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ScienceDirect
Crop Protection 27 (2008) 851–854
Crop Protection
www.elsevier.com/locate/cropro

Short communication

Lagomorph and rodent responses to two protein hydrolysates

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Received 4 May 2007; received in revised form 10 July 2007; accepted 11 July 2007

Abstract

Various species of rodents and lagomorphs were used in bioassays to determine the effectiveness of protein hydrolysates (specifically hydrolyzed casein and gelatin) as herbivore repellents. Mixed sex groups of captive rabbits, pocket gophers, voles, and mountain beavers were offered hydrolyzed casein or gelatin test diets in single-choice tests following a training period with a hydrolysate-free diet. The effectiveness of either hydrolyzed casein or gelatin was dependent on the species. Hydrolyzed casein was identified as an effective repellent for mountain beavers and pocket gophers as these species showed a strong avoidance of hydrolyzed casein diets. Rabbits demonstrated slightly higher avoidance of the gelatin diets versus hydrolyzed casein. However, hydrolyzed casein and gelatin displayed little potential as repellents for voles as both products were readily consumed. Rather than universal application for all pest herbivores, test diet preferences suggest that repellent application depends on the pest species.
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Keywords: Gelatin; Gopher; Herbivore; Hydrolyzed casein; Mountain beaver; Rabbit; Vole; Wildlife damage management

1. Introduction

Repellents deter herbivores by exploiting their fear of novel stimuli (shiny objects, odor sachets, predator odors, etc.; Sunnucks, 1998; Nolte and Wagner, 2000; Kimball and Nolte, 2006), altering the palatability of the food resource (Andelt et al., 1994; Lutz and Swanson, 1995; Mason et al., 1999; Nolte and Wagner, 2000; Kimball and Nolte, 2006), or producing a conditioned taste aversion (Nolte and Wagner, 2000; Baker et al., 2007). Each of these mechanisms may be effective, depending on the motivation of the individual herbivore. Habituation to novel stimuli may pose a problem when no negative consequences are experienced by the animal during the encounter with the novel stimuli (Nolte, 1999). Products that promote taste aversions offer the best efficacy even in highly motivated herbivores since their consumption may cause negative postingestive consequences (i.e. toxic malaise). However, these repellents may not be practical as toxic agents are unlikely to be approved for many commodities, particularly food products.

Hydrolyzed casein (HC) has recently been demonstrated to significantly reduce browsing by deer, even when alternative food choices are minimized (Kimball et al., 2005). Relative to commercial herbivore repellents, HC is inexpensive, readily available, and food safe. In addition, HC is exempt from US Environmental Protection Agency requirements for residue tolerance. Gelatin (GE) is another example of a protein hydrolysate (from the hydrolysis of collagen, the primary protein found in connective tissues of mammals). GE was also used in this study because of the observation that laboratory mice avoided GE to a greater extent than HC (K. Field, personal communication).

Preliminary two-choice tests suggested that rabbits and voles avoided GE versus control to a greater extent than HC, while pocket gophers and mountain beavers avoided HC relative to the control more so than GE. However, two-choice tests are not sufficient for predicting repellency because the repellent must not only be avoided when

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0261-2194/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.cropro.2007.07.004

alternative foods are available, but also reduce the intake of foods when no alternatives are present. Thus, we conducted a series of single-choice tests as a more appropriate test to evaluate the repellency of HC with pocket gophers and mountain beavers and the repellency of GE with rabbits and voles.

2. Methods

2.1. Subjects

Mixed-gender, captive subjects of each species were used in single-choice tests. The species tested were New Zealand white rabbit (*Oryctolagus* spp.), mountain beaver (*Aplodontia rufa*), Western pocket gopher (*Thomomys mazama*), and Townsend's vole (*Microtus townsendii*). Animal procedures were approved by the National Wildlife Research Center Institutional Animal Care and Use Committee. Single-choice tests were conducted during the period of July–September 2006.

