POTENTIAL NEWER CONTROL METHODS

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With the advent of widespread anthelmintic resistance, the risk of production losses due to gastrointestinal nematode (GIN) infection has become a major issue for small ruminants. Reliance on drug/chemical control can no longer be considered the standard approach and alternative methods need to be available as part of an integrated control program.

Copper oxide wire particles (COWP) are marketed as a supplement for use in areas where copper levels in forages are insufficient and copper deficiency issues (ataxia due to degeneration of the spinal cord, spastic paralysis in newborns, anemia, color changes in pigmented wool or hair, etc.) are present. It is also known that COWP treatment results in a decrease in fecal egg counts (FEC) where *Haemonchus contortus* is the predominant GIN (Bang et al., 1990a). *Ostertagia ostertagia* and *Teladorsagia circumcincta* are also abomasal GIN, but COWP have been shown to have limited efficacy against these parasites, possibly due to the increase in abomasal pH associated with the pathology of these nematodes (Bang et al, 1990; Lawton et al, 1996; Dimander et al, 2003). COWP can be administered orally in gelatin capsules or mixed in feed (Terrill et al., 2012). Efficacy is similar for the two methods (Burke et al., 2010). Once in the rumen, the particles mix with the ingesta and pass through the GI tract. In the abomasum, particles adhere to the mucosa and the acidity causes dissolution of copper ions from the particles (Patten, 2006; Vatta et al., 2009). The peak concentration of COWP is at 4 days but can persist for as long as 10 days before decreasing (Bang et al., 1990). Studies have shown that the efficacy of COWP in reducing FEC was as high as 97% in *H. contortus* infected sheep and goats (Burke et al., 2004; Burke and Miller, 2006; Burke et al., 2007; Soli et al., 2010; Burke et al., 2012). Reduction in worm fecundity and larval contamination of grazing areas has also been observed (Knox, 2002). With the reduction of *H. contortus,* an increase in blood packed cell volume also occurs, thus signs of anemia dissipate (Burke et al, 2004; Soli et al, 2010). However, limited efficacy was seen for small and large intestine GIN. Sheep are highly susceptible to copper toxicity and the margin of safety is very narrow. Copper accumulates in the liver and toxicity occurs when the liver cannot accumulate any more. The excess copper remains in the blood, causing a hemolytic crisis and resultant anemia which can lead to poor production and mortality. Therefore, with copper sensitivity issues in mind, the least amount of COWP that results in an acceptable level of control, without toxicity, should be used. Low doses of COWP (0.5 g or 1.0 g) have been given multiple times (6 week intervals) over a summer grazing season and liver copper concentration was within the normal range, but treated animals did have higher liver copper concentration than untreated animals (Burke and Miller, 2006). Monitoring of liver enzymes (sorbitol dehydrogenase, aspartate aminotransferase and gamma-glutamyl transferase) is important if multiple treatments are administered (Solaiman et al, 2001; Burke and Miller, 2006). Copper sulfate has also been associated with control of *H. contortus*, but that form of copper is more readily absorbed than COWP, therefore, toxicity may be more of an issue. Copper sulfate added to supplement feed or administered orally as a drench was shown not to be very effective for controlling *H. contortus* (Burke and Miller, 2008). The mode of action is not known, but it has been speculated that copper changes the ideal abomasal environment for *H. contortus* leading to death and/or expulsion (Burke et al., 2004). In addition, recent scanning and transmission electron microscope imagery revealed physical damage to the cuticle of *H. contortus* recovered from COWP treated animals (Moscona, 2013; V. Kelly, unpublished observations). This may result in disruption of vital processes that are necessary to maintain viability. In areas where *H. contortus* is present in high numbers, COWP may be an effective means of controlling infection while reducing the need for anthelmintics, but regulation of copper supplementation to avoid toxicity is important.

