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Rearing *Tenebrio molitor* L. (Coleptera: Tenebrionidae) in the "Lunar Palace 1" during a 105-day multi-crew closed integrative BLSS experiment

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ABSTRACT

Yellow mealworm (*Tenebrio molitor* L.) is one of the animal candidates for space bioregenerative life support systems. In this study, *T. molitor* was involved in a 105-day multi-crew closed integrative BLSS experiment for a tentative rearing study. The results showed that the overall bioconversion rate (ratio of *T. molitor* gained to the total feed consumed) of *T. molitor* reared in the closed system was 8.13%, while 78.43% of the feed was excreted as frass. *T. molitor* reared in the closed system had a good nutritional composition. The eight essential amino acids (EAAs) in *T. molitor* larvae accounted for 41.30% of its total amino acids, and most EAA contents were higher than the suggested amino acid pattern recommended by the FAO/WHO. *T. molitor* sample obtained in this work was high in polyunsaturated fatty acids, and low in saturated fatty acids, indicating that the composition of fatty acids was beneficial to human health. In the open environment outside the experimental system, we simultaneously reared three parallel groups of larval *T. molitor* using the same feeding regime and temperature condition. Compared with *T. molitor* reared in the open environment, larvae reared in the closed system grew slower. With the course of time t, the growth rate of *T. molitor* in the open environment was $0.839e^{0.017t}$ times that of larvae in the closed system. This paper can provide data for future design and improvement of BLSS containing a *T. molitor* rearing unit.

1. Introduction

Human long-term habitation on the Moon and subsequently on the Mars in the future will rely on a Bioregenerative Life Support System (BLSS) for in-situ regeneration of oxygen, water and food. A BLSS integrates biotechnology and engineering control technology based on the principle of ecological system. It is an artificial ecosystem which consists of plants, animals and microorganisms. Essential substances needed by the crew members are regenerated in the system. Thus, it provides the crewmembers with a life support environment similar to the ecosystem on the Earth (Liu et al., 2012).

A series of studies on theories and technologies of BLSS has been conducted by researchers around the world. Before those technologies are applied to a space life support system, they should be firstly tested in an integrated BLSS experimental system with human participation built on the Earth, such as the experimental systems BIOS-3 in Russia (Gitelson et al., 2002) and LMLSTP in the U.S. (Lane et al., 2002) built in earlier years. However, in these two systems, rearing animals for the purpose of providing animal nutrients to humans as well as treating inedible plant biomass was not involved.

The idea of introducing insects into a BLSS for providing animal protein to astronauts was first put forward by Yu et al. (2008). There has been a series of study on rearing silkworms (*Bombyx Mori* L.) in BLSS (Tong et al., 2012; Tong and Liu, 2011; Yang et al., 2009). Our recent study reported that rearing yellow mealworm (*Tenebrio molitor* L.) in a BLSS is feasible and important (Li et al., 2013). *T. molitor* has a good nutritional profile with high protein value and can reach a relatively high growth rate and a high economic coefficient while adapting to higher rearing den-

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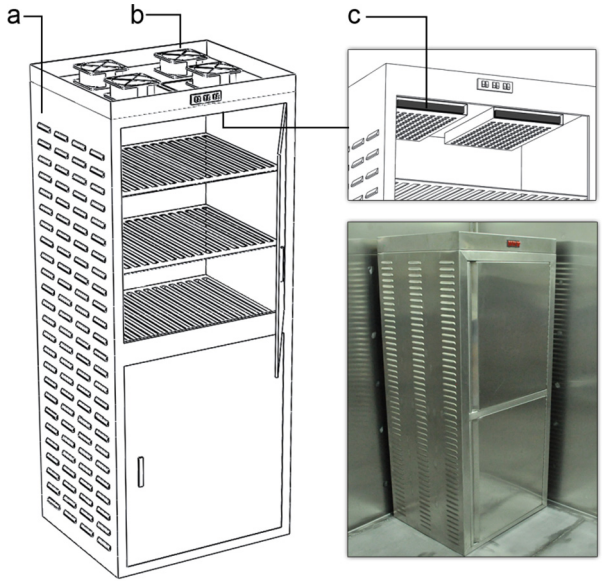

Fig. 1. Design and photo of the rearing chamber for *T. molitor*. a. Perforated stainless steel incubator. b. Exhaust fans. c. Air filters.

sities (Vantomme et al., 2012). It is insusceptible to disease, and requires less labor for its management (Vantomme et al., 2012). Most importantly, this insect can be fed on inedible plant biomass in a BLSS, and thus is an important link in the waste treatment process (Li et al., 2013).

