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Effect of Chlorella Supplementation on Survival and Larval Growth of the Edible Beetles, *Protaetia brevitarsis* and *Allomyrina dichotoma*

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Edible insects are reported to be rich in protein, minerals and vitamins, and much attention has been paid to them as a future food source. In Korea, they were massively reared and sold. In order to enhance the market value of edible insects for industrialized mass production, it is important to develop the safe and nutritious feed sources for rearing them are needed. In this study, a chlorella-free control feed (Exp1) and six experimental feeds supplemented with 0.5~2.0% liquid or powder types of chlorella were formulated. *Protaetia brevitarsis* and *Allomyrina dichotoma*, registered as food ingredients in Korea, were fed with the designed feeds and parameters of growth including larval survivorship, larval body weight, and larval period were analyzed. When chlorella added, larval survivorship was increased 2~13%(p>0.05) and 9~22%(p<0.05) in each beetle compared to the control. Interestingly, the larval period of chlorella powder-added groups was shortened by 24 days (Exp3, p<0.05) in *P. brevitarsis* and 19 days (Exp4, p<0.01) in *A. dichotoma*. Meanwhile, some parameters, crude protein, crude fiber, copper, zinc, potassium, magnesium, and phosphorous, in chlorella-added groups of *P. brevitarsis* were also higher than the control group. Therefore, chlorella could promote the larval growth performance of these two beetles and be used as a feed additive in rearing them.

Key words : *Allomyrina dichotoma*, chlorella, edible insect, growth, *Protaetia brevitarsis*

Introduction

The world population is expected to reach 9 billion by the year of 2050[7]. It was thought that global agriculture will need to increase output over 70% compared with current food requirements. Thus, alternative sources of protein such as insects will be required [1, 7, 8, 9]. Insects are considered highly nutritional [5, 20, 21, 23, 31, 32]. They have short generation times [31], require little land or water [13], and produce small amounts of greenhouse gas emissions [21, 31].

Protaetia brevitarsis and *Allomyrina dichotoma* are part of the family of Scarabaeidae belonging to the order Coleoptera. The larvae of them have been traditionally used for treatment of inflammatory disease, breast cancer, and liver-related diseases such as hepatic cancer, liver cirrhosis, and hepatitis [14, 24, 30, 34]. Several researches demonstrated that *P. brevitarsis* and *A. dichotoma* have pharmacological functions, including anti-cancer, anti-cytotoxic, and anti-oxidant activities [15, 28, 29, 35]. In Korea, they have been approved as general food ingredients by Ministry of Food and Drug Safety [17, 18]. The Korean government deregulated legislation on the edible insects and then domestic edible insect market is significantly growing. The domestic insect market is expected to expand 1.8 times, from 300 billion won in 2015 to 500 billion won in 2020. However, almost farmers rearing edible insects are small business. First of all, standardization of insect quality is essential in order to produce massively. Feed is considered to be an important factor for the developmental and reproductive performance of insects [27].

Chlorella is a genus of unicellular green algae, belonging to the phylum Chlorophyta. It contains high levels of important nutrients, including essential amino acids, protein, chlorophyll, vitamins, minerals, and bioactive substances [2, 4, 25]. Chlorella is popularly supplemented as a nutritious feed additive for animals and showed antioxidant, anti-inflammatory, antilipidemic, and antiatherosclerotic activities [10, 22, 24, 26]. These benefits of chlorella could show great potential as a source of insect feeds. In addition, no studies have attempted to mix chlorella with fermented oak sawdust

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as an additive of insect feeds to improve larval growth. Thus, the present study investigated the effect of dried powder or liquid type of chlorella on larvae growth of *P. brevitarsis* and *A. dichotoma*.

Materials and Methods

Insects

The larvae of *P. brevitarsis* and *A. dichotoma* in experiments were purchased from private sellers (Gimje-si, Jeollabuk-do, Korea and Siheung-si, Kyunggi-do, respectively). They were kept in insect rearing facilities at National Institute of Agricultural Science at 25±1℃ with 40~60% relative humidity. For *P. brevitarsis,* we individually reared newly hatched larvae to the adult on different dietary treatments and checked once a week. The larvae were used the third instar of *A. dichotoma* because of longer larval period compared to the first or second instar and checked every ten days. For each group of two beetles, 20 larvae were tested and each treatment was repeated three times.

