The effectiveness of copper oxide wire particles as an anthelmintic in pregnant ewes and safety to offspring

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Abstract

The objective of the experiment was to determine the effectiveness of copper oxide wire particles (COWP) in pregnant ewes and safety to lambs. COWP have been used recently as an anthelmintic in small ruminants to overcome problems associated with nematode resistance to chemical dewormers. Doses of COWP (≤ 4 g) have been used in lambs without clinical signs of copper toxicity. Use in pregnant ewes has not been examined. Mature Katahdin ewes were administered 0 ($n = 14$), 2 ($n = 15$), or 4 ($n = 15$) g of COWP 33 ± 1.6 days before lambing in late March 2004. Fecal egg counts (FEC) and blood packed cell volume (PCV) were determined between Days 0 (day of COWP administration) and 35. Lambs were weighed within 24 h after birth, at 30 and 60 days of age, and in mid-September (~120 days of age). Blood was collected from lambs within 24 h after birth and at 30 days of age for determination of the activity of the liver enzyme, aspartate aminotransferase (AST) in plasma. Within 7 days after COWP administration, FEC decreased by 1308 and 511 eggs/g (epg) in the 2 and 4 g groups, respectively, compared with an increase of 996 epg in the control group ($P < 0.02$). PCV was similar among groups between Days 0 and 35. Lamb plasma AST activity at birth increased with increasing dose of COWP in dams ($P < 0.001$). Plasma AST activity at 30 days of age was similar for lambs from ewes treated with 0 and 2 g COWP, but was slightly greater in lambs from ewes treated with 4 g COWP ($P < 0.02$). Birth weights decreased with increasing COWP ($P < 0.003$). By 30 (COWP × birth type, $P < 0.02$) and 60 (COWP × birth type, $P < 0.02$) days of age, weight of multiple-born lambs decreased with increasing COWP, while weight of single-born lambs was similar among treatments. In mid-September (~120 days of age) weights of multiple-born lambs from ewes treated with 4 g COWP tended to be lightest compared with lambs from ewes treated with 0 or 2 g COWP or single-born lambs ($P < 0.09$). Lamb survival to 30, 60, or 120 days of age was not affected by COWP treatment to ewes. Administration of 4 g COWP to late pregnant ewes may negatively impact multiple-born offspring, but the 2 g appears to be safe for production.
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1. Introduction

Options for gastrointestinal parasite control for small ruminants are limited because of the rapidly developing resistance of nematodes to chemical dewormers (Miller and Craig, 1996; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003). Other means of parasite control have become necessary. Copper oxide wire particles (COWP) have been used in lambs to reduce infection with *Haemonchus contortus* (Bang et al., 1990; Knox, 2002; Burke et al., 2004). No clinical signs of copper toxicity have been reported when administering less than 6 g of COWP to lambs or 10 g COWP to ewes although liver concentrations of copper were elevated in ewes (Suttle, 1987). There was a linear increase with dose of COWP (0–6 g) in concentrations of copper in the liver, but all levels were considered within a safe zone (Puls, 1988; Gartrell and Beetson, 2004). Liveweight gains were similar (Burke et al., 2004) or elevated, likely in response to decreased parasite infection (Langlands et al., 1983; Knox, 2002).

COWP have been used to treat sheep with copper deficiency without any clinical signs of copper toxicity at doses lower than 10 g (Dewey, 1977; Whitelaw et al., 1980; Suttle, 1981, 1987). Maternal copper from COWP can be transported to the fetus of pregnant ewes (Langlands et al., 1982) and milk of lactating ewes (Whitelaw et al., 1980). Fetal and conceptus concentrations of copper increased in ewes treated with COWP in early pregnancy (Langlands et al., 1982).

Growth of lambs or potential toxicity to lambs from COWP-treated ewes considered to be normocupretic and use of COWP in mature sheep for parasite control has not been examined. The objective of the current experiment was to examine the efficiency of COWP in reducing a mixed parasite infection in mature pregnant ewes and determine whether there were any adverse effects on their offspring.

