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Research paper

Diet selection and trade-offs between condensed tannins and nutrients in parasitized sheep

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ABSTRACT

Foraging behavior by parasitized herbivores can be interpreted as a decision-making process where individuals are faced with trading-off the ingestion of nutrients with the ingestion of potentially medicinal -and toxic- plant secondary compounds. We determined how parasitized sheep prioritize selection of crude protein, energy and a medicinal plant secondary compound (quebracho tannins-QT). Foraging preferences were tested in 40 lambs before experiencing a parasitic infection (Phase 1), during an infection (Phase 2; 10,000 L_3 *Haemonchus contortus* per lamb) and after chemotherapy (Phase 3). Lambs were assigned to four groups (10 lambs/group) such that animals in Group 1 (Control) could choose between foods of high (HEP) or low (LEP) energy to protein ratios. The other groups received the same choice, but QT were added (4%) to HEP (Group 2), to LEP (Group 3) or to both foods (Group 4). All groups under a parasitic infection (Phase 2) increased their preference for HEP (from 0.44 to 0.66 ± 0.042; $P < 0.05$) and intake of digestible energy (from 0.106 to 0.126 ± 0.007 Mcal/kg BW; $P < 0.05$) relative to Phase 1, a pattern that remained during Phase 3. Only lambs receiving QT in HEP increased their intake of QT from Phase 1 to Phase 2 ($P < 0.05$). Fecal egg counts and blood parameters revealed a parasitic infection ($P < 0.05$) in Phase 2 that subsided in Phase 3, although no differences were detected among groups ($P > 0.05$). The importance of protein nutrition on parasitized animals has been highlighted before, but these results suggest that lambs prioritized the ingestion of energy-dense over protein-dense foods or medicinal condensed tannins when challenged by gastrointestinal parasitism. Consumption of medicinal tannins represented a side-effect of the preference manifested for energy-dense foods during testing.

1. Introduction

Herbivores are constantly challenged by changes occurring in their feeding environment at different temporal and spatial scales (Provenza et al., 2003). They respond to these challenges by generating physiological responses and behaviors that contribute to the achievement of a dynamic state of homeostasis (Villalba and Provenza, 2009). Many of these behaviors emerge as adaptive responses to a variety of environmental stressors and involve adjustments in food intake and selectivity, allowing the animal to cope with the challenge (Villalba and Provenza, 1997; Provenza and Villalba, 2006). For instance, small ruminants are able to reduce nutrient imbalances (Wang and Provenza, 1996) and to avoid toxic compounds (Ginane et al., 2005), via an associative learning process (Provenza, 1995). However, herbivores are faced with a multidimensional and changing feeding environment where each plant represents a diverse array of chemicals with both potential beneficial and deleterious effects in a dose-dependent manner. From the standpoint of plant secondary compounds like condensed tannins (CT), they may on one hand promote toxic/antinutritional effects (i.e., a cost) (Lozano, 1998), and on the other supply medicinal effects at different –typically lower- concentrations (i.e., a benefit) (Crozier et al., 2009; Villalba et al., 2017). Because of this dual action, selection of some plant secondary compounds by herbivores becomes a decision-making process where individuals balance the ingestion of medicinal compounds such as CT as a function of these chemicals' negative and positive post-ingestive consequences (Villalba et al., 2017).

Recent studies have shown that sheep can increase their preference for medicinal CT-rich foods when infected with gastrointestinal parasites (Lisonbee et al., 2009; Juhnke et al., 2012). These results suggest that mammalian herbivores are able to self-select plants with bioactive medicinal compounds in response to a parasitic challenge. Nevertheless, CT are not consumed alone as these compounds are ingested

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with other nutrients present in the target plant and in other plants available in the community, shaping the intensity of the costs and benefits of CT. Indeed, nutrients and plant secondary compounds interact in the gastrointestinal tract enhancing or depressing the post-ingestive actions of single chemicals (Provenza et al., 2003; Provenza and Villalba, 2006). Additionally, parasitism influences an herbivore's nutrient needs by causing tissue and epithelial damage, as well as blood losses which influence nutrient absorption and requirements (Knox et al., 2006). It is known that parasitized ruminants increase their need for protein given the enhanced demands triggered by the immune response (Min et al., 2004), blood losses and requirements to maintain the integrity of tissues (Hoste et al., 2015). For instance, protein supplementation improved goats' responses to parasites, represented by a reduction in fecal egg counts (FEC) and an increase in milk production (Chartier et al., 2000). In addition, when associated with condensed tannins, proteins can form strong complexes (Mueller-Harvey, 2006), which ensure protection against ruminal degradation and subsequent release in the duodenum at greater pH values (Waghorn, 2008). Thus, protein supplementation can be interpreted as beneficial for herbivores challenged by gastrointestinal parasites (Coop and Kyriazakis, 1999; Hoste et al., 2012). However, in contrast to proteins, less is known regarding energy needs in parasitized ruminants (Hoste et al., 2008).

In this study, we aimed at enhancing our understanding about herbivores' interactions with their always-changing feeding environment by focusing on the interplay between CT and nutrients when sheep are challenged by a parasitic infection. When confronted to selecting from an array of different sub-optimal foods, an herbivore is expected to build a diet that entails the best possible compromise under the available conditions and that provides different chemicals in proportions that are as close as possible to the individual's multiple needs (Simpson and Raubenheimer, 1995). We thus offered sheep choices of two sub-optimal foods, one high in energy and the other high in protein with or without the addition of condensed tannins. Considering the antiparasitic properties of tannins (Hoste et al., 2006), that condensed tannins improve protein utilization in ruminants (Waghorn, 2008) and that protein nutrition enhances the hosts' immune responses (Min et al., 2004), we predicted that herbivores would increase the consumption of condensed tannins when parasitized, and that such selection would be influenced by the nutritional composition of the foods on offer. Our objective was to determine how sheep prioritize selection of crude protein, energy and a medicinal CT when experiencing or not a parasitic burden.

2. Methods

2.1. Ethical note

The experiment was conducted in accordance with the United States of America laws and regulations and with approval of the Utah State University Institutional Animal Care and Use Committee (Approval number 2669). Lamb manipulation during exposure and experimental tests was within limits of regular husbandry procedures.

2.2. Study site

The experiment was conducted at the Green Canyon Ecology Center, located at the Utah State University in Logan, Utah, under procedures approved by the Utah State University Institutional Animal Care and Use Committee (Approval #2669). Forty commercial Finn-Columbia-Polypay-Suffolk crossbred lambs (3 month-old; 36.9 ± 4.4 kg initial body weight) were housed in individual, adjacent pens measuring 2.4 x 3.6 m under a protective roof with free access to fresh water and trace mineral salt blocks throughout the study. All lambs were familiarized with their pens and feeders for 1 month before the onset of the experiment.

Table 1
Chemical composition of the experimental feeds.

