

## Brewers' Dried Grain as a Feed Additive for the Korean Rhinoceros Beetle, *Allomyrina dichotoma*

Myung-Ha Song, Heui-Sam Lee and Kwanho Park\*

Industrial Insect Division, National Institute of Agricultural Sciences, Rural Development Association, 166 Nongsaengmyeong-ro, Wanju, Jeollabuk-do 55365, Korea

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Edible insects have recently been increasingly promoted as a source of protein. As the number of farms rearing these insect increases, it is important to develop safe and nutritious feed sources to improve their commercial quality. The aim of the current study was to determine the effect of food by-products as feed supplements for the Korean rhinoceros beetle, *Allomyrina dichotoma*, which has been registered as a general food ingredient in Korea. We compared the effects of waste citrus peel, soybean curd cake, soybean oil meal, and brewers' dried grain on the growth of third instar larvae of *A. dichotoma*. Groups of larvae were fed with fermented sawdust and nine different combinations of the above by-products and the effects on their growth were measured until pupation. The highest survival rate was with feed supplemented with 10% brewers' dried grain (66.7%,  $p < 0.05$ ), and these larvae were also 26% heavier ( $p < 0.05$ ) than the control group that received no supplementation. In the 10% brewers' dried grain group, the larval period of third instar was shortened by almost 28 days ( $p < 0.01$ ) compared to the control group. Of all the groups, only that which was fed the brewers' dried grain supplement showed more than a 90% pupation rate ( $p < 0.05$ ). Therefore, brewers' dried grain may be useful as a source of feed for *A. dichotoma*.

**Key words** : *Allomyrina dichotoma*, Brewers' dried grains, by-product, Edible insect, growth

### Introduction

Edible insects have been recently considered as potential new protein sources [7]. Insect rearing is more environmentally friendly than livestock production because of lower greenhouse gas emissions and reduced water and soil pollution [26]. Insect rearing uses land more efficiently as large numbers of eggs are produced and rates of growth and production are high [16, 25]. Also, insects are high in nutritional value because of their high content of vitamins, minerals, and unsaturated fatty acids as well as proteins [2, 28].

The Korean rhinoceros beetle, *Allomyrina dichotoma* is the family Scarabaeidae belonging to the order Coleoptera. The larvae have been traditionally used for the treatment of liver-related diseases and diabetes [24]. A number of studies demonstrated that *A. dichotoma* have various biological func-

tions such as antineoplastic, anticytotoxic, and antioxidant properties [12, 29]. As the larvae of this species have been listed as a general food ingredient, interest in commercial rearing has increased [15, 21]. It is needed to develop standard techniques for mass-rearing of the beetle and investigate feed supplements with rich nutrients and low cost to improve their commercial quality [13, 20, 23].

Generation of large-scale food waste occurs during food processing and can cause adverse effects on the environment and additional processing costs for the waste treatment because of their poor biological and oxidative stability [1, 17]. Therefore, the management of these waste materials is one of the major concerns in food processing industry. However, they contain potentially valuable nutrients including proteins, fats, fibers, and bioactive compounds [9] and have been used as components of animal feeds and in the production of both natural flavonoids and fuel [1, 14]. Soybean meal, distillers dried grains, and corn gluten feed have already been used as alternative protein sources for livestock production [27] and there is increasing interest in efficient use of a range of food by-products for animal feeds [4, 18].

Several studies have indicated that by-products, either individually or in combination, have a positive influence on animals [6, 13, 22]. However, the agricultural and industrial

#### \*Corresponding author

Tel : +82-63-238-2994, Fax : +82-63-238-3833

E-mail : nicegano@korea.kr

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by-products, having effects of reduced feed costs and environmental pollution, are mostly used in poultry or livestock production. To improve the insect quality, food by-products such as citrus peel, soybean curd cake, soybean oil meal, and brewers' dried grain were added to standard commercial feed for rearing *A. dichotoma*, mainly consisting of fermented oak sawdust.

