NUTRIENT ANALYSIS OF LESS COMMON INVERTEBRATE PREY ITEMS

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Abstract

A total of 11 different types of invertebrate prey items [Banded crickets *(Gryllodes sigillatus)* (¼ and ½ inch), harvester ants (*Pogonomyrmex occidentalis*), termites (*Reticulitermes flavipes)* (workers), bloodworms *(Chironomus plumosus*), red wigglers (possible mix of *E. foetida* and *E. andrei*, *Lumbricus rubellus*, and/or *Aporrectodea caliginosa),* tobacco hornworms (*Manduca sexta*), bean beetles (*Callosobruchus maculatus*), and fruit flies (*Drosophila melanogaster* and *Drosophila hydei)*] were analyzed by the Fort Worth Zoo. The selection of invertebrates encompassed different species and sizes that are increasingly fed to insectivorous animals at the Fort Worth Zoo, but for which there is minimal published nutrient composition information. Items were analyzed for proximate, mineral, and vitamins A and E content. The resulting data were compared to published values for similar items and to estimated target nutrient ranges for amphibians. These less common invertebrate prey items showed considerable variation in nutrient content between and within species compared to published data. The majority of nutrients, including Ca:P ratio and vitamins A and E, of the prey items did not achieve the proposed nutrient levels, which reinforces the rationale for supplementation of invertebrate prey items to meet estimated nutrient targets.

Introduction

Invertebrate prey items are widely used in the diets of many captive insectivorous species to model diets consumed in the wild. The process of selecting appropriate prey items for insectivorous animals, especially when increasing variety of items offered, is limited by knowledge of their nutrient profiles. Published nutrient analysis exists for commonly used invertebrate prey items, including crickets (Barker *et al*., 1998; Finke, 2002; Finke, 2007) mealworms (Bernard & Allen, 1997; Finke, 2002) superworms (Barker *et al*., 1998; Finke, 2015), waxworms (Bernard & Allen, 1997; Barker *et al*., 1998; Finke, 2002), silkworms (Barker *et al*., 1998; Finke, 2002), bloodworms (Bernard & Allen, 1997), *Drosophila melanogaster* fruit flies (Bernard & Allen, 1997; Oonincx & Dierenfeld, 2011), and earthworms (Bernard & Allen, 1997). However, published nutrient values are lacking for less common invertebrate prey items fed in captivity, such as bean beetles, hornworms, termites, *Drosophila hydei* fruit flies, and harvester ants.

The purpose of this study was to determine the nutrient content of invertebrate prey items for which data does not readily exist in order to help determine their overall adequacy by comparing to suggested nutrient targets for amphibians (Ferrie *et al*., 2014).

Materials & Methods

Invertebrate Prey Items

Banded crickets (*Gryllodes sigillatus*), ½-inch and ¼-inch, were obtained from Phat Jack's Farms (Grand Prairie, TX); they were fed Purina Game Bird Startena 5419 prior to arrival. Harvester ants (*Pogonomyrmex occidentalis*) were obtained from Life Studies LLC (Hurricane, Utah); they arrived with pieces of fresh apple as food. Termites (*Reticulitermes flavipes* workers; primarily nymph stage) were obtained from Carolina Biological Supply Company (Burlington, NC); they arrived with soil and damp paper towels as substrate. Frozen bloodworms (*Chironomus plumosus*) were obtained from San Francisco Bay Brand (San Francisco, CA). Red wigglers (possible mix of *E. foetida* and *E. andrei*, *Lumbricus rubellus*, and/or *Aporrectodea caliginosa*) were obtained from Uncle Jim's Worm Farm (York, PA); worms arrived in soil substrate. Tobacco hornworms (*Manduca sexta*) were obtained from Great Lakes Hornworm (Romeo, MI); they arrived with a proprietary media, of unknown nutrient content, as a food source. Bean beetles (*Callosobruchus maculatus*) were obtained from Carolina Biological Supply Company (Burlington, NC) as starter cultures. Fruit flies (*Drosophila melanogaster* and *Drosophila hydei*) were obtained from Flymeat.com (Port Orchard, WA) as starter cultures.

