas.
efficacy of using COWP as an adjunctive treatment in cameldids. The purpose of the study reported here was to determine whether COWP administered as an oral bolus effectively decreased fecal gastrointestinal nematode (GIN) egg counts in adult alpacas. We hypothesized that alpacas treated with COWP (2 g, PO, twice at a 45-day interval) would have significantly reduced FECs compared to alpacas receiving a placebo.

Materials and Methods

Animal welfare

All animal procedures used in this study were approved by the University of Tennessee Institutional Animal Care and Use Committee (protocol no. 2681). Fifty-seven adult alpacas located on 2 privately owned farms were enrolled: 30 animals were located at farm 1 and 27 were located at farm 2. All animals used had not received anthelmintic treatment 1 month prior to the start of the study.

Experimental design and protocol

This study was performed during the months of April through August of a single calendar year in the eastern region of Tennessee in 2019. Power analysis was performed prior to enrollment of animals in this study with a focus on determining a difference in FEC between treatment groups over time. The alpacas were blocked based on sex and randomized into a placebo or experimental treatment group (CTRL and COWP groups, respectively) between the 2 farm locations using a computerized spreadsheet program (Excel 2016, version 16.0; Microsoft Corp). Treatment groups were cohoused on 4 pastures between the 2 farm locations. This study was performed as a double-blind trial from the perspective of the research observers and producers.

To determine baseline values (day 0) and monitor health during the study, physical examinations, including FAMACHA scores and body condition scores (BCS), were performed for each animal in both the CTRL and COWP groups. During every examination, each animal was assigned a FAMACHA score on a scale of 1 (not anemic; ideal) through 5 (severely anemic), as described by Storey et al. and a BCS on a scale of 1 (thin) through 5 (obese), as described by Van Saun, with a BCS of 3 being ideal. A single-blinded observer assigned both FAMACHA score and BCS throughout the study period. The study timeline included administering 2 g of COWP or a placebo control capsule PO on day 0 and day 45; collecting and analyzing fecal samples (Modified McMaster fecal egg counts [FECs]) at 15-day intervals; performing physical examinations, including FAMACHA scoring and body condition scoring (BCS) at 15-day intervals; and measuring Hcts at 30-day intervals (Appendix 1).

Blood samples were collected from the jugular vein of each animal using an 18-gauge, 1.5-inch needle and an evacuated 2-mL glass tube with EDTA added (BD Life Sciences). Blood was kept at 4 °C until processing, and Hct was performed within 24 hours of collection. Briefly, microhematocrit tubes were filled with whole blood and centrifuged at 1,000 X g for 5 minutes. Hct concentrations were obtained using a digital reader (HemataStat II; EKF Diagnostics). Two Hct concentrations were obtained for each animal at each sampling time point, and the coefficient of variation (CV) was determined. If the CV was greater than 15%, the Hcts were repeated. For each animal, the mean of the 2 Hct readings obtained was used for statistical analysis.

Fecal egg counts

Fecal egg counts were performed using a Modified McMaster’s technique. Fecal samples were collected digitally from the rectum or by taking a representative sample of an observed bowel movement. Feces were stored at 4°C until analysis within 24 hours of collection. For analysis, 2 g of fecal matter was weighed out into a paper cup, 28 mL of sodium nitrate solution was weighed out into a graduated cylinder, the sodium nitrate solution was mixed into the fecal sample, and then the solution was well stirred until the fecal matter was dissolved. Next, a plastic transfer pipette was used to fill each chamber of the McMaster slide (FEC Source) with 0.15 mL of the fecal solution. The slide was read on the 10X objective and the total number of eggs found in both chambers was multiplied by 50 to determine eggs per gram (EPG). While all findings were noted, only GIN eggs were counted to obtain the EPG.

Statistical analysis

The effects of treatment, day, sex, and paddock on FAM, FEC, BCS, and Hct were analyzed using mixed model analysis for repeated measures with day as the repeated factor and treatment as the between-subject effect. The initial FEC at day 0 was significantly different between treatment groups and was included in the model as a covariate. Diagnostic analysis was conducted to examine model assumptions for normality using the Shapiro-Wilk test. Ranked data transformation was applied if diagnostics analysis exhibited violation of normality. Post hoc multiple comparisons were performed with Tukey’s adjustment. Statistical significance was identified at the level of P < .05. Analyses were conducted using commercially available statistical software (SAS, version 9.4; SAS Institute Inc).
normal limits throughout the trial and no clinical side effects associated with the placebo or COWP treatments were detected.

Mean FEC

Sex (P = .36) and paddock (P = .11) did not have a significant effect on FEC. Time (P < .001) and treatment (P = .02) did have a significant effect on FEC. The mean ± SD FEC on day 0 was significantly (P < .001) higher for the COWP group (300 ± 543.47 EPG), compared with the CTRL group (108.93 ± 113.08 EPG; Figure 1); therefore, mean FEC was included in the statistical model as a covariate. COWP were administered on day 0 and day 45 of the trial. On day 15 following treatment administration, animals in the COWP group had a significantly (P = .01) lower mean FEC (73.22 ± 170.77 EPG) compared to animals in the CTRL group (107.14 ± 153.18 EPG). As the study and summer months progressed, the mean FEC of the CTRL group continued to trend upwards. Although the COWP group’s mean FEC also trended upwards, the mean FEC of the COWP group at day 60 (57.15 ± 86.82 EPG) remained significantly (P < .001) lower than that of CTRL group (208.93 ± 273.2 EPG). On day 75, the mean FEC remained significantly (P < .001) lower for alpacas in the COWP group (110.71 ± 121.98 EPG), compared with the CTRL group (328.57 ± 449.16 EPG).