Experimental feeds

The dried powder and liquid *Chlorella* were purchased from Daesang Corp., Seoul, Korea. *Chlorella* contains 60.0% crude protein, 1.8% crude fat, 13.2% crude fiber, 5.4% crude ash, 166.7 mg% potassium, 3,128.5 mg% phosphorus. Liquid type is produced by removing the precipitate by lowering the pH so as not to produce the precipitate, and then, hot water extraction and filtration. Powder type is produced by extracting hot water without pretreatment and then concentrating, filtration, and freeze drying. After chlorella mixed with fermented oak sawdust, they were fermented for longer than 1 month. Fermented oak sawdust was used as the basic feed for rearing the larvae. Seven combinations of powder or liquid type of chlorella and fermented oak sawdust were formulated at different ratios based on Table 1.

Proximate analysis

The moisture, protein, fat, fiber, and ash were based on standard methods of the Association of Official Analytical Chemists (AOAC) [3]. The moisture content was determined by drying the samples in an air-circulating oven at 105℃. The crude protein content was determined by the Kjeldahl method. The crude fat content was determined using the Soxhlet fat extraction method. Crude fiber was measured after boiling 5 g of defatted sample in sulphuric acid and sodium hydroxide. Ash content was estimated by burning the samples at 550℃ overnight. The carbohydrates content was calculated with the following formula: total carbohydrates (% FW) = 100 - (% moisture + % protein + % fat + % ash). The results show total carbohydrates as g/100 g FW.

Analyses of minerals and heavy metals

Mineral and trace elements were determined by direct current plasma emission spectrometry. And dry sample was digested by adding HNO_3 and HF and used for analysis of heavy metals by atomic absorption spectroscopy.

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 survivorship was checked until all larvae pupated or died. The mortality of *P. brevitarsis* and *A. dichotoma* larvae for the seven experimental feeds was shown in Table 2. The survival rate of all the chlorella-treated groups from two beetle species was higher than that of each control group. This showed that chlorella-mixed feeds were useful for rearing both of two beetles.

For *P. brevitarsis*, the larval weight for the group with

Table 1. Composition of different feed ingredients

Name of feed	Ingredients
Exp1	100% fermented oak sawdust
Exp2	99.5% fermented oak sawdust + 0.5% dried chlorella powder
Exp3	99% fermented oak sawdust + 1% dried chlorella powder
Exp4	98% fermented oak sawdust + 2% dried chlorella powder
Exp5	99.5% fermented oak sawdust + 0.5% chlorella in fresh water
Exp6	99% fermented oak sawdust + 1% chlorella in fresh water
Exp7	98% fermented oak sawdust + 2% chlorella in fresh water

Table 2. Mortality of two species of larvae by experimental feeds

Feed	No. of *P. brevitarsis* larvae		% mortality	No. of *A. dichotoma* larvae		% mortality
	Start of the trial	End of the trial		Start of the trial	End of the trial	
Exp1	60	53	11.7	60	46	23.3
Exp2	60	54	10	60	50	16.7
Exp3	60	54	10	60	52	13.3
Exp4	60	56	6.7	60	56	6.7
Exp5	60	54	10	60	42	30
Exp6	60	54	10	60	48	20
Exp7	60	60	0	60	52	13.3

Initial number of larvae in each treatment was 20. Each treatment contained three replicates.

chlorella powder (Exp2, 3, and 4) was significantly higher than that for the control group (Exp1) during the experimental period (Fig. 1A). However, the larval weight for the Exp5, 6, and 7 were lower than that for Exp1. This was probably due to the loss of some nutrients in the pretreatment for the production of liquid type of chlorella. The larval weight of *A. dichotoma* with chlorella addition was highly increased compared to the control during the experimental period (Fig. 1B). Especially, Exp4 in both beetles showed the highest body weight as 3.87 g (p<0.05) and 31.78 g per individual (p<0.05), respectively.

In both two beetles, all groups added chlorella had shorter larval period than the control group, Exp1 (Table 3). When chlorella powder-mixed feeds were fed to *P. brevitarsis* (Exp2 to 4), the larval period was 12~20% less than the chlorella liquid-treated groups and the control group (p<0.05). For the larval period of *A. dichotoma*, all chlorella-added groups (Exp2 to 7) showed 0.80~0.91 times shorter than the control (p<0.05). And the pupation rate was 100% for all treatments. Therefore, feed with chlorella would be beneficial for farms rearing these beetles to reduce the rearing costs.

