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Research paper

Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Pasture larval studies in horses, cattle and goats

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ABSTRACT

A series of placebo-controlled trials were conducted in horses, cattle and goats in different seasons and bioclimatic regions of New South Wales and Queensland, Australia, to evaluate the ability of BioWorma®, a feed supplement containing the spores of *Duddingtonia flagrans* IAH 1297, to reduce the larval development of parasitic gastrointestinal nematodes (GIN) and their subsequent migration from faeces onto the surrounding pasture.

In each trial, faeces were collected from animals harbouring a burden of nematode parasites following a period of supplementation with a placebo and again after supplementation with BioWorma. The faeces were manually placed onto pasture plots at one or two distinct geographical sites and the effect of treatment was determined by subsequent monitoring the numbers of parasitic larvae on the pasture surrounding the faecal pats at two weekly intervals over an eight week period. The results for these studies showed that administration of BioWorma at a minimum daily dose of 3×10^4 spores/kg bodyweight reduced parasite larvae in the pasture surrounding the faeces by 53–99 % over an eight week post treatment period in horses, cattle and goats in a range of bioclimatic zones and in different seasons.

Overall, the studies with BioWorma show substantial reductions in GIN infectivity of pasture surrounding faeces of treated horses, cattle and goats ($P < 0.05$). Results indicate that the use of BioWorma in these host species would lead to decreased levels of GIN infection in animals grazing pasture when this product is used and would provide an alternative means of controlling parasitic nematodes.

1. Introduction

Gastrointestinal nematodes (GIN) are important parasites of grazing animals worldwide, having a negative impact on productivity, reproductive performance and animal welfare. In extreme cases, parasitism can lead to death of the host animal. The problem has been exacerbated by the parasites' acquisition of resistance to the anthelmintic chemicals traditionally used to control them (Kaplan and Vidyashankar, 2012). The widespread problem of anthelmintic resistance has led to greater emphasis being placed on non-chemotherapeutic means of parasite control (Gill and Le Jambre, 1996; Knox et al., 2012). In addition, for meat and milk producing livestock, increasing numbers of consumers are requiring products that are derived from systems using minimal chemical interventions (Will, 2015).

One novel approach to controlling GIN is the potential to use nematophagous fungi to reduce free living larval stages. *Duddingtonia flagrans* has been widely studied and produces robust chlamydospores that can be added to animal feed (Mendoza de Gives et al., 2006). After being consumed in feed, the chlamydospores pass through the animal's digestive tract and inoculate the faeces where they germinate. *D. flagrans* then forms a network of hyphae throughout the faecal mass which inhibits the free living stage of larvae from completing their development by producing sticky traps which capture and destroy the larvae (Fontenot et al., 2003; Ojeda-Robertos et al., 2009; Paz-Silva et al., 2011). Therefore, *D. flagrans* reduces nematode populations on pasture surrounding the faecal mass and consequently lowers the incidence of

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Table 1
Summary of animal numbers, seasons, dates, trial sites and average daily temperatures at the trial sites.

Trial Code	Number of animals	Seasons (calendar months and year)	Locations of pasture phases	Average daily temperatures (°C) (Max. / Min.)
Horse Trial 1	5	Autumn (March – May 2009)	Armidale, NSW	20.8 / 9.2
Horse Trial 2	6	Spring (September – November 2010)	Armidale NSW Nimmitabel NSW	19.5 / 8.7 20.0 / 5.3
Horse Trial 3	6	Autumn (April – June 2011)	Armidale NSW Dayboro QLD	16.4 / 4.5 24.0 / 13.1
Cattle Trial 1	6	Spring (October – December 2010)	Armidale NSW Nimmitabel NSW	21.6 / 10.6 22.0 / 8.8
Cattle Trial 2	6	Autumn (April – June 2011)	Armidale NSW Dayboro QLD	15.3 / 3.9 22.9 / 12.3
Goat Trial 1	6	Spring (October – December 2010) =	Armidale NSW Nimmitabel NSW	21.5 / 10.3 21.8 / 8.2
Goat Trial 2	12	Autumn / Winter (May – July 2011)	Armidale NSW Dayboro QLD	13.6 / 2.2 21.6 / 10.0
Goat Trial 3	12	Spring/ Summer (November 2011 – January 2012)	Dayboro QLD Nimmitabel NSW	28.3 / 17.8 23.7 / 8.8

infection of animals grazing that pasture (Waller et al., 1994; Baudena et al., 2000).

