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Growth Performance and Nutrient Composition in the White-spotted Flower Chafer, *Protaetia brevitarsis* (Coleoptera: Scarabaeidae) Fed Agricultural By-product, Soybean Curd Cake

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Insects are gaining recognition as an alternative source of protein. As a result, more and more domestic farms have begun mass rearing of edible insects. In order to produce high quality insects, studies on the development of safe and nutritious feed sources are needed. Given the cost of rearing insects, agricultural and industrial by-products are good sources for feed. The efficient utilization of these by-products can help in reducing the cost of production and preventing environmental pollution. In the current study, *Citrus unshiu* peel (CP), soybean curd cake (SCC), soybean oil meal (SOM), and brewers dried grain (BDG) were investigated for their effects on larval growth and development of *Protaetia brevitarsis*. Interestingly, the feed with 10% SCC increased larval weight by 3.5 times. For the larval period, the group of 10% SCC was significantly shorter than the control. Furthermore, minerals such as Zn, Ca, K, Mg, Na, and P were recorded to be high in 10% SCC. A total of 17 amino acids were present in 10% SCC, of which tyrosine and arginine were predominant. The heavy metal contents were very small amounts or not detected in any of the investigated groups. These findings provided a scientific basis for the utilization of soybean curd cake as a nutritional feed source to promote larval growth and produce quality insects.

Key words : Agricultural by-products, edible insect, growth, nutrition, *Protaetia brevitarsis*

Introduction

The white-spotted flower chafer, *Protaetia brevitarsis* (*P. brevitarsis*), are part of the family of Scarabaeidae belonging to the order Coleoptera and primarily distributed throughout Eastern Asia [7, 22]. The larvae of *P. brevitarsis* have been traditionally used for treatment of inflammatory disease, breast cancer, and liver-related diseases such as hepatic cancer, liver cirrhosis, and hepatitis [13, 29]. They were listed as a general food ingredient by the Korean Ministry of Food and Drug Safety in 2016 [17, 18]. As the interest in rearing of *P. brevitarsis* increased, it is necessary to build a foundation for stable and cost-effective mass-rearing systems [26]. Especially, agricultural by-products were the best choice with the low cost. Recently, research and development of various natural resources such as agriculture and food processing by-products has been found to improve livestock use, resulting in increased utilization of by-products, decreased animal feed costs, and environmental preservation [1, 23, 25].

In the food industry, large amounts of by-products such as peel, pulp, and seeds are produced and they are sometimes regarded as waste. These by-product wastes have been used as materials for animal feed and fuel production [4, 14, 16].

Soybean curd cake is surplus material when tofu is made from soybeans. Since high levels of nutrients in this tofu wastes after processing, it can be used as a material for livestock feed or fertilizer [1, 15, 19].

Soybean oil meal is primarily the by-product of soybean oil extraction and the primary protein source in poultry rations. A variety of studies have been conducted to enhance the nutrient value and acceptability of soybean oil meal in poultry feeding [8, 9, 21].

Brewers dried grains are readily available and cheap by-products from brewery industries [24]. When compared with soybean protein used in poultry rations, net protein

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ratio and protein efficiency ratio values of brewers dried grains were similar [6].

However, the agricultural and industrial by-products, having effects of reduced feed costs and pollution, are mostly used in poultry or livestock production. Therefore, the present study was conducted to find out the effects of feeding four kinds of agricultural by-products, such as *Citrus unshiu* peel, soybean curd cake, soybean oil meal, and brewers dried grain, in *P. brevitarsis* widely rearing as edible insects.

Materials and Methods

Insects

The second instar larvae of *P. brevitarsis* were purchased from a private seller (Hapcheon-gun, Gyeongsangnam-do, Republic of Korea). The purchased beetles were kept in insect rearing facilities at National Institute of Agricultural Science at 25±1℃ with 40~60% humidity. Larvae of *P. brevitarsis* were maintained in the plastic box (30 mm long × 48 mm wide × 27 mm high) with enough fermented sawdust. The third instar larvae were collected based on their head capsule size for this experiment.

Feed with different additives

Nine types of the feeds were formulated in different proportions of ingredients. To test the effects agricultural and industrial by-products, we added *Citrus unshiu* peel, soybean curd cake, soybean oil meal, and brewers dried grain based on Table 1. After these feed additives were mixed with fermented oak sawdust, they were fermented for longer than 1 month.