Discussion

In this study, no clinically notable adverse side effects were associated with COWP or placebo administration based on subjective and quantifiable physical examination parameters. There was no significant difference in mean BCS or FAMACHA score observed between treatment groups. This may have been due to the relatively small range of
measurement for both the FAMACHA score and BCS, both with a measurable range of 1 through 5. With the narrow scoring range, a much larger sample size may be needed to obtain statistical differences in these values. FEC had a significant effect on Hct, but treatment did not. Animals with a higher FEC had a lower Hct; however, no animals became anemic during the study period, likely due to the overall low parasite burden. Toward the end of the study, FECs gradually increased with the progression of summer months. If the study was continued through August and September, the parasite burden may have continued to increase allowing investigators to observe a significant difference in Hct between groups.

Both herds enrolled in this study were from well-managed farms that instituted strategic parasite management practices and handled animals regularly. Animals included in the trial were apparently healthy adults at an ideal BCS and relatively low FEC at the start of the study. Both the COWP and CTRL groups started with low initial FEC. By day 15 following the first administration of the oral boluses, the COWP group had a significantly lower FEC. Both groups had an increase in FEC from days 15 to 45, consistent with previous small ruminant studies that demonstrated that the effects of COWP boluses are short lived. This decrease in FEC was observed again in the animals receiving an additional dose of COWP administration on day 45. On day 60, the COWP group had a significantly lower FEC when compared to the CTRL group. The CTRL group’s mean FECs increased throughout the study duration, which could have been associated with a higher prevalence of GIN, associated with the late summer months. Although the COWP group’s mean FECs also increased between treatments, it remained significantly lower than that of the CTRL group on day 60. The increase in mean FEC by 30 days after treatment with COWP was likely associated with pasture exposure to GIN and re-infection with grazing.

At the initiation of the study, the COWP group had a significantly higher mean FEC compared to the animals in the CTRL group. Ideally, FECs would have been performed on each individual animal prior to the start of the trial in order to minimize FEC variation between groups. The investigators took this variation in starting FECs into account in the statistical analysis, using FEC at day 0 as a covariate in the analysis. All alpacas enrolled had a relatively low GIN burden throughout the trial period. Goats and sheep have a relatively high parasite burden as demonstrated with average EPG which is quantified in several parasite investigations. This trial used the Modified McMaster methodology to determine FEC, with the lowest detectable concentration at 50 EPG. Using a more sensitive methodology such as the Modified Stoll test may have allowed the investigators to identify more subtle changes in FEC at lower concentrations. Clinical evidence of heavy endoparasitism was not observed in any animal enrolled nor was any interventionally anthelmintic administration required. At the University of Tennessee Veterinary Medical Center, most blood transfusions to treat H. contortus-associated anemia in small ruminants and camelds occur during the fall months of August and September (unpublished data). The present study ended in July, prior to the season when the parasite burden may be at its peak in the region.

A limitation of the present study was that copro-culture was not performed. Larval fecal cultures would have determined the specific parasites within the herds and observed changes in species prevalence with treatment. Regarding the GIN parasites that affect small ruminants and camelds in temperate regions, H. contortus is the most relevant and pathogenic. In small ruminants, parasitism with H. contortus was found to decrease following treatment with COWP while other common GIN parasites were not affected. COWP has been found to decrease the egg-producing fecundity of H. contortus as well as kill adult worms. This makes the therapeutic use of COWP bolus administration beneficial in minimizing both the internal and environmental worm burden. In the current study, the FEC was significantly lower at time points following COWP administration. This information taken together allowed the authors to presume that H. contortus was likely present in the study population and affected by COWP treatment; however, further studies are required to confirm this suspicion.

Alpacas treated with oral administration of 2-g COWP boluses at a 45-day interval had significantly lower GIN FECs, compared to alpacas receiving a placebo. Although the effects were short lived, this treatment appeared to be safe and efficacious. Further research is required to evaluate the direct effect of COWP on specific GIN parasites, long-term effects of use, and safety at higher dosing or increased frequency in adult alpacas.

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### Appendix 1

Timeline for conducting physical examinations (PE) that included body condition scores and FAMACHA scores, performing fecal egg counts (FEC), and determining Hcts for 57 healthy, client-owned adult alpacas that received either a 2-g bolus of copper oxide wire particles (COWP; COWP group; n = 29) or placebo (CTRL group; 28) on day 0 and day 45 on 2 participating farms in a clinical trial conducted between April and August 2019.

| Study day/outcome measured | Treatment |
|----------------------------|-----------|
| 0  | PE         | COWP or placebo  |
|    | FEC        |             |
|    | HCT        |             |
| 15 | PE         |             |
|    | FEC        |             |
| 30 | PE         |             |
|    | FEC        |             |
|    | HCT        |             |
| 45 | PE         | COWP or placebo  |
|    | FEC        |             |
| 60 | PE         |             |
|    | FEC        |             |
|    | HCT        |             |
| 75 | PE         |             |
|    | FEC        |             |
| 90 | PE         |             |
|    | FEC        |             |
|    | HCT        |             |