

Research article

Effect of post-gut loading time on the macro-nutrient content of three feeder invertebrate species

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Abstract

Techniques for increasing the nutritional value of feeder invertebrates include dusting the prey items with a vitamin and mineral powder supplement, or providing the invertebrates with nutrient-rich food, called gut loading, prior to feeding to insectivores. However, the delay between gut loading of prey and consumption by insectivores varies according to feeding regime and may be considerable if prey is left in the vivarium. This study aimed to determine the effect of time post gut loading on the nutritional composition of three species of invertebrates, mealworms (*Tenebrio molitor* larvae, approximately 23–30 mm), giant African land snails (*Achatina fulica*, shell size 10–35 mm) and dubia cockroaches (*Blattella germanica*, approximately 20–30 mm). Feeder invertebrates were maintained on a dry commercial gut-loading diet, supplemented with fresh vegetables for 48 hours, after which the diet was removed. Samples of invertebrates (120 g of mealworms, 80 g of snails and 100 g of cockroaches) were taken for proximate analysis (dry matter, ash, crude fat and crude protein) at 0.5 h (excluding cockroaches), 1 h, 6 h and 12 h (excluding snails) using standard laboratory procedures. A one-way analysis of variance was performed to test for differences in nutrient content between time points in each species. Crude protein and fat contents of snails were significantly greater at 6 h compared to 0 h ($P < 0.01$). However, no other significant differences in nutrient content of snails or other species were detected at any time points. It is feasible that the increases in crude protein and fat of snails were a result of nutrient accumulation within the body. These findings indicate that in the species tested, with the exception of snails, delays of up to 12 hours between gut loading of the prey and their consumption by a carnivore are unlikely to affect the concentrations of macro-nutrients (protein, fat, dry matter and ash).

Introduction

The term invertebrate applies to a number of taxa, from insects to molluscs and annelids, which make up to 80% of known species (Spevak and Dierenfeld 2000). Invertebrates are a staple component of many zoo-housed animals' diets, but in particular are essential sources of nutrition for insectivores (Hunt et al. 2001; McClements et al. 2003; Finke 2002, 2013).

For insectivores to be successfully managed, bred and rehabilitated in captivity, zoos and private owners must ensure that captive individuals receive sufficient nutrition from the limited commercially available feeder invertebrate species (Allen and Ullrey 2004; Finke and Winn 2004), from in-house production or harvesting free-living invertebrates.

The majority of feeder invertebrates available to captive facilities, such as house crickets (*Acheta domesticus*), locusts (*Locusta migratoria*) and mealworms (*Tenebrio molitor* larvae), are considered to provide sufficient water, protein, fat, carbohydrate and micro-minerals to meet the needs of the commonly held insectivorous species (Barker et al. 1998; Dierenfeld 1989). However, most feeder invertebrates are a poor source of select macro-minerals and vitamins, such as calcium and fat-soluble vitamins (A, D and E), consequently leading to deficiencies and nutritional disorders. Disorders frequently seen include metabolic bone disorder (MBD), malnutrition and hypovitaminosis A, D and E (Barker et al. 1998; Dierenfeld 1989; Klasing et al. 2000; Finke 2002, 2003; Hernandez-Divers 2006; Michaels et al. 2014). Nutritional deficiencies that are left

unaddressed can lead to muscular and neurological dysfunction, poor growth and poor reproductive success (Allen et al. 1993; Bernard et al. 1997; Anderson 2000; Finke 2002; Dierenfeld and Fidgett 2003; McWilliams 2005; Donoghue 2005; Vaughan and Browne 2009).

Nutritional disorders have not been documented in insectivores *in situ*. This has been attributed to the variety of invertebrate prey species available to free-ranging insectivores, the variability of the prey's diet, and the predator's ability to self-supplement (McClements et al. 2003; Finke 2003, 2013; Finke and Winn 2004; Ogilvy et al. 2012; Allen et al. 1993). Insectivores have been reported to exhibit self-supplementation in the form of geophagy and coprophagy. Box turtles (*Terrapene ornate*) have been reported to actively consume soil, which is believed to reduce nutritional deficiencies in minerals including calcium (Kramer 1973; Beyer 1994; Hiu 2004). However, opportunities for self-supplementation in captivity are limited and nutritional disorders are responsible for high morbidity and mortality and limited fecundity in many insectivore collections (Oonincx and Dierenfeld 2012). Therefore, suboptimal nutrition can negatively impact breeding and conservation programmes, as well as compromising the health of captive insectivores (Dierenfeld 1989; Allen and Ullrey 2004; Finke and Winn 2004; Hernandez-Divers 2006).

Nutritional deficiencies in insectivores can be successfully prevented or at least minimised using a variety of techniques, with the two most common involving either the dusting of prey with a multi-vitamin and mineral powder, and/or gut loading invertebrate prey (Bernard et al. 1997; Anderson 2000; Hunt et al. 2001; Finke 2003; Michaels et al. 2014). Dusting typically requires the prey to be fed immediately after dusting (McClements et al. 2003) since the powder is thought to fall off due to invertebrate movements, as well as being actively removed by grooming (Michaels et al. 2014). Therefore, the amount of supplement powder consumed by the insectivore is often considered to be variable, unquantifiable and unreliable (Bernard et al. 1997; Hunt et al. 2001). In contrast, Michaels et al. (2014) reported that dusting crickets with a calcium supplement resulted in a significant increase in calcium content, which was maintained for 5.5 hours, indicating that dusting may be an efficacious method of insectivore supplementation.

