Research Article

Amino acid, fatty acid, and carbohydrate metabolomic profiles with ginsenoside-induced insecticidal efficacy against *Ostrinia furnacalis* (Guenee)

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A B S T R A C T

Background: Previous studies have shown the insecticidal efficacy of ginsenosides. In the present study, we aimed to investigate the metabolic mechanism related to the inhibitory effect of panaxadiol saponins (PDSs) against the Asian corn borer *Ostrinia furnacalis* (Guenee).
Methods: Third instar larvae of *O. furnacalis* were fed normal diets with different concentrations of PDSs for 4 days. The consumption index, relative growth rate, approximate digestibility, and conversion of ingested and digested food were recorded. A targeted gas chromatography–mass spectrometry assay was performed to detect the profiles of amino acids, fatty acids, and carbohydrates in larvae of *O. furnacalis*. In addition, the activity of detoxification-related enzymes was determined.
Results and Conclusions: PDSs decreased the consumption index, relative growth rate, approximate digestibility, and conversion of ingested and digested food in the 3rd instar larvae of *O. furnacalis* in a dose-dependent manner. PDSs decreased 15 free amino acids, 16 free fatty acids, and 5 carbohydrates and increased the levels of palmitoleic acid, palmitic acid, and 9-octadecenoic acid in the 3rd instar larvae. The activity of detoxification-related enzymes, such as acetylcholinesterase, glutathione S-transferase, cytochrome P450, carboxylesterase, trehalase, acid phosphatase, and alkaline phosphatase, was reduced in a dose-dependent manner in the 3rd instar larvae exposed to PDSs. These data confirmed the inhibitory effect of PDSs against growth, food utilization, and detoxification in the 3rd instar larvae of *O. furnacalis* and the potential for using PDSs as an efficient tool for insect pest management for *O. furnacalis* larvae.
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1. Introduction

Ginsenosides can limit the oviposition, feeding, and egg viability of insects, thus indicating the efficacy of ginsenosides as control agents against insect pests [1–4]. Ginsenosides include approximately 60 triterpene saponins and panaxadiol saponins (PDSs) and are found in *Panax ginseng* [5]. Clinical and preclinical trials in humans and animals have shown various pharmacological properties of PDSs, including antiinflammatory, antifatigue, antiallergic, and antistress properties [6–10]. These studies might also show

that ginsenosides can affect the accumulation and metabolism of secondary metabolites in insects and other mammals.

As a high-throughput technique, metabolomics can identify small molecules, based on which novel insights into biological processes associated with insecticide toxicology and resistance can be revealed [11–13]. The metabolism of carbohydrates, amino acids, and lipids in insect pests is responsive to insecticides and pesticides [11]. It has been reported that resistance to neonicotinoids, such as imidacloprid, in *Drosophila* larvae is metabolic, proteomic based, and associated with changes in metabolic-related enzymes, including cytochrome P450 (CYP450) enzymes [4,12–14].

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Ginsenosides have been shown to regulate the metabolism of amino acids, glucose, and lipids in rats [9,15,16]. Wang et al [9] showed that total ginsenosides, PDSs, and panaxatriol saponins changed the urine metabolites, including neurotransmitters, amino acids, organic acids, and gut microbiota metabolites, in Wistar rats. Our previous reports have confirmed the antifeedant, oviposition-deterring, and enzymatic inhibitory activities of total ginsenosides from *P. ginseng* on *Mythimna separata* and *Pieris rapae* [3,4]. We determined the inhibitory activities of acetylcholinesterase (AChE) and glutathione S-transferase (GST) by total ginsenosides in the management of *M. separata* larvae. Yang et al [17] showed that the administration of *P. ginseng* total ginsenosides obviously reduced the activity of AChE, carboxylesterase (CarE), and GST and increased mixed-function oxidase activity in *Plutella xylostella* (Linnaeus), suggesting the antidetoxification capacity of total ginsenosides in insect pests. Accordingly, we speculated that the efficient preventative effects of ginsenosides against pest infestation might be related to impaired metabolism within the pest. However, the understanding of the underlying metabolomics mechanism

China (obtained by chromatography of water extract on D101 macroporous resin). The alcohol extract was then dissolved in 10% NaOH and reextracted using butyl alcohol. The NaOH fraction was used for PDS extraction using alcohol sedimentation. PDS was incorporated into normal diets for final concentrations of 0 (control), 0.5, 1.0, 2.0, 5.0, and 10 mg/g, and each experiment was replicated 20 times. Dry diets were stored at 4°C before the experiments.

2.2. Insect food consumption and utilization

The 3rd instar larvae (10 days after hatching) were fasted for 4 h and then evenly spaced in plastic dishes with prepared diets (n ≥ 20 for each concentration) for 4 days. Food was replaced daily. Insect feces and surplus food were collected daily and dried separately (80°C, 48 h). Food consumption, relative growth rate, digestibility, and conversion efficiency of digested food in the 3rd instar larvae were calculated according to the following formulas:

$$\text{Consumption index}(\%) = \frac{Dry\ weight\ of\ food\ eaten}{Duration(day) \times mean\ dry\ weight\ of\ larva} \times 100\%$$

$$\text{Relative growth rate}(\%) = \frac{Gained\ dry\ weight}{Duration(day) \times mean\ dry\ weight\ of\ larva} \times 100\%$$

$$\text{Approximate digestibility}(\%) = \frac{Dry\ weight\ of\ digested\ food}{Dry\ weight\ of\ ingested\ food} \times 100\%$$

$$\text{Conversion of ingested food}(\%) = \frac{Gained\ dry\ weight}{Dry\ weight\ of\ ingested\ food} \times 100\%$$

$$\text{Conversion of digested food}(\%) = \frac{Gained\ dry\ weight}{Dry\ weight\ of\ digested\ food} \times 100\%$$

associated with ginsenosides is limited.

