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RESEARCH ARTICLE

Complete Nutrient Content of Four Species of Commercially Available Feeder Insects Fed Enhanced Diets During Growth

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Commercially raised feeder insects used to feed captive insectivores are a good source of many nutrients but are deficient in several key nutrients. Current methods used to supplement insects include dusting and gut-loading. Here, we report on the nutrient composition of four species of commercially raised feeder insects fed a special diet to enhance their nutrient content. Crickets, mealworms, superworms, and waxworms were analyzed for moisture, crude protein, fat, ash, acid detergent fiber, total dietary fiber, minerals, amino acids, fatty acids, vitamins, taurine, carotenoids, inositol, and cholesterol. All four species contained enhanced levels of vitamin E and omega 3 fatty acids when compared to previously published data for these species. Crickets, superworms, and mealworms contained β-carotene although using standard conversion factors only crickets and superworms would likely contain sufficient vitamin A activity for most species of insectivores. Waxworms did not contain any detectable β-carotene but did contain zeaxanthin which they likely converted from dietary β-carotene. All four species contained significant amounts of both inositol and cholesterol. Like previous reports all insects were a poor source of calcium and only superworms contained vitamin D above the limit of detection. When compared to the nutrient requirements as established by the NRC for growing rats or poultry, these species were good sources of most other nutrients although the high fat and low moisture content of both waxworms and superworms means when corrected for energy density these two species were deficient in more nutrients than crickets or mealworms. These data show the value of modifying the diet of commercially available insects as they are growing to enhance their nutrient content. They also suggest that for most insectivores properly supplemented lower fat insects such as crickets, or smaller mealworms should form the bulk of the diet. Zoo Biol. 34:554–564, 2015.

Keywords: insects; β-carotene; fatty acids; vitamin E; minerals; vitamins; amino acids

INTRODUCTION

Nutrient analysis of commercially bred insects is now available because of their role as food for captive insectivores kept in zoos or as pets by hobbyists [Jones et al., 1971; Martin et al., 1976; Pennino et al., 1991; Barker et al., 1998; Finke, 2002; Oonincx and Dierenfeld, 2011; Finke, 2013]. Nutritional analysis of commercially bred insects suggests they are excellent sources of most nutrients including minerals, amino acids, fatty acids, and vitamins. Nutrients which appear to be low across most species of commercially bred insects include calcium, vitamins A, D, E, thiamin, and omega-3 fatty acids. Additionally some other nutrient concentrations like pyridoxine and several minerals are low in certain species of commercially breed insects. This is supported by reports of proven or suspected nutrient deficiencies in captive bred insectivores including calcium, vitamin A, vitamin D, and thiamin. [Ferguson et al., 1996; Miller et al., 2001; Pessier et al., 2005; Crawshaw, 2008; Hoby et al., 2010; Oonincx et al., 2010; Feldman et al., 2011]. Recently, it has been suggested that in addition to more traditional ways of supplementing the base nutrient content of insects such as dusting and gut-loading, their

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nutritional content might be improved by altering the diet used by the supplier to grow the insect. [Ferrie et al., 2014; Livingston et al., 2014]. So, the purpose of this study is to provide a nutrient analysis of four species of commercially bred insects fed a special feed designed to enhance their nutrient content. While the focus of this study is on the nutrients that were targeted for dietary modification (β-carotene, vitamin E, omega-3 fatty acids [all species], and thiamin [crickets and superworms only]) the insects were analyzed for all known nutrients for comparison to previously published data for these species.

METHODS

Cricket (late instar *Acheta domestica* nymphs), mealworms (late instar larva of the beetle *Tenebrio molitor*) superworms (late instar larva of the beetle *Zophobas mori*), and waxworms (late instar larva of the moth *Galleria mellonela*) were obtained from Timberline Live Pet Foods (Marion, IL 62959). Insects fed special diets and marketed for enhanced nutrient content (Vita-bugs®) were used in this analysis. For crickets and superworms the base diet was modified to increase omega-3 fatty acids (via flaxseed, canola oil, and fish oil), lutein (via corn gluten meal and a yellow carotenoids supplement – Oro Glo™) and vitamin E, thiamin and β-carotene using commercial supplements. For mealworms the base diet was modified to increase omega-3 fatty acids (via canola oil and fish oil), and vitamin E and β-carotene using commercial supplements. For waxworms the base diet was modified to increase omega-3 fatty acids (via flaxseed, canola oil, and fish oil), and vitamin E and β-carotene using commercial supplements. Twenty five individuals of each species were weighed to the nearest mg to determine average weight. For each species tested approximately 500–800 g of live insects were shipped to a commercial analytical laboratory (Covance Laboratories, Madison, Wisconsin 53707) for analysis of moisture, protein, fat, ash, acid detergent fiber (ADF), total dietary fiber (TDF), minerals, amino acids, fatty acids, vitamins, cholesterol, inositol, and selected carotenoids. The insects were shipped live and kept frozen at −70°C upon receipt until analyzed. All insects were fasted for approximately 24 hr prior to analysis to minimize the effects of food retained in the gut. All values reported are the result of a single analysis and the analytical methods used to analyze these insect samples are shown in the appendix. Nitrogen free extract (NFE) was calculated as 100 minus the sum of moisture, crude protein, crude fat, ash, and ADF. Metabolizable energy (kcal/kg) was calculated using standard calculations ([g of crude protein $\times$4.0] + [g of crude fat $\times$9.0] + [g of NFE $\times$4.0]). Protein recovery was calculated as the sum of the amino acids plus taurine divided by crude protein (nitrogen times 6.25). Fatty acid recovery was calculated as sum of the fatty acids divided by crude fat. The nutrient content of these insects was compared with NRC recommendations for both the laboratory rat (growth) and for domestic poultry (0–3 week old broiler chickens) [NRC, 1994; NRC, 1995] since these are likely close to true requirements and can serve as reasonable models for many captive insectivores. All comparisons were adjusted for insect energy density (nutrients/1,000 kcals). Detailed nutrient data on an as is, dry matter and per 1,000 kcal basis and comparisons to the requirements of the laboratory rat, broiler chickens, trout, growing dogs, and growing cats are shown in appendix Tables 1–4 [NRC, 1994; NRC, 1995; NRC, 2006; NRC, 2011].

