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Effect of High-Energy Food On Body Mass Regulation in Yunnan Red-Backed Vole, *Eothenomys miletus*

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WLZ and XMY conceived and designed the study. YG performed the experiments and acquired the data. WLZ and YG wrote the manuscript. All authors have read and approved the final manuscript.

Key words
***Eothenomys miletus*, Food quality, Body mass regulation, Energy metabolism, Digestive tract morphology, Adaptation strategies**

ABSTRACT

Quality or quantity of food in the environment can affect the energy metabolism of wild small mammals. In order to clarify the effect of high-energy food on the body mass, thermogenic capacity, and body composition in *Eothenomys miletus* from the Hengduan mountain region, and further determine the role of high-energy food on body mass regulation. The present experiment investigated the effects of a control group fed with standard feed and an experimental group fed with high-fat and high-sugar food on energy metabolism last for 28 days acclimation in *E. miletus*, body mass, resting metabolic rate (RMR), food intake, organ masses, and changes in digestive tract morphology in *E. miletus* were measured. The results showed that after 28 days of acclimation, body mass of the high-energy food group in *E. miletus* increased, and similar trends were observed in stomach weight, small intestine weight, and large intestine weight, but had no effect on RMR and food intake. All of the above results indicated that in a high-energy food environment, *E. miletus* maintain energy balance by increasing body mass and digestive tract weights, suggesting that *E. miletus* can enhance their adaptability to the environment by changing body mass and physiological indicators under different food quality conditions.

INTRODUCTION

The focus of research in the field of ecology is to determine how different environmental factors will affect the survival and distribution of mammals (Guan *et al*., 2018). Small mammals face many environmental factors in the wild, such as temperature, photoperiod, precipitation, etc. Among them, the stress of food quality or quantity cannot be ignored (Zhu *et al*., 2022). Quality of food will directly affect the energy intake and energy consumption of small mammals in the wild, and the nutritional composition of food determine the quality of food, generally, foods with high sugar and fat contents are considered high-quality, while foods with high fiber content are considered low-quality (Cui *et al*., 2020). When facing different food qualities, different species will choose different regulatory methods to adapt to changes in the environment. For example, *Apodemus chevrieri* facing high-fat and low-fat foods, the total weight with contents or without contents of the digestive tract, the weight without content and dry weight of the stomach, and some indicators of the large intestine and cecum were significantly higher in the low-fat food group than that of in the high-fat food group. By adjusting the morphology of the digestive tract, energy balance is maintained (Gao *et al*., 2013). Male rats domesticated with high-sugar and high-fat foods can alter the expression of hypothalamic neuropeptides, increase food intake, and lead to obesity (Fleur *et al*., 2010). But there is also a situation where wild species have not changed their body mass and body fat mass when faced with high-sugar and high-fat, such as *Phodopus sungorus* (Wade and Bartness, 1983), *Siberian hamsters* (McElroy *et al*., 1986). Under normal circumstances, small mammals reduced energy expenditure while increasing energy utilization to maintain their energy homeostasis when facing low-quality food (Yang *et al*., 2016).

Phenotypic plasticity refers to the ability of animals

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to change their phenotype in response to environmental changes, mainly manifested in morphology, physiology, behavior or other aspects (Rafael *et al*., 2013). Small mammals need to regulate their energy intake and expenditure to maintain their survival in the wild, mainly manifested in aspects such as body mass and food intake (Zhu *et al*., 2016). Because changes in animals mass can reflect their nutritional status and some adaptive adjustments made with environmental changes, and it has important effects on various parameters such as physiology, morphology, and behavior of animals (Nagy, 1995). Among the environmental factors that affect animals body mass, food quality is one of the important factors; the regulation of body mass in the face of different food quality has species specificity. High-fat foods can significantly increase the mass of *Meriones unguiculatus* (Liu and Liu, 2005). Compared to the low-fat group fed *Cricetulus barabenis*, the high-fat group adapted to changes in food by reducing food intake and increasing body fat (Bi *et al*., 2018). After feeding high-fiber rabbit feed, *Niviventer lotipe* did not increase their food intake to compensate for the decrease in digestion rate, the weight and the length of the digestive tract, especially the small intestine and cecum, did not increase adaptively. Although animals can save energy by reducing basal metabolic rate and non-trembling heat production, their energy utilization efficiency is too low to maintain energy balance, resulting in a significant weight loss (Chi *et al*., 2023). Moreover, changes in body mass and morphology of organs and digestive tract are another pathway for animals to face changes in food in the wild, according to the integrated processing hypothesis, when food quality changes, herbivorous species will alter their food intake, alter the length and weight of the digestive tract to alter food retention time, and maintain energy homeostasis (Batzli *et al*., 1994). It compared the relationship between the digestive tract and food quality of 19 rodents and found that dietary habits were one of the key factors affecting the morphology of the digestive tract (Perrin and Curtis, 1980). In the wild, changes in the size of the digestive tract had a significant impact on energy stability, and the ability of the digestive tract to quickly adjust under the influence of food quality was crucial for the survival of animals.

