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Research Article

Estimate of Chitin in Raw Whole Insects

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Insects contain significant amounts of fiber as measured by crude fiber, acid detergent fiber (ADF) or neutral detergent fiber (NDF). It has always been assumed that the fiber in insects represents chitin based on the structural similarity between cellulose and chitin and the fact that the ADF fraction from insects contains nitrogen. In this study, a number of insect species that are raised commercially as food for insectivores were analyzed for moisture, crude protein (nitrogen × 6.25), fat, ash, NDF, ADF, and amino acids. Additionally, the ADF fraction was analyzed for nitrogen and amino acids to determine if proteins might be present in the ADF fraction. The ADF fraction contained a significant amount of amino acids accounting for 9.3–32.7% of the ADF (by weight). The presence of amino acids in the ADF fraction means that using ADF to estimate insect chitin results in an overestimation of insect chitin content. Using ADF adjusted for its amino acid content, the estimated chitin content of these insect species ranged from 2.7–49.8 mg/kg (as is) and 11.6–137.2 mg/kg (dry matter basis). Additionally, these data suggest that for the species measured here the amount of chitin nitrogen is quite small (as a % of total nitrogen) and that crude protein (nitrogen × 6.25) provides a reasonable estimate of the true protein for most species of insects. Zoo Biol 26:105–115, 2007. © 2007 Wiley-Liss, Inc.

Keywords: insect; chitin; ADF; protein; amino acids

INTRODUCTION

Insects serve as a food source for a variety of animals. Published studies show that whole insects contain variable but significant amounts of fiber as measured by crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF) [Finke, 1984, 2002; Pennino et al., 1991; Barker et al., 1998]. For plant-based foods the makeup of the various components of these fibers is well established. ADF is composed typically of

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cellulose and lignins whereas NDF is composed of cellulose, lignin, and hemicellulose [Van Soest and Robertson, 1977]. Although insects contain significant amounts of both ADF and NDF, the components that make up these fibers are unknown. Various authors have suggested that the fiber in insects represents chitin because chitin (linear polymer of β-(1→4) *N*-acetyl-D-glucosamine units) is similar structurally to cellulose (linear polymer of β-(1→4)-D-glucopyranose units) and because the ADF fraction has been shown to contain nitrogen [Finke, 1984, 2002; Barker et al., 1998]. Several evaluations of the nutritional value of various shellfish products (shrimp meal, crab meal, and crayfish meal) have concluded that either ADF or CF provide reasonable estimates of shellfish chitin [Lovell et al., 1968; Watkins et al., 1982; Stelmock et al., 1985]. Studies that have reported the digestibility of chitin by various species of birds in reality actually determine the digestibility of ADF or CF fractions of shellfish or shellfish meal [Jackson et al., 1992; Weiser et al., 1997; Akaki and Duke, 1999].

Chitin exists rarely in a pure form in nature but instead is usually in a complex matrix with other compounds. The cuticle of crustaceans such as crabs, shrimp, and crayfish is composed usually of chitin in a matrix with protein and minerals (mostly calcium) [Johnson and Peniston, 1982, No et al., 1989]. In contrast, the cuticle of insects is composed of chitin in a matrix with cuticular proteins, lipids, and other compounds [Kramer et al., 1995; Nation, 2002]. Although most insects contain only insignificant amounts of minerals in their cuticle, there are some species such as the pupae of the face fly (*Musca autumnalis*) and larvae of the black soldier fly (*Hermetia illucens*) that contain significant amounts of calcium in their cuticle [Dashefsky et al., 1976; Roseland et al., 1985; Tomberlin et al., 2002]. Although ADF or CF may be an accurate estimate of shellfish chitin, the structural differences between insect cuticle and shellfish cuticle means that using ADF or CF may not result in an accurate estimate of insect chitin. Little quantitative data exists concerning the chitin content of whole insects but using an enzymatic assay Cauchie [2002] reported that aquatic insect larvae contained between 2.9–10.1% chitin on a dry weight basis. Klasing [1998] stated that the proportion of chitin in arthropods ranges from 18–60% but because no references are cited, the data is impossible to verify. The values in Klasing are likely too high for insects based on quantitative analysis of their cuticle and the fact that chitin is found only in the insects endocuticle and exocuticle [Kramer et al., 1995, Nation, 2002].