RESULTS

Insect weights, proximate analysis, and metabolizable energy content are shown in Table 1. As expected all four insect species were fairly high in protein and fat with the superworms and waxworms containing much more fat than mealworms and crickets. As a result of the high fat and low moisture content waxworms contained only 86% of the protein recommended for broiler chickens. All four species contained similar levels of fiber as measured by both ADF and TDF. As expected, the ash content of all four insect species was low. Energy content (as is) varied widely with cricket nymphs containing the least amount of energy while waxworms contained the most.

Mineral analyses are shown in Table 2. All four species were a poor source of calcium containing less than 50% of

TABLE 1. Average weight and proximate analysis of selected insect species on an as is basis

	Crickets	Mealworms	Superworms	Waxworms
Weight (mg/insect)	349	78	558	235
Moisture (g/kg)	725	689	630	641
Crude Protein (g/kg)	165	186	186	144[a]
Crude Fat (g/kg)	79	82	14.4	19.4
NFE (g/kg)	1	9	7	−2
Total Dietary Fiber (g/kg)	10.9	12.9	14.4	<7.5
Acid Detergent Fiber (g/kg)	17.8	22.3	23.4	15.2
Ash (g/kg)	12.2	11.3	9.3	8.0
Metabolizable Energy (kcal/kg)	1,375	1,520	2,069	2,322
Metabolizable Energy (cal/insect)	480	119	1,154	546

If no superscript is shown the insect meets the requirement of both rats and broiler chickens.
[a]Value is 50–100% of the NRC requirements of 0–3 week old broiler chickens.

TABLE 2. Mineral content (mg/kg) of selected insect species on an as is basis

Mineral	Crickets	Mealworms	Superworms	Waxworms
Calcium	366[a,c]	156[a,c]	262[a,c]	203[a,c]
Phosphorus	2,190	2,640	2,090[d]	1,930[d]
Magnesium	193[d]	620	435	266[b,d]
Sodium	1,110	225[c]	385[c]	<123[a,c]
Potassium	2,850	3,350	2,860	2,310
Chloride	2,210	1,760	1,630	760[d]
Iron	17.5[d]	20.7[d]	19.9[c]	9.6[a,c]
Zinc	54.3	49.5	30.2	25.9[d]
Copper	6.3	8.3	3.6[d]	3.3[d]
Manganese	8.7[c]	3.2[b,c]	3.7[b,c]	2.7[a,c]
Iodine	0.145	<0.10[a,c]	<0.10[a,c]	<0.10[a,c]
Selenium	0.133	0.123	0.103	0.177

[a]Value is <50% of the NRC requirement of rats for growth.
[b]Value is 50–100% of the NRC requirement of rats for growth.
[c]Value is <50% of the NRC requirements of 0–3 week old broiler chickens.
[d]Value is 50–100% of the NRC requirements of 0–3 week old broiler chickens.

the requirements for both rats and poultry. Iron levels met the requirements established for growing rats in all species except waxworms but would be considered deficient for poultry ranging from 17% to 54% of the requirement. Manganese was low in all species with only crickets meeting the requirement of the growing rat whereas no insect species met the requirement of poultry. Only crickets contained detectable levels of iodine. Other mineral deficiencies observed were mostly restricted to waxworms and to a lesser degree superworms as a result of the high fat and energy content of these two species.

Amino acid analyses are shown in Table 3. These insects appear to be a good source of most of the essential amino acids. Both waxworms and superworms contained only 53% and 74% of the sulfur amino acid (methionine plus cysteine) requirement of poultry. Only crickets contained detectable amounts of taurine. When compared to NRC requirements for rats or poultry the first limiting amino acid for all four species of insects appears to be the sulfur amino acids methionine and cystine. Protein recovery for all species of insects tested was excellent ranging from 95.5% to 103.2%.