Alternatively, gut loading involves providing the feeder invertebrates with a nutrient dense diet, with the intention of filling the prey's gastrointestinal tract with essential nutrients which would otherwise be lacking, and promoting nutrient absorption and accumulation in the invertebrate. This method is considered to provide a reliable method of delivering a balanced diet to the insectivore (Bernard et al. 1997; Hunt et al. 2001; Mayntz and Toft 2001; Dierenfeld and Fidgett 2003).

Different aspects of the nutritional value of feeder invertebrate species have been reported, including the proximate composition, vitamin, mineral and carotenoid content, and amino acid and fatty acid compositions (Finke 2007, 2013; Oonincx and van der Poel 2011; Ogilvy et al. 2012). Previous research into the suitability of feeder invertebrate species as prey items for captive insectivores have focused on either the unsupplemented nutritional composition of invertebrates, or the ability to manipulate their nutritional value by provision of alternative gut-loading diets over different periods of time immediately prior to analysis. Whilst gut loading studies have reported variable results in improving feeder invertebrate nutritional composition, the chemical composition of gut-loading material has been shown to be influential in this regard for a number of prey species (Allen and Oftedal 1989; Anderson 2000; Oonincx and van der Poel 2011). However, many feeder invertebrates are yet to be studied. Additionally, in captivity, live feeder invertebrates may be left inside vivaria without a food supply for undefined periods until they are consumed by the resident insectivore. Dusting may also be ineffective, particularly

in complex environments, where supplements may be removed by the invertebrate (Michaels et al. 2014). Therefore, variable and often unknown time delays may be encountered between supplementation and consumption by the insectivore. This delay has the potential to result in a change in the nutrient composition of the feeder invertebrate, either via excretion of the gut-load diet, nutrient absorption and accumulation (Jones and Raubenheimer 2001; Raubenheimer and Jones 2006) or the metabolic effects of starvation.

The findings of this study will be used to determine the period of food deprivation recommended for feeder invertebrates, without compromising nutritional value to the insectivore. This information will be of value when managing feeding schedules and protocols for a variety of insectivorous species held in zoological institutes and private collections.

Methods

Specimens

Three different species of feeder invertebrates were used in this study, each treated as separate experiments. One kilogram of mealworms (*Tenebrio molitor* larvae; approximately 23–30 mm), 400 g of giant African land snails (*Achatina fulica*; shell size 10–35 mm) and 500 g of Dubia cockroaches (*Blaptica dubia*; approximately 20–30 mm) were obtained from Livefoods Direct Ltd (Sheffield, UK), Paignton Zoo (Devon, UK) and Ricks Livefood (UK) respectively. All specimens were provided with water in the form of soaked cotton wool, but no food was offered for 24 hours in order to clear their gastrointestinal tract of previous diet as per Finke (2002). The mealworms were selected due to their popularity as feeder invertebrates and as their nutritional content has previously been studied. Cockroaches and snails were selected as, although their nutrition has not been widely studied, they remain popular food items due to their high fecundity. Snails may also be provided to specialist insectivores and molluscivores. Prior to arrival at our laboratory, the mealworms and cockroaches were raised by breeders on dry gut-load diet ("Dry diet"; Bug Grub, ProRep, Essex.), supplemented with potatoes and apple. Likewise, the snails were reported to have been raised on leaf litter and supplemented with a variety of lettuces and cucumber ("Wet diet") prior to entry into the study.

Treatments

On arrival, each species was released into separate, ventilated plastic boxes measuring 190 mm (height) x 590 mm (width) x 390 mm (depth) with air holes in the lid. All setups had a thermogradient of 20–31° C, set to fluctuate around the 27° C reported by Bernard et al. (1997) as optimal for gut loading, controlled by an underfloor heating mat (12w, EuroRep, Middlesex) and regulated by a Microclimate Pulse Thermostat (B2 model, 600w, Microclimate International, Wolverhampton). Enclosure air temperatures were measured using an infrared temperature gun (Electronic Temperature Instruments, Worthing) and humidity (measured with a Reptiland digital hygrometer (TRIXIE Heimtierbedarf GmbH & Co. KG, Germany)) ranged from 50–80% during the study. All feeder invertebrates were subjected to a 12:12h artificial light cycle. Each experimental setup contained cardboard egg crates in order to increase the surface area of the tanks, in order to prevent overcrowding and to reduce invertebrate mortality associated with stress.

Each group was provided with a diet of commercially produced dry gut-load diet, "Bug Grub" (Prorep, Essex, UK; Table 1), and a wet diet of approximately 1 cm³ diced potato in standardised quantities in bowls (51 mm diameter x 6 mm depth) 24 hours after arrival. Both wet and dry diets were provided due to the differing feeding strategies of the invertebrates chosen for analysis (Finke

Table 1. Proximate composition of diets fed to feeder invertebrates (mean \pm SEM). Results shown as % DM (except DM).

Diets	Dry matter	Ash	Protein	Fat
Mealworm 60g dry ^a , 20g wet ^b	71.33 \pm 0.14	76.15 \pm 0.29	19.34 \pm 0.08	1.32 \pm 0.49
Cockroach 60g dry ^a , 20g wet ^b	70.59 \pm 0.66	75.15 \pm 0.28	18.87 \pm 0.02	1.44 \pm 0.53
Snail 20g dry ^a , 60g wet ^b	35.365 \pm 0.47	75.96 \pm 0.08	15.43 \pm 0.39	1.44 \pm 0.35
Bug Grub	N/A	12.8	18.6	N/A

^aCommercial Bug Grub Diet (ProRep, Essex).

