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Rearing *Tenebrio molitor* in BLSS: Dietary fiber affects larval growth, development, and respiration characteristics

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ABSTRACT

Rearing of yellow mealworm (*Tenebrio molitor* L.) will provide good animal nutrition for astronauts in a bioregenerative life support system. In this study, growth and biomass conversion data of *T. molitor* larvae were tested for calculating the stoichiometric equation of its growth. Result of a respiratory quotient test proved the validity of the equation. Fiber had the most reduction in mass during *T. molitor*'s consumption, and thus it is speculated that fiber is an important factor affecting larval growth of *T. molitor*. In order to further confirm this hypothesis and find out a proper feed fiber content, *T. molitor* larvae were fed on diets with 4 levels of fiber. Larval growth, development and respiration in each group were compared and analyzed. Results showed that crude-fiber content of 5% had a significant promoting effect on larvae in early instars, and is beneficial for pupa eclosion. When fed on feed of 5–10% crude-fiber, larvae in later instars reached optimal levels in growth, development and respiration. Therefore, we suggest that crude fiber content in feed can be controlled within 5–10%, and with the consideration of food palatability, a crude fiber of 5% is advisable.

1. Introduction

Artificial rearing of yellow mealworm (*Tenebrio molitor* L.) mainly serves to fulfill the demands of feed for pets and livestock; however, there is considerable interest in the use of this insect as a potentially valuable source of food for humans [1]. In bioregenerative life support systems (BLSSs) for future long-term and far-distance manned space missions, *T. molitor* is a potential source of animal food for astronauts [2]. A BLSS is an artificial closed ecosystem similar to the Earth's biosphere [3]. The necessities for crew life support (e.g. plant food, animal food, air, water, etc.) circulate in the system using bioregenerative processes combined with physico-chemical technologies to greatly save launch costs, as well as significantly decreasing waste products. It has been discussed that rearing insects in a BLSS has some advantages [4], and recent research has reported that rearing *T. molitor* in a BLSS as a source of food for astronauts is feasible and important [2]. *T. molitor* has a good nutritional profile with high protein value, a good composition of essential amino acids, is unsusceptible to disease, and can reach a relatively high growth rate and a high economic coefficient while adapting to higher rearing densities. [1]. In addition, it

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requires only light labor for rearing, which saves crew's working time. Most importantly, this insect can be fed on inedible plant biomass in a BLSS, e.g. straw, bran, vegetable roots, etc., so as to act as an important link in the waste treatment process [2].

To introduce *T. molitor* into a progressive BLSS, the first concern is using limited inedible plant biomass to fulfill *T. molitor*'s requirement for food. Nearly 50% of inedible plant biomass in a conceptual BLSS is composed of crop straw [5], which is quite different with the types of feed widely used in terrestrial agriculture, i.e. feed from a BLSS contains a much higher proportion of lignocelluloses. A proper diet made from a suitable proportion of ingredients with an appropriate degree of microbial fermentation could provide substances and energy for *T. molitor* rearing. For reducing cost in terrestrial agriculture, feed could also be obtained by means of microbial fermentation of crop straw.

It is noteworthy that the midgut of *T. molitor* contains enzymes and micro-organisms capable of lignocellulose degradation [6]. Therefore, we hypothesize that the oxidation of fiber is an important part in the metabolic activity of *T. molitor*, as it provides material and energy for *T. molitor*'s life activities. However, considering feed ingestion and digestion, high fiber content in feed would lead to increased hardness (difficulty of chewing), and thus decreases feed palatability. Therefore, either too high or too low in feed fiber content could both lead to growth inhibition.

Quantitative analysis of the oxidation of substrates during *T. molitor* metabolic growth can be made by calculating the stoichiometric equation, which gives a quantitative interpretation of the metabolic process [5].

In order to reveal the role that oxidative decomposition of fiber plays in *T. molitor*'s metabolic activity, and subsequently find out a proper dietary fiber content for *T. molitor*, the stoichiometric equation was calculated, and the effect on growth, development and respiratory metabolism of different levels of feed fiber content was examined in this study.

