

# Complete Nutrient Composition of Commercially Raised Invertebrates Used as Food for Insectivores

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A variety of invertebrates are commonly fed to insectivorous animals by both zoos and hobbyists, but information as to the nutrient composition of most commercially raised species is limited. Adult house crickets, house cricket nymphs (*Acheta domesticus*), superworms (*Zophobas morio* larvae), giant mealworm larvae, mealworm larvae and adult mealworms (*Tenebrio molitor*), waxworm larvae (*Galleria mellonella*), and silkworm larvae (*Bombyx mori*) were analyzed for moisture, crude protein, crude fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals, amino acids, fatty acids, and vitamins. Earthworms (*Lumbricus terrestris*) were analyzed for moisture, crude protein, crude fat, ash, ADF, NDF, minerals, amino acids, and vitamins A and D<sub>3</sub>. Proximate analyses were variable, with wide ranges found for moisture (57.9–83.6%), crude protein (9.3–23.7%), crude fat (1.6–24.9%), ADF (0.1–7.4%), NDF (0.0–11.5%), and ash (0.6–1.2%). Energy content ranged from a low of 674 kcal/kg for silkworms to 2,741 kcal/kg for waxworms. Using an amino acid scoring pattern for rats, the first limiting amino acid for all invertebrates tested was the total sulfur amino acid methionine+cystine. Deficiencies by nutrient (% of samples deficient vs. NRC requirements for rats on a dry matter (DM) basis) were as follows: calcium (100%), vitamin D<sub>3</sub> (100%), vitamin A (89%), vitamin B<sub>12</sub> (75%), thiamin (63%), vitamin E (50%), iodine (44%), manganese (22%), methionine-cystine (22%), and sodium (11%). Deficiencies by invertebrate species (number of nutrients deficient vs. the NRC requirements for rats on a DM basis) were as follows: waxworms (9), superworms (8), giant mealworm larvae (7), adult mealworms (6), mealworm larvae (5), adult house crickets (4), house cricket nymphs (4), silkworms (4), and earthworms (4). These data provide a basis for determining nutrient intake of captive insectivores, and will aid in the development of gut-loading diets to provide captive insectivorous animals with appropriate levels of necessary nutrients. Zoo Biol 21:269–285, 2002. © 2002 Wiley-Liss, Inc.

**Key words:** insects; minerals; vitamins; amino acids; fatty acids

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## INTRODUCTION

Insects are an important food source for both animals and humans, and references to their nutritional value are found in a number of articles across a range of scientific disciplines [for reviews, see DeFoliart, 1989; Bukkens, 1997]. While most of these studies focused on the protein and energy content of insects, some included more extensive analyses of the minerals, vitamins, fatty acids, and amino acids found in insects.

Since the 1970s insects have been evaluated as a potential feedstuff for poultry and other food-producing animals. The insects evaluated include house flies [Calvert et al., 1969; Teotia and Miller, 1974; Ocio and Vinaras, 1979; Onifade et al., 2001], Mormon crickets [Finke et al., 1984; Finke et al., 1987], silkworm larvae [Ichhponani and Malik, 1971; Fagoonee, 1983; Lin et al., 1983; Rao, 1994], house crickets [Nakagaki et al., 1987; Finke et al., 1989], and several other species [Phelps et al., 1975; Landry et al., 1986; Finke et al., 1989]. Most of these data evaluate the nutritional value of dried insect meals, not intact insects; but the data pertaining to protein quality evaluations are important as they represent evaluations based both on amino acid analysis and animal feeding trials using both poultry and laboratory rats.

More recently the nutritional content of selected species of cultured insects has been studied because of their role as food for captive insectivorous reptiles, birds, and mammals kept either in zoos or as pets by hobbyists [Jones et al., 1972; Martin et al., 1976; Frye and Calvert, 1989; Pennino et al., 1991; Barker, 1997]. Most of these studies have concentrated on analyses of moisture, protein, fat, ash, calcium, and phosphorus, although some more extensive analyses have been published [Barker et al., 1998].

Unlike many free-ranging animals that feed on a variety of different species, animals in captivity may be fed only one or two species of invertebrates. As such they are likely to be more prone to nutritional deficiencies, especially since these insects are probably fed a standardized diet. For these reasons it was decided to do a complete nutritional analysis of invertebrates commercially raised as food for captive animals and compare these values to requirements established for the laboratory rat. In addition to providing valuable information concerning the nutrient intake of captive species fed these invertebrates, these data will also provide a valuable baseline from which to develop diets designed to enhance the nutritional value of these prey species.

## METHODS

Adult house crickets, house cricket nymphs (*Acheta domesticus*), superworms (*Zophobas morio* larvae), giant mealworm larvae (obtained by treating mealworm larvae with juvenile hormone to prevent them from molting into adults), mealworm larvae and adults (*Tenebrio molitor*), waxworms (*Galleria mellonella* larvae), and cultured earthworms (*Lumbricus terrestris*) were obtained from a commercial supplier (Timberline Industries Inc., Marion, IL). All individuals of these species were fasted for approximately 24 hr to clear their gastrointestinal of any residual food. Silkworm eggs (*Bombyx mori*) were obtained from Mulberry Farms Inc. (Fallbrook, CA). The silkworm larvae were fed an artificial diet until they reached a weight of approximately 1,000 mg (about 20–25% of their maximum size), at which time they were frozen. Silkworm larvae were not fasted prior to freezing. Ten to 25 individuals

