

RESEARCH ARTICLE

An Investigation Into the Chemical Composition of Alternative Invertebrate Prey

D.G.A.B. Oonincx^{1*} and E.S. Dierenfeld²

¹Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands

²Department of Animal Health and Nutrition, Saint Louis Zoo, St. Louis, Missouri

The aim of this study was to determine the chemical composition of eight invertebrate species and evaluate their suitability as alternative prey. The species selected were rusty red cockroaches (*Blatta lateralis*), six-spotted cockroaches (*Eublaberus distantis*), Madagascar hissing cockroaches (*Gromphadorhina portentosa*), fruit flies (*Drosophila melanogaster*), false katydids (*Microcentrum rhombifolium*), beetles of the mealworm (*Tenebrio molitor*), and superworm beetles (*Zophobas morio*), as well as woodlice (*Porcellio scaber*). Dry matter (DM), crude protein, crude fat, neutral detergent fiber, acid detergent fiber, ash, macro and trace minerals, vitamins A and E, and carotenoid concentrations were quantified. Significant differences were found between species. Crude protein content ranged from 38 to 76% DM, fat from 14 to 54% DM, and ash from 2 to 8% DM. In most species, calcium:phosphorus was low (0.08–0.30:1); however, *P. scaber* was an exception (12:1) and might prove useful as a dietary source of calcium for insectivores. Vitamin E content was low for most species (6–16 mg/kg DM), except for *D. melanogaster* and *M. rhombifolium* (112 and 110 mg/kg DM). The retinol content, as a measure of vitamin A activity, was low in all specimens, but varied greatly among samples (0.670–886 mg/kg DM). The data presented can be used to alter diets to better suit the estimated requirements of insectivores in

The current address of D.G.A.B. Oonincx is Laboratory of Entomology, Department of Plant Sciences, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands.

The current address of E.S. Dierenfeld is Novus International, Inc., 20 Research Drive, St. Charles, MO 63304.

*Correspondence to: D.G.A.B. Oonincx, Laboratory of Entomology, Department of Plant Sciences, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands.
E-mail: dennisooincx@hotmail.com

Received 31 July 2009; Revised 24 December 2010; Accepted 7 January 2011

DOI 10.1002/zoo.20382

Published online in Wiley Online Library (wileyonlinelibrary.com).

2 Oonincx and Dierenfeld

captivity. Future research on the topic of composition of invertebrate prey species should focus on determination of nutrient differences owing to species, developmental stage, and diet. *Zoo Biol* 29:1–15, 2011. © 2011 Wiley-Liss, Inc.

Keywords: chemical composition; insect; invertebrate; insectivory

INTRODUCTION

In most zoos and private collections, only a limited selection of invertebrates is offered as feeder animals. This selection often depends on the availability and acceptance by the predator. However, other factors should also be taken into account when formulating an optimal diet, such as natural feeding ecology, dietary requirements of the predator, and the chemical composition and potential digestibility of the prey offered. For the commonly fed invertebrates, information on chemical composition is available [Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002; Jansen and Nijboer, 2003; Oonincx et al., 2010]. Although readily available, these species may not be the most suitable prey for insectivorous species to meet optimal nutritional demands or fulfill behavioral needs. The aim of this study was to determine the chemical composition of a selection of alternative invertebrate species that may be used to complement or improve the diet for insectivorous animals in zoos and private collections.

MATERIALS AND METHODS

Animals

Seven species of potential feeder insects and one species of crustacean were examined in this investigation. Because earlier studies on insects have shown diet to be a major determinant influencing their chemical composition [Oonincx and van der Poel, 2010; Ramos-Elorduy et al., 2002; Simpson and Raubenheimer, 2001], information on the provided diet is detailed where available.

Rusty red cockroaches (*Blatta lateralis*: Dictyoptera; Blattidae) of two sizes, small (second instar nymphs; 0.9–1.3 cm) and medium (third instar nymphs; 1.3–1.9 cm), were provided by a commercial supplier (TheBugpros.com). They were offered a small amount of water upon arrival and sampled within 24 hr of receipt via overnight shipment.

Six-spotted cockroaches (*Eublaberus distantis*: Dictyoptera; Blaberidae) of three sizes, small (1.5–3.0 cm), large (4.5–5.0 cm), and adult nymphs (4.5–5.0 cm), were provided by Agama International (Montevallo, AL). Upon arrival, they were housed on a substrate of woodland soil and provided with apple and dog food (Purina HiPro, Nestlé Purina PetCare Company, St. Louis, MO) and sampled within 24 hr of receipt via overnight shipment.

Madagascar hissing cockroaches (*Gromphadorhina portentosa*: Dictyoptera; Blattidae) of two sizes, small (1.5–3.5 cm) and adult (4.0–6.0 cm), were reared at the St. Louis Zoo on a diet of laboratory rodent biscuit (Rodent Block, Purina Mills, St. Louis, MO) and dry dog food (Purina HiPro), supplemented with lettuce, apples, and sweet potatoes. Animals were sampled immediately after removal from their enclosure.

Fruit flies (*Drosophila melanogaster*: Diptera; Drosophilidae) were purchased from Carolina Biological Supplies (Burlington, NC) and reared for two generations on Formula 424 (Carolina Biological Supplies). Adult *D. melanogaster* were chilled in their rearing containers at 7°C for approximately 15 min and sampled immediately after removal from their enclosure.

False katydids (*Microcentrum rhombifolium*: Orthoptera; Tettigoniidae) were provided by the St. Louis Zoo Insectarium where they were reared on a diet of firethorn (*Pyracantha* spp.), supplemented with raspberries, blackberries (*Rubus* spp.), and lettuce (*Lactuca* spp.) leaves. Only adults were sampled, immediately after removal from their enclosure.

Common rough woodlice (*Porcellio scaber*: Isopoda; Porcellionidae) were provided by the St. Louis Zoo's Insectarium and reared at the Orthwein Animal Nutrition Center (OANC) at the zoo for 6 weeks. They were housed in plastic bins on a substrate of wood chip mulch, leaf litter, and well-rotted wood, and supplemented with sweet potato, carrot, and apple. Only adults were sampled, immediately after removal from their enclosure.

