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Feed resource selection of Criollo goats is the result of an interaction between plant resources, condensed tannins and *Haemonchus contortus* infection

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ABSTRACT

The tropical deciduous forest (TDF) is an ecosystem with a heterogeneous array of plant species containing different condensed tannin (CT) content. Recent studies explored the effect of polyethylene glycol (PEG) or *Haemonchus contortus* infection on the intake and feed resource selection of goats. These factors have not been investigated simultaneously. The present study evaluated the role of infection with the abomasal parasitic nematode *Haemonchus contortus* and the effect of neutralizing CT with PEG on the feed resource selection of Criollo goats in a cafeteria trial. A group of 22 Criollo goats (31.7 ± 4.8 kg) habituated to browse the TDF, were assigned to four groups: two groups were artificially infected with *H. contortus* (6000 L_3 *per os*) and received PEG (50 mg/day) (INF + PEG group) or no PEG (INF group) and the other two groups were kept free of infection with moxidectin (0.4 mg/kg BW sub-cutaneously) and received either PEG (MOX + PEG) or no PEG (MOX). Intake and resource selection of four shrub plant species (*Havardia albicans*, *Gymnopodium floribundum*, *Leucaena leucocephala* and *Piscidia piscipula*) by goats, and the dynamics of faecal *H. contortus* egg excretion were determined daily. Intake varied depending on the interaction between plant resource and the infection level ($P < 0.001$), and between plant resource and whether goats were dosed with PEG ($P < 0.001$). Excretion of *H. contortus* eggs was decreased by 69% and 72% for groups with and without PEG administration, respectively. Infected groups had a higher CT intake. The use of PEG reduced intake of *L. leucocephala* and increased the selection of *H. albicans,* a high-CT and high crude protein (CP) resource. Animals infected with *H. contortus* ingested more high-CT resources. Furthermore, CP intake was lower in PEG dosed goats, possibly to reduce the energetic cost associated with urinary nitrogen excretion. Selection of feed resources varied depending on parasite infection, PEG dosage and plant resource. We concluded that biological interactions influencing intake and selection of feed resources in goats are complex, rather than arising from a single factor such as parasite infection or secondary compounds such as CT. These findings support that goats' resource selection is a complex process aimed at nutritional optimization.

1. Introduction

Goats can display selective behaviour towards feed resources both under grazing and pen-controlled conditions (Duncan and Young, 2002; Egea et al., 2016; Manousidis et al., 2016). The selection behaviour implies that goats consume plant resources disproportionately to their availability (Johnson, 1980; Manly et al., 2004). Gastrointestinal nematode (GIN) infections and the condensed tannin (CT) content of plants have been proposed to modify the intake and selection of feed resources by ruminants. For example, GIN infection changed diet selection (Hutchings et al., 2006) and triggered self-medication behaviour that was either therapeutic (Huffman, 2003) or prophylactic (Amit et al., 2013; Villalba et al., 2016). Feed selection involving plants containing CT presents a duality based on its anti-nutritional effect

Abbreviations: TDF, tropical deciduous forest; CT, condensed tannins; PEG, polyethylene glycol; CP, crude protein; GIN, gastrointestinal nematode; FB, fresh basis; LW, live weight; DM, dry matter; EPG, eggs per gram of faeces; MSI, Manly selection index; NDF, neutral detergent fiber; ADF, acid detergent fiber; TP, total phenols; TT, total tannins

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(Makkar, 2003; Frutos et al., 2004) and its beneficial effect on nutrition (Min and Hart, 2003; Mueller-Harvey, 2006; Piluzza et al., 2014) and health (Hoste et al., 2012, 2015). Previous studies performed with Criollo goats under controlled cafeteria conditions showed that CT content was not the main factor that modified the intake of tannin rich plants (Alonso-Díaz et al., 2008, 2009). Furthermore, neutralizing CT with polyethylene glycol (PEG) did not modify the ingestion of a tannin-rich plant by goats (Brunet et al., 2008) or the selection of different tannin-rich resources (Hernández-Orduño et al., 2012). There is also evidence suggesting that a mixed natural GIN infection (Ventura-Cordero et al., 2017a) or a high artificial *Haemonchus contortus* infection (Ventura-Cordero et al., 2018a) did not influence intake or feed resource selection in Criollo goats. However, no previously published work has explored the resource selection of plants with different CT content using an experimental design where animals are simultaneously infected with *H. contortus* and PEG is used to neutralize CT. This is important because parasitic infections and CT are invariably present under natural grazing/browsing conditions in heterogeneous vegetation such as the tropical deciduous forest (TDF) (Novelo-Chi et al., 2014). Thus, it is important to study secondary compounds in conjunction with gastrointestinal nematodes to broaden our understanding of goat feeding behaviour (Muir, 2011). Given the importance of understanding the fundamental processes affecting the ingestive behaviour of goats, the present study evaluated the role of infection with the abomasal parasitic nematode *H. contortus* and the neutralization of CT on intake and resource selection of Criollo goats in a cafeteria trial.

