TITLE PAGE

- Korean Journal for Food Science of Animal Resources -

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ARTICLE INFORMATION	Fill in information in each box below
Article Title	Nutritional Composition of White-spotted Flower Chafer (*Protaetia brevitarsis*) Larvae Produced from Commercial Insect Farms in South Korea
Running Title (within 10 words)	Nutritional composition of white-spotted flower chafer larvae
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by the research invigoration program of 2020 Gyeongnam National University of Science and Technology. In addition, this study was evidently conducted to obtain data for public interest purposes and the results cannot be used for commercial promotion of a certain farm. Therefore, the actual name of participating insect farms is not disclosed.
Author's contributions (This field may be published.)	
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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Abstract

This study was conducted to compare the nutritional composition of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from five commercial insect farms in South Korea. The feeding sources of larvae were different as follows: Farm A, fermented oak sawdust; Farm B, fermented oak and scrub sawdust; Farm C, commercial feed; Farm D, private fermented feed; and Farm E, byproduct from mushroom compost. Drying yield significantly varied by insect farm, ranging from 14.12% to 27.28%. However, there was only small difference (5.14-7.38 g/100 g) in moisture content of dried larvae powder ($p<0.001$). The larvae produced from Farm A, B, and D presented higher protein content and lower lipid content compared to those from Farm C and E ($p<0.05$). No significant differences in total and essential amino acid contents were found, regardless of the insect farms. Phosphoserine, taurine, and gamma-aminobutyric acid, well-known physiological useful compounds, were detected in form of free amino acids. The major fatty acids in the *P. brevitarsis* larvae were oleic acid, palmitic acid, palmitoleic acid, and linoleic acid. The larvae from Farm A, B, and E exhibited higher oleic acid content than those from Farm B and C ($p<0.05$). Moreover, the larvae from Farm A presented the lowest saturated fatty acid/unsaturated fatty acid ratio. Although the underlying mechanisms of the nutritional composition differences are not yet clearly understood, this study suggests that the Farm A production system, using only oak feed, could be potentially beneficial in increasing the protein content and decreasing SFA/UFA ratio in *P. brevitarsis* larvae.

Keywords: commercial edible insect, amino acid profile, fatty acid profile, feeding source, nutritional composition

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Introduction

Recently, as the global demand for sustainable protein sources has been increasing, apart from conventional edible meat sources, edible insects have been suggested as an emerging food protein source (Patel et al., 2019). With the recent world trend, in South Korea, an interest in edible insects has also been growing constantly, and the scale of edible insect farming and the related commercial markets has been increasing rapidly (Ghosh et al., 2017). Fifteen insect species have been legally registered as 'livestock' by the Ministry of Agriculture, Food and Rural Affairs in July 2020 (MAFRA, 2020). In addition, nine insect species including *Allomyrina dichotoma* larvae, *Apis mellifera* L., Bombycis corpus, *Bombyx mori* L., *Gryllus bimaculatus*, *Oxya japonica* Thunberg, *Protaetia brevitarsis* larvae, *Tenebrio molitor* larvae, and *Zophobas atratus* larvae are registered as general food ingredients in the Korea Food Code (MFDS, 2020).

The larvae of white-spotted flower chafer (*P. brevitarsis*) have been used as a traditional medicine to treat inflammation, hepatic disease, and breast cancer in South Korea (Song et al., 2017). In practice, various physiological benefits of the *P. brevitarsis* larvae, such as antioxidant, antibacterial, anticancer, and antithrombotic effects, have been already proven scientifically (Lee et al., 2017; Yoon et al., 2003). With the registration of *P. brevitarsis* larvae as a general food ingredient, recent studies have noted that of the proximate composition of *P. brevitarsis* larvae varied considerably: moisture (3.99-7.98%), protein (42.46-57.86%), fat (7.33-26.70%), ash (3.96-8.45%), and carbohydrate (10.56-23.71%) (Chung et al., 2013; Ghosh et al., 2017; Jeong et al., 2020; Kim et al., 2017, Yeo et al., 2013). Regarding the large variation in proximate composition, Choi et al. (2019) have suggested that the nutritional composition of the *P. brevitarsis* larvae could be affected by feeding sources, similarly to conventional livestock. Moreover, it has been reported that differences in feeding sources have a greater impact on the nutritional composition of *P. brevitarsis* larvae compared to the conventional livestock, since it has more short and simple digestive system (Yoon et al., 2020). Furthermore, as the whole larvae including a digestive tract are generally consumed and

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processed, it is known that fasting methods could be one of the most important factors affecting the nutritional value of edible insect larvae (Noh et al., 2015).