	Experimental feeds			
	HEP	HEP + QT	LEP	LEP + QT
Beet pulp (%)	88	57.5	28	24
Barley (%)	7	35.5	3	12
Soybean Meal (%)	3	1	32	32
Barley Straw (%)	1	1.5	35	26
CaHPO4 (%)	1	1	2	2
Quebracho Tannins (%)	0	4	0	4
DM (g/kg fresh matter)	927	919	927	925
DE (Mcal/kg)[1]	3.4	3.4	3.05	3.07
CP (%)	10.3	10.2	19.9	20.4
ADF (% DM)	23.8	19.1	33.2	27.1
NDF (% DM)	37.9	32.2	46	37.5

HEP = High Energy feed; LEP = High Protein feed; QT = Quebracho tannins. DM = dry matter; DE = digestible energy; CP = crude protein.

[1] Determined by NRC (1985) tables.

2.3. Experimental approach

Animals were drenched with the anthelmintics levamisole and albendazole at 7.5 mg/kg BW so that animals had no parasitic burdens at the beginning of the study. This was assessed by determining fecal egg counts (FEC) in all lambs 2 days before the study began.

Experimental foods were formulated using a mix of beet pulp, soybean meal, barley grain and barley straw in different proportions such that all animals were offered two suboptimal but complementary foods regarding their energy and protein content (Table 1). All ingredients were ground to a similar particle size (1–2 mm). Lambs were weighed and then randomly assigned to four groups (10 animals/group) and 40 pens, considering the variation of gender and weight, which resulted in a uniform distribution of animals among groups. Lambs in the Control Group (Group 1) received a simultaneous offer of a food with a high energy to protein ratio (HEP), and a food with a low energy to protein ratio (LEP; Table 1). The rest of the groups received the same choice but quebracho tannins (QT) (extracted from the quebracho tree *Schinopsis lorentzii*; 76% condensed tannins; Silva Team, Ontario, CA, USA) were added in a 4% concentration to HEP (Group 2; choice between HEP + QT and LEP), to LEP (Group 3; choice between HEP and LEP + QT) or to both HEP and LEP (Group 4; choice between HEP + QT and LEP + QT). All foods were offered in separate plastic containers (1400 g per container) and the placement of each container (left vs right) was randomized across days.

On day 1 of the study, all lambs were familiarized during 7 consecutive days with their respective test foods before the beginning of the study (Table 2). Depending on their treatment group, lambs received LEP or LEP + QT during days 1, 3 and 5 and HEP or HEP + QT on days 2, 4 and 6. On day 7 all lambs received their respective choice between the two assigned foods.

2.4. Testing phases

2.4.1. Testing before a parasitic infection (Phase 1)

From 0900–1600 and on a daily basis, lambs were offered ad libitum weighed amounts of their respective rations in a 2-way choice test. At 1600, refusals were collected and weighed and food intake was recorded. No other food was offered until the following day. Exposure to these foods lasted for 10 days (D8-D17).

2.4.2. Testing during a parasitic infection (Phase 2)

After Phase 1, all lambs were offered alfalfa pellets in ad libitum amounts for 7 days (D18-D23) such that all condensed tannins were eliminated from the gastrointestinal tract. Subsequently, lambs in all groups were artificially infected with an oral drench which delivered

Table 2
Feeding protocol and determinations during the study.

Procedure	Feeds offered	Days
Habituation	Experimental feeds	1–7
FEC		*6*
PCV		*7*
Phase 1: Testing before a parasitic infection		
Preference tests	Experimental feeds	8–17
Tannins free period	Alfalfa pellets	18–23
Phase 2: Testing during a parasitic infection		
Infection		24
Parasitic development	Alfalfa pellets	24–49
Preference tests	Experimental feeds	50–59
FEC		*49, 59*
PCV		*59*
Phase 3: Testing after a parasitic infection		
Drench anthelminthics		60
Wash out period	Alfalfa pellets	60–73
Preference tests	Experimental feeds	74–83
FEC		*84, 73*
PCV		*84*

FEC = fecal egg counts; PCV = packed cell volume.

10,000 of infective larvae (L_3) of *H. contortus* from an inoculum consisting of 90% *H. contortus* and 10% other nematode species (*Trichostrongylus*, *Cooperia* and *Oesophagostomum*). Lambs were offered *ad-libitum* amounts of alfalfa pellets during this period (D24-D49). After 25 days, when L_3 reached the adult stage and animals experienced a parasitic burden, all groups were given access to their respective experimental foods. The protocol of feeding was as described for Phase 1 and exposure to the experimental foods lasted 10 days (D50-D59).

2.4.3. *Testing without a parasitic infection (Phase 3)*

The day after Phase 2 ended (D60), all animals were drenched with levamisole and albendazole at 7.5 mg/kg BW so their parasitic infection was eliminated, assessed by fecal egg counts and blood parameters (see below). Subsequently, all lambs were offered ad libitum amounts of alfalfa pellets for 14 days to allow for the excretion of all tannins ingested during testing (Silanikove et al., 1994), and to allow for recovery from the parasitic burdens (D60-D73). Subsequently, all groups were given access to their respective experimental foods as described before. Exposure to the experimental foods lasted 10 days (D74-D83).

2.5. *Measurements*

Fecal and blood samples were taken at sequential intervals corresponding to the testing phases described above (Table 2). Blood was collected via jugular venipuncture at 0800 from all individuals before infecting the animals and at the end of Phases 2 and 3. Total blood cell count was determined during the same day of collection at the Utah Veterinary Diagnostic Laboratory (Logan, UT) using a Hematology Analyzer (Advia 120 Hematology Analyzer; Siemens Healthcare Diagnostics, Tarrytown, NY).

Fecal samples were collected at 0800 directly from the rectum of each subject and analyzed for fecal egg counts during the same day of collection to assess the number of eggs per gram of feces (EPG) according to the Mac Master methodology. Samples were collected at the beginning of the study, 25 days after infection (beginning of Phase 2), at the end of Phases 2 and 3 (Table 2).

Animals were weighted with a 12 h fasting period before being infected and every 14 days after infection.

Daily ration intake was measured by subtracting the amounts of food offered from those refused. Preference for a specific food during the choice tests was estimated as: (Intake of the test food / Overall intake) x100.

2.6. *Chemical analyses*

The foods offered were sampled for nutritional analyses during days 1, 5 and 10 of each testing phase and then composited for analyses (Table 1).

Composited samples were analyzed for dry matter (DM), and crude protein (CP) according to AOAC (1995), and neutral (NDF) and acid (ADF) detergent fiber according to Goering and Van Soest (1970). Digestible energy (DE, Mcal/Kg) of the feeds was estimated from values obtained from their ingredients according to NRC (1985).

