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The Effect of Different Nutrient Compositions on the Larval, Pupal and Pre-Adult Developmental Periods and Adult Weight of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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Research Article

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Abstract

In this study the effect of different types of nutrient mixtures on *Tenebrio molitor*'s larval, pupal and pre-adult developmental periods and adult weight were investigated. The trials were conducted under continuous dark laboratory conditions of 27±2°C temperature and 60±5% relative humidity. 9 different nutrients (dry yeast, corn flour, fine white flour, whole wheat flour, wholemeal flour, wheat flour, wheat germ, oatmeal and rye flour) were mixed in different amounts and 5 different nutrient compositions were used in the trials. As a conclusion, pre-adult total developmental period was 122.93±12.13 days in the first nutrient, 96.77±7.65 days in the second nutrient, 125.42±2.14 days in the third nutrient, 100.47±9.85 days in the fourth nutrient and 143.90±1.21 days in the fifth nutrient compositions, respectively. The shortest developmental period was found to occur in the second nutrient composition. No significant difference was found between pupal periods in different nutrient compositions. The shortest larval periods were found in the second nutrient composition as 90.66±6.57 days and in the fourth nutrient composition as 93.99±9.93 days, respectively. In adult weights, the differences between the second and fourth nutrient compositions and the differences between the first and fifth nutrient compositions were statistically insignificant. The highest adult weight was found in the third nutrient composition (121.35±3.3 mg).

Keywords: *Tenebrio molitor*, developmental period, different nutrient compositions, adult weight

Farklı Besin Bileşimlerinin *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)'un Larva, Pupa ve Ergin Öncesi Gelişim Dönemi ve Ergin Ağırlığı Üzerine Etkisi

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Öz

Bu çalışmada farklı besin karışımlarının *Tenebrio molitor*'un larva, pupa ve ergin öncesi gelişim zamanları ile ergin ağırlığına etkisi araştırılmıştır. Denemeler 27±2°C sıcaklık ve %60±5 bağıl nem koşullarında sürekli karanlık laboratuvar şartlarında yürütülmüştür. 9 farklı besin maddesi (kuru maya, mısır unu, galeta unu, tam buğday unu, kepekli un, buğday unu, ruşeym, yulaf ezmesi ve çavdar unu) farklı miktarlarda karıştırılmış ve denemelerde 5 farklı besin maddesi karışımı kullanılmıştır. Sonuç olarak, ergin öncesi toplam gelişme süresinin birinci besin karışımında 122.93±12.13 gün, ikinci besin karışımında 96.77±7.65 gün, üçüncü besin

karışımında 125.42±2.14 gün, dördüncü besin karışımında 100.47±9.85 gün ve beşinci besin karışımında ise 143.90±1.21 gün olduğu ve en kısa gelişme süresinin ikinci besin karışımında gerçekleştiği tespit edilmiştir. Farklı besin karışımlarında böceklerin pupa dönemleri arasında önemli bir fark bulunmamıştır. En kısa larval dönem ikinci besin karışımında 90.66±6.57 gün, dördüncü besin karışımında ise 93.99±9.93 gün olarak bulunmuştur. Ergin ağırlıklarında ikinci ve dördüncü besin maddesi karışımları arasındaki farklar ile birinci ve beşinci besin maddesi karışımları arasındaki farklar istatistiksel olarak önemsiz bulunmuştur. En yüksek ergin ağırlığı üçüncü besin karışımında (121.35±3.3 mg) bulunmuştur.

Anahtar Kelimeler: *Tenebrio molitor*, gelişim zamanı, farklı besin kompozisyonları, ergin ağırlığı

