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Self-selection of plant bioactive compounds by sheep in response to challenge infection with *Haemonchus contortus*

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ABSTRACT

Plant bioactives can potentially benefit herbivores through their effects on health and nutrition. The objective of this study was to determine the importance of polyphenols and terpenes on the ability of lambs to self-select these compounds when challenged by a parasitic infection and the subsequent impact on their health and productivity. Thirty-five lambs were housed in individual pens and assigned to five treatment groups (7 animals/group), where they received: 1) A basal diet of beet pulp:soybean meal (90:10) (CONTROL); 2) The same diet, but containing 0.3% of bioactive natural plant compounds extracted from grape, olive and pomegranate (BNP); 3) A simultaneous offer of the diets offered to the Control and BNP groups (Choice-Parasitized; CHP-1); 4) The Control diet, and when lambs developed a parasitic infection, the choice described for CHP-1 (CHP-2); and 5) The same choice as CHP-1, but animals did not experience a parasitic burden (Choice-Non-Parasitized; CHNP). Lambs, except CHNP, were dosed with 10,000 L_3 stage larvae of *Haemonchus contortus*. Infected lambs under choice treatments (CHP-1 and CHP-2) modified their feeding behavior in relation to the CHNP group as they increased their preference for the feed containing polyphenols and terpenes, interpreted as a behavior aimed at increasing the likelihood of encountering medicinal compounds and nutrients in the environment that restore health. This change in behavior corresponded with an improvement in feed conversion efficiency. However, an increased preference for the diet with added plant bioactives did not have an effect on parasitic burdens, hematological parameters, blood oxidation, or serum concentration of IgE.

1. Introduction

Bioactives in plant tissues represent a significant benefit for the nutrition, health and welfare of ruminants grazing diverse plant communities. There are multiple studies showing the positive effects of plant secondary compounds on different aspects of sheep production such as growth [1–3], gastrointestinal parasite control [4], enteric methane production [5] and meat quality [6]. In addition, there is evidence suggesting that herbivores modify their food selection and preference as a function of their physiological needs [7–9] and biochemical characteristics of the diet [1]. For instance, herbivores increase their preference for bioactive-containing plants and rations in order to improve their health [10, 11]. Self-medication stemming from an integration of individual needs with the chemical characteristics of food is an adaptive behavior that contributes to improve the fitness of livestock challenged by disease [12, 13].

Condensed tannins have been used as a model to explore self-medicative behaviors in ruminants [14–16] given that these compounds have direct negative impacts on the parasite, mainly through: (1) lower establishment of the infective third-stage larvae (L_3) in the host, (2) lower excretion of eggs by adult worms and (3) impaired development of eggs into L_3 [17]. In addition to the direct negative impacts of condensed tannins on endoparasites, other plant secondary compounds may provide direct and/or indirect medicinal effects to the host through their antioxidant or immune enhancing properties. This is because parasitic infections increase reactive oxygen species in the host, causing lipoperoxidation, cellular damage and inflammation [18–20]. Thus, self-selection of bioactive compounds other than condensed tannins

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may provide a significant mechanism leading to protection against the oxidative stress or inflammation caused by endoparasites. Previous research demonstrates that provision of an antioxidant (i.e., vitamin E) to lambs infected with *Haemonchus contortus* led to a decrease in parasitic burdens when compared to control (unsupplemented) animals [21].

Several plant secondary compounds like polyphenols and terpenoids have established antioxidative, anti-inflammatory and gene regulatory properties [22–24] with the potential to attenuate parasitic infections in herbivores. Moreover, antioxidant compounds like polyphenols and triterpenic acids have been implicated in gut health and growth performance [25, 26]. Nevertheless, the concept of "nutraceutical" self-medication has not been applied to these bioactive natural plant compounds in mammalian herbivores.

Parasitism induce negative internal states, which may trigger changes in foraging behavior that enhance the likelihood of finding prophylactic plant secondary compounds in the environment [11, 27]. However, it is unknown whether parasitized ruminants are more likely to increase their preference for the novel orosensorial dimensions promoted by the presence of polyphenols and terpenes in a feed.

We hypothesized that gastrointestinal parasitism in sheep causes deviations from homeostasis, which enhance intake of feeds containing bioactive compounds and result in health and productivity improvements. Thus, the objective of this study was to determine the importance of bioactive polyphenols and terpenes on parasitized lamb performance, ingestive behavior and gastrointestinal parasite burdens.

2. Material and methods

The study was carried out at the Green Canyon Ecology Center, Utah State University, located in Logan, Utah (41° 44′ 76″ N; -111° 50′ 3.80″ W) between June 22nd and August 23rd, 2016.