of each species were weighed as a group. This was repeated 10 times to determine the average weight for the individuals of each species. For each species tested, approximately 1 kg of material was frozen for 48 hr, packed in dry ice, and shipped to a commercial analytical laboratory (Covance Laboratories, Madison, WI) for nutrient analysis. The data presented is from a single analysis and is expressed on an as-is basis. Nitrogen-free extract (NFE) was calculated as 100 minus the sum of moisture, crude protein, crude fat, ash, and acid detergent fiber (ADF). Metabolizable energy was calculated using standard calculations ( $[\text{g of protein} \times 4.0] + [\text{g of fat} \times 9.0] + [\text{g of NFE} \times 4.0]$ ). Protein recovery (as is) was calculated as the sum of the amino acids plus taurine and ammonia divided by total crude protein ( $[\text{nitrogen} \times 6.25]$ ). Protein recovery (corrected) was calculated as protein recovery (as is) minus 13.7% to correct for the molecule of water added to each amino acid during hydrolysis of the peptide bond. Fatty acid recovery (as is) was calculated as the sum of the fatty acids divided by total fat. Fat recovery (corrected) was calculated as fat recovery (as is) minus 10% to correct for the molecule of water added to each fatty acid and for the loss of glycerol during the conversion of triglycerides to free fatty acids. The nutrient content of these invertebrates was compared with National Research Council (NRC) [1995] recommendations for the laboratory rat for growth on a dry matter (DM) basis.

## RESULTS

Animal weights, proximate analysis, and metabolizable energy content are shown in Table 1. As expected, all invertebrates were a good source of protein and contained sufficient quantities to meet NRC recommendations. While all invertebrates contained sufficient fat to meet NRC recommendations, silkworm larvae and earthworms contained quite low levels (8–10% fat, DM basis), whereas mealworm larvae, giant mealworm larvae, superworms, and waxworms all contained high levels of fat (35–60%, DM basis). As expected, invertebrates contained little if any carbohydrates (as determined by NFE), with the exception of silkworm larvae. Since the silkworm larvae were not fasted prior to analysis, most of the carbohydrate probably represents food remaining in the gastrointestinal tract. The silkworm diet contained approximately 60% carbohydrates on a DM basis. Earthworms contained almost no detectable fiber as measured by both NDF and ADF. In contrast, all insect species contained significant quantities of fiber, with the highest levels found in adult mealworms and the lowest levels found in silkworm larvae. Adult crickets contained 40% more NDF than cricket nymphs, but a similar concentration of ADF. Energy content varied widely between species, ranging from 674 kcal/kg for silkworms to 2,741 kcal/kg for waxworms (as is), and 3,796 kcal/kg for adult mealworms to 6,619 kcal/kg for waxworms (DM basis).

Mineral analyses are shown in Table 2. As expected, all invertebrates were poor sources of calcium, with all insect species having less than one-third the dietary levels recommended by the NRC. Earthworms contained slightly higher levels but were still deficient. All invertebrates contained sufficient quantities of phosphorus, and calcium : phosphorus ratios ranged from 1:3.6 for earthworms to 1:16.9 for mealworm larvae. Likewise, all invertebrates provided adequate magnesium, sodium, potassium, and chloride to meet dietary levels recommended by the NRC, with the exception of waxworms, which were slightly deficient in sodium (72% of NRC on a

**TABLE 1. Average weight and proximate analysis of selected invertebrates on an as is basis**

	Superworms <i>Zophobas morio</i>	Giant mealworm (larvae) <i>Tenebrio molitor</i>	Mealworm (larvae) <i>Tenebrio molitor</i>	Mealworms (adult) <i>Tenebrio molitor</i>	Waxworms <i>Galleria mellonella</i>	Silkworms <i>Bombyx mori</i>	Crickets (adult) <i>Acheta domesticus</i>	Crickets (nymph) <i>Acheta domesticus</i>	Earthworms <i>Lumbricus terrestris</i>
Weight (mg/insect)	610	304	126	136	314	1,045	465	97	204
Moisture (g/kg)	579	610	619	637	585	827	692	771	836
Protein (g/kg)	197	184	187	237	141	93	205	154	105
Fat (g/kg)	177	168	134	54	249	14	68	33	16
NFE (g/kg)	11	1	27	-14	-14	44	-8	9	36
Neutral detergent fiber (g/kg)	39	29	57	115	81	11	68	36	ND
Acid detergent fiber (g/kg)	27	25	25	74	34	11	32	22	1
Ash (g/kg)	10	12	9	12	6	11	11	11	6
Metabolizable energy (kcal/kg)	2,423	2,252	2,056	1,378	2,747	674	1,402	949	708

ND, not detected.

