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# Development and Testing of a Matrix for Mongoose Toxic Bait: Nontoxic Bait Acceptance Cage Trials

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**ABSTRACT:** The only pesticide currently registered for mongoose control is a product developed for rats that consists of a hard-cereal bait block. Although the active ingredient (diphacinone) is known to be highly effective for mongoose, previous studies indicate that carnivorous and omnivorous mongooses do not readily consume the hard bait matrix designed for gnawing rodents. A palatable bait matrix with a consistency more appropriate to mongoose dentition and feeding behavior will be required to develop a more effective mongoose pesticide. We evaluated the acceptance and consumption of nontoxic versions of four candidate bait matrices: FOXECUTE® and FOXSHIELD® (Animal Control Technologies, Australia; ACTA); HOGGONE® (ACTA); and a potted pork shoulder loaf containing artificial dead mouse scent developed by WS-NWRC as a bait for invasive brown treesnakes (hereafter ‘BTS bait’). We offered test groups of six mongooses one of the candidate bait matrices alongside dry dog kibble dog food as a challenge diet for five days. Because the potential active ingredients para-aminopropiophenone and sodium nitrite require accumulation of the toxicant within a relatively brief period of time to affect lethal toxicity before they are metabolized, we conditioned mongooses to feeding within only a four-hour window rather than slowly sampling the bait throughout the day. We estimated rate and amount of consumption through review of time-lapse photography of feeding trials and measured total consumption by weighing uneaten portions of bait. From the first day offered, most mongooses readily consumed ample amounts of all four bait matrices and consumed almost no challenge diet. Overall, consumption was highest and most consistent with the BTS bait. Although this trial did not clearly discriminate an optimal bait matrix, this result is highly encouraging in that we have multiple palatable options. The final selection will be based on other characteristics of the bait matrix such as longevity in the field, compatibility with the selected toxicant, and ease of manufacture, storage, and use. We provide an overview of some of these characteristics for each candidate bait type.

**KEY WORDS:** bait acceptance, *Herpestes auro punctatus*, invasive species, small Indian mongoose, vertebrate pesticide development

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## INTRODUCTION

Introduced small Indian mongooses (*Herpestes auro punctatus*) are serious predators of native wetland, seabird and upland forest avian species in the Hawaiian Islands (Hays and Conant 2007), as well as in other introduction sites worldwide (Nellis and Everard 1983, Yamada and Sugimura 2004). Mongooses are well established across most of the main Hawaiian Islands (Hawaii, Oahu, Maui, and Molokai) where they pose a threat to the eggs and nestlings of native ground-nesting birds (Hays and Conant 2007). The threat of accidental or intentional introductions to other mongoose-free islands in the Hawaiian chain (e.g., Kauai, Lanai) and other Pacific locations highlights the need for a comprehensive menu of control techniques, including attractive and palatable baits and effective toxicants, to quickly respond to reported sightings or incipient mongoose populations under a diversity of scenarios (Phillips and Lucey 2016). Mongooses also present a health risk to humans as hosts of leptospirosis in Hawaii (Wong et al. 2012) and the Caribbean (Everard et al. 1976), and as a rabies reservoir on several islands in the Caribbean (Zieger et al. 2014).

Eradication of introduced mammals is a powerful conservation tool (Howald et al. 2007); however, mongoose eradication has been attempted only on few occasions and with limited success. A known total of eight eradication campaigns and many control campaigns have been

conducted to remove or reduce island mongoose populations (Barun et al. 2011). However, even with their limited scope, these attempts probably delayed or prevented further declines or even extirpations of native species. Very few teams have the technical expertise to remove mongooses successfully, even from small islands. Lack of expertise is reflected by past failures and little progress beyond local trapping control programs. In Amami-Oshima, Japan, over 10 years of intensive trapping reduced mongoose populations island-wide; however, alternative methods such as toxicants are being considered and tested to eradicate remnant mongooses in difficult-to-trap areas. In Hawaii, live-traps (Tomahawk Live Trap, Tomahawk, WI) and registered 50 ppm diphacinone wax block baits applied within bait stations are employed (SLN No. HI-980005; Smith et al. 2000, Barun et al. 2011). However, these methods have been less successful in areas with low mongoose density or high alternate prey density.