Hengduan Mountain is located at the intersection of the ancient northern and eastern regions, belonging to the transitional zone from the eastern plain hills to the Qinghai Tibet Plateau. With significant elevation differences and a complex and variable climate environment, it has become one of the regions with the richest vegetation diversity in China and even the world (Zhang *et al*., 2016). Moreover, this area has the characteristics of small annual temperature difference, large daily temperature difference, and abundant food resources, making it one of the most abundant areas for mammals. *Eothenomys milletus* is a rodent belonging to the family Cricetidae, endemic to China and an inherent species in the Hengduan Mountains. It is a nocturnal animal and lives in camping caves. In previous studies conducted in our laboratory, we investigated the effects of high sugar foods on energy metabolism and digestive tract in *E. milletus* under different altitude conditions (Gong *et al*., 2021) and explored the adaptive regulation of physiological characteristics such as metabolic rate and digestive tract morphology in *E. milletus* under different environmental conditions (Chen *et al*., 2023; Ren *et al*., 2020; Zhu and Gao, 2017; Gao *et al*., 2013). However, there is still no knowledge on *E. milletus* in the same region will regulate their body mass and energy metabolism when facing high-sugar and high-fat foods, as well as their digestive tract morphology to adapt to the environment, therefore, the aim of this experiment was to explore the effects of high-sugar and high-fat foods on the survival strategies of *E. milletus* in the Jianchuan area of Dali, and further understand their survival strategies when facing different food conditions. Based on the above, we believed that when faced with high-sugar and high-fat foods, *E. milletus* may adjust their body mass and digestive strategies to adapt to changes in food quality and maintain survival.

MATERIALS AND METHODS

Experimental design

E. milletus was caught in shrubs and farmland in Jianchuan County, Dali City (26 ° 43'95 "N, 99 ° 75'03" E, altitude 2590 m, the annual average temperature is 12.3 ℃, and the annual average rainfall is 731.1 mm), after disinfecting and killing fleas, bring them back to the animal room of Yunnan Normal University for single box feeding (the size of the mouse box is 26×16×15cm). The feeding environment has a photoperiod of 12L: 12D, and the temperature is controlled at around 25 ± 1 ℃. All animals were fed with standard feed (produced by Kunming Medical University), with free access to water and drinking water. The animals used in this experiment are all adult individuals in the non-reproductive period. After two weeks of adaptation in the laboratory, the voles were randomly divided into two groups: high sugar and high fat group (n=7;♀:♂=4:3), acclimation temperature of 25 ± 1 ℃, moderate light, fed with high-sugar and high-fat feed and acclimated for 28 days; the control group (n=7;♀:♂=4:3), with an ambient temperature of 25 ± 1 ℃ and moderate light exposure, was fed with standard feed (Table I) and acclimated for 28 days. After the experiment, the resting metabolic rate (RMR) and food intake of the animals were measured. Then, the animals were euthanized, and their body mass, organ weight, and digestive tract weight were measured. There was no significant difference in body mass between the experimental group and the control group before the experiment ($P>0.05$).

Table I. High fat and high sugar feed and standard feed compositions.

Food ingredients	Standard food	High fat and sugar
Sucrose (%)	2.5	23.4
Crude fat (%)	6.2	21.4
Fibre (%)	9	6.2
Starch (%)	17.5	3.2
Crude protein (%)	20.8	16.8
Neutral washing fiber (%)	21.5	10.5
Acid washing fiber (%)	12.5	10.5
Ash content (%)	10	8
Calorific value (kj/g)	17.5	27.5

Measurement of body mass and food intake

Body mass was measured using an analytical balance (Switcherland, AB204-S type) to the nearest 0.01 g. According to the food balance method, replace the rat box with a clean one at 9am on the same day and add 10 ± 1g of dried feed. After 24 h, collect the feed from the mouse box at 9am the next day and dry it to a constant weight, calculate by subtracting the remaining feed from the weight of the feed added the day before, and during this period, *E. milletus* can freely consume the feed.