Vitamin analyses are shown in Table 4. None of the insects contained detectable levels of vitamin A/retinol or vitamin D_3, and only superworms contained detectable levels of vitamin D_2. Vitamin E content was high ranging from 53.7 to 163.0 IU vitamin E/kg. Only crickets contained vitamin K above the threshold for detection (50 mg/kg). All insects tested contained substantial quantities of most of the B-vitamins and choline, although lower levels of both thiamin (50–100% of the requirements) and vitamin B_{12} (<50% of the requirements) were found in mealworms, superworms, and waxworms. Only crickets contained sufficient thiamin and vitamin B_{12} to meet the requirements of both rats and poultry. All species contained significant quantities of inositol.

Table 5 shows the fatty acid composition of the various insect species. All insects contained adequate levels of the essential fatty acid linoleic acid (18:2 n-6) to meet NRC recommendations for rats. In addition, all insects also

TABLE 3. Amino acid content (g/kg) of selected insect species on an as is basis

Amino acid	Crickets	Mealworms	Superworms	Waxworms
Alanine	15.0	16.4	14.4	11.8
Arginine	13.6	13.8	12.9	11.8
Aspartic acid	13.0	15.2	16.2	14.3
Cystine	1.61	1.63	1.75	1.09
Glycine	8.83	10.0	9.29	7.94
Glutamic acid	18.9	21.3	24.4	18.2
Histidine	3.64	5.59	5.92	3.17
Isoleucine	6.65	8.35	8.81	6.58
Leucine	11.7	14.0	13.6	10.8
Lysine	9.56	10.7	10.7	8.56
Methionine	2.74	2.55	2.55[a,b]	2.40[a,b]
Phenylalanine	5.87	6.54	7.40	5.80
Proline	9.86	12.8	10.6	10.4
Serine	6.67	8.60	8.12	9.48
Threonine	6.21	7.57	7.47	5.99
Tryptophan	1.44	2.16	2.03	1.63
Tyrosine	10.7	11.9	13.1	9.10
Valine	9.84	12.8	12.3	9.53
Taurine	0.18	<0.1	<0.1	<0.1
Methionine + Cystine	4.35	4.18[b]	4.30[a,b]	3.49[a,b]
Phenylalanine + Tyrosine	16.6	18.4	20.5	14.9
Protein Recovery (as is)	95.5%	97.8%	97.6%	103.2%

[a]Value is 50–100% of the NRC requirement of rats for growth.
[b]Value is 50–100% of the NRC requirements of 0–3 week old broiler chickens.

TABLE 4. Vitamin, choline, and inositol content of selected insect species on an as is basis

Vitamin	Crickets	Mealworms	Superworms	Waxworms
Vitamin A (IU/kg - from retinol)	<1,000[a,c]	<1,000[a,c]	<1,000[a,c]	<1,000[a,c]
Vitamin D_2 (IU/kg)	<40[a]	<40[a]	531	<40[a]
Vitamin D_3 (IU/kg)	<40[a,c]	<40[a,c]	<40[a,c]	<40[a,c]
Vitamin E (IU/kg)	53.7	36.2	163.0	63.3
Vitamin K (mg/kg)	78.4	<50[a,c]	<50[a,c]	<50[a,c]
Vitamin C (mg/kg)	92.0	99.0	101.0	90.0
Thiamin (mg/kg)	2.0	1.1[b]	1.7[b]	1.2[b,d]
Riboflavin (mg/kg)	16.6	8.7	11.2	9.3
Pantothenic Acid (mg/kg)	20.3	15.6	7.0	32.8
Niacin (mg/kg)	29.5	46.5	35.3	33.6
Pyridoxine (mg/kg)	2.13	6.90	3.55	1.74[a,d]
Folic Acid (mg/kg)	1.07	1.55	0.64	0.61
Biotin (mg/kg)	0.21	0.43	0.38	0.29
Vitamin B_{12} (μg/kg)	193.0	1.3[a,c]	9.9[a]	<1.2[a,c]
Choline (mg/kg)	1,020	1,410	1,240	1,550
Inositol (mg/kg)	345	267	223	236

[a]Value is <50% of the NRC requirement of rats for growth.
[b]Value is 50–100% of the NRC requirement of rats for growth.
[c]Value is <50% of the NRC requirements of 0–3 week old broiler chickens.
[d]Value is 50–100% of the NRC requirements of 0–3 week old broiler chickens.

contained significant levels of linolenic acid (18:3 n-3) and lesser amounts of eicosapentaenoice acid (20:5 n-3). The three dominant fatty acids in all four species were oleic acid (18:1), palmitic acid (16:0), and linoleic acid (18:2). Fatty acid recovery was good ranging from 84.8% to 87.9%. Cholesterol levels ranged from 513 to 985 mg/kg.

