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# Evaluation of Potential Food Items as Challenge Diets in 2-Choice Tests with Feral Swine

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**ABSTRACT:** Registration of a new toxicant for feral swine in the United States requires meeting test standards under the Federal Insecticide, Fungicide, and Rodenticide Act as regulated by the U.S. Environmental Protection Agency. A primary requirement is to compare the palatability and efficacy of the toxicant to a non-toxic challenge diet in a 2-choice test; however, no standardized challenge diet exists for feral swine. We conducted a series of 2-choice tests to examine four potential challenge diet items for preference by feral swine. We found that feral swine consumed the most and spent the most time feeding on rough rice (i.e., seed rice), although dog food and rough rye could not be wholly discounted as potential challenge diets. Rough rice was preferred, provided adequate nourishment, and is readily consumed by free-ranging feral swine. Therefore, we conclude that rough rice is an appropriate challenge diet for incorporating into 2-choice tests to meet test standards for evaluating the effectiveness of an oral toxicant for feral swine.

**KEY WORDS:** bait, challenge diet, food preference, *Sus scrofa*, toxicant, vertebrate pest control, wild pig

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## INTRODUCTION

Feral swine (*Sus scrofa*) were introduced to the United States centuries ago, but in recent decades their populations have rapidly increased in abundance and distribution (Barrios-Garcia and Ballari 2012). As a result, the U.S. feral swine population is currently estimated to be >6 million with 36 states having established populations (Mayer 2014), making feral swine the most abundant introduced ungulate in the U.S. (Sweeney et al. 2003). Coinciding with the increase in abundance and distribution of feral swine, damage and control costs have rapidly increased over the same time span. An estimated \$1.5 billion is spent annually on costs associated with crop damage, depredation of livestock, spread of disease, and labor and equipment for control efforts (Pimentel et al. 2000). In addition to these costs, numerous negative ecological consequences are also associated with feral swine, including predating on sensitive species (Taylor and Hellgren 1997, Jolley et al. 2010), habitat and nest destruction (van Riper and Scott 2001), and reductions in plant species diversity (Hone 2002).

Current control methods such as trapping and shooting can reduce feral swine populations on a local scale (Coblentz and Baber 1987, Choquenot et al. 1993, Mayer and Brisbin 2009, West et al. 2009, Campbell et al. 2010), but these methods are having limited success on large scales. Given the limited ability of conventional control measures, new technology and methods are needed to complement current techniques that are used to control feral swine. Development of a new orally-delivered

toxicant may provide a more cost-effective method for controlling feral swine on a larger scale. Currently, there are no toxicants registered for use on feral swine in the U.S. Therefore, research conducted by the National Wildlife Research Center, U.S. Department of Agriculture, in Fort Collins, CO; the Invasive Animal Cooperative Research Center, University of Canberra, Australia; and the Texas Parks and Wildlife Department is focused on developing a feral swine-specific toxicant. The current formulation is an oil-based matrix that conceals a 10% concentration of micro-encapsulated sodium nitrite (Snow et al. 2016).

Registration of a new toxicant requires meeting test standards under the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. §136 et seq.) regulated by the U.S. Environmental Protection Agency. A primary requirement is to determine the efficacy and palatability of the compound using 2-choice feeding trials, in which the animals have a choice between a non-toxic food item and the toxicant bait (Schneider 1982, European Community 2009). These trials require that a standard challenge diet be offered as a non-toxic food item to ensure that the toxicant bait is preferred and lethal in the presence of alternative options (Schneider 1982). For example, when testing new rodenticides with rodents, a standard challenge diet consists of a mixture of cornmeal, rolled oat groats, sugar, and corn oil. In essence, this mixture provides a moderately to highly-palatable and nutritious food item as an alternative to a toxic bait. It also represents food items that rodents are accustomed to eating (European Community 2009).

An appropriate challenge diet has not been established for use in 2-choice toxicant studies with feral swine. Ongoing development of a toxicant bait for feral swine (Snow et al. 2016) necessitates this test. Thus, our objective was to evaluate various food items that feral swine could be accustomed to eating for appropriateness as a challenge diet. Specifically, we examined preference by feral swine for four potential food items relative to a placebo bait. Results from this study will support the required test standards for registering a new toxicant for feral swine in the U.S.

