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Original Article

Growth response to population density in larval stage of darkling beetles (Coleoptera; Tenebrionidae) *Tenebrio molitor* and *Zophobas atratus*

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ABSTRACT

Insect farming efficiency is deterred by the complications associated with a high population density, such as competition and stress. Darkling beetles are farmable insect candidates as they are well adapted to survive at a high larval density. This study tested the effect on productivity of the larval density in two species of darkling beetle, *Tenebrio molitor* Linn. and *Zophobas atratus* Fab. Larval weight gain was measured by feeding larvae with wheat (*Triticum aestivum* Linn.) bran and leaves of ivy gourd (*Coccinia grandis* (L.) Voigt.). Larvae were also abstained to test weight loss at different larval densities. The results showed similar weight gains in both species ($p = 0.7858$). During abstaining periods, *Z. atratus* had significantly higher weight loss ($p < 0.001$), and *Z. atratus* reared at a higher larval density also had a higher weight loss ($p < 0.001$). Per capita weights at different densities were not significantly different in either species (*T. molitor* $p = 0.749$; *Z. atratus* $p = 0.841$). The lower survival rate in *Z. atratus* was assumed to be due to cannibalism which was independent of population density. *T. molitor* seemed to be more suitable for farming at high larval density because it had not only less weight loss during the abstaining period, but also a higher survival rate.

Introduction

The increasing food demand due to an increasing human population has led to edible insects being considered as a potential future diet because they can be efficiently produced (Van Huis, 2013). Various insects already form a part of diets, such as crickets (Orthoptera), grubs and beetles (Coleoptera), caterpillars (Lepidoptera), as well as ant and bees (Hymenoptera) (Ramos-Elorduy et al., 1998). Insects provide relatively higher proportions of protein, unsaturated fatty acid and essential minerals than more conventional animal or plant sources (Banjo et al., 2006; Finke, 2002; Yang et al., 2014). Secondly, insects convert feed to their biomass more efficiently than conventional livestock. A kilogram of pork, for instance, requires approximately twice the feed needed to produce 1 kg of mealworm (Oonincx and de Boer, 2012; Oonincx et al., 2015; Smil, 2002). In addition, insects are estimated to use less water, energy, and land for production (Nakagaki and DeFoliart, 1991; Oonincx and de Boer, 2012; Van Huis et al., 2013).

Complications emerge when insects are farmed at a larger scale, as a high population density leads to a lower per capita yield. For example, Orlando strain housefly, *Musca domestica* Linn., reared at a higher population density yielded relatively lighter dried pupal weight (Sullivan and Sokal, 1963). Mosquito, *Aedes aegypti* Linn., reared at a higher population density was also reported to have a slower larval developing rate as well as a reduction in pupal weight (Barbosa et al., 1972). Larval growth in *Tenebrio molitor* Linn. was also negatively influenced by larval density, presumably due to lower feeding opportunity due to increased competition (Morales-Ramos and Rojas, 2015). In addition, contagious diseases can spread more easily as the population density increases (Steinhaus, 1958). Hence, insect farming should consider utilizing a species or strain that can tolerate a high population density.

Darkling beetles, such as *T. molitor* and *Zophobas atratus* Fab., are candidates for large-scale farming because of several advantages. First, both insects are reported to have faster growth when reared at a high population density (Quennedey et al., 1995; Weaver and McFarlane, 1990). Second, they are well-adapted to a drought environment; *T. molitor*, for example, has developed a mechanism to collect water from the atmosphere (Machin, 1975). In addition, *T. molitor* is well-adapted to conditions of high larval density, as it is

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capable of boosting its immunity under crowded population conditions to prevent infection (Barnes and Siva-Jothy, 2000). However, the relationship between population density and total biomass conversion is a new perspective for insect farming that could lead to efficient land use and predictable productivity. Hence, larvae of both species were reared at different larval densities to record their responses and overall biomass production.

From a conventional livestock perspective, animal welfare is important, consisting of freedom from hunger, thirst, and stress, if not only in a moral sense, but also for improved quality and productivity (Blokhuis et al., 2003; Brambell, 1965). Hence, the response of darkling beetles during starvation was studied by measuring weight loss as another welfare parameter. In addition, the survival rate was also investigated, since both darkling beetles species were reported to have higher cannibalism as population density increased (Ichikawa and Kurauchi, 2009).