Ostrinia furnacalis (Guenée) is a major insect pest of maize worldwide, especially in Asia [18]. The purpose of this study was to investigate the effect of ginsenosides (PDSs) on the amino acid, lipid, and carbohydrate profiles in the 3rd instar larvae of *O. furnacalis*. Targeted gas chromatography−mass spectrometry (GC−MS) was used to analyze the metabolomics profiles before and after exposure to ginsenosides in *O. furnacalis*. The impacts of ginsenosides on the growth, food consumption and utilization, and metabolic-related enzyme activity in *O. furnacalis* were also determined. To the best of our knowledge, this is the first study to investigate the metabolic-based mechanism associated with ginsenoside-mediated pest infestation against corn. This study provides novel implications into the ginsenoside-induced molecular toxicology and agricultural management of the Asian corn borer.

2. Materials and methods

2.1. Preparation of diet formulation

Total ginsenosides (75% alcohol extract, 90% purity, UV) from the leaf and stem of *P. ginseng* were obtained from the National Ginseng Engineering Research Center of Jilin Agricultural University, Jilin,

"Duration" indicates the feeding period (4 days); "mean dry weight of larvae (g)" was determined by taking the value of dry larval body weight at the beginning and end; "gained dry weight (g)" was determined by calculating the difference in dry larval weight at the beginning and end; "dry weight of digested food (g)" was determined by using the difference between dry weight of ingested food and feces (g). Conversion of digested food is the efficiency of digested food conversion to body substance in the 3rd instar larvae. Each experiment was replicated 20 times.

2.3. Analysis of feces

After feeding for 72 h, the feces of the 3rd instar larvae were collected, dried, and prepared for analysis. The qualitative and quantitative analyses of ginsenosides Rb1, Rc, Rb2, Rb3, and Rd in feces were performed using an HPLC system. Standards of Rb1, Rc, Rb2, Rb3, and Rd were obtained from the National Ginseng Engineering Center of Jilin Agricultural University, Jilin, China. Each experiment was replicated 10 times.

2.4. GC−MS analysis

Metabolomics profiles of 18 free amino acids (FAAs), 16 free fatty acids (FFAs), and 5 carbohydrates in the 3rd instar larvae of

O. furnacalis in response to PDS treatment were detected using targeted GC−MS techniques. Dried larvae were ground, and samples were incubated in trimethylsilane solutions [pyridine:hexamethyldisilazane:trimethylchlorosilane = 9:3:1 (v/v/v)] at 100°C for 30 min. Samples were then derivatized under nitrogen and dissolved into dichloromethane for analysis. GC−MS analysis was performed using a DB-5MS GC column (30 m × 0.25 mm, 0.25-μm film thickness; J&W Scientific, Folsom, CA, USA) with a temperature increase from 80°C to 280°C and a flow rate of 1.0 ml/min. The carrier gas was helium (He). GC−MS analysis was performed using a Thermo Scientific Trace GC ultra ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a quadrupole mass selective detector on electro-impact mode (70 eV). Based on these analyses, metabolic compounds were identified by comparing them to the standards in the national Mass Spectral Librarie (NIST 2008 database: www.sisweb.com/software/ms/nist.htm). The percentages were calculated using the peak area normalization method. Each experiment was replicated 5 times.

2.5. Enzyme-linked immunosorbent assay

At the end of the feeding period, larvae were collected, dried, ground, and centrifuged. Samples were quantified using the Coomassie Blue G250 method [19] and were then used to assess the contents of GST, CarE, CYP450, AChE, acid phosphatase (ACP), alkaline phosphatase (ALP), and trehalase (TH) using enzyme-linked immunosorbent assay kits (R&D, Minneapolis, Minnesota, USA) on a microplate reader (BIO680; Bio-Rad, Hercules, CA, USA). The inhibitory rates of enzymes were calculated as follows: inhibitory rate (%) = (control − test) × 100%/control. Each experiment was replicated 10 times.

2.6. Statistical analysis

Statistical analyses were performed using SPASS 22.0 (IBM, USA) software. All data were expressed as mean ± SD, and differences among groups were analyzed using one-way analysis of variance and multiple least significant difference tests. Differences were considered to be significant when $p < 0.05$

3. Results

3.1. Effects of PDSs on food consumption and utilization

PDSs inhibited the consumption, growth, digestibility, and conversion of food in the 3rd instar larvae in a dose-dependent manner. At a concentration of 10 mg/g, the consumption index, relative growth rate, approximate digestibility, conversion of ingested food, and conversion of digested food of the 3rd instar larvae decreased by 19.12%, 11.75%, 17.76%, 25.8%, and 35.88%, respectively, compared with the control ($p < 0.05$, Table 1).

3.2. Contents of PDSs in the feces

Table 2 shows that the content of saponin monomers (Rb1, Rc, Rb2, Rb3, and Rd) in the feces of the 3rd instar larvae increased with increase in PDS in a dose-dependent manner. The total content of all saponin monomers in the feces of the 3rd instar larvae fed with diets containing 10.0 mg/g PDS reached 37.45%, which was significantly higher than that in the feces of larvae consuming diets containing 5.0 mg/g PDS (11.10%) ($p < 0.001$, Fig. 1).