USDA WS-NWRC Hawaii Field Station researchers have conducted field studies evaluating various potential lures, attractants, and bait types (Pitt et al. 2015). Mongooses in this study foraged over a wide area (mean home range estimates were 21.9 and 28.8 ha at two study sites), and readily investigated the various novel food baits, including fish, beef and egg-baited stations with revisits over multiple days. However, long-lasting lures and palatable baits still need to be developed and trialed in the field.

A recent WS-NWRC cage trial of several candidate toxicants, including commercial rodenticide formulations, novel toxicants [sodium nitrite (SN) and para-aminopropiophenone (PAPP)], and minced-chicken formulations with diphacinone, demonstrated potential for development of a highly effective toxic bait for mongoose control (Sugihara et al. 2018). These findings also indicated that the relative inefficacy of the commercial rodenticide formulations was likely due to the hard consistency of grain-based pellets and blocks which are not appropriate to the dentition and feeding modes of mongooses. Additionally, a toxicant registration evaluation was recently produced for mongooses in Hawaii by WS-NWRC (Ruell et al. 2019). The results of this review indicate that sodium nitrite, PAPP, diphacinone, and bromethalin all have potential to be registered as toxicants for mongoose control for use in bait stations if suitable toxicant/bait matrix combinations can be identified, with a diphacinone bait being the least expensive and fastest to register. A diphacinone bait could also potentially be registered for limited uses outside of bait stations. Development of an effective mongoose bait will require a softer, palatable matrix that can be paired with an effective toxicant.

## OBJECTIVE

In this pilot phase of mongoose toxic bait development, we evaluated bait acceptance of selected nontoxic bait matrices for mongooses, a necessary first step before incorporating toxicants. By identifying potential nontoxic bait matrices that are palatable to mongooses and ruling out those that are not, we ultimately minimized the number of trials, and thus animals, necessary to conduct subsequent palatability trials involving various combinations of bait matrices and toxicants. The objective of this pilot phase is to simply gauge which of the candidate matrices have adequate bait acceptance rate (i.e., are consumed in sufficient amounts) to warrant future consideration as a toxicant matrix.



**Figure 1. Camera field of view, with the test bait (nontoxic FOXECUTE®) pinned on the bait station wire bar and dog kibble challenge diet within the tray underneath.**

We assessed acceptability and consumption of four nontoxic versions of the following bait matrices (Figure 1):

- 1) nontoxic FOXECUTE® and FOXSHIELD® are semi-soft blocks of meat- and fish-flavored bait,

- respectively, produced by Animal Control Technologies, Australia (ACTA). Commercial versions in Australia have a sausage-like casing and are formulated with PAPP for invasive fox control;
- 2) nontoxic HOGGONE® (ACTA) is a peanut paste-based bait. A 10% SN version of HOGGONE was recently registered in Australia for control of feral swine. A modified HOGGONE formulated with 5% SN is currently in development for feral swine control in the U.S.;
- 3) ‘BTS bait’ is a processed pork shoulder loaf formulated with synthetic lipids mimicking the scent profile of dead mice. This product was developed by WS-NWRC as a cost-effective alternative to dead newborn mice as a vehicle to deliver acetaminophen to invasive brown treesnakes.

## METHODS

### Mongoose Capture

Wild small Indian mongooses were trapped in Hilo, Hawaii and surrounding areas, and transported to and individually housed in the WS-NWRC research facility per standard internal protocols (SOP AC 005.00). Upon arrival, sex and body mass were recorded for each animal.

Animals were dusted for ectoparasites with Drione® (1.0% pyrethrin) before entering the test facility. A bellows duster was used to lightly coat the nape and dorsal areas of the mongooses, avoiding the eyes, nose, and mouth, while still in the trap.

Any animals with injuries, sustained aggressive behavior, or poor body condition (pelage mange, worn or missing teeth) were immediately euthanized by carbon dioxide inhalation (SOP AC/HI 002.01). Twenty-four animals were used, including three of each sex for each of the four nontoxic bait matrices trialed. An additional 4-6 mongooses were housed as spare animals to replace animals deemed unfit for inclusion in trials. We randomly assigned mongooses to test groups while ensuring a relatively equal sex ratio within each group.