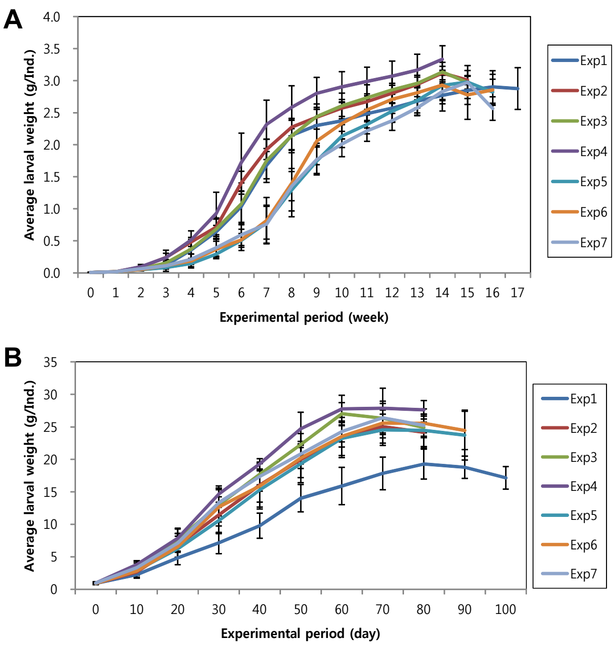

Fig. 1. Larval body weight of *P. brevitarsis* (A) and *A. dichotoma* (B) over the duration of the study. Larvae were fed with liquid or powder of chlorella treated with 0.5, 1, 2% w/w. Values presented were the mean ± SD of three independent experiments.

Nutritional analysis results were summarized in Table 4. Chung et al. [6] reported that *P. brevitarsis* contained 57.86% crude protein per 100 g of dry weight. Considering that the nutritional analysis performed in this study was based on the wet weight, the protein contents of Exp1 was estimated to be similar to the reported results. The crude protein content of Exp2, 4, 6, and 7 was higher than that of eggs, determined to be 9~14%[33]. In particular, Exp7 had 1.3 times more proteins than the control Exp1. And Exp4 including 2% of chlorella powder had the highest moisture and crude fiber content.

Table 3. Average length of the larval period from different feeds

Feed	Larval period of 3rd instar (day)	
	P. brevitarsis	*A. dichotoma*
Exp1	79.4±9.7^{a}	94.5±10.2^{a}
Exp2	66.2±6.2^{b}	83.3±4.8^{ab}
Exp3	55.6±12.6^{c}	77.5±6.6^{c}
Exp4	67.9±12.9^{b}	75.4±7.8^{c}
Exp5	70.1±4.6^{b}	86.4±5.1^{b}
Exp6	77.2±4.6^{b}	83.1±4.5^{ab}
Exp7	67.8±6.3^{b}	85.5±4.2^{b}

Values presented were the mean ± SD of three independent experiments. The mean values followed by different superscript letters within each column indicate that they were significantly different (Tukey's multiple comparison test, p< 0.05).

Table 4. Proximate composition and mineral contents of *P. brevitarsis* with different feeds

Components		Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	Exp7
Nutrient (%)	Moisture	71.70	71.96	72.32	75.63	75.16	72.55	73.17
	Crude protein	13.16	15.11	13.82	14.42	13.91	15.73	16.41
	Crude fat	0.67	0.79	1.16	0.67	1.31	0.80	0.67
	Crude fiber	1.58	2.07	2.45	2.58	2.35	2.27	1.69
	Crude ash	4.43	2.31	2.42	1.53	1.44	1.76	1.44
	Carbohydrate	8.46	7.76	7.83	5.17	5.83	6.89	6.62
Minerals (mg/kg)	Copper	0.12	0.4	0.43	0.51	0.48	0.66	0.56
	Zinc	1.58	2.76	2.29	2.09	2.12	2.83	2.64
	Calcium	152.81	125.99	119.92	123.05	150.36	145.38	115.91
	Potassium	169.11	184.6	212.01	304.01	258.18	279.93	300.41
	Magnesium	57.42	55.4	59.11	65.13	61.7	58.93	64.24
	Phosphorus	139.35	146.94	146.95	160.75	108.5	116.95	131.74

Among minerals, Cu and Zn were the highest in Exp6 (0.66 and 2.83 mg/kg, respectively). The highest contents of K, Mg, and P in Exp4 were found individually to be 304.01, 65.13, and 160.75 mg/kg. Minerals are involved in multiple biological processes including energy utilization, protein metabolism, inflammation, oxygen transport, and immune function [19]. All chlorella-added groups contained high levels of minerals except for calcium, and thus chlorella is a beneficial feed additive for *P. brevitarsis*. Moreover, these larvae feeding chlorella-added feeds could act as a food ingredient for new food and functional food products.