2. Material and methods

2.1. Study area

The study was conducted at a small ruminant farm belonging to the Faculty of Veterinary Medicine and Animal Science, Universidad Autónoma de Yucatán (FMVZ-UADY) (19°30'N, 87°30'W), in October and November of 2016. The climate of the area is classified as tropical sub-humid with summer rainfall (AW_0) and a mean annual temperature of 27 °C. The mean annual rainfall is 940 mm with a mean annual relative humidity of 72% (Flores and Espejel, 1994).

2.2. Experimental animals

The authors are aware of ethical standards based on the published guidelines of Sherwin et al. (2003) and declare that all experimental procedures complied with these ethical standards. Additionally, the ethical committee of FMVZ-UADY approved the experimental protocol used in the study (reference number: CB-CCBA D-2014-003).

Initially, 22 Criollo goats, all with grazing/browsing experience in the TDF, were selected. GIN natural infection in goats was verified and removed by the application of 12 mg/kg of levamisole (L-Vermizol®, Aranda, Querétaro, México) and 10 mg/kg of albendazole sulfoxide (Parzen® 2.5%, Parfarm, Cd. México, México). Following this treatment, all animals were confined in two 15 × 15 m pens with concrete flooring where they were fed a complete diet consisting of 2.2 kg fresh basis (FB) of chopped *Pennisetum purpureum* grass (representing 2.0% of live weight [LW] in dry matter), along with 300 g FB of grain-based feed supplement (16% crude protein [CP] and 1.8 MCal metabolizable energy [ME]/kg dry matter [DM]) (representing 1.0% of the LW in dry matter), and fresh water *ad libitum*. The latter allowed goats to fulfil their requirements for maintenance with low activity level (AFRC, 1993).

2.3. Artificial infection protocol

After 10 days inside the pens, the absence of patent GIN infection was confirmed in all experimental animals by respective flotation procedures (MAFF, 1986). Then, goats were artificially infected. This procedure lasted for 4 days, during which daily doses of 1500 *H. contortus* infective larvae (L_3), "Paraíso" isolate, were daily administered to reach an infective dose of 6000 L_3 per animal.

The L_3 were obtained from four donor goats with respective monospecific *H. contortus* infection. Donor faeces were obtained daily and were processed at the FMVZ-UADY Parasitology Laboratory. Fresh faeces from donors were mixed and included in faecal cultures that were maintained for 5 days at 28 °C. The L_3 were harvested using a Baermann apparatus. Then, clean larvae were counted and infection doses were prepared for each goat on a daily basis.

2.4. Infection development and experimental group formation

After the artificial infection protocol, goats developed infection and patency was confirmed when the excretion of worm eggs was detected in goat faeces. On day 15 post-infection, animals were placed in individual pens (2 × 2 m) to adapt to handling related to the cafeteria trial. Faecal samples from all goats were obtained daily from day 21 post-infection onwards to identify the presence of *H. contortus* eggs using the flotation technique as described by MAFF (1986), and the modified McMaster technique described by Rodríguez-Vivas and Cob-Galera (2005). Infection was confirmed on days 28, 29 and 30 after artificial infection.

Twelve goats with low mean faecal egg excretions (< 700 eggs per gram of faeces [EPG]) were dewormed with 0.4 mg/kg of injectable moxidectin administered sub-cutaneously (Cydectyn® NF, Fort Dodge Saudé Animal, Campinas, Brazil), to maintain them free of *H. contortus* infection. Then, they were assigned to one of two non-infected groups (n = 6 animals per group). The ten goats with highest faecal excretion (> 2200 EPG) were selected for inclusion into the two infected groups (n = 5 animals per group). One of the infected and dewormed groups was subsequently dosed with PEG. The four experimental groups were: 1) MOX: non-infected goats (n = 6); 2) INF: goats infected with *H. contortus* (n = 5); 3) MOX + PEG: non-infected goats dosed with PEG (n = 6); and 4) INF + PEG: goats infected with *H. contortus* and dosed with PEG (n = 5). The four experimental groups were balanced according to LW and age.

2.5. Cafeteria trial design

After forming the experimental groups, a three-day adaptation period helped to establish the amount of each plant resource offered to experimental goats (400 g FB). This quantity was used as the baseline amount of foliage offered to goats. The cafeteria trial lasted eight days (day 31 to day 38 post-infection). During this time, the voluntary intake and feed resource selection of four plant species (*Havardia albicans, Gymnopodium floribundum, Leucaena leucocephala* and *Piscidia piscipula*) was measured for each experimental goat.

2.5.1. Feed resources

The foliage used in the present study included two plants species with high CT content (*G. floribundum,* and *H. albicans*) and two with low CT content (*L. leucocephala* and *P. piscipula*) (Ventura-Cordero et al., 2018b). These plant species are shrubs commonly consumed by goats in the TDF (Ríos and Riley, 1985; Novelo-Chi et al., 2014; González-Pech et al., 2015; Ventura-Cordero et al., 2018b). Fresh foliage of these plant species was harvested daily from 06:00 h to 08:00 h from bushes and trees in an area adjacent to the grazing circuits of the small ruminant flock of the FMVZ-UADY.

2.5.2. Feeding and observation schedules

A grain-based feed was provided as a supplement at a rate equivalent to 0.5% of their LW as DM (09:00 h). Leaves (400 g FB) of the four plant resources were kept in respective plastic containers before the observations started. The cafeteria trial started at 13:00 h and lasted for one hour. This time was based on previous cafeteria trials using tropical

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