## Materials and Methods

### Insects

The second instar larvae of *A. dichotoma* were purchased from a private seller (Gyeongsan-si, Gyeongsangbuk-do, Korea). The purchased beetles were kept in insect rearing facilities at 27±1°C with 60% humidity. The larvae were maintained with sufficient fermented sawdust and the third instar larvae were collected based head capsule size. For this experiment, the third instar larvae were used because they have a longer larval period and eat a lot of feed compared to the first or second instar larvae.

### Feed with different contents

The larvae were fed fermented oak sawdust feed, also called basic feed, purchased from a commercial supplier (Wanju-gun, Jeollabuk-do, Republic of Korea) including 88~97.5% of oak sawdust, 1~5% of wheat bran, 1~5% of sugar, and 0.5~2% of effective microorganisms (EM) fermentation broth. The food by-products such as citrus peel, soybean curd cake, soybean oil meal, and brewers' dried grain were food wastes or residues after production of citrus-based drinks, tofu, soybean oils, and beer, respectively. They were each obtained from food processing companies and added with different amounts by its weight to make feeds for *A. dichotoma* larvae after drying in an oven at 60°C. Chemical composition of by-products from Korean Feed Ingredients Association was given in Table 1. To test the effects of feed additives, nine combinations of by-products and fermented

sawdust were prepared in the following way:

Control: 100% basic feed;

Exp1: 95% basic feed + 5% citrus peel;

Exp2: 90% basic feed + 10% citrus peel;

Exp3: 95% basic feed + 5% soybean curd cake;

Exp4: 90% basic feed + 10% soybean curd cake;

Exp5: 95% basic feed + 5% soybean oil meal;

Exp6: 90% basic feed + 10% soybean oil meal;

Exp7: 95% basic feed + 5% brewers' dried grain;

Exp8: 90% basic feed + 10% brewers' dried grain.

After mixing, they were fermented for longer than a month in an indoor fermentation room.

### Rearing condition

The third instar larvae were reared in plastic containers (420 mm long × 240 mm wide × 220 mm high) with various feeds and the rearing room was maintained at 27±1°C and 60% relative humidity under 8 hr light and 16 hr dark photoperiod. The feeds were sufficiently fed to the larvae and changed once a week. Each group of larvae fed on feeds supplemented with food by-products was compared its survival rate, larval body weight, duration for third instar larvae, pupal weight, and pupation rate. Survival rates of the larvae were recorded when all the larvae had pupated and body weight of them was checked once per 10 days. The length of larval period was determined as days between the beginning of the third instar and the beginning of the prepupal period. Twenty of the third instar larvae were used in each test and each treatment was repeated three times.

### Statistical analysis

The mean and standard deviation of each group were compared to the 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 survival rate for different ratios of food by-products mixed with basic feed was shown in Fig. 1. The mean survival rates of larvae fed both citrus peel (5%;  $p=0.0734$ , 10%;  $p=0.0629$ ) and brewers' dried grain (5%;  $p=0.0533$ , 10%;  $p=0.0177$ ) was higher than that of control (48.3±7.6%). Especially, the survival rate for addition of brewers' dried grain at 10% w/w was the highest as 66.7±2.9%. However, there was 100% mortality for feed supplemented

Table 1. Chemical composition of four by-products

| Components | Contents of components (%) |                   |                  |                      |
|------------|----------------------------|-------------------|------------------|----------------------|
|            | Citrus peel                | Soybean curd cake | Soybean oil meal | Brewers' dried grain |
| Moisture   | 66.3                       | 4.7               | 11.5             | 72.1                 |
| Protein    | 9.3                        | 34.8              | 45.7             | 8.1                  |
| Fat        | 2.7                        | 17.0              | 1.7              | 1.9                  |
| Fiber      | 17.0                       | 39.6              | 5.7              | 6.4                  |
| Ash        | 4.7                        | 3.9               | 6.5              | 1.8                  |