2. Materials and methods

Katahdin ewes between 2 and 4 years of age were bred in December 2003 to one of two Katahdin rams. Ewes were diagnosed for pregnancy using transrectal ultrasonography (Aloka SSD 500 V ultrasound scanner equipped with a 7.5 MHz linear array prostate transducer; Aloka Co. Ltd., Japan). In late March pregnant ewes were randomly assigned to receive 0 ($n = 14$), 2 ($n = 15$), or 4 ($n = 15$) g COWP (Copasure; Animax Veterinary Technology, UK) in a gelatin capsule administered per os 33 ± 1.6 days before lambing. Only four ewes had been dewormed with moxidectin (Cydectin®; 0.2 mg/kg oral administration) between December and March prior to COWP treatment. Ewes grazed bermudagrass (*Cynodon dactylon*) overseeded with rye (*Secale cereale*) as a single group throughout the experiment except for supplementation with bermudagrass hay while lambing in the barn. Ewes had continuous access to trace mineralized salt devoid of copper (Land O'Lakes Sheep and Goat Mineral, Shoreview, MN) and water. Ewes were supplemented with 500 g corn/soybean (4.4:1.0) 19 days before COWP administration until 28 days post-lambing. Ewes lambed within a 30 day period between mid-April and mid-May.

Fecal egg counts (FEC), as determined by a modified McMaster technique (Whitlock, 1948), and blood packed cell volume (PCV) were determined between Days 0 (day of COWP administration) and 35. FEC and PCV of lambs was determined 7 days after weaning. Body weight of ewes was determined post-lambing. Lambs were weighed within 24 h after birth, at 30 and 60 days of age, and in mid-September (~120 days of age). Blood was collected from lambs within 24 h after birth and at 30 days of age for determination of the liver enzyme, aspartate aminotransferase in plasma (AST; Booneville Community Hospital, Booneville, AR). Plasma AST activity is a measurement of liver copper status of lambs (Buckley and Tait, 1981). Between birth and 30 days of age three lambs died (one multiple from a control ewe was laid on and two multiple-born from 2 g COWP-treated ewes; one was mis-grouped and the other died from unknown causes) and five lambs were orphaned because dams had mastitis with little or no milk (three multiple-born from a control ewe and two multiple-born from a 2 g COWP-treated ewe). Lambs were weaned at approximately 60 days of age. At that time 11 lighter weight lambs (2 g COWP, $n = 7$; 4 g COWP, $n = 4$; all multiple-born) were culled from flock. It is common practice for this flock to cull the bottom 15% in terms of live weight. Three lambs died after weaning (one single- and one multiple-weaned from control ewes and one multiple-weaned from a 4 g COWP-treated ewe; two died from haemonchosis and one died for unknown reasons).

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.

Data were analyzed using the mixed models procedure of SAS (1996). The mathematical model used for PCV and FEC included COWP treatment, date, COWP by date, 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 data were log transformed: $\ln(\text{FEC} + 1)$. Statistical inferences were made on transformed data and untransformed LS means were presented. Body weights of ewes and lambs, AST activity, PCV and FEC of lambs were analyzed by GLM with dam COWP treatment, sex, birth or weaning type, and sire of lamb (lamb weights), and interactions. LS means and standard errors of the mean of all response variables were presented. Regression analysis was used to evaluate the relationship between plasma AST activity of lambs at birth and body weight of ewes adjusted for dam COWP treatment and birth type of lamb. Regression was used to evaluate the relationship between ewe COWP treatment and lamb birth weight adjusted for type of birth. The relationships were determined to be linear. For all analyses, lambs born as twins and triplets were analyzed as a single class of multiple-born for statistical analyses. If multiple-born lambs were reared as a single (i.e., sibling died), weaning type was considered single. A single-bearing ewe raised a twin from a twin-bearing ewe, so that weaning type was reversed for these lambs.

