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RESEARCH

Nutritional Composition and Protein Quality of the Edible Beetle *Holotrichia parallela*

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ABSTRACT. The adult edible beetle *Holotrichia parallela* Motschulsky (Coleoptera: Scarabaeoidea) represents a traditional food source in China. Based on nutritional analyses, adult *H. parallela* is high in protein (70%) and minerals and low in fat. *H. parallela* contained approximately 10% chitin; the corrected protein content was 66%. Oleic acid and linoleic acid were the most abundant fatty acids. Of the total amino acids in *H. parallela*, 47.4% were essential amino acids. The amino acid scores were 87 and 100, based on the corrected crude and net protein contents, respectively; threonine was the limiting amino acid. In vitro protein digestibility was 78%, and the protein digestibility-corrected amino acid score was 89 based on the net protein content. Adult *H. parallela* may be a potential source of proteins and minerals for humans and animals.

Key Words: beetle, *Holotrichia parallela*, nutritional composition, protein quality, amino acid composition

Edible insects have received considerable attention. Insect consumption, which is generally regarded as safe, has been documented for thousands of years in many parts of the world (DeFoliart 1989, Verkerk et al. 2007). Studies have reported that insects are good sources of proteins and minerals and contribute to the daily requirements of these nutrients in certain developing countries (Ladron de Guevara et al. 1995, Bukkens 1997, Ramos-Elorduy et al. 1997, Banjo et al. 2006, Elemo et al. 2011). Additionally, insects have a high biodiversity with a higher feed conversion efficiency than cattle (Labandeira and Sepkoski 1993, Finke and Winn 2004, Verkerk et al. 2007). Considering that protein-energy malnutrition remains a widespread problem, insect consumption represents an inexpensive method of alleviating the food shortage crisis (DeFoliart 1992, 1999). Therefore, a thorough assessment of the nutritional composition and protein quality of insects is of utmost importance.

In China, insect consumption has been practiced in many areas of the country for thousands of years. Currently, insects are considered to be popular sources of food and medicine (Namba et al. 1988, Feng et al. 2009). Although certain insects are sold throughout the country, a few insect species are collected and sold locally as in the case of the adult *Holotrichia parallela* Motschulsky (Coleoptera, Scarabaeoidea). *H. parallela* is a crop pest; it belongs to the Melolonthidae subfamily of the Scarabaeoidea family. In the summer season, indigenous individuals collect these beetles from fields and consume them when fried. The *H. parallela* larva has been used in traditional medicine (Dong et al. 2008). Information on the human consumption of adult *H. parallela* has been reported; however, limited data are available on the nutritional composition of this beetle (Hu et al. 2010). According to our previous study, ethanol and water extracts of adult *H. parallela* have antioxidant properties (Liu et al. 2012b). However, there is no information on the nutritional composition and protein quality of this insect.

In this study, the chemical composition, fatty acid profile, and amino acid composition of the adult *H. parallela* were assessed. Additionally, the protein digestibility-corrected amino acid score (PDCAAS) of this insect was calculated.

Materials and Methods

Sample Preparation. Adult *H. parallela* were collected in July from peanut fields in the suburbs of Qingdao (Shandong Province, China). The beetles were starved for 48 h to empty their gut contents, washed with water, and killed by exposing them to freezing temperatures. The frozen samples were allowed to thaw at room temperature and were air-dried at 50°C for 2 d. After removing the wings and legs, the dried samples were ground in a mill, passed through a 50-mesh screen, and stored at 4°C in air-tight containers.

Composition. Moisture, ash, and fat contents were determined according to the Association of Official Analytical Chemists methods 934.01, 942.05, and 920.39, respectively (Association of Official Analytical Chemists [AOAC] 2006). Crude protein was determined by the Kjeldahl method (984.13) using the nitrogen conversion factor of 6.25. Chitin was extracted according to the method reported by Liu. (Liu et al. 2012a) Carbohydrate content was calculated by the following equation:

$$\text{Carbohydrate} = 100 - \left(\begin{matrix} \text{moisture} + \text{ash} + \text{crude fat} \\ + \text{crude protein} + \text{chitin} \end{matrix} \right) \times 100\%$$

Fatty Acid Analysis. Fatty acid methyl esters were prepared as reported by Chen et al. (2010). Fatty acid determination was performed in a gas chromatograph (7890A GC/5975C MS, Agilent, Santa clara USA), equipped with an HP-5MS capillary column (30 m by 0.25 mm by 0.25 μm). The injector port and detector temperatures were held at 260°C and 280°C, respectively. The injected volume was 1 μl. The temperature of the column was held at 50°C for 1 min, increased to 190°C at a rate of 20°C/min, then increased to 240°C at a rate of 4°C/min, and finally increased to 280°C at a rate of 10°C/min. The final temperature was held for 2 min. Fatty acids in the samples were identified by comparing their retention times to those of fatty acid methyl ester standards (Sigma, Santa Clara USA).

