

Growth performance of the edible mealworm species, *Tenebrio molitor* (Coleoptera: Tenebrionidae) on diets composed of brewer's yeast

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Abstract

Yellow mealworms (*Tenebrio molitor* Linnaeus) are very promising insects for the food and feed industry. Because mealworms are in the spotlight as an alternative protein source in the future, it is necessary to develop efficient rearing techniques for mass production. To evaluate the effects of brewer's yeast (BY) on the growth of mealworms, *Tenebrio molitor* Linnaeus, the mealworms were fed with wheat bran (WB) diets containing different levels of BY (0, 10, 30, 50, and 70%). Larval survival, larval weight, development time, pupal weight and eclosion rate were monitored for 12 weeks. The results showed that mealworms fed on the diets containing 30% and 50% of BY have significantly higher weight gain, specific growth rate and daily weight gain, and lower larval duration than fed the control diet (100% WB) and other BY diets (10% and 70% BY). Larval survival on the diets containing 30% and 50% of BY was higher than on control diet. Pupal weight and eclosion rate were not significantly different among all diets. In conclusion, we suggest feeding the diet containing 30% of brewer's yeast with wheat bran in order to increase the production of mealworms.

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Int. J. Indust. Entomol. 39(2), 54-59 (2019)

Received : 5 Nov 2019
Revised : 4 Dec 2019
Accepted : 6 Dec 2019

Keywords:

Tenebrio molitor,
yellow mealworm,
larvae,
brewer's yeast.

Introduction

Yellow mealworms, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), are very promising insects for the insect food and feed industry (Van Huis and Tomberlin, 2017). They have interesting characteristics in terms of nutritional composition, are easier to rear than many other insect species and their safety aspects are also well studied (Van Huis and Tomberlin, 2017). Additionally, they are very well adapted to grow in a dry environment as compared to other insects for which higher water inputs are required likely have higher total virtual water content (Koutsos *et al.*, 2019). Furthermore, compared to other animal and plant protein

sources, they require considerably less land (Ooninx and De Boer, 2012; Van Huis, 2013; Koutsos *et al.*, 2019) and emit less greenhouse gases (Ooninx *et al.*, 2010). It makes them a more sustainable and alternative source of animal protein. In Ynsect (Évry, France), *T. molitor* was selected based on a screening of 15 insect species for the most suitable species on the animal feed market (Hubert, 2016). Recently, it has been shown that hospital meals containing mealworms are effective for improving nutritional status of cancer patients after surgery in Korea (dietary calories and protein intake increased 1.5 times and 1.6 times, respectively, Kim *et al.*, 2016a). Therefore, because the demand for mealworms as a dietary supplement is expected to increase in the near future,

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it is important to develop efficient rearing techniques for mass production. Optimization of mealworm production is necessary to make it more cost competitive to more established protein sources.

The nutritional composition of diets can influence insect growth rate and nutritional quality (Anderson, 2000; Davis and Sosulski, 1974). This provides opportunities to improve productivity and modify the nutritional composition of mealworms to better suit the needs of consumers (Van Broekhoven *et al.*, 2015). As one of the common natural yeast products, brewer's yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) are able to improve animal growth by promoting digestion and absorption of nutrients and stimulating immune responses and relieving stress (Jin *et al.*, 2018). Brewer's yeast contains various immunostimulating compounds such as β -glucans, nucleic acids and mannan oligosaccharides (White *et al.*, 2002), and it was shown to be capable of enhancing immune responses (Siwicki *et al.*, 1994; Ortuño *et al.*, 2002), making it an excellent health promoter for fish culture. Besides, due to their richness in crude proteins, vitamins and peptides, yeast was used as a promising candidate for partial replacement of fish meal in aquatic feeds (Guo *et al.*, 2019; Nguyen *et al.*, 2019; Pongpet *et al.*, 2016). Compared to aquaculture, the growth performance of mealworms was partially examined by diets composed of various sources including brewer's yeast (Davis and Sosulski, 1974; Van Broekhoven *et al.*, 2015). Yellow mealworms were also used as an experimental model for insect molecular biology, and brewer's yeast was also used as a protein source when raising experimental insects for molecular biology (Urrejola *et al.* 2011; Lardies *et al.* 2014; Tindwa *et al.* 2015).

