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Effects of condensed tannin on natural coccidian infection in goat kids☆

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ABSTRACT

This study was carried out to test the efficacy of a particular type of Quebracho extract, rich in condensed tannins on coccidian infections and growth performances of kids naturally infected with *Eimeria* spp. Thirty-nine male kids aged 52–81 days were randomly allocated in 4 groups: A control group (CG, 9 kids) and three tannin-treated groups (TG, 10 kids each), that received a feed supplement containing Quebracho tannin (Silvafeed BYPRO Q). During the 45-day study period, kids received the treated feed, regularly once a day according to a varying schedule: For a week in a month (TG1), for a day every 5 days (TG2) and for 3 days every 15 days (TG3). Faecal samples were taken from each kid at 0, 5, 10, 15, 30, 35, 40, 45 days and individual oocyst counts per gram (OPG) were performed using the double FLOTAC® technique. In addition, animals were weighed at 15, 30 and 45 days of the trial. The results showed that the number of *Eimeria* oocysts in the treated groups decreased since the first 5 study days and was significantly different ($P < 0.0001$) from study 15-day period onwards. The percentage of oocyst reduction higher than 95% was found in 58% of the kids on study day 5. Comparison in OPG counts obtained from TG groups highlighted that in order to obtain a significant control of the *Eimeria* population, a high dose or a prolonged administration of Quebracho was required. In fact, OPG counts recorded in the TG1 kids receiving CT for 14 days resulted in the lowest oocyst count. Further, kids from TG2 recorded a lower least-square mean (LSM) value for OPG than the TG3 group showing that a single and more frequent administration of CT (TG2) seems to be more effective than treatments supplied repeatedly for 3 days (TG3). All kids consuming the CT weighed significantly heavier than the kids of the control group, and weight gain of kids from TG2 was higher than those of the other tannin-treated groups.

1. Introduction

Coccidiosis is one of the most ubiquitous and widespread enteric diseases of goats worldwide. *Eimeria* infections can show severe clinical signs in kids, and loss of animals has been recorded (Khodakaram Tafti and Mansourian, 2008; Chartier and Paraud, 2012). Currently, chemotherapy is extensively being used to control the infection, however the rapid and widespread development of drug resistance coupled with the increasing lack of trust by consumers for meat from chemotherapically treated animals call for alternative methods of control of this disease (Sangster, 2001; Min and Hart, 2003). Tannins are a group of phenolic compounds whose possible nutritional and physiological actions are under investigation. These compounds can be grouped into condensed and hydrolysable tannins, widely distributed in the plant

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kingdom. Condensed tannins are polymers of flavan-3-ols, flavan-3,4-diols or related flavanol residues linked via carbon—carbon bonds (Min and Hart, 2003). Over the last 15 years, the direct antiparasitic activity of condensed tannins (CT) from several plants, such as *Hedysarum coronarium*, has been observed on a few gastrointestinal nematodes in goats and in sheep (Niezen et al., 1995; Athanasiadou et al., 2000, 2001; Molan et al., 2000; Min et al., 2009; Osoro et al., 2009). As part of their effects on the impact of gastro-intestinal parasitism, CT has improved the live weight gain, wool production and reproductive efficiency in sheep and has influenced environmental issues, such as reduction of nitrogen pollution (Waghorn, 2008; Vasta and Luciano, 2011). On the other hand, there is evidence that high levels of tannins can produce adverse effects in animals. It depends on the animal species, metabolic stage, age and level of exposure and on the structure of tannins themselves (Makkar, 1993).

To date, a few surveys have shown the activity of forage containing CT against protozoa of the genus *Eimeria*. Hur et al. (2005) investigated the efficacy of CT from pine needles and oak leaves in goats naturally infected with coccidia and showed that they significantly reduced the number of oocysts excreted through the faeces. Molan et al. (2009) observed the effect of *Pinus radiata* bark extract on the sporulation of three species of *Eimeria* in poultry. More recently, the anticoccidial effect of the legume sainfoin and the foliage from lentisk was considered in lambs and kids, respectively, with promising results (Markovics et al., 2012; Saratsis et al., 2012).