Minerals Analysis. Minerals were analyzed at the Analytical Center of the Institute of Oceanology of the Chinese Academy of Sciences. The samples were subjected to a dry-ashing procedure at 550°C according to the AOAC method (AOAC 2006). Mineral content was determined according to the method reported by Fernandez et al. (2002) using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Thermo Fisher Scientific, USA) and calibration curves of mineral standards.

Amino Acid Analysis. Amino acid analysis was performed by ion-exchange chromatography with an automatic amino acid analyzer (Hitachi L-8800, Hitachi, Japan) in Shandong Academy of

Agriculture Science (China) according to the method reported by Cuevas-Rodriguez et al. (2006). The amino acid score (AAS) was calculated using the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO)-suggested pattern of amino acid requirements for children of preschool age (2–5 yr).

Sequential In Vitro Protein Digestibility Procedure. In vitro protein digestibility was assessed as described by Tang et al. (2009) with some modifications (Tang et al. 2009). Ground sample (2.0 g) was suspended in 50 ml of distilled water and adjusted to pH 2.0 with 12 N HCl. Pepsin (10% w/w, Sigma) was added, and the mixture was incubated at 37°C for 2 h. The sample pH value was adjusted to 5.3 with 0.9 M $NaHCO_3$ and to 7.5 with 1.0 M NaOH. Pancreatin (10% w/w, Sigma) was added, and the mixture was incubated at 37°C for 2 h. To inactivate the enzymes, the test tubes containing the digests were submerged in boiling water for 10 min. The sample digests were cooled to room temperature and centrifuged (10,000 × *g* for 20 min). Blanks containing only enzymes were simultaneously run. The digestibility value was calculated (after subtracting the blank) by dividing the supernatant nitrogen content by the total nitrogen content in the 2-g sample and multiplying the value by 100.

PDCAAS. PDCAAS was calculated using the following formula:

$$\text{PDCAAS} = (\text{mg of the limiting amino acid in 1g protein of the sample} \times \text{digestibility})/\text{mg of same amino acid in 1g of a reference pattern}$$

Results and Discussion

Composition. The results, shown in Table 1, reveal that adult *H. parallela* is an excellent protein source. The protein content of adult *H. parallela* was approximately 24%, which is higher than the 16% protein content of silkworm (Longvah et al. 2011). On a dry weight (DW) basis, *H. parallela* contained 70.57 g of protein/100 g, which is comparable to the protein content of beef and pork (40–75 g of protein/100 g DW; Bukkens 1997). Compared with other insects, the protein content of *H. parallela* was within the normal range (40–75 g/100 g DW; Verkerk et al. 2007). The protein content of *H. parallela*, however, was higher than that of other Coleoptera beetles, which have a crude protein content of 21–54 g/100 g DW (Ramos-Elorduy et al. 1997). Adult *H. parallela* collected from different locations have very similar protein contents (70.27% DW; Hu et al. 2010).

It has been reported that insects contain different amounts of chitin, which contribute to the nitrogen content of the insect (Ozimek et al. 1985, Zhang et al. 2000, Majtan et al. 2007). By using a nitrogen conversion factor of 6.25, there may be an overestimation of the actual protein content of the insect. Chitin was extracted from adult *H. parallela* by demineralization and deproteination methods (Zhang et al. 2000, Liu et al. 2012a). The chitin content in adult *H. parallela* was approximately 10.5%. The crude fiber content of this species has been reported to be 10.36% (Hu et al. 2010). Therefore, chitin was the main constituent of crude fiber. In comparison, chitin content in honeybee is 11.1% (Ozimek et al. 1985) and in silkworm pupae and larvae is 15–20% (Zhang et al. 2000). In this study, the nitrogen content of the extracted chitin was 6.4%. Because the theoretical nitrogen content of completely acetylated chitin is 6.89%, it is possible that insignificant amounts of protein remain following chitin extraction. When using this value to correct for the nitrogen content in chitin, the corrected protein content of adult *H. parallela* was 66.4%, i.e., 4% lower than the crude protein content. This nitrogen value was still within the normal range and was used for the amino acid composition analysis.

Adult *H. parallela* had a low fat content (3.8 g of fat/100 g DW). The fat content of insects varies widely among species; fat content ranges from 7 to 77 g/100 g DW (Verkerk et al. 2007). Because of its high protein content, adult *H. parallela* has a high protein to fat ratio, comparable to that of lean beef.