Rearing condition

The third instar larvae were reared in a plastic container (200×130×130 mm) with designated feeds. For each group, 20 larvae were tested and each feed was changed once a week. The rearing room was regulated at 25±1℃ with 40~60% humidity under 12:12(L:D) light condition. We measured larval weight, larval period, and pupation rate. Each treatment was repeated three times.

Table 1. Composition of nine different feeds with four additives

Name of feed	Ingredients
Control	100% Basic feed
5% CP	5% *Citrus unshiu* peel + 95% Basic feed
10% CP	10% *Citrus unshiu* peel + 90% Basic feed
5% SCC	5% Soybean curd cake + 95% Basic feed
10% SCC	10% Soybean curd cake + 90% Basic feed
5% SOM	5% Soybean oil meal + 95% Basic feed
10% SOM	10% Soybean oil meal + 90% Basic feed
5% BDG	5% Brewers dried grain + 95% Basic feed
10% BDG	10% Brewers dried grain + 90% Basic feed

Analysis of proximate, amino acids, minerals, and heavy metals composition

The proximate composition (moisture, protein, fat, fiber, and ash) of *P. brevitarsis* larvae fed with each feed was determined by following standard methods of the Association of Official Analytical Chemists (AOAC) [2]. Briefly, moisture content was determined by drying the sample at 105℃ to constant weight. The crude protein content was determined by the Kjeldahl method and calculated by multiplying the nitrogen content using a factor of 6.25. Crude fat was extracted from each sample in a Soxhlet extractor with ethyl ether. The content of fiber was determined according to the AOAC enzymatic gravimetric method. The ash content was estimated by dry-ashing in furnace at 550℃ for 5 hr.

A dry ashing procedure was used to prepare the samples for mineral analysis. Copper (Cu), zinc (Zn), calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), and phosphorus (P) contents of the samples were determined by direct current plasma emission spectrometry.

Amino acids were determined in freeze-dried samples hydrolyzed with 6 M HCl by an automated Amino Acid Analyzer (Beckman 6300, CA, USA).

Dry sample was digested by adding HNO_3 and HF in an autodigester at 180℃ and filtered. This sample was used for analysis of heavy metals including arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) by atomic absorption spectroscopy and final concentrations of heavy metals were determined.

Statistical analysis

The mean and standard deviation (SD) of each experiment were compared to control. Statistical differences at $p>0.05$ between the groups were analyzed by one-way ANOVA analysis followed by Tukey's multiple comparison test.

Results and Discussion

The larval weight was observed for 13 weeks with different feeds. The groups adding agricultural and industrial

