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Reduced neophobia: A potential mechanism explaining the emergence of self-medicative behavior in sheep

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HIGHLIGHTS

- We explored the selection of novel foods by parasitized and healthy lambs.
- Parasitized lambs modified their feeding behavior relative to non-parasitized lambs.
- Ingestive responses were influenced by the type of novel food and flavor on offer.
- Diverse diets and risk proneness may increase the likelihood of finding medicinal plants.

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ABSTRACT

Gastrointestinal helminths challenge ruminants in ways that reduce their fitness. In turn, ruminants have evolved physiological and behavioral adaptations that counteract this challenge. For instance, emerging behavioral evidence suggests that ruminants self-select medicinal compounds and foods that reduce parasitic burdens. However, the mechanism/s leading to self-medicative behaviors in sick animals is still unknown. We hypothesized that when homeostasis is disturbed by a parasitic infection, consumers should respond by increasing the acceptability of novel foods relative to healthy individuals. Three groups of lambs (N = 10) were dosed with 0 (Control—C), 5000 (Medium—M) and 15 000 (High—H) L_3 stage larvae of *Haemonchus contortus*. When parasites had reached the adult stage, all animals were offered novel foods and flavors in pens and then novel forages at pasture. Ingestive responses by parasitized lambs were different from non-parasitized Control animals and they varied with the type of food and flavor on offer. Parasitized lambs consumed initially more novel beet pulp and less novel beet pulp mixed with tannins than Control lambs, but the pattern reversed after 9 d of exposure to these foods. Parasitized lambs ingested more novel umami-flavored food and less novel bitter-flavored food than Control lambs. When offered choices of novel unflavored and bitter-flavored foods or different forage species to graze, parasitized lambs selected a more diverse array of foods than Control lambs. Reductions in food neophobia or selection of a more diverse diet may enhance the likelihood of sick herbivores encountering novel medicinal plants and nutritious forages that contribute to restore health.

1. Introduction

Free-ranging herbivores face many challenges. In addition to avoiding predators, they must cope with disease and acquire a balanced diet from foods that differ greatly in nutritional and toxicological composition [1,2]. Many plant tissues contain plant secondary metabolites (PSMs) which have long been recognized as defensive chemicals that deter herbivory through their aversive orosensory and toxic effects [3]. In contrast to these negative actions, recent evidence suggests that some PSMs can contribute to increase animal fitness if they are ingested at appropriate doses [4,5]. A good example of such duality is represented by condensed tannins, a group of PSM which can have both antinutritional and medicinal effects on consumers [6,7].

Herbivores have evolved mechanisms to maintain homeostasis and contemporarily learn to avoid or prefer certain foods because they lower [8] or increase [9] their fitness, respectively. Medicines like some PSMs raise fitness by reducing disease and thus are expected to be preferred during some instances of the lifetime of the individual [10,5]. In a seminal study, Huffman and Seifu [11] observed that wild chimpanzees suffering from parasite-related diseases consumed the

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bitter pith of the plant *Vernonia amygdalina*, which contains sesquiterpene lactones and steroid glucosides with antiparasitic activity at the doses consumed by the animals [12]. Since these pioneering results, additional evidence pointing to the use of PSMs to recover or to maintain health has been reported for ruminants [13,14], insects [15] and birds [16]. In addition to PSMs, mammalian herbivores self-medicate with substances such as polymers and clays to attenuate the negative impacts of toxicosis and ruminal acidosis [17]. The study of self-medication in animals led to the emergence of the word "zoopharmacognosy," meaning the process by which animals use PSMs or other non-nutritional substances to treat and prevent disease [18].

While past research has uncovered many examples of self-medicative behavior in different animal species, there is a lack of basic understanding of how the behavior is triggered. Learning self-medicative behavior involves consuming a medicine with potential toxic [3] effects to consumers which may also make the food unpalatable [19]. What are the mechanisms by which sick mammalian herbivores would be willing to incorporate potentially toxic and unpalatable PSMs into their diets at the expense of – or in addition to – consuming their required amounts of nutrients?

When physiological requirements are met and the animal is in a homeostatic state – such as when balanced rations are available ad libitum – ruminants eat only small amounts of novel foods, i.e., they are neophobic [20]. This neophobic behavior likely evolved to decrease the likelihood of consuming harmful foods which reduce animal fitness. However, departure from homeostasis (e.g., when there is an inadequate or unbalanced supply of nutrients) may reduce neophobia [20].

Birds on a positive energy budget are averse to risk, but prone to it when experiencing a shortage in the supply of energy to their bodies [21]. This is because individuals which have acquired abundant reserves of energy have more potential fitness to lose from taking risks (e. g., selecting novel foods, exploring new places) than individuals which have not [22].