Mealworm beetles (*Tenebrio molitor*: Coleoptera; Tenebrionidae) were provided by Timberline Fisheries (Marion, IL). Upon arrival, they were housed on a substrate of wheat bran, as provided by the supplier, and a fresh slice of potato was provided for moisture; beetles were sampled within 24 hr of arrival.

Superworm beetles (*Zophobas morio*: Coleoptera; Tenebrionidae) were provided by the same supplier, and housed and sampled identically as described above for mealworm beetles.

Laboratory Analyses

For all species except woodlice (quantity insufficient), fresh tissues ($n = 1-5$ samples) were homogenized in a food processor and subsamples (0.5 g, in duplicate) were taken for vitamin A, E, and carotenoid extraction, according to Barker et al. [1998]. After evaporation under N₂ gas, extracts were sealed in cryovials and stored at -20°C until overnight shipment to Arizona State University for HPLC analysis [McGraw et al., 2006]. Dry matter (DM) content of remaining tissues was determined via freeze drying at the OANC Nutrition Laboratory until a stable weight was reached. Dried samples were ground in a laboratory mill and sent to Dairy One Forage Laboratory (Ithaca, NY) for proximate (crude protein, crude fat, detergent fiber fractions, ash) and macro (calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), and sodium (Na)) and trace mineral (iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and molybdenum (Mo)) determinations. Invertebrates were pooled to provide a minimum of 10 g DM per sample for each set of analyses.

Statistics

Proximate and mineral assay data, except for the *M. rhombifolium* and woodlice ($n = 1$), were analyzed by MANOVA using SPSS version 15.0 to determine whether species effects on composition were present. Within the cockroaches, a MANOVA was used to determine the effect of developmental stage. For specific differences, the data were analyzed by analyses of variance (ANOVA) followed by a Tukey's Honestly Significant Difference test. Differences between mean values were considered significant at $\alpha \leq 0.05$.

RESULTS

Average values of proximate, mineral, and vitamin analyses are presented in Tables 1–3, respectively. Where applicable, standard deviations and the stage of development are shown. The MANOVA indicated that species had a significant effect (Pillai's trace $F = 9.424$, $P < 0.001$) on all analyzed nutrients, except for Mo.

Cockroaches

The three cockroach species differed distinctively in chemical composition. Six-spotted roaches contained the highest DM content (~40–50%) of the three species. Stage of development significantly affected most nutrients (Pillai's trace $F = 5.129$, $P = 0.007$), as well as DM content, with the exception of Fe, Mo, and S.

All roaches contained high concentrations of crude protein (38–76% DM basis; Table 1), similar to values found in literature on American cockroaches (*Periplaneta americana*; 54% DM) [Bernard and Allen, 1997].

Cockroaches contained moderate-to-high concentrations of crude fat. In the earlier stages of development (small vs. large or adult stages), they contained more protein and less fat than larger specimens of the same species, as is true for most animals (a notable exception being neonatal rodents). Crude fat percentage increased with age in *B. lateralis* (from 14 to 27% DM) and *G. portentosa* (from 20 to 25% DM), but that same pattern was not present in *E. distanti*. The reported fat content of American cockroaches (28.4%) was slightly higher than the first two species, but lower than *E. distanti* [31–54% DM; Bernard and Allen, 1997].

In terms of dietary “fiber” content, both neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were similar for *B. lateralis*, averaging about 12% of DM. Approximately 60–90% of ADF in insects is chitin provided by the exoskeleton [Barker et al., 1998; Finke, 2007; Oyarzun et al., 1996]. The ADF content of *G. portentosa* was 10–13% of DM. However, NDF in this species was considerably higher (~36% of DM) and may represent true dietary fiber from vegetables in the digestive tract. Both body and gut content, especially in species with a relatively large gut or consuming high fiber diets, contribute to the nutrient content of feeder prey species. Thus, diet may provide essential nutrients otherwise unavailable from the insect with an empty gut [Finke, 2003; Klasing et al., 2000].

Total ash content of *E. distanti* was significantly lower (2–4% DM) than in *B. lateralis* (7–8% DM; $P < 0.001$) and *G. portentosa* (4–8% DM; $P = 0.007$), similar to American cockroaches [3.3% DM; Bernard and Allen, 1997]. Mineral content among the three cockroach species differed greatly (Table 2). As expected [Barker et al., 1998; Finke, 2002; Studier and Sevick, 1992], an inverse Ca:P ratio was found in cockroaches. Therefore, if using cockroaches as a feeder species, Ca supplementation is necessary to achieve a Ca:P of 1:1 [Donoghue and Langenberg, 1994]. Larger invertebrates (adult or large nymphs) contained lower concentrations of most minerals (Ca, P, Mg, K, Na, Zn, Cu, Mn, and Mo) compared with smaller sized individuals of the same species. Nonetheless, roaches seem to be an excellent dietary source of Zn and Cu. Fe content in *E. distanti* and *G. portentosa* increased with age. Because excess dietary Fe can contribute to Fe storage diseases in several species of birds and mammals [Bonar et al., 2006; Farina et al., 2005; Sheppard and Dierenfeld, 2002; Williams et al., 2008], it is important to know all contributory factors for Fe intake.