Heavy metals and their accumulation has become a public problem [12]. Arsenic, cadmium, mercury, and lead were not detected in all investigated samples. This meant that the larvae fed chlorella-added feed were safe, and thus farms would immediately be able to apply the formulating feed with chlorella to rearing this beetle. In addition, it would be possible to utilize the larvae of *P. brevitarsis* with chlorella-added feed as sources for human foods.

Microalgae are rich in proteins and vitamins, and can serve as the alternative protein source for poultry and other food-producing animals. In the present study, we found that the dietary adding chlorella could enhance the growth performance of the beetles, e.g. the body weight of *P. brevitarsis* and *A. dichotoma* larvae nearly increased 135% and 127%, respectively, in the group of adding 2% chlorella powder. Meanwhile, the larval weights in chlorella liquid-mixed groups were lower than those in the control. So we recommended that the dried chlorella powder was appropriate for rearing both of two beetles.

Many reports were proved that chlorella in feeds could have the anti-oxidant and immune modulating properties in rat or rabbit [10, 22, 24, 26]. The larvae with chlorella were rich in minerals including copper, zinc, potassium, magnesium, and phosphorus. Copper is a constituent of various oxidizing enzymes [11]. Zinc has a wide range of functions such as enzyme activity, structure, and regulation of gene expression [16]. Phosphorus and magnesium are important components of bone and muscle. Therefore, the larvae fed chlorella-added feeds might be a useful source of dietary minerals. However, to the best of our knowledge, this is first study about the effect of chlorella supplementation in rearing edible insects. There needs to be investigated further studies on fecundity from the studied generation and succeeding generations.

In conclusion, our results demonstrated that supplementation of dried chlorella powder at the level of 2% could promote the growth and nutrient value in rearing the larvae of *P. brevitarsis* and *A. dichotoma* when compared with the control without its supplementation. Dietary chlorella might be useful as a feed additive in rearing the beetles because of its effect of promoting larval growth.

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초록 : 흰점박이꽃무지와 장수풍뎅이 유충에 대한 사료 첨가제로서 클로렐라의 효과

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국제연합식량농업기구(FAO)의 보고에 따르면, 미래의 식량문제 해결 방안으로 식용곤충을 지목하였다. 식용곤충은 단백질을 비롯한 무기질과 비타민 등의 함량이 풍부하여 영양학적 가치가 높은 것으로 평가된다. 따라서 국내 곤충산업의 확대를 위해서는 안정적인 대량사육 시스템의 개발과 안전한 식용곤충 전용 사료의 개발 등에 대한 연구가 요구된다. 본 연구에서는 액상과 분말 클로렐라를 식용곤충종인 흰점박이꽃무지와 장수풍뎅이의 사료 첨가제로 이용하여 각 유충의 생육에 미치는 효과를 분석하였다. 두 곤충 모두에서 클로렐라 액상과 분말을 첨가했을 때 유충의 생존율이 대조구보다 높았다(흰점박이꽃무지; $p>0.05$, 장수풍뎅이; $p<0.05$). 클로렐라 분말을 첨가한 사료를 급여했을 때 흰점박이꽃무지와 장수풍뎅이 유충의 3령 기간이 각각 최대 24일($p<0.05$)과 19일($p<0.05$) 단축되어 곤충 사육농가의 노동력 절감 효과를 기대할 수 있을 것으로 생각된다. 또한, 장수풍뎅이는 주로 학습애완용으로 판매되는 것에 비해 식용 소재로 활발히 활용되고 있는 흰점박이꽃무지 실험구에 대하여 영양성분 분석을 실시하였다. 그 결과, 클로렐라 액상 및 분말 첨가 처리구에서 수분, 조단백질, 조섬유 및 구리, 아연, 마그네슘과 같은 무기질의 함량이 높았고, 납과 카드뮴, 수은 및 비소 등 중금속은 모든 실험구에서 검출되지 않았다. 이러한 연구결과를 바탕으로 흰점박이꽃무지와 장수풍뎅이 사육 시 사료 첨가제로서 클로렐라가 활용될 수 있을 것으로 기대된다.