2.7. *Statistical analyses*

Intake of DM, CP, DE and preference for HEP were analyzed as 2 × 2 factorial (Food: LEP, LEP; Tannin presence: Yes, No) split-plot design with lambs (random factor) nested within Food and Tannin presence (fixed factors) and Day and Phase (Phase 1, Phase 2 and Phase 3) as the repeated measures, using the Mixed procedure of SAS (SAS ® 9.4 Foundation for Microsoft Windows for x64, Cary, NC: SAS Institute Inc.). Crude protein and DE intakes were estimated as: [$\text{HEP}_{\text{intake}}$ x (CP or DE concentration)$_{\text{HEP}}$ + $\text{LEP}_{\text{intake}}$ x (CP or DE concentration)$_{\text{LEP}}$]. We tested for a normal distribution of the error residuals and homogeneity of variance. Means were analyzed using pairwise differences of least square means and the variance-covariance structure used were those that yielded the lowest Bayesian Information Criterion.

Blood parameters, fecal egg counts and evolution of body weight were analyzed as a mixed model with repeated measures (Phase) and lambs (random factor) nested within groups. We compared FEC at the beginning and end of Phase 2 “Testing during a parasitic infection” with lambs (random factor) nested within groups.

Cartesian bi-coordinate plots of CP vs. DE intake (Simpson and Raubenheimer, 2001), were used to observe the lambs’ selection of CP and DE in Cartesian nutritional space across the 3 phases of the study. Within the Cartesian space, foods were represented as lines radiating from the origin of coordinates with set concentrations of CP and DE that determined the angle of the line with the x and y axis (Simpson and Raubenheimer, 2001).

3. Results

3.1. *Growth*

Lambs' body weight throughout the study progressively increased from 36.9 ± 4.4 kg in Phase 1 to 49.5 ± 1.4 kg in Phase 3 ($P < 0.0001$), with a mean growth rate of 229 ± 3.2 g/d. The daily growth rate did not differ between groups, regardless of experimental phases ($P > 0.05$).

3.2. *Fecal egg counts*

The monitoring of nematode eggs excretion showed an effective infection with FEC that sharply increased from 0 (Phase 1) to an average of 1600 eggs per gram (EPG) at the beginning of the Phase 2 to reach an average peak of 3155 EPG at the end of this Phase, returning to 0 counts during Phase 3 (Phase effect; $P < 0.0001$; Table 3). Even when the increase in FEC from the beginning to the end of Phase 2 was numerically greater for the Control than for the rest of the groups (Table 3), no differences in FEC were detected among groups of lambs ($P > 0.05$; Table 3).

3.3. *Blood tests*

Packed cell volume (PCV) was affected by Phase ($P < 0.001$; Table 4). Globally, PCV in all groups decreased from Phase 1 to Phase 2 ($P < 0.001$), and then this parameter increased from Phase 2 to Phase 3 ($P < 0.001$). Results relative to the other blood parameters (white

Table 3
Fecal egg counts (FEC) in eggs per gram (EPG) in lambs before experiencing a parasitic infection (Phase 1), during an infection with Haemonchus contortus (Phase 2) and after the infection was terminated by chemotherapy (Phase 3). Lambs received a simultaneous choice of a food high in energy (HEP) and another high in protein (LEP) (Control; Gp1). The rest of the groups received the same choice but quebracho tannins (QT) were added (4%) to HEP (Gp2), to LEP (Gp3) or to both HEP and LEP (Gp4).

	Phase 1	Phase 2 - Beginning				Phase 2 - End				Phase 3	SEM	Gp Effect	Phase Effect	GpxPhase
	All Groups	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	All Groups				
FEC (EPG)	0	1038	2011	1539	1495	2913	3494	3288	3100	0	525	NS	***	NS
% increment[1]						59.2	35.4	42.2	30.5		11.7	NS		

[1] % Increment in FEC from beginning to end of Phase 2.

blood cells, mean corpuscular volume and mean corpuscular haemoglobin) are presented in Table 4. The number of white blood cells decreased from Phase 1 to Phases 2 and 3 for all groups ($P < 0.001$) and mean corpuscular volume and mean corpuscular haemoglobin increased from Phase 1 to Phases 2 and 3 ($P < 0.001$). Nevertheless, no differences between groups were detected for any of the blood parameters assessed ($P > 0.05$).

3.4. *Feeding behavior*

3.4.1. *Food intake and preference*

Intake of HEP and preference for HEP increased from Phase 1 (“Testing before an infection”) to Phase 2 (“Testing during an infection”) for all groups of lambs (Fig. 1A, B; $P < 0.05$; Table 5). When parasitic burdens were terminated by chemotherapy (Phase 3), the pattern observed for Phase 2 persisted regardless of the choice presented. The increase in preference for HEP from Phase 1 to 2 was also explained by a decrease in intake of LEP for Groups Control, 3 and 4 (Fig. 1C; $P < 0.05$; Table 5). Lambs that had no tannins in either feed (Control) consumed more LEP in Phases 1 and 2 than animals in the other groups ($P < 0.05$; Fig. 1C; Table 5). Consequently, preference for HEP was lower in Control lambs than in those that had tannins in their choice (Groups 2, 3 and 4) during Phase 2. Groups 2 and 4 increased their overall food intake from Phase 1 to Phase 2 ($P < 0.05$) but intake between Phases 2 and 3 remained stable ($P > 0.05$; Fig. 1D; Table 5).

3.4.2. *Intake of nutrients*

During Phase 1, lambs that did not receive condensed tannins in HEP (Control, Group 3) showed greater CP and DE intake values than animals offered condensed tannins in HEP (Groups 2 and 4; $P < 0.05$; Fig. 2A, B; Table 5). However, lambs presented with condensed tannins in HEP (Groups 2 and 4) increased their intake of CP during Phase 2 relative to Phase 1 ($P < 0.05$; Fig. 2A; Table 5), whereas those receiving HEP without tannins in their choice (Control, Group 3) showed similar values of CP intake across phases ($P > 0.05$). Following the pattern of increased preference for HEP from Phase 1 to Phase 2, intake of DE also increased for all groups during Phase 2 relative to Phase 1, particularly driven by groups that had condensed tannins in HEP (Groups 2 and 4) ($P < 0.05$). In addition, lambs that had condensed tannins available in their choice (Groups 2, 3 and 4) selected a greater DE/CP ratio during Phase 2 than lambs in the Control group ($P < 0.05$) and this pattern continued for Group 4 during Phase 3 (Fig. 2D; Table 5).

3.4.3. *Condensed tannin intake*

The amounts of tannins consumed by animals varied by Group and by Phase. As expected, lambs offered condensed tannins in both foods (Group 4) showed the greatest intake of tannins ($P < 0.05$; Fig. 2C). Lambs consuming condensed tannins with HEP (Groups 2 and 4) increased their tannin intake from Phase 1 to Phase 2, when they experienced a parasitic infection ($P < 0.001$; Fig. 2C), consistent with the increased levels of HEP and DE intakes displayed by these groups during this period. In contrast, lambs that were offered condensed tannins only in LEP (Group 3), reduced their intake of tannins from Phase 1 to Phase 2, when they experienced a parasitic infection ($P < 0.001$; Fig. 2C). Condensed tannin intake remained stable in Phase 3 for all groups relative to Phase 2 ($P > 0.05$; Fig. 2C).