Selected carotenoids are shown in Table 6. Crickets, mealworms, and superworms all contained β-carotene

TABLE 5. Fatty acid (g/kg) and cholesterol (mg/kg) content of selected insect species on an as is basis

Fatty Acid	Crickets	Mealworms	Superworms	Waxworms
Caprylic 8:0	<0.07	<0.07	0.71	<0.07
Capric 10:0	<0.07	<0.07	0.13	<0.07
Lauric 12:0	<0.07	0.11	<0.07	<0.07
Myristic 14:0	0.59	1.43	1.67	0.42
Myristoleic 14:1	<0.07	<0.07	<0.07	<0.07
Pentadecanoic 15:0	0.10	0.16	0.37	<0.07
Pentadecenoic 15:1	<0.07	<0.07	<0.07	<0.07
Palmitic 16:0	17.2	12.3	35.9	59.7
Palmitoleic 16:1	0.85	0.84	1.37	3.95
Heptadecanoic 17:0	0.19	0.22	0.75	0.09
Heptadecenoic 17:1	<0.07	<0.07	<0.07	<0.07
Stearic 18:0	6.54	2.56	11.5	4.09
Oleic 18:1	16.4	27.3	42.8	79.0
Linoleic 18:2	20.7	24.3	26.4	17.6
Gamma Linoleic 18:3	<0.07	<0.07	<0.07	<0.07
Linolenic 18:3	3.49	1.03	3.76	2.91
Octadecatetraenoic 18:4	<0.07	<0.07	0.09	0.19
Arachidic 20:0	0.19	0.18	0.29	0.16
Eicosenoic 20:1	0.14	0.19	0.18	0.10
Eicosadienoic 20:2	<0.07	<0.07	0.08	0.12
Eicosatienoic 20:3	<0.07	<0.07	<0.07	<0.07
Arachidonic 20:4	0.14	<0.07	0.09	<0.07
Eicosapentaenoic 20:5	0.44	0.22	0.34	0.31
Benhenic 22:0	<0.07	<0.07	0.12	0.08
Erucic 22:1	<0.07	<0.07	<0.07	<0.07
Docosapentaenoic 22:5	<0.07	<0.07	<0.07	<0.07
Docosahexaenoic 22:6	<0.07	<0.07	<0.07	<0.07
Lignoceric 24:0	<0.07	<0.07	<0.07	<0.07
Cholesterol	985	513	450	753
Fat Recovery (as is)	84.8%	86.4%	87.9%	87.0%

TABLE 6. Carotenoid content of selected insect species on an as is basis

	Crickets	Mealworms	Superworms	Waxworms
β-Carotene				
mg/kg	2.72	0.076	1.99	<0.20
IU Vitamin A/kg	4,533	126[a,b]	3,317	<333[a,b]
Lutein (mg/kg)	0.204	<0.20	0.284	1.12
Zeaxanthin (mg/kg)	<0.20	<0.20	<0.20	0.594

[a]Value is <50% of the NRC requirement of rats for growth.
[b]Value is <50% of the NRC requirements of 0–3 week old broiler chickens.

although levels were much lower in mealworms. Lutein was detected in crickets, superworms, and waxworms but not mealworms. Only waxworms contained detectable levels of zeaxanthin.

DISCUSSION

The proximate analysis for crickets, mealworms, superworms, and waxworms are similar to previous reports in the literature for these species [Jones et al., 1971; Martin et al., 1976; Pennino et al., 1991; Barker et al., 1998; Finke, 2002]. Although the mealworms analyzed in this study contain 39% less fat than previously reported [Finke, 2002] that is likely a result of their smaller size (78 vs. 126 mg) as lipid content increases with increasing size/age in mealworm larvae [Finkel, 1948]. Although both acid detergent fiber (ADF) and neutral detergent fiber (NDF) have previously been reported for these species this is the first report of total dietary fiber for commercial feeder insects [Pennino et al., 1991; Barker et al., 1998; Finke, 2002].

The low calcium content of these four species of insects is similar to previous reports and is consistent with most other published data for feeder insects [Jones et al., 1971; Martin et al., 1976; Barker et al., 1998; Finke, 2002; Oonincx and Dierenfeld, 2011; Finke, 2013]. In this study, calcium levels in these four species were only 7–21% and 3–9% of the requirements for rats and poultry respectively.

Phosphorus contents were much higher than calcium levels in all species and are similar to those reported previously [Jones et al., 1971; Martin et al., 1976; Barker et al., 1998, Finke, 2002]. Both superworms and waxworms contained insufficient phosphorus to meet the requirements of poultry (72% and 59% respectively) but levels were adequate for growing rats. Phosphorus from insects is likely to be readily available [Dashefsky et al., 1976] which may compensate for the slightly low levels.

Magnesium content of these species of insects is in general similar to that reported [Martin et al., 1976; Barker et al., 1998; Finke, 2002] although the crickets contained only 58% (per 1,000 kcals) that previously seen for both smaller cricket nymphs and adult crickets [Finke, 2002].