### Housing

Mongooses were held in stainless steel rabbit cages (Allentown Caging Equipment Co., Inc., Allentown, NJ), with each individual cage measuring 42 cm tall × 61 cm wide × 64 cm deep (Figure 3) which allowed the full range of natural movement. Mongooses had ad libitum access to water in ball-stoppered bottles attached to the front of the cage at all times throughout all phases.

### Acclimation and Conditioning Phase

Mongooses were subject to an acclimation period of 5-7 days prior to feeding trials. The test room was maintained at 24-25°C and 12:12 h light:dark cycle during the trials. For the first 48 hours of captivity, mongooses had ad libitum access to a maintenance diet (dry cat food pellets) until they exhibited consumption; animals that did not consume cat food during this window were not included in the study. Once they began consuming the maintenance diet, mongooses were conditioned to receiving access to their daily ration, within a limited time window each morning (4 hours; 0800-1200 h) to simulate infrequent food item encounters in the field, such as natural prey or

baits in bait stations. This limited window for consumption is also important for judging whether a bait is a suitable matrix for SN or PAPP, because their modes of action require consuming enough of the toxicant over a short enough window to achieve a lethal effect. Food was provided in the morning, while cage cleaning and maintenance occurred in the afternoon to minimize stress while food was available.

To mimic the presentation of toxic bait in the field and to prevent spillage from falling through the grated cage floor, we used Protecta LP<sup>®</sup> bait stations (Bell Laboratories, Inc., Madison, WI) as feed trays for all phases of this study (Figure 1). We modified bait stations by removing the top cover to allow for monitoring of consumption by video recording.

### **Trial Phase**

We evaluated acceptance and consumption of nontoxic bait matrices via two-choice feeding trials. Test baits were provided along with an equal amount, by mass, of dry dog kibble (Doggy Bag<sup>™</sup>) challenge diet (different than the dry cat kibble maintenance offered during the acclimation phase). To mimic bait block presentation in bait stations, we secured the nontoxic FOXSHIELD·FOXECUTE, and BTS bait within bait stations on the wire rods provided with the commercially available rodenticide bait stations (Figure 1); these rods are intended to prevent removal of the bait block from the bait station. HOGGONE, a paste, was placed on the bait station floor in the tray area intended for loose baits (e.g., pellets). The dry dog kibble challenge diet was also offered in the floor tray directly beneath the rod-mounted baits or beside the paste bait. For each trial, we offered 70 g each of test and challenge diet at the same time. We estimated 70 g as the upper range of what we would expect could be consumed by a mongoose in a single feeding. We conducted each trial in the morning, with baits available for the same 4-hour window allowed during the acclimation period, approximately 0800 to 1200. After each exposure period, we removed the bait stations and test baits. We weighed any uneaten or spilled test or challenge diet remaining in the bait station or on the cage floor or excreta collection tray to assess consumption.

Due to variation in humidity levels in the animal testing room, both the test and challenge diets were expected to gain or lose small amounts of moisture each day during the exposure period. Therefore, two samples each diet were weighed and placed in empty mongoose cages similar to those used for the trials. The moisture control samples were exposed to the same environmental conditions in the same room as the test animals during the exposure period, and were weighed at the same time as the food remaining after the exposure period. The weights of diets offered each day were then adjusted by multiplying a correction factor calculated as the final weight of the environmental control sample divided by the initial weight. The corrected amount offered at the start of the exposure period was used to calculate amount eaten from each feeder (i.e., amount eaten = corrected amount offered minus amount remaining).

We repeated feeding trials, using the same test diet for each treatment group, for 5 days. If any animal exhibited

signs of lethargy and/or illness or was not consuming any food during the trial phase, that animal was offered small amounts of raw chicken pieces as a diet supplement. If any animal continued to show signs of inappetence or distress, it was euthanized and not replaced.

The order of treatment group trials was randomized, with nontoxic FOXSHIELD and HOGGONE trials commencing 29 April 2019; nontoxic FOXECUTE commencing 6 May 2019; and nontoxic BTS bait on 13 May 2019.