3.4.4. *Selection of CP and DE in cartesian space*

Fig. 3 shows how differences in energy and protein consumption were translated into nutritional space. Lambs in Control and Group 3 ate similar amounts of CP and DE across all phases, whereas more variability among phases was observed for Groups 2 and 4. For those groups which received HEP with tannins (Groups 2 and 4), CP intake tended to increase from Phase 1 to Phases 2 (parasitic infection), and 3 (after a parasitic infection) (HEP x Phase; $P = 0.06$; Fig. 3). Digestible energy intake for Phase 1 was lower for lambs that were offered HEP with tannins (Groups 2 and 4) than for those that were offered HEP without tannins ($P < 0.05$). Intake of DE also increased from Phase 1 to Phases 2 and 3 for lambs that had HEP with tannins (Groups 2 and 4) (Phase effect; $P < 0.0001$), such that DE intake during Phases 2 and 3 became similar among all groups of lambs (Fig. 3).

4. Discussion

4.1. *Nematode infection*

Results show animals were infected during Phase 2, evidenced by

Table 4
Blood parameters (PCV: packed cell volume; WBC: white blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin) in lambs before experiencing a parasitic infection (Phase 1), during an infection with *Haemonchus contortus* (Phase 2) and after the infection was terminated by chemotherapy (Phase 3). Lambs received a simultaneous choice of a food high in energy (HEP) and another high in protein (LEP) (Control; Gp1). The rest of the groups received the same choice but quebracho tannins (QT) were added (4%) to HEP (Gp2), to LEP (Gp3) or to both HEP and LEP (Gp4).

	Phase 1				Phase 2				Phase 3				SEM	Gp Effect	Phase Effect	GpxPhase
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4				
PCV (%)	33.5	33.3	34.7	33	32	30.4	32.2	30.6	35.9	36.9	38.4	35.1	0.86	NS	***	NS
WBC (10^3/mm^3)	10.4	9.8	8.8	10.1	8.4	8.2	8.9	7.8	8.8	9.1	8.6	8	0.7	NS	***	NS
MCV (μm^3)	25.5	27	25.6	25.3	28.2	29	28.5	28.1	28.2	29.1	28.5	29.2	0.63	NS	***	NS
MCH (Pg)	9.4	9.9	9.4	9.3	10.3	10.6	10.4	10.3	10.1	10.4	10.1	10.3	0.19	NS	***	NS

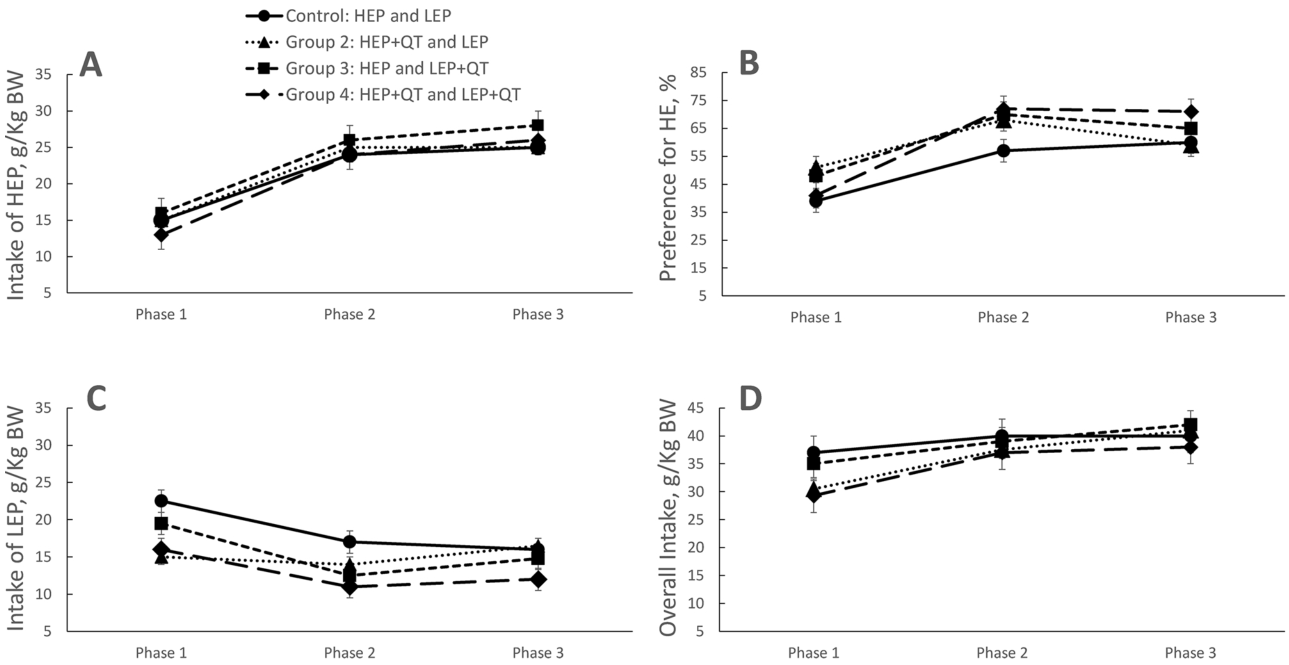

Fig. 1. Feeding behavior by lambs before experiencing a parasitic infection (Phase 1), during an infection with *Haemonchus contortus* (Phase 2) and after the infection was terminated by chemotherapy (Phase 3). Lambs received a simultaneous choice of a food high in energy (HEP) and another high in protein (LEP) (Control; Group 1). The rest of the groups received the same choice but quebracho tannins (QT) were added (4%) to HEP (HEP + QT and LEP; Group 2), to LEP (HEP and LEP + QT; Group 3) or to both HEP and LEP (HEP + QT and LEP + QT; Group 4). Panel A: Intake of HEP, Panel B: Preference for HEP, Panel C: Intake of LEP, Panel D: Overall food intake. Means are averages for 10 lambs/group with SEMs.

FEC values at or above 3000 eggs per gram (EPG) towards the end of the phase. Nevertheless, no significant group effects were detected, although lambs that did not receive condensed tannins (Control) showed the greatest increment in FEC from the beginning to the end of Phase 2. It is likely that the high nutritional quality of the foods on offer, along with the moderate tannin content (4%), were responsible for not observing significant differences in parasitic burdens among groups, since nutrients have significant positive effects on resilience and resistance against gastrointestinal nematode infections (Sykes and Coop, 2001; Retama-Flores et al., 2012).

Blood parameters also confirmed the presence of parasitic burdens during Phase 2, evidenced by a clear decline in PCV values four weeks after infection, caused by the blood sucking activity of *H. contortus* (Mir et al., 2007). An increment in the PCV values for Phase 3 also reveals that animals recovered from the infection experienced during Phase 2. Values for mean corpuscular volume were greater in Phase 2, suggesting a tendency for a macrocytic anemia. Macrocytic red blood cells are large and tend to have higher mean corpuscular hemoglobin, which may also explain the elevated values for this parameter during Phase 2 (Walker et al., 1990), which in conjunction with MCV remained high during Phase 3.