3.3. PDS metabolism of proteins, lipids, and carbohydrates

Fig. 2 shows that the total contents of FAAs, FFAs, and carbohydrates in the metabolic profiles of the 3rd instar larvae of *O. furnacalis* were gradually decreased with increasing PDS consumption in a dose-dependent manner (Fig. 3A, B and C). The total content of 18 FAAs, 5 carbohydrates, and 16 FFAs in the 3rd instar

Table 2
Contents of saponin monomers in the feces of the 3rd instar larvae of *O. furnacalis*

Con (mg/g)	Rb1	Rc	Rb2	Rb3	Rd
CK	0	0	0	0	0
0.5	0	0.12 ± 0.01e	0.30 ± 0.02e	0.56 ± 0.04d	0
1.0	0	0.57 ± 0.04c	2.29 ± 0.02b	0.44 ± 0.03e	0.49 ± 0.04d
2.0	0.15 ± 0.01c	0.49 ± 0.05d	1.57 ± 0.11c	0.82 ± 0.02c	2.60 ± 0.21c
5.0	1.42 ± 0.01b	2.03 ± 0.14b	0.57 ± 0.03d	3.14 ± 0.14b	3.94 ± 0.29b
10.0	3.82 ± 0.03a	4.37 ± 0.32a	3.46 ± 0.06a	6.02 ± 0.32a	19.78 ± 0.24a

CK, control; Con, concentration; LSD, least significant difference.
Different letters indicate significant differences at $p < 0.05$ by LSD analysis.
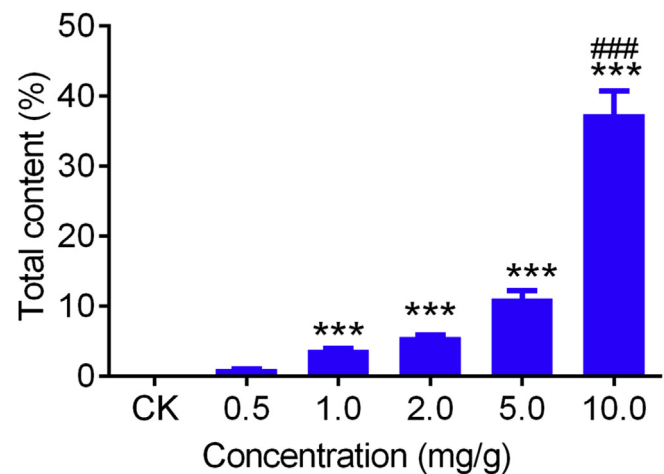

Fig. 1. The total content of ginsenosides in the feces of the 3rd instar larvae of *O. furnacalis*. ***$p < 0.001$ *vs.* 0.5 mg/g. ###$p < 0.001$ *vs.* 5.0 mg/g. CK, control.

Table 1
The indices of food consumption and utilization in the 3rd instar *O. furnacalis* in response to PDS treatment

Con (mg/g)	C.I. (%)	R.G.R. (%)	A.D. (%)	C.I. (%)	C.D. (%)
CK	2.04 ± 0.32a	2.98 ± 0.21a	47.58 ± 3.95a	16.20 ± 0.58a	43.64 ± 10.35a
0.5	1.86 ± 0.53ab	2.94 ± 0.16a	45.47 ± 3.28ab	14.40 ± 2.66ab	39.89 ± 11.64ab
1.0	1.83 ± 0.18ab	2.97 ± 0.11a	43.98 ± 4.73ab	13.78 ± 3.76b	33.90 ± 10.51bc
2.0	1.81 ± 0.18ab	2.89 ± 0.14a	41.50 ± 10.48abc	13.58 ± 1.75b	31.97 ± 5.48c
5.0	1.68 ± 0.23b	2.78 ± 0.55ab	40.13 ± 4.73abc	12.45 ± 2.78b	28.55 ± 10.92d
10.0	1.65 ± 0.45b	2.63 ± 0.41b	39.13 ± 4.33bc	12.02 ± 3.67b	27.98 ± 11.44d