Recently, the specific use of brewer's yeast as an alternative protein source has been of interest in both fish (Pongpet *et al.*, 2016) and shrimp (including prawns; Guo *et al.*, 2019; Nguyen *et al.*, 2019). However, the effects of dietary addition of brewer's yeast on growth has still been poor understood in farming edible insects. Even though a number of studies have examined different by-products in mealworm diets (Kim *et al.*, 2016b; 2016c; Kim *et al.*, 2018), to our knowledge no research has been conducted to evaluate the use of these yeast products in mealworm diets. This study aimed to investigate the effects of the partial replacement of wheat bran with brewer's yeast on the growth performance and feed efficiency.

Materials and methods

Experimental Insects

Newly hatched larvae of *Tenebrio molitor* were obtained from the insect rearing facilities of the National Institute of Agricultural Sciences, Wanju, Republic of Korea, for more than twenty-nine generations.

Diet preparation

Tenebrio molitor larvae were fed 100% wheat bran (WB) on the standard rearing protocols. To test the effects of brewer's yeast (BY) on the growth performance of *T. molitor* larvae, different amounts of BY were mixed with WB (diet abbreviations: Control =100% WB; BY10 = 10% BY + 90% WB; BY30 = 30% BY + 70% WB; BY50 = 50% BY + 50% WB; BY70 = 70% BY + 30% WB). WB and BY were purchased from Hankook Woorimil-Nonghyup (Gwangju, Korea) and Dasomia, Inc (Daejeon, Korea), respectively.

Growth trial on the different diets

150 individuals with 1st instar were collected from the laboratory colony. For the five different diets, 30 individual larvae were isolated in a Petri-dish (35 × 10 mm). After five weeks, each larva was moved to a medium-sized Petri-dish (60 × 10 mm). Laval development was monitored daily and larval weights were measured weekly. During the experiment, insects were maintained in a climate chamber (26°C, 65% relative humidity, a 9:15 h light:dark photoperiod). In addition, each of diets and fresh Chinese cabbage were provided every three days.

Statistical analysis

Statistical analyses were performed using SPSS 25.0 software. Significant differences between samples were analyzed by one-way analysis of variance (ANOVA), and Duncan's tests at a significance level of 0.05.

Results

Larval Survival

Table 1. Larval duration, pupal weight, and pupation rate according to the content of wheat bran (WB) and brewer's yeast (BY) in standard and alternative diets.

Diet	Survival (%)	Weight gain (mg) ¹⁾	SGR ²⁾	Larval duration (days)	Pupal weight (mg)	Eclosion (%)
control	73	93.82 ^b ±35.71	4.48 ^c	76.26 ^c ±5.16	155.64 ±16.49	95
BY10	67	108.87 ^b ±26.90	4.67 ^b	74.67 ^{bc} ±6.35	144.88 ±26.39	100
BY30	87	132.77 ^a ±23.60	4.88 ^a	67.17 ^a ±5.20	147.22 ±19.14	100
BY50	90	135.32 ^a ±33.14	4.89 ^a	68.35 ^a ±6.16	154.69 ±22.34	100
BY70	87	107.23 ^b ±37.47	4.62 ^{bc}	72.46 ^b ±6.80	149.80 ±26.96	96
<i>p</i> Value	0.068	*	*	*	0.482	0.545

Diet abbreviations: control (100% WB); BY10 = 10% BY + 90% WB; BY30 = 30% BY + 70% WB; BY50 = 50% BY + 50% WB; BY70 = 70% BY + 30% WB.

**p* < 0.0001, *p*-value by One-way ANOVA, Values are Mean and Standard Deviation.

^{a,c} different superscript letters are obtained by Tukey's multiple range comparison test at $\alpha=0.05$ level.

¹⁾ Weight gain (mg) = final weight - initial weight. ²⁾ Specific growth rate (SGR) = (ln final weight - ln initial weight) / days.

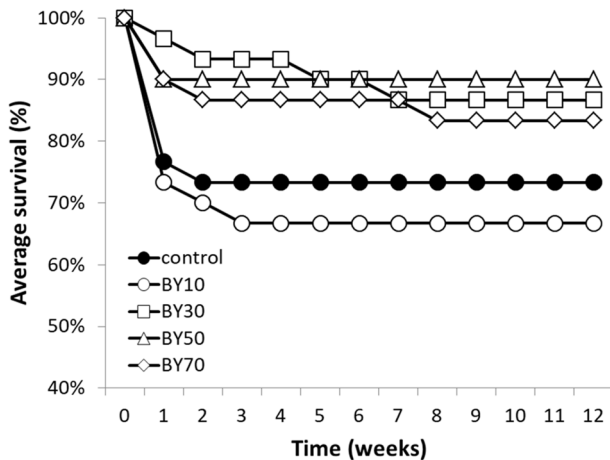


Fig. 1. Average larval survival of *Tenebrio molitor* as percentage of the total number of larvae at week (n=30). *Tenebrio molitor* larval survival on standard or alternative diets with different wheat bran (WB) and brewer's yeast (BY) content. Values are means ± S.D.

