

Analysis of the nutritional components in larvae and pupae of *Hepialus gonggaensis*

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Abstract: *Ophiocordyceps sinensis*, a herbal medicine also named DongChongXiaCao (DCXC), is one of the most valuable medicinal fungus used in traditional Chinese medicine for the treatment of many chronic disease. The natural product of *O. sinensis* is actually a caterpillar entomopathogenic fungus-host larva complex. Host insect is not only the material basis for the formation of *O. sinensis*, but also an important part of the efficacy. The aim of the present paper is to evaluate protein, amino acid, mineral element and water content composition of larvae and pupae of *Hepialus gonggaensis*. Data analysis showed variable values in the content of the protein, amino acid, mineral element and water content composition. The pupae showed good nutritional value than the larvae. Heavy metal were also detected in indoor raised larvae. The Pb, As, Cu and Cd were detected, and the content were under the limits of food in China except for Cu. Furthermore, Hg reported in other literatures was not detected. Therefore, both the larvae and pupae of *H. gonggaensis* have certain edible value and prospect for developing functional foods.

Keywords: *Hepialus gonggaensis*; *Ophiocordyceps sinensis*; Nutritional components

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1. Introduction

Insects are invertebrate animals of the Class Insecta, the largest and most widely distributed taxon within the Phylum Arthropoda on land. They comprise the most numerous and diverse group of animals. Generally, insects are excellent sources of protein and amino acids, they provide an adequate content of mineral salts, vitamins and energy. Edible insect and its consumption is practiced worldwide and has recently been proposed as a potential solution to fight food shortages[1]. Many insects can provide a significant caloric intake, lipids are well represented in these animals, especially in the larval forms[2,3].

Ophiocordyceps sinensis, a larva-fungi complex, is one of the most valuable natural product and herbal medicine in China[4]. The pharmacological activities of *O. sinensis* has been demonstrated, including anti-tumor[5-6], anti-oxidant[7,8], anti-hyperglycemia activities[5] and improve the body's immune function significantly[9-11]. Previous studies have indicated that this medicine may also have a therapeutic effect against chronic kidney disease[12,13] and chronic obstructive pulmonary disease[14]. The *O. sinensis* parasitizes larvae of moths from the order Lepidoptera, particularly *Hepialus/Thitarodes*[15]. Host insect is not only the material basis for the formation of *O. sinensis*, but also an important part of the efficacy. Therefore, it is necessary to determine the nutritional components of host insect of *O. sinensis*. In this study, the protein, fat, amino acid and mineral element of the larvae and pupae of *Hepialus gonggaensis* were analyzed.

2. Materials and Methods

2.1. Animal materials and sample preparation

All larvae and pupae of *H. gonggaensis* were obtained from the breeding bases of Chongqing Academy of Chinese Material Medica, which located in Kangding City, Sichuan Province. They were maintained in glass containers populated with about 200 mature larvae and pupae (100, respectively) at 15 °C and 12 h/12 h day/night cycles prior to detection. The larvae and pupae of *H. gonggaensis* were weighed and recorded using an analytical balance, followed by smash, homogenate. Then the protein, crude fat, amino acid, mineral element and water content were detected according to certain standards.

2.2. Detection and Analysis

The content of water, crude fat, protein, amino acid, ash and mineral elements were detected according to the national standard GB 5009.3-2010, GB/T 5009.6-2003, GB/T 5009.5-2010, GB/T 5009.124-2003, GB/T5009.4-2010 and national agricultural industry standard NY/T1653-2008, respectively[16].

Nutrition analysis were performed as follows: The carbohydrate content (%) = 100 - (crude fat content + protein content + ash content + water content) (1); The energy (kJ/hg) = the protein mass fraction × 17 + the fat mass fraction × 37 + the carbohydrate mass fraction × 17 (2); In formula (1) and (2), all the mass fractions of protein, fat, and carbohydrate were the contents per 100 g of fresh sample[17]. The experimental data were analyzed using SPSS statistics.

