การประเมินอัตราการเติบโตและประสิทธิภาพการใช้รำข้าวสาลีร่วมกับใบตำลึงในการเลี้ยงตัวอ่อนด้วง (*ZOPHOBAS ATRATUS* FABRICIUS) (COLEOPTERA: TENEBRIONIDAE)

EVALUATION OF GROWTH RATE AND FEED CONVERSION EFFICIENCY OF DARKLING BEETLE *ZOPHOBAS ATRATUS* FABRICIUS (COLEOPTERA: TENEBRIONIDAE) ON WHEAT (*TRITICUM AESTIVUM*) BRAN AND IVY GOURD (*COCCINIA GRANDIS*) LEAF

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บทคัดย่อ

การเลี้ยงด้วงดำ (*Zophobas atratus* Fabricius) เพื่อใช้เป็นอาหารทางเลือกกำลังเป็นที่ได้รับความสนใจ เนื่องจากมีข้อดีหลายประการ โดยงานวิจัยนี้ได้ทำการศึกษาประสิทธิภาพการใช้อาหาร (ECI) และอัตราการเติบโต (RGR) ของตัวอ่อนด้วงดำที่เลี้ยงด้วยรำข้าวสาลี (*Triticum aestivum*) และใบตำลึงสด (*Coccinia grandis*) ผลการทดลองแสดงให้เห็นว่าตัวอ่อนด้วงดำมีประสิทธิภาพในการใช้รำข้าวสาลีสูงขึ้นเมื่อได้รับใบตำลึงสดเป็นอาหารเสริม อย่างไรก็ตามประสิทธิภาพการใช้รำข้าวสาลีจะต่ำลงเมื่อตัวอ่อนด้วงดำเข้าสู่ระยะสุดท้ายของตัวอ่อน ส่วนการศึกษาอัตราการเติบโตพบว่าตัวอ่อนด้วงดำสามารถเติบโตได้อย่างน้อยร้อยละ 2.6 ต่อวัน ซึ่งสูงกว่าปศุสัตว์ทั่วไป ดังนั้นการเลี้ยงด้วงดำด้วยรำข้าวสาลีและใบตำลึงสด จึงสามารถสร้างรายได้และช่วยประหยัดเวลาด้วย

คำสำคัญ: ด้วงดำ (*Zophobas atratus*) ประสิทธิภาพการใช้อาหาร อัตราการเติบโต ใบตำลึง (*Coccinia grandis*) รำข้าวสาลี (*Triticum aestivum*)

Abstract

Rearing darkling beetle (*Zophobas atratus* Fabricius) larvae as an alternative food source drew attention due to many advantages. In this study, Efficiency of Conversion of Ingested food (ECI) and Relative Growth Rate (RGR) were measured from *Z. atratus* larvae fed on wheat (*Triticum aestivum*) bran and fresh ivy gourd (*Coccinia grandis*) leaves. ECIs of these larvae fed on wheat bran were improved by supplementation of fresh ivy gourd leaves, but ECIs still declined in the late larval instars. RGR of *Z. atratus* larvae was at least 2.6% per day which was faster than conventional livestock. Therefore, rearing *Z. atratus* with wheat bran and fresh ivy gourd leaves was profitable and time saving.

Keywords: darkling beetle (*Zophobas atratus*), ECI, RGR, ivy gourd (*Coccinia grandis*), wheat (*Triticum aestivum*) bran

Introduction

As a response to the global population rising, insects are brought up as an alternative food source (Huis et al., 2013). Edible insect production is environmentally friendly since it requires lower amount of resources, such as feed, space, and time, to produce the same quantity of food compared to livestock production (Nakagaki & DeFoliart, 1991; Oonincx & de Boer, 2012). It also generates less environmental pollution and even converts organic waste to useful biomass (Oonincx et al., 2010; Veldkamp et al., 2012). In addition, some insects contain higher amount of necessary nutrition (Ramos-Elorduy et al., 1997; Cerritos, 2009; DeFoliart et al., 2009). Therefore, these insects are healthier and more valuable food source.

Several species of darkling beetles are agricultural pests, but the larval forms can be alternative feeds. High biomass conversion efficiency and rapid growth rate are pivotal advantages of rearing darkling beetles as alternative food source (Oonincx & de Boer, 2012). However, their growth is sensitive to various factors. In *Tenebrio molitor*, a well-known species of darkling beetle, its growth and development could be significantly altered by oxygen level, moisture, temperature, population density, and feed nutrition (Loudon, 1988; Weaver & McFarlane, 1990; Greenberg & Ar, 1996; Broekhoven et al., 2015; Oonincx et al., 2015).