The survival of *Tenebrio molitor* larvae grown in the five different feeds was examined until 12 weeks. Although difference of larval survival rate was not statistically significant (*p* = 0.068), the larval survival of *T. molitor* on BY50 was highest among all diets (Table 1, Fig. 1, BY50 > BY30 > BY70 > control > BY10). With the exception of BY10, all diets containing brewer's yeast were higher than the control (Fig. 1). Most of the larval deaths occurred within a week after hatching (Fig. 1). Larvae with BY10 survived only 73% after a week after hatching (Fig. 1).

Larval weight

The larval weight for eight weeks was highest in BY30 and

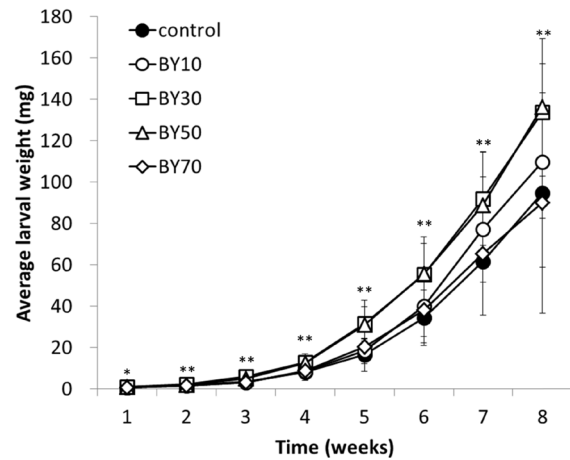


Fig. 2. Average larval weight of *Tenebrio molitor*, determined weekly until the first pupa was observed. Control (wheat bran 100%); BY10 (brewer's yeast 10% + wheat bran 90%); BY30 (brewer's yeast 30% + wheat bran 70%); BY50 (brewer's yeast 50% + wheat bran 50%); BY70 (brewer's yeast 70% + wheat bran 30%), *p*-value by One-way ANOVA (*p* < 0.05 and ** *p* < 0.01)

BY50 among all diets (Fig. 2). The larval weight of mealworms which had been raised with BY 30 was significantly higher than other diets in every week (Fig. 2). Larvae, fed on BY30 and BY50, resulted in higher weight than other diets from third to eighth week (Table 1). In the eighth week, the average weight of the larvae was above 130 mg in both diets (BY30 and BY50). The larval weight of BY10 and BY70 was higher than the control (WB 100%) after the fourth week (Fig. 2). The larval weight measured at the eight week is increasing in the following order: control < BY70 < BY10 < BY30 < BY50 (Table 1).

Development time

The development of larvae grown on BY30 and BY50 differed from that of larvae grown in 100% WB. Diets strongly affected development time ($p < 0.0001$). Developmental duration on feeds BY30 and BY50 was shorter (>69 days) than on control diet (76 days; $p < 0.0001$). Specifically, larvae grown in BY30 had nine days shorter development than larvae grown in 100% WB (Table 1). Furthermore, larvae which had been raised in the BY30 began to pupate at seventh week. In the eight week, pupae were observed under all diets with the exception of control, which was found for the first time in the pupae at the tenth week.

Pupal weight and Eclosion

Although the difference was not statistically significant ($p = 0.482$), the pupal weight of *T. molitor* grown on BY50 was similar to that on a control feed (Table 1, Fig. 3). Pupal weight was higher on feed 100% WB and lower on feed BY10. Eclosion rate was also not significantly different among the groups ($p = 0.545$, Table 1).