4.2. Feeding behavior

4.2.1. Evolution of intake and preference for the experimental foods

Lambs in this study responded to a parasitic infection by increasing consumption of the feed with high energy to protein ratio (HEP), which did not subside after the infection was terminated by chemotherapy (Phase 3). In contrast, parasitized lambs reduced their intake of the feed with the greatest protein content (low energy to protein ratio (LEP)) when infected (Phase 2) relative to the baseline period (Phase 1), and then intake of LEP remained stable during Phase 3. When looking at the overall amount of food consumed during testing, it can be concluded that parasitism did not constrain overall food intake, as it is typically reported for herbivores infected with gastrointestinal nematodes (Kyriazakis et al., 1998; Kyriazakis, 2014). On the contrary, lambs in Groups 2 and 4 (ingesting condensed tannins with HEP) showed a positive trend of food intake from Phase 1 to Phase 2, likely reflecting the increased nutrient demands triggered by parasitism and the availability of nutrient-rich foods that satisfied those needs (Sykes and Coop, 2001). The severity of infection in this study (a single dose of 10,000 L_3) was planned to be below the threshold that leads to clinical diarrhoea and intake depression (Kyriazakis et al., 1998), in order to maintain the

Table 5
Intake of foods and nutrients. Probability values regarding fixed effects in the model and their interactions.

	LEP	HEP	LEPxHEP	Phase	LEPxPhase	HEPxPhase	LEPxHEPxPhase
Preference for HEP	0.2109	0.3045	0.3742	< 0.001	0.0518	0.5924	0.0467
Intake HEP	0.2789	0.5250	0.2453	< 0.001	0.4432	0.783	0.7907
Intake LEP	0.1603	0.0649	0.7592	< 0.001	0.4799	0.0228	0.0683
Total Intake	0.5677	0.0140	0.5327	< 0.001	0.9015	0.1026	0.6280
Intake CP	0.3399	0.0045	0.8258	0.0760	0.9503	0.0591	0.3016
Intake DE	0.6951	0.0152	0.4798	< 0.001	0.8733	0.1238	0.6826
Ratio DE / CP	0.1461	0.0208	0.5068	< 0.001	0.0309	0.6032	0.0455

HEP = High Energy feed; LEP = High Protein feed; DE = digestible energy; CP = crude protein.

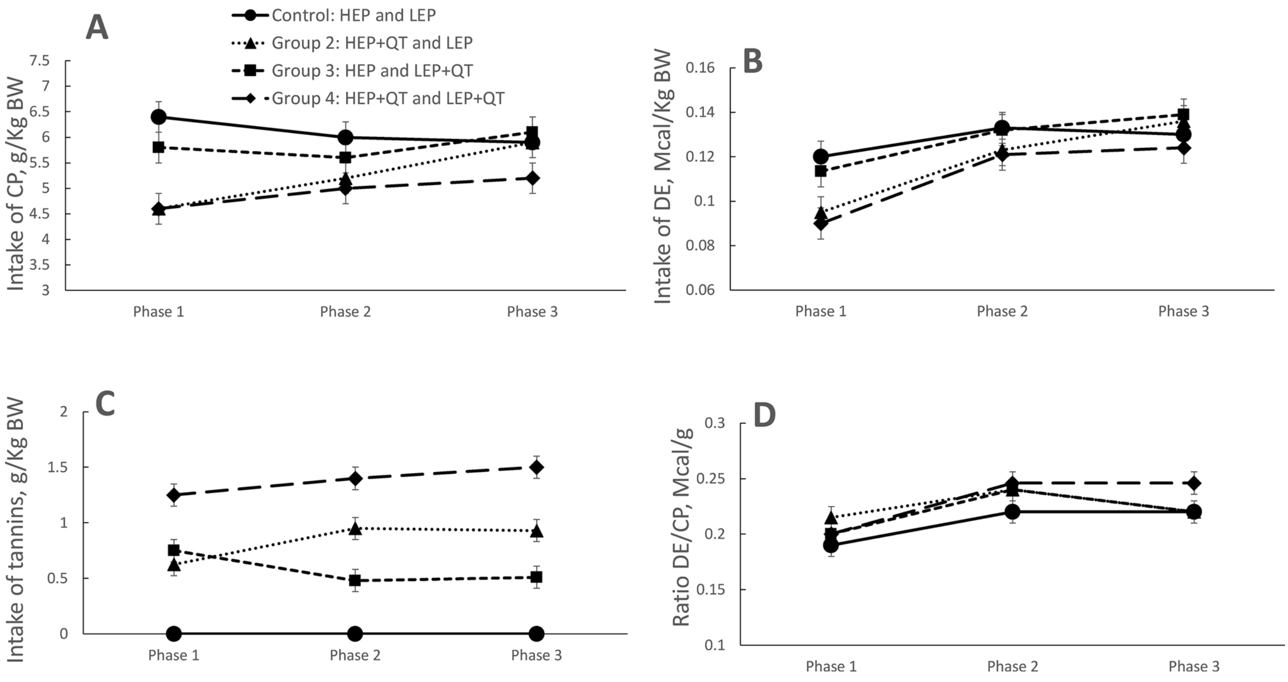

Fig. 2. Intake of crude protein (CP; Panel A), digestible energy (DE; Panel B), condensed tannins (Panel C) and ratio of DE/CP (Panel D) selected by lambs before experiencing a parasitic infection (Phase 1), during an infection with *Haemonchus contortus* (Phase 2) and after the infection was terminated by chemotherapy (Phase 3). Lambs received a simultaneous choice of a food high in energy (HE) and another high in protein (LEP) (Control; Group 1). The rest of the groups received the same choice but quebracho tannins (QT) were added (4%) to HEP (HEP + QT and LEP; Group 2), to LEP (HEP and LEP + QT; Group 3) or to both HEP and LEP (HEP + QT and LEP + QT; Group 4). Means are averages for 10 lambs/group with SEMs.

animals' motivation to select a balanced diet from the 2-way choices presented. A more severe infection could have depressed food intake and motivation to eat (Kyriazakis et al., 1998; Kyriazakis, 2014). Alternatively, the nutritional composition of the foods available in the study likely reduced the extent and duration of anorexia, and enhanced the rate of recovery in food intake during infection as it has been

