Historical Uses and Breeding Technologies of Protaetia brevitarsis surensis (Larvae for Food) for the Edible Insect Industry in South Korea

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

We aim to review Protaetia brevitarsis surensis (PBS) with a specific focus on its historical uses and characteristics, making it one of the most viable food sources or medicinal materials. Ordinary PBS and food insects are characterized by low-emission, especially greenhouse gas, insect farming technologies in Republic of Korea. In addition, PBS is considered essentially acceptable for alternative insect food for human and effective environmental conservation in the near future. Generally, PBS larvae have long been studied the most widely as edible and medicinal insects in Korea compared to the other countries. Almost one thousands farmers are breeding the PBS and the productivity range are very variable from 0.3 to 5.0 tons/person-per year.

Keywords: Protaetia brevitarsis surensis (PBS); breeding technology; medicinal materials; insect food.
1. INTRODUCTION

Protaetia brevitarsis surensis (PBS) is characterized by a relatively small size of adults with 20-25 mm in length and 0.7-1.0 g and 0.8-1.2 g in width for female and male, respectively. The skin of PBS is a flat shape with a hard but shiny exoskeleton that is a coppery black color. There are irregular white or yellowish-white spots on the front chest plate and elytra that appear to be dented. Protaetia brevitarsis gathers around flowers or trees to get some sap out of the trees, especially in 20 years old oak trees. The insect can be found across Korea, Eastern Siberia, and Japan from late Spring to Fall. It is easy to spot these insects in the air since they typically fly without fully stretching front wings and make a heavy sound like a hornet. Additionally, PBS larvae also known as the white-spotted flower chafer, are called by different local names, for example, “Gumbeing-i” in Korean and “Shirahoshi-hanamugun” in Japanese [1]. However, PBS is often mistaken for Dynastinae or rhinoceros beetles, both are mostly called Gumbeing-i among the general people in South Korea. Dynastinae and rhinoceros adults are mostly used for kids’ recreation, and larvae are used for traditional food or medicine sources in the rural area. The larva length is 3 cm and 7 cm long for Protaetia brevitarsis and a coleopteran, respectively. PBS larvae have long been studied the most widely as edible and medicinal insects in Korea compared to other countries. Donguibogam, one of the oldest medical books (published in 1610, Plate 1) in Asia, records its common uses as medicinal materials and food traditionally.

We aim to review PBS with a specific focus on its historical uses and characteristics, making it one of the most viable food sources or medicinal materials.

2. REVIEWS ON HISTORY, LEGISLATION, BREEDING AND THE BIOLOGY OF PBS

2.1 Legislation Governing Food Materials of PBL in Republic of Korea (ROK)

In South Korea, despite its long history, as shown above, the use of Protaetia brevitarsis surensis (PBS) larvae have gained popularity among the public in recent years since the worldwide services and benefits of insects as edible food sources were televised in CHANNEL A as Edible thing X-file 88th on March 3, 2014, produced by Young-Don Lee, (http://www.ichannela.com/program/template/program_refinement.do?cateCode=0501&subCateCode=050100&pgm_id=WPG2140064D).

Then Rural Development of Administration (RDA), academia and rural communities cooperated to persuade the Korean congress in 2014 that led to the passing of a bill that legalized the production of food products made of PBS larvae.

Ministry of Food and Drug Safety (MFDS) issued a temporary food permit in 2014. In 2016, they were registered for food code (specifically "C000400) as a general food ingredient by the Korean MFDS (Welcome to the Ministry of Food and Drug Safety | Ministry of Food and Drug Safety (mfds.go.kr).

In South Korea, using PBS larvae for food requires strict permission that the level of heavy metals should be less than 0.3, 0.1, 0.1 mg/kg for Pb, Cd, As, respectively. With the increased interest in this PBL, it is therefore needed to develop a safe and nutritious feed source to produce high-quality insects [2]. Recently, the use of PBS larvae was found to support the liver and kidneys’ primary functions and help reducing a blood clot [3,4]. The results suggest their potential for the exploitation and utilization of insect resources. In addition, PBS larvae have traditionally been used for preventing inflammatory disease, breast cancer, and liver-related diseases such as hepatic cancer, liver cirrhosis, and hepatitis which was written by HeoJun in the book of Donguibogam (1610, Plate 1).