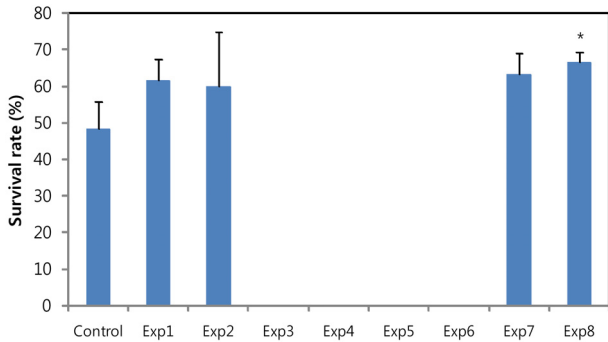


Fig. 1. Survival rates of *Allomyrina dichotoma* larvae using experimental feeds. Citrus peel, soybean curd cake, soybean oil meal, and brewers' dried grain mix were fed to larvae. Control group was fed with fermented sawdust with no supplement. Values are expressed as mean  $\pm$  SD; \*  $p$  values of  $<0.05$  indicate significant difference from the control group.

with soybean-derived by-products; soybean curd cake and soybean oil meal. All of the larvae that were fed soybean oil meal died within 3 weeks without weight gain. The weight of the larvae increased during the first few weeks of feeding feeds with soybean curd cake, but decreased thereafter and the larvae died within 10 weeks. This was probably because of over fermentation in mixture of sawdust and soybean-derived by-products containing high concentrations of protein (Table 1). Over fermentation, which lead to the excessive gas production and heat, carry a potential threat to the survival of insects. And there was reported that alpha-amylase inhibitor present in common bean might closely to the inhibition of the larval growth and alpha-amylase activities in species of bruchids (Coleoptera: Bruchidae) [10, 11]. Ishimoto and co-workers demonstrated that larvae of weevil species could not grow and develop by feeding on the bean containing a proteinous inhibitor against  $\alpha$ -amylase. This would probably suppress growth performance of *A. dichotoma*, however, the exact causes of larval death in the groups supplemented with soybean curd cake and soybean oil meal were unknown. On the other hand, soybeans and soybean meal are a source of isoflavones known to improve growth, promote tissue growth, and prevent diseases. They also contain considerably higher energy and protein, and lower fiber content. These make them an ideal feed ingredient in formulating balanced rations [5, 30]. Our previous study also demonstrated that for larvae of another Scarabid beetle, *Protaetia brevitarsis*, supplementation with soybean curd cake improved growth performance and nutrient value [22]. Nutritional significance of beans remains

controversial. There needs to be investigated further studies on the presence or absence of  $\alpha$ -amylase inhibitor in by-products produced after processing the soybeans, effects of this inhibitor on *A. dichotoma* larvae, or the differences in the overall process of soybean digestion and absorption between *A. dichotoma* and *P. brevitarsis*.

The larval weights in Exp7 ( $p<0.001$ ) and Exp8 ( $p<0.001$ ) groups were significantly higher than that in the control group after the 13th week (Fig. 2). Growth rate in Exp8 was particularly high for 10 weeks after the administration of the feed. Also, the larvae belonging to Exp8 weighted over 20 g at fifth week after feeding, which was the earliest time in the experimental and control groups. Generally, farms rearing the beetle sell third instar larvae weighing about 20 g - 25 g. Taking this into consideration, it might shorten the rearing period and reduce the rearing cost by feeding brewers' dried grain to the beetle.

In Exp 1, 2, 7, and 8, the maximum larval weights were 22.28 g, 23.36 g, 29.59 g, and 31.19 g ( $p=0.0022$ ), respectively, while that of the control was recorded to be 24.73 g. Especially, Exp8 showed the highest increase in weight, 26.12% higher than the control. Not only did the larvae of Exp8 have the highest maximum weight, but they also reached maximum larval weight in the shortest time in approximately 91 days ( $p=0.0002$ ) (Table 2). These results showed that larval body weight was increased by feeding feeds supplemented with brewers' dried grains. As mentioned, brewers' dried grains are rich in protein and fiber, and widely used as protein supplements in diets of pigs and cattle. They also contain vitamins, minerals, and both essential and non-essential amino acids [8, 16]. So it is suitable for animal feed as well as several studies have been con-