In this regard, in the Korean edible insect industry, the establishment of a standard production system has been attempted for stable production and utilization of edible insects as food ingredients with constant quality and safety. However, many edible insect farms in South Korea have been producing by the rearing protocol based on the owners' individual experiences. Thus, in order to establish a potentially applicable production system, it could be primarily necessary to compare the nutritional composition of edible insects produced by various current production systems. Until now, although there are some previous studies determining the nutritional composition of *P. brevitarsis* larvae (Chung et al., 2013; Ghosh et al., 2017; Jeong et al., 2020; Kim et al., 2017, Yeo et al., 2013), but little studies have been compared the nutritional composition of *P. brevitarsis* larvae produced from different commercial farms. Therefore, the objective of this study was to determine the major nutritional composition (proximate composition, amino acid profile, and fatty acid profile) of white-spotted flower chafer (*P. brevitarsis*) larvae, collected from five commercial insect farms in South Korea.

Materials and methods

Rearing information of white-spotted flower chafer larvae

Frozen whole white-spotted flower chafer (*Protaetia brevitarsis*, Coleoptera: Scarabaeidae) larvae, which were harvested at third instar and fasted for 3 days, were kindly provided by five large-scale commercial insect farms located in the Gyeongsang-namdo, South Korea. The frozen and vacuum-packaged samples were placed in an ice cooler and transported to the laboratory. According to the manufacturers' information, the conditions of the rearing room, such as temperature, relative humidity (RH), and lighting control, were similar for guaranteeing maximum profits as follows: average temperature of 25°C, 60% RH, and 16L:8D. However, the feeding sources for *P. brevitarsis* larvae in the insect farms varied as follows: Farm A, fermented oak sawdust; Farm B, fermented oak and scrub sawdust; Farm C,

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commercial feed (Goomlife, Gimhae-si, Gyeongsang-namdo, South Korea); Farm D, private fermented feed (oak sawdust 50%, rice bran 5%, barley bran 5%, molasses 5%, water 25%); and Farm E, the byproduct from mushroom compost. However, the detailed feed composition, manufacturing method, and harvesting methods of the larvae were unfortunately not provided for confidentiality reasons.

Experimental design and sample preparation

The experimental design of this study was a completely randomized block design with three independent replications. The collected *P. brevitarsis* larvae from each farm were separated randomly into three groups (approximately 120 g per group) as a block. The assigned larvae samples were weighed, placed in an aluminum dish, and hot-air dried at 55±1°C for 12 h. The dried samples were re-weighed to determine the drying yield and ground using a food blender (HMF3800SS, Hanil Electric, Seoul, South Korea). The obtained powder was filtered through a 100-mesh sieve, and the filtrate was vacuum-packaged in a polyamide/polyethylene bag and stored at -20°C until further analysis.

Analysis of P. brevitarsis larvae

1. Drying yield

The drying yield of *P. brevitarsis* larvae samples was calculated as follows:

Drying yield (%) = $[(W_b - W_a) / W_b] \times 100$

Where W_b = Weight of sample before the drying process (g), and W_a = Weight of sample after the drying process (g).

2. Proximate composition

The proximate composition of dried *P. brevitarsis* larvae was determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2006). Moisture content (oven air-drying method, 950.46B), fat content (Soxhlet method, 960.69), and ash content (muffle furnace method, 920.153) were expressed as g/100g of dried sample. The

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protein content of dried larval samples was determined by the Dumas method (N × 6.25) using a nitrogen analyzer (Rapid N Cube, Elementar Analysen systeme GmbH, Hanau, Germany).

3. Amino acid profile

Total amino acids in the *P. brevitarsis* larvae samples were determined by the method of AOAC (1998) with some modification as described by Jo et al. (2018). One gram of the sample was hydrolyzed in 15 mL of 6 N HCl at 110℃ for 24 h. The hydrolyzed samples were filtered using glass wool, and the filtrate was concentrated using a vacuum rotary evaporator at 55℃. After removal of the solvent, 10 mL of 0.2 N sodium citrate buffer was added, and the diluted sample was filtered with a 0.45 μm syringe filter before analysis.

Free amino acids were determined following the method of Jo et al. (2018) with modification by the instruction of an amino acid analyzer. Five grams of each sample was homogenized with 25 mL of distilled water for 1 min and it was filled up to 50 mL with distilled water. The homogenate was centrifuged at 7,000×g for 10 min (4℃), and the supernatant was mixed with 12% trichloroacetic acid (TCA) in the same volume ratio (1:1, v/v). After approximately 1 h, the mixture was centrifuged at 7,000×g for 20 min. To remove TCA and lipid components in the supernatant, hexane was added to the mixture at a 1:1 ratio (v/v). The mixture was centrifuged again at 8,960×g for 10 min. The water phase was collected from the bottom and filtered through a 0.2 μm syringe filter. Hydrolyzed amino acids and free amino acids were analyzed with a Biochrom 30 plus amino acid analyzer (Biochrom Ltd., Cambridge, UK) using ninhydrin as the color reactant and a single ion-exchange resin column. The detection wavelength was 440 nm (proline) or 570 nm (all other amino acids), and an external standard was used to calculate the concentration of each amino acid. The results are reported as μg/g dry matter.

