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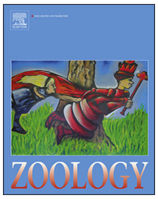

Go with your gut: Digestibility and digestive function of two arid-zone Australian murids, *Pseudomys australis* and *Notomys alexis*

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ABSTRACT

Spinifex hopping-mice (*Notomys alexis*) and plains mice (*Pseudomys australis*) are able to successfully occupy arid zones of Australia. We studied the digestive parameters and energy assimilation of captive spinifex hopping-mice and plains mice. The experiment consisted of six diets fed to the animals for periods of 12 days per food type. On a dry matter basis, the plains mice consumed between 2.5 and 7.2% and the hopping-mice between 5.8 and 9.3% of their body mass in food per day. The body mass of the spinifex hopping-mice increased significantly on the sunflower seed diet, while body mass did not change significantly for the plains mice on any diet. Apparent digestibility of macronutrients was similar in the hopping-mice and plains mice when maintained on the same diet, however digestibility of total micronutrients differed. Maintenance energy requirements for the plains mice were 529 kJ kg$^{-0.75}$ d^{-1} and spinifex hopping-mice 550 kJ kg$^{-0.75}$ d^{-1}. Spinifex hopping-mice and plains mice are able to exploit a range of food items and efficiently digest macronutrients, to ensure they meet their nutritional needs, an ability they require in the variable arid environment. The information gained in this study increases the paucity of information on Australian native murids, specifically their digestive function and energy requirements, and will aid captive murid management. The study will allow future expansion into field studies, to aid the conservation of wild rodent diets and nutrition of arid zone murids.

1. Introduction

Rodents have adaptive digestive strategies they utilise to survive in a wide range of environments and in particular desert ecosystems. Cricetidae rodents, such as Norway lemmings (*Lemmus lemmus*), have specialised caecum-colon structures that efficiently absorb water, sodium and potassium (Lange and Staaland, 1970). The specialised structure is an adaptation to cope with a low mineral diet (Lange and Staaland, 1970). Other desert rodents have an elongated colon indicating a high capacity for water reabsorption (Murray et al., 1995; Hagen et al., 2015), and some have the ability to produce concentrated urine to conserve water (Weisser et al., 1970; Gordge and Roberts, 2008). Desert rodents have comparatively low basal metabolic rates and energy requirements than non-desert rodents (Degen, 1998; Arends and McNab, 2001).

Consuming an omnivorous diet enables rodents to spend less time foraging, while those with specialised diets (granivores and insectivores) are adapted to efficiently digest their foods but spend more time foraging (Schreiber, 1979). Desert species in the family Muridae (murids), such as the Australian *Notomys* spp. and *Pseudomys* spp., are opportunistic omnivores, consuming grasses, seeds and insects, dependent on seasonal availability (Watts, 1970; Murray et al., 1999). More recently Pavey et al. (2016) reported a large proportion of seed (> 55%), smaller volumes of other plant material, and occasional insects in the diet of plains mice (*Pseudomys australis*) throughout the year. The most commonly consumed plant species included the grasses *Dactyloctenium radulans*, *Chloris pectinata* and *Panicum* spp. and two forb species (*Boerhavia schomburgkiana*, *Amaranthus mitchellii*) (Pavey et al., 2016). By having an omnivorous as opposed to a herbivorous diet desert murids can maximise food resources to their advantage, enabling them to thrive in an unpredictable dry environment.

Little is known about the digestive efficiency of arid zone murid species and how nutrient intake is regulated in such an unpredictable environment. Food in the wild undergoes seasonal and temporal variation in availability. Digestibility of diets in captivity has been studied in various Cricetidae rodents, including voles (*Microtus* spp.), lemmings (*Lemmus* spp.), muskrats (*Ondatra zibethicus*) (Drożdż, 1968; Batzli and Cole, 1979; Schreiber, 1979; Campbell and MacArthur, 1994), and Hystricomorpha rodents (Veloso and Bozinovic, 1993; Bozinovic, 1995; Van Zyl et al., 1999). Few studies have been undertaken on diet

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digestibility in Sciuromorpha (Gębczyński et al., 1972; Schreiber, 1979) and Muridae rodents (Drożdż, 1968). Likewise captive and laboratory investigations of nutrition in Australian native murids are rare and little is known about their nutritional ecology in the wild, typically it is limited to diet choice (Watts, 1970; Carron et al., 1990; Murray et al., 1999; Pavey et al., 2016).

The spinifex hopping-mouse (*Notomys alexis*) and plains mouse are small (< 60 g) native Australian murids, and both opportunistic omnivores, consuming mainly plant material and insects when available (Watts 1970; Murray et al., 1999; Pavey et al. 2016), an adaptive strategy particularly useful to arid zone species. Spinifex hopping-mice are categorised as 'least concern' and are distributed in arid zones across central and western Australia (Breed and Ford, 2007; Moseby, 2016). The 'vulnerable' plains mouse occupies west and southwest regions of Lake Eyre (Breed and Ford, 2007; Burbidge and Woinarski, 2016), and live in burrows dug into hard clay, or in swampy/sandy ground (Brandle et al., 1999). Spinifex hopping-mice and plains mice are commonly held in captivity, in zoos and private collections. They are fed as omnivores, on diets of seeds, and/or vegetables, and these diets are made *ad lib* as there are no formal nutritional guidelines for feeding native Australian murids. Although diet choice has been studied in the wild (Watts, 1970; Murray et al., 1999), no nutrition studies have been undertaken on spinifex hopping-mice or plains mice.