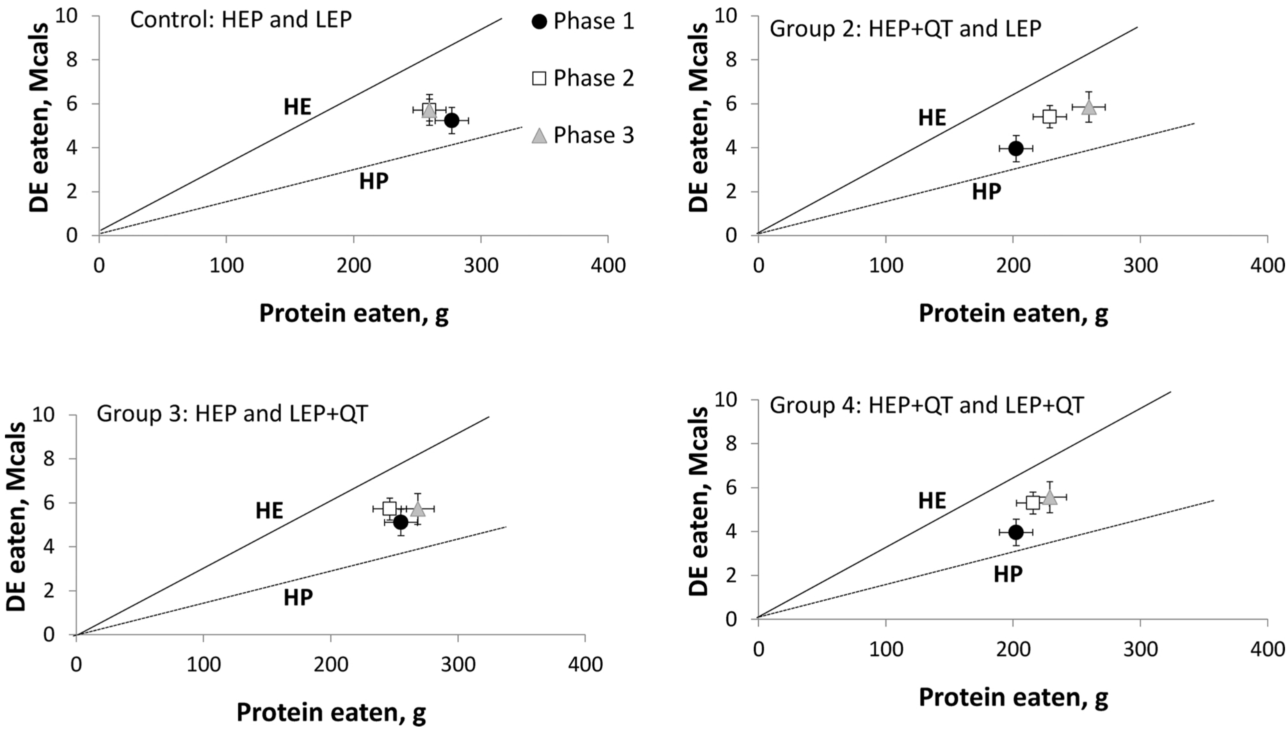

Fig. 3. Bicoordinate plots of mean crude protein (CP) and digestible energy (DE) intake by lambs before experiencing a parasitic infection (Phase 1), during an infection with *Haemonchus contortus* (Phase 2) and after the infection was terminated by chemotherapy (Phase 3). Lambs received a simultaneous choice of a food high in energy (HEP) and another high in protein (LEP) (Control; Group 1). The rest of the groups received the same choice but quebracho tannins (QT) were added (4%) to HEP (HEP + QT and LEP; Group 2), to LEP (HEP and LEP + QT; Group 3) or to both HEP and LEP (HEP + QT and LEP + QT; Group 4). Means are averages for 10 lambs/group with SEMs.

suggested before (Sykes and Coop, 2001; Kyriazakis, 2010). Previous studies also report lack of anorexia in lambs experiencing nematode infections (Méndez-Ortíz et al., 2012; Retama-Flores et al., 2012).

Lambs' feeding responses after infection did not return to baseline values. It is likely that the time allowed for recovery after the infection (14 d) was not long enough for complete recovery. Nevertheless, PCV values, indicative of anaemia, observed during Phase 3 suggest animals recovered from the parasitic infection, although other blood parameters such as mean corpuscular volume and mean corpuscular haemoglobin remained high during Phase 3. Alternatively, it is possible that preferences developed during Phase 2 did not extinguish during Phase 3 because the selected diet promoted benefits to the host that led to enduring and persisting food preferences. Preferences for nutritious foods can cause hedonic shifts, which make conditioned responses resistant to extinction (Arbour and Wilkie, 1988; Villalba and Provenza, 1997; Harris et al., 2004). In addition, reductions in the formation of ruminal ammonia and greater efficiency of protein utilization due to the protein-binding capacity of tannins (Waghorn, 2008) could explain the sustained intake for tannin-containing foods. Persistent preferences for sainfoin, a tannin-rich legume have been observed after sheep recovered from haemonchosis (Costes-Thiré et al., 2018). Finally, animal growth during the study might have influenced diet selection towards Phase 3 of the study (Sahin et al., 2003). Nevertheless, the time elapsed between Phases was not long enough to promote pronounced differences in diet selection due to growth.

4.2.2. *Nutrient intake*

Lambs in this study responded to a parasitic infection by increasing preference for the energy-dense food (HEP), and consequently they increased the ingestion of DE from Phase 1 to Phase 2, particularly for groups that consumed condensed tannins with HEP (Groups 2 and 4). These groups also slightly increased their intake of CP during Phase 2, a result of the greater intake of the energy-dense food (HEP) instead of an increment in the consumption of the protein-rich food (LEP). On the contrary, intake of the protein-rich feed LEP declined from Phase 1 to Phase 2 for Controls and Groups 3 and 4. This pattern is in contrast to previous findings suggesting that herbivores select diets with higher protein content when infected with nematode parasites (Kyriazakis et al., 1994, 1996) in order to accrue amino acids for tissue repair and for mounting an immune response (Houdijk et al., 2000; Knox et al., 2006; Nnadi et al., 2009). Consistent with this notion, sheep supplemented with soybean meal – a protein-rich supplement- showed greater resistance to haemonchosis than non-supplemented counterparts (Wallace et al., 1995). Nevertheless, an augmentation of protein in the diet leads to inefficiencies that require additional energy to excrete the excess N that accumulates in the animals' tissues (Van Soest, 2018), and high levels of N intake lead to ammonia toxicity in ruminants (Lobley and Milano, 1997). These penalties might have reduced ingestion of the protein-rich food in the present study, particularly when lambs were maintained on a basal diet of alfalfa pellets (rich in protein) in-between Phases. On the other hand, gastrointestinal parasitism disrupts the host's energetic metabolism (Sykes and Coop, 1976), substantially reducing the efficiency of utilization of energy for lamb growth (Méndez-Ortíz et al., 2019). This could explain the greater preference for HEP, and the concomitant increase in calorie intake by parasitized lambs during Phase 2. Gastrointestinal parasites impinge both protein and energetic costs in small ruminants and the interaction between parasitic burdens and metabolizable energy intake is the best predictor of live weight change in lambs (Méndez-Ortíz et al., 2019). Likewise, energy supplementation has been found to improve resilience against gastrointestinal nematodes and to increase growth in sheep (Retama-Flores et al., 2012) and goats (Gárate-Gallardo et al., 2015). Parasitized goats browsing in a tropical deciduous forest also preferred energy-rich species that balanced the greater levels of protein present in that plant community (Ventura-Cordero et al., 2018).