Sodium, potassium, and chloride levels reported for these four species are similar to those previously reported although the value for sodium in mealworms is only 57% that previously seen [Jones et al., 1971; Martin et al., 1976; Finke, 2002]. Only crickets contained sufficient sodium, potassium, and chloride to meet the requirements of both rats and poultry. Sodium was below the detection limits in waxworms which is in-line with previous information for waxworms and consistent with reports for wild Lepidoptera (both pupae and adults) [Studier et al., 1991; Studier and Sevick, 1992; Finke, 2002].

Levels of iron, zinc, copper, and manganese were variable but all values were similar to those previously reported for these four species of insects [Martin et al., 1976; Barker et al., 1998; Finke, 2002]. Crickets, mealworms, and superworms met the iron requirements for rats, but were low for poultry (51%, 54%, and 38% the requirement respectively). Waxworms would be considered deficient for both rats and poultry (47% and 17% of the requirements respectively). For manganese only crickets met the requirement for rats (250%) whereas levels were below the rat's requirement for mealworms, superworms, and waxworms (84%, 70%, and 45% of the requirement respectively). None of the four species met the manganese requirements for poultry with values ranging from 8% to 42% of the requirement. Zinc and copper were adequate in all species for both rats and poultry except for waxworms where both zinc and copper were only 89% and 70% of the requirements for poultry respectively. Iodine was only detected in crickets, and levels were sufficient to meet the requirements of both rats (278% of the requirement) and poultry (121% of the requirement). This pattern is similar to that previously observed although in that report mealworms also contained iodine at relatively low levels [Finke, 2002]. All four species contained sufficient selenium to meet the requirements of both rats and poultry. Although the insects analyzed in this report were "fasted," mineral composition in general, is probably a function of the food sources of the insect, both the minerals absorbed from the diet as well as that remaining in the gastrointestinal tract [Finke, 2015; Oonincx and van der Poel, 2010].

The amino acid patterns reported here are consistent with the amino acid profiles previously reported for these species [Finke, 2002; Finke, 2007; Bednarov et al., 2014]. The analytical data suggests that for insects, total sulfur amino acids are first limiting when used to feed rats. Taurine levels in crickets seen here are similar to those previously seen in crickets [Finke, 2002] and in wild grasshoppers

[Finke, 2015]. The lack of taurine in the other three species is consistent with previous analysis and while taurine was previously detected in mealworms (80 mg/kg as is) that is below the detection limit seen in this study [Finke, 2002]. Although there is only limited data available taurine levels appear to be highly variable in insects and are likely a function of both the species and life stage [Finke, 2002; Ramsay and Houston, 2003; Finke, 2013; McCusker et al., 2014]. In *Drosophila melanogaster*, taurine levels increased from approximately 100 mg/kg in larvae to 700–1,100 mg/kg in adult flies [Massie et al., 1989]. The significance of taurine for captive insectivores is currently unknown although several authors have speculated that it may play a role in prey selection for insectivorous birds feeding their nestlings and subsequently affect their development and behavior [Ramsay and Houston, 2003; Arnold et al., 2007].

The excellent recovery of nitrogen/crude protein as amino acids in all species analyzed here suggests that most of the nitrogen in these insects is from amino acids and that only a small amount of the nitrogen is from chitin or other compounds. This is consistent with previous data for these species as well as data for other feeder insects [Finke, 2002; Finke, 2007; Finke, 2013].

No retinol was detected in these four species consistent with previous reports (the low levels reported by Barker and Pennino are below the detection limit of this assay) [Jones et al., 1971; Barker et al., 1998; Pennino et al., 1991; Finke, 2002]. This is consistent with most reports for both commercially raised and wild insects. Significant levels of vitamin A have been reported in only a few species of insects [Pennino et al., 1991; Oyarzun et al., 1996; Finke, 2002]. Locusts fed a grass diet supplemented with wheat bran and fresh carrots contained significantly more retinol than those fed only a grass diet, but the retinol levels (110–190 μg retinol or 366–633 IU Vitamin A/kg dry matter (DM) for all locusts are well below the requirements of the rat [Oonincx et al., 2010]. Much like the results observed by Oonincx for locusts, the retinal content of Vita-bug® crickets was about 50% higher than that for crickets fed a regular commercial cricket diet although both levels were fairly low (320 and 212 IU vitamin A/kg DM respectively) [Finke unpublished data]. Retinoids are only found in the compound eyes of insects where they are synthesized from their carotenoid precursors and the retinoid synthesized (typically either retinal or 3-OH retinal) is species specific [Smith and Goldsmith, 1990; Seki et al., 1998]. For that reason it is not surprising that retinol is rarely detected in whole insects. A better understanding of the vitamin A content of insects and the utilization of various insect retinoids and carotenoids as a source of vitamin A is important as vitamin A deficiency has been reported in several species of captive insectivores [Ferguson et al., 1996; Miller et al., 2001; Pessier et al., 2005; Hoby et al., 2010; Brenes-Soto and Dierenfeld, 2014; Clugston and Blaner, 2014].

