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

Shuangli Liu1, Xiaohui Wang2, Rui Zhang1, Mingjie Song1, Nanqi Zhang1, Wanying Li1, Yingping Wang1, Yonghua Xu1,* Lianxue Zhang1,**

1 National & Local Joint Engineering Research Center for Ginseng Breeding and Application (Jilin), Jilin Agricultural University, China
2 Research Center of Agricultural Environment and Resources, Jilin Academy of Agricultural Sciences, China

ABSTRACT

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.

© 2019 The Korean Society of Ginseng. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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].

* Corresponding author. 2888 Xincheng Street, Changchun City, Jilin Province, China.
** Corresponding author. 2888 Xincheng Street, Changchun City, Jilin Province, China.
E-mail addresses: xuyonghua777@yeah.net (Y. Xu), ZLX863@163.com (L. Zhang).
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-deterrent, and enzymatic inhibitory activities of total ginsenosides from \( \textit{P. ginseng} \) on \( \textit{Mythimna separata} \) and \( \textit{Pieris rapae} \) [3,4]. We determined the inhibitory activities of acetylcholinesterase (AChE) and glutathione S-transferase (GST) by total ginsenosides in the management of \( \textit{M. separata} \) larvae. Yang et al [17] showed that the administration of \( \textit{P. ginseng} \) total ginsenosides obviously reduced the activity of AChE, carboxylesterase (CarE), and GST and increased mixed-function oxidase activity in \( \textit{Plutella xylostella} \) (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 associated with ginsenosides is limited.

\( \textit{Ostrinia furnacalis} \) (Guenée) is a major insect pest of maize worldwide, especially in Asia [18]. The purpose of this study was to investigate the effects of ginsenosides (PDSs) on the amino acid, lipid, and carbohydrate profiles in the 3rd instar larvae of \( \textit{O. furnacalis} \). Targeted gas chromatography–mass spectrometry (GC–MS) was used to analyze the metabolomics profiles before and after exposure to ginsenosides in \( \textit{O. furnacalis} \). The impacts of ginsenosides on the growth, food consumption and utilization, and metabolic-related enzyme activity in \( \textit{O. furnacalis} \) 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 \( \textit{P. ginseng} \) were obtained from the National Ginseng Engineering Research Center of Jilin Agricultural University, Jilin, 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 (\( n \geq 20 \) 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:

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

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

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

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

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

“Duration” indicates the feeding period (4 days); “mean dry weight of larva (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 trimethylsilyl 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 Coo-massie Blue G250 method [19] and were then used to assess the contents of GST, CatE, 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 15.12%, 11.75%, 17.76%, 25.8%, and 35.88%, respectively, compared with the control (p < 0.05, Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Con (mg/g)</th>
<th>R.G.R. (%)</th>
<th>A.D. (%)</th>
<th>C.I. (%)</th>
<th>C.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>2.04 ± 0.32a</td>
<td>2.98 ± 0.21a</td>
<td>47.58 ± 3.95a</td>
<td>43.64 ± 10.35a</td>
</tr>
<tr>
<td>0.5</td>
<td>1.86 ± 0.53ab</td>
<td>2.94 ± 0.16a</td>
<td>45.47 ± 3.26ab</td>
<td>39.89 ± 11.64ab</td>
</tr>
<tr>
<td>1.0</td>
<td>1.83 ± 0.18ab</td>
<td>2.97 ± 0.11a</td>
<td>43.98 ± 4.73ab</td>
<td>33.90 ± 10.51bc</td>
</tr>
<tr>
<td>2.0</td>
<td>1.81 ± 0.18ab</td>
<td>2.89 ± 0.14a</td>
<td>41.50 ± 10.48abc</td>
<td>31.97 ± 5.48c</td>
</tr>
<tr>
<td>5.0</td>
<td>1.68 ± 0.23b</td>
<td>2.78 ± 0.55ab</td>
<td>40.13 ± 4.73abc</td>
<td>28.55 ± 10.92d</td>
</tr>
<tr>
<td>10.0</td>
<td>1.65 ± 0.45b</td>
<td>2.63 ± 0.41b</td>
<td>39.13 ± 4.33bc</td>
<td>27.98 ± 11.44d</td>
</tr>
</tbody>
</table>

A.D., approximate digestibility; Con, concentration; C.D., conversion of digested food; C.I., consumption index; C.L., 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.

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).

### Table 2

<table>
<thead>
<tr>
<th>Con (mg/g)</th>
<th>Rb1 (%)</th>
<th>Rb2 (%)</th>
<th>Rb3 (%)</th>
<th>Rd (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01b</td>
<td>0.49</td>
<td>0.04d</td>
<td>0.26c</td>
</tr>
<tr>
<td>1.0</td>
<td>0.15</td>
<td>0.57</td>
<td>0.03e</td>
<td>0.26c</td>
</tr>
<tr>
<td>2.0</td>
<td>0.14</td>
<td>0.22</td>
<td>0.04e</td>
<td>0.26c</td>
</tr>
<tr>
<td>5.0</td>
<td>0.14</td>
<td>0.22</td>
<td>0.04e</td>
<td>0.26c</td>
</tr>
<tr>
<td>10.0</td>
<td>0.14</td>
<td>0.22</td>
<td>0.04e</td>
<td>0.26c</td>
</tr>
</tbody>
</table>

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.

