Original paper

Changes in chemical composition during the metamorphosis stages of *Tenebrio molitor*

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Abstract In recent years, the demand for alternative proteins has increased with the growth of the global population. *Tenebrio molitor* (*T. molitor*) larvae have been widely used as feed for pets, zoo animals, and livestock. It has four life stages: egg, larva, pupa, and adult. *T. molitor* is often fed as a larva, but it can also be fed as a pupa or adult as feed for large reptiles and birds. In this study, we investigated the chemical composition of *T. molitor* at different metamorphosis stages. The test samples were *T. molitor* larvae, pupae, and adults, and three groups of 20 individuals per group were used at each metamorphosis stage. We measured moisture, crude protein, nitrogen free extracts, crude ash, ether extracts, and fatty acid contents in *T. molitor* at each metamorphosis stage. Moisture and crude protein contents were significantly lower in larvae than in pupae and adults. Ether extracts and nitrogen free extracts contents were significantly higher in larvae than in pupae and adults. Unsaturated fatty acid content was significantly different between all metamorphosis stages ($P < 0.01$). The pupa was the highest, followed by the larvae and adults. These results suggest that the chemical components of *T. molitor* change during metamorphosis.

Key words: fatty acid, mealworm, metamorphosis stage, chemical composition, *Tenebrio molitor*

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Introduction

According to the International Feed Industry Federation [1], given the anticipated growth of the world's population to around 9 billion people by 2050. For this purpose, a double amount of animal protein is required. The Food and Agriculture Organization [2] stresses the importance of finding alternatives to conventional animal feed because of its limited amount. Insects could be a part of the solution [3]: among them, mealworms are one of the promising candidates for alternative feed and they are already produced in relatively large scale farms as pets, zoo

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animals, and livestock feed. In addition, mealworms are not only suitable as animal feed but they are also considered ideal for human nutrition and have even been expected for food are also increasing [4,5]. Mealworms are the larvae of *Tenebrio molitor* (*T. molitor*), which belongs to the Tenebrionidae family, of the Coleoptera order. *T. molitor* undergoes metamorphosis in the following order: egg, larva, pupa, and adult. *T. molitor* is often fed as a larva, but it can also be fed as a pupa or adult as feed for large reptiles and birds. Although there are reports on the nutritional value of *T. molitor* larvae, there are few reports on changes in nutritional value associated with metamorphosis. It is generally said that in holometabolous insects, the internal material composition and metabolic system change significantly during metamorphosis [6]. Furthermore, it has been reported that the amount and composition of ether extracts change as the insect grows after hatching and before it becomes an adult [7]. Therefore, even in *T. molitor*, which is a holometabolous insect, the component composition may change with metamorphosis. In particular, when the amount and quality of lipid, which is one of the main components, varies greatly, it is necessary to take this into account when feeding it to animals. The fatty acid composition of feed is extremely important in maintaining the life and functionality of the animals that intake it. It has been reported that changes in the ratio of dietary n-6 to n-3 fatty acids affect the immune response of rat, broilers, and pigs [8~10]. If the fatty acid composition of *T. molitor* changes as a result of metamorphosis, it is important to design feeding that considers these changes. Thus, in this study, we investigated the chemical composition of *T. molitor* at different metamorphosis stages.