This study was undertaken to increase our understanding of arid zone species biology and aid the conservation of two unique Australian murid species. We aimed to determine the nutritional composition of six food items commonly fed in captivity that replicate wild food items (seeds, nuts, vegetation and insects) to gain insights into their nutritional requirements, hence maximise nutrition, health, and fecundity in captivity, and thus species conservation. We also determined the different digestive parameters and energy assimilation of captive spinifex hopping-mice and plains mice by feeding a range of diets of differing nutritional values and by conducting opportunistic dissections. Specifically, we investigated 1) nutritional composition and digestibility of diets fed in captivity, 2) the nutritional and energy requirements of the two species, and 3) described their gastrointestinal morphology and correlated it to function.

2. Methods

2.1. Animals and housing

The animals used in the nutrition study were from captive colonies housed at Western Sydney University, Hawkesbury Campus. Nine plains mice (four male and five female) and a total of 18 spinifex-hopping mice (16 male and two female) were used. Due to a disease outbreak (Stannard et al., 2017), the spinifex hopping-mice ($n = 8$) used in the sweet potato (*Ipomoea batatas*) trial were different animals to those used in the sunflower seed (*Helianthus annuus*) and corn (*Zea saccharata*) trials. Animals were housed individually in plastic enclosures measuring 54 × 38 × 25 cm, with a section of mesh ventilation in the lid. Cardboard boxes with shredded paper and tubes were provided as housing and enrichment. Animals were held under reverse light cycles 12L:12D and ambient temperatures of 22 ± 3 °C.

2.2. Digestibility experiment

The animals were fed one food item *ad libitum* for twelve days, with the first five days an adjustment and on the following seven days total faecal and leftover food collection occurred. Animals were weighed on day one, five and twelve of the trial to assess changes in body condition. Samples of food, food refused and scats were collected separately and stored at −20 °C until later laboratory analysis. Food items included sweet potato, sunflower seeds, sweet corn kernels, spinach (*Spinacia oleracea*), raw peanuts (*Arachis hypogaea*) and mealworms (*Tenebrio molitor*). Between each food trial animals were returned to their usual captive diet for five days. The usual diet consists of a range of vegetables including silverbeet (*Beta vulgaris*), kale (*Brassica oleracea*), sweet potato, carrot (*Daucus carota*), and broccoli (*Brassica oleracea*), as well as standard rat/mouse pellets and seeds (a mix of millet, wheat, corn and sunflower seeds).

2.3. Nutrient analysis

Food samples, leftover food and scat samples were oven dried to a constant mass prior to analysis and each sample was then ground into a homogenous mixture for further analysis. Ash content was determined using a muffle furnace set to 500 °C for 24 h. Energy composition was determined using an oxygen bomb calorimeter (Parr 6200, Parr Instrument Company, Moline, IL). Nitrogen and protein (protein = N x 6.25) were determined using the Kjeldahl method (Jones, 1991). Lipids were measured by extracting with chloroform/methanol with a 1:1, v/v mixture (Folch et al., 1957). Acid detergent fibre was determined using the AOAC method 973.18 (AOAC, 1990). NFE was calculated as 100-protien-lipids-ash-acid detergent fibre.

2.4. Statistical analysis

Body mass changes were statistically analysed using a repeated measures ANOVA for the plains mice. For the spinifex hopping-mice a repeated measures ANOVA was performed for each trial separately (due to different animals/numbers being used in each trial). Apparent total tract digestibility was calculated by subtracting the nutrient excreted (N_{out}) from the nutrient intake (N_{in}) and expressing it as a percentage of nutrient intake (Robbins, 1983). Digestible energy intake (DEI) was calculated on a metabolic weight basis by scaling intake against body mass to the power of 0.75 to enable comparisons with published data on small mammals. DEI was graphed over the change in body mass (%) to determine maintenance energy requirements as a zero change in body mass (y-axis intercept). One-way ANOVAs were used to determine statistical difference of macronutrient intake and digestibility between the two species when maintained on the same diet.

2.5. Gastrointestinal morphology

Opportunistic gastrointestinal samples were collected from nine plains mice and four spinifex hopping-mice. These animals were not used in the nutrition trial and sourced from another captive colony. Prior to their death animals were housed at the Alice Springs Desert Park, Alice Springs, Northern Territory, Australia. They were fed a variety of seeds and vegetables as well as Barastoc mouse breeder cubes (Ridley Agriproducts, SA) and water *ad libitum*. Animals were routinely treated with anti-parasitic treatments to prevent ectoparasite and endoparasite infestation and were apparently healthy at the time of death, being of good body mass and well hydrated. Animals were euthanased as part of routine husbandry in the captive colony.

Immediately after euthanasia the whole bodies were immersed in 10% neutral buffered formalin. The carcasses were fully opened to ensure complete penetration of fixative into the internal organs. Upon arrival at Macquarie University, North Ryde, NSW, Australia, tissues were dissected from the fixed bodies. Gut length from the stomach to caecum, and the caecum to the anus was measured without stretching for all specimens, and size of the caeca were measured using Vernier callipers.

3. Results

3.1. Nutritional composition and digestibility of diets

Sunflower seed and peanuts had the highest dry matter composition and spinach had the lowest at 9.5% (Table 1). Total mineral (ash) composition was the highest in spinach, 12.5% and below 4% in all other trial diets. Total energy was the highest in the peanut diet that also had the highest lipid composition (Table 1).