Fatty Acid Composition. The fatty acid composition of adult *H. parallela* is shown in Table 2. Oleic acid (40.75%) was the most abundant fatty acid, followed by linoleic acid (35.72%). Oleic acid and linoleic acid accounted for approximately 75% of the total fatty acids. The content of palmitic acid and stearic acid was 13.29% and 7.05%, respectively. These four fatty acids were the most abundant fatty acids in *H. parallela*; the rest of the fatty acids accounted for <5%. Even though adult *H. parallela* had a low fat content, it was a good source of unsaturated fatty acids (2.9 g/100 g DW).

It has been reported that there are differences in the fatty acid composition among insects, even among those within the same taxonomic family (Bukkens 1997). On the other hand, the fatty acid composition of insects collected from the same location is similar, suggesting that the fatty acid composition is affected by diet (Bukkens 1997). Interestingly, the range of concentrations of the fatty acids in adult *H. parallela* was similar to that of peanut kernels (Grosso et al. 2000). In this study, the adult *H. parallela*, which were collected from peanut fields, consumed peanuts and thus had a similar fatty acid composition to that of the legume.

Mineral and Trace Element Contents of *H. parallela*. It has been reported that insects are good sources of minerals (Verkerk et al. 2007). The dried beetle samples contained 5.53 g of ash/100 g DW, which is higher than the 3.61% ash content previously reported for this species (Hu et al. 2010). The ash content is indicative of the mineral content; in insects, ash content is 3–8 g/100 g DW (Verkerk et al. 2007).

Adult *H. parallela* are rich in essential minerals such as potassium, calcium, phosphorus, magnesium, manganese, copper, zinc, and iron (Table 3). Beetles contain a high iron content (28 mg/100 g DW), higher than that of silkworm prepupae and pupae (24 mg/100 g; Longvah et al. 2011). The iron content of *H. parallela* is comparable to that of other insects but higher than that of beef (~6 mg/100 g DW; Bukkens 1997).

Table 2. Related percentages of fatty acid of adult *H. parallela*

Fatty acid	Content (%)
Myristic acid (C14:0)	0.53
Pentadecanoic acid (C15:0)	0.19
Palmitic acid (C16:0)	13.29
Palmitoleic acid (C16:1)	1.1
Heptadecanoic acid (C17:0)	1.08
Stearic acid (C18:0)	7.05
Oleic acid (C18:1)	40.75
Linoleic acid (C18:2)	35.72
Arachidic acid (C20:0)	0.2
Eicosenoic acid (C20:1)	0.61
Behenic acid (C22:0)	0.13
Lignoceric acid (C24:0)	0.08

Table 1. Proximate compositions of adult *H. parallela* (g/100 g)

	Moisture	Ash	Crude protein	Crude lipid	Chititn	Carbohydrate
Samples in this study	3.63 ± 0.04	5.53 ± 0.23	70.57 ± 0.10	3.76 ± 0.12	10.47 ± 0.53	6.04
Other samples[a]	—	3.61	70.27	16.26	10.36 (crude fiber)	—

Values are expressed as means ± standard deviations of triplicate analyses.
[a]Adult *H. parallela* from Zhucheng, Shandong, China (Hu et al. 2010).

Table 3. Mineral composition of adult *H. parallela*

Minerals	Content (mg/kg DW)	Content (mg/kg DW)[a]
Potassium	13,887 ± 326	13,193
Phosphorus	7,379 ± 37	—
Magnesium	2,065 ± 7	2,474
Calcium	1,415 ± 36	1,143
Iron	281 ± 31	329
Zinc	154 ± 16	178
Manganese	68 ± 1	—
Copper	72 ± 0.6	41

Values are expressed as means ± standard deviations of triplicate analyses.
[a]Adult *H. parallela* from Zhucheng, Shandong, China (Hu et al. 2010).

Table 4. Amino acid compositions of adult *H. parallela*

Amino acid	g/100 g dried weight	g/100 g crude protein	g/100 g net protein
Aspartate	4.49 ± 0.08	6.76	8.89
Threonine[a]	1.97 ± 0.12	2.97	3.89
Serine	2.40 ± 0.41	3.61	4.75
Glutamate	6.92 ± 0.76	10.42	13.69
Glycine	4.81 ± 0.97	7.24	9.52
Alanine	3.66 ± 0.77	5.51	7.24
Cysteine + methionine[a]	1.87 ± 0.20	2.82	3.71
Valine[a]	3.41 ± 0.20	5.14	6.75
Isoleucine[a]	2.92 ± 0.14	4.4	5.78
Leucine[a]	4.38 ± 0.06	6.6	8.66
Tyrosine + Phenylalanine[a]	3.74 ± 0.24	5.63	7.4
Lysine[a]	3.59 ± 0.74	5.41	7.09
Histidine[a]	1.41 ± 0.14	2.12	2.78
Arginine	2.40 ± 0.21	3.61	4.74
Proline	1.90 ± 0.40	2.86	3.76
Tryptophan[a]	0.68 ± 0.23	1.02	1.35

Values are expressed as means ± standard deviations of triplicate analyses.
[a]Essential amino acid.