None of the insects sampled contained detectable levels of Vitamins D_3 and only superworms contained Vitamin D_2. Previously vitamin D was not detected in these species but the threshold for detection was 250 IU/kg where in this report the detection limit is 80 IU/kg. Similar to these data Oonincx reported values of 150 IU vitamin D_3/kg DM for mealworms [Oonincx et al., 2010]. The values reported by Oonincx for crickets (934 IU vitamin D_3/kg DM) were much higher than the values reported here although these results may be in part due to the residual food remaining in the crickets gut [Oonincx et al., 2010]. The vitamin D requirements for many species can be met though exposure to specific wavelengths of ultraviolet light so dietary concentration of vitamin D in feeder insects may be less critical.

Vitamin E levels are considerably higher than those previously observed for these species [Pennino et al., 1991; Barker et al., 1998; Finke, 2002]. Published data shows vitamin E contents ranging from 9.6 to 26.9 IU/kg (as is) for crickets, less than 5–33.1 IU/kg (as is) for mealworms, 7.7–13.8 IU/kg (as is) for superworms, and 13–103.9 IU/kg (as is) for waxworms. In a short-term (7 day) feeding trial adding vitamin E to the diet of crickets had no effect on cricket vitamin E content [Pennino et al., 1991]. For mealworms a short-term (7 day) feeding trial resulted in a small but significant increase in vitamin E content in mealworms although it is unclear if these results might simply be due to the residual food in the gut [Pennino et al., 1991]. When swine are fed diets with increased levels of vitamin E during their growth phase elevated amounts of vitamin E were found in various tissues [Asghar et al., 1991a; Asghar et al., 1991b].

Although there is relatively little data available, wild caught insects appear to contain more vitamin E (range approximately 16–171 IU/kg as is) than typical commercial feeder insects and the levels are comparable to those observed here for enhanced feeder insects [Pennino et al., 1991; Oyarzun et al., 1996; Cerda et al., 2001; Arnold et al., 2010]. Dierenfeld has reported vitamin E deficiency in zoo animals and has suggested their diets contain 75–300 IU vitamin E/kg of diet versus 27 and 10 IU/kg diet suggested by the NRC for rats and poultry respectively [Dierenfeld, 1989, 1994]. Given that the vitamin E requirements are a function of both the amount and the type of dietary fat it seems likely that the current NRC recommendations for rats and poultry are probably not appropriate for animals fed high fat insects.

There are no previous reports regarding the vitamin K content of commercial feeder insects. Only crickets contained detectable levels of vitamin K (78.4 mg/kg). The detection limit of this assay is high (>50 mg/kg) relative to the requirements for rats (1 mg/kg diet) or poultry (0.5 mg/kg diet) so it is unclear as to how to properly interpret these results relative to the requirements of insectivores.

The vitamin C values reported here are 4-to more than 10 times those previously reported for these four species [Jones et al., 1971; Finke, 2002]. The reason for this difference is unknown. Although vitamin C is not a required nutrient for rats or poultry it is required for guinea pigs, fish, and some species of primates [NRC, 1995; NRC, 2011]. The vitamin C levels reported here would easily meet the recommendations for trout with values ranging from 775% (waxworms) to 1338% (crickets) of the requirements [NRC, 2011].

For thiamin the large cricket nymphs analyzed in this report contained 400% and 900% more thiamin than that previously reported for adult crickets and small nymphs respectively [Finke, 2002]. Likewise for superworms, values reported here were 173% higher than those reported previously [Finke, 2002]. The data is notable as the diets of both of these two species was modified to include more thiamin in an effort to increase levels in the insect. In contrast the thiamin levels in both mealworms and waxworms were about half that previously reported for these species although their diet was not modified. The reason for this is currently unclear although for waxworms the previous analysis were prepuape and this analysis were larvae. The limit of detection for this assay is 0.1 mg thiamin/kg and the relative standard deviation (RSD) is 3.9% in an egg noodle matrix. Thiamin in feeder insects is of interest since thiamin deficiency has been reported in Puerto Rican Crested Toads (*Bufo lemur*) [Crawshaw, 2008]. In that study, toads recovered when given a supplement containing calcium, glucose, and thiamin while those given a supplement containing calcium and glucose showed no improvement. Presumed thiamin deficiency has also been observed in anoles (*Anolis* sp) [Feldman et al., 2011]. Affected anoles recovered within two days after being injected with a B-vitamin complex. For rats the thiamin requirement increases with increasing levels of dietary carbohydrates so for thiamin, rats or poultry may not be the best model for insectivores consuming a diet containing little carbohydrates. A thiaminase has been reported in both Japanese silkworm larvae (*Bombyx mori*) and African silkworm pupae (*Anaphe* spp) [Nishimune et al., 2000]. The extent to which thiaminases are found in commercially produced feeder insects and their potential effect on insectivores is currently unknown.