**TABLE 2. Mineral content of selected invertebrates on an as is basis**

Mineral	Superworms	Giant mealworm (larvae)	Mealworm (larvae)	Mealworms (adult)	Waxworms	Silkworms	Crickets (adult)	Crickets (nymph)	Earthworms
Calcium (mg/kg)	177 <sup>c</sup>	184 <sup>c</sup>	169 <sup>c</sup>	231 <sup>c</sup>	243 <sup>c</sup>	177 <sup>c</sup>	407 <sup>c</sup>	275 <sup>c</sup>	444 <sup>b</sup>
Phosphorus (mg/kg)	2,370	2,720	2,850	2,770	1,950	2,370	2,950	2,520	1,590
Magnesium (mg/kg)	498	864	801	606	316	498	337	226	136
Sodium (mg/kg)	475	489	537	632	165 <sup>a</sup>	475	1,340	1,350	965
Potassium (mg/kg)	3,160	2,970	3,410	3,400	2,210	3,160	3,470	3,520	1,820
Chloride (mg/kg)	1,520	1,750	1,870	1,910	640	620	2,270	2,220	910
Iron (mg/kg)	16.5	21.5	20.6	21.8	20.9	16.5	19.3	21.2	50.4
Zinc (mg/kg)	30.7	44.5	52.0	46.2	25.4	30.7	67.1	68.0	17.7
Copper (mg/kg)	3.6	6.4	6.1	7.5	3.8	3.6	6.2	5.1	1.5
Manganese (mg/kg)	4.3	3.6	5.2	4.0	1.3 <sup>c</sup>	4.3	11.5	8.9	1.3 <sup>a</sup>
Iodine (mg/kg)	<0.1 <sup>c</sup>	<0.1 <sup>c</sup>	0.17	0.22	<0.1 <sup>c</sup>	<0.1 <sup>c</sup>	0.21	0.28	0.38
Selenium (mg/kg)	0.14	0.13	0.25	0.16	0.11	0.14	0.19	0.10	0.40

<sup>a</sup>Value is 67–100% of the NRC requirements for rats for growth.

<sup>b</sup>Value is 33–67% of the NRC requirements for rats for growth.

<sup>c</sup>Value is 0–33% of the NRC requirements for rats for growth.

DM basis). In general, all species of invertebrates analyzed were good sources of trace minerals, although waxworms and earthworms were deficient for manganese (29% and 71% of NRC on a DM basis). Four insect species—the superworms, giant mealworm larvae, waxworms, and silkworms—contained no detectable iodine.

Amino acid analyses are shown in Table 3. Invertebrates appear to be a good source of essential amino acids, although both superworms and waxworms were slightly below the recommended levels for total sulfur amino acids (methionine+cystine: 79% and 73%, respectively, of the NRC on a DM basis). Crickets contained substantial levels of taurine, while all types and life stages of mealworms contained only a small amount. None of the other invertebrates contained detectable levels of taurine. When compared to NRC requirements for rats, the first limiting amino acid of these invertebrates appears to be the sulfur amino acid methionine+cystine. Except for silkworms, the corrected protein recovery for all species of invertebrates tested was similar, ranging from 72.1% to 86.7%. The reason for the low recovery of protein as amino acids (59.5%) for silkworms is unknown, although it is possible that the food remaining in the gastrointestinal tract may have contributed to these lower recoveries.

Vitamin analyses are shown in Table 4. Silkworms were the only invertebrate tested that contained detectable levels of preformed vitamin A. The origin of the vitamin A is unclear since none was detected in the diet, but it may be a result of conversion of carotenoids in the diet. None of the invertebrates contained detectable levels of  $\beta$ -carotene or vitamin D<sub>3</sub>. None of the mealworms analyzed contained detectable levels of vitamin E. The superworms did not meet NRC recommendations for vitamin E (61% of NRC on a DM basis), although all other insects analyzed met NRC recommendations for rats. All of the invertebrates tested contained substantial quantities of most of the B-vitamins and choline, although the superworms, giant mealworm larvae, adult mealworms, and cricket nymphs and adults did not contain sufficient thiamin to meet NRC recommendations (33%, 72%, 61%, 18%, and 26% of the NRC on a DM basis). No vitamin B<sub>12</sub> was found in waxworms and silkworms, while only low levels were detected in superworms, giant mealworm larvae, mealworm larvae, and adult mealworms (18%, 6%, 22%, and 28% of the NRC on a DM basis, respectively). Both cricket nymphs and adult crickets contained sufficient vitamin B<sub>12</sub> to meet NRC requirements.

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) to meet NRC recommendations for rats. In addition, all insects also contained detectable levels of linolenic acid (18:3), which is required by the rat. Waxworms were unique in that almost 90% of the total fatty acids were either oleic or palmitic. For all other insects tested the three primary fatty acids were oleic, palmitic, and linoleic. Fatty acid recovery (corrected) ranged from 71.3% to 86.1%.

## DISCUSSION

The proximate analyses for earthworms, mealworm larvae, superworms, waxworms, and crickets are similar to previous reports in the literature [Jones et al., 1972; Martin et al., 1976; Pennino et al., 1991; Barker, 1997; Zhenjun et al., 1997; Barker et al., 1998]. As expected, cricket nymphs contained more moisture and less fat and protein than adult crickets. Barker [1997] observed a similar pattern for pro-