The zinc content of *H. parallela* was 15.4 mg/100 g DW, which was higher than that of dried silkworm pupae (Longvah et al. 2011). Adult *H. parallela* contain high amounts of zinc and iron, which are essential for human health. The iron and zinc contents of *H. parallela* previously reported were 33 mg/100 g and 17 mg/100 g DW, respectively.

Amino Acid Composition. The amino acid composition of *H. parallela* is listed in Table 4. To the best of our knowledge, no study has reported the amino acid composition of *H. parallela.* Based on the results, the corrected crude protein content (64%) was higher than the sum of the protein amino acids (50%). It has been reported that the differences in values between crude and net protein content depend on the type of food group (Salo-Vaananen and Koivistoinen 1996). When the nitrogen conversion factor of meat and meat products (i.e., 5.17) was used to calculate the protein content of *H. parallela*, the crude protein content (54.3%) was close to the sum of the protein amino acids (50.56%).

According to the amino acid composition analysis, glutamate was the most abundant amino acid, followed by glycine, aspartate, and leucine, with values of 13.69, 9.52, 8.89, and 8.66 g/100 g of net protein, respectively. The percentage of savory amino acids (i.e., aspartate and glutamate) and sweet amino acids (i.e., glycine and alanine) were 23% and 17%, respectively. These values were similar to those present in silkworm (23% savory amino acids and 12% sweet amino acids; Longvah et al. 2011). *H. parallela* had an adequate amino acid balance, with high levels of essential amino acids (EAAs). *H. parallela* contained 18 of the common amino acids; 47.39% were EAAs, and the ratio of EAAs to non-EAAs was 0.90, which meets the FAO/WHO (1973) requirements of 40% and 0.6, respectively.

Table 5 lists the AAS compared with the FAO/WHO (1985) amino acid requirements for a 2–5-yr-old child. It has been reported that AAS is underestimated when determined by the crude protein content

Table 5. Estimate AAS of adult *Holotrichia parallela* based on the crude and net protein content

Amino acid	FAO/WHO pattern for preschool children	Crude protein	Net protein
His	1.9	112	146
Ile	2.8	157	206
Leu	6.6	100	131
Lys	5.8	93	122
Met and Cys	2.5	113	148
Phe and Tyr	6.3	89	117
Thr	3.4	87[a]	114[a]
Val	3.5	147	193
Trp	1.1	93	123

[a]Limiting amino acid.

Table 6. Estimated PDCAAS of adult *H. parallela* based on the crude and net protein content

	Crude protein	Net protein
AAS	87	100
Digestibility (%)	78.43	78.43
PDCAAS	68.23	89.41

(Zlatanos et al. 2006). In this study, the AAS of *H. parallela* was determined by the crude protein content (66.4%) and net protein content (50.56%). The AASs were 87 and 100, based on the crude protein and net protein content, respectively; threonine was the limiting amino acid. Tryptophan and lysine have been reported to be limiting amino acids in most edible insects; however, limiting amino acids vary widely according to the insect species (Bukkens 1997). Lysine is the limiting amino acid in cereal-based foods (Mokrane et al. 2010); the high lysine content in *H. parallela* (7.09 g/100 g net protein) might help to supplement cereal-based diets, which are generally low in lysine.

PDCAAS of *H. parallela*. Insects with hard exoskeletons may have poor digestibility. Therefore, crude protein content may not be an accurate measure of the biologically available nitrogen. WHO suggested that PDCAAS should be used for the evaluation of protein quality in foods. The in vitro protein digestibility of various insects ranges from 76 to 98% (Ramos-Elorduy et al. 1997). The in vitro protein digestibility of adult *H. parallela* was within this range (78.4%). The PDCAAS calculated for *H. parallela* was 68 and 89 based on the crude protein and net protein content, respectively (Table 6). *H. parallela* PDCAAS is comparable to that of silkworm (86, based on net protein content; Longvah et al. 2011) but higher than those of food plants, such as peanut meal (52) and whole wheat (42; Singh et al. 2008). Additionally, *H. parallela* PDCAAS was comparable to those of seafood; the PDCAAS of cuttlefish, octopus, and squid is 70.9–81.4 based on crude protein content and 89.7–100 based on net protein content (Zlatanos et al. 2006).

Adult *H. parallela* is an edible beetle with high levels of protein (70%) and minerals and low levels of fat. It contained 10% chitin; the corrected protein content was 66%. The amino acid profile revealed that this beetle is a good source of EAAs. The high levels of lysine present in this beetle might help to supplement cereal-based diets, which are low in lysine. The high PDCCAS indicates that beetle protein is of good nutritional value. Therefore, adult *H. parallela* may be a potential source of proteins and minerals for humans and animals.

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