4. Fatty acid profile

To analyze the fatty acid composition in *P. brevitarsis* larvae, fatty acid methyl ester (FAME) was synthesized according to the method of O'Fallon et al. (2007) with some

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modifications. Briefly, 1 g of the dried larvae powder was weighed into a test tube with a screw cap, and 6.3 mL of absolute methanol and 0.7 mL of 10 N KOH were added. For permeating, dissolving, and hydrolyzing the sample, the tubes were heated in a 55℃ water bath for 1.5 h with thorough shaking every 20 min. After cooling in cold water, 0.58 mL of 24 N H_2SO_4 was added to the test tubes and mixed by inversion. Heating and cooling were carried out as described above. Three milliliters of hexane were mixed by vortexing, and the hexane layer was separated. The upper hexane layer containing the FAME was placed into a glass vial and kept at -20℃ until further analysis. FAME analysis was performed using an HP 6890N GC-FID (Hewlett-Packard Co., Wilmington, DE, USA) equipped with a Supelco^{TM} SP-2560 capillary column (100 m×0.25 mm×0.20 μm) (Sigma-Aldrich, St. Louis, MO, USA). One microliter of sample solution was injected into the column and He was used as the carrier gas. The gas flow rate was 1 mL/min, and the oven temperature was held at 140℃ for 5 min, then increased to 240℃ at a rate of 3℃/min, and the temperature was maintained at 240℃ for 10 min. The temperatures of the injector and detector were set at 260℃. Detected FAMEs were identified by comparing the retention times of peaks with those of the standards 37 component FAME mixture (Supelco, Bellefonte, PA, USA), which were analyzed under the same conditions mentioned above.

Statistical analysis

One-way ANOVA was conducted to analyze the collected data using the SPSS program (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was performed to compare significant differences among means ($p<0.05$).

Results and discussion

Drying yield and proximate composition

The drying yield and proximate composition of *P. brevitarsis* larvae produced from commercial insect farms in South Korea are shown in Table 1. The obtained data varied

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considerably depending on the insect farms ($p<0.001$). The drying yield ranged from 14.12 to 27.28%, and the highest yield was observed for the larvae produced from Farm D and E ($p<0.05$). Drying yield is one of the important processing factor directly affecting the profit of the seller, when edible insects are processed as pills and powder. Before harvesting, edible insect larvae are generally fasted for 3-4 days to remove residues in the intestine for better color and flavor (Kwon et al., 2013). According to Noh et al. (2015), fasting for 4 days before harvesting caused 27% weight loss in *P. brevitarsis* larvae. To our knowledge, in some cases, fasting with water immersion is carried out to promote defecation and minimize weight loss. Thus, the evaporation of absorbed water during drying process could greatly reduce the drying yield in the larvae fasted with water. If this speculation is valid, there would be similar moisture content in dried samples, despite the large variation on drying yield.

The difference in moisture content between the highest and lowest values (5.14-7.38 g/100 g) was approximately 2.24 g/100 g ($p<0.05$), which seemed to be relatively smaller than the difference in drying yield. The protein and lipid contents of *P. brevitarsis* larvae were greatly affected by production farms ($p<0.001$), in which changes in the relative content of lipids and proteins were observed. The larvae produced from Farm A, B, and D presented higher protein content, but lower lipid content compared to Farm C and E ($p<0.05$). The lowest ash content was found in larvae from Farm C and E ($p<0.05$).

In general, the large variation observed in the proximate composition of edible insects is mainly related to differences in developmental stages, feeding source, origin, and analytical methods (Rumpold et al., 2013). According to Oonincx et al. (2015), supplementation with a low-protein and high-fat diet decreased the protein content of yellow mealworm larvae but increased total fatty acid content. Moreover, they found no difference in the fatty acid profile of yellow mealworm larvae fed with different diets, despite evident differences in total fatty acid content (Oonincx et al., 2015). In this study, the larvae produced from Farm C and E were fed with commercial feed and the byproduct of mushroom compost, respectively. Thus, it seems that the feeding sources used in Farm C and E might have more digestible nutrients, particularly lipid compounds and/or their precursors, when compared to the other feeding

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199 sources used in Farm A, B, and D. As a result, the increased lipid content in *P. brevitarsis*
200 larvae might cause a relative decrease in protein and ash contents. From the current perspective
201 that edible insect has been primarily focused as an alternative protein source, our results
202 indicate that supplementation of oak only, oak plus scrub, or private fermented feed used in
203 Farm A, B, and D, respectively, could be beneficial in producing the *P. brevitarsis* larvae with
204 high-protein and low-fat contents.
205
206 ***Total and free amino acid profiles***
207 The total amino acid profiles of *P. brevitarsis* larvae produced from commercial insect
208 farms in South Korea are shown in Table 2. No difference in total amino acid content was
209 found ($p>0.05$), regardless of insect farms, in which the essential and non-essential amino acid
210 contents of *P. brevitarsis* larvae were 38.45-42.75% and 57.25-61.55%, respectively. Eight
211 essential amino acids, including histidine (for infants), isoleucine, leucine, lysine, methionine,
212 phenylalanine, threonine, and valine were found in the larvae. Among them, the phenylalanine
213 and methionine contents were greatly affected by insect farms ($p=0.027$ and $p=0.006$,
214 respectively). In particular, the larvae produced from Farm B, which used oak plus scrub feed
215 had higher essential amino acids (methionine) and sulfur-containing amino acid (cysteine)
216 contents compared to those from other farms ($p<0.05$).
217 The obtained data for total amino acids in this study were considerably similar to the
218 previous observation on *P. brevitarsis* larvae (mostly third instar), which was reported by
219 Chung et al. (2013), Noh et al. (2015), and Yoon et al. (2020). In particular, Chung et al. (2013)
220 suggested that *P. brevitarsis* larvae could be a potentially useful source of essential amino acids
221 (methionine, threonine, valine, isoleucine, leucine, phenylalanine, histidine, and lysine) to
222 humans. In addition, Noh et al. (2015) reported that the supplementation of rice bran during
223 fasting could slightly increase the total amino acid content of *P. brevitarsis* larvae. Recently,
224 Yoon et al. (2020) evaluated the supplementary effects of the five natural feeding sources, such
225 as aloe, apple, banana, sweet persimmon, and sweet pumpkin, on the nutritional composition
226 of *P. brevitarsis* larvae, and found that different feeding sources could change the proportion