**TABLE 3. Amino acid content of selected invertebrates on an as is basis**

Amino acid	Superworms	Giant mealworm (larvae)	Mealworm (larvae)	Mealworms (adult)	Waxworms	Silkworms	Crickets (adult)	Crickets (nymph)	Earthworms
Alanine (g/kg)	14.3	13.7	15.4	18.1	9.4	4.2	18.0	13.7	5.4
Aspartic acid (g/kg)	15.8	16.2	15.2	16.6	13.4	6.1	17.2	10.9	9.4
Arginine (g/kg)	9.6	10.3	9.7	10.2	7.1	3.9	12.5	9.4	6.1
Cystine (g/kg)	1.5	1.5	1.6	1.6	1.1	0.8	1.7	1.3	1.5
Glycine (g/kg)	9.5	9.9	10.4	20.0	7.4	5.6	10.4	8.1	5.5
Glutamic acid (g/kg)	24.2	22.8	21.1	22.8	19.5	9.3	21.5	16.0	13.3
Histidine (g/kg)	6.0	6.5	5.9	6.8	3.3	2.4	4.8	3.4	2.3
Isoleucine (g/kg)	9.3	8.6	9.4	10.3	6.3	3.0	9.4	6.6	3.8
Leucine (g/kg)	19.1	14.3	19.9	19.6	12.4	4.9	20.5	14.7	7.5
Lysine (g/kg)	10.3	11.2	10.2	10.5	7.9	4.4	11.0	8.3	6.6
Methionine (g/kg)	2.1	2.6	2.4	3.0	2.2	1.3	3.0	2.0	1.9
Methionine + cystine (g/kg)	3.6 <sup>a</sup>	4.1	4.0	4.6	3.3 <sup>a</sup>	2.1	4.7	3.3	3.4
Phenylalanine (g/kg)	6.8	7.5	6.6	6.2	5.3	2.7	6.5	4.3	3.7
Proline (g/kg)	10.8	12.1	13.0	15.0	9.5	3.2	11.5	8.5	3.5
Serine (g/kg)	9.2	9.1	9.6	9.8	10.5	3.4	10.2	6.4	5.1
Threonine (g/kg)	7.8	6.4	7.7	8.1	5.9	2.9	7.4	5.5	4.7
Tryptophan (g/kg)	1.8	1.7	1.5	2.6	1.2	0.7	1.3	0.8	0.9
Tyrosine (g/kg)	13.7	14.3	13.7	7.9	8.8	2.9	10.0	8.5	3.3
Valine (g/kg)	10.3	12.2	11.0	15.0	6.8	3.8	10.7	7.6	4.3
Taurine (mg/kg)	< 50	50	80	140	< 50	< 50	1,410	810	< 50
Ammonia (g/kg)	2.9	3.5	2.7	4.5	2.4	2.9	3.6	2.3	1.3
Protein recovery (as is)	93.9%	100.2%	100.0%	88.2%	99.6%	72.9%	94.0%	90.3%	85.8%
Protein recovery (corrected)	80.2%	86.5%	86.7%	74.5%	85.9%	59.2%	80.3%	76.6%	72.1%

<sup>a</sup>Value is 67–100% of the NRC requirements for rats for growth.

**TABLE 4. Vitamin content of selected invertebrates on an as is basis**

Vitamin	Superworms	Giant mealworm (larvae)	Mealworm (larvae)	Mealworms (adult)	Waxworms	Silkworms	Crickets (adult)	Crickets (nymph)	Earthworms
Vitamin A (IU/kg)	< 1,000 <sup>c</sup>	< 1,000 <sup>c</sup>	< 1,000 <sup>c</sup>	< 1,000 <sup>c</sup>	< 1,000 <sup>c</sup>	1,580	< 1,000 <sup>c</sup>	< 1,000 <sup>c</sup>	< 1,000 <sup>c</sup>
β carotene (mg/kg)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Vitamin D <sub>3</sub> (IU/kg)	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>	< 256 <sup>c</sup>
Vitamin E (IU/kg)	7.7 <sup>a</sup>	< 5.0 <sup>c</sup>	< 5.0 <sup>c</sup>	< 5.0 <sup>c</sup>	13.3	8.9	19.7	9.6	NA
Vitamin C (mg/kg)	12.0	24.0	12.0	54.0	< 10.0	< 10.0	30.0	18.0	NA
Thiamin (mg/kg)	0.6 <sup>b</sup>	1.2 <sup>a</sup>	2.4	1.0 <sup>a</sup>	2.3	3.3	0.4 <sup>c</sup>	0.2 <sup>c</sup>	NA
Riboflavin (mg/kg)	7.5	16.1	8.1	8.5	7.3	9.4	34.1	9.5	NA
Pantothenic acid (mg/kg)	19.4	14.5	26.2	24.0	20.2	21.6	23.0	26.3	NA
Niacin (mg/kg)	32.3	41.3	40.7	56.4	37.5	26.3	38.4	32.8	NA
Pyridoxine (mg/kg)	3.2	5.8	8.1	8.1	1.3 <sup>b</sup>	1.6	2.3	1.7	NA
Folic acid (mg/kg)	0.66	1.17	1.57	1.39	0.44	0.71	1.50	1.45	NA
Biotin (mg/kg)	0.35	0.37	0.30	0.28	0.29	0.25	0.17	0.05	NA
Vitamin B <sub>12</sub> (μg/kg)	4.2 <sup>c</sup>	1.3 <sup>c</sup>	4.7 <sup>c</sup>	5.6 <sup>c</sup>	< 1.2 <sup>c</sup>	< 1.2 <sup>c</sup>	53.7	87.2	NA
Choline (mg/kg)	1,736	1,712	1,844	2,422	1,641	1,128	1,519	1,094	NA

<sup>a</sup>Value is 67–100% of the NRC requirements for rats for growth.

<sup>b</sup>Value is 33–67% of the NRC requirements for rats for growth.

<sup>c</sup>Value is 0–33% of the NRC requirements for rats for growth.

NA, not analyzed.