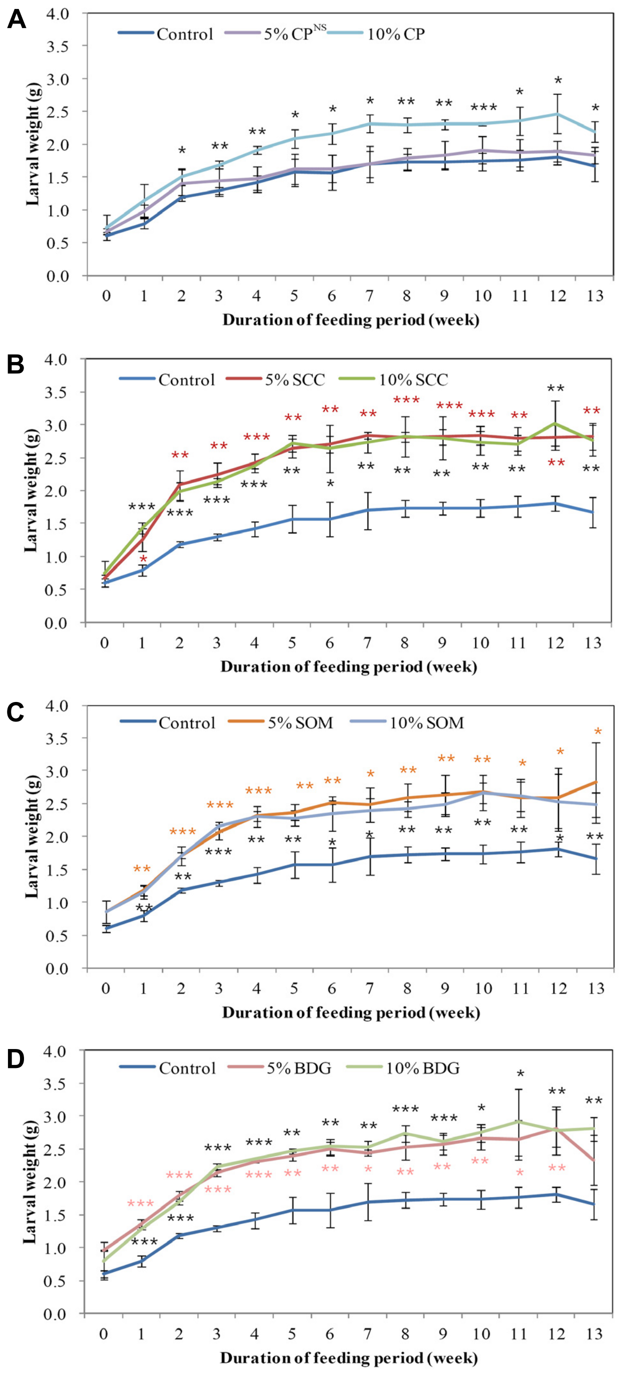

Fig. 1. Body weight changes of *P. brevitarsis* larvae rearing on nine different feed composition. Change in body weight of larvae fed *Citrus unshiu* peel (A), soybean curd cake (B), soybean oil meal (C), and brewers dried grain (D) or the control. Individual larva weight was observed over the 13 weeks of growth in each group. NS, no significant difference; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

by-products showed that the weight of larvae was entirely increased compared to the control (Fig. 1). In particular, the increase rate in the group of 5%(a 170% increase; p=0.00221) and 10% SCC (a 140% increase; p=0.00069) was found to be high in the early stage of third instar larvae (for 2 weeks after feeding). Also the group of 10% SCC (p=0.00124) was found that they reached the fastest (5 weeks after feeding) weight of 2.5 g (Fig. 1B). In general, when the weight of larvae is estimated to be 2.5 to 3 g they can be sold from the insect farms. Given this point, it is believed that feeding soybean curd cake will enable to produce insects efficiently.

Table 2 showed the total larval period from each group. All groups had shorter larval period than the control group. Especially, the group of 10% SCC (p=0.00082) was significantly reduced to 40% of the larval period compared to the control group. This meant that larvae fed diet with 10% soybean curd cake grew faster and somehow it accelerated their development. It could be considered that the utilization of soybean curd cake provided the insect farms with economic benefits.

The pupation rate for all groups was more than 80%. Especially, pupation rate of the groups adding soybean curd cake and brewers dried grain was more than 98%, though there were no significant differences (Table 2).

Proximate nutritional composition of *P. brevitarsis* larvae fed different feeds was given in Table 3. The analyzed samples contained similar amount of each component with low variation, particularly, 10% SCC was found to contain the highest amount of protein, fibre, and ash of the nine samples. The results demonstrated that soybean curd cake was a good nutritional source.

Table 4 showed the results of the mineral contents of 9 samples. All the mineral concentrations were determined on a dry weight basis. Copper contents were found in similarly

Table 2. Larval period and pupation rate with different contents of by-products as a feed supplement

Name of Feeds	Larval period (days)	Pupation rate (%)^{NS}
Control	121.09±5.53^{NS}	84.26±13.7
5% CP	111.77±7.15^{NS}	86.67±2.89
10% CP	81.32±3.05***	89.47±5.26
5% SCC	76.95±6.89***	100±0.00
10% SCC	69.44±8.16***	98.33±2.89
5% SOM	83.39±8.70**	94.81±5.01
10% SOM	79.2±5.27***	98.25±3.04
5% BDG	78.65±9.83**	98.33±2.89
10% BDG	72.05±10.24**	100±0.00

Values are means ± SD. The asterisk is used to indicate the statistical differences according to one-way ANOVA and Turkey's multiple comparison test. NS, no significance; $^{**}p<0.01$; $^{***}p<0.001$.

