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Analysis of nutrients in *Apriona germari* and treatment of diarrhea in mice fed with insect powder

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ABSTRACT

The insect larvae of *Apriona germari*, *Apriona swainsoni* and other closely related species are related to the insects that bore through the stems of the *Caesalpinia decapetala*. Because of therich nutritional as well as medicinal value of edible *A. germari*, it is often used to nourish the body, enhance immunity, and to treat ailments such as diarrhea. The soluble protein content of *A. germari* was determined using Kjeldahl and Soxhlet extraction methods. The crude fat content and the content of various fatty acids was determined by the normalization method, normalization method refers to that after the fat in the sample is extracted by hydrolysis ether solution, it is saponified and methylated under alkaline conditions to generate fatty acids and methyl esters, which are analyzed by capillary column gas chromatography and quantitatively measured by area normalization method. Amino acid content determined using an amino acid analyzer after acid hydrolysis showed 17 kinds of amino acids. A diarrhea mouse model was established by injecting a suspension of *Escherichia coli* into the abdominal cavity of mice. The mice were then fed with *A. germari* fodders at dose concentrations of 12%, 8%, 4% and 0%. The number of *E. coli* was determined from the intestinal tracts of mice; the activities of antioxidant enzymes and digestive enzymes in the serum were determined. Eleven kinds of fatty acids were detected in *A. germari*; unsaturated fatty acids accounted for 71.87% of the total fatty acids. *A. germari* is an edible resource insect with high nutritional value. Mice fed with *A. germari* showed an increase in the activity of antioxidant enzymes and digestive enzymes in the serum, which helped in the effective inhibition and reduction in the total number of intestinal bacteria and *E. coli*. The results indicate that *A. germari* has high nutritional value; *A. germari* also has a certain therapeutic effect on diarrhea caused by *E. coli* in mice.

1. Introduction

Doumi Beetles (also known as cloudworms and thorns in some areas) are cylindrical in shape, which belong to *Apriona germari*, *Apriona swainsoni*, and whole larvae of other closely related insects. However, since the two species of Longhorn beetles, *A. germari* and *A. swainsoni* are the most common inhabitants of *Caesalpinia decapetala* [1], the main biomorphological differences between the larvae of the *A. germari* and the *A. swainsoni* have been analyzed. Buckwheat is a resource insect with both edible nutritional value and medicinal value. It is said that in ancient times, people exchanged a bucket of rice for a worm, hence it got its name [2].

According to Pharmacopoeia records such as "Compendium of Materia Medica" [3] and "Shen Nong's Materia Medica", harvesting and supplementing Doumi Beetles strengthen the body; Doumi Beetles have a therapeutic effect on measles involution, prickly heat rash, liver cirrhosis, blood diseases and other ailments. Doumi Beetles are also known for their unique effects on anorexia and nocturnal bedwetting caused by "chancre accumulation" in young children; they help alleviate the effects on growth retardation, drooling, teeth grinding, hemorrhoids, muscle pain, and bone pain. There are some examples in some parts of East China: there are many patients with indigestion and diarrhea who have improved or even recovered after eating Doumi Beetles. Because of the high nutritional value of fighting rice worms, the tradition of eating fighting rice worms in China has continued till this day.

However, there are few scientific researches on Doumi Beetles in the

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treatment of diarrhea. Therefore, it is urgent to provide theoretical research support for scientific research experiments and papers, and finally provide further theoretical basis for the safe consumption, medicinal effect and health food development of Doumi Beetles.

2. Materials and methods

2.1. Lipid extraction from A. germari using soxhlet method

Total lipids were extracted using petroleum ether as the solvent in a Soxhlet apparatus. The extraction was carried out for about 8 h until there are no traces of oil in the extraction tube, which is an indication for completion of lipid extraction. The resultant lipid sample was then dried in an oven at 25 °C for 24 h, cooled in a desiccator, and weighed. The fatty acid content of the samples were determined using a normalization method. GB5009.168.2016, detect the content of various fatty acids in *B. militaris* [4].

2.2. Preparation of mouse model by injection of E. coli and data processing

A total of 40 healthy, 3–4-week-old normal Kunming male mice with a mass of 20 ± 2 g were selected; five groups of mice, four mice per group were arranged in a random combination. The groups were as follows: the control group, 12.0% buckthorn powder group, 8.0% buckthorn worm powder group, and 4.0% buckeye worm powder group. The mice in the control group were fed with 2 g of the basal diet (air-dried).

The mice were injected intraperitoneally with 20.0 mL/μg of 3×10^8 CFU/mL *E. coli* solution for 5 days. For modeling, the mice in the blank control group were injected with the same volume of sterilized normal saline into the abdominal cavity; each group was fed with basal diet during the period of diarrhea construction.

After the diarrhea model was constructed, the mice were fed with mixed diets for 2 weeks. During the treatment of mice with *B. chinensis* powder, the experimental mice in the 12.0% *B. militaris* powder group, 8.0% B. The additional amount of buckwheat meal was 2 g each of 12.0%, 8.0%, and 4.0% mixed diets; all the experimental mice in the blank control group were made to wait until each group of experimental mice completed the corresponding 2 g mixed diet, after which the mice were finally fed with the basal diet freely.

After the treatment period, three mice with similar health status were selected from the three replicates of each group, and anesthetized with an appropriate amount of ether. Blood samples were collected from the heart with a 2 mL syringe to obtain the serum, which was used to analyze further parameters. Amylase (AMS), lipase (LPS), and lactate dehydrogenase (LDH) activities in serum samples were analyzed using relevant kits. Other enzymes analyzed include Peroxidase (POD), superoxide dismutase (SOD), glutathione sulfur transferase (GST), and catalase (CAT) activities. After blood collection, the intestines of the mice were harvested following anatomical principles and stored at −80 °C until further analysis.