After processing, the PBS larva powder consists of 55-58% protein, 17-18% fat, carbohydrates and ashes. Almost 20 amino acids are composed in the body of a PBS larva, making up both essential and non-essential components, and nearly 75% is unsaturated fat. This nutritional composition of fat is similar to vegetable oils [5-8]. This insect's nutritional composition compares with unsaturated fats and omega-3, -6, and -9 fatty acids that blue-backed fish contains, such as mackerel. Therefore, each PBS larva contains sufficient animal protein and unsaturated fats that
can provide humans with a variety of high-quality food in short term.

2.2 The Life-Cycle of PBS

The PBS's whole life cycle is highly variable, ranging from 90 to 180 days. There are four main stages in the metamorphosis of PBS. All PBS's life start from eggs for 7-8 days, and then the 1\textsuperscript{st} larval stage span for approximately 10 days, 2\textsuperscript{nd} larval stage for the next 20 days, and the 3\textsuperscript{rd} (early, mid, late) larval stage for 60-150 days. In sequence, PBS goes through the cocoon stage (metamorphosis) for 30-35 days and finally as an adult for 60 to 120 days. Each of these stages mostly depends on the temperature and feed nutrition [9].

Plate 1. The original book of Dong-I-Bogam (Broadcasted on Korean SBS TV; The found future from insect)

Fig. 1. The shape and growth stage duration of Protactia brevitarsis surensis (sunday documentary Korean Brodcasting SBS; The life cycle of Protactia brevitarsis surensis)
2.3 Breeding Permission

In Korea, PBS breeding is not permission but just licensed to all people registered by the Agricultural Tech knowledge center of City or Gun, a provincial government. Most PBS farmers operate breeding facilities at a relatively small scale (i.e., about 100-200 m²). Nevertheless, it is possible to produce more than 2 Mg annually at this farm size, if equipped with the complete control of main environmental factors, such as temperature, air, and moisture, as well as multi-racks (more than ten shelves) and with superior seed adults.

2.4 Cultivar and Genetic Diversity of PBS:

Normally, PBS adults share almost the same phenotypic patterns with four other kinds in the genus of Protaetia. However, the most important is its distinct behavior crawling with the back and the abdomen facing the sky when they are in the larval stage. The adults are also characterized brown-black color with white spots on their back (Fig. 1).

Typically, many farmers are breeding these insects from those that they collect from compost piles near their residence or cropland. Nowadays, however, they tend to rely on the use of specialized seed insects. Additionally, farmers are adopting alternative breeding practices to avoid the inferiority caused by succeeding breeding. Han et al. [10] is dominated at many insect farms as the Korean indigenous species and valuable industrial insect resources in South Korea. Additionally, they investigated the genetic diversity specifically within the Korean PBS population and compared it with morphologically closed species. They also examined how many genetically different haplotypes distribute in the reared PBS collected from a total of 14 insect farms and the interspecific genetic difference between congeners using mitochondrial COI gene sequences (DNA barcoding region).

2.5 The Productivity of PBS

In the past until the year 2000, before breeding in constructed modern systems is less common, farmers generally collected PBS larva from compost dumps and used it as medicine, especially for treating or relieving liver cancer and related symptoms. Traditionally, PBS larvae were primarily collected 1) in a decayed oak tree, which was left after cultivation of shiitake, and 2) from the composts of several kinds of straw, especially mixed with rice and barley. The letter has almost 3,000 year-long histories because rice straw mats were the common roofing materials in Korea, covering the roof throughout the year but replaced with new ones every four-five years. Therefore, PBS larvae were collected in the same time intervals (4-5 years) from the decayed straw mats whenever replaced. The timing of covering the roof with new rice straw mats is usually in winter, following rice harvesting, in Korea (Plate 2). From these traditional simple collection methods of PBS larvae (Plate 3), it has been only about 20 years that PBS larvae can be grown in plastic film greenhouse (Plate 4). However, several problems are exits mainly related to inconvenient control methods for temperature and pests, such as mice entering the greenhouse. Maintaining the set temperature is very important for the growth activities of PBS without disease occurring. However, a mouse is a severe pest species capable of killing and eating PBS larvae and should be controlled. The prevention of mouse entrance is considered the most crucial practice for the prevention of mouth entrance in all PBS breeding systems, for example, installing greenhouse base cemented into the ground and building rigid walls without crevices.