Introduction

The mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is an important stored product pest and has been extensively used by researchers in numerous studies. In addition, they are used as a natural feed source for poultry and fish since they include high amounts of protein, fat and fatty acids [1, 2]. In order to succeed in the battle with this insect and use it more effectively as an animal feed source, its biology should be well studied. For example, it is very important to research its nutritional requirements in the laboratory, and to know in which nutrients it is reared quicker, in which nutrients it has a higher fecundity rate and how different types of nutrients influence the biology of the insect. FAO's Animal Feed Sources Information system reports that some insects, including *T. molitor*, are used in feeding animals [3, 4]. The significance of nutrient in insect development and physiology has been determined by a great number of researchers. Nutrients influence many activities such as development rate, fecundity and longevity by changing metabolic activity in insects [5, 6]. The increasing importance of this insect, particularly in animal nutrition requires a more detailed research of the influence of different nutrients on this insect. In our study, 5 different nutrients were researched in detail by using different nutrient mixtures which have not been studied before. The objective of this study is to examine the possibility of rearing *T. molitor*, which is generally reared by using wholemeal flour and flour, by using different nutrients and to compare its developmental periods. The shortest possible developmental period of *T. molitor*, which already has a naturally very long developmental period, will be found, and nutrient alternatives for its development as an increasingly important natural feed will be investigated. It is crucial to rear this insect, which is used both in laboratory studies and as animal feed by producers, with nutrients of reasonable cost to get the fastest fecundity. In insects, development occurs through the stages of egg, larva, pupa and adult. The time from egg to adult emergence is referred to as the pre-adult development period. The duration of pre-adult development in insects is also highly affected by the type of food provided. The biology of the *T. molitor* has been well studied and its optimal conditions are 27ºC at >60% RH [7]. According to Li et al. [7], the egg stage lasts 3-9 days, the larval stage 26-76 days, and the pupal stage 5-17 days. Before emergence, most of the larvae go through typically 15 to 20 instars

[8]. Growing in the most suitable nutrient environments will save time. It will cause the larvae to stay in cold for a shorter period of time.

Materials and Methods

This study investigates the effect of different nutrients on *T. molitor*'s larval, pupal, pre-adult developmental times and its adult weight. The trials were conducted under continuous dark (CD) laboratory conditions with a temperature of 27±2°C and a relative humidity of 60%±5. Five different nutrient compositions were provided in the study. The first nutrient composition consisted of dry wheat, corn flour, fine white flour, whole wheat flour, wholemeal flour, wheat flour, wheat germ, oatmeal and rye flour (in equal amounts, 150 gr in total). The second nutrient composition included only wheat germ (150 gr). The third nutrient composition was composed of wheat flour, rye flour, oat meal and whole wheat flour (150 gr in total, 1/1). The fourth nutrient composition was composed of fine white flour and dry yeast (150 gr in total, 2/1). The fifth nutrient composition consisted of whole wheat flour and corn flour (150 gr in total, 1/1). The studies first started by rearing the insect in all nutrient media mentioned above. The insects were reared in medium size wide plastic containers (30×20×5 cm). Wood dust was added on nutrients to ease their movement and the nutrients were renewed every 10 days. The larvae in the old nutrients were sieved and transferred to the new nutrient. Small pieces (2 for each, 4×4×6 cm) were cut from egg boxes and they were placed in containers. They are known to provide convenience for adults to mate and also preferred to lay eggs. While the plastic containers were covered to prevent contamination by other organisms, small holes were opened on the top side to enable the insects to breathe. Potato was used for humidity (3×3 cm). Potatoes were provided by being wrapped into aluminium foil in order to prevent potato to contact with the nutrient and to decay and wet the nutrient. Potatoes were changed every 3 days for the litter not to get mouldy. Litter for nutrient was adjusted as 4-5 cm high. The research was started with the insect reared for two generations in the nutrient composition studied. Each nutrient composition was prepared in the abovementioned amounts. For each nutrient composition, three sample groups were formed with the insect taken from the population at different times. In each nutrient composition, 10 males and 10 females that were reared on that same nutrient composition and has reached adulthood on that day were placed. The insects were taken from the containers 7 days later. The time until pupa stage was recorded, and the larval period was found for each insect. In addition, pupae which formed in containers were taken into separate containers. The containers were checked every day, and the larvae emerging on the first day were placed in different Petri plates (9×1.5 cm). Petri plates were observed every day and the period from pupa stage to adulthood was recorded. At each adult emergence, the time was calculated and pupal period was found. The period from the first set up of the trial to the adult emergence was calculated and pre-adult total developmental periods were found. The adults were weighed the first day they emerged and their adult weights were recorded. Data Analysis: SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was used to

find out the influence of different nutrient compositions on *T. molitor*'s larval, pre-adult developmental and pupal time and adult weights. In each trial, 50 observations and 3 different repeated measurements were recorded and the analyses were made based on a total of 150 observations. One-way ANOVA was used to compare the repeated measurements of five different nutrient compositions. In the assessment of data, Wilk's Lambda test was used and 0.05 error margin was taken as a basis.