Crickets, ants, and termites were isolated from packaging materials on arrival. Frozen bloodworms were thawed, rinsed and strained. Red wigglers were placed in fresh potting soil after arrival for 48 hours before processing to allow for rehydration to mimic conditions in the Ectotherm Department prior to feeding out to animals; they were isolated from soil prior to analysis. Hornworms were sorted on arrival, with most of those at or below 1-inch in length harvested for sampling. Remaining hornworms were maintained at 24ºC in the plastic containers (with propriety media) in which they came to continue growing for up to one week after arrival. After 5-7 days, hornworms within 1-2 inch length were harvested for sampling. Bean beetle cultures were raised on dried black-eyed peas by Ectotherm Department staff and collected periodically (placed in 0ºC freezer until pooled sample accumulated). Fruit flies were raised on fruit fly media (Formula 4-24 Instant Drosophila Medium, Plain, Carolina Biological, Burlington, NC) by Ectotherm Department staff and collected periodically (placed in 0ºC freezer until pooled sample accumulated). Individual weights were obtained for each prey item, followed by freezing samples at -80ºC until processing for analysis.

Laboratory Analysis

Crickets, ants, termites, fruit flies, and bean beetle samples were pulverized into powder using a coffee grinder (Mr. Coffee® coffee mill, Model 3164, Cleveland, OH). Hornworm, red wiggler and bloodworm samples were first frozen in liquid nitrogen (Airgas, Fort Worth, TX) for 20 minutes, then pulverized into powder using a GRINDOMIX GM 300 knife mill (Retsch, Inc., Newtown, PA).

All samples were split into a 35-50 g portion for proximate and mineral analysis and at least 4g for vitamin analysis. *D. hydei* fruit flies were not analyzed for vitamin content due to insufficient sample size.

Proximate and Mineral Analysis

Samples were ground into homogenous powders, sent to Midwest Laboratories (Omaha, NE) to determine gross energy, proximate (crude protein, crude fat, crude fiber, ash, moisture) and mineral (calcium, phosphorus, magnesium, sodium, potassium, iron, zinc, copper, manganese, and selenium) concentrations according to AOAC (proximates and minerals) and ASTM (gross energy) methods (AOAC, 1996; ASTM, 2013).

Vitamin Analysis

Approximately 0.4 grams of each homogenized ground sample was placed into each of eight test tubes. Samples were extracted according to methods previously described (Stacewicz-Sapuntzakis *et al*., 1987; Coslik *et al*., 2009) and analyzed for retinol and alpha-tocopherol using a Waters™

2695® separations module HPLC, with a Grace/Vydac 201TP54® column and a Waters™ 2487® dual wavelength absorbance detector.

Results and Discussion

Nutritional Composition of Prey Items Analyzed

The name, average item weight, and proximate analysis for each of the invertebrates analyzed are listed in Table 1. Unless otherwise noted, all values are reported on a dry matter basis. Gross energy content varied slightly across species, with bean beetles the highest at 6.8 kcal/g and termites the lowest at 5.6 kcal/g. Crude protein content ranged from 58.2% in bean beetles to 75.3% in fruit flies (*D. melanogaster*). The bean beetle appeared to be the least significant source of moisture, crude protein, and ash, but the highest in fat (26.7%). Macromineral content of the invertebrate items is presented in Table 2. Red wigglers had the highest calcium content (0.69%) and Ca:P ratio (0.71), while bean beetles had the lowest calcium and Ca:P ratio. The trace mineral and vitamin levels of the items analyzed are shown in Table 3. Bean beetles appeared to have the highest zinc and copper content. Bloodworms and red wigglers had the highest selenium content of items in which it was detected. Hornworms of both sizes had some of the lowest trace mineral values despite the appearance of substantially higher ash than the other prey items. Harvester ants appeared to contain more vitamin A (retinol) (2212 IU/kg) compared to the other species and the second highest vitamin E (a-tocopherol) level (175.7 IU/kg). Fruit flies (*D. melanogaster*) were found to have the highest vitamin E (a-tocopherol) content (216.3 IU/kg), while bean beetles contained the least (11.7 IU/kg).