Zophobas atratus (Fabricius), another species of darkling beetle used in food production, is favorable for rearing in crowded conditions (Quennedey et al., 1995). They were first found in bat guano, but also well adapted to arid and artificial environment. Although the life history of *Z. atratus* as an insect model was well documented, factors that influence its growth rate were not yet fully understood (Tschinkel, 1981; Quennedey et al., 1995). According to a recent study, the growth rate of *Z. atratus* larvae was significantly depended on diet quality. When fed with protein-rich diet, they grew rapidly and reached the pupal stage in a short period, while starch-rich diet caused slow growth and low survival rates (Broekhoven et al., 2015). Similar trends were also observed in *T. molitor* and *Alphitobius diaperinus*, from the same study.

Although the effect of nutrition on the growth rate was documented in leftover foods, growth rate of *Z. atratus* fed with wheat (*Triticum aestivum*) bran was worthy studied. Since the bran was enriched with fibers, it was difficult to digest and halted nutrients absorption in the larvae. This could cause food constrain in larvae, so their adaptation to low quality food can be studied. Larvae of *Z. atratus* were also reported relying more on carrot when they were fed with imbalance diet (Broekhoven et al., 2015). This result suggested that the necessity of fresh vegetables in raising larvae was also another interesting subject. For *T. molitor*, availability of carrot in their diet also improved their food-to-biomass conversion in imbalance diet (Oonincx et al., 2015). Fresh and young leaves of ivy gourd were good representatives of fresh vegetables for three reasons. First of all, nutrition of fresh leaf of ivy gourd was lower than both wheat bran and other possible fresh vegetables such as carrot. Therefore, the amount of leaf consumption would not be due to nutritional compensation. Second, the secondary compound and toughness of fresh ivy gourd leaf were low. So, *Z. atratus* larvae could eat as much as they prefer. Finally, the ivy gourd leaf was cheap and free of insecticide. Hence, an investigation of their role in diet could further minimize the cost of *Z. atratus* mass production.

During the diet experiments, the larvae gradually shifted their consumption pattern. Two possible explanations were the leaf-to-larval ratio and the larval ages. An additional experiment was set up to compare larval consumption pattern by control the ratio of wheat bran and fresh ivy gourd leaf in feed for the entire growth period.

Moreover, the comparison between experiments with different ratio could be used to understand the necessity of fresh vegetable for rearing with low quality feeds. The understanding of *Z. atratus* growth rate on wheat bran and fresh ivy gourd would allow the economic evaluation of *Z. atratus* as a potential alternative food source for human.

Materials and methods

Experimental setup and dried weights estimation

Larvae of *Z. atratus* obtained from insectaria were reared with wheat bran and carrot at 25 °C with 12:12 light and dark cycle. Wheat bran had been prepared by incubating at approximately 90 °C for at least 15 min to wipe out pests and fungi that contaminated the bran and was kept in sealed container. Young leaves at the top 30 cm. of ivy gourd (*Coccinia grandis*) were collected, measured the weight and given to larvae as soon as possible. Dried weight and percentage of water in larvae, wheat bran, ivy gourd leaves, and feces were measured by weighting their fresh and dried weights, after being incubated at 65 °C for two days. The measurement was repeated for at least five times, and percentages of dried weights and water content were calculated by the following formulas:

$$\text{Percent of dried weight} = \frac{\text{dried weight}}{\text{fresh weight}} \times 100\%$$

$$\text{Percent of water} = 100\% - \text{Percent of dried weight}$$

Experiments

Experiment I (Effect of fresh ivy gourd leaves on larval growth): Two groups (called A and B) of larvae with weight of 30 to 50 mg per larva, assuming that they were sixth instar larvae (Quennedey et al., 1995), were reared with population of 56 to 62 individuals in cylindrical containers with 9.4 cm² cross section area. The feeds for both groups were alternating between fresh ivy gourd leaves and wheat bran every three days. The leaves were supplied at about 1 g to group A and 1.5 g to group B per feeding cycle, while the wheat bran was provided at 60% of the larval weight in each group. At the end of each three-day period, the leftover from each group was removed and measured its fresh and dried weight. The total larval masses of each group were also measured after removing the leftover. The experiment continued for 66 days to observe the long term effect of fresh leaves in the diets.

The values of ECI, RGR, and FCR were calculated and recorded for both groups every three days to compare their growth rate. In addition, the RGR was also calculated in a six-day interval to cover the whole wheat bran-fresh leaves cycle.