3. Results

FEC were similar among treatments on Day 0. FEC tended to be reduced in ewes that received 2 or 4 g COWP and increased in ewes that received no COWP ($P < 0.07$; Fig. 1A). There were little apparent differences among groups of COWP-treated ewes in PCV over time and PCV increased between Days 0 and 35 (COWP × day, $P > 0.10$; day, $P < 0.001$; Fig. 1B).

All ewes were determined to be pregnant before administration of COWP, but expected lambing rate was
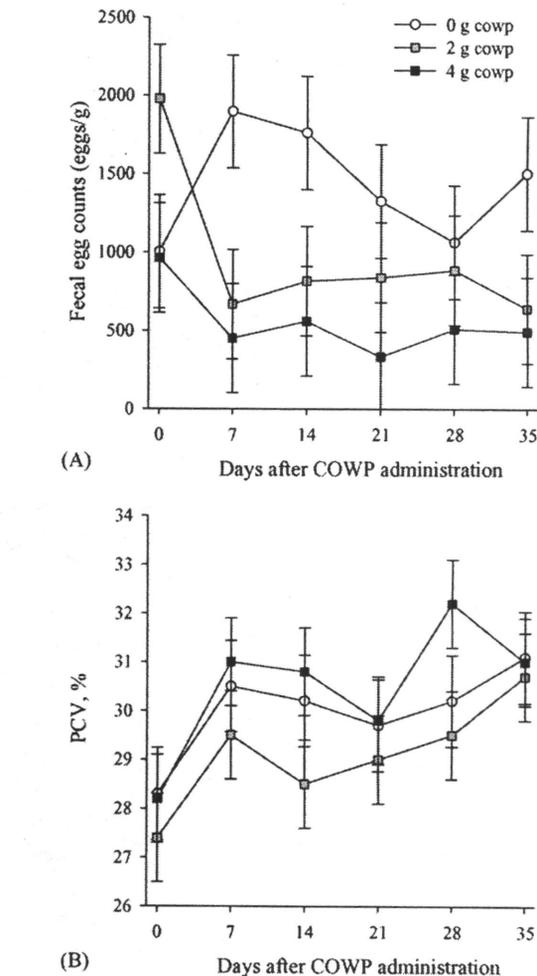

(A)

(B)

Fig. 1. Least squares means and standard errors of fecal egg counts (FEC; A) and packed cell volume (PCV; B) of ewes administered 0 ($n = 14$; open circle), 2 g ($n = 15$; shaded square), or 4 g ($n = 15$; closed square) copper oxide wire particles (COWP) during late pregnancy (Day 0). Effect of COWP over time tended to be significant for FEC ($P < 0.07$).

not determined. Number of lambs born per ewe tended to be greater in COWP compared with control ewes (0 g, 1.42 ± 0.13 lambs/ewe; 2 g, 1.79 ± 0.13 lambs/ewe; 4 g, 1.73 ± 0.13 lambs/ewe; $P < 0.10$).