Different sizes of hornworms, crickets, and fruit flies were analyzed for comparison of effect of size on nutrient content. Hornworms were separated into below 1-inch and 1-2-inch length categories considering these sizes are commonly fed to the ectotherm collection at the Fort Worth Zoo. The hornworm sizes appeared similar in proximate content (apparent 1% or less difference in crude protein, fat, and ash) and some minerals. The smaller size (<1 inch) hornworms appeared to be higher in calcium (0.38% versus 0.26%, respectively), sodium (0.15% versus 0.09%, respectively), iron (180 ppm versus 101 ppm, respectively), and manganese (46.6 ppm versus 23.9 ppm, respectively). They may be similar or not different in vitamin E (80.9 IU/kg versus 92.0 IU/kg, respectively), suggesting no clear trend across all nutrients based on size for hornworms. The moisture, crude protein, and ash values were similar for both sizes of crickets. The ¼-inch crickets appeared to be higher in most other nutrients including fat, calcium, magnesium, iron, manganese, selenium, and vitamin A. Only crude fiber and vitamin E may have been lower in the smaller crickets suggesting a possible effect of size (or stage of growth) on nutrient content in this species of cricket. Other authors have suggested a possible larger gastrointestinal tract compared to overall body size in younger crickets may contribute to higher nutrient levels compared to adults (Hunt *et al*., 2001). Comparing the different sizes of fruit flies, which were different species, both may have been similar in moisture, crude fiber and ash content, but the smaller *D. melanogaster* appeared to be notably higher in iron (707 ppm versus 257 ppm, respectively), zinc (312 ppm versus 217 ppm, respectively), and manganese (22.2 ppm verses 14.7 ppm, respectively) than the larger *D. hydei*. Only fat appeared to be notably lower in the smaller *D. melanogaster* (9.7% versus 18.8%, respectively). There may be an effect of size on nutrient content in fruit flies, although species differences could be a factor in the observed differences between values.

Some limitations were encountered in this study. Multiple samples of each species were not obtained. Several values were not detected by commercial lab analysis. The sample size of fruit flies (*D. hydei*) was too small to allow for vitamin A (retinol) and vitamin E (a-tocopherol) analysis.

Comparison to Literature Values

Comparison to prior published data was limited to only studies of non-gut loaded invertebrates. The goal of this study was determine the inherent nutrition content of each species provided in a captive setting without supplementation. As reviewed by Livingston *et al.* (2014), gut loading and dusting can affect the nutrient content of invertebrates.

The banded cricket was evaluated in this study based on its use (in place of the common domestic cricket) at the Fort Worth Zoo due to the supplier's selection of that species for resistance to a common disease affecting the broadly used *Acheta domesticus*. Although a different species, *G. sigillatus* nymphs (26 mg and 136 mg average weights) have similar protein, fat, and ash content compared to *A. domesticus* nymphs (97 mg average weight) (Finke, 2002). Calcium content in the ½-inch crickets (0.12%) was equal to that reported by Finke (2002) but appeared to be much higher in the ¼-inch size (0.24% versus 0.12%, respectively). The crickets from this study had a higher Ca:P ratio, with ¼ inch having the highest ratio (0.23) and Finke's (2002) the lowest (0.11). Vitamin E appeared to be considerably higher in both the ½- and ¼-inch crickets (77.4 and 48.2 IU/kg, respectively) from this study compared to that reported by Finke (2002) (28.1 IU/kg).

The harvester ants in this study contained similar moisture and gross energy to that reported for *Pogonomyrmex* species by Dimmitt & Ruibal (1980). Compared to the related *P. barbatus* analyzed by Ramos-Elorduy *et al.* (1997), the ants in this study were considerably higher in crude protein (73.6% versus 45.8%, respectively) and fiber (32.7% versus 2.8%, respectively), but much lower in fat (8.4% versus 34.3%, respectively) and ash (4.4% versus 9.3%, respectively). Comparing harvester ants to other omnivorous ants, including black ants (*Carebara vidua*) (Ayieko *et al*., 2012), and two species of velvety tree ants (*Liometopum apiculatum* and *L. occidentale*) (Ramos-Elorduy *et al*., 1998, 2001, 2007), the harvesters appeared to be much higher in protein (73.6% versus 37.3-42.5%, respectively), considerably lower in fat (8.4% versus 36.2-49.5%, respectively), and higher in ash (4.4% versus 1.6-3.1%, respectively). Further, the harvester ants appeared to be higher in calcium (0.44% versus 0.22-0.26%, respectively), Ca:P ratio (0.64 versus 0.21, respectively), and higher or similar in most other minerals. The vitamin A reported for these other species of ants varied widely between them (29.3-25,566 IU/kg), with harvester ants from this study falling within that range (2212 IU/kg). Harvester ants appear to be much higher in vitamin E than black ants (175.7 IU/kg versus 5.9 IU/kg, respectively).