2.1. Animals

Thirty-five lambs (2–3 months of age) of 42 ± 4.0 Kg BW were used during the study. Before the study began, all animals were orally dosed against gastrointestinal parasites with Levamisole and Albendazole at 7.5 mg/kg body weight in order to eliminate the presence of gastrointestinal parasites in their digestive tract.

Animals were housed in individual pens measuring 2.4 × 3.6 m under a protective roof. Pens were made of a metallic grid and fixed with metallic wires without sharp edges. Each pen had a wooden feeder. A water line with automatic nipples provided free access to drinking water. The lambs also had free access to trace salt mineral blocks. The animals were housed in individual cages in a way that they had visual contact with their peers so they did not experience stress due to isolation. The study was conducted according to procedures approved by the Utah State University Institutional Animal Care and Use Committee (approval # 2618).

2.2. Treatments and experimental design

Parasitized lambs were assigned to four treatment groups and offered ad libitum amounts of feeds that differed in the presence of bioactive natural plant compounds: 1) CONTROL, a basal diet (Basal) of beet pulp:soybean meal (90:10) balanced for an average live weight gain of 200 g/day according to NRC [21]; 2) Added bioactive natural plant compounds (BNP), the same ration but containing 0.3% of natural plant compounds (Polyphenols + Terpenes) extracted from grape, olive and pomegranate (GOPE, GOPE-40 ProNutra Solutions S.L., Spain) standardized in polyphenols - 30% proanthocyanidins, 8% ellagic acid, 1,5% gallic acid, 1% hydroxytyrosol, and 6% triterpenic acids (maslinic and oleanolic acid); 3) Choice-Parasitized (CHP-1), a simultaneous offer of Basal and Polyphenols + Terpenes feeds; and 4) CHP-2. The diets were prepared at the research facility by mixing the ingredients into batches that were fed to animals during periods of 7 to 10 days, when new batches were made. The plant bioactives added to the diet were stable during storage [26]. There are no detected levels of polyphenols or terpenes in the ingredients of the basal diet [28]. Animals received the same feed as CONTROL before being infected. However, when they developed a parasitic infection, these animals had the same choice described for CHP-1. A fifth treatment group, Choice-Non-Parasitized (CHNP), received the same choice as CHP-1, but animals did not experience a parasitic burden.

Lambs were randomly distributed across treatments (7 lambs/treatment) and pens, considering the variation of gender (female, ram lambs and wethers) and weight, resulting in a uniform distribution of animals within each treatment group. After 22 days of consuming their respective rations, lambs in groups CONTROL, BNP, CHP-1 and CHP-2 were infected with an oral dose that delivered an inoculum of 10,000 infective larvae (L_3) of *Haemonchus contortus*. All groups continued ingesting the respective rations described above. After three weeks, when animals developed a parasitic infection, group CHP-2 had the same choice as CHP-1 and CHNP. Subsequently, all animals received their respective rations for 18 more days. Thus, this design aimed at exploring the effect of bioactive compounds (BNP vs. CONTROL) on parasitic burdens in sheep as well as the behavior patterns of sheep when given an opportunity to select a bioactive-containing food with the potential to improve their health. The design also allowed to determine the self-selection of plant bioactive compounds by sheep when experiencing (CHP-1) a parasitic infection vs. those animals that were parasite-free (CHNP). Finally, the study determined the benefit and selection of plant bioactives before (CHP-1) or after (CHP-2) lambs experienced a parasitic infection.

Lambs were fed once a day at 0800 and they received their rations in ad libitum amounts. The amounts offered were adjusted every morning so that animals had at least 10% of refusal in their feeders on the ensuing day at 0730. If the animal had < 10% left from the previous day, an additional 500 g of feed was added to the feeders.

2.3. Measurements

2.3.1. Intake

Daily ration intake was measured by the difference between the amounts offered and refused. Preference for groups offered a choice between the basal diet and the bioactive containing diet was estimated as intake of Polyphenols + Terpenes/total food intake.

2.3.2. Diet quality

The rations were sampled according to the batches that were mixed during the study: end of June (before starting the experiment), beginning of July, end of July, beginning of August and end of August (before finishing the experiment). The samples were analyzed for dry matter (DM), crude protein (CP) according to AOAC [29], neutral detergent fiber (NDF) according to Van Soest et al. [30], and acid detergent fiber (ADF) according to Goering and Van Soest [31]. The total digestible nutrients (TDN) and the metabolizable energy (ME) were calculated according to the information in the NRC [28] feed composition tables. There were no significant differences in diet quality composition between different batches, and means are shown in Table 1.

2.3.3. Animal weight gain

Animals were weighed, with a previous liquid and solid fast period of 12 h. The animals were weighed for the first time on the first day of the experiment. After this day, animals were weighed every three weeks.

2.3.4. Animal feed efficiency

Feed Efficiency was calculated by dividing the animal average daily weight gain by the respective daily food intake. This represents the animal gain/kg of ration consumed.