Materials and Methods

For the experiment, out of commercially available mealworms (larvae of *T. molitor*) as feed, healthy individuals of similar size were picked and used. Mealworms were reared in aluminum tray (45 cm long × 30 cm wide × 4 cm deep) for about 2 months, and when they became pupae (1 to 2 weeks) or adults (about 1 month), they were stored frozen at -20°C. They were fed with wheat bran in sufficient quantity, the feeding conditions were controlled at 25°C with 60-70% relative humidity. Three groups of 20 individuals per group were arranged at each metamorphosis stage: larva, pupa, and adult. We measured moisture, crude protein, nitrogen free extracts, crude ash, ether extracts, and fatty acid contents at each metamorphosis stage 20 individuals as one sample. Moisture, crude protein, crude ash and ether extracts contents were determined using the standard methods [11]. Nitrogen free extracts were calculated by subtracting the total percent values of other measurements from 100. Fatty acids were extracted and measured with the hexane and acetone treatments [12]. After derivatization into fatty acid methyl esters, were analyzed using GC-FID (GC-1700; Shimadzu Co., Kyoto). As standard a commercial fatty acid methyl ester (FAME) standard mixture (Supelco 37 Component FAME Mix; Sigma-Aldrich, USA) was used. The temperatures of the injector and detector were set at 250°C and 280°C. Helium was used as a carrier gas at the flow rate of 8.0 ml/min. A DB-WAX capillary column (0.53 mm×30 m×1 μm, Agilent, USA) was used. The oven temperature was programmed as follows: increased from 50°C to 200°C at the rate of 25°C/min, further raised to 230°C at the rate of 3°C/min, and held for 13 min. Using the GC measurement results, the content of each fatty acid per 1 g of *T. molitor* was calculated. All statistical analyses were performed with SPSS statistical software (SPSS Inc., Chicago, IL, USA), and $P < 0.01$ was considered statistically significant. The statistical significance of differences was assessed with one-way ANOVA using SPSS statistical software (SPSS Inc., Chicago, IL, USA) followed by Tukey's test.

Results

The chemical composition of *T. molitor* at each metamorphosis stage is presented in Table 1. The weight per capita was highest for pupae, followed by adults and larvae (P <0.01). The moisture content of larvae was significantly lower than that of pupae and adults (P <0.01). There was a significant difference in crude protein content at all metamorphosis stages (P <0.01), with the crude protein content being lowest in larvae, followed by pupae and adults. There was no significant difference in the crude ash content at any transformation stage. Ether extracts and nitrogen free extracts contents were significantly lower in pupae and adults than in larvae (P <0.01). Table 2 presents the fatty acid content per 1 g of *T. molitor* at each metamorphosis stage. At all metamorphosis stages, the main saturated fatty acid was palmitic acid (C16:0), the monounsaturated fatty acid was oleic acid (C18:1), and the polyunsaturated fatty acid was linoleic acid (C18:2). C16:0, C18:1, and C18:2 were highest in pupae, followed by larvae and adults (P <0.05). The contents of saturated and unsaturated fatty acids (n-3, n-6, and n-9 fatty acids) are shown in Figure 1. There was a significant difference in the fatty acid content between each metamorphosis stage in all items (P <0.01). In all fatty acid contents, pupae had the highest content, followed by adults and larvae. When the ratio of n-6

Table 1 Chemical composition in each metamorphosis stage of *Tenebrio molitor*.

	Larva	Pupa	Adult
Per capita weight (mg)	67.3±2.8^{c}	124.7±0.8^{a}	95.3±1.8^{b}
Moisture (%)	53.9±0.3^{b}	68.5±0.3^{a}	68.5±0.7^{a}
Crude protein (DM%)	49.6±0.3^{c}	62.8±0.4^{b}	65.1±0.1^{a}
Crude ash (DM%)	6.1±0.2	7.0±0.5	5.9±0.4
Ether extracts (DM%)	11.6±0.4^{a}	6.3±0.5^{b}	4.0±0.3^{b}
Nitrogen free extracts (DM%)	32.7±0.5^{a}	23.8±1.3^{b}	25.0±0.4^{b}

Each value represents the average ± standard error (n=3)
^{a,b,c} Means with different superscripts are significantly different (P <0.01)

Table 2 Fatty acid composition in each metamorphosis stage of *Tenebrio molitor*.