Results

The influence of different nutrient compositions on the larval, pupal and pre-adult developmental period of *T. molitor*

Figure 1 shows the influence of different nutrient compositions on *T. molitor*'s larval and pre-adult total developmental period. The shortest larval period was found in the second and fourth nutrient compositions with 90.66±6.57 and 93.99±9.93 days, respectively. The longest larval period was found in the fifth nutrient composition with 136.86±0.89 days. No statistically significant difference was found between the larval period of the second and fourth nutrient and between the first and third nutrient compositions ($P > 0.05$). In the fifth nutrient composition, larval period was found to be very long and it was statistically different from the others ($P=0.000$, F= 46577.500). The shortest pre-adult total developmental period was found in the fourth and second nutrient compositions. The slowest development was found in the fifth nutrient composition. The differences between pre-adult developmental periods were found to be significant in all groups ($P= 0.000$, F= 34411.558).

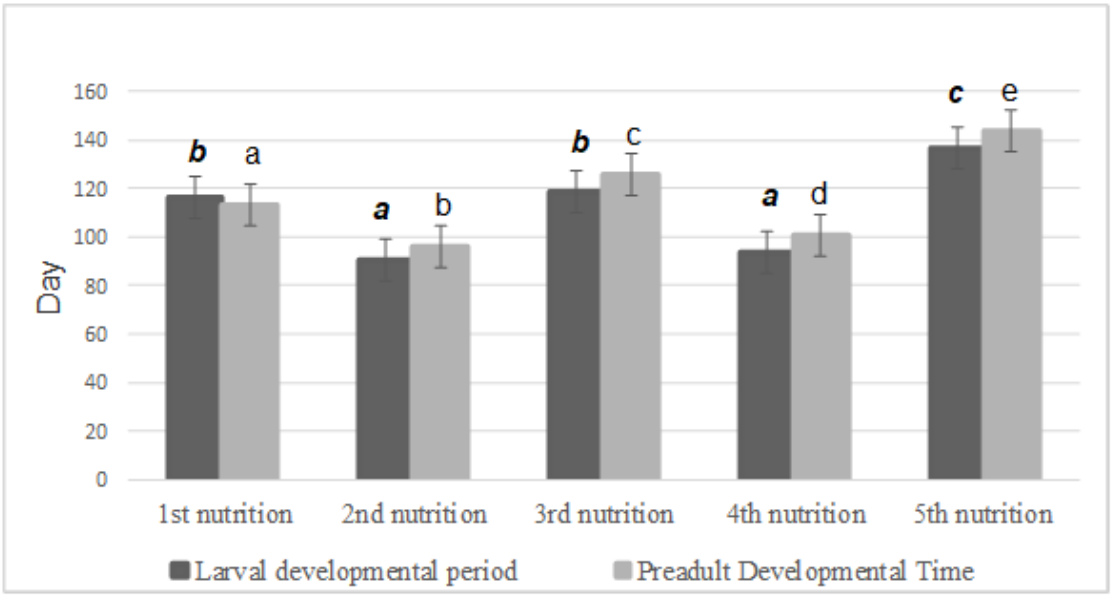

Figure 1. *The influence of different nutrient compositions on the larval and pre-adult total developmental period of Tenebrio molitor (the differences between the values shown with the same lower-case letter are not statistically different p>0.05, Wilks' lambda). A total of 150 individuals were used, with 50 individuals in each trial. Shows the average data from a total of 150 individuals*

Figure 2 shows the influence of different nutrient compositions on *T. molitor*'s pupal developmental period. Pupal periods of the first and fifth nutrient compositions were similar and no statistically significant difference was found ($P>0.05$). The differences between the pupal periods of second, third and fourth nutrient compositions were also insignificant ($P= 0.000$, $F= 4579.389$).