Lamb plasma AST activity at birth increased with increasing dose of COWP in dams (0 g, 55.8 ± 9.0 U/L; 2 g, 78.0 ± 9.0 U/L; 4 g, 108.3 ± 9.2 U/L; $P < 0.001$; Fig. 2). Plasma AST activity was greater in single-born lambs than multiple-born lambs (94.7 ± 10.9 U/L > 67.8 ± 5.2 U/L; $P < 0.04$). At 30 days of age plasma AST was slightly greater in lambs from ewes treated
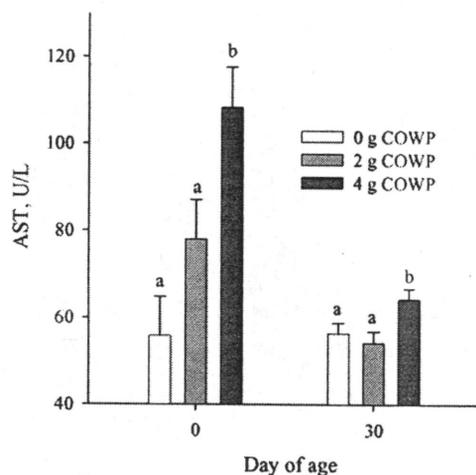
Fig. 2. Least squares means and standard errors of plasma aspartate aminotransferase (AST) activity determined in lambs at birth and 30 days of age born from ewes administered 0 ($n = 21$; white bars), 2 g ($n = 29$; light gray bars), or 4 g ($n = 26$; dark gray bars) copper oxide wire particles (COWP) during late pregnancy. Within an age category least squares means lacking a common letter differ ($P < 0.05$).
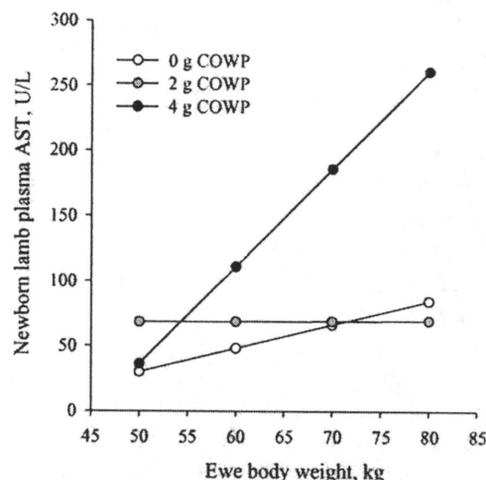

Fig. 3. Regression lines for plasma aspartate aminotransferase (AST) activity determined in lambs at birth born from ewes administered 0 ($n = 21$; white symbols), 2 g ($n = 29$; gray symbols), or 4 g ($n = 26$; black symbols) copper oxide wire particles (COWP) during late pregnancy vs. body weight of dam. Symbols represent predicted values for 50, 60, 70, and 80 kg ewes. Slope and intercept for each COWP treatment are different ($P < 0.001$).

with 4 g COWP (0 g, 56.3 ± 2.4 U/L; 2 g, 54.2 ± 2.7 U/L; 4 g, 64.3 ± 2.4 U/L; $P < 0.02$; Fig. 2). There was no effect of birth type on AST activity. There was a greater increase in AST activity in lambs from ewes treated with 4 g COWP as body weight of dam increased compared with lambs born from dams treated with 0 and 2 g COWP ($P < 0.001$; Fig. 3). Lamb survival to 30 (89.5%), 60 (89.5%), or 120 (birth to 120 days of age, 71.0%; weaning to 120 days of age, 79.4%) days of age was not affected by COWP treatment to ewes.

Birth weights decreased with increasing dose of COWP (0 g, 4.4 ± 0.14 kg; 2 g, 4.0 ± 0.14 kg; 4 g, 3.7 ± 0.14 kg; $P < 0.003$; Fig. 4A) and were greater in single compared with multiple-born lambs ($P < 0.03$). The regression equation was determined to be linear ($y_{\text{single}} = 4.3 - 0.051x$, $y_{\text{multiple}} = 4.1 - 0.206x$, where $y =$ birth weight and $x =$ COWP treatment; $P < 0.03$). By 30 (0 g, 13.7 ± 0.51 kg; 2 g, 11.8 ± 0.38 kg; 4 g, 11.2 ± 0.34 kg; COWP $\times$ birth type, $P < 0.02$; Fig. 4B) and 60 (0 g, 20.3 ± 0.84 kg; 2 g, 16.6 ± 0.63 kg; 4 g, 16.9 ± 0.56 kg; COWP $\times$ birth type, $P < 0.02$; Fig. 4C) days of age, weight of multiple-born lambs decreased with increasing dose of COWP. In mid-September ($\sim$120 days of age) weights of multiple-born lambs from ewes treated with 4 g

COWP tended to be lightest compared with lambs from ewes treated with 0 or 2 g COWP or single-born lambs ($P < 0.10$; Fig. 4D).

The LS mean of PCV (21.9%) and log transformed FEC (untransformed mean, 3953 eggs/g) of post-weaned lambs was similar among ewe COWP groups and weaning type.