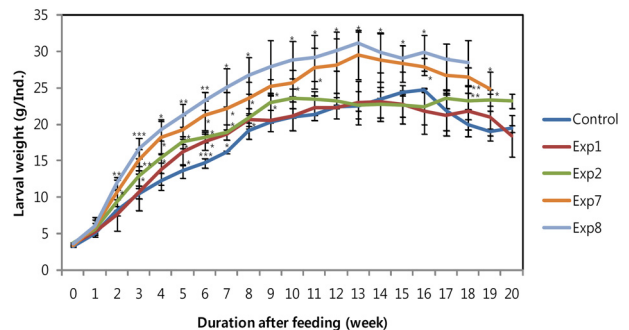


Fig. 2. Body weight changes of the larvae fed by-products. Body weight of the groups fed with brewers' dried grain increased for 13 weeks in a time-dependent manner. The results are mean  $\pm$  SD (\* $p<0.05$ ; \*\* $p<0.01$ ; and \*\*\* $p<0.001$ ). Body weight;  $F(4,97)=3.0268$ ,  $p=0.0213$ .

Table 2. Mean period of larvae with maximum weight and maximum larval weight with different feeds

| Feeds   | Max. larval weight (g) | Duration to reach max. weight (day) | Percentage of Max. larval weight gain (%) |
|---------|------------------------|-------------------------------------|---|
| Control | 24.73±0.25             | 124.14±9.06                         |   |
| Exp1    | 22.28±2.62             | 110.14±9.99*                        | -9.91                                     |
| Exp2    | 23.36±1.49             | 108.14±8.97*                        | -5.54                                     |
| Exp7    | 29.59±3.35             | 93.43±4.93***                       | 19.65                                     |
| Exp8    | 31.19±1.59**           | 91.14±4.67***                       | 26.12                                     |

Data are represented as the mean ± SD. \* indicates statistically significant difference as compared to the control. \* $p<0.05$ ; \*\* $p<0.01$ ; and \*\*\* $p<0.001$ .

ducted to investigate possible usages [3, 19]. Similar to the effect induced by livestock diets supplemented with brewers' dried grains, nutritious components might be able to induce the beetle to grow strongly and fast.

The larval period of third instar supplemented with brewers' dried grain was significantly shorter than that with the control. In Exp8, the average larval period was 28 days shorter than the control (Table 3). This would be beneficial for insect rearing farms as it would shorten the rearing period and reduce the rearing costs.

The pupation rate for all groups was more than 75% (Table 3). For Exp1, the rate of the pupation was higher than the control but the larval weight was lower. As shown in Table 2 and 3, the weight of the larvae when fed with citrus peel was lower than that of the control, but the larval duration was shorter. It seemed that the nutrient contents could help larvae grow fast but do not have much effect on larval size. Only Exp8 had a pupation rate of 90% or more in the experimental and control groups ( $p=0.0314$ ). Except for Exp1, the pupal weight of other groups was relatively heavier than the control (Table 3). In addition, the pupal weight for Exp8 was significantly heavier than the control by 59% ( $p=0.0016$ ).

Table 3. Larval period, pupal weight, and pupation rate with different feed compositions

| Feeds   | Larval period of third instar (day) | Pupal Weight (g) | Pupation rate (%) |
|---------|-------------------------------------|------------------|-------------------|
| Control | 148.2±13.66                         | 4.77±0.60        | 77.08±7.88        |
| Exp1    | 141.60±9.24                         | 4.73±0.65        | 80.18±5.36        |
| Exp2    | 142.60±14.29                        | 4.91±0.69        | 79.93±8.76        |
| Exp7    | 124.20±9.01**                       | 7.28±0.84**      | 88.43±4.65        |
| Exp8    | 119.60±6.88**                       | 7.57±0.48**      | 90.78±2.46*       |

Data are represented as the mean ± SD. \* indicates statistically significant difference as compared to the control. \* $p<0.05$ ; and \*\* $p<0.01$ .