Furthermore, the conversion of traditional collection methods of PBS larvae to a panel house with modern IoT systems has been accelerated. These systems are characterized by automatic control of temperature, moisture, and air with a remote controller, such as in the cellular phone or a control panel in the entrance (Plate 5).

The inputting method for adults has three steps. The 1st step is preparing a living box and filling up 1/3 height such as 10-cm feeds in 30-cm height of living box. The larva feeds should be passed 4mm sieve, which is very important for improving egg numbers. The 2nd step is the input of adults with a 1~3:1 (female: male) ratio. The last step is to feed the adult feed for 1-week feeding. In the 1st step, the larva feed should be checked no re-heating for two days at the breeding room and maintained 50-60% moisture content. In the 2nd step, the breeding adults' numbers 50 in 20-L living box are suitable for increasing egg numbers and which is economically viable for sieving (8-mm mesh) interval (5-7 days) for collecting eggs and separation of adults in the next egging, which will be continued for almost two months. In the 3rd step, the adult feed should be pre-evaluated for the preference of adults and egging pattern for the improving egg numbers in the living box.
Plate 2. Covering the roof with rice straw mats; (http://cafe.daum.net/jh2221/6KyR/15)

Plate 3. Collection of PBS larvae in the rice straw roof dumps

Plate 4. Breeding of PBS larvae in a vinyl film greenhouse, where constant temperature control is challenging
Ten years ago, most farmers were breeding PBS larvae in the bed because it is straightforward for making by a panel with 2 X 4 X 0.5 m for width, length, and height in the vinyl plastic house. Currently, however, this breeding system is not recommended. This is because PBS disease is quickly propagated, especially, *Metarhizium anisopliae*, which could wipe out the entire population within 2-3 months after the incidence of two or three diseases that PBS larvae catch in the breeding bed or box. Therefore, most Korean farmers are breeding them in the individual living box with 5-15 layers for reducing that disease damage.

Larva feed has been changed from yard composts or rice straw to highly nutritious and fully fermented supplements. According to Kang et al. [11] and Moon et al. [12], the new feed sources are manufactured through microbial decomposition of cellulose in the sawdust of oak tree, and the communities of microbes selected coexist with the coleopteran larva. Kang et al. [11] show that these microbes were applied for decomposing organic matter and wheat powder as an energy source, which was changed to molasses lately. More recently, rice and barley brans, and soybean are used for energy. Moon et al. [12] determined that the growth characteristics of PBS larva and egg-laying efficiency by using fermented mulberry sawdust as organic matter. At the growth chamber temperature of 25°C and using wheat bran mixture of 10 or 30%, the weight of female adults and larva growth increased. Moon et al. [12] also stated that the duration of larval growth spans 70-85 days at 25°C, and the number of eggs was the highest in the 5th week after hatching start, and the egging started five days after eclosion. Specifically, the number of eggs reached 73% that was egged between 3 to 8 weeks after eclosion. The larva's growth duration was variable between 65 and 121 days at 25°C, depending on feed sources [12]. For example, it was 71.1 days for the wood dust of oak [11], 65 days in fermented rice straw [9], 121.09 days in sawdust piles of an oak tree [13]. The duration of PBS growth takes nearly 100 days at 20°C [11] but 66-70 days at 30°C [14]. Briefly, the growth duration of PBS was negatively correlated with total digestible nutrients (TDN) which means high nutrients can reducing the growth duration and increasing larva’s weight. The living room temperature should be keeping between 20 to 30°C and the best room temperatures are 25 and 27°C for larva and adult’s growth, respectively.

2.6 Common Insect Pests

In Korea, the mushroom fly is a common insect pest, which harms PBS living in the fermented larva’s feed by eating hypha and indirectly mediating several diseases and mites [15]. The use of insecticides to control the mushroom fly was evaluated, showing that 35% of total PBS larvae were killed due to exposure to insecticides. The results suggest that spraying insecticides is not a safe method while breeding PBS larvae.