## METHODS

This study took place during June-July 2015 in an outdoor captive facility operated by the Texas Department of Wildlife, Kerr Wildlife Management Area (KWMA), Hunt, TX. This is a certified holding facility for feral swine, permitted by the Texas Animal Health Commission. Free-ranging feral swine were live-trapped throughout nearby counties by USDA Animal Plant Health Inspection Service (USDA APHIS) Wildlife Services and private landowners, then brought to KWMA for housing and testing. While at KWMA, feral swine were maintained on Bluebonnet® 18% Sow Ration Pellet (AC Nutrition, LP, Ardmore, OK) provided at 3-5% of group body mass daily and water *ad libitum* from self-maintaining water troughs. Prior to testing, feral swine were group-housed in a 2-ha (5-ac) holding pen with naturally growing vegetation, trees, and shade structures. All experimental methods were approved by the Texas Parks and Wildlife Department Institutional Animal Care and Use Committee (protocol 211072020151).

We selected four food items to test as potential challenge diets, based on literature from domestic swine diets (Owsley and Van Dyke 1994) and discussions with a swine veterinarian (Fred L. Cunningham, USDA NWRC, pers. comm., June 2015). The food items included: 1) meat- and grain-based dog food (Country Dog Food, Platinum Pet Foods LLC, Brownwood, TX), 2) cleaned rough rice (i.e., seed rice, *Oryza sativa*), 3) rough rye (i.e., rye seed, *Secale cereale*), and rice hulls (i.e., husks surrounding grains of rice). Our goal was to select four food items that were moderately-palatable for feral swine.

We conducted a series of 2-choice tests to compare the relative preference of these food items to our placebo bait matrix of peanut paste mixed with crushed grains (Snow et al. 2016). For each replicate of the trial, we randomly selected feral swine and placed them into one of four small pens (~15×15 m) until each pen contained three animals. Inside each pen were two 58-L rubber feeding tubs (Marshalltown Company, Fayetteville, AR) that were staked to the ground to prevent overturning. We placed a RECONYX PC900 remote camera (RECONYX, Inc., Holmen, WI) centered above each feeding tub with the tub in the middle of the frame.

The feral swine were allowed to acclimate to the new pens for one day and provided their maintenance diet and water *ad libitum*. After the day of acclimation, we randomly placed one of the four potential challenge diets into a randomly selected tub inside each pen. In the other tub we placed the placebo bait. All food items were offered at 4% of group body mass within each pen. We

used time-lapse imagery at 15-second intervals to record consumption of food items at each tub. Each 2-choice test lasted for 15 hours, then any remaining food was removed and weighed. This experiment was replicated five times. During each replicate, the potential challenge diets were systematically rotated among pens and tubs to minimize any effect from the pens or tubs. Overall, 60 feral swine were tested.

We compared the relative preferences for each of the potential challenge diets in the presence of placebo bait using two metrics: 1) the amount of each food item consumed and 2) the amount of time spent feeding on each food item as indexed by the imagery. We imported all images into the Colorado Parks and Wildlife Photo Database for image processing (v3.0, Ivan and Newkirk 2016) and recorded the number of feral swine that were feeding on the food items in each image. Both sets of data were analyzed with linear mixed models using package lme4 (Bates et al. 2014) in Program R (R Core Team 2008). We used the proportion of bait remaining and the proportion of feral swine feeding per total number of images as response variables, respectively. We examined how these variables were influenced by the challenge diet offered (i.e., categorical fixed effects) and included a pen random effect. We examined the 95% confidence intervals for a lack of overlap on zero to indicate a statistical and biological difference among treatment types. We used rice hulls as our reference treatment to compare all other treatments.

## RESULTS

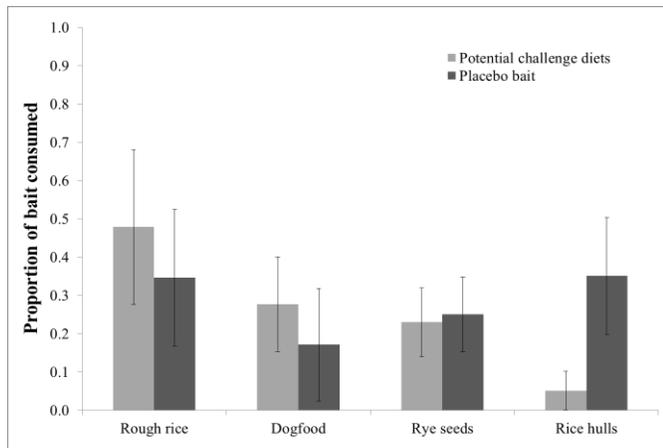
In the presence of placebo bait, we found that feral swine consumed a higher proportion of rough rice ( $\bar{x}$  = 48%;  $\beta$  = 0.43; 95% CI = 0.09-0.76) compared to all other food items (Figure 1). Consumption rates of the other three treatments were similar to each other. The dog food was consumed second most ( $\bar{x}$  = 28%;  $\beta$  = 0.23; 95% CI = -0.11-0.56), rye seeds third most ( $\bar{x}$  = 23%;  $\beta$  = 0.18; 95% CI = -0.16-0.51), and rice hulls least ( $\bar{x}$  = 5%;  $\beta$  = 0.05; 95% CI = -0.18-0.29). The proportion of placebo bait consumed during the feeding trials did not differ among the treatments, although the average consumption was highest when paired with rice hulls and rough rice at 35% each, followed by rye seeds at 25% and dog food at 17%.