Experiment II (Growth rate at different instar): To improve our insight of larval age effect on the growth rate, fifth instar (group C) and seventh instar (group D) larvae were reared in identical containers with population of 31 to 33 individuals respectively. They were weekly supplied with wheat bran and fresh ivy gourd leaves at 120% and 50% of larval weight, respectively. Overall weight in each group was measured weekly for 8 weeks, while individual weight was assessed at the beginning and the end of the experiment.

Analyses

Parameters used for estimating larval growth consisted of Efficiency of Conversion of Ingested food (ECI; Waldbauer, 1968) as calculated by formula

$$\mathrm{ECI} = \frac{\text{dried weight gained by larvae}}{\text{dried weight ingested food}} \times 100\%$$

and Relative Growth Rate (RGR; Tammaru et al., 2004) of larvae calculated by formula

$$\mathrm{RGR} = \frac{\mathrm{Ln}(\text{final weight}) - \mathrm{Ln}(\text{initial weight})}{\text{time}}$$

However, since most of weight gained in leaf consuming was contributed by water, it is more suitable to expressed in term of Feed Conversion Ratio (FCR; Broekhoven et al., 2015) instead of ECI as following

$$\mathrm{FCR} = \frac{\text{weight of ingested food}}{\text{weight gained by larvae}}$$

Data and calculation results were assorted in Microsoft Excel. Comparisons between groups were computed by t-test or Wilcoxon rank sum test in STATISTIX 8 (Analytical Software, 2003).

Results

Proportions of dried weight to fresh weight were extremely different between wheat bran and fresh leaf (Table 1). To some extent, feces from consuming different diet also contained different amount of biomass (Wilcoxon rank sum test, $p = 0.05$).

In experiment I, the initial larval weights of both groups, A and B, were approximately equal (Table 2). However, group B larvae were significantly heavier than group A's at the end of the experiment. During feeding on wheat bran, larvae from both groups equally converted wheat bran biomass into theirs and had similar RGRs. In addition, both groups of larvae had similar FCR from feeding on leaf, but larvae in group B significantly had higher RGRs than group A larvae ($p < 0.05$). In summary of both wheat bran and leaf, larvae from group B significant had higher RGRs than group A larvae ($p < 0.05$).

Table 1 Summary of dried masses and water contents in percentages

	Numbers of repeat	Dried mass (%)	Water content (%)
Wheat bran	5	94.8±0.41	5.2±0.41
Leaf of ivy gourd	6	10.9±1.06	89.1±1.06
Larva	5	38.4±2.00	61.6±2.00
Feces*	22	89.6±3.71	10.4±3.71
Feces**	22	85.2±19.7	14.8±19.7

Remark * = from larvae fed with wheat bran, ** = from larvae fed with fresh leaf

Table 2 Comparison of larval growth parameters from two groups (A and B) of larvae rearing by different amount of leaf. Values were presented by mean ± SD, while comparisons were calculated by two samples t-test and significances were presented in the *p*-value

Group	A	B	*p*-value
Initial larval weight (mg)	44.8±12.2	40.5±13.1	0.0690
Final larval weight (mg)	401±99.1	522±129	<0.0001
ECI of wheat bran (%)	5.06±1.82	5.41±1.31	0.6516
FCR of leaf	1.83±0.85	1.56±0.71	0.4286
RGR of wheat bran (%)	2.37±0.79	2.62±0.63	0.4095
RGR of leaf (%)	2.84±0.99	4.04±1.39	0.0303
RGR of wheat bran and leaf (%)	2.60±0.62	3.33±0.81	0.0278

In experiment II, ECIs were quite stable in group C, within a range of 10.3 to 16.1%, while ECIs in group D was greater than 10% during the first period, but dropped under 10% afterward (Figure 1). Average larval growth in group C was fitted to exponential curve, while growth of group D larvae was less fit to exponential curve (Figure 2).

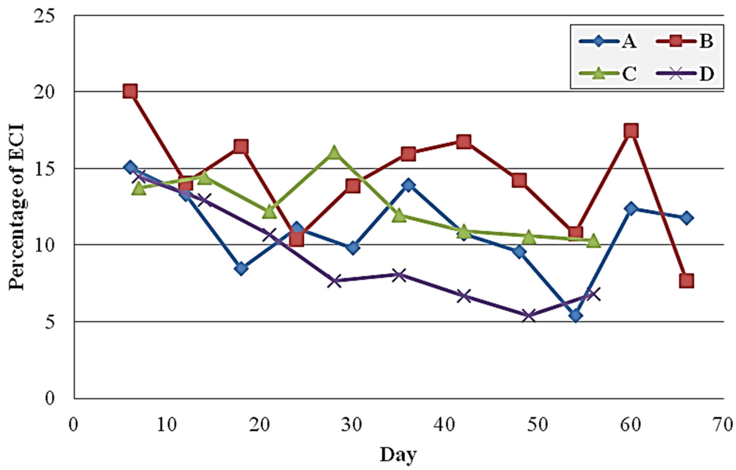

Figure 1 Combined ECIs of bran and leaf in all groups (A, B, C and D) according to experimental times.