Alternatively, it is recommended that high temperature and pressure conditions can effectively reduce insect pests without insecticide application as common. High temperature and pressure need to be maintained before and after fermentation in the process. The critical time that temperature is higher than 70°C is at least longer...
than 24 hours when mixing feed in the fermenter (not published) or UV (ultraviolet) disinfection [16].

Choi et al. [17] studied the importance of proper rearing densities of PBS. This study aimed to provide information and data for the management of insect breeding farms by identifying an appropriate density when rearing PBS larvae. The breeding box of PBS was filled with 2 L of fermented sawdust on a 50 × 35 × 15 cm sheet of plastic, and the density of 200, 300, and 400 PBS individuals in the first, second, and third larval stages. At each rearing density, the treatment was repeated five times, and the sawdust medium was replaced three times (10 intervals). The experiments were conducted for a total of 30 days from March 1, 2020. Among the selected rearing densities, when 200 PBS in the first, second, and third larval stages were reared in the breeding box, there was a higher survival rate and lower mortality rate, thereby leading to efficient production. Choi et al. [17] suggest a practical method that can increase production efficiency through the environmental management of insect breeding farms.

Kwak et al. [18] studied smart farming approaches of insects. Their study informed that Korean insect farms mostly operate as family base working units and develop and sell products altogether. Such insect farming generally occurs within a sandwich panel house for conserving temperature and is equipped with temperature and CO₂ sensors. These methods and systems will be changing to IoT systems near soon because the insect consuming food and feed for companion animals is rapidly increasing. The primary food company requires that as the cheap, standardized sustainable supply, and therefore standardized insect farms are needed at a proper scale.

2.7 Insect-Pest Control Research

Eco-friendly control method using formic acid of mites that are parasitic to PBS adults Lee et al. [3], published that parasitic mites that occur during the rearing of industrial insects such as PBS are challenging to control because chemical pesticides should not be use. To eco-friendly control the parasitic mites on the PBS, formic acid was used. When fumigation for 24 hours of 30 ml of 30~50% solution of formic acid in a 42 L rearing box for PBS, the control value of parasitic mites was more than 90%, and it did not damage the lifespan and the number of spawning of PBS.

As a result of the above, it is expected that the difficulties of industrial insect farmers will be solved, and the positive perception of consumers about edible insects will spread.

Mass breeding of PBS resulted in entomopathogenic fungal infection, usually *Metarhizium anisopliae*. A mixture of microorganisms (*Bacillus subtilis*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*) delayed fungal infection by *M. anisopliae*, which infected fewer PBS when the microorganism mixture was added to sawdust as feed for PBS for 30-d, their mortality rate was approximately 35% less than that of the control group, which was fed sawdust without the EM. In addition, the growth of *M. anisopliae* on agar media spread with each bacterium as inhibited by up to 80% [18].

PBS is sensitive to entomopathogenic fungi, such as *M. anisopliae* [11], which induce disease in intensive breeding systems. The disease tends to propagate quickly in low moisture content, such as below 40%, but it decreases by increasing the moisture content up to 60% of fermented feeds. Additionally, the disease spread occurs easily by worker’s hands, using a living box, air pan, or contaminated feeds and adults. Therefore, feeds should be well fermented and all the tools (i.e., brushes, living boxes, door handles) and insectarium need to be sterilized.

2.8 The Larva of *Protaetia brevitaisis surensis* Feed Research

Sericulture and Entomology Experiment Station, Jeonbuk Agricultural Research & Extension Service Berries (mulberry) mixture marc mixing in mulberry sawdust (mulberry marc: bokbunja marc: blueberry marc = 1: 1: 1) was added to feed the fermented sawdust fermented while breeding PBS larvae (references). The addition ratio was added to the Berries (mulberry) marc 10, 20, and 30% by three treatments. In addition, the fermentation period is 20 days, 40 days, 60 days, and 80 days by four treatments by the addition ratio, the developmental characteristics of the PBS larvae by fermentation period of larva’s feed were investigated. The C/N rate according to the berry mixed marc addition ratio was lower the higher the addition ratio. The development period of PBS larvae is shorter than the control of mulberry fermentation sawdust until the fermentation period of 40 days, but there was no difference from the fermentation period 60
days after the mulberry fermentation sawdust. The higher the fermentation period 40 days, the longer the growth period, the higher the fermentation period of more than 60 days, the growth period tended to be shorter.

