ASSESSMENT OF LEOPARD TORTOISE (*GEOCHELONE PARDALIS*) SCENT PREFERENCE

Kimberly R. Douglass, Cassandra R. Lockhart, BS* and Mark S. Edwards, PhD

California Polytechnic State University, Animal Science Department, San Luis Obispo CA 93407-0255 USA

Abstract

It is suggested tortoises are attracted to certain ingredient scents. The preference for scent of ginger, anise, rose, and a control (water) among leopard tortoises was evaluated. Sixteen leopard tortoises (*Geochelone pardalis*) participated in a series of trials exposing them to pair-wise combinations of the three scents and control. Response criteria evaluated include position (left, right, no decision) and scent selection. Tortoises selected all scents, including the control, in the right position more than making no decision ($P < 0.05$). There was a trend for individuals to select all scents, including the control, in the left position more than no decision ($P = 0.0592$). This suggests animal movement was not random. There was no significant difference between selections made in left and right positions ($P > 0.05$). The percentage of times each scent was chosen across all treatment combinations did not differ significantly from fifty percent ($P < 0.05$). These results suggest there is no preference for ginger, anise, rose scents over each other or a control (water) among leopard tortoises.

Introduction

Tortoises are believed to have an excellent sense of smell, but only across short distances.[3] Their olfactory sense is used when searching for food, recognizing the presence of conspecifics, identifying territory, or selecting nesting sites.[2] Pet turtles have been noted to smell their food before biting or rejecting it.[3] One study discovered box turtles were able to discriminate between two differing food sources based on olfactory senses.[3] Another study demonstrated omnivorous lacertid lizards displayed a preference for overturned cups hiding a piece of apricot over the control, suggesting lizards use airborne odor cues to locate food.[4]

Tortoises are typically herbivorous, consuming a variety of leaves, stems, flowers, and fruits.[1] Many commercially available tortoise diets include ingredients, often in the form of an extract, that exude distinct and strong scents, such as rosemary, garlic, ginger, rose, horseradish, anise, and juniper. Such ingredients may be included, at a significant cost to the manufacturer, to: stimulate animal food acceptance, improved animal food intake, or producing organoleptic qualities appealing to human caregivers, thus promoting continued diet use.

The objective of this study was to evaluate if leopard tortoises (*Geochelone pardalis*) express preference of ginger, anise, rose scent extracts, and a control (water).

Methods

Sixteen five-year-old leopard tortoises (*Geochelone pardalis*), randomly selected from the California Polytechnic State University leopard tortoise colony, were included in this study. Animal husbandry, diets and enclosures are described elsewhere.[5] Use of the animals indicated in this study, protocol #903, has been reviewed and approved by the California Polytechnic State University Institutional Animal Care and Use Committee.

Three liquid extracts were evaluated: anise, ginger, and rose (Star Kay White, Inc., Congers NY, 10920). Drinking water was used as a control. Separate syringes were used to dispense 5 ml of each treatment or control into a treatment dedicated container made of a 3" PVC drain cover and pipe cap. Tortoises were exposed to empty PVC containers in their enclosures 2 weeks prior to the start of testing.

Trials were conducted in a 189.3 L structural foam stock tank (Model #4243, Rubbermaid Commercial Products LLC, Winchester VA, 22601). The tank floor was marked with masking tape to designate fixed subject starting and scent container positions (Figure 1).

Sixteen scent-location treatments were tested: six scent pair combinations: ginger/anise, ginger/ginger, ginger/rose, ginger/control, anise/anise, anise/rose, anise/control, rose/rose, rose/control and control/control, and two scent positions: left or right. Tortoises were randomly assigned to one of sixteen treatment orders. Each animal was tested with the appropriate treatment on testing days. Two hundred fifty six trials were conducted throughout the experiment.

On the day of a preference trial, the test area was set up with all materials except scent containers. For each trial, 5 ml of liquid was placed in each container. Containers were placed in the testing area with the drain cap grating parallel to the long sides of the tub. Before initiating the trial, 1 minute elapsed, allowing the scents to disperse.

The designated tortoise was placed on the starting position (time 0 minutes). Time was started and each individual was allowed 10 minutes to make a "decision". A tortoise was considered to have chosen a particular scent if its nose passed the line at the end of the tub where the scent was placed (Figure 1). If the tortoise's nose did not enter either scent's zone within 10 minutes from time 0, the result was recorded as "no decision".

Upon completion of an individual trial, the tortoise was transferred back to its enclosure. Scent containers were removed and rinsed with tap water and dried in preparation for the following trials. Any materials, including feces or urine, that were introduced to the test area by the study subject were removed and the area cleaned with potable water.

A sign test of median was used to analyze the position data to determine if tortoises were randomly moving within the test area or actively moving towards the scents. Scent choice data was analyzed by testing for a population proportion. Tests resulting in "no decision" were not included in analyses of side and scent preference as they had no bearing on the subject. For all statistical analyses, significance was set at ($P < 0.05$).

