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Effect of the consumption of heather on incoming larvae and established population of *Teladorsagia circumcincta* in experimentally infected Cashmere goats

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ABSTRACT

This study was performed in Cashmere goats that were experimentally infected with *Teladorsagia circumcincta* to investigate the effects of heather consumption on the establishment of incoming infective larvae (experiment 1) and on an adult nematode population (experiment 2). In experiment 1, 24 non-lactating goats were divided into 2 groups: heather-supplemented *vs.* non-supplemented. After 2 weeks of adaptation to the diet, all of the goats were experimentally infected with 6000 infective larvae of *T. circumcincta*. Twelve animals (6 controls and 6 supplemented with heather) were slaughtered at 6 days and at 3 weeks post-infection (pi). After slaughter, the worms were counted and the female worm fecundity and development were determined. Heather consumption was associated with a significant reduction in larval establishment at 6 days ($P = 0.033$) and at 3 weeks ($P = 0.041$) pi. No differences in worm counts between the slaughter times were found. In the goats slaughtered at 3 weeks pi, the number of eggs *in utero* and length of the female worms were significantly ($P < 0.001$) lower than those of control group. In experiment 2, 24 non-lactating goats were experimentally infected with 10,000 *T. circumcincta* infective larvae daily for 5 consecutive days (total infection of 50,000 larvae). After 3 weeks, 2 groups were established: control and heather-supplemented. The faecal egg output of each animal was measured at 2-days interval during the experimental period. The goats were slaughtered after 3 weeks of heather administration for parasitological studies. Heather administration was associated with a significant decrease in egg excretion between 25 and 29 days pi. The worm counts were similar in both groups, but the female length and fecundity were significantly ($P < 0.001$) lower in supplemented goats. These results show that heather consumption reduces the establishment of *T. circumcincta* larvae in goats and the development and fecundity of female adult parasites.

1. Introduction

An increasing number of studies have shown that the consumption of bioactive plants containing secondary metabolites, such as tannins, by small ruminants can regulate their gastrointestinal (GI) nematode population

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(Brunet et al., 2008b; Terrill et al., 2009; Manolaraki et al., 2010), thus reducing their dependence on conventional chemotherapy and supporting the possibility of a sustainable mechanism to control GI parasitism (Waller and Thamsborg, 2004; Hoste et al., 2006; Waller, 2006).

Heather species belong to the Ericaceae family (*Erica* spp. or *Calluna vulgaris*) and have a moderate to high tannin content (ranging from 30 to 100 g tannic acid equivalents per kg dry matter (DM), Moreno-Gonzalo et al., 2012) but low nutritive value (Frutos et al., 2002). Heather is a common component of the natural vegetation found in mountain communities in humid temperate areas. Goat diets include high proportions of heather because goats browse on heathlands even when pastures are available (Celaya et al., 2007). Recently, a number of studies have been conducted to investigate the anthelmintic and nutritional effects of heather supplementation on goats naturally infected by GI nematodes (Osoro et al., 2007a,b, 2009; Frutos et al., 2008; Celaya et al., 2010). The results of these studies suggest that (i) heather supplementation significantly reduces the level of GI nematode egg excretion in grazing goats, (ii) the faecal nematode egg counts (FEC) reduction could be associated with a decrease in worm fertility and/or a reduction in the establishment of incoming third-stage larvae, (iii) the consumption of heather is associated with an apparent increase in the resilience of the goats to GI nematode infections, and (iv) the amount of tannins consumed by goats offered heather does not seem to be associated with substantial anti-nutritional effects, which eventually resulted in a better performance in the animals incorporating these shrubs in their diet.