3. Results

3.1. Fatty acid analysis of A. germari

A total of 11 kinds of fatty acids were detected in *B. chinensis*. Saturated fatty acids (lauric acid, myristic acid, stearic acid, arachidic acid, palmitic acid, pearl fatty acid) accounted for 28.13% of the total fatty acids, and unsaturated fatty acids accounted for 71.87% of the total fatty acids. Among them, monounsaturated fatty acids (oleic acid, palmitoleic acid, arachidonic acid) accounted for 69.69% of the total fatty acids, and polyunsaturated acids (linoleic acid, linolenic acid) accounted for 2.18% of the total fatty acids. Saturated fatty acids are mainly represented by palmitic acid (25.55%); unsaturated fatty acids are mainly represented by oleic acid (63.68%). The specific contents and types are shown in Table 1.

3.2. Bacterial load in the intestines of mice infected with E. coli and fed with A. germari

Analysis of bacterial load in the colon, cecum, and ileum regions of the large intestine in mice in the 4% B. The results are listed in Table 2. The number of *E. coli* CFU in the three intestinal parts of the mice in the control group was significantly different from that in the blank control group and the three groups of B. ($P < 0.05$). The number of *E. coli* in the cecal intestinal tract in the model control group was the highest compared with that of other groups, reaching 20.24×10^6 CFU/mL; *E. coli* colonies in the three intestinal parts of the experimental mice showed no significant difference in the case of 12.0% *B. chinensis* powder group compared with the blank group ($P > 0.05$). Compared with the 4.0% *B. chinensis* powder group and the model group, the *E. coli* load was significantly lower ($P < 0.05$). In the cecum, there was no significant difference between the 8.0% and 12.0% *B. chinensis* group ($P > 0.05$); in the ileum and colon, there was a significant difference between the 8.0% and 12.0% *B. chinensis* group ($P < 0.05$). It can be seen that the effect of inhibiting the number of intestinal *E. coli* is the most significant at a concentration of 12.0%.

4. Discussion

Pathogenic diarrhea is a contagious disease and an important cause of poor health in animal larvae. In this study, the number of *E. coli* and total bacteria in the intestinal tract of mice was detected by feeding A. germari powder to mice suffering from diarrhea. It was found that *B. militaris* powder could effectively inhibit the growth of *E. coli*. In the research on insect inhibition of *E. coli*, Meier J L [4] found that the antibacterial protein extract of *Stomoxys calcitrans* exerted an inhibitory effect on the growth of *E. coli*; Vassilopoulou L [5] found that fly maggot antimicrobial peptides can effectively reduce the number of *E. coli* in mice. In the research on the inhibition of *E. coli* by traditional Chinese medicines, Chen Y et al. [6] found that Huanglian Jiedu decoction had an inhibitory effect on the growth of intestinal *E. coli* in rats. The results of this experiment are consistent with the above research findings, indicating that A. germari has a good bacteriostatic effect on *E. coli* in the intestinal tract of mice.

In this paper, the whole larvae of Doumi beetles were studied, and the effects of Doumi beetles on the blood enzyme activity and intestinal bacteria number of diarrhea individuals were discussed. However, it is not known whether there are special substances in Doumi beetles. Therefore, the functional components contained in the larvae of A. germari can be further explored to explore the functional components contained in Doumi beetles. In addition, Doumi beetles have many effects in ancient Chinese medicine records, such as the treatment of developmental delay, drooling, molars, hemorrhoids and bone pain. Therefore, there are still many aspects that need to be improved in how

Table 1
The content and types of fatty acids in *Apriona germari*.

Types of fatty acids	unit	content	SE
Lauric acid	g/100 g	0.0167	0.3565
myristic acid	g/100 g	0.1095	
Palmitic acid	g/100 g	5.3616	
palmitoleic acid	g/100 g	1.2449	
Pearlescent fatty acid	g/100 g	0.0149	
Stearic acid	g/100 g	0.2636	
Oleic acid	g/100 g	13.3664	
Linoleic acid	g/100 g	0.4098	
arachidic acid	g/100 g	0.137	
linolenic acid	g/100 g	0.0477	
arachidonic acid	g/100 g	0.0167	

Table 2
Effects of Apriona germari powder on intestinal *Escherichia coli* count of diarrhea mice.

Groups	*E. coli* count (CFU· mL^{-1})		
	Cecum ($\times 10^6$)	Ileum ($\times 10^5$)	Colon ($\times 10^5$)
Blank group	4.92 ± 0.39^{d}	2.90 ± 0.40^{d}	5.58 ± 0.62^{d}
Model group	20.24 ± 2.03^{a}	9.57 ± 0.87^{a}	13.57 ± 1.83^{a}
4.0% A. germari powder group	13.05 ± 1.92^{b}	6.75 ± 0.89^{b}	10.03 ± 1.16^{b}

to make better use of the rice worm and better develop the edible and medicinal value of the rice worm.

Animal ethics

The animal experiments complied with the ARRIVE guidelines and be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

All animals were kept in a pathogen-free environment and fed ad lib. The procedures for care and use of animals were approved by the Ethics Committee of the College of Veterinary Medicine (Nov.zffuac2021011) and all applicable institutional and governmental regulations concerning the ethical use of animals were followed. This experiment ignores the influence of sex on the results of the experiment, and the selection of animals also includes males and females to show "gender equality" in animal experiments.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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