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Comparison of clay and charcoal as feed additives for *Protaetia brevitarsis* (Coleoptera: Scarabaeidae)

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Abstract

The white-spotted chafer, *Protaetia brevitarsis* (Coleoptera: Scarabaeidae), has been traditionally used in Korea as a medicine for preventing liver-related diseases and suppressing liver cancer. Therefore, this insect is economically important and is commercially reared and sold in Korea. Recently, *P. brevitarsis* was listed as a temporal food ingredient by the Korean Ministry of Food and Drug Safety. Given the increasing economic importance of this beetle, we have sought to improve rearing conditions for its commercial production. In this study, we compared the effects of two food supplements, clay and charcoal, on the growth of second instar larvae of *P. brevitarsis*. Clay and charcoal are generally known as good adsorbent for removal of contaminating substances in insect feed. We fed second instar *P. brevitarsis* larvae a commercial diet consisting of fermented sawdust with seven different combinations of clay and/or activated charcoal, and measured their effects on weight gain for approximately 17 wk until larvae pupated. We found that addition of clay at 2.5% w/w of the fermented sawdust diet had no negative effect on weight gain of second instar *P. brevitarsis* larvae and thus may improve the quality of *P. brevitarsis* as a commercial food.

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Introduction

The white-spotted chafer, *Protaetia brevitarsis* (Coleoptera: Scarabaeidae), is distributed throughout Japan, Taiwan, Korea, China and parts of Europe (Cho, 1969). The adults are observed from late June through July in Korea (Kim *et al*., 2005; Zhang, 1984). These beetles are holometabolous and undergo three larval instars prior to pupation. Larvae overwinter as third instars in the soil and then pupate. In Korea, *P. brevitarsis* are raised commercially and used as a traditional medicine for the treatment of liver cancer (Park *et al*., 1994; Kang *et al.*, 2001; Yoo *et al*., 2007). They were recently listed as a temporal food ingredient by Korean Ministry of Food and Drug Safety. Therefore, rearing of this beetle has gained increasing attention in Korea, and *P. brevitarsis* has been mass-reared for commercial purposes since the late 1990s. Thus, it is important to optimize rearing conditions for beetles to improve their commercial quality.

Diet and growth conditions have been previously investigated (Kwon, 2009). In this study, we sought to characterize the effect

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of supplementing insect feed with adsorbents such as clay and charcoal because they have been shown to remove contaminants such as heavy metals and bio-waste (Babel and Kurniawan, 2003). To improve the quality of the larvae, these two additives were added to standard commercial diet used for *P. brevitarsis* larvae, consisting primarily of fermented sawdust. We measured body weight change and pupation characteristics of insects fed diet supplemented with seven combinations of clay and/or charcoal.

Materials and Methods

Experimental Animals

Second instar larvae of *P. brevitarsis* (Coleoptera: Scarabaeidae) were reared from a laboratory colony started from insects purchased from a commercial supplier, Teunteun Farm (Siheung-si, Kyeonggi-do, Republic of Korea). The purchased beetles were reared on fermented sawdust at 25°C with ca. 40% humidity for six months prior to use in experiments. From this laboratory colony, second instar larvae were collected based head capsule size for use in subsequent experiments.

Feeds with Different Additives

Second instar larvae were fed fermented oak sawdust diet purchased from a commercial supplier in Hoengseong-gun, Gangwon-do, Republic of Korea. To test the effects of feed additives, charcoal and/or clay were added at the indicated concentrations (Table 1). These additives were suspended in an equal weight of tap water and then mixed with the basic feed as described in Table 1.

Table 1. Seven treatments for feed composition with two additives, charcoal and clay.