A number of field experiments performed with grazing goats have shown that many plants have anthelmintic activity (Kabasa et al., 2000; Min et al., 2004, 2005; Pomroy and Adlington, 2006; Moore et al., 2008; Landau et al., 2010), including heather (Moreno-Gonzalo et al., 2012). However, grazing experiments with goats that harbour mixed GI nematode infections are strongly dependent on climatic conditions, and the number and the development stages of the nematodes present in the GI tract cannot be precisely determined by FEC and coprocultures. Additionally, the effects of the consumption of tannin-containing plants could vary depending on the stage of the parasites upon exposure to the tannins (Paolini et al., 2003c). *Teladorsagia circumcincta* is the most prevalent gastric nematode found in goats in temperate dry and humid areas, such as Central and Northwest Spain (Valcárcel and Romero, 1999; Álvarez-Sánchez et al., 2006). The present study was performed in Cashmere goats experimentally infected with *T. circumcincta* to investigate the effects of heather consumption on the establishment of incoming infective larvae (experiment 1: preventive administration), and on the adult parasite population (experiment 2: curative administration).

2. Materials and methods

2.1. Experimental site

The experiments were conducted at the "El Carbayal" research station in northwest Spain (6°53′W, 43°20′N, Sierra de San Isidro, Illano, Asturias) in accordance with the Spanish Royal Decree 1201/2005 (based on European Council Directive 86/609/ECC) for the protection of animals used for experimental and other scientific purposes.

2.2. Experimental designs

2.2.1. Experiment 1: preventive administration of heather

Twenty-four, 30–50 months old, non-lactating Cashmere does showing <100 trichostrongylid eggs per gram (epg) of faeces were dewormed with ivermectin (Oramec, Merial, Lyon, France) 4 weeks before the experiment start date. The trial lasted for 5 weeks and was divided into 2 successive periods: a 2-weeks period for adaptation to the diet and a 3-weeks experimental period. The goats (showing no nematode eggs in coproscopic examination) were divided into 2 groups of 12 animals each, balanced according to their body weight (32.7 ± 1.85 kg BW): heather supplemented (70% alfalfa hay + 30% heather) *vs.* non-supplemented (alfalfa hay). Diets (30 g DM kg $BW^{-0.75}$ day^{-1}) were offered twice a day (at 9:00 h and 18:00 h), accounting for an average of 410 g DM $goat^{-1}$ day^{-1} as calculated for the goats mean BW. The amounts were below the maintenance requirements of non-lactating, non-pregnant goats and were conceived to avoid refusals. Heather was cut every 3 days in a nearby heathland area belonging to the experimental farm and stored chilled until it was supplied to the animals. *C. vulgaris* accounted for more than 90% of the supplied heather, with scarce amounts of *Erica umbellata* and *Erica cinerea*. Total phenolic and tannin values in heather were 76.5 (±4.9) and 48.2 (±2.3) g tannic acid equivalents per kg DM respectively (assays for heather total phenolics and tannins were conducted following the Folin–Ciocalteu technique in combination with polyvinyl-polypyrrolidone, using tannic acid as the reference standard, Makkar et al., 1993). After 2 weeks of adaptation to the diets, all of the goats were experimentally infected with 6000 *T. circumcincta* infective larvae (supplied by the Moredun Research Institute, Scotland, UK). During the experimental period, the goats were maintained in individual metabolic cages with unrestricted water, and their food intake was monitored. Twelve animals (6 controls and 6 supplemented with heather) were slaughtered at 6 days post-infection (pi) and the other 12 at 3 weeks pi (3 and 5 weeks, respectively, after heather administration in the supplemented group). After slaughter, the worms were counted and the female worm fecundity and development were determined.

2.2.2. Experiment 2: curative administration of heather

Twenty-four, 30–60 months old, non-lactating Cashmere does showing <150 trichostrongylid epg of faeces were dewormed with ivermectin (Oramec, Merial, Lyon, France) 4 weeks before the experiment began. The trial lasted for 6 weeks and was divided into 2 successive periods: a 3-weeks period for the establishment of an adult nematode population and a 3-weeks experimental period including the administration of heather in the supplemented group. All of the does (showing no trichostrongylid eggs in coproscopic examination) were experimentally