^bApproximately 1 cm³ diced new potato.

et al. 2005). Mealworms and cockroaches were provided with 60 g dry diet and 20 g wet diet. The snails were provided 20 g dry diet and 60 g wet diet as per Ademolu et al. (2004). Snails and cockroaches were also provided with a dish (51 mm diameter x 6 mm depth) of cotton wool soaked in cool, boiled water as a further source of moisture, as per Oonincx and Dierenfeld (2012). Mealworms were not given a water source as this is not considered necessary for their husbandry. All diets and water sources were replaced every 24 hours to prevent mould growth and tanks were cleaned out every 24 hours to prevent the consumption of faeces (Finke 2002). The diets and water were provided *ad libitum* for 48 hours, previously demonstrated to be sufficient time for gut loading of mealworms (Allen and Oftedal 1989; Bernard et al. 1997; Hunt et al. 2001). This is in contrast to Finke's (2013) study on cockroaches, in which the test groups were left purposefully unfed before testing; gut loading in snails has not been previously investigated. At the time of preparation, 8 g of the total diet for each species was taken for analysis. Leftover food was removed after 48 hours and the first feeder invertebrate sample (T 0 h) of 120 g of mealworms (approximately 1,300), 80 g (n=55) of snails, and 100 g (n=120) of cockroaches was taken. The diets provided were partially consumed by all the species, but remaining food was not weighed due to contamination with debris. Sample size was not equivalent among species due to limitations in total invertebrates supplied. Subsequently, further samples were taken at 30 minutes (T 0.5 h; excluding cockroaches), 1 hour (T 1 h), 6 hours (T 6 h) and 12 hours (T 12 h; excluding snails). A limited supply of cockroaches and snails resulted in the exclusion of samples for these species at some time points (noted above). The samples of whole invertebrates were then live-frozen at -20°C (as per Barker et al. 1998; Colorado Plateau Biodiversity Center 2012).

Proximate analyses

After samples had been frozen for 24 hours they were weighed and freeze dried. The freeze dried product was homogenised using a pestle and mortar and stored at room temperature prior to proximate analysis. Samples were placed in a freeze drier (Virtis Benchtop™ K Series) until they achieved a constant weight. The remaining sample was weighed again to determine freeze dry matter, and moisture content was calculated as performed by Oonincx and Dierenfeld (2012). Two grams of homogenised sample were incinerated in a muffle furnace at 650° C for 16 hours to determine ash content. Crude protein was analysed using the Kjeldahl method (ISO 2009) using approximately 2 g of dried sample for mealworms and snails. In contrast, 1 g of dried sample was analysed for cockroaches as the samples were

more dense than other invertebrates. Analyses of blanks of 1 g starch were performed each day (as per ISO 2009). The Kjeldahl method-derived nitrogen content of samples was then converted to crude protein content following multiplication by a factor of 6.25. Crude fat was determined by extracting 3 g samples with petroleum ether using Soxhlet apparatus (ISO – standard 6492). Bomb calorimetry (Parr Bomb Calorimeter 1261, Parr Instrument Company, Illinois, USA) was used to determine gross energy content of samples. Standards of benzoic acid were analysed each day to ensure calorimeter accuracy (Parr 2008). The homogenised sample was pelleted prior to testing using a pellet press and drops of distilled water, and then left in an oven at 55° C to dry for 12 hours. The pellets were then weighed and tested in the apparatus. All samples were analysed in quadruplicate.

Statistical analysis

Statistical software SPSS (Version 19, IBM Corp., Armonk, NY, USA) was used to analyse and compare results from each time point. Each species was tested individually, comparing the differences between the various time points for each nutrient separately. The Kormogorov–Smirnov test was used to test for normality of the data. All the data recorded were parametric, therefore a one way analysis of variance (ANOVA) was used to determine significant differences between the time points. Any significant differences were then tested using the post-hoc Bonferroni test. The level of significance was set at $P < 0.05$.

Results

Diet

Diet composition is presented in Table 1. Cockroach and mealworm diets had freeze-dry matter contents of 70.6% and 71.3%, respectively, whereas the snail diet had a freeze-dry matter content of 35.4%. High ash contents were found in all diets at around 75%. Crude protein contents ranged from 15.4 to 19.3%DM in the diets. Crude fat contents were low in all diets at less than 2% DM.

Invertebrate nutrient composition

The mean and standard error of the proximate nutrient composition for each species are shown in Table 2.

Snails

A significant increase in fat, between time points 0 h and 1 h ($P=0.032$) was observed (Fig. 1). Crude protein also increased significantly between time points 0 h and 6 h ($P=0.006$). A positive

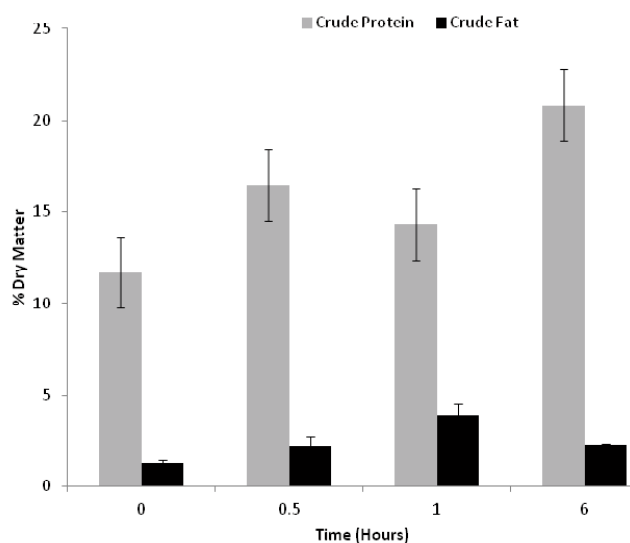


Figure 1. Crude protein and crude fat contents of snails.