Table 3. Proximate composition of *P. brevitarsis* with different feeds

Component	Control	5% CP	10% CP	5% SCC	10% SCC	5% SOM	10% SOM	5% BDG	10% BDG
Moisture	81.82	79.94	79.22	74.17	71.37	76.55	79.02	76.38	75.73
Crude protein	8.13	9.69	10.58	13.35	14.69	12.24	12.02	12.18	13.02
Crude fat	3.54	4.06	3.86	5.16	5.05	3.8	2.87	4.09	4.35
Crude fibre	2.67	2.72	2.68	2.82	3.77	2.95	2.27	3.16	2.91
Crude ash	1.75	1.73	1.64	1.96	2.9	2.02	2.02	2.04	1.92

Table 4. Concentration (mg/kg) of minerals in *P. brevitarsis* with different feeds

Minerals	Control	5% CP	10% CP	5% SCC	10% SCC	5% SOM	10% SOM	5% BDG	10% BDG
Cu	4.94	4.79	5.14	5.62	4.97	4.27	3.93	4.6	4.27
Zn	22.62	21.95	21.89	25.83	43.74	22.12	21.15	21.73	25.24
Ca	1215.1	1079.41	966.3	1106.82	1265.63	1037.74	830.82	1039.27	885.83
K	4474.6	4645.81	4811.84	4756.12	5551.66	4417.94	4433.52	3599.44	4441.27
Mg	692.73	655.77	619.11	669.96	770.34	584.94	536.54	529.67	574.69
Mn	35.35	25.77	25.08	26.41	30.33	20.7	19.8	18.31	17.93
Na	606.56	535.41	523.3	513.04	661.61	464.68	419.96	424.46	458.64
P	1640.25	1662.63	1581.64	1556.81	1854.83	1259.07	1081.53	1042.06	1331.44

small percentages in all the samples analyzed, ranging from 3.93 mg/kg in 10% SOM to 5.62 mg/kg in 5% SCC. The highest levels of Zn, Ca, K, Mg, Na, and P were found in the group of 10% SCC to be 43.74, 1265.63, 5551.66, 770.34, 661.61, and 1854.83 mg/kg, respectively. Of these, Zn is an essential element of nutrition and physiology in animals [20]. The amount of Zn in 10% SCC rose approximately twice as much as that in the control. This high zinc content was expected to be related to stimulating the growth of *P. brevitarsis* larvae.

In the results of amino acid analysis from Table 5, cystein, methionine, serine, tyrosine, phenylalanine, histidine, arginine, and proline were concentrated in 10% SCC. The rate of growth and protein synthesis depends on cysteine and methionine [28]. It is also known that cystine, oxidated dimer form of cysteine, paring with methionine can improve mineral absorption, particularly zinc [5, 11]. Tyrosine and arginine stimulate the secretion of growth hormones [10, 27], individually showed 3.1- and 2-fold-changes in 10% SCC compared to control. These results showed that there was a correlation between the promotion of larval growth and high contents of amino acids in the group of 10% SCC.

Table 5. Amino acid composition of *P. brevitarsis* with different feeds

Amino acid	Control	5% CP	10% CP	5% SCC	10% SCC	5% SOM	10% SOM	5% BDG	10% BDG
Cysteine	0.076	0.087	0.1	0.144	0.169	0.149	0.15	0.153	0.161
Methionine	0.075	0.099	0.119	0.172	0.177	0.154	0.147	0.153	0.162
Aspartic acid	0.475	0.592	0.638	0.839	0.911	0.746	0.734	0.757	0.824
Threonine	0.25	0.32	0.344	0.468	0.496	0.399	0.396	0.419	0.457
Serine	0.332	0.458	0.534	0.729	0.745	0.591	0.58	0.655	0.698
Glutamic acid	0.96	1.142	1.231	1.538	1.687	1.409	1.392	1.378	1.516
Glycine	0.488	0.554	0.579	0.722	0.804	0.66	0.677	0.653	0.726
Alanine	0.442	0.518	0.547	0.609	0.697	0.589	0.564	0.542	0.609
Valine	0.335	0.399	0.44	0.513	0.584	0.488	0.446	0.466	0.512
Isoleucine	0.23	0.286	0.317	0.378	0.426	0.353	0.327	0.339	0.379
Leucine	0.404	0.494	0.543	0.678	0.74	0.611	0.582	0.595	0.666
Tyrosine	0.359	0.568	0.798	1.18	1.102	0.942	0.916	1.058	1.046
Phenylalanine	0.261	0.331	0.38	0.507	0.522	0.441	0.417	0.45	0.483
Lysine	0.421	0.524	0.55	0.66	0.751	0.625	0.592	0.599	0.645
Histidine	0.159	0.214	0.231	0.322	0.328	0.27	0.271	0.291	0.307
Arginine	0.25	0.344	0.374	0.474	0.506	0.398	0.401	0.414	0.476
Proline	0.461	0.648	0.737	1.029	0.991	0.828	0.882	0.903	1.03