The lower intake of digestible energy by groups 2 and 4 (e.g., tannins present in HEP) than by groups 1 and 3 (e.g., tannins not present in HEP) during Phase 1 suggest that condensed tannins in non-parasitized individuals constrained HEP intake. However, this effect was no longer observed during a parasitic infection, suggesting that under this physiological state animals overcame such restriction, likely to ingest more energy which also led to greater levels of tannin consumption (see below). The "movement" in chemical space by individuals during and after a parasitic infection reveals the important role that the food high in energy played in the process.

4.2.3. *Tannin intake and selection of nutrients in chemical space*

We predicted that parasitized lambs would trade-off the ingestion of some nutrients for the ingestion of condensed tannins to a greater extent when parasitized than when healthy. This is because previous research has shown lambs increase their preference for tannin-containing foods when experiencing a parasitic burden, which subsides with the termination of the infection (Villalba et al., 2010; Juhnke et al., 2012). An analysis conducted to explore the self-selection of nutrients and CT in parasitized sheep revealed that parasitized animals that learned to associate CT-containing foods with recovery from parasitic burdens selected more CT, but less protein than control animals (Villalba et al., 2017). This study also shows that parasitism influenced tannin intake but this behavior was influenced by the nutritional composition of the feeding environment, consistent with previous research conducted in herbivorous insects (Simpson and Raubenheimer, 2001). Parasitized lambs in our study increased their intake of CT from Phase 1 (baseline) to Phase 2 (infection) when these chemicals were present in the energy-dense food (Groups 2 and 4), whereas intake of tannins declined when tannins were present in the protein-dense food LEP (Group 3). When looking at the selection of DE in nutritional space it can be observed a clear increase in DE intake (and a concomitant increase in CP intake) for groups that had condensed tannins in the energy-dense food (Groups 2 and 4) from before (Phase 1) to after the infection (Phase 2). Lambs without condensed tannins in their diet (Control) or offered condensed tannins in LEP (Group 3) also reduced their intake of LEP and increased their preference for HEP during Phase 2. Collectively, these results suggest that the energy-to-protein ratio of the food selected was more consequential than the presence of tannins or the availability of high concentrations of protein in LEP. It is possible that an appropriate nutritional plane, which allowed animals to fulfil their requirements and maintain growth, as observed in this study, reduced the need to enhance the consumption of a medicinal plant secondary compound, especially when present at moderate concentrations (4% in our study). The prioritization of nutrients over medicinal CT has also been observed in parasitized goats that received an array of forages of different concentrations of nutrients and plant secondary compounds (Ventura-Cordero et al., 2018).

Some condensed tannins enhance protein nutrition by decreasing ruminal proteolysis, making more high-quality proteins available to repair the damages in the host's mucosa caused by endoparasites (Coop and Kyriazakis, 1999; Hoste et al., 2012). Thus, the incorporation of condensed quebracho tannins in the diets of parasitized animals may have contributed to an efficient utilization of protein needed in response to an *H. contortus* challenge.

5. Conclusion

Our study gives insights into how herbivores may trade off the ingestion of nutrients with the ingestion of medicinal plant secondary compound when experiencing a parasitic burden. Parasitized lambs prioritized selection of an energy-dense food over a protein-dense food and intake of tannins only increased when these chemicals where present in the energy-dense food. Several studies have focused on protein provision in parasitized herbivores to tend for the increased protein demands triggered by gastrointestinal parasitic infections. However, energy supply is a key contributor to resilience and resistance in herbivores challenged by parasitic infections and this study shows that the significance of this variable was reflected in the animals' foraging decisions.

Declarations of interest

None.

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References

AOAC, 1995. Official Methods of Analysis. 16th ed.. Association of Official Analytical Chemists, Arlington VA.

Arbour, K.J., Wilkie, D.M., 1988. Rodents'(Rattus, Mesocricetus, and Meriones) use of learned caloric information in diet selection. J. Comp. Psychol. 102 (2), 177.

Chartier, C., Etter, E., Hoste, H., Pors, I., Mallereau, M.P., Broqua, C., Mallet, S., Koch, C., Masse, A., 2000. Effects of the initial level of milk production and of the dietary protein intake on the course of natural nematode infection in dairy goats. Vet. Parasitol. 92 (1), 1–13.

Coop, R.L., Kyriazakis, I., 1999. Nutrition–parasite interaction. Vet. Parasitol. 84 (3), 187–204.

Costes-Thiré, M., Villalba, J.J., Hoste, H., Ginane, C., 2018. Increased intake and preference for tannin-rich sainfoin (*Onobrychis viciifolia*) pellets by both parasitized and non-parasitized lambs after a period of conditioning. Appl. Anim. Behav. Sci. 203, 11–18.

Crozier, A., Jaganath, I.B., Clifford, M.N., 2009. Dietary phenolics: chemistry, bioavailability and effects on health. Nat. Prod. Rep. 26 (8), 1001–1043.

Gárate-Gallardo, L., de Jesús Torres-Acosta, J.F., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Cámara-Sarmiento, R., Canul-Ku, H.L., 2015. Comparing different maize supplementation strategies to improve resilience and resistance against gastrointestinal nematode infections in browsing goats. Parasite. 22.

Ginane, C., Duncan, A.J., Young, S.A., Elston, D.A., Gordon, I.J., 2005. Herbivore diet selection in response to simulated variation in nutrient rewards and plant secondary compounds. Anim. Behav. 69 (3), 541–550.

Goering, H.K., Van Soest, P.J., 1970. Forage Fiber Analyses (apparatus, Reagents, Prcedures, and Some Applications). USDA Agr Handb.Forage Fiber Analyses (apparatus, Reagents, Prcedures, and Some Applications). USDA Agr Handb.

Harris, J.A., Shand, F.L., Carroll, L.Q., Westbrook, R.F., 2004. Persistence of preference for a flavor presented in simultaneous compound with sucrose. J. Exp. Psychol. Anim. Behav. Process. 30 (3), 177.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M., Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol. 22 (6), 253–261.

Hoste, H., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., 2008. Nutrition–parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? Parasite Immunol. 30 (2), 79–88.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. Vet. Parasitol. 186 (1), 18–27.

Hoste, H., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S.M., Terrill, T.H., 2015. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. Vet. Parasitol. 212 (1), 5–17.

Houdijk, J.G., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2000. Can an increased intake of metabolizable protein affect the periparturient relaxation in immunity against *Teladorsagia circumcincta* in sheep? Vet. Parasitol. 91 (1–2), 43–62.

Juhnke, J., Miller, J., Hall, J.O., Provenza, F.D., Villalba, J.J., 2012. Preference for condensed tannins by sheep in response to challenge infection with Haemonchus contortus. Vet. Parasitol. 188 (1), 104–114.