Table 2. Mean (\pm SEM) nutrient content of feeder invertebrates following food deprivation post-gut loading. Results shown as % DM (excluding gross energy).

Time post-gut loading (h)	Dry matter	Ash	Gross energy kcal/kg	Crude protein	Crude fat
<i>Mealworms (Tenebrio molitor)</i>					
0	41.32 \pm 0.00 ^a	2.98 \pm 0.09 ^a	27.36 \pm 0.13 ^a	44.95 \pm 0.81 ^a	35.39 \pm 0.38 ^a
0.5	41.59 \pm 0.00 ^a	2.92 \pm 0.06 ^a	27.51 \pm 0.23 ^a	47.23 \pm 0.76 ^a	35.24 \pm 0.31 ^a
1	41 \pm 0.00 ^a	2.78 \pm 0.76 ^a	26.99 \pm 0.87 ^a	43.97 \pm 0.73 ^a	35.5 \pm 0.61 ^a
6	41.14 \pm 0.00 ^a	2.8 \pm 0.07 ^a	28.06 \pm 0.08 ^a	46.32 \pm 0.42 ^a	34.22 \pm 1.40 ^a
12	40.40 \pm 0.01 ^a	2.91 \pm 0.04 ^a	27.17 \pm 0.88 ^a	38.8 \pm 5.60 ^a	33.82 \pm 1.60 ^a
<i>Snails (Achatina fulica)</i>					
0	42.93 \pm 0.00 ^a	38.49 \pm 0.67 ^a	5.33 \pm 0.19 ^a	11.69 \pm 1.48 ^c	1.25 \pm 0.18 ^b
0.5	43.97 \pm 0.01 ^a	36.55 \pm 1.48 ^a	6.32 \pm 0.36 ^a	16.47 \pm 1.50 ^a	2.16 \pm 0.58 ^a
1	43.6 \pm 0.01 ^a	37.88 \pm 1.51 ^a	6.36 \pm 0.44 ^a	14.33 \pm 1.32 ^d	3.86 \pm 0.72 ^b
6	42.33 \pm 0.02 ^a	39.18 \pm 1.70 ^a	5.98 \pm 0.29 ^a	20.83 \pm 1.70 ^{cd}	2.27 \pm 0.86 ^a
<i>Cockroaches (Blaptica dubia)</i>					
0	35.59 \pm 0.01 ^a	3.12 \pm 0.04 ^a	ND	51.16 \pm 1.55 ^a	25.55 \pm 1.31 ^e
1	37.47 \pm 0.00 ^a	3.16 \pm 0.13 ^a	ND	46.26 \pm 1.33 ^a	23.07 \pm 1.02 ^a
6	36.31 \pm 0.00 ^a	3.35 \pm 0.02 ^a	ND	48.51 \pm 0.74 ^a	23.55 \pm 2.07 ^a
12	36.55 \pm 0.00 ^a	3.15 \pm 0.03 ^a	ND	48.75 \pm 0.30 ^a	29.24 \pm 0.25 ^e

ND – Not determined.

a-e: Differences indicate within-column differences for each species ($P < 0.05$)

trend in protein content was also identified in snails at 1 h and 6 h ($P = 0.060$). Ash content in the snails was highly variable and no other significant differences were detectable.

Mealworms

No significant differences or changes occurred in any mealworm nutrients between any time point. The least variable was ash (2.8–3.0% DM) which remained low throughout the study, while the most variable nutrient throughout the study was crude protein (ranging from 38.8–47.2% DM; Table 2).

Cockroaches

No significant differences were detectable in cockroach nutrient composition at any time point, although there was a trend towards an increase in fat content ($F = 3.327$, $P = 0.09$) between samples taken at 1 h and 12 h. Cockroaches had the highest crude protein content of all the feeder invertebrates tested (46.3–51.2% DM) and the lowest dry matter values (35.6–37.5%). Crude fat content of cockroaches varied over the course of the study from 23.1 to 29.2% DM (Table 2). Gross energy values were not determined for this species.

Discussion

Our findings have demonstrated that no significant change in macro-nutrient content occurs in mealworms or cockroaches following 12 h without food after gut loading. Further studies regarding the effects on micro-nutrients in unfed invertebrates would be beneficial in formulating effective feeding practices for insectivores. Feeder invertebrates remaining uneaten after 12

hours were not investigated, and may undergo changes in nutrient content. A precautionary approach is therefore recommended whereby either feeder invertebrates should be removed if uneaten after 12 hours, or provided with a source of gut-loading diet within the insectivore's vivarium, as long as this can be protected from the insectivore.