A.D., approximate digestibility; Con, concentration; C.D., conversion of digested food; C.I., consumption index; C.I., conversion of ingested food; CK, control; LSD, least significant difference; R.G.R., relative growth rate.
Different letters indicate significant differences at $p < 0.05$ by LSD analysis.
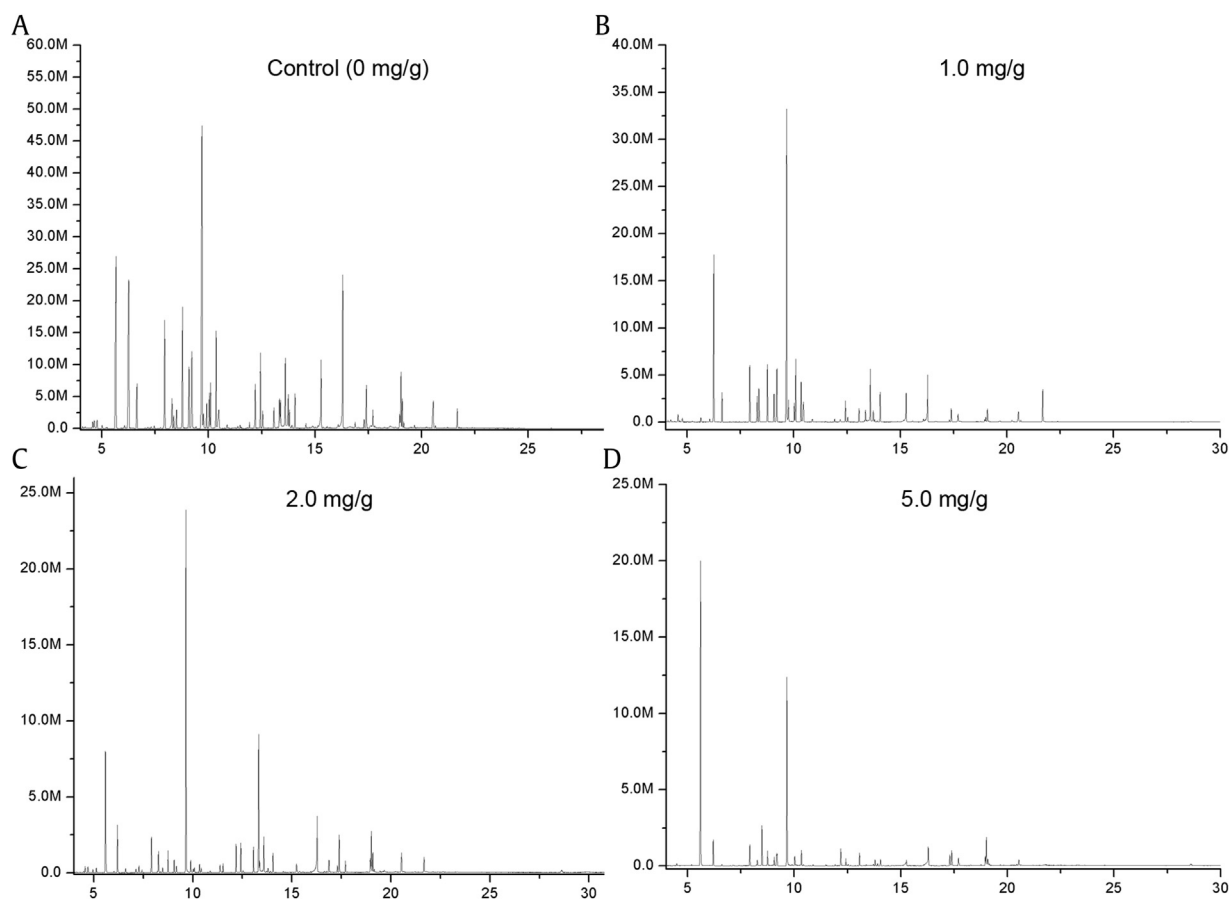
Fig. 2. The chromatographies of GC−MS analysis in the 3rd instar larvae of *O. furnacalis*. (A−D) The chromatogram of the targeted free amino acids (FAAs), free fatty acids (FFAs), and carbohydrates in the 3rd instar larvae consuming diets containing (A) 0 mg/g (control), (B) 1.0 mg/g, (C) 2.0 mg/g, and (D) 5.0 mg/g of PDS. GC, gas chromatography; MS, mass spectroscopy; PDS, panaxadiol saponin.

larvae fed with diets containing 5 mg/g PDS decreased by 64.43%, 73.69%, and 54.67%, respectively. We found the main FFAs in the larvae to be Lys (5.11 ± 0.10%), L-Ala (3.32 ± 0.02%), Gln (2.76 ± 0.01%%), L-Thr (2.18 ± 0.04%), L-Val (2.03 ± 0.04), lactic acid (10.58 ± 0.13%), sebacic acid (3.60 ± 0.52%), palmitic acid (3.30 ± 0.23%), oleic acid (2.42 ± 0.04%), stearic acid (2.35 ± 0.22%), inositol (11.67 ± 0.18%), maltose (8.18 ± 0.14%), and D-glucitol (4.55 ± 0.05%). The sum amount of lactic acid and C16-C18 FFAs accounted for 80.91% of total FFAs.

Data shown in Table 3 suggest that PDS treatment decreased all FAAs and carbohydrates and most FFAs (13 of 16) in the 3rd

instar larvae in a dose-dependent manner. For instance, the percentages of L-Ala, L-Leu, L-Thr, Gln, and Lys in the 3rd instar larvae feeding on a diet with 5 mg/g PDS were reduced by 20.46−71.62% and were significantly lower than those in the control group (2.64 ± 0.01 *vs.* 3.32 ± 0.02; 1.17 ± 0.02 *vs.* 1.55 ± 0.01; 0.53 ± 0.02 *vs.* 2.18 ± 0.04; 0.12 ± 0.01 *vs.* 2.76 ± 0.01; 1.45 ± 0.03 *vs.* 5.11 ± 0.10; p < 0.05) (Table 3). The levels of lactic acid, oleic acid, and stearic acid were reduced by 61.43−95.00%, which was lower those in the control group (4.08 ± 0.34 vs. 10.58 ± 0.13; 0.63 ± 0.03 vs. 2.42 ± 0.04; 0.18 ± 0.01 vs. 3.60 ± 0.52; p < 0.001). The contents of ribitol, D-glucitol, inositol, and
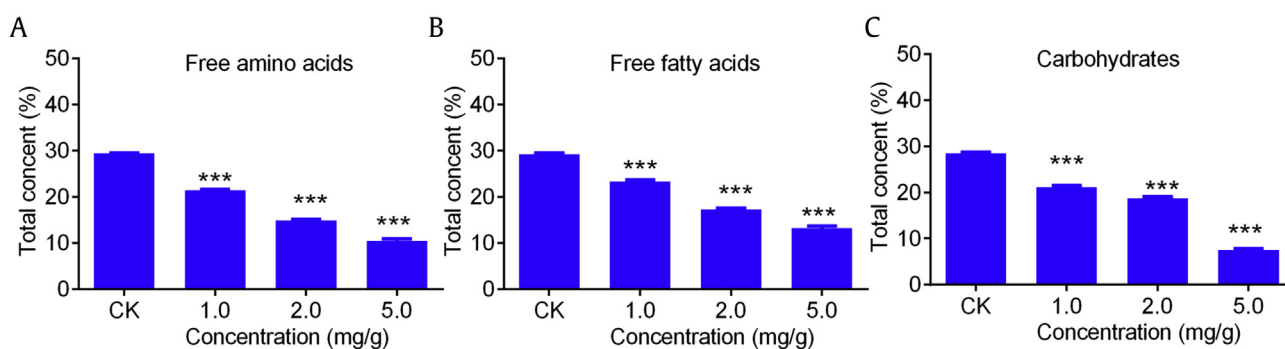

Fig. 3. The profile of (A) free amino acids, (B) free fatty acids, and (C) carbohydrates in the 3rd instar larvae of *O. furnacalis* consuming diets containing PDS. ***p < 0.001 *vs.* CK (control, 0 mg/g). CK, control; PDS, panaxadiol saponin.