Results and discussion

The number of times tortoises selected a scent in the right zone, left zone or no decision across the entire study is summarized in Figure 2. Using a one sample sign test, a true population median of the differences between left or right and no decision equal to 0 would indicate tortoises were randomly moving within the test area. A true population median of the differences significantly higher than 0, would indicate tortoises were actively moving to the left or right. The population median of the differences was significantly higher than 0 for the right zone when subtracting no decision ($P < 0.05$) and only a trend for the left zone when subtracting no decision ($P = 0.0592$). There is evidence to show tortoise movement was not random within the test area, but animals were actively moving into the left and right zones (i.e., actively participating in the trial).

By testing for a population proportion, the number of times scents in the left and right zones were chosen did not differ significantly from fifty percent ($P > 0.05$). There is no reasonable evidence to support a preference of scent position; therefore, further analysis excludes position.

The total number of times each scent was selected across individuals independent of location is provided in Figure 3. By testing for a population proportion, the number of times each scent was chosen in all of the treatments did not differ significantly from fifty percent (Figure 4) ($P > 0.05$). There is no evidence to support the subjects in this study expressed a preference between extracts of ginger, anise, rose, or the control (water).

The lack of an observed preference of *G. pardalis* for a particular scent in this study may suggest extracts do not increase food acceptance or intake. Preferences for a scent extract may be more accurately evaluated by offering *G. pardalis* all extracts at the same time.

Factors that may have contributed to these results include weather, management, motion, noise disturbance and animal health. Variations in temperature and air movement may have disrupted the tortoises' decision-making process; ambient temperature should be controlled during future testing. Although testing was to occur prior to feeding, on some occasions the tortoises were accidentally fed prior to testing. However, none of the animals were in the process of eating when removed from their enclosures for testing. Animal caretakers working around the testing area during the study may have caused some motion or noise disturbances.

Conclusions

1. Leopard tortoises were actively participating in the preference trial, moving to the left and right scent zones in the test area.
2. Leopard tortoises had no preference for the left or right scent zones (position).
3. Leopard tortoises had no preference among scents of ginger, anise, rose, or a control (water).

Acknowledgements

The authors wish to express their sincere appreciation to the following: the Smithsonian National Zoological Park for donation of animals to the California Polytechnic State University teaching

and research program; PMI Nutrition International / Mazuri® for their contributions supporting the California Polytechnic State University Comparative Animal Nutrition program; Dr. Jeffrey Sklar, California Polytechnic State University Statistics Department for assistance with data analysis, and the California Polytechnic State University Animal Science students that provide daily expert animal care.

LITERATURE CITED

1. Boycott, R.C. 1988. The South African Tortoise Book. Southern Book Publishers (Pty) Ltd., Johannesburg.
2. Ferri, V. 1999. Turtles & Tortoises. Firefly Books Ltd., Willowdale, Ont.
3. Fitch, A.V. 1965. Sensory Cues in the Feeding of the Ornate Box Turtle. Transactions of the Kansas Academy of Science 68 (4): 522-532.
4. Cooper, W.E. and Valentín, P. 2001. Location of fruit using only airborne odor cues by a lizard. Physiology & Behavior 74: 339– 342.
5. Higgins, C. and Edwards, M.S. 2009. Application of allometric field metabolic rate equations to predict energy and food requirements of leopard tortoises (*Geochelone pardalis*). Proceedings of the American Zoo and Aquarium Association Nutrition Advisory Group. Tulsa OK.

Figure 1. Structural foam stock tank used as testing arena for scent preference evaluation by leopard tortoises (*Geochelone pardalis*). Positions marked in white on tank floor were indicated to facilitate repetitive placement of scent containers and the test subject. Other labels and markings were added to the image for descriptive purposes. Initial orientation of test subject is indicated by the small arrow (center); diagonally hatched areas (ends) indicate zones into which an animal would move to indicate a selection had been made.