In contrast to mealworms and cockroaches, snail nutrient content was shown to vary over time, and demonstrated a significant increase in crude protein content by the 6 h time point. Fat content had also increased at the 1 h time point, but differences were no longer detectable at later time points. The increase in these nutrients was somewhat unexpected given that food deprivation is generally associated with protein and fat loss as body reserves are mobilised for energy production. Although the snails were provided with a different diet to the other species, their diet was determined to provide equivalent amounts of protein and fat. As such, it is unlikely that differences in protein and fat during the period without food can be explained by the preceding dietary intake. However, snails were not tested at 12 h due to sample size constraints, at which point further changes in nutrient content may have occurred.

When compared to the findings of Aboua (1990), who analysed snail meat, shell and whole snail compositions separately, our whole snail values for dry matter (42.3–43.8%) were lower compared to those previously reported (53.5%). As freeze drying does not result in all of the moisture being removed from the samples, the results from the current study may underestimate the true dry matter values and explain this discrepancy. Whole snails were tested in this study as they are often presented whole to an insectivore in captivity and certain predators may consume

molluscs whole, including the shell, such as red knots (*Calidris canutus*), and select species of reptiles, in particular terrapins (Davenport et al. 1992; Dekinga and Pierma 1993; Van Gils et al. 2003).

The ash and protein content found during the current study (38.5–39.2 and 11.7–20.8% DM, respectively) were also lower than those found by Aboua (1990), who reported ash content of 43.7% DM and protein contents varying between 38.5 and 40.5% DM. This may reflect differences in the age of individuals used, diet provided or the environmental conditions. For example, Aboua (1990) used wild-caught animals with an unknown diet and therefore it is unknown what effect the diet may have had on the individuals tested. It is feasible that differences in species, age or environmental conditions of the snails included in our study may explain their divergent nutrient content, and likewise may have contributed to the novel response to the period of food withholding.

No effect of food deprivation was apparent in mealworms. In terms of nutrient contents determined here, our findings are similar to previous reports for dry matter and crude protein. Previous studies have determined that the dry matter content of mealworms varies from 35.0% to 42.3% DM (Jones et al. 1972; Barker et al. 1998; Finke 2002), which is similar to that found in the current study (40.4–41.6% DM). Crude protein levels for mealworms in the current study (38.8–47.2% DM) were also found to be within the range reported in other studies (35.0–57.5% DM; Jones et al. 1972; Pennino et al. 1991). The fat content of mealworms in the current study (33.8–35.5% DM) fell within that found by the previous studies, although our results were considerably less variable than previous reports (12.4%–60% DM; Jones et al. 1972; Pennino et al. 1991). The standardised diet and use of an initial gut emptying period employed in the current study may have reduced variability.

Ash content in mealworms has been reported to range from 0.6% DM for non-gut loaded individuals to 8% for well-fed insects (Barker et al. 1998; Finke 2003; Finke and Winn 2004; Oonincx and Dierenfeld 2012). The results from the current study ranged from 2.8–3.0% DM, which is at the lower end of the reported range. As the diet provided here was predicted to be high in mineral content (where ash content is used as a proxy for mineral content), this may have reduced consumption of the diet by feeder invertebrates. High calcium diets have been shown to increase mortality of feeder invertebrates, and high mineral diets have resulted in reduced voluntary feed intake (Allen and Oftedal 1989; Michaels et al. 2014). However, husbandry and dietary information is missing from many of the previous studies reporting mealworm proximate analysis (Jones et al. 1972; Allen and Oftedal 1989), such that further comparison and interpretation of our findings was not possible.

Although energy content is important for diet formulations, few studies have measured the gross energy of invertebrates. High energy values were found for mealworms (6,446 kcal/kg) in the current study, similar to that found by Jones et al. (1972; 6,530 kcal/kg), and is likely to reflect their high fat content. Therefore, mealworms offer an energy-dense food source for insectivores (Barker et al. 1998), which may be of particular benefit to insectivores such as shrews and desert species (Heard 2014), which have a high metabolic water requirement, given the high water yield following oxidation of fat in contrast with the oxidation of carbohydrate-rich invertebrates (D'Agostino 2014; Downer and Matthews 1976).

No significant changes in nutrient content were detectable in cockroaches following food deprivation. To the authors' knowledge, this is the first experimental study to investigate the nutrient composition of *Dubia* cockroaches. The values found for *Dubia* cockroach protein differed considerably between our

study and previous studies using other species of cockroach. Crude protein values of 46.26–51.16% DM were determined in *Dubia* cockroaches here, whereas other species have ranged from 38.3 to 76.1% DM (Oonincx and Dierenfeld 2012), indicating that the cockroaches used here are within the normal range of other cockroaches used as live prey. Fat content measured here (23.1–29.2% DM) was also found to be within the ranges found by Oonincx and Dierenfeld (2012) and higher than that found by Finke (2013) for *B. lateralis*. Prey diets, study conditions and life stage of the cockroaches could not be compared between the studies.

A possible explanation for an increase in protein content in cockroaches is an accumulation of uric acid post gut loading (Cochran 1985). The build-up of this excretion product, with its high nitrogen content, could have resulted in an overestimate of crude protein content when using the Kjeldahl method (which measures nitrogen) employed here (Pilbarot and Pilard 2012). As such, future studies investigating uric acid production would be beneficial (Cochran 1985; Jones and Raubenheimer 2001; Raubenheimer and Jones 2006). Furthermore, the chitin within the exoskeleton of adult cockroaches and mealworms contains substantial concentrations of nitrogen. This would also contribute to our calculation of protein content since the adults used for testing here have a high chitin:meat ratio compared to smaller individuals (Finke 2007). The study by Oonincx and Dierenfeld (2012) provides further evidence that life stage may affect nutritional composition as indicated by the difference in *B. lateralis* and *E. distantis* micro- and macro-nutrient results. As such, the instar of cockroaches should be considered when evaluating their suitability for inclusion in an insectivorous diet.