Dried whole honey bees contained 11.1% chitin when analyzed using an assay published for shellfish [Ozimek et al., 1985]. Using this value to correct for the nitrogen in the chitin the authors reported that the corrected protein content of dried honeybees (52.0%) was 8.5% lower than the crude protein content (56.8%). Interestingly the sum of the amino acids (and neither cystine nor tryptophan were measured) was 106% that of the corrected crude protein content suggesting that chitin in honey bees as measured by this assay contained some amino acids. In this study, a number of species of insects used commonly as food for insectivores are analyzed for ADF, nitrogen, and amino acids to determine the contribution of amino acids to insect ADF.

MATERIALS AND METHODS

Crickets (*Acheta domesticus*; nymphs and adults), waxworms (*Galleria mellonella*; larvae), mealworm adults (*Tenebrio molitor*; beetles), and giant mealworms (*Tenebrio molitor*; larvae) were obtained from Timberline Industries

(Marion, IL). Silkworms (*Bombyx mori*; larvae) were obtained from Mulberry Farms Waxworm Tarvae, Inc. (Fallbrook, CA). Crickets, adult mealworms/beetles, giant mealworm larvae, waxworm larvae, and silkworm larvae were all fasted for 24 hr to clear their gastrointestinal tract of any residual food. Bee brood (*Aphis melifera*) were obtained from the United States Department of Agriculture bee laboratory in Tucson, Arizona. The brood consisted mostly of bee pupae but also contained some mature larvae (<10%). All samples were 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 sample.

Whole insects were analyzed for nitrogen (Association of Official Analytical Chemists [AOAC] method = 955.04), moisture (AOAC method = 926.08), fat (AOAC method = 945.02), ash (AOAC method = 923.03), amino acids (AOAC method = 982.30), ADF, and NDF [United States Department of Agriculture, 1970; Association of Official Analytical Chemists, 1995]. ADF is the average of two separate assays; all others are the result of a single analysis. The ADF fractions were analyzed for nitrogen (AOAC method = 955.04) and most of the amino acids (AOAC method = 982.30). The amino acids methionine, cystine, and tryptophan were not determined because it was thought that the acid hydrolysis step used to determine ADF would result in the partial or complete destruction of these amino acids resulting in artificially low values for these amino acids. The proximate and amino acid analysis of whole mealworm adults and bee brood shown have been reported previously [Finke, 2002, 2005].

RESULTS

Proximate analysis of whole insects is shown in Table 1. As expected the primary components of most insects was moisture, protein, and fat with smaller amounts of ash and fiber (both ADF and NDF).

The amino acid composition of whole insects and ADF fractions are shown in Table 2. As expected the amino acid patterns for whole insects and ADF fractions were quite different. The amino acid pattern found in the ADF fraction likely reflects the specific cuticular proteins present in insects. Many of these proteins are rich in specific amino acids that helps give them their unique properties.

DISCUSSION

There are numerous proximate analyses of a variety of both wild caught and cultured insects. The values reported here for moisture, protein, fat, ash, ADF, and NDF are similar to those reported earlier for these species [Pennino et al., 1991, Barker et al., 1998, Finke, 2002]. The ADF content of adult mealworms is much higher than that for the other species but is comparable to values obtained for adult Mormon crickets (*Anabrus simplex*) [Finke, 1984].

The amino acid patterns reported are consistent with the amino acid profiles published previously for these species [Finke, 2002]. The primary exceptions are the values for the amino acids methionine and cystine for waxworm larvae, giant mealworm larvae, and adult crickets. In these three instances, even after corrections for minor differences in crude protein content, methionine, and cystine, values here are 15–25% (adult crickets), 25–28% (giant mealworm larvae), and 40–43%

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TABLE 1. Analysis of selected whole insects (all values are mg/g - as is)

	Cricket nymphs	Adult crickets	Giant mealworm larvae	Mealworm adults[a]	Waxworm larvae	Silkworm larvae	Bee brood[b]
Moisture	752.0	682.0	648.0	637.0	585.0	874.0	768.0
Crude protein[c]	175.0	225.0	174.0	237.0	161.0	88.0	94.0
Fat	44.0	59.0	134.0	54.0	243.0	12.0	47.0
ADF	22.5	26.0	23.0	74.0	21.0	9.0	3.0
NDF	37.0	52.0	33.0	115.5	37.0	7.5	2.0
Ash	12.0	16.0	10.0	12.0	9.0	14.0	8.0

[a]Data from Finke (2002).
[b]Data from Finke (2005).
[c]Crude protein measured as $N \times 6.25$.