### **Consumption Rate Monitoring**

We monitored frequency and duration of feeding events by video recording using GoPro<sup>®</sup> cameras (Hero 5 Black and Hero 7 Silver models; San Mateo, CA). We mounted cameras approximately 23-30 cm directly above the bait on a flat aluminum bar secured to the vertical rear wall of the bait station. From this perspective, the cameras could capture the full view of the test bait and challenge diet and visitation/sampling by the mongoose (Figure 1). To accustom mongooses to the presence of cameras during the trial phase, we painted wooden blocks black to mimic cameras and mounted them in the same position during the acclimation phase. Because of battery capacity limitations, the Hero 5 Black models did not capture the entirety of each feeding period and were used to record only the nontoxic HOGGONE feeding trials.

We analyzed videos of each feeding trial and recorded the duration of each feeding event and visually estimated the amount of bait matrix that was consumed during each event. Videos were recorded at two frames/sec and rendered at 29 frames/sec. We calculated the real-time duration of each feeding event using the formula  $((x*29)/2)$ , where  $x$  = video duration of feeding event in seconds. We visually estimated the amount of bait matrix consumed during any given feeding event as a percentage of the total mass that was offered. We obtained the actual total mass eaten by weighing the remaining diet at the end of the exposure period. We used the estimated percentages eaten from observations and the measured total consumption to estimate the mass of bait eaten during each feeding event.

## **RESULTS**

Acceptance and consumption of all test baits was high. All baits were very highly preferred over the dry dog kibble challenge diet, with many mongooses consuming none of the dry dog kibble on most days. Daily and average consumption of test material and challenge diet are tabulated in Siers et al. (2020).

Consumption rates estimated from video observations are depicted in Figures 2-5. These represent the maximum amount of the bait matrix that was consumed during any 30- or 60-minute sliding window of time throughout each 4-hour feeding session. The entire amount consumed during the feeding session is also depicted. The dosage of active ingredient consumed during any such period can be estimated from the amount of matrix consumed, the concentration of the toxicant in the matrix, and the mass of the mongoose.

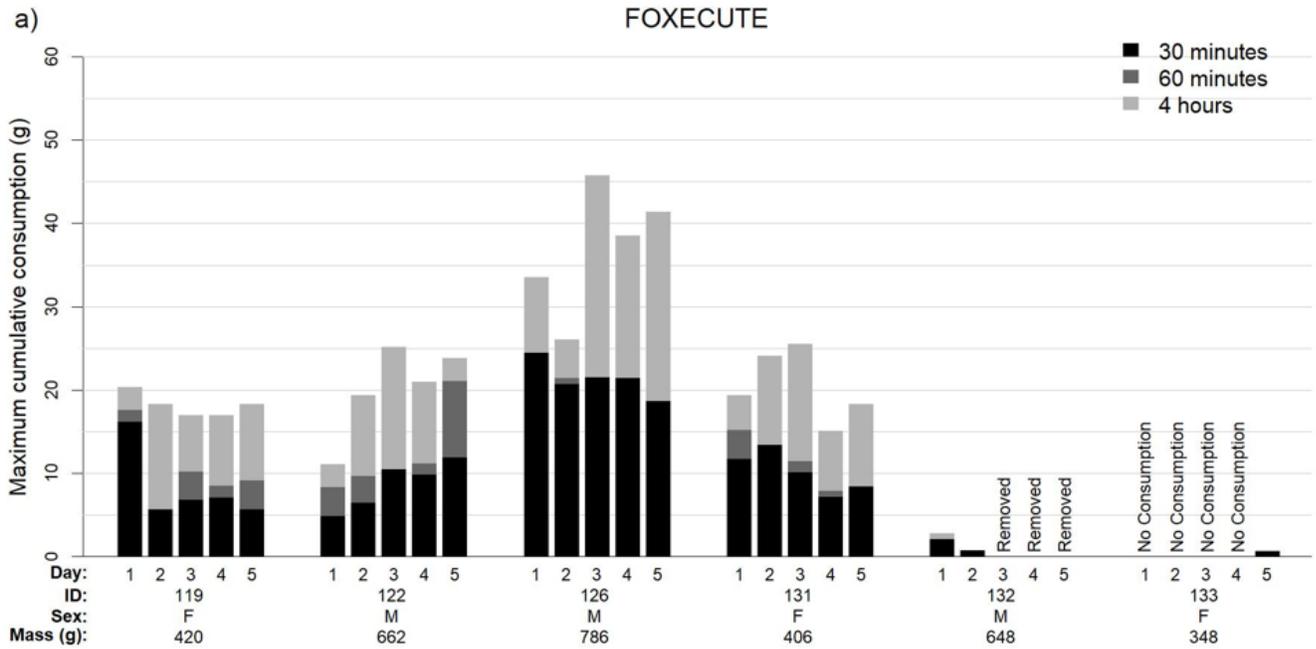


Figure 2. Time-bound consumption rates of nontoxic FOXECUTE estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