Materials and methods

Larvae of both species were initially reared in insectaria (Faculty of Science, Mahidol University, Bangkok, Thailand). Insects were fed with wheat bran and carrot at 25 °C, 70–80% relative humidity and a 12:12 h light-to-dark cycle. Twelfth-to-thirteenth instars of *T. molitor* were individually weighed and randomly divided into two groups at initial densities of 7.38 and 14.0 individuals per cm^2 (TmLD and TmHD, respectively). Sixth-to-seventh instars larvae of *Z. atratus* were also reared at densities of 2.1 and 6.2 individuals per cm^2 (ZaLD and ZaHD, respectively). The low population density conditions were based on the maximum larval density reported in other studies (Morales-Ramos and Rojas, 2015; Quennedey et al., 1995). Because larval tolerance needed to be tested at even higher population densities, the population densities were increased about two-to-threefold of the low population densities. All groups were reared in identical containers, which were covered with ventilation mesh. Wheat (*Triticum aestivum* Linn.) bran was heat sterilized before being fed to larvae. Fresh leaves of ivy gourd (*Coccinia grandis* (L.) Voigt.) were given to larvae as a water source. Every larval group was alternatively switched between “feeding” and “abstaining” periods, each lasting 3 d. During the feeding period, bran and leaves were given at 60% and 50% of larval weight, respectively. Larvae were left in the empty container during an abstaining period. Larvae were counted and weighed at the end of each period. The experiment terminated after five successive repeats in *T. molitor* because the results were relatively constant (Table 1). Experiments in *Z. atratus* were extended to eight cycles as variation among repeats was high and it was necessary to study the effect of larval densities on survival rate.

Measurements of larvae weight change were carried out in terms of the relative growth rate (RGR; Tammaru et al., 2004), during a feeding period according to Eq. (1):

$$\text{RGR} = \left[\text{Ln}\left(\text{w}_\text{f}\right) - \text{Ln}(\text{w}_\text{i})\right] \Big/ \text{t} \tag{1}$$

where w_f is the final weight and w_i is the initial weight after time t.

During an abstaining period, RGR was modified to compensate for the weight decrease and re-termed as the relative weight loss (RWL) according to Eq. (2):

$$\text{RWL} = -\left[\text{Ln}\left(\text{w}_\text{f}\right) - \text{Ln}(\text{w}_\text{i})\right] \Big/ \text{t} \tag{2}$$

The growth rate was estimated from the overall weight change using an exponential model, as described to be the best fit (Von Bertalanffy, 1951) according to Eq. (3):

$$\text{w}_\text{t} = \text{w}_\text{i}\text{e}^{\text{rt}} \tag{3}$$

where r is the growth rate, t is the time, w_i is the initial weight and w_f is the final weight.

The RGR, RWL, growth rate, and mortality rate were calculated using Microsoft Excel (Version 2007; Microsoft; Redmond, WA, USA). All statistic analyses were computed using the STATISTIX 8 software package (Analytical Software, 2003). Differences in the RGR and RWL were tested using one-way analysis of variance, the least significant difference (LSD) test and a two-sample t test. The mortality rate in *Z. atratus* at different periods was compared using a chi square test.

Results

There were no significant differences between the per capita weights within each species, at the beginning of experiment (*T. molitor*: $t = 1.71$, $df = 204$, $p = 0.089$; *Z. atratus*: $t = 0.924$, $df = 78$, $p = 0.358$) till the end (*T. molitor*: $t = 0.321$, $df = 204$, $p = 0.749$; *Z. atratus*: $t = 0.202$, $df = 41$, $p = 0.841$). Average RGRs were relatively higher in *T. molitor*, but they were not different between species nor larval densities (Table 1) ($F = 0.36$, $df = 3, 18$, $p = 0.7858$). The RWL was significantly higher in *Z. atratus* than *T. molitor* ($t = -10.39$, $df = 21.7$, $p < 0.001$). Changes in the population density resulted in significant differences in RWLs ($F = 57.10$, $df = 3, 22$, $p < 0.001$). However, there were no significant differences in the RWL in *T. molitor* (LSD test, $p > 0.05$). Significant differences in *Z. atratus* occurred between ZaLD and ZaHD (LSD test, $p < 0.05$) (Table 1).

The overall growth rate was influenced by both factors of species and population density (Table 1, Fig. 1). Growth rates in *T. molitor*

Table 1

Summary of experimental data: Averages per capita weight and growth rate.