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of essential amino acids, but did not affect the total amino acid content. Consequently, it is expected that the enrichment of some essential amino acids could be possible through dietary feeding control, but which might have little to no impact on the total amino acid content of *P. brevitarsis* larvae.

A total of 33 free amino acids, including 8 essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, and valine), were detected in five larval samples from different production farms (Table 3). Except for cystathionine, the contents of all free amino acids of *P. brevitarsis* larvae significantly differed by insect farms. The content of essential amino acids in detected free amino acids ranged from 4,073 to 5,6 μg/g, in which the highest content was observed for the larvae from Farm A. Moreover, free amino acids such as phosphoserine, taurine, and γ-amino-butyric acid (GABA), which are well-known to provide physiological benefits to human health (Diana et al., 2014; Huxtable, 1992; Mcmahon and Oommen, 2008), were detected, depending on production farms.

Phosphoserine acts as a calcium stabilizer, which is rich in casein residues in milk proteins, and in turn contributes to improvement in calcium absorption (Mcmahon and Oommen, 2008). According to Jarboe and Mabrouk (1974), moreover, aqueous beef extract contained 1.84 mg of phosphoserine per 100 g of sample, as a form of free amino acid. In this study, it was observed that the larvae from Farm A, B, and C included 1,001, 1,153, and 773 μg of free phosphoserine per gram of dry matter. However, opposite results have been reported by Yoon et al. (2020), who reported no detection of free phosphoserine in *P. brevitarsis* larvae fed with oak-fermented sawdust plus aloe, apple, banana, sweet persimmon, or pumpkin. However, given that the free phosphoserine was detected in the larvae fed with oak (in the case of Farm A and B in this study), it could be thought that the free phosphoserine content might also be affected by other rearing conditions.

Taurine, 2-aminoethane sulfonic acid, has been well-known to have positive effects on osmoregulation, calcium modulation, antioxidation, radioprotection, and energy production in the mammalian body (Huxtable, 1992). In this study, except for the larvae from farm C, 25.29-44.11 μg of taurine per gram of dry matter was detected, which was similar to the previous

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finding (Yoon et al., 2020). It has been reported that beef (*semitendinosus* muscle) and lamb (*longissimus lumborum* muscle) contained 38.6 and 31.0 mg of taurine/100 g, respectively (Purchas et al., 2004). Considering that the larvae sample was analyzed as a dried form in this study, it seems that the taurine content of *P. brevitarsis* larvae might be lower compared to conventional meat sources.

Recently, gamma-aminobutyric acid has received a great interest in the food industry, due to its various physiological effects on blood pressure control, activation of liver function, and improvement in brain function etc. (Diana et al., 2014). In this study, *P. brevitarsis* larvae contained 10.36-99.12 μg of GABA per gram of dry matter sample. GABA is generally found in fermented foods, since lactic acid bacteria produce glutamic acid decarboxylase for catalysis of L-glutamic acid to GABA. In this regard, the observed GABA content in white-spotted flower chafer larvae was potentially comparable to those of fermented goat's milk (28 mg/kg; Minervini et al., 2009) and fermented pork sausage enriched with GABA through lactic acid bacteria fermentation (0.124 mg/kg; Li et al., 2009). Consequently, our results show that white-spotted flower chafer larvae are not only an excellent resource for supplying essential amino acids, but also that they could be a useful food source for supplying some free amino acids (e.g. phosphoserine, taurine, and GABA) to promote physiological activity.