The termites in this study were compared to published data from workers of related species *Armitermes euamignathus (*Redford & Dorea, 1984*)*, *Nasutitermes sp.* (Redford & Dorea, 1984; Oyarzun *et al*., 1996), and *Termes sp.* (Raksakantong *et al*., 2010) based on those species' similar feeding ecology to *R. flavipes*. Compared to the range of data from those species, the termite workers in this study were on the high end of protein content (63.5% versus 20.9%-66.7%, respectively), moderate-to-high in crude fat (23.9% versus 2.2%-36.6%, respectively), moderate-to-low in ash (4.7% versus 2.3%-46.1%, respectively), and higher in crude fiber (13.2% versus 6.1%, respectively).

The bloodworms in this study (*Chironomus plumosus*) appeared to be higher in crude protein (63.4% versus 52.8%, respectively), lower in fat (6.2% versus 9.7%, respectively), lower in ash (5.3% versus 11.3%, respectively), and considerably lower in calcium (0.12% versus 0.38%, respectively) and Ca:P ratio (0.12 versus 0.42, respectively) than *Chironomus sp.* analyzed by Bernard & Allen (1997). Among the other minerals, the greatest apparent difference was the much higher iron content reported by Bernard & Allen (1997) than found in this study (2940 ppm versus 758 ppm, respectively).

The fruit flies (*D. melanogaster*) analyzed in this study appeared moderately higher in crude protein (75.3% versus 56.3-70.1%, respectively), lower in fat (9.7% versus 12.6-19.0%, respectively), and of similar ash content (6.2% versus 4.5-7.2%, respectively) compared to published values (Bernard & Allen, 1997; Barker, 1998; Oonincx & Dierenfeld, 2011) The fruit flies in this study appear to be higher in calcium than published values (0.25% versus 0.10-0.17%, respectively), resulting in a slightly higher Ca:P ratio in comparison to the literature (0.19 versus 0.010-0.13, respectively). As in this study, vitamin A was not detected in *D. melanogaster* by Barker et al (1998), but was detected at a low concentration (2.2 IU/kg) by Oonincx & Dierenfeld (2011). Vitamin E appeared to be higher in *D. melanogaster* in this study than reported in literature (216.3 IU/kg versus 23-166 IU/kg, respectively).

Comparison to Amphibian Nutrient Targets

Comparison of the data from this study (on an equal energy basis) to the proposed target nutrient recommendations for amphibians (Ferrie *et al*., 2014) appears to indicate none of the 11 invertebrate items analyzed met the recommended minimum calcium target nor achieved a positive Ca:P ratio. Of the other minerals analyzed, both cricket sizes, bean beetles and 1-2 inch hornworms did not meet the iron target; both sizes of hornworms did not meet the copper target; bean beetles and *D. hydei* fruit flies did not meet the manganese target; and termites, bean beetles, hornworms (both sizes) and fruit flies (both species) did not meet the selenium target (not detected in any of these). None of the items met the proposed vitamin A target, although harvester ants came close. Only harvester ants and *D. melanogaster* fruit flies met the proposed vitamin E target. Overall, the bean beetle appeared to be the most dilute, but highest-energy food item. Consequently, eating to meet an energy need suggests they would be a poor source of most nutrients. In general, harvester ants came closest to hitting nutrient targets. Horned lizards in captivity consuming diets comprised of entirely harvester ants appear to thrive in captivity.

Thus, feeding most of these items (or combinations of these items) would result in a diet deficient in many nutrients including calcium, vitamin A, and vitamin E, which supports routinely supplementing these invertebrates to help provide a nutritionally-complete diet to meet minimum target nutrient levels.

Conclusion

The data from this study add to the growing knowledge base of nutrient content of invertebrate prey items and their contribution to captive insectivore diets. Comparison of the invertebrate species shows varied nutrient composition among them, which supports the approach of incorporating a combination and/or a variety of prey items to provide a broader range of nutrients in captive diets. Comparing these prey items to suggested amphibian target ranges reveals that many items do not provide an adequate diet. This supports the notion that insects raised in captivity are innately deficient in the proper amounts of nutrients suggested to be necessary for health of amphibians. Gut-loading and dusting invertebrate food items to improve their nutritional profiles appears necessary. Future research is needed to expand upon the variety of captive-fed invertebrate prey items analyzed for nutritional content, both in their natural state and supplemented, and to further elucidate the true nutrient requirements of primarily insectivorous species.