Quebracho is a well-known source of condensed tannin that originates from hardwood trees, mainly belonging to the Anacardiaceae family, native from South America. The extract has shown a direct effect against single or mixed infections by *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep (Athanasiadou et al., 2000; Butter et al., 2000; Max et al., 2005).

The aim of this study was to investigate the possible effects of condensed tannins from Quebracho on coccidian infections and growth performances in kids, naturally infected by the *Eimeria* spp.

2. Material and methods

The study was conducted in a goat farm (AZGC) of the University of Milan located in a mountain area of northern Italy (44°43′0″N-9°2′46″E), at 715 m above sea level. The flock was managed following the intensive system and the kids were housed indoors in small groups and maintained on artificial suckling, until weaning (35–40 days).

2.1. Study design

Thirty-nine kids, aged 52–81 days of the Alpine breed selected for genetic purposes and naturally infected by *Eimeria* were allocated to the study. The animals were randomly allocated in four groups ranked by body weight, age and the number of *Eimeria* oocysts per gram of faeces (OPG) on day −4. Nine kids were allocated to the control group (CG) and 30 were allocated to 3 tannin-treated groups (TG; 10 kids per group). Each group was kept in a pen with a concrete wall. All groups received lucerne hay and water ad libitum and cereal flakes (300–400 g/day) and were allowed to adapt to their surroundings for 4 days before the trial was started. On day 0 for 45 days, kids in the TG groups received a supplementation of Quebracho tannin (QT) in their diet in the early morning, according to a specific schedule, i.e. once a day for 1 week in a month (TG1: from day 0 to day 7 and from day 30 to 37; total of 14 days of treatment); for 1 day every 5 days (TG2: total of 9 daily treatments) and for 3 days every 15 days (TG3: total 9 daily treatments). The QT source was given as a commercially available (Silvafeed BYPRO Q) feed supplement that was obtained as a single batch from one supplier (Silvateam, San Michele Mondovi, Piedmont, Italy). This food additive contained QT extracted from the heartwood of the tropical tree *Schinopsis lorentzii*, containing a minimum of 70% of cold-soluble condensed tannins, as certified by the supplier. Kids were offered the moist brown powder QT ready-mix with cereal flakes formulated to supply a 5% of CT per kg of dry matter. The study was approved by the Milan University Institutional Animal Care and Use Committee.

2.2. Data collection and parasitological analyses

Faecal samples were taken directly from the rectum of each kid on days 0, 5, 10, 15, 30, 35, 40 and 45 (D0, D5, D10, D15, D30, D35 and D45). All faecal samples were stored at 4 °C until examined. Individual OPG counts were carried out using the double FLOTAC® technique, using a flotation solution (Magnesium Sulphate, s.g.1.280) (Cringoli, 2006). With the double FLOTAC® technique, the reference unit was the single flotation chamber (volume of 5 ml; corresponding to 0.5 g of faeces). The analytic sensitivity of the technique was 2 OPG. Animals were weighed on days 15, 30 and 45 of the study.

In order to identify the *Eimeria* species infecting the kids, a pooled sample of faeces for each group was mixed in 2% potassium dichromate and the oocysts allowed to sporulate for 7 days at room temperature. The mixture was centrifuged at 1200 rpm for 10 min, the supernatant discarded and the sediment placed into a vial filled with a saturated sodium chloride solution. The oocysts were identified according to the criteria of Eckert et al. (1995).

2.3. Statistical analysis

A faecal oocyst count reduction test was carried out and the percentage of reduction was calculated as $\% = 100^*[1-(T/C)]$, where T was the OPG counts in the treated kids and C the OPG counts in control kids on days 5, 10, 15, 30, 35, 40 and 45.

OPG were analyzed as repeated measures, assuming an autoregressive covariance structure. The following model was fitted:

$$y_{ijklmn} = \mu + T_i + D_j + \mathrm{TD}_{ij} + W_k + A_l + e_{ijklmn}$$

where y_{ijklmn} is the dependent variable, μ is the population mean, T_i is the fixed effect of the ith treatment ($i = 1, 4$), D_j the fixed effect of the jth day of measure ($j = 1,8$), W_k is the fixed effect of the kth weight class ($k = 1, 3$), TD_{ij} is the interaction effect between treatment and day of measure,