Parameter	*Tenebrio molitor*		*Zophobas atratus*	
	TmLD	TmHD	ZaLD	ZaHD
Initial				
Per capita weight* (g)	40.2 ± 11.9	37.1 ± 12.6	40.3 ± 10.4	43.0 ± 11.6
Population density (individuals per cm^2)	7.38	14.04	2.08	6.24
Final				
Per capita weight* (g)	77.4 ± 23.9	78.5 ± 23.1	175.3 ± 49.7	172.0 ± 45.7
Population density (individuals per cm^2)	7.38	14.04	1.14	3.33
RGR (% g/d)*	6.059 ± 2.801	7.022 ± 3.065	5.709 ± 1.809	5.63 ± 2.061
RWL (% g/d)*	0.865 ± 0.126^{c}	0.764 ± 0.128^{c}	1.512 ± 0.153^{b}	1.969 ± 0.266^{a}
Growth rate (g/d)	00253	0.0306	0.0229	0.018

TmLD = *Tenebrio molitor* at low density; TmHD = *Tenebrio molitor* at high density; ZaLD = *Zophobas atratus* at low density; ZaHD = *Zophobas atratus* at high density; RGR = relative growth rate; RWL = relative weight loss.

* = Values are presented as mean ± SD.

$^{a, b, c}$ = different superscript letters between columns indicate significant differences ($p < 0.05$) using the least significant difference test.

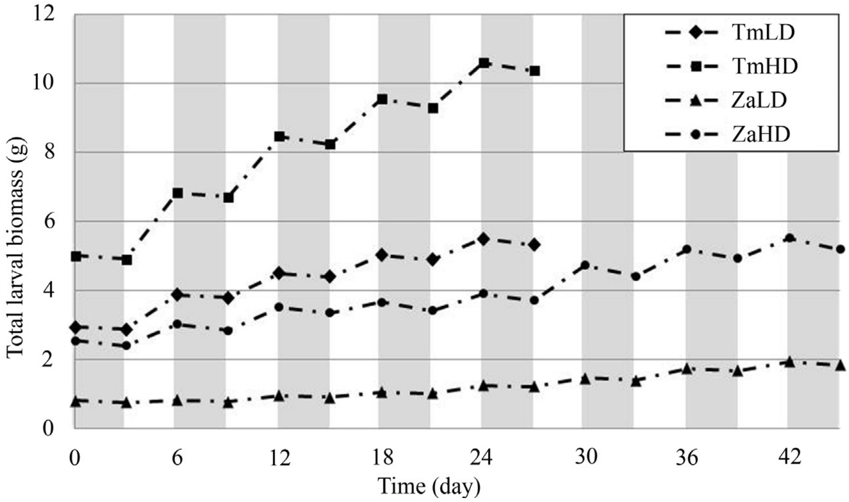

Fig. 1. Total larvae mass in 3-day intervals for two species: Tm (*T. molitor*) and Za (*Z. atratus*) under two conditions: LD (low density), and HD (high density), where gray and white areas represent abstaining and feeding periods, respectively, in each experimental cycle.

were relatively higher and the higher larval density seemed to have a higher growth rate (Table 1). However, in *Z. atratus*, the overall growth rate at low larval density was higher. The survival rates of *T. molitor* were 100% in both groups. Populations of *Z. atratus* gradually decreased to about half at the end of the experiment, regardless of the density (Fig. 2). The numbers of *Z. atratus* decreased differently with different population densities ($\chi^2 = 6.88$, $df = 1$, $p < 0.01$). In ZaLD, the survival rate during a feeding period was lower than during an abstaining period, while survival rates were equal during both feeding and abstaining periods in ZaHD.

Discussion

RGRs of 6–7% in both species indicated their potential as a farmable product compared to conventional livestock which has been reported to have an RGR of 2% per day (Ittner and Hughes, 1938; Kesbi and Tari, 2014). Despite documentation of lower growth rates when larvae of both species were reared at a low population density (Quennedey et al., 1995; Weaver and McFarlane, 1990), the RGRs were not influenced by population density, at least in the studied population densities. The RGR in all groups was relatively lower than that reported on a diet of human leftovers (Van Broekhoven et al., 2015), which suggested that wheat bran and leaf were not the ideal diet for rearing both species.