Table 6. Heavy metal contents of *P. brevitarsis* with different diets

Heavy metals	Control	5% CP	10% CP	5% SCC	10% SCC	5% SOM	10% SOM	5% BDG	10% BDG
As	0	0	0	0	0	0	0	0	0
Cd	0	0	0	0	0	0	0	0	0
Hg	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Pb	0	0	0	0	0	0	0	0	0

Heavy metal contamination in environmental and food samples has harmful effects such as toxicity and accumulation and contamination in biological systems [12]. The heavy metal contents were analyzed after 48 hour starvation for each group. Hg content was very low and As, Cd, and Pb were not detected in all investigated samples (Table 6). These results of analysis indicated that *P. brevitarsis* larvae fed various by-products were safe. It was also considered that the feed adding by-products could be readily available in farms rearing these beetles.

Generally, by-products that can be produced approximately 20% of weight after agricultural and industrial processing contain diverse nutritional factors such as protein, fibers, minerals, and vitamins [3]. They could serve as an alternative energy source in animal feeds with the reduced cost. As analyzing the effects of feeds adding different by-products including *Citrus unshiu* peel, soybean curd cake, soybean oil meal, and brewers dried grain, a feed with 10% soybean curd cake showed the best effect on the larval growth and nutritional values of *P. brevitarsis*.

Farms rearing insects have been expanded and grown for using them as additives of food as well as animal feeds. Furthermore, *Gryllus bimaculatus* and the larvae of *Tenebrio molitor*, *P. brevitarsis*, and *Allomyrina dichotoma* were listed as general food ingredients by Korean Ministry of Food and Drug Safety last year. Accordingly, there were increasing interest and mass rearing facilities of those edible insects. Thus, it is needed to investigate affordable and nutritional food sources for insects and develop mass rearing system for the continued expansion of the insect industry. In this study, four agricultural by-products with high nutrition values were tested. Considering the results above, it could be stated that supplying 10% SCC (10% of soybean curd cake and 90% of basic feed) was contributed to improve the quality of insects and reduce the costs of rearing.

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초록 : 비지박 첨가 먹이원 급여에 따른 흰점박이꽃무지 유충의 생육과 영양성분 변화

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세계적으로 곤충시장이 매년 성장하는 가운데 우리나라에서도 곤충산업이 미래 유망 산업으로 주목 받고 있다. 뿐만 아니라, 최근 갈색거저리와 쌍별귀뚜라미, 흰점박이꽃무지 및 장수풍뎅이가 일반식품원료로 등록됨에 따라 식용곤충에 대한 관심이 늘고 곤충의 대량사육이 전국적으로 이루어지고 있다. 이에 안전하고 고영양성분이 포함된 식용곤충 전용 먹이원 개발 연구가 필요하다. 최근 들어 각종 부존자원의 사료화는 부존자원의 활용, 사료비 절감, 환경보존 등의 효과를 거두고 있는 것으로 나타나고 있다. 따라서 식용곤충으로 많이 사육되고 있는 흰점박이꽃무지의 생육을 촉진하면서 경제적이고 안정적인 사육을 도모하기 위해 감귤박, 비지박, 대두박, 맥주박 등 4종의 부존자원을 이용하여 효과를 조사하였다. 10% 비지박을 급여한 유충의 무게가 대조구 대비 약 3.5배 증가하였고, 유충기간 또한 40% 이상 단축되었다. 또한, 10% 비지박 첨가 실험구에서 일반조성분과 미네랄 및 아미노산의 함량이 전체적으로 높은 것으로 나타났다. 중금속 함량 분석 결과, 납과 카드뮴 및 비소는 모든 실험구에서 검출되지 않았다. 본 연구결과는 흰점박이꽃무지 유충의 생육 촉진 효과를 가지는 비지박이 곤충 먹이원의 소재로 활용될 수 있음을 보여준다.