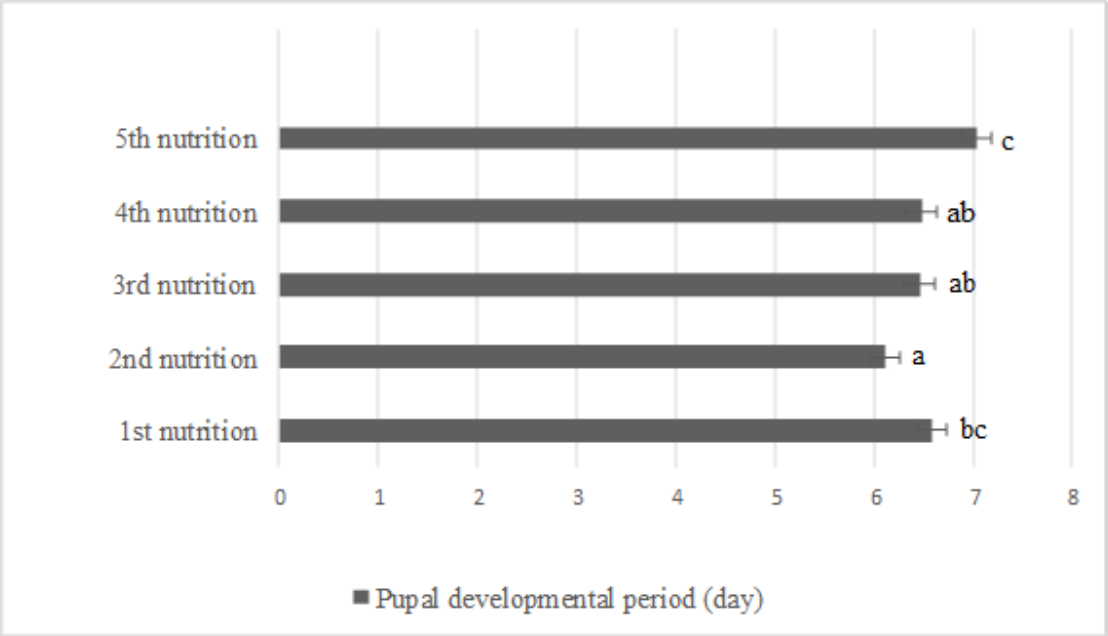

Figure 2. *The influence of different nutrient compositions on the pupal developmental period of Tenebrio molitor (the differences between the values shown with the same lower-case letter are not statistically different p>0.05, Wilks' lambda). A total of 150 individuals were used, with 50 individuals in each trial. Shows the average data from a total of 150 individuals.*