**TABLE 5. Fatty acid content of selected invertebrates on an as is basis**

Fatty acid	Superworms	Giant mealworm (larvae)	Mealworm (larvae)	Mealworms (adult)	Waxworms	Silkworms	Crickets (adult)	Crickets (nymph)
Lauric 12:0 (g/kg)	< 0.2	0.6	< 0.2	< 0.1	< 0.2	< 0.2	< 0.2	< 0.2
Myristic 14:0 (g/kg)	1.7	5.2	2.9	0.8	0.4	< 0.3	0.4	0.2
Pentadecanoic 15:0 (g/kg)	0.4	0.2	< 0.2	< 0.1	< 0.2	< 0.4	< 0.2	< 0.2
Palmitic 16:0 (g/kg)	52.8	25.5	22.9	8.5	79.6	1.7	15.6	6.1
Palmitoleic 16:1 (g/kg)	0.7	4.8	3.5	0.6	5.1	0.1	0.9	0.3
Heptadecanoic 17:0 (g/kg)	0.7	0.2	< 0.2	0.2	< 0.2	< 0.2	0.2	0.1
Heptadecenoic 17:1 (g/kg)	0.6	0.2	0.3	0.2	0.3	< 0.2	< 0.1	0.1
Stearic 18:0 (g/kg)	12.6	4.0	3.9	2.6	3.4	1.2	5.8	2.9
Oleic 18:1 (g/kg)	66.0	66.4	53.9	17.9	124.0	3.2	15.4	6.4
Linoleic 18:2 (g/kg)	32.9	49.0	34.8	13.7	15.2	3.5	22.9	11.0
Linolenic 18:3 (g/kg)	1.1	2.2	1.4	0.4	1.1	1.4	0.6	0.4
Arachidic 20:0 (g/kg)	0.4	0.2	0.3	0.2	0.3	0.1	0.4	0.3
All others (g/kg)	0.2	< 0.2	0.2	< 0.1	< 0.2	0.1	0.5	0.1
Fat recovery (as is)	96.1%	94.0%	93.0%	83.5%	92.1%	81.3%	92.2%	84.4%
Fat recovery (corrected)	86.1%	84.0%	83.0%	73.5%	82.1%	71.3%	82.2%	74.4%

tein and fat in a comparison of pinhead and adult crickets. The only data available for silkworm larvae are those of Frye and Calvert [1989], who reported a lower moisture and higher protein and fat content than was seen in this study. The larvae analyzed by Frye and Calvert were much larger than those analyzed here (approximately 3,600 mg vs. 1,000 mg), and they may have evacuated their gut contents in preparation for pupation, thereby decreasing their moisture content. This relationship is in contrast to that reported by Studier et al. [1991] for eastern tent caterpillars. There is no published data with which to compare the results for adult mealworms.

While it is often assumed that soft-bodied insects contain less fiber than those with a hard exoskeleton, Bukkens [1997] reported similar crude fiber values (DM basis) for crickets, termites, grasshoppers, and caterpillars. Despite the differences in fiber measurements (crude vs. ADF) the present study suggests insects with a hard exoskeleton do contain more fiber. Silkworms contained 6.4% ADF (DM basis) similar to that of the other insect larvae, superworms, mealworms, giant mealworms and waxworms (6.3–8.4% ADF DM basis). Cricket nymphs and adult crickets contained intermediate levels of fiber (9.6 and 10.2% ADF DM basis). Adult mealworms contained high levels of ADF (20.4% DM basis).

Analysis of insect cuticles showed that chitin represents only 25–40% of the dry weight of the insect cuticle, while protein usually represents in excess of 50% of the dry weight of the cuticle [Richards, 1978]. Although there is extensive protein-chitin binding, sclerotized protein is more important than chitin in determining the physical characteristics (hardness) of the insect cuticle. It seems likely that ADF represents both chitin and protein, since chitin is structurally similar to cellulose [Stelmock et al., 1985], additionally, amino acids have been detected in the ADF residue using high performance liquid chromatography (HPLC) analysis (Finke, unpublished results). In contrast to the results obtained for ADF, the NDF value (DM basis) for silkworms was much lower than that of any other insect species, while adult mealworms and waxworms were highest. This suggests that many of the proteins in the insect's cuticle might also be contained in the NDF fraction.

The data on calcium content of these invertebrates are consistent with many previous studies of these species [Jones et al., 1972; Martin et al., 1976; Strzelewicz et al., 1985; Nakagaki et al., 1987; Frye and Calvert, 1989; Barker, 1997; Barker et al., 1998]. Most wild-caught insects also appear to be low in calcium, although in general the values are somewhat higher than those reported for captive-raised insects. Insects that have been shown to contain substantial quantities of calcium include eastern tent caterpillar cocoons (1.8%, DM basis) [Studier et al., 1992], stoneflies (1.15%, DM basis) [Studier and Sevic, 1992], and housefly pupa (0.93%, DM basis) from larvae raised in poultry manure containing 5.1% calcium [Teotia and Miller, 1974]. Presumably wild insectivores obtain sufficient calcium by varying the prey species consumed, by feeding on insects which contain additional calcium in their gastrointestinal tract and by ingesting soil particles adhering to their prey [Bilby and Widdowson, 1971].

Phosphorus content was much higher than the calcium level in all species tested, and was similar to those reported previously [Jones et al., 1972; Martin et al., 1976; Nakagaki et al., 1987; Frye and Calvert, 1989; Barker et al., 1998]. Unlike plant-based phytate phosphorus, the phosphorus in insects is likely to be readily available, as was shown for facefly pupa [Dashefsky et al., 1976].