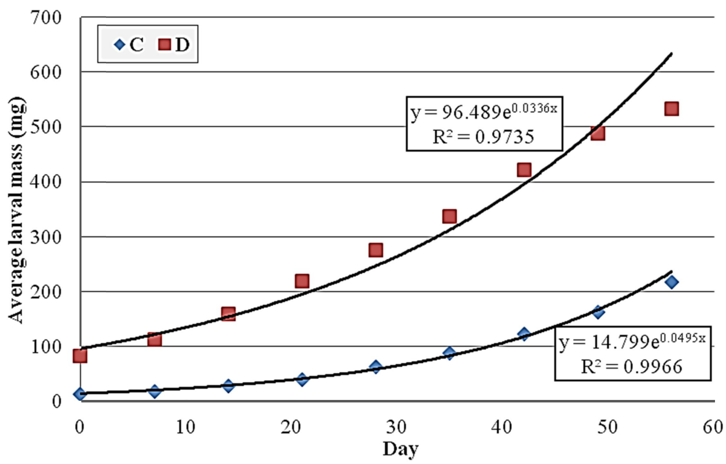

Figure 2 Average larval weights in groups C and D were measured weekly and compared to exponential curves predicted from data; formulae and coefficient of determinations were presented above the curves.

Discussions

Despite the fact that these larvae relied mainly on wheat bran, percentage of water in bran was much less than in the larvae. It was less than 6% in wheat bran while there was about 60% in larvae (Table 1). Moreover, the larvae were also lost water with ejected feces, as water percentage in feces from digested wheat bran was significantly greater than in undigested wheat bran ($p < 0.01$). On the other hand, larvae of *Z. atratus* seemed to receive water mainly from ivy gourd leaves since water content in digested ivy gourd leaf was much less than the fresh leaves (Table 1). Regardless of possible vitamins and minerals, water seems to be the major mineral provided by leaf of ivy gourd and other vegetables. Therefore, the lack of water in bran or other feed could be a factor that induced larvae to feed more on vegetables.

Although larvae in both groups, A and B converted approximately equal amount of bran and leaf to their masses, group B larvae were heavier at the end of the experiment as more leaf was provided. In addition, the graph of larval ECIs on overall feed indicated that larval biomass conversion performance was gradually declined throughout the experiment (Figure 1). The decline of ECIs could be due to smaller portion of leaf was consisted in their diet as they grew larger. Another possible reason was the decrease in insect growth rate in late instars.

The hypotheses were proved by experiment II. As leaf was given to larvae at the same proportion, ECIs in young larvae (group C) were relatively constant, indicated the necessity of leaf in their biomass conversion (Figure 1). In other studies, larvae of *Z. atratus* that fed on high starch diet also consumed more carrot (Broekhoven et al., 2015), while larvae of *T. molitor* showed higher ECIs on identical diet when carrot was available (Oonincx et al., 2015), suggesting consuming vegetation assist in digestion. On the other hand, older larvae (group D) achieved less than10% ECIs in most part of experiment which means that more fresh leaf and bran were leftover as they grew older. Low ECIs in group D were likely because of declination of larval growth rate as shown in Figure 2. RGRs in smaller larvae were about 4 to 5%, while there were 2 to 3% in larger larvae. Average growths of group C and D were similar to the early work on this species (Quennedey et al., 1995). Larvae took about 50 to 60 days to grow from newly hatched larvae to reach 200 mg in weight, and took another 30 days to make another 400 mg. Therefore, the decline of ECIs in late instars was likely due to change in their growth rate.