3. Results

3.1. Fresh weight, dry weight and water content

The detection results were shown in Table 1. Average weight of fresh mature larva of the *H.*

gonggaensis achieved 0.465 g. The water content and dry weight in mature larva were 0.361 g and 0.104 g, and could reach 77.63% and 23.36% of its body weight, respectively. The average weight of fresh

pupa could reach 0.483 g. The water content and dry weight in pupa were 0.366g and 0.117g, and came up to 75.77% and 24.22%.

Table 1. Fresh weight, dry weight, water content of larva and pupa of *Hepialus gonggaensis*

Developmental stage	Fresh weight	Fresh weight	Water content
larva	0.465	0.104	77.63
pupa	0.483	0.117	75.77

3.2. Contents and energies of protein, crude fats, ash and carbohydrate

The detection results were shown in Table 2. The results indicated that the protein contents in the larvae and pupae of *H. gonggaensis* were 100.2mg/g and 118.6mg/g, respectively, which were far below than that of *Tenebrio molitor* [18], and were not much different than the protein content in the eggs[19], which was slightly higher than that in the bamboo insects[20]. The crude fat content in the larvae and pupae of *H. gonggaensis* were 47.50mg/g and 46.30mg/g, respectively, which were lower than those in their contrasting objects. The ash contents in the larvae and pupae of *H. gonggaensis* were 15.40 mg/g and 21.90 mg/g, respectively, which were

higher than those in other contrasting objects. The carbohydrate contents in the larvae and pupae of *H. gonggaensis* were 55.50mg/g and 58.40mg/g, respectively, which were higher than those in other contrasting objects. The energies per unit mass in the larvae and pupae of *H. gonggaensis* were 5.10KJ/g and 4.60KJ/g, respectively, which were lower than those in other contrasting objects such as the eggs. The contents of proteins, ash and carbohydrates in the pupae of the *H. gonggaensis* were slightly higher than those in larvae of the *H. gonggaensis* except for the fact that the crude fat content in the pupae of the *H. gonggaensis* was lower than that in the larvae of the *H. gonggaensis*, and the conventional nutrient contents of both larvae and pupae were similar.

Table 2. Content of protein, fat, ash, carbohydrate and energy in larva, pupa of *H. gonggaensis* and in other foods

Species	Protein content (mg/g)	Fat (mg/g)	Ash (mg/g)	Carbohydrate (mg/g)	Energy (KJ/g)
<i>H. gonggaensis</i> larva	100.20	47.50	15.40	55.50	5.10
<i>H. gonggaensis</i> pupa	118.60	46.30	21.90	58.40	4.60
<i>Tenebrio molitor</i> (L.) larva	210.30	141.90	15.70	14.30	9.07
<i>Tenebrio molitor</i> (L.) pupa	248.50	116.00	15.10	12.70	8.73
<i>Chilo fruscidentalis</i> Hampson	99.00	210.00	5.00	24.00	9.86
Egg	147.00	90.00	10.00	15.00	5.74

Table 3. Content of amino acids in larva and pupa of *Hepialus gonggaensis*

Amino acids	Larva's content(%)	Pupa's content(%)	Amino acids	Larva's content(%)	Pupa's content(%)
ASP	3.18	3.52	ILE*	1.50	1.93
THR*	1.41	1.56	LEU*	0.58	2.06
SER	2.26	2.35	TYR	1.77	2.15
GLU	4.10	5.22	PHE*	1.48	1.62
GLY	1.83	2.33	LYS*	1.22	2.47
ALA	2.39	3.10	HIS	0.97	1.28
CYS	0.30	0.48	ARG	1.77	2.36
VAL*	2.13	2.54	(E+N)	31.24	38.71
MET*	0.64	1.65	E(N)	8.96(22.28)	10.53(28.18)
PRO	1.71	2.09	E/(E+N)	28.68	27.20
TRP*	—	—	E/N	0.40	0.37

Note: * is essential amino-acid; E is Essential amino acid total content; N is non-essential amino acid total content; - is not detected.