TABLE 2. Amino acid and ammonia analysis of whole insects and the ADF fraction of whole insects (all values are mg/g)

	Cricket nymphs		Adult crickets		Giant mealworm larvae		Mealworm adults		Waxworm larvae		Silkworm larvae		Bee brood	
	Whole insect	ADF fraction	Whole insect	ADF fraction	Whole insect	ADF fraction	Whole insect[a]	ADF fraction	Whole insect	ADF fraction	Whole insect	ADF fraction	Whole insect[b]	ADF fraction
Alanine	17.7	15.7	17.3	13.8	14.0	13.6	18.1	26.3	11.5	41.7	3.6	9.3	4.5	3.7
Arginine	12.4	3.3	12.9	5.6	10.5	4.7	10.2	8.7	8.2	7.5	3.8	<0.1	4.0	3.3
Aspartic acid	13.9	4.6	19.1	13.1	16.4	9.0	16.6	11.8	14.9	18.2	6.1	5.3	7.6	1.3
Cystine	1.6	NA	2.2	NA	1.9	NA	1.6	NA	2.1	NA	0.8	NA	2.0	NA
Glutamic acid	20.5	10.1	23.5	25.5	23.1	14.1	22.8	15.6	19.5	18.7	9.0	8.1	12.9	1.9
Glycine	10.6	8.2	10.2	6.9	10.4	11.3	20.0	29.7	9.3	29.3	5.1	7.7	4.1	14.1
Histidine	4.5	7.2	5.1	4.4	6.6	6.9	6.8	11.9	3.6	2.9	2.6	3.6	2.0	7.0
Isoleucine	7.1	2.7	8.2	10.8	8.6	9.4	10.3	12.7	6.7	15.1	2.9	2.9	4.3	12.8
Leucine	12.7	5.5	15.0	17.0	14.3	11.8	19.6	21.4	11.4	19.9	4.3	4.0	6.6	0.1
Lysine	10.9	4.9	11.5	9.2	11.3	5.9	10.5	4.9	9.2	6.0	4.4	3.6	5.8	21.9
Methionine	2.7	NA	4.4	NA	3.4	NA	3.0	NA	4.4	NA	1.1	NA	2.0	NA
Phenylalanine	5.6	2.1	6.8	7.3	7.6	7.0	6.2	4.9	6.0	8.3	2.5	1.3	3.3	3.1
Proline	10.7	11.7	12.2	14.6	12.9	12.0	15.0	18.6	12.4	11.0	3.1	<0.1	5.7	0.1
Serine	7.5	4.0	11.7	12.5	9.5	8.0	9.8	9.2	12.4	35.6	3.4	5.1	3.3	5.8
Threonine	6.8	2.7	7.0	5.6	7.1	5.0	8.1	7.8	5.8	4.8	2.5	3.4	3.1	1.6
Tryptophan	1.1	NA	1.7	NA	1.8	NA	2.6	NA	1.4	NA	0.6	NA	0.9	NA
Tyrosine	11.0	6.8	9.9	10.8	13.9	14.4	7.9	11.6	8.7	11.4	3.0	3.9	4.1	3.5
Valine	10.5	12.9	10.9	15.7	12.0	15.8	15.0	25.5	8.4	16.3	3.5	9.1	4.9	5.9
Ammonia	3.5	45.5	4.0	27.6	3.6	26.5	4.5	18.7	2.8	17.4	2.4	19.9	1.9	44.1

ADF fraction, analysis of ADF residue; NA, not analyzed; Whole insect, analysis of whole insects.
[a]Data reported previously (Finke, 2002).
[b]Data reported previously (Finke, 2005).

(waxworm pupae) higher than those previously reported [Finke, 2002]. The reason for these higher values is unknown. The analytical data presented here supports previous data from both chemical analyses and animal feeding trials that suggest that for growing rats, insects are typically first limiting in total sulfur amino acids (methionine and cystine) [Goulet et al., 1978; Ryan et al., 1983; Ozimek et al., 1985; Finke et al., 1987; Onifade et al., 2001; Finke, 2002].