Fatty acid(mg/g DM)	Larva	Pupa	Adult
C11:0	2.70±0.12	2.92±0.40	2.25±0.35
C12:0	1.02±0.69	0.38±0.10	0.80±0.50
C13:0	ND	0.04±0.03	0.33±0.14
C14:0	9.88±0.06^{b}	8.73±0.07^{c}	12.15±0.35^{a}
C14:1	0.39±0.06	0.26±0.02	0.34±0.01
C15:0	0.42±0.21	0.54±0.01	0.61±0.01
C15:1	ND	ND	0.04
C16:0	71.01±0.27^{b}	126.45±4.88^{a}	56.21±1.29^{c}
C16:1	7.15±0.11^{b}	25.67±3.20^{a}	9.08±2.83^{b}
C17:0	0.68±0.13	0.87±0.21	0.73±0.05
C17:1	1.01±0.09	1.11±0.04	0.82±0.06
C18:0	14.42±0.27	11.90±1.31	9.97±1.71
C18:1	231.11±1.64^{b}	272.53±2.76^{a}	174.28±2.54^{c}
C18:2	137.57±3.03^{b}	176.34±2.41^{a}	105.76±2.27^{c}
C18:3	4.62±0.07^{b}	7.10±0.34^{a}	3.65±0.21^{b}
C20:0	0.53±0.02^{a}	0.50±0.05^{a}	0.15±0.07^{b}
C20:1	0.43±0.16	0.33±0.30	0.16±0.09
C20:2	0.21±0.08^{b}	2.17±0.12^{a}	0.17±0.06^{b}
C21:0	ND	0.05±0.03	0.01±0.01
C20:3	ND	0.11±0.00	0.05±0.04
C20:4	ND	0.06±0.04	0.01±0.00
C20:5	ND	0.01±0.00	ND
C22:0	ND	0.05±0.02	ND
C22:1	ND	0.00±0.00	0.01±0.01
C22:2	0.83±0.11^{b}	10.30±0.32^{a}	0.12±0.12^{b}
C23:0	ND	5.11±0.15	ND
C24:0	ND	0.02±0.01	ND
C24:1	ND	0.01±0.01	0.01
C22:6	ND	2.49±0.50	ND

Each value represents the average ± standard error (n=3). ND, Not detected.
^{a,b,c} Means with different superscripts are significantly different (P <0.05)

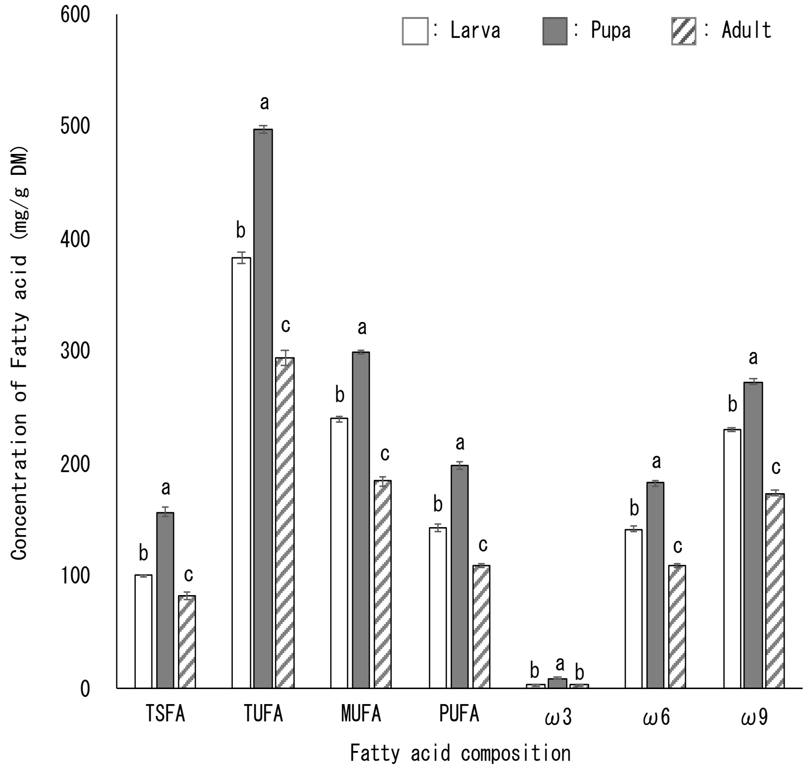

Figure 1 Fatty acid composition in each metamorphosis stage of *Tenebrio molitor*.
Each value represents the average ± standard error (n=3)
[a,b,c] Means with different superscripts are significantly different ($P<0.01$)
TSFA, total saturated fatty acid; TUFA, total unsaturated fatty acid; MUFA, mono-unsaturated fatty acid (C14:1, C16:1, C17:1, C18:1, C20:1); PUFA, poly-unsaturated fatty acid (C18:2, C18:3, C20:2); ω3 is include C18:3. ω6 is include C18:2 and C18:3. ω9 is include C18:1 and C20:1.

to n-3 fatty acids was calculated using the values of n-3 and n-6 fatty acids, it was 31 for larvae, 30 for adults, and 19 for pupae.