3.3. Amino acid analysis

As can be seen from Table 3, both the larvae and pupae of *H. gonggaensis* contained 17 kinds of amino acids, the total amino acid content was 38.71% in the pupae, which was higher than 31.24% in the larvae, and the essential and non-essential amino acid contents were 10.53% and 28.18% respectively in the pupae, both of them were higher than 8.96% and 22.28% in the larvae. The proportion of essential amino acids in total amino acids was 27.2% in the pupae, which was slightly lower than 28.68% in the larvae, and the ratio of essential amino acids to non-essential amino acids was 0.37 in the pupae, which was slightly lower than 0.40 in the larvae. Among 17 kinds of amino acids in the larvae, the contents of the glutamic acid and asparaginic acid were relatively higher, which were 4.10% and 3.18%, respectively. The contents of cystine and methionine were relatively lower, which were 0.30% and 0.64%,

respectively. The contents of glutamic acid and asparaginic acid in the pupae were relatively higher, which were 5.22% and 3.52%, respectively. The contents of cystine and histidine in the pupae were relatively lower, which were 0.48% and 1.28%, respectively.

3.4. Heavy metal analysis

It can be seen from Table 4 that the content of heavy metal elements in the pupae of *H. gonggaensis* was slightly higher than that in the larvae, and the metal Hg was not detected in both of them. The content of the metal element Cu was slightly higher than the maximum limit of heavy metals in the foods specified (Chinese Food Laws $Pb \leq 1\text{mg/kg}$, $Cd \leq 0.2\text{mg/kg}$, $As \leq 2.0\text{mg/kg}$, $Hg \leq 0.1\text{mg/kg}$ and $Cu \leq 10\text{mg/kg}$). The contents of the metal elements As, Cd and Pb were well within the maximum limit of heavy metals (Chinese Food Laws).

Table 4. Content of heavy metal in larva and pupa of *Hepialus gonggaensis*

Stage	Element ($\mu\text{g/g}$)				
	Pb	Cd	As	Hg	Cu
larva	0.332	0.098	1.192	N.D.	21.751
pupa	0.415	0.156	1.325	N.D.	22.085

4. Discussion

The *H. gonggaensis*, as a host insect of *O. sinensis*, has been artificially bred in large quantities. The nutrition of *O. sinensis* comes mainly from its host *H. gonggaensis* larvae. The purpose of this study was to determine the nutritional components of the larvae and pupae of *H. gonggaensis* under the artificial breeding condition. In order to provide basic data for the study of nutrition transformation in the forming process of *O. sinensis* and also provide reference, they have given the values for food development. The results of this study indicated that the larvae and pupae of *H. gonggaensis* are rich of proteins, and the P/G value of crude protein and crude fat has reached 2.0 (insects with $P/G < 2$ are called high-fat insects)[21]. In terms of amino acids, the content of amino acids in foods, especially the contents of 9 kinds of essential amino acids and the structural proportion of amino acids are the main factors that determine the nutritional values of proteinogenic amino acids. The larvae and pupae of *H. gonggaensis* have all kinds of amino acids and the contents are relatively abundant and comprehensive. 17 amino acids were detected in both larvae and pupae. The essential amino acids and semi-essential amino acids show relatively high-quality proteins. Therefore, the larvae and pupae of *H. gonggaensis* have a high nutritional value for food development.

The heavy metal contents in the larvae and pupae of artificially-bred *H. gonggaensis* are much lower

than those in medicinal materials specified in the Chinese Pharmacopoeia. The heavy metal contents are not exceed the specified range for Chinese foods, while the nutrition (including heavy metal contents) of *O. sinensis* comes from the host larvae. It may be due to the fact that the foods of the larvae of indoor-bred *H. gonggaensis* are sufficient and simple. The larvae of *H. gonggaensis* can feed on humus soil under the condition. There is no food for feeding[22]. The heavy metal contents in the soil of the Qinghai-Tibet Plateau are generally higher, especially for the metal arsenic content[23]. It can be inferred that the higher heavy metal content in the larvae of the *H. gonggaensis* in the wild leads to slightly higher heavy metal content in *O. sinensis*.

5. Conclusion

Our analysis results also indicated that the content of heavy metals in *O. sinensis* feeding on indoor-bred *H. gonggaensis* larvae was much lower than those in the *O. sinensis* feeding on *H. gonggaensis* larvae in the wild. Therefore, the content of heavy metals in *O. sinensis* can be improved by reducing the heavy metal content in the larval breeding environment and feed.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial

relationships that could be construed as a potential conflict of interest.

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