Rabbits and mountain beavers were individually housed and tested in $8' \times 8' \times 10'$ and $8' \times 8' \times 4'$ outdoor pens, respectively. Test diets were offered to rabbits and mountain beavers in individual 8″ plastic food bowls. Pocket gophers were individually housed and tested in $11'' \times 14'' \times 7''$ plastic rat cages equipped with wire bar lids. Voles were individually housed and tested in similar plastic mouse cages measuring $8'' \times 12'' \times 5''$ equipped with wire bar lids. Rodent cages were maintained in indoor environments kept at 68 °F (±2 °F) with a 9 h light cycle where the test diet could be accessed through the wire bar lids.

2.2. Test diets

Test diets were prepared with a kitchen-type bowl mixer. The appropriate ingredients were placed in the mixer and water was added to produce a dough consistency (Table 1). Each diet consisted of test protein (HC, GE, or cellulose) added to a foundation diet. The foundation ingredients were corn starch (Dyets Inc., Bethlehem, PA), whole-wheat flour (King Arthur®, Norwich, VT), sucrose (Kroger®, Cincinnati, OH), corn oil (Crisco®, J.M. Smucker Co., Orrville, OH), salt mix (AIN-76; Dyets Inc.), and vitamin mix (AIN-76A; Dyets Inc.). The volume of water required to prepare the dough was dependent on the test material added. The dough was spread with a rolling pin to produce a thickness of 2.0 cm. Blocks measuring $8 \times 4 \times 2$ cm were cut and then dried at 105 °C in a food dehydrator (Sausage Maker Inc., Buffalo, NY) until a consistent mass was obtained. One batch tended to produce between 12 and 18 bars.

Table 1
Description of ingredients used in the preparation of various test diets

Diet	Ingredients
Foundation	250 g corn starch 150 g whole-wheat flour 300 g sucrose 61 mL corn oil 35 g salt mix 10 g vitamin mix
Hydrolyzed casein	800 g foundation 200 g casein hydrolysate
Gelatin	800 g foundation 200 g gelatin
Cellulose (control)	800 g foundation 200 g cellulose

The training diet consisted of foundation and whole-wheat flour. The control diet was prepared with foundation and cellulose (Dyets Inc.); the HC diet consisted of foundation and casein hydrolysate (HCA-411; American Casein Company, Burlington, NJ). GE diets were similarly prepared with foundation and GE (PolyPro 5000; PB Leiner Co., Jericho, NY).

2.3. Training

Subjects were adapted to the testing schedule with a 4-day training period. Subjects were food deprived overnight (14 h) after which they were offered the training diet for 4 h. Intake of training diet (g) was determined by mass difference. Following the 4 h test period, the test diets were removed and the subjects were given access to their basal ration for 6 h—until the beginning of the next deprivation period. Subjects had ad libitum access to water throughout the experiment. The basal/maintenance diet for rabbits consisted of Purina® Rabbit Chow Complete Blend (PMI Nutrition International, Henderson, CO), supplemented with apple slices, until the study was completed. The three rodent species received Lab Diet #5012 (PMI Nutrition, Inc.) supplemented with apple. Mountain beavers and pocket gophers were additionally provided alfalfa cubes in addition to rat diet and apple.

Following the training period, 16–18 subjects of each species were assigned to two treatment groups according to training diet intake (mass) such that mean intake and standard deviation were similar between treatments (8 or 9 subjects per treatment).

2.4. Single-choice tests

For all species, subjects in the protein treatment group were offered only the hydrolysate-containing diet and subjects in the control group were offered only the control diet for 4 consecutive days according to the schedule established in the training phase. Intake (mass) was recorded daily for each subject. Rabbits and voles assigned to the treatment group were offered GE diets, while mountain beavers and pocket gophers assigned to the treatment group were offered HC in the single-choice study. Control group subjects were offered the control diet, regardless of species.