Measurement of RMR

RMR was measured through an 8-channel FMS portable respiratory system (Sable Sys tems International, Inc). Fasting for 2-4 h before measurement, placed the animal in a metabolic chamber (1.5 L) with an air flow rate of 200 mL/min, used an artificial climate box (SPX-300 type, Shanghai Boxun Medical Equipment Factory) to control the experimental temperature at 25.0 ± 0.5 °C, animals adapt to a resting state in the metabolic chamber for 30 min, and open the Experiment Data software to measure their metabolic rate for 4 rounds, each lasting 15 min. Data for every 5 min were recorded for a total of 60 min. After the experiment, export the experimental data and select the lowest oxygen consumption as its RMR.

Measurement of organ weights and digestive tract morphology

Quickly dissect animals, carefully separated the liver, heart, kidneys, cecum, large intestine, small intestine, and stomach, and weigh them in a timely manner, measured the length of each digestive tract, transferred to a low-temperature refrigerator at -80 ℃ for storage and backup.

Statistical analysis

Using SPSS 26.0 software package for data analysis, all data conforms to normal distribution and homogeneity of variance. Because there is no significant difference in the heat production indicators between males and females, the data was combined for use. All data were analyzed using independent sample t-tests; and perform linear regression analysis between organ weight, digestive tract weight, and body mass. The results were expressed as mean ± standard error, with $P<0.05$ indicating significant differences.

RESULTS

Body mass and organ weights

Compared with the control group, the experimental group showed a significant increase in body mass of the *E. milletus* (t=-5.473, P>0.01), But its liver weight (t=-2.075, P>0.05), heart weight (t=-0.398, P>0.05), and kidney weight (t=-0.197, P>0.05) had no significant difference between two groups (Fig. 1A, B). And a linear correlation analysis was conducted between body mass and the weight of various organs, and the results also showed that body mass had no relation to liver weight (Y=0.698+0.026X, P>0.05), heart weight (Y=0.290+0.003X, P>0.05), or kidney weight (Y=0.331+0.002X, P>0.05).

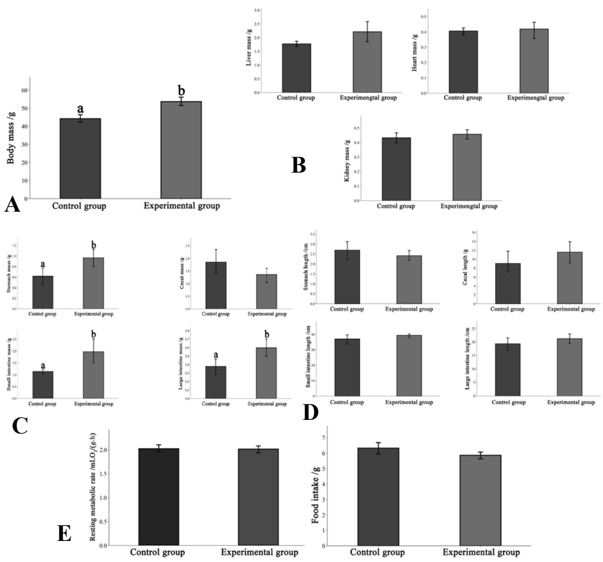

Fig. 1. Effect of high energy food on body mass (A), organ (liver, heart and kidney) (B), digestive tract (stomach, small intestine, large intestine and cecum) mass (C), digestive tract (stomach, small intestine, large intestine and cecum (D), RMR and food intake (E) of *E. milletus.*

Weights of the digestive tract

The weight of the digestive tract of *E. milletus* fed with high-energy food showed significant differences from the control group, with the stomach weight of the experimental group significantly higher than that of the control group (t=-2.801, P>0.05), And the small intestine (t=-2.867, P>0.05), and the large intestine (t= -3.013, P>0.05) the weight showed the same result. However, the cecum is different. Compared with the control group, the experimental group showed a decrease in cecum weight, but did not reach a significant level (t=1.699, P>0.05) (Fig. 1C). Linear analysis was also conducted on the correlation between mass and digestive tract weight, and it was found that the increase in body mass in the high-fat and high-sugar group was positive related to stomach weight (Y= -0.799+0.32X, P>0.05), and large intestine weight (Y= -0.315+0.016X, P>0.05) (Fig. 2).