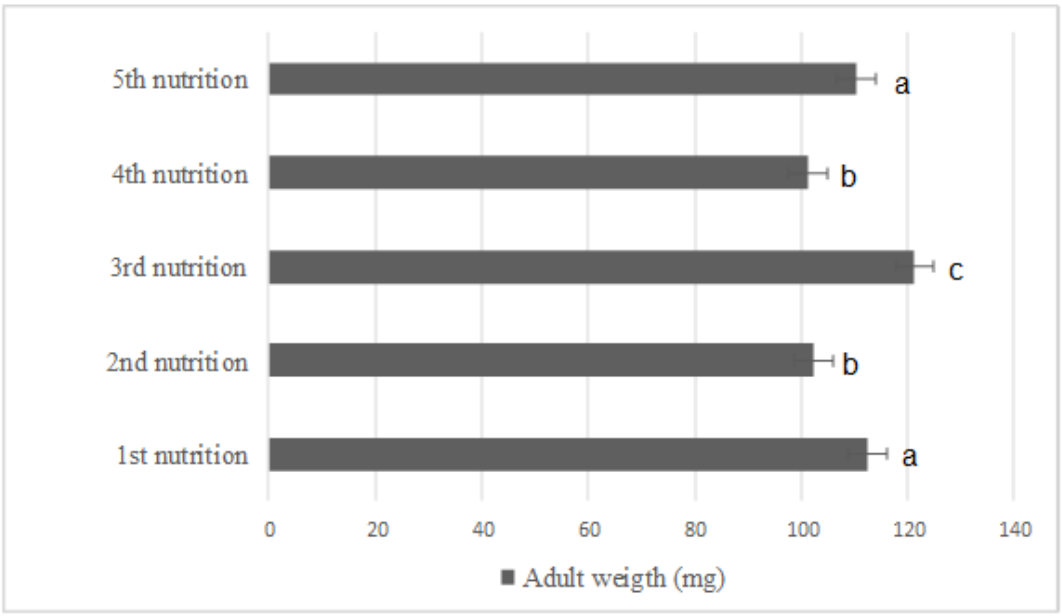

Figure 3. *The influence of different nutrient compositions on the adult weight of Tenebrio molitor (the differences between the values shown with the same lower-case letter are not statistically different p>0.05, Wilks' lambda). A total of 150 individuals were used, with 50 individuals in each trial. Shows the average data from a total of 150 individuals.*

The influence of different nutrient compositions on the adult weight of *T. molitor*

Figure 3 shows the influence of different nutrient compositions on *T. molitor*'s adult weight. While the highest weight was found in the third nutrient composition with 121.35±3.39, the lowest adult weight was found in the fourth nutrient composition with 101.45±6.22 mg. In adult weights, the differences between the second and fourth nutrient compositions and the differences between the first and fifth nutrient compositions were statistically insignificant (P>0.05). The third nutrient composition with the highest average weight was found to be different from the other groups (P= 0.000, F=41331.827).

Discussion

In *T. molitor*'s life cycle, nutrient has a very important role [8-11]. There are a great number of studies on nutrient's influence on its developmental period [12-14], number of larvae [15] and survival rate [16]. Rho and Lee [12] reported that optimum protein carbohydrate ratio should be 1:1 in the life cycle of *T. molitor.* It has been reported by some researchers that the most important components that should be available in the nutrient of *T. molitor* are proteins including nutrients and yeast, and that the use of these nutrients reduces both mortality rate and the developmental period [12, 17]. The fact that wheat germ has high carbohydrate and protein content is in parallel with the result of our study that the shortest development took place in the second nutrient composition. The high protein, fatty acid and energy content of larvae and pupae of mealworm causes many advantages in the nutrition of poultry and fish [18]. In their study conducted with *T. molitor,* Özsoy et al. [19] found that increasing the amount of fat added in the ration led to an increase in the total body fat ratio. 3% fat addition to ration caused an average of 1.6% increase in the body raw oil of the larvae, while 5% fat addition resulted in an increase of 3.2%. Urrejola et al. [15] used different nutrient concentrations which had wheat flour and nutritional yeast and formed groups which contained low and high protein levels. They found that different nutrient concentrations did not cause a significant change in the developmental period and only the number of eggs was found to be higher in those containing high protein. One of the attention-grabbing nutrients in studies about *T. molitor* is yeast. There are studies in which yeast is used as a source of protein, in which yeast activates nutrition and in which casein and lactalbumine are added as sources of protein [20, 21]. In some studies, soy has been used as a protein source [11]. Carbohydrate content is very influential in the development of this insect and a low carbohydrate content has been reported to slow the insect's development [18]. While vitamin B complexes are important in the development of mealworm, it has been found that other vitamins are not very effective [20]. In studies about insect development and physiology, it has been found that the type of nutrient can change metabolic activity and influence the speed of development and thus change a great number of activities such as fecundity, longevity and amount of substances included [8, 22]. In our study, the longest pre-adult developmental period was found in the fifth nutrient composition with 143.90±1.21 days. In the fifth nutrient composition, not only pre-adult total developmental period but also larval period was found to be very long. In this nutrient