TABLE 1. Chemical Composition (Mean ± SD) of Selected Invertebrates

Species	Sample size	Dry matter %	Crude protein	Crude fat	NDF %DM	ADF	Ash
Roaches							
<i>Blatta lateralis</i> (S)	2	20.82 ± 0.64	76.05 ± 0.21	14.45 ± 0.21	11.41 ± 0.60	10.87 ± 2.68	7.88 ± 0.81
<i>B. lateralis</i> (M)	2	28.27 ± 0.27	62.85 ± 0.92	26.50 ± 0.28	12.76 ± 3.00	12.75 ± 2.77	6.89 ± 0.01
<i>Eublabeus distanti</i> (S)	1	42.78	52.1	43.1	N/A	N/A	2.98
<i>E. distanti</i> (L)	5	49.22 ± 1.62	38.28 ± 0.98	54.48 ± 1.30	N/A	N/A	1.96 ± 0.15
<i>E. distanti</i> (A)	2	43.45 ± 0.73	60.50 ± 10.47	31.25 ± 6.43	N/A	N/A	3.80 ± 0.47
<i>Gromphadorhina portentosa</i> (S)	2	30.83 ± 0.78	63.35 ± 4.31	20.30 ± 0.85	36.54 ± 4.88	13.12 ± 7.59	8.49 ± 0.68
<i>G. portentosa</i> (A)	5	38.95 ± 2.99	62.52 ± 3.05	24.56 ± 5.67	34.61 ± 5.81	10.22 ± 2.34	4.06 ± 0.26
Other species							
<i>Drosophila melanogaster</i> (A)	2	30.94 ± 1.87	68.00 ± 1.13	19.00 ± 0.141	17.66 ± 10.51	10.14 ± 0.13	7.20 ± 0.30
<i>Microcentrum rhombifolium</i> (A)	1	36.20	77.8	9.00	41.14	19.39	9.10
<i>Porcellio scaber</i> (A)	1	32.18	41.20	11.50	14.02	5.04	32.69
<i>Tenebrio molitor</i> (A)	2	38.83 ± 1.88	67.65 ± 0.35	17.70 ± 3.11	32.05 ± 1.34	17.63 ± 5.59	4.5 ± 0.13
<i>Zophobas morio</i> (A)	4	38.21 ± 1.61	68.05 ± 0.62	14.25 ± 1.15	50.14 ± 0.93	32.06 ± 1.55	6.16 ± 1.85

S, small; M, medium; L, large; A, adult; N/A, not available; NDF, neutral detergent fiber; ADF, acid detergent fiber.

TABLE 2. (a) Macro Mineral Composition (Mean \pm SD) of Selected Invertebrates and (b) Trace Mineral Composition and Ca:P (Mean \pm SD) of Selected Invertebrates

Species	Sample size	Ca	P	Mg	K	Na	Ca:P
		%DM					
(a)							
Roaches							
<i>Blatta lateralis</i> (S)	2	0.24 \pm 0.00	1.22 \pm 0.01	0.21 \pm 0.01	1.60 \pm 0.00	0.53 \pm 0.00	0.20
<i>B. lateralis</i> (M)	2	0.19 \pm 0.01	0.95 \pm 0.00	0.15 \pm 0.01	1.18 \pm 0.01	0.37 \pm 0.00	0.19
<i>Eublaberus distantii</i> (S)	1	0.08	0.46	0.08	0.75	0.40	0.17
<i>E. distantii</i> (L)	5	0.06 \pm 0.01	0.37 \pm 0.02	0.08 \pm 0.01	0.57 \pm 0.03	0.27 \pm 0.02	0.08
<i>E. distantii</i> (A)	2	0.10 \pm 0.01	0.55 \pm 0.03	0.10 \pm 0.01	0.77 \pm 0.15	0.34 \pm 0.04	0.18
<i>Gromphadorhina portentosa</i> (S)	2	0.25 \pm 0.00	0.93 \pm 0.01	0.24 \pm 0.00	1.24 \pm 0.02	0.33 \pm 0.01	0.27
<i>G. portentosa</i> (A)	5	0.17 \pm 0.05	0.57 \pm 0.05	0.17 \pm 0.02	0.87 \pm 0.13	0.21 \pm 0.04	0.30
Other species							
<i>Drosophila melanogaster</i> (A)	2	0.17 \pm 0.01	1.32 \pm 0.05	0.17 \pm 0.00	1.41 \pm 0.00	0.31 \pm 0.02	0.13
<i>Microcentrum rhombifolium</i> (A)	1	0.24	0.90	0.11	1.15	0.17	0.27
<i>Porcellio scaber</i> (A)	1	14.38	1.22	0.47	0.93	0.81	11.79
<i>Tenebrio molitor</i> (A)	2	0.06 \pm 0.01	0.79 \pm 0.03	0.19 \pm 0.00	1.05 \pm 0.01	0.19 \pm 0.01	0.07
<i>Zophobas morio</i> (A)	4	0.06 \pm 0.01	0.71 \pm 0.03	0.15 \pm 0.02	0.97 \pm 0.02	0.18 \pm 0.01	0.08
		Fe	Zn	Cu	Mn	Mo	
mg/kg DM							
(b)							
Roaches							
<i>Blatta lateralis</i> (S)	2	102.0 \pm 4.2	214.0 \pm 1.4	39.0 \pm 0.0	25.0 \pm 1.4	0.6 \pm 0.0	
<i>B. lateralis</i> (M)	2	89.5 \pm 9.2	164.5 \pm 2.1	33.5 \pm 0.7	18.0 \pm 0.0	0.6 \pm 0.2	
<i>Eublaberus distantii</i> (S)	1	55.0	124.0	12.0	5.0	0.4	
<i>E. distantii</i> (L)	5	91.5 \pm 7.9	83.0 \pm 3.4	15.0 \pm 0.0	22.0 \pm 1.8	1.0 \pm 0.3	
<i>E. distantii</i> (A)	2	111 \pm 41	215.5 \pm 33.2	25.5 \pm 5.0	5.5 \pm 0.7	0.5 \pm 0.1	
<i>Gromphadorhina portentosa</i> (S)	2	153.5 \pm 43.1	202.0 \pm 38.2	22.5 \pm 0.7	10.0 \pm 1.4	0.3 \pm 0.1	
<i>G. portentosa</i> (A)	5	216.0 \pm 89.3	168.2 \pm 19.5	18.8 \pm 1.6	6.4 \pm 1.5	0.4 \pm 0.1	
Other species							
<i>Drosophila melanogaster</i> (A)	2	400.5 \pm 58.7	223.0 \pm 7.1	16.0 \pm 1.4	16.5 \pm 0.7	0.8 \pm 0.1	
<i>Microcentrum rhombifolium</i> (A)	1	131.0	166.0	32.0	93.0	0.3	
<i>Porcellio scaber</i> (A)	1	434.0	170.0	129.0	85.0	<0.1	
<i>Tenebrio molitor</i> (A)	2	89.0 \pm 5.7	144.0 \pm 7.1	21.0 \pm 0.0	15.0 \pm 0.0	1.0 \pm 0.2	
<i>Zophobas morio</i> (A)	4	91.5 \pm 7.9	83.0 \pm 3.4	15.0 \pm 0.0	22.0 \pm 1.8	1.0 \pm 0.3	

S, small; M, medium; L, large; A, adult.