4. Discussion

FEC reduction in response to COWP was 66% and 53% in ewes treated with 2 and 4 g, respectively. Effectiveness of COWP to reduce parasite infection in these ewes was less than that reported in lambs (Burke et al., 2004) possibly due to the seasonal difference in populations of nematode species present in the gastrointestinal tract. COWP has been reported to be more effective in reducing abomasal nematodes than intestinal (Bang et al., 1990; Knox, 2002). Species of nematodes was not determined in the present study, but fecal cultures from a group of lambs measured the previous month were 94% *H. contortus* and 6% *Trichostrongylus* spp. (Burke and Miller, unpublished data). The season (spring) may have influenced the
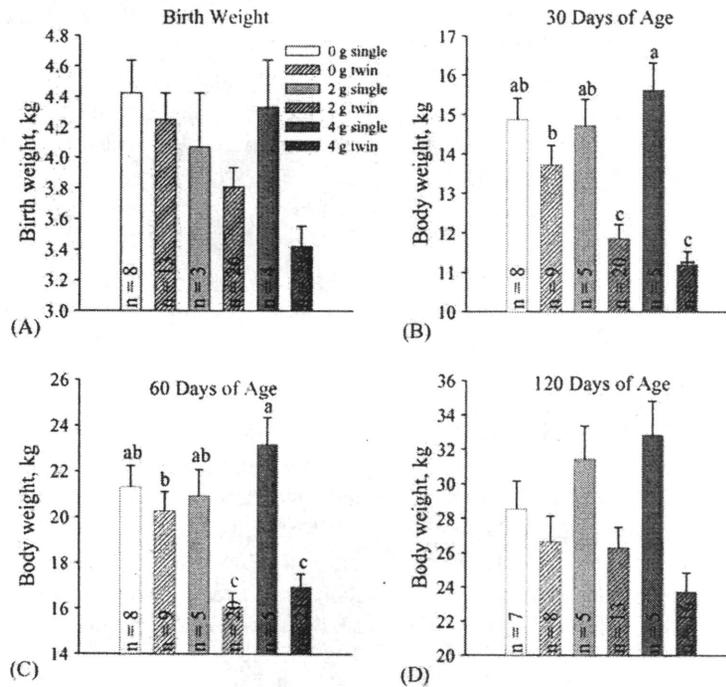
Fig. 4. Least squares means and standard errors of body weight of lambs born from ewes administered 0 (white bars), 2 g (light gray bars), or 4 g (dark gray bars) copper oxide wire particles (COWP) during late pregnancy. Body weights of single (no hatch) and multiple (hatched bars) born lambs are presented at birth (A), 30 (B), 60 (C), and 120 (D) days of age. Within an age category least squares means lacking a common letter differ ($P < 0.05$). Numbers of lambs for each group are included within bars. Between birth and 30 days of age three lambs died and five lambs were orphaned. At 60 days of age 11 lambs were culled and three lambs died after weaning.

types of gastrointestinal nematodes present compared with sheep grazing during the summer. For example, *H. contortus* is considered to be a warm season nematode, while *Trichostrongylus* spp. is considered a cool season nematode (Levine, 1968). The moderate infection level on Day 0 may not have warranted anthelmintic treatment, especially in consideration of the relatively high PCV levels. Katahdin ewes are reported to be more resistant to nematode parasites and less susceptible to a peri-parturient rise than most wool breeds (Burke and Miller, 2002). The increase in PCV as ewes approached lambing was likely due to the increased nutrition received during that period. Others have reported decreased gastrointestinal nematode infection in lambs fed an increased level of protein (Roberts and Adams, 1990; Datta et al., 1999).

Although number of lambs born per ewe tended to be greater in COWP-treated ewes, this difference may have been independent of treatment as number of embryos per ewe was not determined before COWP administration.