During this study, feeder invertebrates were provided with diets consisting of both dry and wet components, the standard gut-loading procedure in previous studies, and used by insect breeders (Anderson 2000; Oonincx and van der Poel 2011; Oonincx and Dierenfeld 2012). Likewise, all the diets consisted of similar fat and protein contents to previous studies (Anderson 2000; Dierenfeld and Fidgett 2003), indicating that our findings will have relevance to typically implemented invertebrate feeding regimes. The uneaten portion of the diet was not analysed so an accurate assessment of nutrient intake by the feeder invertebrates could not be conducted but may provide further understanding of the results presented here.

To enable the generation of recommendations to overcome dietary limitations that may be experienced by some facilities (e.g. availability of raw materials or supplements), testing using different diet compositions would be beneficial. The invertebrates chosen for analysis are taxonomically different, the African land snail a Mollusca, the mealworms and cockroaches representing different orders of Insecta. During the current study alternative feeding strategies or food preferences may have affected the diet consumption. For future studies and in general feeding practices, providing a varied diet and homogenisation of diets may be recommended so that mandible size and strength is not a factor in diet consumption (Finke et al. 2005).

As with many of the previous studies conducted, small sample sizes and limited replicates are a common pitfall. However, this study utilised larger sample sizes than many previously published studies such as Oonincx and Dierenfeld (2012). Nonetheless, samples were taken from the same tank and the sample sizes were restrictive and prohibited the use of additional sampling time points. The use of larger sample sizes without appropriate replication of tanks could actually have introduced further errors by including different sized individuals within the same sample. As shown by Oonincx and Dierenfeld (2012), size and life stage can affect the nutritional content of a species. Finke (2013) increased the sample size (rather than replicates) in order to reduce variation, but separating species into size or life stage categories would also

allow for more accurate and reliable values. Nonetheless, the system of housing feeder invertebrates in large single-species groups is likely to reflect typical husbandry practices in zoos and private collections and to the authors knowledge keepers do not separate feeder invertebrates according to size of life stage prior to offering to insectivores. Therefore, the variability determined here may mirror that seen in insectivore diets.

However, essential micro-nutrients were not investigated in the current study but should be evaluated in future studies, especially minerals such as calcium and phosphorus which are often the nutrients of primary concern when gut loading feeder invertebrates (Michaels et al. 2014). Investigation of any effect of food deprivation on micro-nutrient, amino acid and fatty acid compositions would be beneficial to ensure these essential nutrients are still provided to insectivores in adequate supply by invertebrates that are unfed.

Samples supplied in this study originated from different sources, which may have affected the values found; obtaining samples from multiple sources would allow comparisons between sources. McClements et al. (2003) reported that environmental conditions and feeding schedules affected Ca to P ratios. Consequently an invertebrate's nutritional content may differ between live food breeders as a result of the conditions it is raised in. Initial samples after the invertebrates arrived were not taken as they were all provided with feed during transit (the snails on leaf litter, the mealworms and cockroaches on bran), and therefore the samples would not have represented unfed invertebrates. Analysing unfed samples in future studies may be beneficial in order to observe the gut-load effect. Finke (2002) analysed mealworms that were unfed for 48 hours; the values reported for crude fat and crude protein were less than those found in the current study.

The method of freeze drying used in this study may have resulted in underestimates of dry matter values. The same methods were used by Oonincx and Dierenfeld (2012) and Barker et al. (1998) and therefore the data from the studies are comparable with the results in the current study. Furthermore, a previous study has determined no significant difference between DM values derived from freeze drying and oven drying of biological samples (Jacobs et al. 2011).

More research is required into the effects of longer periods of food deprivation to determine how long zoos can leave feeder invertebrates in an insectivore's enclosure and to formulate effective gut loading and feeding regimes. As an increasing variety of feeder invertebrates are becoming available to zoos and private keepers, the essential fatty acid, amino acid, vitamin and mineral compositions of each species need to be investigated. Previous studies have recognised that a species nutritional composition can differ depending on life stage and diet. Therefore testing multiple species at different life stages, and over extended periods without food, is warranted.

Conclusion

The current study shows that, for up to 12 hours post gut loading, there was no significant change in macro-nutrient values of Dubia cockroaches or mealworms. In contrast, significant changes were demonstrated in fat and protein contents in unfed African land snails, indicating that this species should be consumed soon after gut loading in order to ensure the insectivore's intake is as predicted from typically reported snail body composition analyses. Our findings demonstrate that mealworms and cockroaches can be left uneaten in insectivore enclosures for periods of 12 hours without the need for further gut loading. However, further studies regarding the effect of food deprivation on other nutrients, especially minerals and vitamins, would be beneficial as these may be affected differently.