Fatty acid profile

A total of 17 fatty acid methyl esters (FAME) were found in the larvae produced from commercial insect farms (Table 4), in which all larvae samples showed a higher proportion of unsaturated fatty acids (UFA, 76.0-81.2%) compared to saturated fatty acids (SFA, 18.8-24.0%). The major fatty acids contained in the white-spotted flower chafer larvae were oleic acid ($C_{18:1}$, 51.6-59.5%), palmitic acid ($C_{16:0}$, 14.1-19.5%), palmitoleic acid ($C_{16:1}$, 6.6-11.9%), and linoleic acid ($C_{18:2}$, 5.4-12.9%), and these fatty acids accounted for approximately 90% of the total fatty acids (minimum 88.1 and maximum 92.0%). This finding was in good agreement with the results from previous studies, which have reported that oleic acid is the major lipid composition of white-spotted flower chafer larvae (Chung et al., 2003; Noh et al., 2015; Yoon

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et al., 2020). In the previous studies, oleic acid was shown to be effective in improving cardiovascular disease and lowering cholesterol levels in the blood, a high content of oleic acid has been suggested as a nutritionally good indicator in the white-spotted flower chafer larvae (Chung et al., 2003).

In this study, the larvae from Farm A (oak feed), B (oak plus scrub feed), and E (mushroom byproduct feed) showed higher oleic acid content than those from Farm B and C ($p<0.05$). However, the contents of essential fatty acids, such as linoleic acid ($C_{18:2}$) and α-linolenic acid ($C_{18:3n-3}$), were higher in the larvae from Farm C (commercial feed) than in those from the other insect farms ($p<0.05$). There were no significant differences in the contents of arachidic acid ($C_{20:0}$, one of the essential fatty acids) and cis-4,7,10,13,16,19-docosahexaenoic acid ($C_{22:2}$, DHA). Recently, Yoon et al. (2020) suggested that the fatty acid composition of white-spotted flower chafer larvae could be changed by feeding sources. In addition, Noh et al. (2015) noted that supplementation with aloe, rice bran, or pumpkin during 4 days of fasting could alter the content of oleic acid, from 62.5 to 67.1%. Thus, it could be expected that the fatty acid composition of *P. brevitarsis* larvae could be modified by the supplementary feed during fasting as well as basal feeding during production.

The saturated-to-unsaturated fatty acid ratio (SFA/UFA) of *P. brevitarsis* larvae ranged from 0.23 to 0.32. It has been well documented that a decrease in SFA/UFA positively contributes to the improvement in the nutritional value of foods (Vural and Javidipour, 2002). Based on the SFA and UFA contents previously reported by Zotte and Szendrő (2011), the SFA/UFA of pork loin, beef loin, and chicken breast was calculated as approximately 0.63, 0.86, and 0.52, respectively. In this regard, it could be presumed *P. brevitarsis* larvae provides better SFA/UFA values to human health compared to conventional meat sources. To the best of our knowledge, although there have been no studies on the physiological benefits of edible insect oils in the human body, some recent animal studies have found the potential benefits of insect oil intake on digestibility (Kierończyk et al., 2018) and fatty acid profiles in liver and muscle tissues (Benzertiha et al., 2019). Thus, it seems that *P. brevitarsis* larvae from Farm A

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(only oak feed), which showed higher oleic acid content and the lowest SFA/UFA value, could be the most beneficial source of lipids for human health.

Conclusion

In conclusion, this study confirmed that the white-spotted flower chafer (*P. brevitarsis*) larvae could be an excellent food alternative to supply high-quality protein and lipids. Moreover, phosphoserine, taurine, and GABA, which are known to be physiologically useful, were detected in the form of free amino acids. The contents of the bioactive compounds and the proximate composition were greatly affected by the farms where the larvae were produced. Although the underlying mechanisms of the different nutritional compositions have not yet been clearly understood, this study suggests that the production system of Farm A, using only oak feed, could be potentially beneficial in increasing protein content and decreasing SFA/UFA ratio in *P. brevitarsis* larvae.

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Acknowledgements

This work was supported by the research invigoration program of 2020 Gyeongnam National University of Science and Technology. In addition, this study was evidently conducted to obtain data for public interest purposes and the results cannot be used for commercial promotion of a certain farm. Therefore, the actual name of participating insect farms is not disclosed.

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Table Legends

Table 1. Drying yield and proximate composition of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Table 2. Total amino acid profile of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Table 3. Free amino acid profile of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Table 4. Fatty acid profile of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

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Table 1. Drying yield and proximate composition of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Traits	Farm A[1)]	Farm B	Farm C	Farm D	Farm E	Significance of p value
Drying yield (%)	14.12±0.48^{d}	16.70±0.34^{c}	26.11±0.21^{b}	27.28±0.05^{a}	26.84±0.04^{a}	<0.001
Proximate composition (g/100 g)						
Moisture	5.15±0.05^{cd}	5.14±0.23^{d}	5.97±0.03^{b}	7.38±0.12^{a}	5.38±0.07^{c}	<0.001
Protein	66.82±0.41^{a}	66.02±0.33^{a}	54.48±0.26^{b}	67.07±0.66^{a}	54.16±1.28^{b}	<0.001
Lipid	9.91±0.08^{e}	11.88±1.31^{d}	18.06±0.64^{b}	16.34±0.07^{c}	19.38±0.27^{a}	<0.001
Ash	8.48±0.23^{a}	7.35±0.10^{b}	5.48±0.05^{d}	6.76±0.15^{c}	5.48±0.07^{d}	<0.001

All values are presented as mean ± standard deviation of triplicate. (n =3).
[a-e]Means with different superscripts indicate significant difference within a row ($p<0.05$).
[1)]Farm A, *Protaetia brevitarsis* larvae fed with oak only; Farm B, *Protaetia brevitarsis* larvae fed with oak and scrub; Farm C, *Protaetia brevitarsis* larvae fed with commercial feed; Farm D, *Protaetia brevitarsis* larvae fed with private fermented feed; Farm E, *Protaetia brevitarsis* larvae fed with by-product from mushroom compost.