In the presence of placebo bait, we also found that feral swine spent the most time feeding on rough rice ( $\beta$  = 0.11; 95% CI = 0.03-0.18; Figure 2). The amounts of time spent consuming all other treatments were similar to each other, including dog food ( $\beta$  = 0.05; 95% CI = -0.01-0.13), rye seeds ( $\beta$  = 0.04; 95% CI = -0.02-0.12) and rice hulls ( $\beta$  = 0.01; 95% CI = -0.04-0.06). The amount of time spent feeding the placebo bait was lower than all challenge diet items except for rice hulls.

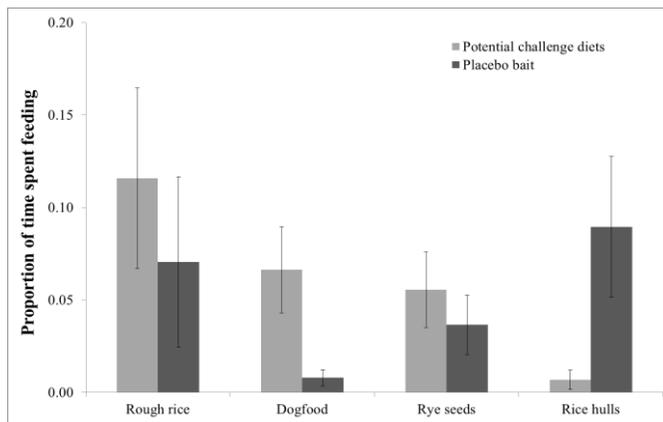
## DISCUSSION

Feral swine consumed the highest proportion and spent the most time feeding on rough rice compared to all other potential challenge diet food items, indicating that rough rice is a preferred food choice when compared to the other food items. It was expected that feral swine would readily consume rice, given the high degree of damage caused by feral swine feeding on fields of rice in the southern U.S.

(Seward et al. 2004) and other parts of the world (Tisdell 1982). Furthermore, rough rice provides a realistic challenge diet for feral swine because it represents an abundant food item to which feral swine are exposed throughout the world.



**Figure 1. Proportions of food items consumed by groups of three captive feral swine (*Sus scrofa*) during 15 hr feeding bouts in pens at the Kerr Wildlife Management Area, Hunt, TX.**



**Figure 2. Proportions of time spent feeding by groups of three captive feral swine (*Sus scrofa*) during 15 hr feeding bouts in pens at the Kerr Wildlife Management Area, Hunt, TX.**

Our results also indicated that feral swine will consume moderate quantities of dog food and rye seeds when placebo bait is present. Feral swine consumed approximately 0.9% and 0.7% of their combined body weight of dog food and rye seeds, respectively; this is comparable to the required maintenance diet for feral swine (i.e., 1.1% of combined body weight daily). Therefore, these food items cannot be entirely discounted as potential challenge diets for future studies. Rice hulls represented the only food item that can be conclusively eliminated as a challenge diet, because it was not preferred by feral swine.

Consumption of the placebo bait did not significantly vary depending on the potential challenge diet items offered simultaneously, indicating these four challenge

items did not influence consumption of placebo bait. Interestingly, the proportion of time spent feeding on the placebo bait varied with the type of challenge diet offered, perhaps indicating some preference for dog food, and non-preference for rice hulls, when compared to placebo bait. The dog food was most similar to the maintenance diet that feral swine routinely ate, which may help explain the higher degree of interest by feral swine. Rough rice did not lead to a reduction in consumption of placebo by feral swine during the feeding trials.

We conclude that rough rice represented an appropriate challenge diet for use in 2-choice tests with feral swine. Rough rice was moderate-highly preferred, provided adequate nourishment, and is readily consumed by free ranging feral swine (Tisdell 1982). To our knowledge, these findings represent the first attempt at identifying a suitable challenge diet to meet test standards for evaluating the effectiveness of an oral toxicant for feral swine (Schneider 1982).

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