We hypothesized that when homeostasis is disturbed by a parasitic infection (which imposes metabolic constraints), consumers should increase the acceptability of novel foods relative to healthy individuals. Sick individuals may have less potential fitness to lose from taking risks (e.g., selecting novel foods) than healthy ones. Additionally, reduced neophobia represents the potential benefit for the consumer of increasing the likelihood of encountering medicinal compounds and nutrients that restore health. Once the individual experiences the benefit of medicines and nutrients, associative learning (i.e., associations between taste and relief experienced after ingesting a medicinal food) will maintain and/or reinforce self-medicative behaviors. To test this hypothesis we induced different magnitudes of parasitic infections (*Haemonchous contortus*) in sheep (*Ovis aries*) and then determined the animals' feeding responses to novel flavors, feeds, and forages.

2. Methods

We conducted the study at the Green Canyon Ecology Center (pen study), and at the Pasture Research Facility (grazing study) at Utah State University, Logan, Utah, U.S.A.

Research protocols for the study were approved by the Utah State University Institutional Animal Care and Use Committee (IACUC No. 2227).

2.1. Animals and infection

Thirty lambs of both sexes [commercial crossbreds, 2–3 months old; live weight (LW) of 26 ± 0.5 kg (average ± SEM)] had free access to sodium chloride with trace mineral blocks and fresh water throughout the study. One month before the study, lambs were weaned, penned individually and received alfalfa pellets in ad libitum amounts and barley grain (300 g/lamb/day). The dietary experience of the lambs' mothers was limited to a basal diet of alfalfa hay and barley grain. The lambs were kept outdoors under a protective roof in adjacent pens measuring 2.4 × 3.6 m.

Lambs were blocked by LW and randomly assigned to three treatment groups (n = 10). Seven weeks before the start of the experiment (June 8, 2013), sheep were drenched with the anthelmintics pyrantel pamoate and albendazole at 25 mg/Kg and 7.5 mg/Kg LW, respectively, delivered in separate doses. Ten days later (June 18, 2013), fecal samples were taken at 0800 from the rectum of each animal, stored in an ice chest and analyzed for fecal egg counts (FEC) during the same day using the MacMaster technique to ensure that animals had very low to nil [<100 eggs per gram (epg)] parasitic burden before the start of the study. Subsequently (June 19, 2013), lambs of each group were dosed orally with infective third-stage (L_3) larvae of *H. contortus* in the following amounts: 0 (Control group: C), 5000 (Medium group: M) and 15 000 (High group: H) L_3/lamb.

2.2. Novel foods and flavors

2.2.1. General protocol

At 0800 all lambs were presented with a three-bucket choice of a novel feed containing 3 concentrations of a novel flavor. Refusals were collected every 20 min, weighed and offered again for a period of 2 h to determine their eating pattern. The position of the buckets was distributed at random inside each individual feeder every day. Lambs were then offered 2 kg of alfalfa pellets from 1200 to 1400 daily; refusals were collected and weighed. No other feed was offered until the next day. The order of presentation of novel flavors was: 1—condensed tannins, 2—umami, 3—bitter, and 4—coconut, maple and apple flavors. Since animals were offered choices among three alternatives, the protocol was devised to give animals enough time of exposure (7 to 9 days) to sample all options. In addition, the design allowed for assessing initial responses to the novel flavored foods relative to ingestive responses occurring when animals gained more experience at ingesting these foods.

All lambs were weighed on August 1 (38 ± 0.8 kg average LW) and August 30 (44 ± 0.8 kg average LW) and they were fed ad libitum amounts of alfalfa pellets in-between tests. Test duration was variable, as it depended on stable intake values in three consecutive days (average intake values within ±5%).

Blood samples were drawn from all lambs by venipuncture of the jugular vein 20 days before the start of the study, and on days 1, 20 and 40 of the study for analysis of red and white cell parameters using a Hemavet HV950FS (Drew Scientific, Oxford, UK) hematology analyzer. Fecal samples were collected as described before during July 19 and 25, August 1 and 21 and September 16 to determine FEC using MacMaster technique.

2.2.2. Condensed tannins

Fecal samples were taken a month after larval dosage (July 19), and a week later (July 25, 2013) and FEC suggested parasites had reached the adult stage (Table 1). Thus, tests with condensed tannins were

Table 1
Fecal egg counts (FEC; epg) in 3 groups of lambs (n = 10) exposed to novel foods and flavors (July 26 to August 30, 2013) and novel pastures (August 31 to September 12, 2013). Lambs of each group were dosed orally with infective third-stage (L_3) larvae of *Haemonchus contortus* in the following amounts: 0 (Control group: C), 5000 (Medium group: M) and 15 000 (High group: H) L_3/lamb.

Group[1]	June 18	July 19	July 25	August 1	August 21	September 16
C	0	0^a	0^a	0^a	0^a	0^a
M	0	1235^b	2350^b	3095^b	3165^b	3906^b
H	0	2415^c	6245^c	8165^c	7985^c	8240^c

[1] Means in a column with different superscripts differ (group effect; $P < 0.10$). SEM = 699.