Aversion to phenylthiocarbamide in mature Targhee and Rambouillet rams

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INTRODUCTION

Overgrowth of sagebrush in the western United States can lead to a reduction in plant diversity, carrying capacity, and wildlife abundance (Launchbaugh, 2003). Suppression of sagebrush with grazing sheep may be a sustainable method of control. The dietary preference for sagebrush in sheep has a calculated heritability of 0.28 (Snowder et al., 2001), suggesting that breeding selection for low-palatable compounds in sheep may be achievable. Ferreira et al. (2013) identified a set of novel genes for bitter taste receptors in sheep, suggesting that sheep may be genetically predisposed to select or avoid plants with bitter or noxious tastes.

Sheep are adaptive selective grazers (Launchbaugh et al., 2001) with varying dietary preference for consuming sagebrush (Snowder et al., 2001). Early research on the primary taste groups in sheep suggested bitterness was the most sensitive (Goatcher and Church, 1970b). Additional studies indicated that sheep can taste and (or) sense bitterness when mimicked by addition of compounds, such as quinine, when added to drinking water (Goatcher and Church, 1970a; Favreau et al., 2010), and lithium chloride, when added to forages (Launchbaugh et al., 1993).

Phenylthiocarbamide (PTC) mimics the bitter tastes found in food and has been used in bitter taste research in humans (Fox, 1932) and mice (Nelson et al., 2003). On the basis of PTC sensitivity, humans have been categorized as tasters and nontasters (Blakeslee and Salmon, 1935) with heritable ($h^2 = 0.55$) PTC thresholds (Morton et al., 1981). Avoidance of PTC may also be influenced by post-digestive factors (Nelson et al., 2003), similar to preferences of sheep grazing bitter/toxic forages (Launchbaugh et al., 2001).

We hypothesized that sheep could detect bitter-tasting compounds and the individual sensitivity would vary. The objective of this study was to determine whether sheep could detect the bitter-tasting compound, PTC, and if so, what PTC concentration would elicit an avoidance response.

MATERIALS AND METHODS

Animals

All animal procedures were approved by an Institutional Animal Care and Use Committee (U.S. Department of Agriculture [USDA], Agricultural Research Service [ARS], Dubois, ID) in accordance with the USDA, Animal and Plant Health Inspection Service Animal Welfare Regulations (2013; 9 C.F.R. § 2.30-2.38 2013) and the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Two trials were conducted at the USDA, ARS U.S. Sheep Experiment Station, Dubois, ID, in the spring of 2018. Trials consisted of mature Rambouillet and Targhee rams ($n = 15$ per trial). Feed and water intake were monitored under controlled conditions of 10 °C with a 12 h light to...
dark cycle. In addition, feed and water were withheld from rams from 1700 to 0700 h each day during the trials. Rams were randomly allotted within breed to alternate pens throughout the barn. Trials were divided into two phases: an acclimation phase, where rams were adjusted to the pens, daily feed and fluid delivery routines, and a testing phase, where the PTC treatments were delivered.

**Experimental Design**

Trials were conducted as a crossover design consisting of five PTC treatments. Rams were randomized to five testing blocks consisting of three rams each. Each block was randomly assigned a PTC-testing sequence, which consisted of the order rams received PTC treatments over the five test days.

Each trial consisted of a 5-d acclimation phase followed by PTC testing. During the acclimation phase of both trials, rams received alfalfa pellets (17.4% crude protein) at a rate of 1.9% of body weight (as fed basis) at 0700 h. Thirty minutes after feed delivery, feed was removed, and two buckets filled with water (1.5 kg, trial 1; 3.0 kg, trial 2) were placed in each pen, and rams were allowed access to the water for 1 h in trial 1 and 2 h in trial 2. Buckets were then removed, and water refusals weighed for each bucket. For the remainder of the day, rams then received alfalfa pellets at a rate of 2.8% of body weight (as fed basis), 45 g of a mineral mix, and additional water (9 kg trial 1; 8 kg trial 2).

PTC was used because it has been demonstrated to elicit a strong bitter taste in human research and has been suggested to be linked to preference of bitter-tasting foods (Blakeslee and Salmon, 1935). It is unknown what PTC concentration mimics the degree of bitterness in plants; therefore, PTC concentrations for trial 1 were chosen over a considerable range and adjusted for trial 2 to better meet the objectives of the study.

The test phase for both trials consisted of test days where PTC-solutions and water (in separate buckets) were delivered after the morning feeding, and each test day was followed by a rest day where only water was delivered in order to minimize potential carryover effects of PTC from the previous test day. On test days, each ram block received one of the five concentrations of PTC-solutions (trial 1: 0.20, 0.56, 1.57, 4.39, or 12.29 mM delivered in a total volume of 1.5 kg; trial 2: 0.20, 0.43, 0.94, 2.03, or 4.39 mM delivered in a total volume of 3.0 kg) in one bucket, and water only (trial 1: 1.5 kg; trial 2: 3.0 kg) in the other bucket. The location (left or right) of the PTC-solution bucket was alternated between test days. For both trials, PTC (Sigma P7629, Sigma-Aldrich, Sant Louis, MO) was dissolved in absolute ethanol and diluted with tap water to the desired concentrations for delivery.