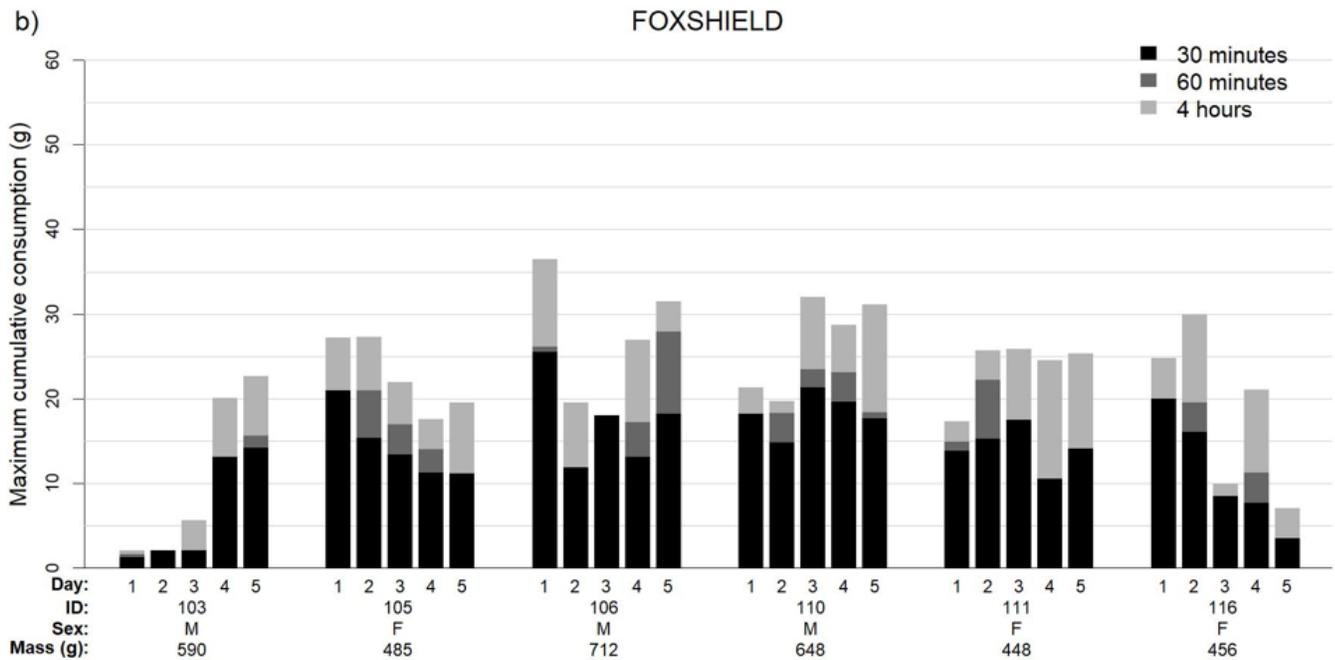


Figure 3. Time-bound consumption rates of nontoxic FOXSHIELD estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