Values for all of the other B-vitamins were similar to those previously reported for these species with only a couple of minor exceptions. Both crickets and superworms contained more vitamin B_{12} (crickets 212% and superworms 135%) than previously reported although no specific alteration in dietary vitamin B_{12} content was made. For superworms and mealworms pantothenic acid levels were much lower (superworms 64% and mealworms 41%) than those reported previously [Finke, 2002]. The reason for these changes is unclear although it may represent biological variation, diet variation since natural ingredients are used or the variation inherent in the assay (the limit of detection for this assay is 0.4 mg pantothenic acid/kg and the RSD is 4.1% in an infant formula matrix). These data in conjunction with previous research suggests that most insect species are a relatively good source of most B-vitamins although the high fat content of many insect larvae may mean that when values are expressed on a per unit energy basis they would be considered deficient.

There is limited information regarding the choline content of insects but the data available suggests insects are rich sources of choline [Finke, 2002, 2013, 2015]. Choline values reported here range from 316% to 489% and 148% to 228% of the requirement for rats and poultry respectively.

There are no previous reports regarding the inositol content of commercial feeder insects. Although inositol is not a required nutrient for rats or poultry it is required for fish. The inositol levels reported here would meet the recommendations for trout with values ranging from 136% (waxworms) to 335% (crickets) of the requirements [NRC, 2011].

The fatty acid pattern observed for these insects differs from that previously observed primarily with regard to linolenic and eicosapentaenoic acid. These data are consistent with previous literature reports that the fatty acid content of insects and other monogastric animals like pigs and poultry can be modified by dietary means. [Thompson and Barlow, 1972; Cookman et al., 1984; St-Hilaire et al., 2007]. When expressed as a percentage of the total fatty acids to compensate for differences in fat content between studies, insects in this study contained a much higher proportion of linolenic acid (LNA - 18:3 n-3) than previously reported (crickets 5.2 versus 1.0%; mealworms 1.5% vs. 1.1%; superworms 3.0% vs. 0.6%, and waxworms 1.7% vs. 1.5%) [Finke, 2002]. Also unlike previous reports all four species of insects in this study contained eicosapentaenoic acid (EPA -20:5 n-3) with levels ranging from 0.2% to 0.7% of the total fatty acids. No docosahexaenoic acid (DHA -22:6 n-3) was detected in these insects even though their diets contains significant quantities of DHA [Finke unpublished results]. EPA and DHA are not typically found in terrestrial insects but usually make up a significant proportion of the total fatty acids in aquatic insects [Sushchik et al., 2003; Gladyshev et al., 2011; Zinchenko et al., 2014]. It has been speculated that aquatic insects may serve an important function in transferring long chain omega-3 fatty acids from aquatic to terrestrial environments [Gladyshev et al., 2011]. The increased levels of both LNA and EPA means the omega-6:omega-3 fatty acid ratio of these insects is much lower than previously reported (crickets 5 vs. 39; mealworms 19 vs. 25; superworms 6 vs. 30, and waxworms 4 vs. 14) [Finke, 2002]. The amount of omega-3 fatty acids and the omega-6 to omega-3 ratio has been implicated as being beneficial in a large number of species due to their role in cell membrane function, gene expression, and inflammation [Schmitz and Ecker, 2008]. It is unclear if they might confer similar benefits to insectivores.

There are no previous reports regarding the cholesterol content of commercial feeder insects. The values seen here range from 450 mg/kg (superworms) to 985 mg/kg (crickets). These values are similar to beef and pork but lower than poultry (beef 650–750 mg/kg; pork 680–720 mg/kg; poultry 1,040–1,430 mg/kg) [USDA, 2015].

As shown in Table 6 unlike previous analysis of these insects, crickets, mealworms, and superworms all contained β-carotene whereas waxworms did not [Jones et al., 1971; Finke, 2002]. The reason for the much lower levels of β-carotene found in mealworms is unclear but may be a function of their age/size. In Drosophila carotenoid absorption is facilitated by the NinaD gene which encodes for a scavenger receptor in the midgut [Voolstra et al., 2006]. In Drosophila NinaD is expressed during late larval stage, so if mealworms have a similar pattern of expression older larger mealworm larvae may contain more carotenoids than younger smaller larvae. The ability of most insectivores to convert β-carotene to retinol is unknown but the gene involved in the cleavage of β-carotene into two molecules of retinal (BCMO1 -β-carotene 15, 15′-monooxygenase) is widely conserved across the animal kingdom including being detected in several species of insectivores [Sayers et al., 2009]. Although the efficiency which insectivores might convert β-carotene to retinol is unknown, using typical conversion efficiencies of 1 International Unit (IU) of vitamin A equals 0.6 μg of β-carotene shows crickets, mealworms, and superworms would contain 4,533, 126, and 3,317 IU of vitamin A respectively. This would be equivalent to 566%, 14%, and 275% of the vitamin A requirements of the rat and 703%, 18%, and 342% of the vitamin A requirements of poultry. In a study with adult cane toads *Bufo marinus*, and Cuban tree frogs, *Osteopilus septentrionalis*, McComb was unable to show any β-carotene 15,15′-monooxygenase activity in either liver or intestinal tissues [McComb, 2010]. The tissues in this study were frozen prior to analysis which may have affected these results.