Treatments	Composition
Control	100 % Basic Feed
Charcoal 25	97.5 % Basic Feed + 2.5 % Charcoal
Clay 25	97.5 % Basic Feed + 2.5 % Clay
Charcoal 25 + Clay 25	95 % Basic Feed + 2.5 % Charcoa + 2.5 % Clay
Charcoal 50	95 % Basic Feed + 5.0 % Charcoal
Clay 50	95 % Basic Feed + 5.0 % Clay
Charcoal 50 + Clay 50	90 % Basic Feed + 5.0 % Charcoal + 5.0 % Clay

Rearing Condition and Experimental Design

Second instars were reared in round petri dishes (98 mm diameter x 15 mm depth) with sufficient amount of the designated feed at 25 °C with ca. 40 % humidity and a 12:12 h (L:D) photoperiod. We measured the body weight of each larva, and provided feed once per wk. Ten larvae were randomly selected for each feed treatment and maintained for approximately 17 wk until most second instars had pupated. Seven different treatments of different additive combinations (10 larvae each) were replicated three times. Larvae were weighed once per wk for 17 wk and development stage of each individual was checked until emerging adults. Larval weight, weight gain per wk, cumulative gain, weight prior to pupation, and time of pupation were calculated. The significance of each treatment was determined and compared to the control treatment using a *t*-test.

Results and Discussion

Larval body weights generally increased 0.105 ± 0.004 g (mean ± S.D.) per wk over the 17-wk experiment (Table 2). However, larvae gained less weight during the latter part of the larval period (Fig. 1). Three treatments – clay 25, charcoal 25 + clay 25, and clay 50 – showed no significant differences compared to the control feed containing no adsorbent (Fig. 2). The maximum weight increase was observed at wk 3 for two treatments (control and charcoal 50 + clay 50) and at wk 4 for five treatments (charcoal 25, clay 25, charcoal 25 + clay 25, charcoal 50, and clay 50) (Table 2). After that time point, weight gain gradually decreased. Moreover, weight decreases were also observed after wk 10, which may be attributed to the secretion of special proteins to form the pupal cell as a defense mechanism against unfavorable environmental factors in preparation of pupation (Lee *et al.*, 2002; Shapiro-Ilan and Russell, 2015). Weight loss prior to pupation is commonly observed in many insect species that under the soil (Ichikawa *et al.*, 2012; Johns *et al.*, 2014).

Larval body weight prior to pupation was also analyzed (Fig. 3), because it is an important correlate of pupation rate and ultimate size of the adult beetle and therefore, the weight and

Table 2. Mean and standard deviation of larval weight increases based on seven different feeds compositions with two feed additives, charcoal and clay