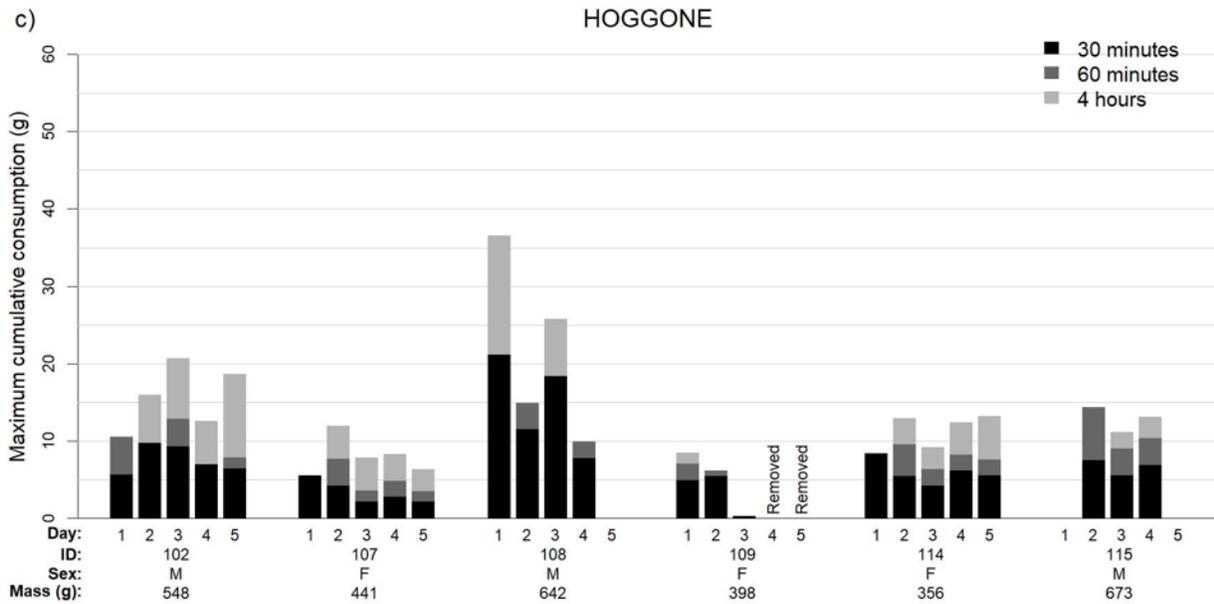


Figure 4. Time-bound consumption rates of nontoxic HOGGONE estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

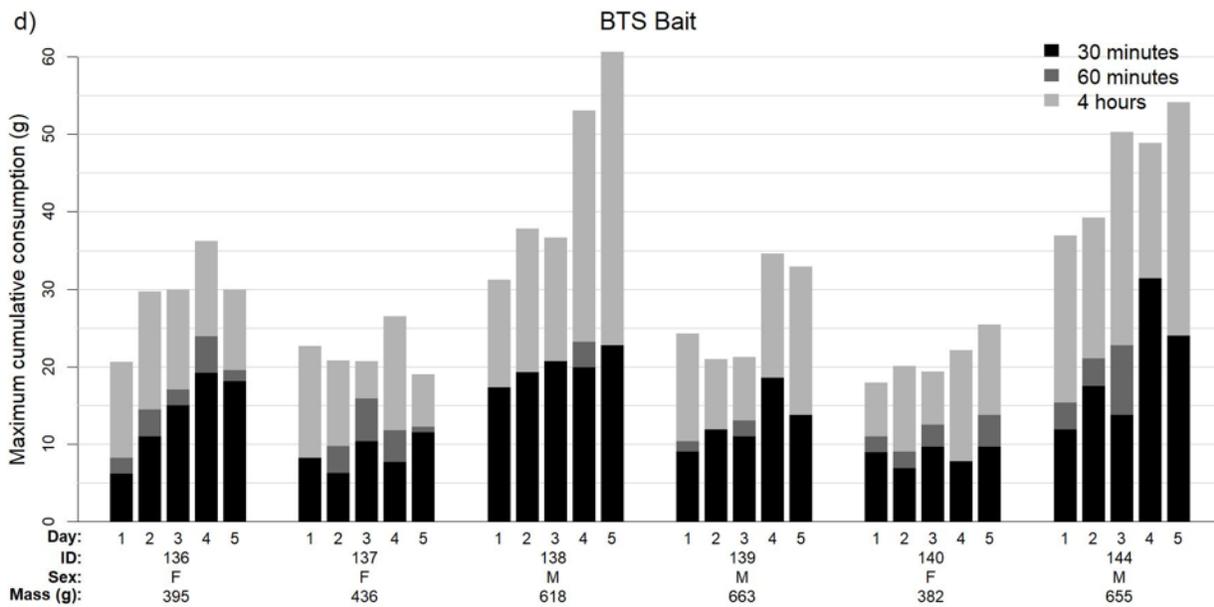


Figure 5. Time-bound consumption rates of nontoxic BTS bait estimated from video observations. Values for 30 and 60 minutes represent the maximum amount consumed during a sliding window of the respective time period. The 4-hour value is the total consumption during the feeding trial.