Compare to conventional livestock, *Z. atratus* is a potential species in term of food production since its RGR could be as much as 5.8% in small instars when sufficient leaf was fed, while their average RGR was at least 2.6% per day in groups A, B, and D. By applying calculation based on ECI, larvae of *Z. atratus* were estimated to grow up 1 kg, when 16.55 kg of wheat bran and 2.6 kg of leaf of ivy gourd were provided. High amount of wheat bran requirement is likely due to its poor nutrition, containing high fibers (40% of dried weight) and low protein (16%) (Ranhotra et al., 1990; Noort et al., 2010). In high protein diet (39.1%), only 3 kg of feed was needed to gain 1 kg of larval weight. They would take less than 80 days to reach 700 mg larvae (Broekhoven et al., 2015). In addition, rearing *Z. atratus* even in poor nutritious wheat bran could be more efficient than beef or swine (Lebret, 2008; Huis, 2013).

Despite of being a drought tolerate insect, *Z. atratus* seems to require vegetables to compensate water loss during feeding on dried substance, such as bran and human leftover. Moreover, vegetables may even play a role in digestion and biomass conversion, as ECIs on bran were improved as more leaf was given to larvae. On the other hand, ECIs declined as larvae grew older, implying alternation in their physiology. *Z. atratus* was a higher potential food source compare to livestock, since it achieves higher ECI compare to livestock.

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References

Analytical Software. *STATISTIX 8 for Windows*, Tallahase, FL, USA; 2003.

Broekhoven S van, Oonincx DGAB, Huis A van. et al. Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. *Journal of Insect Physiology*. 2015; 73: 1-10.

Cerritos R. Insects as food: an ecological, social and economical approach. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. Wallingford, UK: CABI; 2009: 1-10.

DeFoliart G, Dunkel FV, Gracer D. *The Food Insects Newsletter Chronicle of Changing Culture*. Salt Lake City: Aardvark Global Publishing; 2009.

Greenberg S, Ar A. Effects of chronic hypoxia, normoxia and hyperoxia on larval development in the beetle *Tenebrio molitor*. *Journal of Insect Physiology*. 1996; 42: 991-996.

Huis A van. Potential of insects as food and feed in assuring food security. *Annual review of Entomology*. 2013; 53: 563-583.

Huis A van, Itterbeeck J van, Klunder H. et al. *Edible Insects Future Prospects for Food and Feed Security*. Rome: Food and agriculture organisation of the United Nations; 2013.

Lebret B. Effect of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal*. 2008; 2: 1548-1558.

Loudon C. Development of *Tenebrio molitor* in low oxygen levels. *Journal of Insect Physiology*, 1988; 34: 97-103.

Nakagaki BJ, DeFoliart GR. Comparison of diets for mass-rearing *Acheta domesticus* (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *Journal of Economical Entomology*. 1991; 84: 891-896.

Noort MWJ, Haaster D van, Hemery Y. et al. The effect of particle size of wheat bran fractions on bread quality – evidence for fiber-protein interactions. *Journal of Cereal Science*. 2010; 52: 59-64.

Oonincx DGAB, de Boer IJM. Environmental impact of the production of mealworms as a protein source for humans - a life cycle assessment. *PLoS ONE*. 2012; 7: e51145.

Oonincx DGAB, Broekhoven S van, Huis A van. et al. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-product. *PLoS ONE*. 2015; 10: e0144601.

Oonincx DGAB, Itterbeeck J van, Heetkamp MJW. et al. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE*. 2010; 5: e14445.

Quennedey A, Aribi N, Everaets C. et al. Postembryonic development of *Zophobas atratus* Fab. (Coleoptera: Tenebrionidae) under crowded or isolated conditions and effects of juvenile hormone analogue applications. *Journal of Insect Physiology*. 1995; 41: 143-152.

Ramos-Elorduy J, Moreno JMP, Prado EE. et al. Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis*. 1997; 10: 142-157.

Ranhotra GS, Gelroth JA, Astroth K. et al. Distribution of total and soluble fiber in various millstreams of wheat. *Journal of Food Science*. 1990; 55: 1349-1351.

Tammaru T, Nylin S, Ruohomäki K. et al. Compensatory responses in lepidopteran larvae: a test of growth rate maximization. *Oikos*. 2004; 107: 352-362.

Tschinkel WR. Larval dispersal and cannibalism in a natural population of *Zophobas atratus* (Coleoptera: Tenebrionidae). *Animal Behaviour*. 1981; 29: 990–996.

Veldkamp T, Duinkerken G van, Huis A van. et al. *Insects as a sustainable feed ingredient in pig and poultry diets: a feasibility study*. Wageningen Netherlands: Wageningen UR Livestock Research; 2012.

Waldbauer GP. The consumption and utilization of food by insects. *Advances in Insect Physiology*. 1968; 5: 229-288.

Weaver DK, McFarlane JE. The effect of larval density on growth and development of *Tenebrio molitor*. *Journal of Insect Physiology*. 1990; 36: 531-536.