Week	Feeds						
	Control	Charcoal 25	Clay 25	Charcoal 25 +Clay 25	Charcoal 50	Clay 50	Charcoal 50 +Clay 50
1 wk	0.141 ± 0.082	0.212 ± 0.157	0.303 ± 0.154	0.151 ± 0.077	0.177 ± 0.072	0.186 ± 0.121	0.180 ± 0.117
2 wk	0.260 ± 0.117	0.255 ± 0.124	0.362 ± 0.116	0.230 ± 0.119	0.212 ± 0.125	0.369 ± 0.146	0.197 ± 0.137
3 wk	0.357 ± 0.130	0.237 ± 0.111	0.258 ± 0.144	0.298 ± 0.087	0.199 ± 0.093	0.274 ± 0.150	0.213 ± 0.080
4 wk	0.344 ± 0.104	0.291 ± 0.076	0.323 ± 0.090	0.313 ± 0.085	0.286 ± 0.096	0.355 ± 0.130	0.210 ± 0.101
5 wk	0.210 ± 0.114	0.217 ± 0.079	0.169 ± 0.079	0.268 ± 0.062	0.215 ± 0.078	0.141 ± 0.151	0.162 ± 0.106
6 wk	0.106 ± 0.149	0.078 ± 0.095	0.100 ± 0.122	0.102 ± 0.082	0.099 ± 0.114	0.164 ± 0.137	0.119 ± 0.094
7 wk	0.248 ± 0.092	0.177 ± 0.089	0.170 ± 0.118	0.174 ± 0.120	0.188 ± 0.082	0.120 ± 0.141	0.110 ± 0.085
8 wk	0.143 ± 0.215	0.101 ± 0.103	0.098 ± 0.065	0.086 ± 0.076	0.075 ± 0.102	0.154 ± 0.144	0.077 ± 0.120
9 wk	0.034 ± 0.222	0.074 ± 0.100	0.003 ± 0.137	0.135 ± 0.068	0.080 ± 0.078	-0.033 ± 0.122	0.097 ± 0.088
10 wk	0.064 ± 0.103	0.066 ± 0.099	0.018 ± 0.061	0.079 ± 0.101	0.087 ± 0.074	0.050 ± 0.138	0.035 ± 0.098
11 wk	-0.040 ± 0.092	-0.047 ± 0.137	-0.020 ± 0.052	0.002 ± 0.075	-0.002 ± 0.079	0.010 ± 0.087	0.028 ± 0.082
12 wk	-0.012 ± 0.133	0.009 ± 0.224	0.076 ± 0.054	-0.124 ± 0.126	0.024 ± 0.121	0.003 ± 0.116	0.029 ± 0.223
13 wk	0.073 ± 0.060	0.065 ± 0.193	-0.029 ± 0.090	0.052 ± 0.085	0.042 ± 0.093	0.034 ± 0.099	-0.002 ± 0.260
14 wk	0.023 ± 0.069	0.024 ± 0.085	0.032 ± 0.103	0.080 ± 0.105	0.019 ± 0.104	0.039 ± 0.080	-0.008 ± 0.150
15 wk	0.050 ± 0.114	-0.050 ± 0.134	0.049 ± 0.114	0.054 ± 0.096	0.006 ± 0.096	-0.009 ± 0.160	0.052 ± 0.092
16 wk	0.037 ± 0.124	-0.008 ± 0.138	-0.001 ± 0.224	-0.066 ± 0.147	-0.036 ± 0.108	0.025 ± 0.087	-0.001 ± 0.082
17 wk	-0.057 ± 0.128	-0.057 ± 0.204	0.037 ± 0.148	0.033 ± 0.199	-0.051 ± 0.204	-0.024 ± 0.159	0.042 ± 0.075

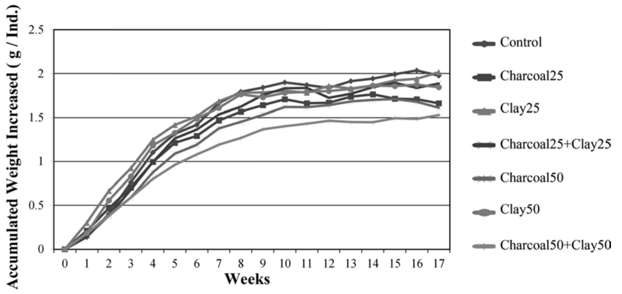

Fig. 1. Means of accumulated larval weight based on seven different feeds compositions with two feed additives, charcoal and clay.

size of prepupal larvae can be used as an indicator of adult insect quality (Sehnal, 1985: Leather, 1988). Although not significant, four of the treatments resulted in lower pupal weights compared to the control treatment. The treatments with clay 25 and clay 50 were similar to the control treatment (Fig. 3). In addition, the time for pupation was also compared for seven treatments (Fig. 4), larvae reared on charcoal 50 required the longest time for pupation (17.7 wk) whereas those reared on clay 25 required the