## DISCUSSION

Two mongooses, 1M:1F, were removed from the study due to prolonged failure to feed on either diet offered. For what little they did eat, both preferred their test diet (nontoxic FOXECUTE and HOGGONE) over the challenge diet. Given the reliable consumption by others in their treatment groups, we believe that their failure to thrive was independent of the treatment and likely due to physiological or psychological factors, and should not

reflect poorly on the suitability of the bait matrix.

Of the 24 test animals, only one, a female in the nontoxic FOXECUTE treatment group, preferred the dry dog kibble challenge diet and ate almost no treatment diet. Preference ratios of the other animals in the same test group ranged from 18:1 to 115:1, indicating this individual as an outlier. Again, it appears unlikely that this anomaly indicated reduced suitability of nontoxic FOXECUTE as a bait matrix.

Excluding these three outliers, average daily consumption of baits, ranked from highest to lowest, were: nontoxic BTS bait ( $31\text{g} \pm 11.75\text{ SD}$ ) > nontoxic FOXECUTE ( $24\text{g} \pm 13.01$ ) > nontoxic FOXSHIELD ( $22\text{g} \pm 8.63$ ) > nontoxic HOGGONE ( $15\text{g} \pm 7.40$ ). The highest exceeded the lowest by a factor of two.

Our results indicate that we are in the fortunate circumstance of having several bait matrix options that are palatable to wild-caught mongooses. The selection of a bait matrix for formulation in a registered product will likely be on the basis of other characteristics such as longevity in the field, compatibility with the selected toxicant, and ease of manufacture, storage, and use. The four candidate toxicants for pairing with a preferred bait matrix are diphacinone, bromethalin, SN, and PAPP (Ruell et al. 2019). Below we discuss our results in light of other matrix and toxicant characteristics:

### **Nontoxic FOXECUTE and FOXSHIELD**

Both products performed well in feeding trials. Nontoxic FOXECUTE was preferred to the dog kibble by a factor of 46, while the preference ratio for nontoxic FOXSHIELD was inestimable in that four of the six mongooses in the treatment group ate no challenge diet and fed exclusively on FOXSHIELD. However, average daily consumption of FOXECUTE was slightly higher, though not likely significantly, than FOXSHIELD. FOXECUTE is meat flavored, while FOXSHIELD is fish flavored. Fish products (sardines, oils) are routinely used as mongoose trap baits and lures and have been shown to be very attractive to mongooses, with extended attractiveness to lure mongooses from afar. Due to regulation of importation of animal products into the United States from Australia, the fish-flavored FOXSHIELD would likely have a lower barrier to importation. Although both products will require import permits from USDA APHIS Veterinary Services, the import of FOXECUTE for commercial distribution and use would likely be subject to additional livestock disease status certification requirements. Both baits are commercially formulated in Australia with PAPP as the active ingredient. There are no registered PAPP pesticide products in the United States and the barriers to registration are the highest of the candidate toxicants we consider (Ruell et al. 2019). These baits are not likely to be easy to formulate with SN, because of their moisture content and the current inability to reliably microencapsulate SN. Current microencapsulation formulations quickly degrade when exposed to moisture, exposing the sodium (causing high saltiness) and causing the release of noxious nitric oxides. The manufacturer (ACTA) does not currently formulate any products containing diphacinone or bromethalin. It is currently undetermined whether ACTA would invest in the equipment and regulatory approvals required to incorporate new toxicants into these matrices for a relatively niche application like mongooses. Thus, a second manufacturing step in the U.S. may be required. As for field usability, FOXECUTE and FOXSHIELD are currently in field use for fox control in Australia, and are formed in easily-handled discrete units and likely have favorable storage and longevity characteristics that would make them highly suitable as a matrix for a mongoose bait.