Various authors have suggested that a significant amount of nitrogen from insects might be contributed by chitin and so estimating protein using nitrogen × 6.25 might result in an overestimate of an insect's true protein content (i.e., the sum of the amino acids). In this study, however, the protein recovery (by weight as amino acids) for these species is relatively high with an average of 92.4% (range = 73.5–110.6%). Of note is the relatively low recovery for silkworm larvae seen here (73.5%) that is similar to that reported previously [Finke, 2002]. The reason for the consistently low recovery of nitrogen as amino acids from silkworm larvae is unknown. In most cases when insects are analyzed for amino acids and where all amino acids are reported the relatively high recovery of nitrogen as amino acids suggests the nitrogen from chitin is a relatively small fraction of the total nitrogen content of the insect. Chitin is present only in the insect's exocuticle and endocuticle and in most insects studied, protein, not chitin, is the predominant compound in the cuticle [Kramer et al., 1995]. Additionally when mealworm exuviae were analyzed for amino acids 65% of the exuviae by weight was accounted for by the amino acids [Finke, unpublished data]. All of these data support the fact that chitin nitrogen represents a fairly small fraction of the insect's total nitrogen. Although detailed amino acid analysis is preferred, it seems that nitrogen × 6.25 provides for a reasonable estimate of total protein for most insects.

From the amino acid composition of the ADF fraction the percentage of ADF that is composed of amino acids can be estimated. As seen in Table 3 an average of 16.6% (range = 6.7% [silkworms] to 32.7% [adult mealworms]) of the ADF by weight is composed of amino acids. By correcting for the amino acid content of the ADF fraction and assuming the remainder of the ADF fraction is chitin a more accurate estimate of insect chitin content can be calculated (Table 3). In reality, this estimate is probably somewhat high because the amino acids methionine, cystine, and tryptophan were not measured in the ADF fraction. For some of the softer bodied insects like silkworm larvae, bee brood, and cricket nymphs the low amino content of the ADF fraction means the estimated chitin content is similar to the value for ADF. In contrast for most of the other species measured there is a significant discrepancy between chitin estimated via ADF and amino acid corrected chitin content with the greatest difference shown for adult mealworms/beetles. These data show that the average chitin content for these species is estimated to be 19.7 mg/kg as is (range = 2.7 mg/kg [bee brood] to 49.8 mg/kg [adult mealworms]) and 65.6 mg/kg on a dry matter basis (range = 11.6 mg/kg [bee brood] to 137.2 mg/kg [adult mealworms]). These values are similar to those for the larvae of aquatic insects (range = 29 to 101 mg/kg dry matter basis) using an enzymatic method [Cauchie, 2002].

There are suggestions in the literature and numerous popular press reports that soft-bodied insects like silkworm larvae contain less chitin and are more digestible than other commonly used feeder insects [Frye and Calvert, 1989]. The data presented shows that on a dry matter basis the estimated chitin content of silkworm larvae is similar to that of crickets (both nymphs and adults) and mealworm larvae.

TABLE 3. ADF content, amino acid content of ADF, and estimated insect chitin content

	Cricket nymphs	Adult crickets	Giant mealworm larvae	Mealworm adults	Waxworm larvae	Silkworm larvae	Bee brood
ADF (mg/kg)	22.5	26.0	23.0	74.0	21.0	9.0	3.0
Recovery of ADF (as amino acids) %[a]	10.3	17.3	14.9	32.7	24.7	6.7	9.3
Estimated chitin (as is mg/kg)[b]AQ5	20.2	21.5	19.6	49.8	15.8	8.4	2.7
Estimated chitin (dry matter basis mg/kg)	81.5	67.6	55.7	137.2	38.1	66.6	11.6

[a]Calculated by summing the weight of the amino acids in ADF and dividing that by the total weight of ADF.
[b]Calculated by multiplying insect ADF times non-amino acid ADF content.

All of the chitin estimates obtained in this study and those for insects in the literature are well below the values reported by Klasing [1998]. The extremely high amino acid content of the ADF fraction for adult mealworms also supports the current thinking that the hardness of insect cuticle is primarily a function of the degree of sclerotization and the amino acid content of the cuticular proteins rather than its chitin content [Nation, 2002].

The amino acids measured accounted for an average of 31.5% (range = 10.0% [bee brood] to 55.1% [adult mealworms]) of the nitrogen found in the ADF fraction. As expected, the recovery of ADF nitrogen with ammonia is much higher than recoveries without ammonia (Table 4). Accurate amino analysis is a result of three separate analyses. Most of the amino acids are analyzed after protein hydrolysis in 6 N hydrochloric acid. This procedure results in the complete destruction of tryptophan and, unless protected, the partial destruction of methionine and cystine. In addition, this procedure converts any glutamine and asparagine to glutamatic acid and aspartic acid, respectively. Typically the ammonia reported during amino acid analysis is thought to be generated by the destruction of these amino acids and the conversion of glutamine and asparagine to glutamatic acid and aspartic acid. Calculations show that even if all of the glutamic acid, aspartic acid, methionine, cystine, and tryptophan found in the insects whole body were attributed to the ADF fraction this could still not account for all of the ammonia recovered in the ADF fraction. This suggests that the bulk of the ammonia recovered in the ADF fraction is from non-protein sources and is likely the result of the breakdown of chitin during acid hydrolysis. Although ammonia is used typically in calculating amino acid recoveries, including ammonia when calculating amino acid recovery from the ADF fraction seems to be unwarranted.