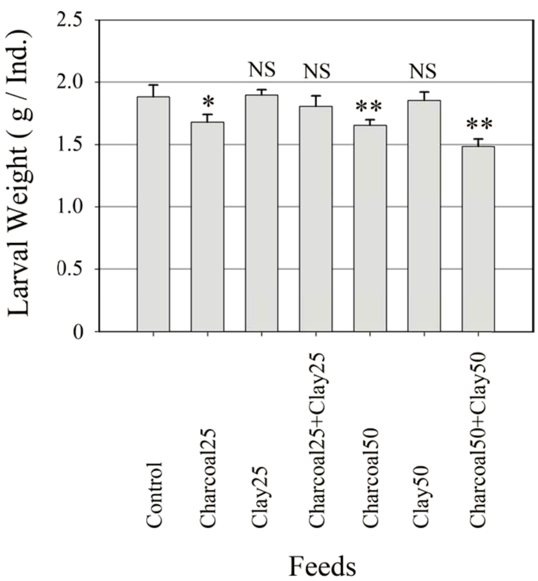

Fig. 2. Means of final larval weight after 17-wk rearing on seven different feeds compositions with two feed additives, charcoal and clay. The error bar indicates the standard errors. The means and standard errors were compared to control by t-test (NS: not significant, * : $p < 0.10$, and **: $p < 0.05$)

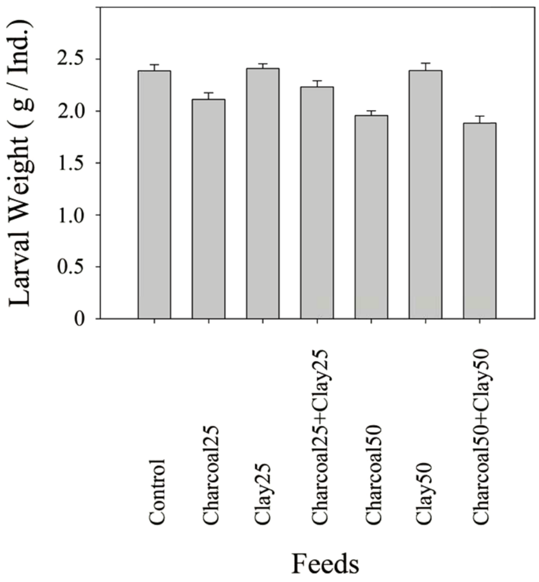

Fig. 3. Means of larval weight just before pupation based on seven different feeds compositions with two feed additives, charcoal and clay. The error bar indicates the standard errors.

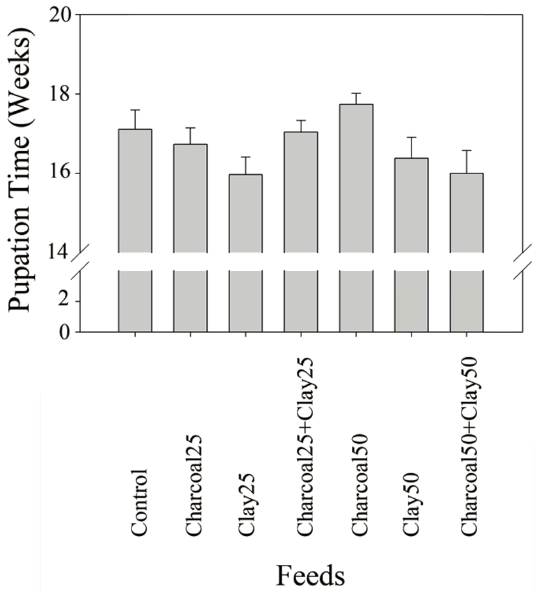

Fig. 4. Means time for required for second instar larvae to pupate based on seven different feeds compositions with two feed additives, charcoal and clay. The error bar indicates the standard errors.

least time for pupation (16.0 wk).

Among six different feeding regimens, clay 25 was determined to be the best for rearing *P. brevitarsis* larvae because it did not adversely affect the larval growth and development relative to the control treatment. Clay is not an organic substituent and appeared to pass through the alimentary tract of the beetles without adverse effects. Given the assumption that clay would have no nutritional value, adding clay might have been a negative factor for rearing insect larvae. However, negligible effects were detected in the larvae reared on the feed with low concentrations of clay in terms of the growth parameters studied. Thus, addition of adsorbent to the diet during commercial production may improve the nutritional quality of this important beetle.

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