Acknowledgements

The authors wish to thank the Fort Worth Zoo Ectotherm Department for their assistance in culturing and collecting some of the invertebrate items analyzed in this study.

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Table 1. Average weight, gross energy and proximate content (dry matter basis) of invertebrate prey items analyzed at Fort Worth Zoo.

Item	**Avg Weight (mg)**	**Gross Energy (kcal/g)**	**Moisture (%)**	**Crude Protein (%)**	**Fat (%)**	**Crude Fiber (%)**	**Ash (%)**
Banded Cricket (1/4 inch)	26	6.3	74.7	68.0	14.3	2.0	4.4
Banded Cricket (1/2 inch)	136	nr[1]	73.9	66.3	10.8	9.3	4.1
Harvester Ant	10	6.0	68.2	73.6	8.4	32.7	4.4
Termite worker	1.5	5.6	77.0	63.5	23.9	13.2	4.7
Bean Beetle	2	6.8	54.3	58.2	26.7	24.5	2.8
Bloodworm	15	nd[2]	83.9	63.4	6.2	nd	5.3
Red Wiggler	308	6.3	82.5	66.8	12.2	nd	7.9
Tobacco Hornworm (<1 inch)	284	nd	86.7	71.0	10.4	nd	8.5
Tobacco Hornworm (1-2 inch)	876	nd	88.3	72.0	9.5	nd	9.1
Fruit Fly (*D. melanogaster*)	0.6	5.9	76.1	75.3	9.7	6.0	6.2
Fruit Fly (*D. hydei*)	1.8	5.8	74.2	62.4	18.8	7.6	4.9

[1] nr, not reported by lab
[2] nd, not detected by analysis

Table 2. Macromineral content (dry matter basis) of invertebrate prey items analyzed at Fort Worth Zoo.

Item	Ca (%)	P (%)	Ca:P	Mg (%)	K (%)	Na (%)
Banded Cricket (1/4 inch)	0.24	1.03	0.23	0.12	1.34	0.40
Banded Cricket (1/2 inch)	0.12	0.92	0.13	0.08	1.15	0.38
Harvester Ant	0.44	0.69	0.64	0.22	0.82	0.19
Termite worker	0.39	0.91	0.43	0.17	1.00	0.35
Bean Beetle	0.07	0.72	0.10	0.13	0.63	nd[1]
Bloodworm	0.12	0.99	0.12	0.12	0.93	0.93
Red Wiggler	0.69	0.97	0.71	0.11	1.14	0.57
Tobacco Hornworm (<1 inch)	0.38	1.73	0.22	0.45	3.46	0.15
Tobacco Hornworm (1-2 inch)	0.26	1.54	0.17	0.43	3.33	0.09
Fruit Fly (*D. melanogaster*)	0.25	1.3	0.19	0.17	1.42	0.29
Fruit Fly (*D. hydei*)	0.27	1.01	0.27	0.16	1.28	0.16

[1] nd, not detected by analysis

Table 3. Trace mineral and vitamin content (dry matter basis) of invertebrate prey items analyzed at Fort Worth Zoo.

Item	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Se (ppm)	Vit A (IU/kg)[1]	Vit E (IU/kg)[2]
Banded Cricket (1/4 inch)	93	169	34.0	63.2	1.18	1106	48.2
Banded Cricket (1/2 inch)	58	158	34.9	30.6	0.61	91.5	77.4
Harvester Ant	216	224	20.4	93.7	1.86	2212	175.7
Termite worker	145	139	27.8	138	nd[3]	43.5	79.9
Bean Beetle	92	346	57.1	15.1	nd	65.6	11.7
Bloodworm	758	98.1	17.4	21.1	3.48	186	29.5
Red Wiggler	447	118	17.1	24.6	2.57	1560	20.3
Tobacco Hornworm (<1 inch)	180	123	12.8	46.6	nd	nd	80.9
Tobacco Hornworm (1-2 inch)	101	98.3	10.2	23.9	nd	nd	92.0
Fruit Fly (*D. melanogaster*)	707	312	28.0	22.2	nd	nd	216.3
Fruit Fly (*D. hydei*)	257	217	25.2	14.7	nd	nm[4]	nm

[1] Vitamin A as retinol
[2] Vitamin E as alpha-tocopherol
[3] nd, not detected by analysis
[4] nm, not measured due to insufficient sample