A number of studies have been published where efficacy of *D. flagrans* has been demonstrated after being fed to horses (Larsen et al., 1996; Fernandez et al., 1999a; Hernandez et al., 2016), cattle (Grønvold et al., 1993; Nansen et al., 1995), goats (Wright et al., 2003; Sanyal et al., 2008) and sheep (Knox and Faedo, 2001; Fontenot et al., 2003; Healey et al., 2018). Efficacy was assessed by reductions in larval burdens on pasture or by reduced total GIN numbers in tracer animals after grazing.

Here we report results of a series of studies conducted to determine the effectiveness of BioWorma, a product containing the chlamydospores of *D. flagrans* strain IAH 1297, in reducing larval migration of GIN from faeces of horses, goats and cattle onto the surrounding pasture.

2. Materials and methods

2.1. Experimental protocol

For each trial, animals harbouring a burden of nematode parasites were selected from a larger group of animals on the basis of worm species present and individual faecal egg counts Larval differentiation (Thienpont et al., 1979; van Wyk et al., 2004) was conducted following group bulk coproculture (50 g sample size) and individual FECs were conducted in triplicate according to a modified McMaster method (Hutchinson, 2009) with sensitivity of 40 eggs per gram (2.5 g samples examined). Resistance status of natural infections was determined from the results of testing of the parent flock by the Diagnostic Services Laboratory of Invetus Pty Ltd. The animals were housed in individual pens with no access to pasture to prevent infection from pasture-based larvae and to assist with supplementation and faecal collection. In some cases the naturally-acquired infections were replaced by, or augmented with, artificial infections. The animals were fed placebo (Livamol®, a product made of molasses, protein and oilseed meals, fish oil, and vitamins and minerals, made by International Animal Health Products Pty Ltd) for 5–7 days then their faeces were collected. Each animal's faeces (Control samples) were kept separate and mixed until homogeneous and faecal egg counts (triplicate) were determined. Four "pats" per sample (matched by weight) were then transported within 24 h of collection by overnight transport for manual placement on day of arrival onto the centre of randomly-allocated 85 cm x 85 cm pasture plots at one or two distinct geographical sites, maintaining an 85 cm distance between plots. The pasture in all trials was typical of that used for grazing animals in the region and had not been grazed for more than 12 months to ensure freedom from infective larvae. The pasture was newly-cut to a height of approximately 10 cm prior to placement of the faecal pats.

The same animals were then fed an equivalent amount of Livamol containing BioWorma (Investigational Veterinary Product, manufactured by International Animal Health Products Pty Ltd) for 5–7 days, providing 3×10^4 chlamydospores *D. flagrans* strain IAH 1297/kg bodyweight (b.w.)/day, and their faeces were again collected and tested as above. The "treated" faeces (BioWorma samples) were then tested, prepared, transported and placed onto pasture plots as above at the same sites used for the Control samples. The total number of faecal pats deposited at each site was 48 = 6 animals x 2 treatments x 4 samples.

The trial dates, seasons and locations of the pasture phases in these trials are shown in Table 1. The trial sites and their bioclimatic zones (Taylor and Hodge, 2014) were Armidale, New South Wales (NSW) in the Northern Tablelands zone; Nimmitabel, NSW in the Southern and Central Slopes / Tablelands zone and Dayboro, Queensland (Qld) in the Subtropical Coastal Qld zone. Trials were conducted predominantly in the spring and autumn, as these are the times when parasite buildups are likely to occur (Donald et al., 1978; Barger et al., 1983).

At 2 weekly intervals from the date of placement (from week 2 through to 8 weeks post placement) for each animal at each trial site the herbage in a 40 cm circle under and around a randomly-selected Control and BioWorma faecal pat was collected down to the ground level using electric clippers. Pasture washings were conducted according to a method modified from Heath and Major, 1968. Briefly, the grass clippings were placed in a 350 µm mesh sealable bag within a pasture washer (metal conical vessel with gated valve at the bottom). The mesh bag was immersed in 90 L of warm water (30 °C) containing Pyroneg detergent (5 g, Diversey, Inc.) to facilitate separation of the larvae from the grass clippings. The contents were agitated for 5–10 min after 1, 3 and 5 h soaking time. After a total of 8 h, two 1 L aliquots from the sedimented washings were collected and cooled at 4 °C overnight. The supernatant was subsequently removed to reduce the volume to a level suitable for counting nematode larvae for each host species (enumeration performed by examination of 5 x 10 µL aliquots and with differentials performed as previously described). From the data the number of infective larvae in each grass sample was calculated.