The amount of nitrogen recovered in the ADF fraction (as a % of whole body nitrogen) averaged 6.0% and ranged from 1.9% (silkworm larvae) to 15.2% (adult mealworm/beetles) (Table 4). These values are similar to those reported by Barker et al. [1998] for mealworm larvae, crickets (both nymphs and adults), waxworm larvae, superworm larvae (*Zophobas morio*), and fruit flies (*Drosophila melanogaster*). Their data showed ADF nitrogen values (as a % of total nitrogen) ranged from 5.2–11.1%. In this study, however, when the nitrogen from the amino acids in the ADF is subtracted out the non-protein ADF nitrogen accounted for an average of only 3.7% of the whole body nitrogen (range = 1.3–6.8%). Barker et al. [1998] did not analyze the ADF fraction for amino acids so the amount of non-protein ADF nitrogen can not be determined from that study. These calculations provide additional evidence that the amount of nitrogen in chitin is a relatively small percentage of the total nitrogen in insects and that 6.25 is a reasonable estimate of the protein content of most insects.

These data do not address the issue of protein and amino acid availability especially for the amino acids from proteins that are either highly sclerotized or which may be bound to chitin. Quantitative data on the digestibility of nitrogen/crude protein of insects are limited. The earliest report by Phelps et al. [1975] showed relatively poor protein digestibility of fried termites with values ranging from 40–50%. The high heat during frying may have affected protein digestibility. More recent work using dried whole insects have resulted in somewhat higher values (62% for dried honey bees; 73% and 75% for dried mealworm larvae, and 85% for dried larvae of the mopanie moth, *Conimbrassia belina*) [Goulet et al., 1978; Dreyer and

TABLE 4. Nitrogen content of whole insects and ADF fraction and recovery of ADF and ADF nitrogen as amino acids

	Cricket nymphs	Adult crickets	Giant mealworm larvae	Mealworm adults	Waxworm larvae	Silkworm larvae	Bee brood
Whole body N (mg/g)	28.0	36.0	27.8	37.9	25.8	14.1	15.0
ADF-N (mg/g)	55.0	87.9	64.4	78.0	83.5	30.0	143.0
Recovery of ADF N (as amino acids) %[a]	27.3	25.5	31.1	55.1	40.7	30.7	10.0
Recovery of ADF N (as amino acids+ammonia) %[b]	96.3	51.3	64.9	74.8	57.8	85.2	35.3
ADF-N (% of whole body N) %[c]	4.4	6.3	5.3	15.2	6.8	1.9	2.4
Non protein ADF-N (% of whole body N) %[d]	3.2	4.7	3.7	6.8	4.0	1.3	2.2

[a]Calculated by summing the nitrogen in the amino acids from the ADF and dividing that by the ADF-N.
[b]Calculated by summing the nitrogen in the amino acids plus ammonia from the ADF and dividing that by the ADF-N.
[c]Calculated by dividing ADF-N by whole body N.
[d]Calculated by dividing ADF-N – ADF amino acid N by whole body N.

Wehmeyer, 1982; Ozimek et al., 1985; van Tets and Hulbert, 1999]. Based on the results presented here, one might expect that the digestibility of protein from insects might be highly variable. Insects that have a larger proportion of their amino acids in the ADF fraction might be expected to have a lower nitrogen/protein digestibility than those with a lower proportion of their amino acids in the ADF fraction.

CONCLUSION

In summary, this study suggests that the fiber content of insects (measured as ADF) consists not only of chitin but also significant amounts of amino acids that likely represent cuticular proteins. Insects with "harder" cuticles do not seem to contain significantly more chitin than softer bodied insects but rather their ADF fraction seems to contain a much higher proportion of amino acids than softer bodied insects. These data also suggest that although amino acid analysis are the preferred means of determining total protein content, nitrogen content $\times$ 6.25 (crude protein) is a reasonable estimate of the true protein content for most of the insect species studied.

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