Table 3
Contents of metabolites in the 3rd instar larvae of *O. furnacalis* in response to PDSs

Metabolite	Control	PDS concentration		
		1 mg/g	2 mg/g	5 mg/g
Free amino acids (mg·100 mg^{-1})				
L-alanine	3.32 ± 0.02a	2.77 ± 0.01b	2.70 ± 0.02bc	2.64 ± 0.01c
Valine	0.04 ± 0.01	/	/	/
Glycine	0.34 ± 0.02a	0.32 ± 0.02a	0.36 ± 0.01a	0.32 ± 0.02a
L-valine	2.03 ± 0.04a	1.82 ± 0.03a	1.47 ± 0.08b	1.39 ± 0.05c
L-leucine	1.55 ± 0.01a	1.52 ± 0.02a	1.19 ± 0.03b	1.17 ± 0.02b
L-isoleucine	1.00 ± 0.02a	0.96 ± 0.03a	0.73 ± 0.02b	0.74 ± 0.02b
Serine	1.44 ± 0.02a	0.62 ± 0.03b	0.47 ± 0.02c	0.39 ± 0.01d
L-threonine	2.18 ± 0.04a	1.06 ± 0.02b	0.98 ± 0.03b	0.53 ± 0.02c
Alanine	0.04 ± 0.01	/	/	/
Homocysteine	1.72 ± 0.02a	1.25 ± 0.03b	1.10 ± 0.02c	0.77 ± 0.01d
L-aspartic acid	1.65 ± 0.02a	0.89 ± 0.01b	0.89 ± 0.02c	0.48 ± 0.01d
Methionine	0.18 ± 0.01	/	/	/
Norvaline	1.75 ± 0.03a	0.93 ± 0.02b	0.68 ± 0.01c	0.11 ± 0.01d
L-proline	1.12 ± 0.02a	1.01 ± 0.02a	0.43 ± 0.01b	0.18 ± 0.01c
Glutamine	2.76 ± 0.01a	2.33 ± 0.02b	0.71 ± 0.01c	0.12 ± 0.01d
L-phenylalanine	0.93 ± 0.01a	0.36 ± 0.01b	0.21 ± 0.01c	0.09 ± 0.01d
DL-ornithine	2.22 ± 0.12a	0.79 ± 0.03b	0.48 ± 0.02c	0.07 ± 0.01d
Lysine	5.11 ± 0.10a	4.81 ± 0.16a	2.51 ± 0.05b	1.45 ± 0.03c
Total	29.38 ± 0.12a	21.44 ± 0.15b	14.91 ± 0.21c	10.45 ± 0.25d
Carbohydrates				
Ribitol	2.48 ± 0.02a	1.61 ± 0.01b	1.05 ± 0.02c	0
Rhamnose	1.59 ± 0.03	/	/	/
D-glucitol	4.55 ± 0.05a	1.65 ± 0.02b	1.66 ± 0.01b	1.40 ± 0.02c
Inositol	11.67 ± 0.18a	11.10 ± 0.19b	9.98 ± 0.23c	1.46 ± 0.03d
Maltose	8.18 ± 0.14a	6.79 ± 0.32b	6.08 ± 0.26c	4.63 ± 0.12d
Total	28.47 ± 0.32a	21.15 ± 0.38b	18.77 ± 0.29c	7.49 ± 0.33d
Free fatty acids (%)				
Lactic acid	10.58 ± 0.13a	9.20 ± 0.29b	7.87 ± 0.23c	4.08 ± 0.34d
Glycolic acid	0.64 ± 0.02a	0.22 ± 0.01b	0.09 ± 0.01c	0.06 ± 0.01d
Butyrate	0.08 ± 0.01d	1.77 ± 0.02a	0.52 ± 0.01c	0.72 ± 0.01b
Oxalic acid	0.81 ± 0.01a	0.46 ± 0.01b	0.16 ± 0.01c	0.08 ± 0.01d
Succinic acid	1.84 ± 0.22a	0.96 ± 0.16b	0.45 ± 0.02c	0.28 ± 0.02d
2-Hydroxyglutaric acid	2.19 ± 0.18a	1.52 ± 0.19b	0.60 ± 0.01c	0.38 ± 0.01d
Palmitoleic acid (C16:1)	0.51 ± 0.02c	0.55 ± 0.02c	0.61 ± 0.01b	0.72 ± 0.03a
Palmitic acid (C16:0)	3.30 ± 0.23c	3.24 ± 0.16c	3.96 ± 0.03b	4.61 ± 0.02a
9-octadecenoic acid (C18:1)	0.37 ± 0.03d	0.47 ± 0.04c	0.99 ± 0.05b	1.13 ± 0.11a
Oleic acid (C18:1)	2.42 ± 0.04a	2.65 ± 0.02b	1.13 ± 0.15c	0.63 ± 0.03d
Stearic acid (C18:0)	2.35 ± 0.22a	1.44 ± 0.13b	0.57 ± 0.03c	0.25 ± 0.02d
Linolenic acid (C18:2)	0.34 ± 0.02a	0.26 ± 0.01b	0.14 ± 0.01c	0.09 ± 0.01d
Sebacic acid	3.60 ± 0.52a	2.06 ± 0.32b	0.21 ± 0.02c	0.18 ± 0.01d
Monolinolein (C18:2)	0.07 ± 0.01	/	/	/
Hexadecyl carbonate (C16:0)	0.05 ± 0.01	/	/	/
Monoolein (C18:1)	0.06 ± 0.01	/	/	/
Total	29.21 ± 0.29a	23.93 ± 0.37b	17.30 ± 0.28c	13.24 ± 0.43d