The RWLs in *T. molitor* were relatively lower than in *Z. atratus*, which could be explained by the relatively slower growth rate in *Z. atratus*, even though the RGRs were not different (Table 1). Metabolism was expected to be the main cause of weight loss, since *T. molitor* could regain water from the air in this study environment (Machin, 1975), and many Tenebrionids are well-adapted to preserve water (Ahearn, 1970). In addition, the RWL in ZaHD was significantly higher than in ZaLD leading to a deduction of more energy being required for living in a higher population density. *Z. atratus* probably was more active at the higher population density to avoid being targeted by cannibalism, since being more active at the pupal stage also increased the survival rate (Ichikawa and Kurauchi, 2009; Tschinkel, 1981). Therefore, *Z. atratus* required more feed to compensate for daily weight loss relative to *T. molitor*. Compared to conventional livestock, the weight loss in insects is negligible, since insects use less energy to maintain body temperature (Lofgreen and Garrett, 1968; Mount, 1968).

The disappearance of *Z. atratus* in sealed containers, with leftovers of small pieces of mandible and legs, leads to an assumption of

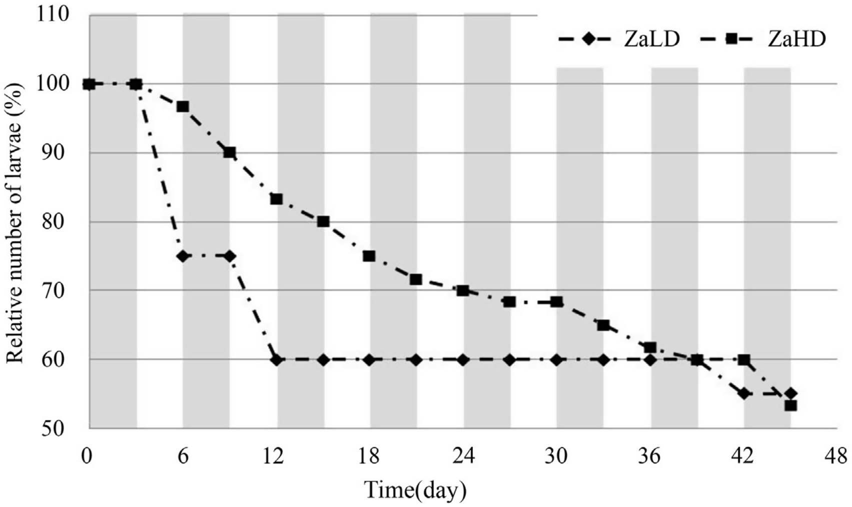

Fig. 2. Relative survival of *Z. atratus* larvae at different densities throughout the experiment, where gray and white areas represent abstaining and feeding periods, respectively, in each experimental cycle.

cannibalism. Although it was expected to be higher as the larval density increased, the results suggested that the rate of cannibalism was independent of the larval density. In *T. molitor*, all larvae survived throughout the experiment, but larvae of *Z. atratus* were gradually lost to cannibalistic behavior. Previous studies of cannibalistic behavior in these species suggested that immobilized individuals were targeted (Ichikawa and Kurauchi, 2009; Tschinkel, 1981). One of the reasons that cannibalism occurs more often in *Z. atratus* could be that they are less active for a longer period during each molting. Therefore, the difference in cannibalistic patterns in ZaLD and ZaHD might also depend on larvae molting behavior instead of food accessibility (Fig. 2). The study supported similar cannibalistic degrees in ZaLD and ZaHD at different population densities.

At the end of the experiment, the per capita weights of larvae were not significantly different within each species, indicating that population density did not influence the individual growth rate. This result was contrary to previous studies in which *T. molitor* were documented as having lower weights in the late larval stage (Weaver and McFarlane, 1990), and the growth performance of *Z. atratus* was also linked with population density (Quennedey et al., 1995). The conflicting results could have been due to the previous studies being conducted under very extreme conditions, while the current study was more practical and within a smaller range of conditions. However, all studies agreed that larval growth is more influenced by some other factors, such as larval stage, feed nutrition and humidity (Greenberg and Ar, 1996; Morales-Ramos and Rojas, 2015; Quennedey et al., 1995; Van Broekhoven et al., 2015).

Although both species had similar biomass conversion rates (RGRs), the RWLs in *T. molitor* were less compared to *Z. atratus* (Table 1). In addition, cannibalism in *Z. atratus* reduced the growth rate (Fig. 1, Table 1) and led to a reduction in the feed-to-weight conversion. Therefore, from both production and economic efficiency perspectives, *T. molitor* is more suitable for farming.

Conflict of interest

The authors declare that there are conflicts of interests.

Acknowledgments

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