Magnesium content is similar to those reported for various captive-bred and wild-caught insects [Martin et al., 1976; Finke, 1984; Nakagaki et al., 1987; Oyarzun

et al., 1996; Barker et al., 1998]. The values for sodium and potassium for superworms and all types and life stages of mealworms (shown in Table 2) are similar to those reported by Jones et al. [1972] for mealworm larvae, and Martin et al. [1976] for mealworm larvae and adults. The values for potassium for house crickets are also similar to those reported by Nakagaki et al. [1987]. In contrast, the sodium content of house crickets in this study was less than half that reported by Nakagaki et al. [1987], which is a result of the high sodium diet used in their study, and the fact that these crickets were fasted prior to analysis. There are no other published data on the sodium or potassium content of other captive-bred insects, although data from wild-caught insects are comparable to those reported here [Levy and Cromroy, 1972; Studier et al., 1991; Studier and Sevick, 1992; Oyarzun et al., 1996]. No published data were found concerning the chloride content of any insects.

The levels of iron, zinc, copper, and manganese were variable, but all values were similar to those previously reported for these invertebrates [Martin et al., 1976; Barker et al., 1998]. However, in general the copper levels shown here were somewhat higher. No published reports of iodine analysis of invertebrates were found, but the data reported here suggest that further research is warranted, since silkworms, giant mealworm larvae, superworms, and waxworms did not contain detectable levels of iodine, and mealworm larvae and adult mealworms contained only small amounts. Only earthworms and both young and adult crickets contained substantial quantities of iodine. All invertebrates contained selenium, and the levels were similar to those reported for termites [Oyarzun et al., 1996]. In general, mineral composition probably largely reflects the food sources of the insect. Studies of wild insects show both seasonal variation [Studier et al., 1992] and variations between different populations of the same species living in the same general area [Finke, 1984].

The amino acid patterns reported here are consistent with the amino acid profiles reported for housefly larvae and pupa, house crickets, silkworm pupa, termites, Mormon crickets, and earthworms. The analytical data presented here suggest that in insects and earthworms total sulfur amino acids are first limiting for rats. This was confirmed in three feeding trials in which methionine was shown to be the first limiting amino acid for rats fed a purified diet containing Mormon cricket meal or house fly larvae [Goulet et al., 1978; Finke et al., 1987; Onifade et al., 2001]. In contrast, when a Mormon cricket-based purified diet was fed to growing chicks, methionine and arginine were shown to be colimiting [Finke, 1984]. Unlike birds, reptiles, and fish, mammals are ureotelic, excreting urea as an end product of protein metabolism. Arginine is synthesized during this process, which is why the arginine requirement for growing rats is low relative to that for birds or fish. In contrast, because poultry are uricotelic and excrete uric acid as an end product of protein metabolism, they have a limited ability to synthesize arginine and thus their requirements are much higher. Because of differing energy and protein requirements between species, this is most easily demonstrated by comparing the arginine requirement relative to that for lysine. For leghorn chicks, catfish, and rats the arginine/lysine ratios are 1.18, 0.84, and 0.47, respectively, showing that rats require little arginine compared to poultry and fish [National Research Council, 1993; 1994; 1995]. Since reptiles are also uricotelic, they may have an increased requirement for arginine relative to that of the laboratory rat. This suggests that while total sulfur amino acids are first limiting when fed to mammals, when fed to uricotelic animals such as birds and reptiles, or ammoneotelic animals such as fish, arginine may also be important.

In general, feeding trials using house fly larvae, pupae, Mormon crickets, house crickets, various lepidoteran larvae, and earthworms show good growth for both rats and chickens, and suggest that insect protein is readily available, with protein-quality values similar to or slightly higher than those of fish meal or soybean meal [Calvert et al., 1969; Teotia and Miller, 1974; Ocio and Vinaras, 1979; DeFoliart et al., 1982; Dreyer and Wehmeyer, 1982; Lin et al., 1983; Finke et al., 1984, 1987, 1989; Phelps, 1985; Landry et al., 1986; Nakagaki et al., 1987; Zhenjun et al., 1997]. However, in five separate studies (three using silkworm pupal meal [Ichhponani and Malik, 1971; Fagoonee, 1983; Rao, 1994], one using house fly larval meal [Onifade et al., 2001], and one using eastern tent caterpillar meal [Finke, 1984]), dried-insect meals produced poor results.

In four of these studies the poor growth appears to be a result of low food consumption. These results may be due to the presence of oxidized fat, since insect meals are generally high in unsaturated fatty acids, which makes them susceptible to oxidation if not treated with an antioxidant. Since oxidation would take place during the drying and storage of these dry meals, fat oxidation would not be an issue for animals fed live invertebrates. The other possibility is the presence of a compound in the insect meal that negatively affects palatability. In the wild, many insect species are known to sequester compounds from their food-plants that cause them to be unpalatable or toxic [Harborne, 1982]. Mulberry leaves, the food-plant of silkworms, are known to contain a number of compounds that might be sequestered by silkworms, including essential oils, flavenoids, and terpenoids [Harborne, 1982]. In this regard, it is important to note that all three authors working with silkworm pupal meal from silkworm larvae fed mulberry leaves commented on the odor of the material [Ichhponani and Malik, 1971; Fagoonee, 1983; Rao, 1994].