The weight of PBS larvae did not vary depending on the fermentation period, but the higher the addition rate, the heavier the weight and survival rate did not change. However, the number of larvae 2.6 g or more, the longer the fermentation period, the higher the addition ratio tends to increase, the faster the growth rate tends compared to mulberry fermentation sawdust.

This study evaluated the efficacy of a blueberry cake mixed diet on larval growth of PBS. When fed oak fermented sawdust with 3, 5, and 10% of blueberry cake, the cumulative proportion of larvae over 2.5 g was 81.6% at 95 days, 82.6% at 75 days, and 89.0% at 55 days after rearing at 25±1°C, respectively. When fed mulberry fermented sawdust with 3, 5, and 10% of blueberry cake, the cumulative proportion of larvae over 2.5 g was 85.2% at 95 days, 84.3% at 85 days, and 85.7% at 55 days after rearing at 25±1°C, respectively. The survival rate of PBS larvae was 88.2%~92.7% when the cumulative ratio of 2.5 g was over 80%.

For maintaining or raising the selling price, the shipment should be even in the 12 months or adjusted by the needs requirements, so storing the larvae before the processing is essential because, after processing, that storage duration is limited for one year in South Korea. So many farmers are adjust the productivity by the selling amount and the best way for maintain or keeping the larva growth is winter sleeping or storing which method can delay the growth and stage development. Park et al. (2020) published that for storing the larvae, the storing temperature is usually set at 8°C, and the storage duration of one month is economically viable because the death rate is the minimum compared to long term storing. However, if the temperature further decreases up to 4°C and the duration increases to 70 days, the mortality rate would increase. Additionally, the adaptable storing weight was 1.8-2.1 g per larva which mortality was only 3%.

The relationship between fecundity and body size in mated adult female PBS was investigated in laboratory conditions. Strong relationships between fecundity and adult weight were found. Larger females were more productive than smaller ones. Male size did not show any effect on fecundity or hatchability. The fecundity of female PBS increased linearly with body size. Total fecundity, expressed by the number of eggs deposited into the sawdust, correlates significantly with female body weight and size (p<0.001). But the size and weight of females did not show any effects on hatchability. This study strongly suggests that relatively large females produce more offspring than smaller females. These results have significant implications in mass rearing by determining conditions likely to improve production

Changes in characteristics of development and oviposition of PBS (Coleoptera: Cetoniidae) by different mixing ratios of fermented mulberry sawdust Lim et al. (2020) publish that by crushing the pruning branches of the berry plant mulberry and blueberries to replace a PBS feeding source, they investigated PBS’s growth and oviposition characteristics affected by different sawdust mixing ratios. Specifically, the mixing ratio of mulberry sawdust and blueberry sawdust, respectively, 90:10, 80:20, and 70:30 compared to oak sawdust, PBS larvae did not show any difference in the growth period and survival rate. However, the larva weight increased by more than twice the ratio of the shipping reference 2.5 g or more. By mixing ratio at 80:20, the larva weight and the number of oviposition were the greatest, compared to oak sawdust.

3. CONCLUSION

In conclusion, the breeding of Protaetia brefitarsis surensis (PBS) is essential for reduction of greenhouse gas emission and supply nutrients, especially protein and unsaturated animal fat to humans. PBS larvae contained high protein and additionally have many medicinal effects, especially liver-related diseases. So, the authors are estimate that PBS productivity from 500 in 2020 to 50,000 tons in 2030 and farmers from one thousands in 2020 to 5,000 in 2030 by the intake 1kg/person as food such as noodle, cake, hamburg. Additionally, the consumption is increasing rapidly nowadays in South Korea and we anticifate the breeding farmers will be increasing in many countries of animal breeding problem and protein needed country altogether.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

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