Discussion

All diets containing brewer's yeast (BY) presented shorter development time than control (100% of wheat bran). In

particular, the diets containing 30% and 50% of brewer's yeast (BY30 and BY50) significantly shortened larval duration than other diets. Larvae fed on the BY30 and BY50 diets had a shorter larval duration than control by 9.1 and 7.9 days, respectively (Table 1). Furthermore, it was about 5.4 weeks shorter than the development time of larvae fed the diet containing 30% of brewers spent grain and 70% of wheat bran (Kim *et al.*, 2016b), and 5.3 weeks shorter than that of distillers dried grain (DSG) with wheat bran (Kim *et al.*, 2016c). Since the above two studies started from larvae 60 days after hatching, we compared the experimental values which added approximately 8.6 weeks of 60 days (Kim *et al.*, 2016b; 2016c). In terms of annual production, control can supply 4.79 generations a year, while BY30 and BY50 can supply 5.43 and 5.34 generations a year, respectively, in which mealworms could be supplied more than five times a year.

Compared to larval weight for each diet, larvae fed BY30 and BY50 were 38.95 mg and 41.5 mg heavier than the control, respectively, and were statistically supported as a group to gain more weight (Table 1). Furthermore, these two diets (BY30 and BY50) increased body weight at the fastest rate [Table 1, specific growth rate (SGR) = 4.88 and 4.89, respectively] and were statistically supported as the fastest growing group. Brewer's yeast is known as a rich source of proteins. Proteins were shown to be capable of enhancing growth rate of mealworms (Zhang *et al.*, 2019). Although faster growth rates were observed compared to control, it should be concerned about commercially available to rear mealworms using this brewer's yeast after taking the costs of BY30 (210% of wheat bran) into account. On the other hand, the diet containing brewer's yeast can lower labor costs due to faster growth rate. However, a cost-economic analysis should be conducted in the future to evaluate the commercial viability.

In the case of the pupal weight, control was heaviest, but was not statistically supported (Table 1, Fig. 3). Recently, the specific use of brewer's yeast as an alternative protein source has been of interest in both fish (Pongpet *et al.*, 2016) and shrimp (including prawns; Guo *et al.*, 2019; Nguyen *et al.*, 2019). Furthermore, brewer's yeast supplementation could improve growth performance, enhance innate immunity, and ammonia nitrogen resistance of Pacific white shrimp (Jin *et al.*, 2018) and represents a possible substitute for fishmeal in giant freshwater prawn diets (Nguyen *et al.*, 2019).

In conclusion, we found that feeding brewer's yeast with wheat bran (BY30 and BY50) yielded higher weight gains,

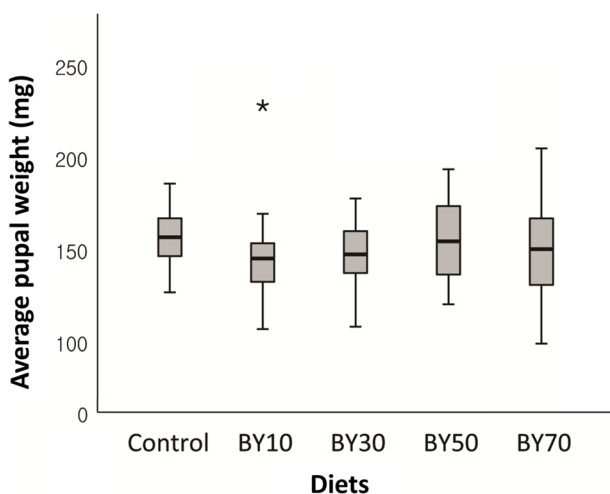


Fig. 3. Boxplot showing pupal weight for *Tenebrio molitor*. The middle line in the box is the median and the bottom and top are the first and third quartiles. The whiskers show minimum and maximum values and asterisks above the whiskers are outliers. Outliers are also included in the analysis.

faster growth, and consequently faster production processes. The diets containing 30% and 50% of brewer's yeast with wheat bran has proven to be able to produce large quantities of mealworms at a faster rate. In this study, we suggest feeding 30% of brewer's yeast with wheat bran rather than 50% to reduce the cost of brewer's yeast. Our results showed that these two diets were strongly supported as equally a high-ranked group in terms of larval duration, weight gain, and specific growth rate. Further research is needed to determine how feeding the brewer's yeast affects the spawning, hatching and nutritional composition of mealworms.

Acknowledgment

We are grateful to O.J. Kim (Department of Agricultural Biology, National Institute of Agricultural Sciences, Wanju) for her valuable assistance in maintaining colonies of yellow mealworms. This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No.PJ01355902)" Rural Development Administration, Republic of Korea.

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