TABLE 3. Vitamins E and A, and Select Carotenoids Content (Mean ± SD) of Selected Invertebrates

Species	Sample size	[Vit E] IU/kg DM ^a	mg/kg DM										[Retinol] IU/kg DM ^b
			[α-Toc]	[DHL]	[Lut]	[Zea]	[AHL]	[Bery]	[Bcar]	[Bery]	[AHL]	[Retinol]	
Roaches													
<i>Blatta lateralis</i> (S)	5	21.7±2.8	14.5±1.9	0	8.9±1.8	7.8±1.5	12.0±1.4	4.2±0.6	3.0±0.6	35.9±5.2	120±17		
<i>B. lateralis</i> (M)	5	17.0±2.1	11.4±1.4	0	5.0±0.8	4.7±0.9	9.3±1.6	4.1±0.9	1.7±1.5	24.8±3.7	83±12		
<i>Eublaberus distanti</i> (S)	1	18.4	12.4	0	13	1.8	0	0	42.9	57.7	192		
<i>E. distanti</i> (L)	5	21.6±5.2	14.5±3.5	0	10.3±9.0	1.0±0.6	0	0	48.2±18.0	59.5±27.0	198±90		
<i>E. distanti</i> (A)	2	20.1±6.3	13.5±4.3	0	5.5±1.3	3.0±0.7	0	0	54.7±0.8	63.2±2.7	211±9		
<i>Gromphadorhina portentosa</i> (S)	6	23.9±5.5	16.0±3.8	11.0±7.2	16.3±9.3	3.1±2.5	0	1.7±0.7	22.7±13.4	54.7±24.7	182±82		
<i>G. portentosa</i> (A)	9	21.1±7.7	14.2±5.2	18.2±12.3	24.2±14.0	3.7±3.4	0	3.3±2.4	66.3±54.8	115.8±72.0	386±240		
Other species													
<i>Drosophila melanogaster</i> (A)	5	166±31	112±21	0	0.4±0.3	0	0	0.2±0.4	0.1±0.2	0.7±0.6	2.2±2.0		
<i>Microcentrum rhombifolium</i> (A)	5	164±54	110±36	0	155±129	59.0±12.3	33.3±14.4	490±114	148±99	886±275	2953±918		
<i>Tenebrio molitor</i> (A)	4	9.0±2.1	6.0±1.4	0	0.2±0.3	0	0	0	3.4±4.0	3.6±4.2	12±14		
<i>Zophobas morio</i> (A)	5	17.8±5.6	12.0±3.8	0	2.4±2.0	0	0	0.1±0.1	9.6±6.2	12.0±7.8	41±26		

S, small; M, medium; L, large; A, adult; Vit E, vitamin E, α-Toc, α-tocopherol; DHL, dehydrolyutein; Lut, lutein; Zea, zeaxanthin; AHL, anhydrolyutein; Bery, β-cryptoxanthin; Bcar, β-carotene; Vit A, vitamin A.

^aVitamin E activity calculated as 1 mg α-toc = 1.49 IU.

^bVitamin A activity calculated as 0.3 μg retinol = 1 IU.

8 Oonincx and Dierenfeld

Vitamin E content of cockroaches was relatively low (11–16 mg/kg DM; Table 3), providing approximately 20 IU vitamin E/kg DM (1 mg = 1.49 IU). Pennino et al. [1991] found almost 10-fold higher concentrations of vitamin E (179 IU/kg DM) in wild-caught cockroaches. Retinol content varied from 25 to 116 mg/kg DM; therefore, calculated vitamin A activity (0.3 µg retinol = 1 IU) was low (<400 IU/kg DM) compared with estimated requirements, using domestic felids as a carnivore model for insectivores (~5,000 IU/kg DM maintenance; 9,000 IU vitamin A/kg DM, growth, and reproduction; [NRC, 2006]). As with vitamin E, free-ranging cockroaches reported by Pennino et al. [1991] contained considerably more vitamin A (1,000 IU/kg DM) than the cockroaches in this study. Lutein, zeaxanthin, and β-carotene was found in all samples. Although dehydrolutein (DHL) and anhydrolutein (AHL) are metabolites of lutein, DHL was not quantifiable in *B. lateralis* or *E. distanti*, and AHL was only found in *E. distanti* samples. Both β-carotene (Bcar), found in all three cockroach species, and β-cryptoxanthin (Bcry) have provitamin A activity in many species [McGraw et al., 2006]. Owing to the widely varying molecular structures of carotenoids, there might be species-dependant differences in the ability of vitamin A synthesis from these compounds. Because vitamin A deficiency has been reported for insectivores fed unsupplemented invertebrates [Ferguson et al., 1996], vitamin A metabolism could be explored among different cockroach species fed identical diets to evaluate synthetic pathways, and determine optimal dietary regimens/ingredients for production of feeder insects with the most appropriate vitamin A levels.

Compared with mealworm and superworm larvae, rusty red roaches (*B. lateralis*) and hissing cockroaches (*G. portentosa*) provide high protein, lower fat alternative food items for insectivores—more similar to cricket proximate nutrient composition [Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002; Jansen and Nijboer, 2003; Oonincx et al., 2010; Pennino et al., 1991]. Six-spotted cockroach nymphs (*E. distanti*), on the other hand, tended to be higher in fat and may be a poorer source of protein than either the other roach species, crickets, or beetle larvae. Owing to their high fat content, they may be considered a high-calorie treat item or could prove useful for improving body condition of insectivores. Mineral content of roaches was variable, depending on species, size, and diet, but all roaches examined still contained inverse Ca:P ratios, in the same ranges as the more commonly fed invertebrate prey [Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002; Jansen and Nijboer, 2003; Oonincx et al., 2010]. Other macrominerals were found in concentrations that would be considered adequate to meet known nutritional requirements of domestic felids [NRC, 2006], considered to be the most suitable physiologic model for insectivores. Conversely, some microminerals, particularly Fe, could be excessive. Small hissing cockroaches are similar in body size to adult house crickets, and may provide a suitable nutritional substitute for crickets in insectivore diets (if consumed by the insectivore).