Finke [1984] also reported extremely poor growth of rats fed purified diets containing high levels of defatted eastern tent caterpillar meal, and attributed this to factors other than amino acid composition. Rats fed high levels (greater than 30%) of eastern tent caterpillar meal had reduced food intake, and exhibited chronic diarrhea and abnormally distended gastrointestinal tracts. These results and those from animals fed diets containing silkworm pupae suggest caution when using invertebrates fed natural food-plant diets.

Previously it has been suggested that a significant amount of nitrogen from insects might be from chitin, and thus estimating protein using [nitrogen  $\times$  6.25] might result in an overestimate of true protein. The fairly high recovery of nitrogen as amino acids in all species except silkworms suggests that the nitrogen from chitin is relatively small. Amino acid recoveries (corrected) calculated from other studies are as follows: house fly pupae 62% [Calvert et al., 1969] and 86% [Teotia and Miller, 1974]; house fly larvae 67% [Ocio and Vinaras, 1979]; termites 72% [Phelps et al., 1975], 71% [Ukhun and Osasona, 1985], and 38%, and 75% [Oyarzun et al., 1996]; Mormon crickets 85% [Finke, 1984]; house crickets 77% [Nakagaki et al., 1987]; three genera of aquatic insects 86% [Bergeron et al., 1988]; and silkworm pupa 68% [Lin et al., 1983] and 90% [Rao, 1994]. The number of amino acids reported in these studies ranged from 16 to 18. Ammonia was reported in only two of these, which helps explain some of the lower recoveries. While detailed amino acid analysis is preferred, in general it appears that [nitrogen  $\times$  6.25] may provide a reasonable estimate of total protein in most invertebrates.

While Jones et al. [1972] detected no vitamin A in mealworm larvae, both Barker

et al. [1998] and Pennino et al. [1991] showed mealworm larvae, superworms, waxworms, crickets, and earthworms to contain extremely low levels of vitamin A (161–972 IU vitamin A/kg DM). Since the levels reported in those two papers are well below the detection limit of the assay used here (1,000 IU vitamin A/kg), it may be that these samples also contained extremely low levels of vitamin A. An analysis of several subsequent samples using a more sensitive assay found no detectable vitamin A in mealworm larvae, and levels of 300–900 IU/kg DM in crickets and waxworms (Finke, unpublished results). Using an HPLC procedure, Pennino et al. [1991] reported that commercially raised honeybees contained fairly low levels of vitamin A (2,800–3,100 IU vitamin A/kg DM). In contrast, using an older colorimetric assay, Hocking and Matsumura [1960] reported that honeybee larvae and pupae contained substantial quantities of vitamin A (165,400–517,400 IU vitamin A/kg DM). It should be noted that this procedure is susceptible to interference from sterols and carotenoids, which may explain the high values reported. An analysis of both bee pupae and larvae detected no  $\beta$ -carotene or preformed vitamin A (Finke, unpublished data). Barker et al. [1998] observed that commercially raised earthworms contained only 14% of the vitamin A seen in wild-caught earthworms. Wild-caught termites contained variable levels of vitamin A, averaging 24,733 IU/kg DM [Oyarzun et al., 1996]. The detection of significant quantities of vitamin A in silkworm larvae is noteworthy in light of the fact that four other species of wild-caught lepidopteran larvae have been reported to contain both  $\beta$ -carotene and retinol [Dreyer and Wehmeyer, 1982; Kodondi et al., 1987]. It seems likely that lepidopteran larvae fed diets consisting primarily of leaves are capable of converting  $\beta$ -carotene to preformed vitamin A.

None of the invertebrates sampled contained detectable levels of vitamin D<sub>3</sub>. The only report of vitamin D analysis of invertebrates is that of Hocking and Matsumura [1960], who showed that honeybee larvae and pupae contained extremely high levels of vitamin D<sub>3</sub> (17,013,400–32,304,300 IU vitamin D/kg DM). Again, this procedure is also susceptible to interference from other sterols, which may explain the high values reported. A low level of vitamin D<sub>3</sub> (1,000 IU/kg DM) has been detected in honeybee larvae (Finke, unpublished data).

Vitamin E analyses appear to fall within the values reported for these species by both Pennino et al. [1991] and Barker et al. [1998]. Dierenfeld [1989, 1994] reported vitamin E deficiency in zoo animals, and suggested that their diets contain 50–200 mg vitamin E/kg of diet (as compared to the 18 mg suggested by the NRC for rats). While wild termites were found to contain sufficient vitamin E (93 mg/kg DM) to meet the minimum recommendations of Dierenfeld, the highest level found in this present study was the 42 mg/kg DM found in adult crickets. The NRC recommends higher levels of vitamin E for high-fat diets, and the DM fat content ranged from 8.1% for silkworm larvae to 60.0% for waxworms, all values higher than the 5% fat diets recommended by the NRC. Another factor is the unsaturated fatty acid content of some of these insect species, which would also warrant a higher requirement for vitamin E. Given what we currently know, it appears that for vitamin E, the current NRC recommendations for rats are probably not appropriate for animals fed these high-fat invertebrates.