In a long term feeding trial Oonincx was also able to enhance the β-carotene content of locusts fed a grass diet supplemented with wheat bran and fresh carrots compared to those fed a grass only or grass and wheat bran diet [Oonincx and van der Poel, 2010]. Since the locusts were not fasted prior to analysis it is unclear how much of the β-carotene was incorporated into the tissue of the insects and how much was simply due to residual food in the gut. The fasted cricket nymphs analyzed in this study contain 38% more β-carotene (2.72 vs. 1.97 mg/kg as is) than the gut-loaded locust nymphs fed grass plus carrots [Oonincx and van der Poel, 2010]. Likewise in a short-term (4 day) feeding trial Oglivy was able to enhance the carotenoid content of three species of crickets fed vegetables or a commercial fish food although it appears most of the enhancement in carotenoid content was a result of the food retained in the gut [Ogilvy et al., 2011]. In contrast, the insects in this study were fasted so the enhancement in β-carotene content is mostly or entirely a result of incorporation into the insect's tissues.

The lack of β-carotene in waxworms is likely a result of conversion of dietary β-carotene to lutein and zeaxanthin. The chromophore used for visual function by insects is species specific [Smith and Goldsmith, 1990] with Orthopetera (including crickets) and Coleoptera (including the adults of both mealworms and superworms) using retinal which can be synthesized by cleaving one molecule of β-carotene into two molecules of retinal. In contrast, Lepidoptera (including waxworms) use 3-OH retinal as their chromophore which is synthesized from zeaxanthin. So for insect species like waxworms and Drosophila that use 3-OH retinal as their chromophore, dietary β-carotene is first converted to zeaxanthin which is then used for 3-OH retinal synthesis [Giovannucci and Stephenson, 1999; Voolstra et al., 2010]. In insects retinoid synthesis from carotenoid precursors only occurs in the compound eye so insect larvae, which lack compound eyes, do not contain retinoids [Giovannucci and Stephenson, 1999; Voolstra et al., 2010]. Drosophila accumulate carotenoids during the larval stage which are then converted to retinoids during the pupal stage when the compound eyes are formed [Voolstra et al., 2010; Von Lintig, 2012]. Although retinal has significant vitamin A activity it is unclear if 3-OH retinal would be a viable source of vitamin A for insectivores.

In addition to β-carotene, crickets, and superworms also contained lutein but no zeaxathin whereas waxworms contained relatively high levels of both lutein and zeaxanthin. The lutein in crickets and superworms is likely accumulated from their diet whereas the lutein and zeaxanthin found in waxworms is likely a combination of accumulation from the diet and synthesis from dietary β-carotene. Wild caught insects contain significant amounts of a variety of carotenoids some of which may serve as a source of vitamin A for insectivores [Arnold et al., 2010; Eeva et al., 2010; Helmer et al., 2015; Newbrey et al., 2013]. In addition to their role as precursors for vitamin A carotenoids may play other important roles in coloration, immune response, and reproduction in insectivores [McGraw and Toomey, 2010; Ogilvy et al., 2012; Brenes-Soto and Dierenfeld, 2014].

CONCLUSIONS

Commercially raised feeder insects are in most cases likely fed a least-cost diet designed to maximize growth and reproduction at the lowest cost without regard to the nutrient content of the feeder insect. These data clearly show that by changing the diet fed to the insect during growth the nutrient content of the feeder insect can be substantially altered. Although not all nutrients (i.e. calcium) can easily be changed these data suggest that the fatty acid composition, vitamin E concentrations, carotenoid content, and perhaps some B-vitamin concentrations in live insects can be altered by changing the diet fed to the insect while it is actively growing. In many cases, the nutrient content of insects fed enhanced diets closely mimics the nutrient content of wild insects (i.e. vitamin E, carotenoids, and fatty acids profiles). This technique has the potential to substantially improve the nutritional value of commercial feeder insects when used as food for captive insectivores.

These data also provide additional guidance regarding the use of certain species in captive feeding programs

[Sincage, 2012]. While high fat insect larvae like waxworms contain high levels of many nutrients when evaluated on an as is or DM basis, their high fat content means that when nutrients are adjusted for energy density they would likely be deficient in many nutrients. As such they should probably not form the bulk of a diet for most healthy captive insectivores unless properly supplemented. High fat/low moisture content insect larvae may, however, be appropriate as part of a mixed diet or as the main component of a diet for an unhealthy animal where the primary nutritional goal is to increase energy intake. Thus, a mixed diet using a variety of different insect species that have been properly “gut loaded” or “dusted” would seem to offer the best hope of providing the appropriate nutrition to captive insectivores.

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APPENDIX

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