Fruit Flies

D. melanogaster samples contained high levels of crude protein along with moderate levels of crude fat, dietary fiber, and ash (Table 1). Ca:P ratios were imbalanced (0.13:1), but *D. melanogaster* seems to provide adequate levels of other macro- and microminerals measured; Fe and Zn concentrations were particularly high. In this and in Barker et al.'s study [1998], the same commercial diet was used, which may underlie the high Fe content as well as the higher fat content compared

with the other published values [18–19 vs. 13% of DM; Table 1; Jansen and Nijboer, 2003; Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002].

In contrast, vitamin E in this study (112 mg/kg DM or 166 IU/kg DM) was considerably higher than levels reported by Barker et al. [1998; 23 IU/kg DM]. Only small traces of lutein, β -cryptoxanthin, β -carotene, and vitamin A were found in *D. melanogaster*. Barker et al. [1998] reported undetectable levels of vitamin A in fruit flies. Insectivores eating a relatively large proportion of *D. melanogaster* might benefit from dietary vitamin A and/or carotenoid supplementation.

Regarding size, *D. melanogaster* may be an alternative size option for species that consume pinhead crickets (*A. domesticus*). Fruit flies are similar in protein content as pinhead crickets (68 vs. 55–68% of DM, respectively) and have a higher crude fat and vitamin E content [Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002]. Ash content is similar between the two insects (7 vs. 5–9% DM), but there are compositional differences in specific minerals which may be of consequence (i.e. Na (0.31 vs. 0.43–0.59% DM), Fe (401 vs. 93–200 mg/kg DM), and Mg (17 vs. 30–39 mg/kg DM). Additionally, vitamin E found in *D. melanogaster* differed greatly from that reported in pinhead crickets [167 vs. 40–70 IU/kg DM; Finke, 2002; Barker et al., 1998].

Katydid

Native *M. rhombifolium* contained the highest percentage of crude protein, the lowest crude fat, and the highest dietary fiber content of all invertebrates evaluated in this study (Table 1). High concentrations of ash and most minerals are noted, but the Ca:P ratio was still low (0.27:1), and Na was low compared with the other investigated species. *M. rhombifolium* also contained very high levels of vitamin E and carotenoids (with the exception of DHL; Table 3). Vitamin A content was high compared with the other investigated species and literature data, except for silkworms [Barker et al., 1998; Finke, 2002]. These high ash and vitamin values are likely owing to the diet of fresh green plant materials consumed, as was observed with other herbivorous insects [Dierenfeld, 2002; Dierenfeld and Fidgett, 2003]. Therefore, *M. rhombifolium* may be an important source of carotenoid pigments, fat-soluble vitamins, and minerals for insectivorous species. However, high fiber content may limit palatability and/or bioavailability of some nutrients.

Woodlice

P. scaber was strikingly different in composition compared with all other invertebrates analyzed in this study. Protein (41% DM), crude fat (12% DM), and fiber (<15% DM) concentrations were relatively low compared with data from other studies on woodlice species [protein 40–80% DM; Pokarzhevskii et al., 2003]. The ash content of woodlice was exceptionally high (33%). As a terrestrial crustacean, woodlice have a mineralized exoskeleton and contain high levels of Ca, Mg, Na, Fe, and Cu (Table 2). The exoskeleton can contain up to 24% Ca [Becker et al., 2005]; similar concentrations (10–15% Ca) have been described for other woodlice species [Bureš and Weidinger, 2003; Graveland and Vangijzen, 1994; Pokarzhevskii et al., 2003; Reichle et al., 1969]. The calculated Ca:P ratio of 12:1 is high; this species can possibly be used to correct dietary Ca deficiencies in insectivore diets. Bioavailability and palatability studies are recommended. Woodlice exoskeletons, however, were not found in the feces of two lizard species (*Pogona vitticeps* and *Diploglossus warreni*) during woodlice acceptability trials (personal observation).

10 Oonincx and Dierenfeld

For the isopod *Armadillum vulgatum*, concentrations of Fe (3,170–3,390 mg/kg DM), Zn 332–341 (mg/kg DM), Cu (347–410 mg/kg DM), and Mn (15–16 mg/kg DM) are reported [Peters et al., 2005]. In the same species, a high Mo concentration (0.3 mg/kg DM) has been reported, which is more than threefold the concentration of *P. scaber* in this study [Anke et al., 2007]. Whether these differences are owing to species, diet, or substrate is unknown. Nonetheless, it is apparent that mineral concentrations in woodlice are variable and highly dependent on soil conditions. For example, the Zn concentration in *Oniscus asellus* varied between 54 and 499 mg/kg DM, in which the extremely high concentration was found in specimens originating from a Zn mine [Hopkin and Martin, 1982]; Cu concentration varied from 82.1 to 163 mg/kg DM in the same study.

The concentration of K in isopods measured in this study (0.93% DM) agrees with reported concentrations for other species of woodlice 0.87–1.32% DM [Pokarzhevskii et al., 2003; Reichle et al., 1969]. *P. scaber* contained a fairly high concentration of Na (0.81% DM), similar to the woodlouse *Ligidium blueridgensis* (0.72% DM) [Reichle et al., 1969]. For the woodlouse *Philoscia muscorum*, a concentration of 0.19% Mg DM was reported [Pokarzhevskii et al., 2003], which is much lower than *P. scaber* in this study (0.47% DM).

Beetles

Tenebrio beetles and *Zophobas* beetles contained more protein than literature values reported for their larval counterparts (51 and 43% DM, respectively). This might be associated with a higher degree of sclerotization in beetles, which could negatively influence digestibility [Finke, 2007]. Additionally, the *Tenebrio* and *Zophobas* beetles contained one-half to one-third of the fat content of their larval counterparts, respectively, as well as a higher ash content (Table 1) [Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002]. In general, macromineral concentrations in beetles were similar to, or slightly lower than, published values for larval stages of these beetles [Table 2; Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002], whereas trace mineral concentrations in the beetles studied were consistently higher than previously reported values for both larval and adult stages. These data provide the first published information on mineral content of adult *Z. morio*. Furthermore, feeding trials conducted separately indicate beetles are readily accepted by certain lizard species in captivity (Oonincx and Dierenfeld, unpublished).