Knox, M.R., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. Vet. Parasitol. 139 (4), 385–393.

Kyriazakis, I., Oldham, J.D., Coop, R.L., Jackson, F., 1994. The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. Br. J. Nutr. 72, 665–677.

Kyriazakis, I., Anderson, D.H., Oldham, J.D., Coop, R.L., Jackson, F., 1996. Long-term subclinical infection with *Trichostrongylus colubriformis*: effects on food intake diet selection and performance of growing lambs. Vet. Parasitol. 61, 297–313.

Kyriazakis, I., Tolkamp, B.J., Hutchings, M.R., 1998. Towards a functional explanation for the occurrence of anorexia during parasitic infections. Anim. Behav. 56 (2), 265–274.

Kyriazakis, I., 2010. Is anorexia during infection in animals affected by food composition? Anim. Feed Sci. Technol. 156 (1–2), 1–9.

Kyriazakis, I., 2014. Pathogen-induced anorexia: a herbivore strategy or an unavoidable consequence of infection? Anim. Prod. Sci. 54 (9), 1190–1197.

Lisonbee, L.D., Villalba, J.J., Provenza, F.D., Hall, J.O., 2009. Tannins and self-medication: implications for sustainable parasite control in herbivores. Behav. Process. 82 (2), 184–189.

Lobley, G.E., Milano, G.D., 1997. Regulation of hepatic nitrogen metabolism in ruminants. Proc. Nutr. Soc. 56 (2), 547–563.

Lozano, G.A., 1998. Parasitic stress and self-medication in wild animals. Adv. Study Behav. 27, 291–317.

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. Small Rumin. Res. 51 (3), 279–283.

Mir, R.A., Chishti, M.Z., Zarger, M.A., Tak, H., Ganie, S.A., 2007. Clinicopathological changes in sheep experimentally infected with Haemonchus contortus. World J. Agric. Sci. 3, 562–566.

NRC, 1985. Nutrient Requirements of Sheep. 6th revised ed.. National Academy Press, Washington, DC, pp. 2–19.

Méndez-Ortíz, F.A., Sandoval-Castro, C.A., Torres-Acosta, J.F.J., 2012. Short term consumption of *Havardia albicans* tannin rich fodder by sheep: effects on feed intake, diet digestibility and excretion of *Haemonchus contortus* eggs. Anim. Feed Sci. Technol. 176 (1–4), 185–191.

Méndez-Ortíz, F.A., Sandoval-Castro, C.A., Vargas-Magaña, J.J., Sarmiento-Franco, L., Torres-Acosta, J.F.J., Ventura-Cordero, J., 2019. Impact of gastrointestinal parasitism on dry matter intake and live weight gain of lambs: a meta-analysis to estimate the metabolic cost of gastrointestinal nematodes. Vet. Parasitol. 265, 1–6.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86 (13), 2010–2037.

Nnadi, P.A., Kamalu, T.N., Onah, D.N., 2009. The effect of dietary protein on the productivity of West African Dwarf (WAD) goats infected with Haemonchus contortus. Vet. Parasitol. 161 (3), 232–238.

Provenza, F.D., 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. Rangeland Ecology & Management/Journal of Range Management Archives. 48 (1), 2–17.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Rumin. Res. 49 (3), 257–274.

Provenza, F.D., Villalba, J.J., 2006. Foraging in domestic herbivores: linking the internal and external milieu. In: Bels, V.L. (Ed.), Feeding in Domestic Vertebrates: From Structure to Function. CABI Publ., Oxfordshire, UK, pp. 210–240.

Retama-Flores, C., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Aguilar-Caballero, A.J., Cámara-Sarmiento, R., Canul-Ku, H.L., 2012. Maize supplementation of Pelibuey sheep in a silvopastoral system: fodder selection, nutrient intake and resilience against gastrointestinal nematodes. Animal. 6 (1), 145–153.

Sahin, A., Keskin, M., Biçer, O., Gül, S., 2003. Diet selection by Awassi lambs fed individually in a cafeteria feeding system. Livest. Prod. Sci. 82, 163–170.

Silanikove, N., Nitsan, Z., Perevolotsky, A., 1994. Effect of a daily supplementation of poly (ethylene glycol) on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. J. Agric. Food Chem. 42 (12), 2844–2847.

Simpson, S.J., Raubenheimer, D., 1995. The geometric analysis of feeding and nutrition: a user's guide. J. Insect Physiol. 41 (7), 545–553.

Simpson, S.J., Raubenheimer, D., 2001. The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. Ecology. 82 (2), 422–439.

Sykes, A.R., Coop, R.L., 1976. Intake and utilization of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformis* larvae. J. Agric. Sci. Cambridge 86, 507–515.

Sykes, A.R., Coop, R.L., 2001. Interaction between nutrition and gastrointestinal parasitism in sheep. N. Z. Vet. J. 49 (6), 222–226.

Van Soest, P.J., 2018. Nutritional Ecology of the Ruminant. Cornell university press.

Ventura-Cordero, J., González-Pech, P.G., Jaimez-Rodriguez, P.R., Ortiz-Ocampo, G.I., Sandoval-Castro, C.A., Torres-Acosta, J.F.J., 2018. Feed resource selection of Criollo goats artificially infected with *Haemonchus contortus*: nutritional wisdom and prophylactic self-medication. Animal 12 (6), 1269–1276.

Villalba, J.J., Provenza, F.D., 1997. Preference for wheat straw by lambs conditioned with intraruminal infusions of starch. Br. J. Nutr. 77 (2), 287–297.

Villalba, J.J., Provenza, F.D., 2009. Learning and dietary choice in herbivores. Rangel. Ecol. Manag. 62 (5), 399–406.

Villalba, J.J., Provenza, F.D., Hall, J.O., Lisonbee, L.D., 2010. Selection of tannins by sheep in response to gastrointestinal nematode infection. J. Anim. Sci. 88 (6), 2189–2198.

Villalba, J.J., Costes-Thiré, M., Ginane, C., 2017. Phytochemicals in animal health: diet selection and trade-offs between costs and benefits. Proc. Nutr. Soc. 76 (2), 113–121.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—progress and challenges. Anim. Feed Sci. Technol. 147 (1), 116–139.

Walker, H.K., Hall, W.D., Hurst, J.W., 1990. Clinical Methods: the History. Physical, and Laboratory Examinations, 3rd ed. Butterworths, Boston, USA.

Wallace, D.S., Bairden, K., Duncan, J.L., Fishwick, G., Gill, M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear, M.J., 1995. Influence of supplementation with dietary soyabean meal on resistance to haemonchosis in Hampshire down lambs. Res. Vet. Sci. 58 (3), 232–237.

Wang, J., Provenza, F.D., 1996. Food deprivation affects preference of sheep for foods varying in nutrients and a toxin. J. Chem. Ecol. 22 (11), 2011–2021.