2. Materials and methods

2.1. Materials

2.1.1. Larval *T. molitor*

T. molitor larvae for testing growth and calculating stoichiometric equation were a laboratory-reared breed (No. TA × TA) obtained from the Institute of Environmental Biology and Life Support Technology, Beihang University. *T. molitor* larvae for testing the effect of different fiber content on larval growth, development and respiration were obtained from Super Cricket Cricket Farm, Inc., Canada.

2.1.2. Feed preparation

Mixed fermentation of feed: Feed used in the cultivation experiment for calculating stoichiometric equation of *T. molitor* growth and in the RQ testing was made through fermentation of a 1:1 mixture of wheat bran and wheat straw. The microbial inoculants were obtained from Beijing Healthhead Science & Technology Co., Ltd., China. The mixture was fermented in a sealed bag for anaerobic fermentation, and was fermented under 28 °C for 48 h. After fermentation, the feed was dried in a 80 °C dryer to lower its moisture content to approximately 18%.

Feeds with four levels of crude-fiber: these feeds were made from natural, food-grade materials. Wheat flour and wheat bran were obtained from Bulk Barn Foods Limited, Canada. Straw was not used in this test to eliminate the potential interference of allelochemicals [7] which may have an effect on *T. molitor* [6]. Instead, the wheat bran was washed to reduce starch and to subsequenty raise the fiber content. We determined the crude fiber of wheat flour, wheat bran and washed wheat bran by the Weende method (Operating Manual of Velp Scientifica [8] and Chinese standard No. GB/T 6434-2006 [9]. Crude protein was determined by the Kjeldahl method [10] and crude fat was determined by acid hydrolysis (Chinese standard No. GB/T5009.6-2003 [11]. The results are shown in Table 1. Flour, bran and washed bran were then mixed according to the ratios shown in Table 1, resulting in four levels of crude-fiber content. Since the four crude-fiber levels are close to the values of 0%, 5%, 10% and 20%, they were designated FD-0, FD-5, FD-10 and FD-20, respectively.

2.2. *T. molitor* rearing for calculating stoichiometric equation

T. molitor larvae were reared for testing growth and chemical composition of each biomass in the process of bioconversion. Results were used to calculate the stoichiometric equation of larval growth. In addition, the respiratory quotient was tested to verify the validity of the equation.

2.2.1. Rearing regime

Three parallels of 200 ± 20 eggs were reared in a stainless steel rearing container (15 cm diameter) under 28 °C until pupation occurs. For every 5 days, 5 g of fermented feed was added in each group. After the rearing, total fresh weight and larval moisture content were measured. The dry mass of feces production and the remaining feed were weighed.

2.2.2. Chemical analyses

Neutral detergent fiber (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL), acid insoluble ash (AIA) in fermented feed and mealworm frass were analyzed according to the methods described by Van Soest et al.

Table 1
Composition of feeds with different levels of crude fiber.

Feed number	Flour: bran ratio	Crude fiber, %	Crude protein, %	Crude fat, %
FD-0	1:0	0.3 ± 0.05	14.36 ± 0.29	1.45 ± 0.02
FD-5	4:3	5.15 ± 0.21	15.25 ± 0.23	3.05 ± 0.05
FD-10	1:6	9.69 ± 0.38	16.15 ± 0.16	4.64 ± 0.08
FD-20	0:1[a]	20.3 ± 0.90	15.59 ± 0.20	5.27 ± 0.02
Wheat flour	–	0.3 ± 0.05	14.36 ± 0.29	1.45 ± 0.02
Wheat bran	–	11.62 ± 0.43	16.45 ± 0.14	5.18 ± 0.09
Washed bran	–	20.3 ± 0.90	15.59 ± 0.20	5.27 ± 0.02

[a] Bran used here was washed wheat bran.