The concentration of the measured fat-soluble nutrients was low in the beetles. However, large differences were apparent.

The *Zophobas* beetles contained twice the vitamin E, 3.5 times the concentrations of vitamin A and β -carotene, and 10 times more lutein compared with *Tenebrio* beetles, and thus may provide a more significant source of these nutrients. It might be interesting to evaluate these nutrients further in all life stages of the two species, to better understand metabolic pathways and ultimate storage as a source of these particular nutrients for insectivores.

DISCUSSION

Little or no reliable information regarding specific dietary requirements of insectivores is available at this time; thus, information and interpretations can only be extrapolated from known requirements of domestic carnivores, as well as inferences from clinical health issues of insectivorous species.

Water

Whole invertebrate prey may comprise the sole source of water for certain insectivores in nature; certainly, dietary sources are primary for many carnivores. Species included in this summary contained 51–79% water, on an as-fed basis, similar to more commonly fed prey invertebrates [Barker et al., 1998; Finke, 2002; Oonincx and van der Poel, 2010]. In many species, body water content decreases with age; that same pattern may not hold for these invertebrates as larval stage water content (from published data) was similar to adults of the two beetles examined in this study. Size (subjectively assessed), as a covariate with age (physiologic stage), seemed more closely related to feeder prey body water content within the roach species evaluated, but sample numbers were limited.

Protein

Protein and fat, as fractions of whole body mass, are inversely related. Overall, whole invertebrate prey fed to captive insectivores provide crude protein in excess of the estimated requirements of domestic carnivores [15% of dietary DM for dogs at maintenance, to 32% for reproduction and growth in cats; NRC, 2006]. Protein and essential amino acid requirements of insectivores have not been specifically determined, and reports on the amino acid composition of commonly fed whole invertebrate prey are limited [Finke, 2002; Oyarzun et al., 1996]. Although sulfur amino acids may be at first limiting [Finke, 2002], given the high concentration of crude protein in prey and the lack of reports of overt amino acid deficiencies [with the possible exception of taurine; Wilson et al., 2003], it is probable that amino acid requirements will be met by the invertebrates assayed. Feeding a mixture of species may help remedy potential imbalances until further data are available.

Fat

The crude fat content of most whole prey, invertebrates included, is considerably higher than the recommended minimum dietary levels needed by domestic carnivores. Dogs and cats, at various stages of growth, reproduction and maintenance, require approximately 5–9% fat in the diet [NRC, 2006]. Again, no specific requirements for dietary fat have been defined for insectivores; however, obesity can be a health problem for insectivores in captivity [Graffam et al., 1998]. Life stage may be as important a consideration as species of invertebrate; larvae of both *T. molitor* and *Z. morio* contain considerably higher crude fat concentrations compared with adults, as measured in this study. Although total fat content can be a source of readily available energy for insectivores, essential fatty acid requirements seem to have not been investigated. Minimum dietary requirements of domestic cats for linoleic and arachidonic acids have been set at 0.5 and 0.2% of DM, respectively [NRC, 2006]. Requirements for ω -3 fatty acids, such as α -linolenic or longer chain fatty acids, have not been defined. Assuming that insectivores, as obligate carnivores (species that rely on fats and protein as primary energy sources), have fatty acid requirements similar to those of the domestic cat, the few studies in which fatty acid composition of insects has been reported [Finke, 2002; Oyarzun et al., 1996] suggest that fatty acid supplies would be adequate. Furthermore, no overt deficiency symptoms have been identified in insectivores.

Fiber

Utilization of dietary fiber by insectivores has not been extensively investigated [Graffam et al., 1998]; even accurate determination of appropriate fiber fractions is limited [Finke, 2002; Oyarzun et al., 1996; Pennino et al., 1991]. Certainly, the larger roaches and the sclerotized exoskeleton of adult *T. molitor* and *Z. morio* contained a higher fiber content than previously reported for typical feeder insects. These fiber fractions can be useful if dilutions of energy and/or nutrient content in diets designed for weight control or reduction are needed.

Macrominerals

With the exception of Ca and possibly Na (at least for some species of prey), macromineral requirements (% of dietary DM) for growing carnivore and poultry species [Ca, 0.4–1.2%; P, 0.3–0.6%; Mg, 0.03–0.1%; K, 0.2–1.4%, and Na, 0.05–0.4%; NRC, 1994, 2006] seem to be met by whole prey analyzed in this report and values are similar to previously reported species [Bernard and Allen, 1997; Finke, 2002]. The insects contained an inverse Ca:P ratio that could result in clinical Ca deficiency without supplementation; this imbalance has been previously and consistently reported in other species [Barker et al., 1998; Bernard and Allen, 1997; Finke, 2002; Pennino et al., 1991]. The Ca:P ratio required for insectivores could be investigated further, because the assumed requirement is based on extrapolation.

However, feeding studies suggest a minimum of 1:1 for the dietary ratio of Ca to P is needed to maintain appropriate health, growth, and reproduction [Bernard and Allen, 1997]. Deficiencies of other substances, such as vitamin D, which regulate the selective absorption of both Ca and P, might also be linked to the frequently encountered symptoms within the metabolic bone disease complex [Donoghue and Langenberg, 1994] and should not be discounted. The finding of high levels of Ca present in woodlice in this study provides an inexpensive, practical, and palatable source of dietary Ca for managed insectivores. Diets containing one part woodlice to eight parts (weight:weight) mealworm larvae, for example, result in a 1:1 ratio of Ca:P (1.5% Ca, 1.4% P, DM basis) without gut loading or supplemental dusting. Studies need to be conducted to determine adequacy, palatability, and bioavailability of this mineral source for a variety of insectivorous species, particularly amphibians and reptiles.