LSD, least significant difference; PDS, panaxadiol saponin.
Different letters indicate significant differences at p < 0.05 by LSD analysis.

maltose in the PDS 5 mg/g group were reduced by 43.40—100%, which were significantly lower than those in the control group (0 vs. 2.48 ± 0.02; 1.40 ± 0.02 vs. 4.55 ± 0.05; 1.46 ± 0.03 vs. 11.67 ± 0.18; 4.63 ± 0.12 vs. 8.18 ± 0.14, respectively; p < 0.01). In addition, PDS treatment completely blocked the production of 3 essential amino acids, that is, Val, Ala, and Met; one glycoside (i.e., rhamnose); and 3 FFAs, that is, monolinolein, hexadecyl carbonate, and monoolein, in the 3rd instar larvae.

By contrast, we found PDSs increased the levels of palmitoleic acid (C16:1), palmitic acid (C16:0), and 9-octadecenoic acid (C18:1) in a dose-dependent manner. The sum content of palmitoleic acid (C16:1), palmitic acid (C16:0), and 9-octadecenoic acid (C18:1) accounted for 14.31% of FFAs in the control group and 48.79% of FFAs in larvae consuming diets containing 5.0 mg/g PDS. Taken together, the PDS-responsive profiles in the 3rd instar larvae of *O. furnacalis* suggest that the administration of PDSs prevented metabolism of *O. furnacalis*.

3.4. PDS inhibits the activity of metabolic enzymes

We further investigated the effect of PDS on the activities of enzymes related to metabolism and detoxication in the 3rd instar larvae of *O. furnacalis*. Table 4 shows that the enzymatic activities of GST, CYP540, CarE, AChE, TH, ACP, and ALP in the 3rd instar larvae were inhibited by the administration of PDSs in a dose- and time-dependent manner. After 72 h, the activity of GST, CYP540, AChE, TH, ACP, and ALP in the 3rd instar larvae consuming diets containing 5.0 mg/g PDS was maximally decreased by 51.97%, 34.75%, 48.44%, 46.42%, 45.87%, and 35.55%, respectively, and the activity of CarE was decreased by 32.69% at 24 h.

Table 4
The activities of 7 key enzymes in the 3rd instar larvae of *O. furnacalis* in response to PDSs

Enzyme	Time	Control	PDS concentration		
			1 mg/g	2 mg/g	5 mg/g
GST (IU/L)	0 h	39.37 ± 8.01a	36.99 ± 9.71a	35.61 ± 9.56a	37.35 ± 6.41a
	24 h	35.00 ± 4.17a	33.89 ± 5.21a	30.49 ± 5.79ab	28.06 ± 2.39b
	48 h	35.86 ± 4.09a	28.18 ± 3.12b	26.20 ± 3.25bc	22.71 ± 2.89c
	72 h	36.64 ± 7.74a	23.71 ± 3.50b	20.81 ± 2.88bc	17.94 ± 2.42c
CYP450 (pmol/L)	0 h	19.10 ± 4.38a	21.17 ± 2.17a	18.96 ± 3.62a	19.21 ± 4.13a
	24 h	20.08 ± 3.87a	17.89 ± 2.68ab	15.88 ± 3.62b	14.90 ± 3.45b
	48 h	18.21 ± 3.07a	15.91 ± 3.25ab	13.62 ± 2.25b	13.57 ± 1.93b
	72 h	18.74 ± 4.17a	15.91 ± 2.14b	12.73 ± 1.50b	12.48 ± 2.95b
CarE (U/L)	0 h	115.14 ± 9.17a	117.10 ± 10.23a	116.92 ± 8.43a	114.73 ± 5.74a
	24 h	117.10 ± 10.14a	103.53 ± 9.22b	91.23 ± 7.32c	77.23 ± 5.24d
	48 h	114.96 ± 9.32a	102.92 ± 8.57b	98.01 ± 9.84b	83.36 ± 7.50c
	72 h	116.58 ± 9.21a	107.13 ± 8.92a	103.68 ± 9.11a	96.62 ± 7.63b
AChE (U/L)	0 h	143.11 ± 14.94a	147.86 ± 11.71a	142.62 ± 10.79a	139.17 ± 12.81a
	24 h	133.59 ± 13.24a	123.12 ± 11.36a	99.64 ± 13.26b	87.47 ± 7.95b
	48 h	138.66 ± 10.53a	110.03 ± 11.17b	87.91 ± 7.52c	81.41 ± 6.34c
	72 h	146.8 ± 7.31a	104.06 ± 8.22b	75.04 ± 6.93c	71.76 ± 6.83c
TH (ng/L)	0 h	40.89 ± 7.48a	39.52 ± 3.04a	39.32 ± 1.46a	40.13 ± 1.87a
	24 h	39.5 ± 7.06a	33.72 ± 3.62b	30.88 ± 3.03bc	29.04 ± 1.98c
	48 h	38.87 ± 7.68a	31.85 ± 4.61b	27.2 ± 3.43bc	25.95 ± 3.65c
	72 h	42.26 ± 7.91a	28.54 ± 3.38b	24.45 ± 3.61bc	21.50 ± 3.78c
ACP (IU/L)	0 h	67.19 ± 5.68a	68.40 ± 3.59a	69.45 ± 5.92a	66.93 ± 9.64a
	24 h	64.60 ± 3.43a	61.66 ± 5.98a	58.95 ± 9.59a	47.71 ± 2.65b
	48 h	65.44 ± 4.49a	57.39 ± 4.38ab	50.90 ± 7.66b	41.18 ± 3.50c
	72 h	66.86 ± 9.91a	55.24 ± 4.75b	49.31 ± 5.08b	36.23 ± 4.89c
ALP (IU/L)	0 h	192.50 ± 22.04a	194.00 ± 19.24a	195.31 ± 12.88a	193.03 ± 13.74a
	24 h	195.64 ± 15.02a	180.36 ± 11.45a	151.53 ± 12.24b	138.73 ± 6.03b
	48 h	194.67 ± 14.83a	168.25 ± 14.18b	141.78 ± 12.24c	125.14 ± 6.06d
	72 h	199.47 ± 12.57a	156.54 ± 10.74b	129.67 ± 9.68c	116.69 ± 6.41c