**Statistical Analysis**

For fluid intake analysis during the test phase within a trial, data were analyzed using PROC MIXED procedures of SAS (Statistical Analysis System, SAS Institute Inc., Version 9.4, Cary, NC). The model included treatment (PTC concentration), sequence (order PTC concentrations were administered to rams), and period (day that PTC was administered within the sequence) with a random statement that included ram within sequence. Trial means are reported as least squares means and are presented in Figures 1 and 2. Mean comparisons were made using the pair-wise contrasts (PDIFF). Significance was set at $P \leq 0.05$, and tendencies were determined if $P \leq 0.10$ for all analysis.
There was considerable variation among rams in sensitivity to PTC observed within and across PTC concentrations for each trial, which are represented in the scatterplots of Figures 3 and 4. Consequently, individual rams were further classified into intake groups based on total PTC intake (gram) over the five test days. Intake group differentiation was determined by 0.5 SD of the population mean to divide rams into high (≥0.5 SD), medium (<0.5 to >−0.5 SD), or low (≤−0.5 SD). One objective of this study was to determine variation among individuals; in an effort to display the variation observed, linear regression was chosen using PROC GLM for analysis by intake group. Planned paired and orthogonal contrasts were used to compare regression lines (slope and intercept analyzed together), slopes, and intercepts between PTC intake categories using the CONTRASTS option of SAS.

**RESULTS AND DISCUSSION**

We hypothesized that sheep could detect PTC when mixed in drinking water and that sensitivity would be different among individuals. Unlike in human studies (Fox, 1932; Blakeslee and Salmon, 1935), rams are unable to verbally express if they can detect PTC. However, at all concentrations the PTC-solution was consumed less than the water, and negative behavioral reactions were observed during the study (e.g., smacking lips and shaking their head after tasting the PTC-solution), particularly with the highest PTC concentrations (data not shown).

In trial 1 (Figure 1), as each PTC-solution increased in concentration, the intake of PTC-solution decreased ($P < 0.0001$). In trial 2 (Figure 2), there was also a treatment effect ($P < 0.01$) on PTC-solution intake but slightly different than observed in trial 1. The intake of the 0.20, 0.43, 0.94, and 2.03-mM concentrations were all similar ($P > 0.05$), but the intake of the 4.39-mM concentration was different than the rest ($P ≤ 0.05$). This may be due to the smaller differences between PTC concentration levels for trial 2 and (or) the increase in total morning fluid offered. The greatest decrease in percentage of PTC-solution intake was observed between 1.57 and 4.39 mM (58%) in trial 1 and 2.03 and 4.39 mM (72%) in trial 2. Individual behavioral
reactions of rams to PTC-solutions and differences in intakes of PTC-solutions, taken together, suggest that rams could detect PTC, and that individual rams varied in sensitivity to detect PTC.

A great deal of variation in PTC-solution intake was observed between individual rams (Figures 3 and 4). In trial 1, the ram with the greatest intake of PTC consumed 9.7-fold more PTC than the ram with the lowest intake (1.06 vs. 0.109 g, respectively). For trial 2, the magnitude of difference was much greater at 60-fold (2.10 vs. 0.0348 g PTC, respectively). On the basis of the variation between individuals within each trial, rams were grouped according to total (gram) PTC intake into high-, medium-, and low-intake groups. Moreover, regression analyses were performed on each intake group within a trial based on percentage of PTC-solution intake of total morning fluid intake (Figures 3 and 4). The slopes of each group within each trial did not differ ($P > 0.05$), suggesting that the rate of aversion between groups was not different. However, most of the intercepts differed across groups (Figures 1 and 2), which suggested that the point of avoidance is different between groups as PTC concentration increases.

Similar to the sensitivity to PTC observed across rams in this study, sensitivity to consuming bitter shrubs has also been observed in grazing sheep. Snowder et al. (2001) determined percentage of sagebrush consumed in the diet of 549 ewes was 10.3% to 31.9% for September and 23.7% to 42.3% for October. The September and October measurements were highly correlated ($r^2 = 0.91$), where the highest consumers in September were also the highest consumers in October (Snowder et al., 2001). Similar to this study, individuals in the high consumer group consistently consumed the most PTC-solution.

**IMPLICATIONS**

Utilizing sheep as a grazing tool to reduce sagebrush canopy has been suggested to entail long-term and high-intensity grazing applications (Seefeldt, 2005); however, sheep grazing may be a good tool for suppressing sagebrush canopy growth and decrease shrub encroachment on grasslands. Because sagebrush selection is moderately heritable in sheep ($h^2 = 0.28$; Snowder et al., 2001), selection for sheep that have a greater tolerance
for bitter-tasting compounds, such as PTC, may translate to suppression of sagebrush canopy on rangelands, and thus, extend the optimal ecological productivity-time period beyond 5 to 15 years post-fire (Moffet et al., 2015).

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Conflict of interest statement: None declared.

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