Trace Minerals

Much variability among and within prey species in trace element composition is apparent owing to various factors, such as small sample sizes, the influence of differing dietary trace mineral levels, species-specific metabolism, varying accuracy of sampling and/or analytical techniques, and possible contamination with substrate materials. Fe requirements of the insectivores would likely be met by any of the species analyzed (32–100 mg/kg DM; up to 200 mg/kg, in published data). Again, some prey may contain inappropriately high levels of Fe for species with a tendency to develop Fe storage disease. Recommended dietary levels of Zn [10–50 mg/kg DM; NRC, 1994, 2006] would be met by all the invertebrate prey analyzed in this study, as well as those summarized from previous reports. Mean Cu concentrations range from 12 to 129 mg/kg DM. Dietary requirements for Cu are estimated between 3 and 8 mg/kg for a variety of domestic carnivores and poultry [NRC, 1994, 2006]. Based

on physiologic requirements of domestic carnivores as models for insectivores, it is likely that (unknown) requirements of insectivores would be met by all prey analyzed in this study. It may be prudent to exercise caution in exclusive use of high-Cu prey as food for insectivores, owing to possibilities of Cu toxicity. Estimated Mn needs (5 mg/kg) would likely be met by any of these prey items, although poultry require higher levels of Mn for growth [67 mg/kg; NRC, 1994]. Similar requirements may apply to reptiles, but studies have not been conducted and no reports of Mn deficiency in reptiles or amphibians were found in the literature. Mo content of the species studied is within the range of earlier studies of invertebrates (0.2–1.0 mg/kg DM). Whether this could be problematic is unknown; however, insectivores store significantly more Mo in their body than rodents [Anke et al., 2007].

Fat-Soluble Vitamins

Regarding fat-soluble vitamin content of feeder insects, few comparative data are available [Pennino et al., 1991; Barker et al., 1998; Finke, 2002]. Except for *D. melanogaster* and *M. rhombifolium*, vitamin E levels were lower in the species examined (9–22 IU/kg DM) than in most commonly fed insects reported previously [18–81 IU/kg DM; Pennino et al., 1991; Barker et al., 1998; Finke, 2002]. Primary Vitamin E deficiency has not been reported for insectivorous species [Dierenfeld, 1989]. Estimated dietary requirements for domestic dogs and cats are about 30 IU/kg DM when fed diets with moderate levels of polyunsaturated fatty acids (PUFAs) [NRC, 2006]. High dietary levels of PUFAs may increase the vitamin E requirement five-fold. Additionally, reproduction and/or optimal function of the immune system may require higher dietary levels of vitamin E than normally needed to prevent typical signs of vitamin E deficiency.

Vitamin A deficiency as well as toxicities may cause a problem in insectivore health [Dierenfeld et al., 1995; Miller et al., 2001]. Insects, in general, contain low concentrations of vitamin A as do the species in this study. For certain fish, mammalian, and avian species, it is known that certain carotenoids can be converted to active forms of vitamin A [Klasing, 1998; Olson, 1989; Robbins, 1993; Wang et al., 1991]. Carotenoid metabolism in insectivores, such as most amphibians and reptiles, is unknown and deserves further study both from a nutritional as well as from a coloration perspective.

In order to optimize insectivore diets, more knowledge is needed on nutrient digestibility and metabolism in both prey and predator species. The data in this article aid in composing diverse insectivore diets based on extrapolated nutrient requirements, supporting prevention of deficiencies and toxic effects.

CONCLUSIONS

1. The chemical composition of invertebrate prey species varies widely, owing to species, diet fed, and stage of development.
2. Based on requirements of domestic carnivores as physiologic models, total protein, fat, most minerals (with the exception of Ca), and vitamin E requirements of insectivorous species are expected to be met by any of the alternative prey species in this report. Vitamin A concentrations were low in all species examined; carotenoids were quite variable across species.

- P. scaber* contained a high Ca content, and may provide a suitable source of this nutrient to balance a lack of Ca in other invertebrate prey species when fed as part of a mixed diet to insectivores.

ACKNOWLEDGMENTS

The authors thank the St. Louis Zoo Insectarium staff for the provision of invertebrates used in the determinations as well as husbandry assistance, especially Ed Spevak and Bob Merz. We appreciate analytical counsel from Kevin McGraw. Also, we thank Bryan W. Cole of The Bugpros.com, Todd Goodman of Timberline Fisheries, and Bert Langerwerf of Agama International who contributed the invertebrates they produce. Special thanks to Phil and Vicky Sonderman for their help and support during the project.

REFERENCES

- Anke M, Seifert M, Holzinger S, Muller R, Schafer U. 2007. The biological and toxicological importance of molybdenum in the environment and in the nutrition of plants, animals and man—Part 2: molybdenum in animals and man. *Acta Biol Hungarica* 58:325–333.
- Barker D, Fitzpatrick MP, Dierenfeld ES. 1998. Nutrient composition of selected whole invertebrates. *Zoo Biol* 17:123–134.
- Becker A, Ziegler A, Eppele M. 2005. The mineral phase in the cuticles of two species of Crustacea consists of magnesium calcite, amorphous calcium carbonate, and amorphous calcium phosphate. *Dalton Transactions* 10:1814–1820.
- Bernard JB, Allen ME. 1997. Feeding captive insectivorous animals: nutritional aspects of insects as food. Fact sheet 003: 1–7. Nutrition Advisory Group handbook.
- Bonar CJ, Trupkiewicz JG, Toddes B, Lewandowski AH. 2006. Iron storage disease in tapirs. *J Zoo Wildl Med* 37:49–52.
- Bureš S, Weidinger K. 2003. Sources and timing of calcium intake during reproduction in flycatchers. *Oecologia* 137:634–641.
- Dierenfeld ES. 1989. Vitamin-E-deficiency in zoo reptiles, birds, and ungulates. *J Zoo Wildl Med* 20:3–11.
- Dierenfeld ES. 2002. Some preliminary observations on herbivorous insect composition: nutrient advantages from a green leaf diet? *Symp Comp Nutr Soc* 4:253.
- Dierenfeld ES, Fidgett AL. 2003. Herbivorous insect composition: you are what you eat? *EAZA Newsl* 3:25–27.
- Dierenfeld ES, Barker D, McNamara TS, Walberg JA, Furr HC. 1995. Vitamin A and insectivore nutrition. *Verhandlungsbericht Erkrankungen Zootiere* 37:245–249.
- Donoghue S, Langenberg J. 1994. Clinical nutrition of exotic pets. *Aust Vet J* 71:337–341.
- Farina LL, Heard DJ, LeBlanc DM, Hall JO, Stevens G, Wellehan JFX, Detrisac CJ. 2005. Iron storage disease in captive Egyptian fruit bats (*Rousettus aegyptiacus*): relationship of blood iron parameters to hepatic iron concentrations and hepatic histopathology. *J Zoo Wildl Med* 36:212–221.
- Ferguson GW, Jones JR, Gehrman WH, Hammack SH, Talent LG, Hudson RD, Dierenfeld ES, Fitzpatrick MP, Frye FL, Holick MF, Chen TC, Lu Z, Gross TS, Vogel JJ. 1996. Indoor husbandry of the panther chameleon *Chamaeleo [Furcifer] pardalis*: effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. *Zoo Biol* 15:279–299.
- Finke MD. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biol* 21:269–285.
- Finke MD. 2003. Gut loading to enhance the nutrient content of insects as food for reptiles: a mathematical approach. *Zoo Biol* 22:147–162.
- Finke MD. 2007. Estimate of chitin in raw whole insects. *Zoo Biol* 26:105–115.
- Graffam WS, Fitzpatrick MP, Dierenfeld ES. 1998. Fiber digestion in the African white-bellied hedgehog (*Atelerix albiventris*): a preliminary evaluation. *J Nutr* 128:2671S–2673S.
- Graveland J, Vangijzen T. 1994. Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. *Ardea* 82:299–314.
- Hopkin SP, Martin MH. 1982. The distribution of zinc, cadmium, lead and copper within the woodlouse *Oniscus-Asellus* (Crustacea, Isopoda). *Oecologia* 54:227–232.
- Jansen WL, Nijboer J, editors. 2003. Zoo animal nutrition tables and guidelines. Amsterdam: European Zoo Nutrition Centre.
- Klasing KC. 1998. Comparative avian nutrition. New York: CAB International. 350p.
- Klasing KC, Thacker P, Lopez MA, Calvert CC. 2000. Increasing the calcium content of mealworms (*Tenebrio molitor*) to improve their