In October, 2013, our team integrated the theories and technologies of BLSS obtained in our previous works, and constructed a closed integrative experimental facility, the Lunar Palace 1 (Stage I). From January to May, 2014, a 105-day multi-crew closed integrative BLSS experiment in Lunar Palace 1 (Stage I) was carried out successfully (Dong et al., 2015). In this 105-day multi-crew closed integrative BLSS experiment, *T. molitor* was involved into the closed system for a tentative rearing study. Biomass accumulation, bioconversion, elemental composition and nutritional quality of *T. molitor* larvae are presented. A comparison of growth curve model between *T. molitor* reared in this system and in an open environment was also made. This paper can provide reference data for future design and improvement of BLSS containing a *T. molitor* rearing unit.

2. Materials and methods

2.1. The T. molitor rearing chamber

Stage I of Lunar Palace 1 consisted of a plant cabin and a comprehensive cabin, in which there was a waste treatment room. As a link of waste treatment, the *T. molitor* rearing chamber was located in the waste treatment room. Temperature in this room was maintained at approximately 28 °C, which is fit for *T. molitor*'s growth. Hence, in order to save space and energy, temperature of the rearing chamber was not controlled separately. As shown in Fig. 1, the *T. molitor* rearing chamber composed of the perforated stainless steel incubator, exhaust fans and air filters (2-cm-thick activated charcoal filter). Air outside the rearing chamber flew through the grilles on both sides of the chamber under the negative pressure produced by the exhaust fans, and then returned to the atmosphere through the air filters.

2.2. Feed preparation

Feed for *T. molitor* came from three sources, i.e. pure wheat bran, mixed-fermented feed and fresh inedible plant biomass. Pure wheat bran served as the main feed for the first 30 days of rearing, and mixed-fermented feed was used instead afterwards. Fresh inedible plant biomass, such as peanut leaves, soybean leaves, strawberry leaves and old vegetable leaves, were used as supplementary feed for supplying moisture and nutrition to *T. molitor*.

The wheat bran as well as the straw were obtained from the common wheat (*Triticum aestivum* L.) planted in the Lunar Palace 1 (Dong et al., 2014a, 2014b), after harvesting, threshing and flour milling. The mixed-fermented feed was made through fermentation of a 1:1 mixture of wheat bran and straw, with the addition of 1% of microbial inoculants (Beijing Healthhead Science & Technology Co., Ltd., China). The moisture of the mixture was then adjusted to 60%. The mixture was sealed in a polyethylene bag for facultative anaerobic fermentation under 28 °C for 48 h, and then was dried in an 80 °C dryer, making the moisture decrease to approximately 18%.

2.3. Experimental procedure

In this study, during the last 30 days of the system start-up phase and the 105-day closed experiment of "Lunar Palace 1", one group of *T. molitor* eggs was sent into the system every 5 days. The rearing experiment lasted for 135 days, and thus 27 groups of *T. molitor* with different rearing durations were obtained. Larvae, frass, and the residual feed in each group were separated for dry weight determinations immediately after the experiment. According to these dry weight data, biomass accumulation and bioconversion of larval *T. molitor* in each group were then calculated. Lignocellulose components (cellulose, hemicellulose, and lignin) in feed and frass were analyzed for understanding the digestion of inedible biomass by *T. molitor*. Analyses of elemental composition and nutritional quality (including protein and fat contents, and amino acids and fatty acids components) in the harvested *T. molitor* larvae were carried out.

2.3.1. Rearing regime

In this study, because of the particularity of the whole closed experiment, we could only test the growth and bioconversion parameters of *T. molitor* immediately when the closed experiment ended. Thus, we observed the growth of *T. molitor* by creating differences in rearing duration among groups. Starting in the start-up phase, 30 days before the 105-day closed experiment began, one group of sterilized eggs was sent into the system every 5 days. The groups were numbered from group 1 to group 27 in time sequence.

T. molitor used in this experiment was a laboratory-reared breed (No. TA × TA) kept by our lab. The adults were kept in a 28 °C incubator for egg collection. The adults lay about 200 ± 50 eggs every 5 days on a 15-cm-diameter filter paper. Egg sterilization was performed on a clean bench. The eggs were washed off the filter paper into a meshed stainless steel tray with sterile distilled water. The tray of eggs was then shaken in a 2% Virkon® S disinfectant (Dupont China Holding Co., Ltd, China) for 10 minutes, followed by being washed five times with sterile distilled water. Then the tray was wrapped with a sterile polyethylene film and was sent into the Lunar Palace 1 through an airlock window.

For feeding, the crew members were asked to follow the regime and record strictly the type of inedible biomass added and the actual mass of feeding. The feeding regime for each group was as follows: 1) for the first 30 days of rearing, 0.5 g of wheat bran was added every 5 days, 2) after 30 days, 5 g of fermented feed was added every 5 days, and 3) as supplementary feed, fresh inedible plant biomass were added quantitatively and recorded: for 5–30 day, 0.2 g per group, and after that, 2 g per group. After the 105-day of closed experiment, larvae, frass, and the remaining