AChE, acetylcholinesterase; ACP, acid phosphatase; ALP, alkaline phosphatase; GST, glutathione S-transferase; CarE, carboxylesterase; CYP450, cytochrome P450; LSD, least significant difference; PDS, panaxadiol saponin; TH, trehalase.
Different letters indicate significant differences at p < 0.05 by LSD analysis.

Accordingly, the inhibitory rates of PDSs against all enzymes, except for CarE, gradually increased with an increase in time and PDSs (Fig. 4). The PDS-induced inhibition of CarE was dose dependent but was negatively correlated with higher concentrations of PDSs, and the inhibitory rates against 6 other enzymes increased over time and with increase in PDS concentrations (Fig. 4). The maximum inhibitory rates of PDSs against GST, CYP540, AChE, TH, ACP, and ALP reached 51.04%, 33.35%, 48.44%, 49.12%, 45.81%, and 41.50% at 72 h, respectively; and the maximum inhibitory rate against CarE reached 34.05% at 24 h.

4. Discussion

Insecticide resistance in insects, including anthropogenic genetic and metabolic-based resistance, seriously threatens the efficiency of insect pest management [16]. Changes in
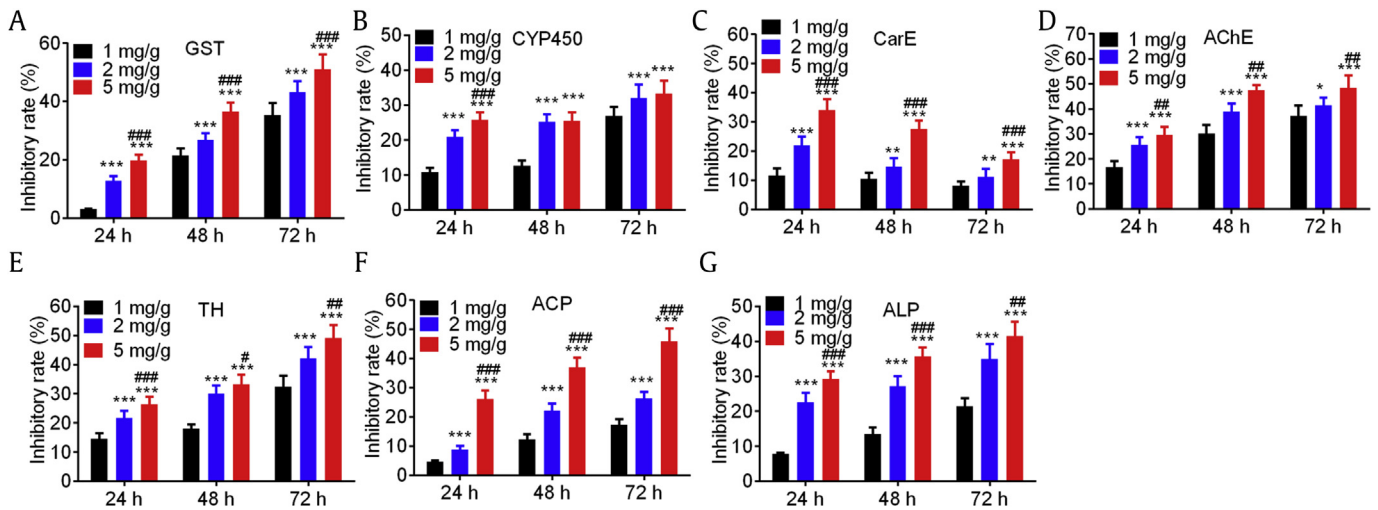

Fig. 4. The inhibitory effect of PDS against enzymes in the 3rd instar larvae of *O. furnacalis*. (A–G) The inhibitory effect of PDS against GST, CYP450, CarE, AChE, TH, ACP, and ALP, respectively. Detection was performed using ELISA analysis. Inhibitory rate (%) = (control − test) × 100%/control. *, **, and *** notes p < 0.05, p < 0.01, and p < 0.001 vs. 1.0 mg/g, respectively. #, ##, and ### notes p < 0.05, p < 0.01, and p < 0.001 vs. 2.0 mg/g, respectively. AChE, acetylcholinesterase; ACP, acid phosphatase; ALP, alkaline phosphatase; CarE, carboxylesterase; CYP450, cytochrome P450; ELISA, enzyme-linked immunosorbent assay; GST, glutathione S-transferase; PDS, panaxadiol saponin; TH, trehalase.

metabolomics in insects induced by insecticides and pesticides challenge the growth and development of insect larvae. The present study shows that diets containing PDSs could inhibit the growth, food consumption, and conversion in the 3rd instar larvae of *O. furnacalis*. Furthermore, we found that PDS consumption changed the metabolomic profiles of FAAs, FFAs, and carbohydrates and altered the activities of metabolic-related enzymes in the 3rd instar larvae. These results suggest a metabolic-based mechanism associated with ginsenoside-mediated pest infestation against *O. furnacalis*.