composition, larval developmental period took longer than in all other nutrient compositions (Figure 1). The fifth nutrient composition was made from equal amounts of whole wheat flour and corn flour. In addition, as in all nutrient groups, potato is provided for humidity. In this case, it can be said that the prolonged developmental period of *T. molitor* in this combination can result from the fact that the insect cannot meet the required substances for optimum development and therefore it should not be preferred in studies. The fact that carbohydrate ratio in whole wheat flour was approximately five times higher than the protein amount and similarly very high carbohydrate rate contrary to a very low protein rate in corn flour may have caused the developmental period to slow down. The similar components of both nutrients caused *T. molitor* larvae not to meet the nutrients they required and the developmental period was prolonged. It will take a very long time for *T. molitor* to develop and it will be a disadvantage to work with this costly nutrient. As is known, nutrient type in the pre-adult stage of especially holometabolic insects is effective in developmental periods. Insufficient nutrients generally cause an extended pre-adult developmental period. Saadiya and El Defrawy [23] fed yellow mealworm larvae with wheat, oats, chicken feed and a mixture of these diets in a study they conducted. In general, yellow mealworm larvae fed with wheat, oats, chicken feed and a mixture of these diets showed good results in terms of growth rate and nutritional composition. Şah et al. [24] fed mealworm larvae with 10 different diets. They found that wheat bran, the natural diet of larvae, was the best food for mealworm production. Adding wheat bran as a supplement helped to increase the number of mealworm offspring produced. Wheat bran, both alone and in combination, is recommended for mass mealworm production. In our study, the nutrient which resulted in the shortest development time was the nutrient in which only wheat germ was used. In this nutrient, pre-adult total developmental period and larval period also took longer than the other nutrient compositions (Figure 1). The results obtained from the group fed with wheat germ were closest to those of the group in the fourth nutrient composition. The content of fourth nutrient composition was fine white flour and dry yeast. In the second and fourth nutrient compositions, adult weights were also found to be very close to each other (Figure 3). The protein rate of wheat germ in the nutrient content is very high and it is above 23 in 100 grams. The rate of carbohydrate is around 42%. This rate met the needed nutrients by *T. molitor* and probably high protein and carbohydrate amounts were a reason of preference as stated by the other researchers. Wheat germ is a very important nutrient source which has been increasingly used in recent times [25, 26]. The other group of dry yeast and fine white flour which had a fast development can be advised for *T. molitor*. Probably the combination of dry yeast and fine white flour met the nutritional needs of the insect and the higher carbohydrate amount in fine white flour and over 50% protein content of dry yeast was enough for its development. In the third nutrient composition, pre-adult developmental period was 125.42±2.14, while the total developmental period was 122.93±12.13 days in the first nutrient composition which was costly with too many components (Figure 1 and Figure 2). First and third nutrient compositions yielded similar results; however, they were not as effective as the second and fourth nutrient compositions. Since too

many nutrients were used in the first nutrient composition, the amount of carbohydrate was excessively high in their content. The amount of protein was insufficient and with the increase in fat ratio, it was not as effective as the second and fourth nutrient compositions. In adult weight, the results of the first and fifth and the results of the second and fourth nutrient compositions were found to be very similar. The highest adult weight was found in the third nutrient composition. The fact that too many nutrients were used in the first nutrient composition may have caused some increase in this weight. In the third nutrient composition, the mixture of wheat flour, rye flour, oatmeal and whole wheat flour caused a high carbohydrate ratio and adult weights increased. In general, there are no significant differences between weights (Figure 3). It is not preferred for insects to have excessive weight and it has been stated that low rates of fat in a nutrient is important since it does not restrict the movements of *T. molitor* [26].

Conclusions

In this study, the result that wheat germ significantly shortened the developmental period show that it is the most suitable nutrient. Additionally, development was very fast in the fourth nutrient composition which consisted of dry yeast and fine white flour. These nutrients are suitable for rearing this insect and there is no need to use too many types of nutrients. The shortest pre-adult total developmental period was found in the fourth and second nutrient compositions. The slowest development was found in the fifth nutrient composition. The excessive amount of nutrients as in the first nutrient composition showed no positive effect on the development of the insect and it was found to be disadvantagous due to its high cost.

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Conflicts of Interest The authors declare that they have no conflict of interest.

Authors Contribution The authors contributed equally to this article.

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