Therefore, brewers' dried grain was useful as a feed supplement for rearing *A. dichotoma*.

*A. dichotoma* has a life cycle of about one year. It is necessary to develop economical rearing techniques for efficient production of this beetle. Many environmental parameters such as temperature, humidity, and photoperiod are major influencing factors while rearing insects. Most farms rearing insects have controlled temperature to regulate the insect productions. However, there was some evidence that food can influence physiological traits [13, 20, 22]. Accordingly, many studies need to be performed focusing on formulating feeds for this beetle and its effects on their life cycle.

Substantial amount of food by-products present environmental and economic problems [1] but have high nutritional components including proteins, fibers, vitamins, and minerals [9]. Most of the by-products are inexpensive and easy to obtain, and have been used as a source of fuel or animal feed [1, 27]. However, by-products were mainly used as a source of feeds during livestock production.

As the interest in edible insects increased, more and more farms were massively rearing them. The larvae of *A. dichotoma* were registered as a general food ingredient by the Korean Ministry of Food and Drug Safety. Eventually researches for mass rearing technique and nutritious feed sources were needed. The application of food by-products could lead to economic benefits for insect farms.

While analyzing the effects of feeds with four kinds of by-products including citrus peel, soybean curd cake, soybean oil meal, and brewers' dried grain, brewers' dried grain showed improvement in growth of *A. dichotoma* larvae. Given the assumption of adverse effect of soybean-derived by-products as feed additives, adding soybean curd cake and soybean oil meal have been negative factors for rearing larvae of the beetle. Therefore, brewers' dried grains have potential to be used as a source of feeds for *A. dichotoma* by reducing the rearing costs and promoting insect quality and production.

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## 초록 : 사료첨가제로서 맥주박이 장수풍뎅이 유충의 생육에 미치는 영향

송명하 · 이희삼 · 박관호\*

(농촌진흥청 국립농업과학원 곤충산업과)

장수풍뎅이 유충은 쌍별귀뚜라미와 갈색거저리 및 흰점박이꽃무지 유충과 함께 국내 식품의약품안전처의 식품 공전에 일반식품원료로 등록되었다. 이에 곤충에 대한 종합적인 관리 시스템 개발이 요구되고, 이와 함께 양질의 곤충을 대량생산하기 위한 위생적이고 안전한 식용곤충 전용 먹이원 개발 등과 관련된 연구를 지속적으로 수행할 필요가 있다. 식품가공 부산물은 단백질, 지질, 무기질 및 기타 여러 가지 생리활성 물질을 함유하고 있어 가축 등 동물 사료의 대체 에너지원으로 활용되고 있다. 따라서 본 연구에서는 감귤박, 비지박, 대두박, 맥주박 등 4종의 식품가공 부산물을 첨가한 먹이원 급여에 따른 장수풍뎅이 유충에 미치는 효과를 비교하였다. 장수풍뎅이 3령 유충에 10% 맥주박을 첨가한 먹이원을 급여했을 때 가장 높은 생존율을 보였고, 유충의 무게 또한 대조구 대비 약 26% 증가된 것으로 나타났다. 유충기간에 있어서도 10% 맥주박을 첨가했을 때 약 120일로 대조구보다 28일 이상 단축되는 것을 확인하였다. 뿐만 아니라 10% 맥주박 첨가 실험구의 용무게와 용화율이 가장 높게 나타나는 것으로 보아 맥주박이 장수풍뎅이 사육용 사료 소재로서 충분히 활용될 가치가 있을 것으로 생각된다. 더불어 맥주박은 시중에서 쉽고 저렴하게 구매할 수 있어 곤충사육 농가에서 손쉽게 활용할 수 있으며, 식품가공 부산물을 친환경적으로 처리하고 식용곤충을 안전하고 경제적으로 사육하는 데 도움이 될 것으로 기대된다.