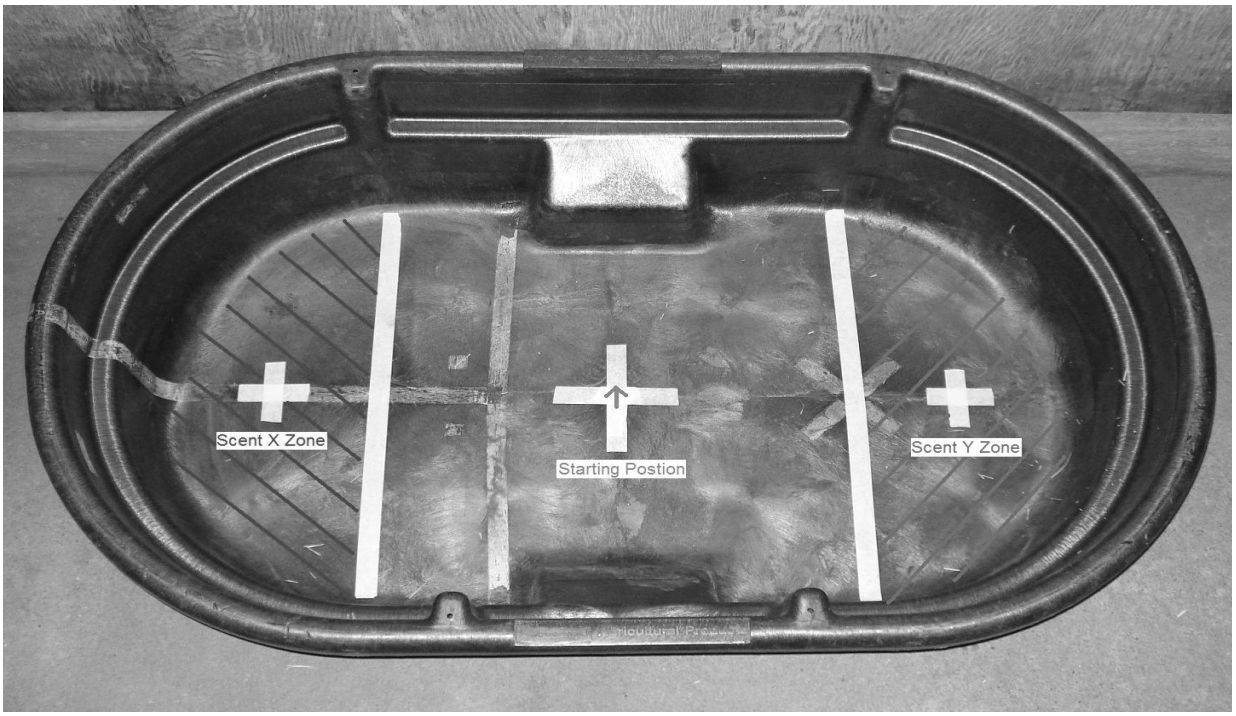

Figure 2. Frequency leopard tortoises (*Geochelone pardalis*) chose to move to the right and left ends of a given test area (zones), and frequency they remained in the middle (no decisions) (n = 256).

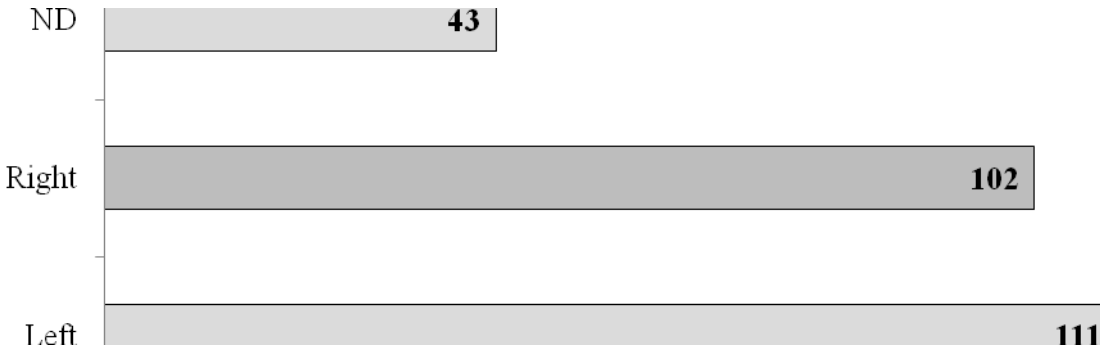

Figure 3. Frequency of times leopard tortoises (*Geochelone pardalis*) chose each scent-ginger, anise, rose, and the control (water); 213 total choices.

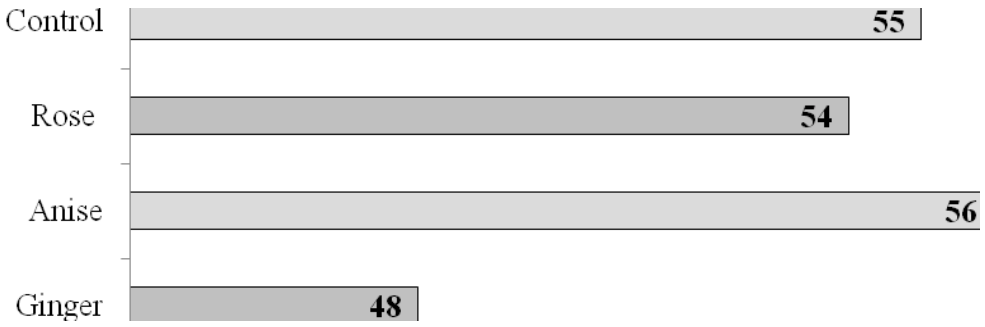

Figure 4. Frequency of times leopard tortoises (*Geochelone pardalis*) chose each scent- ginger, anise, rose, and the control (water) for all possible two-scent combinations (treatments).