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References

- Aboua F. (1990) Chemical composition of *Achatina fulica*. *Tropicicultura* 8: 121–122.
- Ademolu K.O., Idowu A.B., Mafiana C.F., Osinowo O.A. (2004) Performance, proximate and mineral analyses of african giant land snail (*Archachatina marginata*) fed different nitrogen sources. *African Journal of Biotechnology* 3: 412–417.
- Allen M.E., Ullrey D.E. (2004) Relationships among nutrition and reproduction and relevance for wild animals. *Zoo Biology* 23: 475–487.
- Allen M.E., Oftedal O.T., Ullrey D.E. (1993) Effect of dietary calcium concentration on mineral composition of fox geckos (*Hemidactylus garnoti*) and Cuban tree frogs (*Osteopilus septentrionalis*). *Journal of Zoo and Wildlife Medicine* 24: 118–128.
- Allen.M.E., Oftedal O.T. (1989) Dietary manipulation of the calcium content of feed crickets. *Journal of Wildlife Medicine* 20: 26–33.
- Anderson S.J. (2000) Increasing calcium levels in cultured insects. *Zoo Biology* 19: 1–10.
- Barker D., Fitzpatrick M.P., Dierenfeld E.S. (1998) Nutrient composition of selected whole invertebrates. *Zoo Biology* 17: 123–134.
- Bernard J.B., Allen M.E., Ullrey D.E. (1997) Feeding captive insectivorous animals: nutritional aspects of insects as food. *Nutrition Advisory Group Factsheet 003*. Available at: <http://nagonline.net/wp-content/uploads/2014/01/NAG-FS003-97-Insects-JONI-FEB-24-2002-MODIFIED.pdf>
- Beyer W.N., Connor E.E., Gerould S. (1994) Estimates of soil ingestion by wildlife. *Journal of Wildlife Management* 58: 375–382.
- Chapman R.F. (1998) Alimentary canal, digestion and absorption; nutrition. In: Simpson S.J, Douglas A.E. (eds). *The Insects: Structure and Function*, 4th edn. Cambridge: University of Cambridge, 46–80.
- Cochran D.G. (1985) Nitrogen excretion in cockroaches. *Annual Review of Entomology* 30: 29–49.
- Colorado Plateau Biodiversity Center (2012) *Euthanizing Insects*. Available from: www.youtube.com/watch?v=6Wvr6E44nBIU (accessed 7 May 2015).
- D'Agostino J. (2014) Mammal groups: insectivores (Insectivora, Macroscelidea, Scandentia). In: Miller E.R., Fowler M.E. (eds). *Fowler's Zoo and Wild Animal Medicine, Volume 8*. St Louis: Elsevier Saunders, 275–278.
- Davenport J., Spikes M., Thornton S.M., Kelly B.O. (1992) Crab-eating in the diamondback terrapin *Malaclemys terrapin*: dealing with dangerous prey. *Journal of the Marine Biological Association of the United Kingdom* 72: 835–848.
- Dekinga A., Piersma T. (1993) Reconstructing diet composition on the basis of faeces in a mollusc-eating wader, the knot *Calidris canutus*. *Bird Study* 40: 144–156.
- Dierenfeld E.S. (1989) Vitamin E deficiency in zoo reptiles, birds and ungulates. *Journal of Zoo and Wildlife Medicine* 20: 3–11.
- Dierenfeld E.S., Fidgett A.L. (2003) Herbivorous insect composition: you are what you eat? *EAZA News, Zoo Nutrition Special Issue* 3: 25–27.
- Donoghue S. (2005) Nutrition. In: Mader D.R. (ed.), *Reptile Medicine and Surgery*. St Louis: Saunders Elsevier, 251–258.
- Downer R.G.H., Matthews J.R. (1976) Patterns of lipid distribution and utilisation in insects. *American Zoologist* 16: 733–745.
- Famobuwa O.E. (2004) Proximate and chemical analyses of *Archachatina marginata* and *Achatina fulica* (Sulvatica). MSc thesis. Akure: Federal University of Technology.
- Finke M. (2002) Complete nutrient composition of commercially raised invertebrates commonly used as food for insectivores. *Zoo Biology* 21: 269–285.
- Finke M. (2003) Gut loading to enhance the nutrient content of insects as food for reptiles: A mathematical approach. *Zoo Biology* 22: 147–162.
- Finke M.D. (2007) Estimate of chitin in raw whole insects. *Zoo Biology* 26: 105–115.
- Finke M.D. (2013) Complete nutrient content of four species of feeder