There is little published information concerning the B-vitamin levels of live invertebrates, except for the data of Jones et al. [1972]. In contrast to the analysis reported here, Jones et al. (using traditional microbiological methods) showed that

mealworm larvae contained no detectable thiamin. The data reported here shows that mealworm larvae, waxworms, and silkworms contained adequate thiamin to meet NRC recommendations, but that superworms, giant mealworm larvae, adult mealworms, and cricket nymphs and adults were all deficient. The values for riboflavin, pyridoxine, and folic acid reported by Jones et al. [1972] are similar to those found in this study. Rao [1994] reported that dried silkworm pupae contained 11.7 mg nicotinic acid/kg DM, compared to the 152 mg niacin/kg DM seen in the present study for silkworm larva. The data concerning the B-vitamin content of these species of invertebrates are in agreement with those of various other authors [Santos Olivera et al., 1976; Bukkens, 1997; Dreyer and Wehmeyer, 1982; Kodondi et al., 1987], although the data are not directly comparable since much of that data represents analysis of insects that were prepared for human consumption.

Mealworm larvae, giant mealworm larvae, and superworms all contained high levels of oleic, linoleic, and palmitic acid, similar to earlier reports for mealworm larvae [Martin et al., 1976; Jones et al., 1972], although Jones reported substantially higher levels of myristic and palmitoleic acid than those found in this study. The results seen in Table 5 for crickets are similar to the fatty acid levels reported by Calvert et al. [1969], except that fly pupae contained high levels of palmitoleic acid while the levels in crickets were quite low. The fatty acid profile for silkworms was similar to that reported for silkworm pupal meal by Rao [1994] except for the levels of linolenic acid, which were much higher than those seen here. While there were also some similarities with the fatty acid levels reported by Oyarzun et al. [1996] for termites, the termites in that study contained much higher levels of both linolenic and arachidonic acid than those seen in this study. Bukkens [1997] also reported no consistent pattern in fatty acid profiles for a variety of insect species, although all species contained significant quantities of both linoleic and linolenic acid (as was found in the present study). These fatty acid profiles most likely reflect the fatty acid composition of the insects' food, which is commonly seen in most monogastric animals and has also been reported for lacewings [Kaplan et al., 1986].

The use of the nutrient requirements of the laboratory rat is unlikely to be appropriate for all insectivores. However, without data concerning the nutrient requirements of specific insectivores, it does provide an initial basis for comparison. Comparisons with endothermic animals are likely to underestimate the requirements of ectothermic animals since there is a reduced demand for energy, and hence a decrease in food intake. Thus, in order to meet these requirements the food for ectothermic animals must be more nutrient-dense (i.e., a higher nutrient content per unit of energy). While the energy requirements per unit body weight of most ectothermic terrestrial species are likely to be lower than those of endothermic terrestrial mammals, such as rats, they are unlikely to be as low as those seen for fish. There are three reasons for this: First, the body temperature of most fish is lower than that of most reptiles, especially reptiles that spend significant amounts of time basking in the sun. Second, because of the density of water, fish need to expend far less energy maintaining their posture in water than do terrestrial animals on land. Third, freshwater fish are ammonotelic, so they expend much less energy excreting excess nitrogen than do uricotelic (birds and reptiles) and ureotelic (mammals) animals.

Another potential error is that previously published literature has compared invertebrate nutrient contents to those published for other species on a DM basis. These requirements are based on a metabolizable energy content of 3,200 and 3,950 kcal/kg

for poultry and rat diets, respectively, while the energy content (DM basis) of the invertebrates in this study ranged from a low of 3,796 to 6,619 kcal ME/kg. Because of their high energy density, waxworms would have to have a nutrient concentration of more than 160% of the requirements on a DM basis in order to meet the requirements when expressed on an energy density basis. Using high-fat waxworms as an example, nine nutrients (calcium, sodium, manganese, iodine, vitamin A, vitamin D, pyridoxine, and vitamin B<sub>12</sub>) would be considered low on a DM basis. However, when expressed on an isocaloric basis, an additional seven nutrients (phosphorus, magnesium, potassium, iron, thiamin, folic acid, and tryptophan) would be considered deficient. In contrast, for lower-fat invertebrates such as silkworms, crickets, adult mealworms, and earthworms, comparisons on a DM basis or an isocaloric basis yield similar results. These data clearly demonstrate the potential for error when comparing high-fat, energy-dense food sources on a DM basis rather than an isocaloric basis. There is little detailed information available concerning the nutrient requirements of most insectivorous animals. This suggests that for many invertebrate species, minor adjustments to express nutrient content on an isocaloric basis are unlikely to offer any additional benefit. However, for high-fat, energy-dense species (mealworm larvae, giant mealworm larvae, superworms, and waxworms), adjustments appear to be warranted.

These data are most valuable in that they form the baseline for diet development for “gut loading” in order to improve the nutritional value of these invertebrates when fed to captive reptiles, mammals, and birds [Allen and Oftedal, 1989; Strzelewicz et al., 1985; Ferguson et al., 1996; Anderson, 2000; Klasing et al., 2000]. These data suggest that in addition to calcium, insect diets designed for gut loading should also contain substantial quantities of vitamin A, vitamin D (depending on the quality and quantity of ultraviolet light), and vitamin E. Depending on which invertebrates are being used in the feeding program, supplementation of other nutrients may be warranted. One should also be careful not to categorize certain species as inappropriate for feeding programs. While waxworms were deficient in 16 nutrients on an isocaloric basis when compared to the NRC recommendations for rats, a high-fat diet such as waxworms may be appropriate for a sick animal, for which the primary goal is to increase the intake of energy. Thus, a mixed diet using a variety of “gut-loaded” invertebrates would appear to offer the best hope of providing the appropriate nutrition to captive animals.

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