### **Nontoxic HOGGONE**

Although preferred over dry dog kibble by a factor of 33, nontoxic HOGGONE had the lowest average daily consumption at 15 g. This might not be surprising: while the other baits are meat based or flavored formulations designed for carnivores, HOGGONE is based on peanut and cereal products which would probably be considered less attractive to a carnivorous mammal. Typically formulated with SN for feral swine control, the amount and rate of consumption are important in achieving sufficient circulating levels of toxicant to achieve lethal intoxication before being metabolized out of the system. Nontoxic HOGGONE had some of the lowest time-bound and overall consumption rates, suggesting that mongooses would be somewhat less likely to achieve a sufficient circulating dosage to affect lethal intoxication than with other products. This could potentially be overcome by a higher concentration of toxicant in the matrix. Although SN is not an active ingredient in any registered pesticides in the U.S., USDA and collaborators have generated or contracted all of the registration data required for registration of SN as part of the development of HOGGONE as a toxic bait for feral swine (Ruell et al. 2019). If HOGGONE is registered in the U.S. for feral swine, it could be relatively easy to register the same formulation for mongooses. As a matter of practicality, HOGGONE presented the lowest ease of use in our trials. Being a paste, residues were fairly resistant to easy cleaning of bait stations. Reliable formulation of HOGGONE is troubled by the same SN encapsulation difficulties as mentioned above. Likewise, as an ACTA product, availability of the HOGGONE paste matrix formulated with diphacinone or bromethalin is questionable and may require a secondary manufacturing step in the U.S.

### **BTS Bait**

In our trials, mongoose consumed the WS-NWRC pork loaf with artificial mouse carrion scent most reliably and copiously at an average daily consumption of >30 g. The intent of the mouse scent is to act as an attractant to draw the nuisance predator to the bait; it has not yet been evaluated whether the mouse scent affects palatability to mongooses. It is clear that palatability with the scent is not an issue, and future determinations of whether to incur the additional expense of the mouse scent will depend on whether the scent draws mongooses to the bait stations from further away. This bait matrix is currently experimental and being manufactured in small batches at the WS-NWRC Hawaii Field Station in Hilo. Manufacture involves grinding and mixing of pork shoulder and other constituents, then sealing and cooking loaves within a foil pouch. As prepared, pouches of bait are shelf-stable. Field stability has not yet been evaluated, though studies are underway. As currently produced, convenience of use in the field may not be optimal because the pork loaf, of a consistency very similar to the SPAM<sup>®</sup> (Hormel Foods Corporation, Austin, MN) potted meat product, must be removed from the pouch and manually cut into shapes and amounts suitable for deployment in bait stations. Slightly wet with free-form fats and extruded scent lipids, frequent cleaning of hands and equipment will be required. If adopted as a mongoose bait matrix, the manufacturing

process for the scented pork product may be adapted to produce sausage forms that would improve the ease of use. A major advantage is that this product requires no special equipment not available for commercial kitchens. Currently formulated in-house at WS-NWRC, we would be at liberty to incorporate any registered technical material as an active ingredient, provided that the Hawaii facility became registered as a pesticide-producing establishment and that the end product was registered as a pesticide. Beyond very small batches, manufacture could be transferred to the WS Pocatello Supply Depot, the primary WS facility for manufacturing and providing specialized wild-life damage management pesticides and other products that are not readily available from commercial sources.

Video monitoring of bait consumption provided additional insight into rates of consumption that would not have been available from only measuring remaining bait after the entire feeding period. This rate of consumption is particularly important with active ingredients that must be ingested in a large bolus because they metabolize quickly, such as PAPP and SN. Our results will be useful in evaluating the potential for lethal intoxication with one of these toxicants. Actual dosage would be a function of the feeding rate, the concentration of toxicant in the matrix, and the mass of the animal consuming the bait.

As a final usage note, the purpose of the pins or rods in a bait station are to prevent entire pesticide blocks from being removed from the bait station where they are exposed to consumption by nontarget species and are no longer available to other target species visiting the bait station. When the bait is suspended on horizontal rods, mongooses will consume the top surface of the bait; as more bait is consumed, the rod is exposed and the weight of unconsumed bait will keep the bait mass below the rod, which may cause the bait to sag and fall off, leaving a large portion of the bait free to be carried off. We recommend that future bait station designs maintain blocks on vertical retainer rods, reducing the tendency of the mass of bait to remain in a position less accessible for feeding and to fall off of the rod in large quantities.

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