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Table 2. Total amino acid profile of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Traits (μg/g dry matter)	Farm A[1)]	Farm B	Farm C	Farm D	Farm E	Significance of p value
Valine[2)]	220.32±57.10	243.62±46.91	189.28±16.52	186.54±24.09	197.52±16.44	NS[4)]
Isoleucine[2)]	125.73±38.63	147.08±29.35	102.92±9.82	106.73±14.76	119.95±10.10	NS
Leucine[2)]	328.50±102.99	403.43±84.82	278.30±27.62	278.73±42.92	314.24±27.08	NS
Lysine[2)]	359.65±122.29	445.47±92.75	312.05±29.17	327.56±60.59	344.15±31.10	NS
Threonine[2)]	208.63±60.23	249.79±50.11	186.08±19.77	179.26±29.74	203.85±17.93	NS
Phenylalanine[2)]	661.59±200.32^{ab}	886.77±195.66^{a}	488.94±53.35^{b}	516.87±71.57^{b}	578.26±34.88^{b}	0.027
Methionine[2)]	60.93±15.36^{b}	113.34±16.91^{a}	93.19±8.19^{a}	94.61±11.53^{a}	97.66±8.69^{a}	0.006
Histidine[3)]	237.24±79.54	292.15±56.70	205.03±16.68	194.30±39.64	197.57±16.05	NS
Tyrosine	498.54±153.67	747.59±158.79	591.54±47.69	448.67±78.53	648.08±72.68	NS
Argnine	299.77±87.58	332.07±71.69	217.46±20.52	222.89±35.36	221.50±79.81	NS
Aspartic acid	551.68±162.93	669.52±142.97	460.67±48.02	464.99±76.04	495.73±44.72	NS
Glutamic acid	964.95±257.69	1192.25±259.42	820.91±82.85	724.40±116.04	906.22±76.69	NS
Serine	425.28±118.14	533.13±116.43	380.07±37.34	320.84±56.18	408.48±38.93	NS
Glycine	683.50±178.03^{a}	739.82±163.84^{a}	365.30±32.41^{b}	380.80±53.40^{b}	415.83±36.82^{b}	0.004
Alanine	363.22±102.00	443.22±95.35	283.28±26.90	320.95±45.09	295.27±24.69	NS
Cysteine	69.56±15.41^{b}	98.74±18.51^{a}	68.78±5.78^{b}	69.83±8.61^{b}	69.78±6.15^{b}	0.048
Proline	469.25±103.30^{c}	556.06±113.19^{bc}	647.08±85.44^{bc}	1103.52±152.65^{a}	748.53±83.86^{b}	<0.001
Total	6686.61	8256.22	5797.70	6067.84	6370.71	
Essential amino acid	2701.12 (40.40%)	3529.24 (42.75%)	2447.33 (42.21%)	2333.26 (38.45%)	2701.31 (42.40%)	
Non-essential amino acid	3985.49 (59.60%)	4726.98 (57.25%)	3350.38 (57.79%)	3734.58 (61.55%)	3669.41 (57.60%)	

All values are presented as mean ± standard deviation of triplicate. (n =3).

[a-c]Means with different superscripts indicate significant difference within a row ($p<0.05$).

[1)]Farm A, *Protaetia brevitarsis* larvae fed with oak only; Farm B, *Protaetia brevitarsis* larvae fed with oak and scrub; Farm C, *Protaetia brevitarsis* larvae fed with commercial feed; Farm D, *Protaetia brevitarsis* larvae fed with private fermented feed; Farm E, *Protaetia brevitarsis* larvae fed with by-product from mushroom compost.

[2)]Indicates essential amino acids for infants.

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[3)]Indicates conditional essential amino acid for adult human.
[4)]NS: non-significance ($p \geq 0.05$).