- nutritional value for bone mineralization of growing chicks. *J Zoo Wildl Med* 31:512–517.
- McGraw KJ, Nolan PM, Crino OL. 2006. Carotenoid accumulation strategies for becoming a colourful House Finch: analyses of plasma and liver pigments in wild moulting birds. *Funct Ecol* 20:678–688.
- Miller EA, Green SL, Otto GM, Bouley DM. 2001. Suspected hypovitaminosis A in a colony of captive green anoles (*Anolis carolinensis*). *Contemp Top Lab Anim Sci* 40:18–20.
- NRC. 1994. Nutrient requirements of poultry. Washington, DC: National Academy Press.
- NRC. 2006. Nutrient requirements of dogs and cats. Washington, DC: National Academy Press.
- Olson JA. 1989. Provitamin-a function of carotenoids—the conversion of beta-carotene into vitamin-A. *J Nutr* 119:105–108.
- Ooninx DGAB, van der Poel AFB. 2010. Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). *Zoo Biol* 30:9–16.
- Ooninx DGAB, Stevens Y, van den Borne JJGC, van Leeuwen JPTM, Hendriks WH. 2010. Effects of vitamin D-3 supplementation and UVb exposure on the growth and plasma concentration of vitamin D-3 metabolites in juvenile bearded dragons (*Pogona vitticeps*). *Comp Biochem Phys B Biochem Mol Biol* 156:122–128.
- Oyarzun SE, Crawshaw GJ, Valdes EV. 1996. Nutrition of the tamandua. 1. Nutrient composition of termites (*Nasutitermes spp*) and stomach contents from wild tamanduas (*Tamandua tetradactyla*). *Zoo Biol* 15:509–524.
- Pennino M, Dierenfeld ES, Behler JL. 1991. Retinol, alpha-tocopherol, and proximate nutrient composition of invertebrates used as food. *Int Zoo Yearb* 30:143–149.
- Peters EL, Kaufmann-Daszczuk B, Brant HA, Jagoe CH, Richter R. 2005. Element concentrations and fluctuating asymmetry in a terrestrial isopod (*Armadillidium vulgare*) in metropolitan Chicago parklands. Society of Environmental Toxicology and Chemistry. 26th Annual Meeting Baltimore.
- Pokarzhevskii AD, van Straalen NM, Zabojev DP, Zaitsev AS. 2003. Microbial links and element flows in nested detrital food-webs. *Pedobiologia* 47:213–224.
- Ramos-Elorduy J, Gonzalez EA, Hernandez AR, Pino JM. 2002. Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. *J Econ Entomol* 95:214–220.
- Reichle DE, Shanks MH, Crossley DA. 1969. Calcium potassium and sodium content of forest floor arthropods. *Ann Entomol Soc Am* 62:57.
- Robbins CT. 1993. Wildlife feeding and nutrition, 2nd ed. San Diego: Academic Press, Inc. 352p.
- Sheppard C, Dierenfeld E. 2002. Iron storage disease in birds: Speculation on etiology and implications for captive husbandry. *J Avian MedSurgery* 16:192–197.
- Simpson SJ, Raubenheimer D. 2001. The geometric analysis of nutrient—allelochemical interactions: a case study using locusts. *Ecology* 82:422–439.
- Studier EH, Seveck SH. 1992. Live mass, water-content, nitrogen and mineral Levels in some insects from South-Central Lower Michigan. *Comp Biochem Physiol A Physiol* 103:579–595.
- Wang XD, Tang GW, Fox JG, Krinsky NI, Russell RM. 1991. Enzymatic conversion of beta-carotene into beta-apocarotenals and retinoids by human, monkey, ferret, and rat-tissues. *Arch Biochem Biophys* 285:8–16.
- Williams CV, Junge RE, Stalis IH. 2008. Evaluation of iron status in lemurs by analysis of serum iron and ferritin concentrations, total iron-binding capacity, and transferrin saturation. *J Am Veterinary Med Assoc* 232:578–585.
- Wilson ED, Dunker F, Garner MM, Aguitar RF. 2003. Taurine deficiency associated dilated cardiomyopathy in giant anteaters (*Myrmecophaga tridactyla*): preliminary results and diagnostics. Proceedings of the American Association of Zoo Veterinarians, Minneapolis, Minnesota. p 155–159.