Discussion

In this study, the moisture content of pupae and adults was higher than that of larvae. It has been reported that the moisture content in a diet affects the palatability and weight gain of rats [13,14]. Therefore, when feed-ing *T. molitor* at different stages of metamorphosis, it is necessary to consider differences in moisture content. Protein is an extremely important nutrient for animal development and live-stock productivity, and insects such as meal-worms are also used as an alternative protein source [5]. In this study, adult *T. molitor* worms contained the most protein, and *T. molitor* adults can be expected to be used as an alternative protein source compared with feeding during the larval or pupal stages. However, since the weight per capita of adults is less than that of larvae, it is necessary to consider productivity, feeding timing, and other chemical composition as well. Insects are covered with an exoskeleton mainly composed of chitin, a polysaccharide [15]. Mealworm shells are representative chitin-containing organisms [16]. In this study, the larvae had the highest nitrogen free extracts content; therefore, the larvae may have contained a higher proportion of chitin. It has been reported that chitin can be degraded and utilized in mice and chickens [16]. Carbohydrates are the main energy source for animals, and chitin could be used as feed for animals that can digest it. However, because it has been reported that the digestibility of protein varies depending on the chitin content and metamorphosis stage [17], further study of chitin content and digestibility is also required.

The pupa had the highest fatty acid content in all items, and the pupa had the lowest ratio of n-6/n-3 fatty acids. It has been reported that lowering the ratio of n-6/n-3 fatty acids in the feed improves the immune response of broilers [9]. Therefore, feeding *T. molitor* pupae to broilers may improve immune responsiveness. In addition, there are concerns that the ratio of n-6/n-3 fatty acids in the foods may affect the hatch success rate in green sea turtles [18]. The foods of captive turtles on a pellet diet contain a large amount of C18:2, an n-6 fatty acid, and the foods (*Thalassia testudinum*) of wild turtles contain a large amount of C18:3, an n-3 fatty acid. There are concerns that this difference may affect the hatching success rate. Therefore, it is extremely important to consider the ratio of n-6/n-3 fatty acids in food for many animals. Appropriate feeding may be possible by feeding *T. molitor* at different metamorphosis stages in ratio and frequency according to the animal being fed.

In this study, we investigated the chemical composition of *T. molitor* at different metamorphosis stages. These results suggest that chemical components change depending on the metamorphosis stage. Therefore, when feeding *T. molitor* at each metamorphosis stage, such as larva and pupa, it is necessary to consider changes in chemical components. However, there are many unknowns regarding the causes of changes in chemical components associated with metamorphosis, and the mechanism of these changes will need to be investigated in the future.

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Original Paper

Tenebrio molitor の成長過程に伴う成分組成の変化

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要　約　近年、世界人口の増加に伴い、代替タンパク質の需要が高まっている。ミールワームとして家畜や伴侶動物等の飼料に広く用いられているチャイロコメノゴミムシダマシ（*Tenebrio molitor*）は完全変態を行う昆虫であり、卵、幼虫、蛹、成虫の順に形態を変化させる。チャイロコメノゴミムシダマシは幼虫であるミールワームの状態で給与される場合が多いが、大型の爬虫類や鳥類用の飼料として、蛹や成虫といった状態で給与される場合もある。ミールワームの飼料化に関連する一連の研究の中で、今回の試験では TM の形態変化が一般成分および脂肪酸組成に与える影響について検討した。供試動物には、市販ミールワームのうち、目視で活力のある個体のみを選抜した。試験では、各成長過程まで室温で飼育し、幼虫、蛹および成虫 20 個体 ×3 群を得た。測定項目は、各成長過程の一般成分（水分、タンパク質、脂質、炭水化物および灰分含量）および脂肪酸組成とした。水分およびタンパク質含量は、蛹および成虫に比べ幼虫が有意に低く（$P<0.01$）、脂質および炭水化物含量は、蛹および成虫に比べ幼虫が有意に高かった（$P<0.01$）。不飽和脂肪酸含量は、いずれの成長過程においても有意な差が認められ、蛹が最も高く、次いで幼虫、成虫の順であった（$P<0.01$）。これらのことから、TM は、成長過程に伴い一般成分および脂肪酸組成が変化することが示唆された。

キーワード : 脂肪酸、ミールワーム、成長段階、化学組成、*Tenebrio molitor*

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