Ginsenosides are triterpene saponins from *P. ginseng* with active pharmacological properties, including antiinsect activity. Our previous studies had shown the antiinsect activity of total ginsenosides from the leaf and stem of *P. ginseng* [3,4]. The antifeedant and oviposition-deterring activities suggested the inhibitory effect of total ginsenosides against the growth, development, oviposition rate, and egg hatchability of insects [3,4]. In the present study, we detected that food consumption, growth, digestibility, and conversion of food in the 3rd instar larvae of *O. furnacalis* were inhibited in a dose-dependent manner by diets containing PDSs (Table 1). According to the aforementioned data, we confirmed that PDSs could inhibit growth and food consumption in the 3rd instar larvae of *O. furnacalis* by suppressing the digestibility and conversion efficiency of digested food.

Targeted metabolomics analysis showed that PDS treatment suppressed the metabolism and synthesis of amino acids, lipids, and glucoses in the 3rd instar larvae of *O. furnacalis* in a dose-dependent manner (Figs. 2 and 3 and Table 3). In particular, the production of 3 essential amino acids (including Val, Ala, and Met), rhamnose, and 3 FFAs (including monolinolein, hexadecyl carbonate, and monoolein) were undetectable in the 3rd instar larvae of *O. furnacalis* consuming diets containing PDSs. Digestible carbohydrates provide the body with monosaccharides, the primary source of energy needed for metabolism and activities [20]. Deficiencies of amino acids and lipids are associated with shorter life span, poor digestion, decreased body function, growth, oviposition, and reproduction in humans, fish, and insects [21—26]. For instance, Lertratanangkoon et al [27] revealed that glutathione deficiency in Syrian hamsters reduced the production of Met, which subsequently impaired the DNA methylation level and induced hepatic injury or necrosis [28]. The lower amounts of carbohydrates, amino acids, and lipids suggested inhibitory effects of PDSs against the normal metabolism and physical functions in the 3rd instar larvae of *O. furnacalis*, which was consistent with the reduced growth rate and food consumption and utilization in *O. furnacalis* seen in our present study.

Interestingly, we found the contents of palmitoleic acid (C16:1), palmitic acid (C16:0), and 9-octadecenoic acid (C18:1) were gradually increased in the 3rd instar larvae of *O. furnacalis* consuming diets containing PDSs. The sum content of these 3 FFAs accounted for 14.31% of total FFAs in larvae consuming normal diets and was increased to 48.79% in larvae consuming diets containing 5.0 mg/g PDS. Previous studies of animals and humans have shown that elevated levels of palmitoleic acid, palmitic acid, and 9-octadecenoic acid correlated with increased risk for cardiovascular incidents, polymyositis, lipogenesis, type 2 diabetes, insulin resistance, inflammation, lipotoxicity, and metabolic syndrome [29—33]. Pardo et al [29] showed that palmitic acid induced lipotoxicity, endoplasmic reticulum stress, and insulin resistance in hepatocytes and oleic acid could attenuate these symptoms. Our present study showed that the content of oleic acid in the 3rd instar larvae of *O. furnacalis* was decreased with an increase in PDS consumption. This might indicate that PDS consumption resulted in metabolic syndromes and other disorders in the 3rd instar larvae of *O. furnacalis*.

Consistent with the abnormal metabolic syndrome and decreased metabolites, we found the activities of 7 enzymes, including AChE, GST, CYP540, CarE, TH, ACP, and ALP, in the 3rd instar larvae of *O. furnacalis* gradually decreased with increase in PDS concentrations. These enzymes are vital for metabolism and detoxification. GST confers resistance in insects (i) directly by regulating the metabolism of toxins or catalyzing the detoxification of endogenous organic epoxides, peroxides and lipid hydroperoxides, and exogenous insecticides via mediating glutathione (GSH) conjugation [34] and (ii) indirectly by protecting against insecticide-induced oxidative stress [35]. CYP540 plays important roles in the detoxification and resistance to exogenous insecticides by the epoxidation and/or hydroxylation of insecticides [12—14]. AChE hydrolyzes a neurotransmitter: acetylcholine. AChE could be inhibited by CYP enzymes [12]. The inhibition of AChE by insecticides always induces the accumulation of acetylcholine and associated neurotoxicity syndromes or cholinergic symptoms [36,37]. Insect TH were hydrolyzed into substrates, which forms the major hemolymph sugar in insects and provides energy for their development and flight [38]. The inhibition of ACP and ALP by various insecticides had been reported [39,40]. Our previous studies have shown the enzymatic inhibitory activities of total ginsenosides from *P. ginseng* against AChE and GST in *M. separata* larvae [4]. In our present study, we confirmed that PDSs inhibited the activities of AChE, GST, CYP540, CarE, TH, ACP, and ALP in a dose-dependent manner, suggesting (i) an inhibitory effect against detoxification and insecticide resistance, (ii) the induction of neurotoxicity syndrome, and (iii) energy deficiency and growth inhibition in the 3rd instar larvae of *O. furnacalis*, which was inconsistent with the decreased metabolomics profiles of FAAs, FFAs, and carbohydrates by PDSs.

5. Conclusions

Our study confirmed the inhibitory effect of PDS against growth, food utilization and conversion, and detoxification in the 3rd instar larvae of *O. furnacalis*. Using the targeted GS—MS technique, we identified that PDSs decreased the profiles of FAAs, FFAs, and carbohydrates in the 3rd instar larvae of *O. furnacalis*, which was inconsistent with the decreased detoxification capacity. We concluded that PDS treatment might be an effective insect pest management technique. In addition, our data provide novel implications into PDS-mediated insect toxicology in *O. furnacalis*.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

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