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 larvae of O. furnacalis is shown in Table 3.
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 ± 1.31%), 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...
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.
Different letters indicate significant difference; PDS, panaxadiol saponin; TH, trehalase. #, ##, and ### notes are significant differences at p < 0.05, p < 0.01, and p < 0.001 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.

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Time</th>
<th>Control</th>
<th>PDS concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/g</td>
<td>2 mg/g</td>
</tr>
<tr>
<td>GST (IU/L)</td>
<td>0 h</td>
<td>39.37 ± 8.01a</td>
<td>36.99 ± 9.71a</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>35.00 ± 4.17a</td>
<td>33.89 ± 5.21a</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>35.86 ± 4.09a</td>
<td>28.18 ± 3.12b</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>36.64 ± 7.74a</td>
<td>23.71 ± 3.50b</td>
</tr>
<tr>
<td>CYP450 (pmol/L)</td>
<td>0 h</td>
<td>19.10 ± 4.38a</td>
<td>21.17 ± 2.17a</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>20.08 ± 3.87a</td>
<td>17.89 ± 2.68ab</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>18.21 ± 3.07a</td>
<td>15.91 ± 3.25ab</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>18.74 ± 4.17a</td>
<td>15.91 ± 2.14b</td>
</tr>
<tr>
<td>CarE (U/L)</td>
<td>0 h</td>
<td>115.14 ± 9.17a</td>
<td>117.10 ± 10.23a</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>110.70 ± 10.14a</td>
<td>103.53 ± 9.22b</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>114.96 ± 9.32a</td>
<td>102.92 ± 8.57b</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>116.58 ± 9.21a</td>
<td>107.13 ± 8.92a</td>
</tr>
<tr>
<td>ACP (IU/L)</td>
<td>0 h</td>
<td>40.89 ± 7.48a</td>
<td>39.5 ± 3.06a</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>39.5 ± 7.06a</td>
<td>33.72 ± 3.62b</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>38.87 ± 7.68a</td>
<td>31.85 ± 4.61b</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>42.26 ± 7.91a</td>
<td>28.54 ± 3.38b</td>
</tr>
<tr>
<td>TH (ng/L)</td>
<td>0 h</td>
<td>146.8 ± 7.31a</td>
<td>104.06 ± 8.22ab</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>139.5 ± 7.06a</td>
<td>39.52 ± 3.04a</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>138.87 ± 7.68a</td>
<td>33.72 ± 3.62b</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>42.26 ± 7.91a</td>
<td>28.54 ± 3.38b</td>
</tr>
<tr>
<td>ACP (IU/L)</td>
<td>0 h</td>
<td>67.19 ± 5.88a</td>
<td>68.40 ± 3.59a</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>64.60 ± 3.43a</td>
<td>61.66 ± 5.98a</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>65.44 ± 4.49a</td>
<td>57.39 ± 4.38ab</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>66.86 ± 9.91a</td>
<td>55.24 ± 4.75b</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>0 h</td>
<td>192.50 ± 22.04a</td>
<td>194.00 ± 21.24a</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>195.64 ± 15.02a</td>
<td>180.36 ± 14.5a</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>194.67 ± 14.83a</td>
<td>168.25 ± 14.18b</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>199.47 ± 12.57a</td>
<td>156.54 ± 10.74b</td>
</tr>
</tbody>
</table>

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.

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](image_url)
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-deterrent 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 glucose 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, Lerrataranakoon 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

This study was financially supported by the Natural Science Fund Project of Jilin Province Science and Technology Department (20190202163JC) and the National Natural Science Fund of China (31470420).

References

Ruan T, Li L, Lyu Y, Luo Q, Wu B. Effect of methionine de
Barazzoni R, Nep D, Biolo G, Bischoff S, Boirie Y, Cederholm T, Cuerda C,
Humbert B, Bleis P, Martin L, Dumon H, Darmaun D, Nguyen P. Effects of di-
Bradford MM. A rapid and sensitive method for the quantitation of microgram
Abdelazim A, Khater S, Ali H, Shalaby S, Almaghrabi OA. Panax ginseng improves glucose metabolism in
Guo J, He K, Bai S, Zhang T, Zhao J, Wang Z. Physiological responses induced by
Hoi KK, Daborn PJ, Battlay P, Robin C, Batterham P, O
Feyereisen R. Insect P450 inhibitors and insecticides: challenges and oppor-
Zhang N, Na LI, Jia-Lin LI, Kun MA, Cui L, Pharmacy CO, University B. Hypo-
Yang XD, Yang YY, Ouyang DS, Yang GP. A review of biotransformation and
Zhang AH, Tan SQ, Zhao Y, Lei FJ, Zhang LX. Effects of total ginsenosides on the
Bilal M, Freed S, Ahsan Z, Zaka SM, Khan MB. Activity of acetylcholines-