- insect. *Zoo Biology* 32: 27–36.
- Finke M., Winn D. (2004) Insects and related arthropods: a nutritional primer for rehabilitators. *Journal of Wildlife Rehabilitation* 27: 14–27.
- Finke M., Dunham S., Kwabi C. (2005) Evaluation of four commercial gut loading products for improving the calcium content of crickets, *Acheta domesticus*. *Journal of Herpetological Medicine and Surgery* 15: 7–12.
- Fraenkel G. (1950) The nutrition of mealworm, *Tenebrio molitor* L. (Tenebrionidae, Coleoptera). *Physiological Zoology* 23: 92–108.
- Heard D. (2014) Insectivores (hedgehogs, moles, and tenrecs). In: West G., Heard D., Caulkett N. (eds). *Zoo Animal and Wildlife Immobilization and Anesthesia*, 2nd edn. Oxford: John Wiley & Sons, 529–531.
- Hernandez-Divers S.M. (2006) Insects as food: nutritious and delicious. The North American Veterinary Conference 2006: 1794–1795.
- Hui C.A. (2004) Geophagy and potential contaminant exposure for terrestrial vertebrates. *Reviews of Environmental Contamination and Toxicology* 183: 115–134.
- Hunt A., Ward A., Ferguson G. (2001) Effects of a high calcium diet on gut loading in varying ages of crickets (*Acheta domesticus*) and mealworms (*Tenebrio molitor*). In: Edwards M., Lisi K.J., Schlegel M.L., Bray R.E. (eds). *Proceedings of the Fourth Conference on Zoo and Wildlife Nutrition*. Lake Buena Vista, FL: AZA Nutrition Advisory Group, 94–99.
- International Organisation for Standardization (ISO) (2009) Standard 5983–2:2009 *Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content*. Available from: <https://www.iso.org/obp/ui/#iso:std:iso:5983:-2:ed-2:v1:en> (accessed 30 January 2013).
- Jacobs B.M., Patience J.F., Stalder K.J., Dozier W.A., Kerr B.J. (2011) Evaluation of drying methods on nitrogen and energy concentrations in pig feces and urine, and on poultry excreta. *Iowa State University Animal Industry Report* 2011.
- Jones L.D., Cooper R.W., Harding R.S. (1972) Composition of mealworm *Tenebrio molitor* larvae. *The Journal of Zoo Animal Medicine* 3: 34–41.
- Jones S.A., Raubenheimer D. (2001) Nutritional regulation in nymphs of the german cockroach, *Blattella germanica*. *Journal of Insect Physiology* 47: 1169–1180.
- Klasing K.C., Thacker P., Lopez M.A., Calvert C.C. (2000) Increasing the calcium content of mealworms (*Tenebrio molitor*) to improve their nutritional value for bone mineralization of growing chicks. *Journal of Wildlife Medicine* 31: 512–517.
- Kramer D.C. (1973) Geophagy in *Terrapene ornate* Agassiz. *Journal of Herpetology* 7: 138–139.
- Li H., Vaughan M.J., Browne R.K. (2009) A complex enrichment diet improves growth and health in the endangered Wyoming toad (*Bufo baxteri*). *Zoo Biology* 28: 197–213.
- Mayntz D., Toft S. (2001) Nutrient composition of the prey's diet affects growth and survivorship of a generalist predator. *Oecologia* 127: 207–213.
- McClements R.D., Lintzenich B.A., Boardman J. (2003) A zoo-wide evaluation into the current feeder insect supplementation at the Brookfield Zoo. In: Ward A., Brooks M., Maslanka M. (eds). *Proceedings of the Fifth Conference on Zoo and Wildlife Nutrition*. Minneapolis, MN: AZA Nutrition Advisory Group.
- McWilliams D.A. (2005) Nutrition research on calcium homeostasis. I. Lizards (with recommendations). *International Zoo Yearbook* 39: 69–77.
- Michaels C.J., Antwis R.E., Preziosi R.F. (2014) Manipulation of the calcium content of insectivore diets through supplementary dusting. *Journal of Zoo and Aquarium Research* 2: 77–81.
- Ogilvy V., Fidgett A.L., Preziosi R.F. (2012) Difference in carotenoid accumulation among three feeder-cricket species: implications for carotenoid delivery to captive insectivores. *Zoo Biology* 31: 470–478.
- Oninxc D.G.A.B., Dierenfeld E.S. (2012) An investigation into the chemical composition of alternative invertebrate prey. *Zoo Biology* 31: 40–54.
- Oninxc D.G.A.B., Van Der Poel A.F.B. (2011) Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). *Zoo Biology* 30: 9–16.
- Otchoumou A., Dupont-Nivet M., Ocho Anin Atchibri L., Dosso H. (2010) Body proportions and chemical composition of wild and reared edible snails of Ivory Coast. *Italian Journal of Food Science* 1: 105–112.
- Parr (2008) *Procedures for Standardization of Parr Oxygen Bomb Calorimeters (2008) Bulletin No. 101 – Calibration of Oxygen Bomb Calorimeters*. Available from: http://www.siamzim.com/pdf/calorimeters/TN_101.pdf (accessed 30 January 2013).
- Pennino M., Dierenfeld E.S., Behler J.L. (1991) Retinol, alpha-tocopherol, and proximate nutrient composition of invertebrates used as food. *International Zoo Yearbook* 30: 143–149.
- Pilbarot P., Pilard S. (2012) Analysis of urea in petfood matrices: comparison of spectro-colorimetric, enzymatic and liquid chromatography electrospray ionization high resolution mass spectrometry methods. *American Journal of Analytical Chemistry* 3: 613–621.
- Pizzi R. (2010) Invertebrates. In: Meredith A., Johnson-Delaney C. (eds). *BSAVA Manual of Exotic Pets*, 5th edn. Quedgely, Gloucester: BSAVA, 373–385.
- Raubenheimer D., Jones S.A. (2006) Nutritional imbalance in an extreme generalist omnivore: tolerance and recovery through complementary food selection. *Animal Behaviour* 71: 1253–1262.
- Spevak E.M., Dierenfeld E.S. (2000) Dietary husbandry of commonly exhibited terrestrial invertebrates. In: Nijboer J., Hatt J. (eds). *Zoo Animal Nutrition I*. Fürth: Filander Verlag, 21–31.
- Van Gils J.A., Piersma T., Dekinga A., Dietz M.W. (2003) Cost-benefit analysis of mollusc-eating in a shorebird II. Optimizing gizzard size in the face of seasonal demands. *Journal of Experimental Biology* 206: 3369–3380.