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Table 3. Free amino acid profile of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Traits (μg/g dry matter)	Farm A[1)]	Farm B	Farm C	Farm D	Farm E	Significance of p value
Valine[2)]	1368.69±69.00[b]	891.82±10.82[c]	1470.54±25.93[a]	1459.79±12.06[a]	1518.16±36.22[a]	<0.001
Isoleucine[2)]	431.90±16.33[b]	277.69±1.67[d]	369.80±9.52[c]	376.03±5.22[c]	510.59±10.36[a]	<0.001
Leucine[2)]	126.47±5.54[d]	112.47±0.97[e]	134.82±3.35[c]	167.78±3.36[b]	201.30±5.29[a]	<0.001
Lysine[2)]	1168.64±56.31[a]	792.90±6.82[b]	655.66±19.66[c]	552.99±9.52[d]	638.15±14.01[c]	<0.001
Tryptophan[2)]	65.51±113.46[c]	ND[4)]	465.37±2.89[a]	222.78±4.35[b]	221.66±7.02[b]	<0.001
Phenylalanine[2)]	62.57±5.48[c]	54.21±1.75[d]	113.59±1.00[a]	102.13±4.16[b]	110.60±0.33[a]	<0.001
Methionine[2)]	21.81±1.11[a]	13.54±0.46[c]	8.30±0.30[d]	19.07±0.39[b]	22.07±0.54[a]	<0.001
Histidine[3)]	2388.76±53.45[a]	1944.83±14.40[b]	1546.20±38.85[c]	1307.52±5.49[d]	986.86±37.79[e]	<0.001
Tyrosine	674.83±28.98[b]	629.00±6.13[c]	533.85±19.72[d]	341.92±7.41[e]	718.50±17.98[a]	<0.001
Arginine	2662.03±172.10a	1927.53±27.42[b]	1667.22±40.52[c]	1030.30±9.67[d]	2058.50±53.13[b]	<0.001
Glutamic acid	218.26±8.16[a]	176.74±3.51[b]	109.53±2.98[d]	ND	143.09±3.37[c]	<0.001
Serine	295.83±11.45[c]	171.08±0.69[d]	567.25±13.01[a]	494.55±7.80[b]	555.71±14.88[a]	<0.001
Glycine	176.18±152.61[b]	ND	747.42±30.15[a]	ND	734.72±11.82[a]	<0.001
Alanine	652.40±11.76[e]	1569.25±7.61[d]	2242.63±76.54[b]	3671.79±32.94[a]	1679.52±28.81[c]	<0.001
Cystine	120.09±4.49[d]	274.75±2.73[c]	334.82±10.68[a]	294.78±3.73[b]	290.75±10.96[b]	<0.001
Proline	1419.10±2457.95[b]	4023.17±32.40[a]	ND	ND	ND	0.004
Phosphoserine	1001.76±33.97[b]	1153.72±10.18[a]	773.77±22.32[c]	ND	ND	<0.001
Taurine	25.29±2.48[c]	44.11±3.67[a]	ND	30.93±0.27[b]	34.03±0.77[b]	<0.001
Phosphoethanolamine	251.96±17.07[a]	240.74±22.16[a]	34.10±0.92[b]	39.12±1.34[b]	49.44±2.84[b]	<0.001

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Urea	4810.24±726.27[a]	3723.01±15.04[b]	1127.56±39.51[c]	915.88±4.39[c]	1180.26±27.17[c]	<0.001
α-aminoadipic acid	20.86±2.19[c]	24.23±1.17[b]	35.84±0.77[a]	23.23±0.71[b]	35.09±0.24[a]	<0.001
Citrulline	ND	ND	ND	4518.23±25.88	ND	<0.001
α-amino-butyric acid	14.63±0.50[b]	27.00±0.05[a]	12.76±1.07[c]	11.73±0.38[d]	ND	<0.001
Cystathionine	78.61±3.32	94.11±9.83	97.09±10.80	98.96±0.84	72.21±2.26	NS[5)]
β-alanine	303.74±23.76[a]	267.28±9.72[b]	182.98±12.70[c]	192.88±13.50[c]	166.11±2.60[c]	<0.001
β-aminoisobutyric acid	77.88±10.03[a]	29.75±1.20[b]	27.84±1.61[b]	ND	22.23±6.64[b]	<0.001
γ-amino-butyric acid	63.10±3.25[b]	99.12±1.20[a]	10.36±8.99[d]	24.80±0.76[c]	12.34±10.69[d]	<0.001
Ethanolamine	ND	ND	ND	9.95±0.27	ND	<0.001
Ammonia	407.66±54.21[c]	489.47±15.11[b]	446.16±5.27[bc]	583.32±3.64[a]	470.20±7.69[b]	<0.001
δ-hydroxylysine	29.33±1.81[b]	24.90±0.67[c]	11.19±0.96[e]	432.75±2.03[a]	20.14±0.57[d]	<0.001
Ornithine	96.07±2.72[b]	213.52±3.52[b]	70.93±1.67[b]	448.61±5.27[a]	135.64±178.97[b]	0.001
1-methyl-L-histidine	150.87±1.98[b]	157.21±2.04[a]	104.09±1.88[c]	36.70±0.93[e]	91.43±1.46[d]	<0.001
3-methyl-L-histidine	28.42±7.04[a]	11.43±0.64[b]	ND	ND	ND	<0.001
Total	19213.48	19458.55	13901.71	17408.52	12679.30	

All values are presented as mean ± standard deviation of triplicate. (*n* =3).
[a-c]Means with different superscripts indicate significant difference within a row ($p<0.05$).
[1)]Farm A, *Protaetia brevitarsis* larvae fed with oak only; Farm B, *Protaetia brevitarsis* larvae fed with oak and scrub; Farm C, *Protaetia brevitarsis* larvae fed with commercial feed; Farm D, *Protaetia brevitarsis* larvae fed with private fermented feed; Farm E, *Protaetia brevitarsis* larvae fed with by-product from mushroom compost.
[2)]Indicates essential amino acids for adult human.
[3)]Indicate conditional essential amino acid for infants.
[4)]ND: not-detected.
[5)]NS: non-significance ($p\geq0.05$).