Surprisingly, COWP treatment of pregnant ewes influenced birth weight and liver integrity of newborn lambs. Copper from the COWP can be absorbed into the blood, transported to the liver, and become incorporated as ceruloplasmin, a transport protein (for review, Linder et al., 1998), as evidenced by increased plasma ceruloplasmin in ewes (McPhee and Cawley, 1988). Ceruloplasmin-copper is taken up by most tissues in the body, including the placenta, and amniotic fluid (Linder, 1991). In a copper deficient flock, lambs born from dams treated with COWP had increased levels of copper compared with lambs from untreated dams (McPhee and Cawley, 1988). Ceruloplasmin may also be found in milk and may be available to the nursing animal (Linder et al., 1998). Newborn lambs were protected from hypocupremia when dams were supplemented with COWP, although the protection was short-lived (Whitelaw et al., 1980). Results in the current study are consistent with copper being transported to fetuses from COWP-treated dams and thus increasing AST activity at birth. Even 30 days

later, lambs from the 4 g COWP-treated ewes had slightly elevated AST activity, which may suggest that these lambs were receiving copper through the milk or levels of copper in the liver were still elevated.

It has been reported that heavier ewes gave birth to greater total lamb birth weight, placental weight, and livers were larger in newborn lambs (Clarke et al., 1997). This may explain why AST activity increased in lambs from 4 g COWP-treated ewes as body weight of dam increased. There may have been more excess copper in these ewes that could have diffused into fetal tissues leading to greater fetal copper levels. The AST activity in lambs was similar between 30-day-old lambs from control and 2 g COWP-treated dams.

It is unclear why AST activity at birth was greater in single compared with multiple-born lambs. By 30 days of age there was no difference in AST activity of single and multiple born lambs although single-born lambs continued to gain more than multiple-born lambs. This is further evidence that copper was transported in utero and less available in milk.

It is not obvious why birth weights were lighter or multiple-born lambs from COWP-treated ewes gained less than control multiple-born lambs. There have been no other reports of elevated copper leading to lighter weights or decreased gains in ruminant animals. However, by 120 days of age this difference in multiple-born lambs may have disappeared. The 120 days weight does not account for the improved population of lambs from the 2 g and 4 g COWP-treated ewes resulting from culling lighter lambs. Although multiple-born lambs from COWP-treated ewes were lighter, survivability was similar to other groups of lambs. A lighter weight did not predispose lambs to greater parasite infection as FEC and PCV was similar among groups of lambs. Similarly, ewe COWP treatment during pregnancy did not affect level of gastrointestinal parasitism in weaned lambs.

There are risks associated with the use of COWP in sheep, especially in geographical areas susceptible to copper toxicity. Sources of copper that can lead to copper toxicity in sheep include cattle feed or mineral, water (copper pipes), or feed mill contamination. Excess copper can lead to copper toxicity in sheep; signs include diarrhea, anorexia, dehydration, and hemolytic crisis (Aiello, 1998). Low levels of dietary molybdenum, sulfate, or zinc may increase absorption or retention of copper. Hemolytic crisis in chronically toxic animals may be precipitated by stress, poor nutrition, and lactation. 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). Copper metabolism may differ between wool breeds and hair breeds, such as Katahdin used in the present study.

The peri-parturient rise in FEC may necessitate deworming in pregnant ewes, especially during warmer months conducive to *H. contortus*. Producers must weigh the risk of supplemental COWP to that of level of *H. contortus* infection in pregnant ewes. At this time 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).

5. Conclusion

Effectiveness of COWP as an anthelmintic in mature ewes did not appear to have been as great as that previously observed with lambs. This may have been due to the season in which the study occurred as COWP is only effective on abomasal nematodes (i.e., *H. contortus* and *Teladorsagia circumcincta*) and in the spring higher levels of non-abomasal nematodes are usually present. Additional research is necessary to examine effects of COWP on known populations of nematodes in mature sheep. COWP administration to ewes in late gestation and placental transfer of copper may have an effect on liver integrity in newborn lambs but this appeared to be short-lived. Survivability and body weight by 120 days of age was similar among all groups of lambs.

information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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