Nematophagous fungi are potential candidates for biological control of GIN. These fungi are found worldwide and occur naturally in soil and other environments that are rich in organic matter, i.e. feces (Jackson and Miller, 2006). There are over 200 species and are divided into three groups: nematode trapping, endoparasitic, and those that parasitize nematodes within cysts and plant root knots. All of these are saprophytic soil dwellers with the ability to utilize nematodes as a source of energy. Cyst and root knot fungi produce vegetative hyphae that penetrate eggs within a cyst or root knot (Vianene and Abawi, 2000). Endoparasitic fungi spores are ingested by the host nematode where they germinate and disseminate throughout the body of the nematode and eventually penetrate the cuticle forming new spores (De and Sanyal, 2009). Nematode trapping fungi form specialized structures that ensnare (trap) the host nematode. The morphology of these trapping structures is diverse (Grønvold et al., 1993; Grønvold et al., 1996b). A successful candidate for biological control must be able to access the fecal pat in order to have contact with the developing larvae. Trapping activity has been examined and *Arthrobotrys* spp. consistently yielded reductions in infective larvae (L3) when mixed into feces (Waller and Faedo, 1993; Larsen et al., 1994). However, when spores were fed to sheep, the harsh abomasal environment rendered the thin-walled spores non-viable (Larsen et al., 1994). *Duddingtonia flagrans* thick-walled spores were assessed for the ability to survive the GI tract as well as retain predatory activity. The advantage of *D. flagrans* is its rapid growth rate and affinity for trapping and digesting larvae as evidenced by the number of L3 recovered from feces being reduced by over 80% (Larsen et al., 1998; Waller et al., 2006). *D. flagrans* uses hyphal nets and rings which constrict and ensnare L3. The most important factor to induce trap formation is contact of the mycelium by moving larvae as they will serve as a food source (Nordbring-Hertz et al., 2006). Once a larvae is anchored by the trapping structures, hyphae then penetrate the larval cuticle and digest it from within (Grønvold et al., 1996a). Maximum trap formation was achieved at 30°C when induced with L3 of *Ostertagia ostertagi* (Grønvold et al., 1996b). This study also revealed that trap formation occurred between 10°C to 35°C and gradually dropped off after 2 to 3 weeks. Studies have been conducted using *D. flagrans* spores in oral suspensions and in feed supplements administered to sheep, goats, and cattle with mixed species GIN infection (Mendoza de gives et al., 1998; Knox and Faedo 2001; Waller et al., 2001; Peña et al., 2002; Waghorn et al., 2003; Fontenot et al., 2003; Dimander et al., 2003; Terrill et al., 2004; Waller et al., 2006; Ojeda-Robertos et al., 2008). Efficacy of *D. flagrans* is determined by a reduction of L3 in feces (or on forage around feces) and by a reduction in infection in tracer animals (Ketzis et al., 2006). In sheep, Peña et al. (2002) reported dosages in the range of 50,000-1,000,000 spores per kg body weight fed for 7 days resulted in 76.6 to 100% reduction of L3 in feces. Fontenot et al. (2003) reported a dose of 500,000 spores per kg of body weight fed for 18 weeks to grazing ewes resulted in a 78.9-99.1% reduction of L3 in feces, as well as reduced pasture infectivity, and a 96.8% reduction of GIN infection in tracer animals. Similarly, Waghorn et al. (2003) reported that lambs and kids fed 250,000 or 500,000 spores per kg body weight resulted in an efficacy of approximately 78% and there was no bias to parasite species or host animal. A study using Spanish meat goats with mixed species infection reported that every day feeding was almost as good as every other day, and both were better than every third day. Therefore, missing a day did not take away from adequate control (Terrill et al., 2004). Based on these data, the recommended dose for sheep and goats was 250,000-500,000 spores/kg of body weight and for cattle 1,000,000 spores/kg of body weight (Ketzis et al., 2006). Larval reduction using *D. flagrans* usually occurs within 7 days after beginning the treatment, and some level of reduction can last up to 4-5 days post treatment (Mendoza de Gives et al., 1998; Burke et al., 2005). The shelf life of the spores varies depending on environmental conditions. Moist environments simulate germination and can shorten shelf life to less than a week (Larsen, 2006). In contrast, dried spores have a shelf life of more than 20 months (Grønvald et al., 1996b). An additional concern is the potential adverse effects of *D. flagrans* on the environment, particularly beneficial soil nematodes. Free-living saprophitic nematodes, arthropods and other nematode-trapping fungi were not adversely affected by *D. flagrans* (Knox et al., 2002). The main goal of using *D. flagrans* is for long term reduction of pasture contamination as there is no anthelmintic effect on adult GIN in the animal (Ketzis et al., 2006). It is known that copper is an active fungicide and if COWP are used to control *H. contortus*, there may be an interaction that affects viability of the fungus in the feces. This possible scenario was evaluated, and there was no adverse effect of COWP on *D. flagrans* L3 reduction (Burke et al., 2005). Of particular note, L3 were reduced by COWP, and there was an additional reduction by *D. flagrans*. This shows promise for using both of these biological alternatives together in a control strategy. In spite of the many benefits of *D. flagrans*, one concern is the daily feeding of animals. Possible solutions include delivering spores in feed blocks and sustained release systems (Waller et al., 2004; Ketzis et al., 2006). These methods have been evaluated, but unfortunately not been pursued. Therefore, daily feeding is still the best method for delivery. In that regard, International Animal Health Products (IAHP) in Australia has established a strain of *D. flagrans* that can provide control at a dose of 30,000 spores/kg body weight, which is much lower than previous recommendation. This has made the cost of commercialization reasonable. It is anticipated that IAHP will have their feed additive product available in the near future.

Overall, incorporating COWP and/or *D. flagrans* into an integrated control program for GIN is warranted.

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