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Table 4. Fatty acid profile of white-spotted flower chafer (*Protaetia brevitarsis*) larvae produced from commercial insect farms in South Korea

Fatty acid components (%)		Farm A[1)]	Farm B	Farm C	Farm D	Farm E	Significance of p value
Myristic acid	$C_{14:0}$	0.64±0.03^{d}	0.70±0.04^{cd}	0.73±0.03^{bc}	0.88±0.06^{a}	0.79±0.05^{b}	<0.001
Pentadecanoic acid	$C_{15:0}$	0.55±0.03^{b}	0.69±0.03^{ab}	0.80±0.02^{a}	0.22±0.19^{c}	0.21±0.04^{c}	<0.001
Palmitic acid	$C_{16:0}$	14.07±0.35^{d}	16.42±0.30^{b}	15.14±0.14^{c}	16.16±0.54^{b}	19.46±0.64^{a}	<0.001
Heptadecanoic acid	$C_{17:0}$	0.47±0.03^{bc}	0.57±0.18^{b}	0.91±0.03^{a}	0.29±0.25^{bc}	0.21±0.18^{c}	0.002
Stearic acid	$C_{18:0}$	2.73±0.02^{a}	2.26±0.08^{b}	1.65±0.04^{d}	1.76±0.02^{c}	2.73±0.48^{a}	<0.001
Arachidic acid	$C_{20:0}$	0.34±0.30	0.16±0.28	ND[2)]	0.27±0.24	0.60±0.010)	NS[3)]
Myristoleic acid	$C_{14:1}$	2.52±0.13^{a}	1.71±0.08^{b}	1.18±0.04^{c}	0.25±0.22^{e}	0.67±0.03^{d}	<0.001
cis-10-Pentadecanoic acid	$C_{15:1}$	0.69±0.02^{b}	0.81±0.02^{b}	1.14±0.02^{a}	0.19±0.17^{c}	0.29±0.01^{c}	<0.001
Palmitoleic acid	$C_{16:1}$	8.07±0.26^{cd}	9.40±0.23^{bc}	10.95±0.25^{ab}	11.93±0.44^{a}	6.63±2.32^{d}	0.001
cis-10-Heptadecanoic acid	$C_{17:1}$	0.48±0.01	0.45±0.39	0.31±0.27	ND	0.57±0.02	NS
Oleic acid	$C_{18:1}$	58.69±0.52^{a}	58.71±1.43^{a}	51.55±0.80^{b}	51.71±0.91^{b}	59.48±1.45^{a}	<0.001
Linoleic acid	$C_{18:2}$	7.29±0.05^{c}	5.38±0.02^{d}	12.85±0.30^{a}	12.19±0.21^{b}	5.70±0.15^{d}	<0.001
α-Linolenic acid	$C_{18:3\ n-3}$	0.35±0.30^{c}	0.53±0.46^{bc}	0.98±0.02^{ab}	1.05±0.01^{a}	0.56±0.01^{bc}	<0.001
cis-11-Eicosenoic acid	$C_{20:1}$	ND	ND	ND	0.38±0.32^{a}	ND	0.034
cis-11,14,17-eicosatrienoic acid	$C_{20:3\ n-3}$	0.84±0.01^{a}	0.17±0.30^{b}	ND	ND	ND	<0.001
cis-13,16-Docosadienoic acid	$C_{22:2}$	0.17±0.29	0.18±0.31	0.20±0.35	0.48±0.41	0.54±0.01	NS
cis-4,7,10,13,16,19-docosahexaenoic acid + cis-15-tetracosenoic acid	$C_{22:6}$ + $C_{24:1}$	2.10±0.12	1.86±0.21	1.61±0.52	2.25±0.31	1.58±0.09	NS
Saturated fatty acids (SFA)		18.80	20.80	19.23	19.58	24.00	
Unsaturated fatty acids		81.20	79.20	80.77	80.42	76.00	

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(UFA)					
SFA/UFA	0.23	0.26	0.24	0.24	0.32

All values are presented as mean ± standard deviation of triplicate. (n =3).
[a-d]Means with different superscripts indicate significant difference within a row ($p<0.05$).
[1)]Farm A, *Protaetia brevitarsis* larvae fed with oak only; Farm B, *Protaetia brevitarsis* larvae fed with oak and scrub; Farm C, *Protaetia brevitarsis* larvae fed with commercial feed; Farm D, *Protaetia brevitarsis* larvae fed with private fermented feed; Farm E, *Protaetia brevitarsis* larvae fed with by-product from mushroom compost.
[2)]ND: not-detected.
[3)]NS: non-significance ($p\geq0.05$).

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