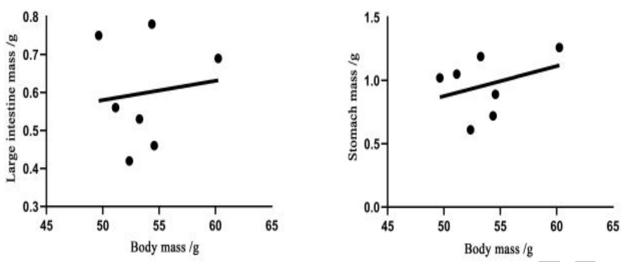

Fig. 2. Linear regression analysis between body mass, stomach mass, and large intestine mass in *E. milletus*.

Digestive tract lengths

Although the digestive tract weight of *E. milletus* fed with high-fat and high-sugar groups showed differences from the control group, in terms of digestive tract length, except for stomach length, the digestive tract length of the control group was smaller than that of the experimental group, but did not reach a significant level of difference (stomach: t=0.933, P>0.05; small intestine: t=-1.354, P>0.05; large intestine: t=-1.137, P>0.05; cecum: t=-1.149, P>0.05) (Fig. 1D).

RMR and food intake

There was no significant difference in the resting metabolic rate between the experimental group and the control group of *E. milletus* (t=0.163, P>0.05). Although the food intake of the high fat and high sugar group of *E. milletus* decreased, it did not reach a significant level (t=2.048, P>0.05) (Fig. 1E).

DISCUSSION

The nutritional niche of animals consists of energy and nutrient requirements, food selection, digestion strategies, and other aspects (Hume, 2002). The change in mass is the most intuitive physiological response of animals to changes in the external environment. With changes in the environment and acclimation conditions, the mass of animals also changes accordingly. For small mammals, maintaining a stable weight will affect their survival and reproductive choices. There are many factors that affect the body mass of small mammals in the wild, and food quality is one of the most important factors. Generally, when faced with changes in food quality, animals will also adapt to the environment by changing body mass, such as high-fat foods can reduce the intake and increase digestion rate of *Lasiopodomys brandtii,* but do not significantly affect body mass, basal metabolic rate, non-shivering thermogenesis (NST), uncoupling protein 1 (UCP1) content, and serum leptin; serum leptin is not correlated with energy intake, but is positively correlated with body fat content (Zhao *et al*., 2008). *N. lotipes* experienced significant body mass loss when faced with low-quality foods such as high-fiber foods due to the inability to maintain energy balance (Chi *et al*., 2023). *A. chevrieri* fed high-fat foods increase body fat and maintain stable weight to enhance survival ability (Gao *et al*., 2013). In this experiment, *E. milletus* fed with high-fat and high-sugar also chose to gain body mass to cope with high-quality food, which showed the same results as those fed with high-sugar food (Gong *et al*., 2021). Moreover, the changes in the weight of internal organs and digestive tract have a significant impact on the mass of animals and can serve as important indicators for adapting to the environment (Wang and Wang, 2001). In our study, the weight of organs such as the liver and kidneys, as well as the digestive tracts such as the large intestine and stomach, also increased. Through linear analysis, it was found that the increase in body mass was related to the increase in stomach and large intestine weight. In addition, when facing high-quality food, small mammals usually choose to reduce their food intake to reduce energy intake, or increase their metabolic rate to increase energy support, all of which are aimed at maintaining energy balance. The same goes for *E. milletus*, although their metabolic rate did not increase when fed high-sugar and high-fat foods, their food intake also decreased similarly. Therefore, in this experiment, the increasing of body mass was caused by a synergistic increase in the masses of digestive and liver organs, although there was no increase in energy expenditure, in order to avoid excessive mass gain, we still chose to reduce energy intake to reduce the risk of predation.

According to the integrated processing response hypothesis, when there is a change in food quality, animals will maintain digestive intake by changing intestinal retention time, digestibility, and other methods (Yin *et al*., 2019). It summarized the digestive adaptability characteristics of herbivorous animals when facing food changes: firstly, increasing the overall capacity of the digestive tract; secondly, relative changes in the length of the small intestine, and thirdly, relative changes in the length of the cecum (Vorovtsov, 1962). The longer small intestine of *Niviventer niviventer* is designed to better absorb high-quality food (Chen *et al*., 2014). The small intestine length and total digestive tract length of *Passer montanus* that consume high-quality food were longer than those that consume low-quality food, which was beneficial for increasing the time for food to be digested and absorbed in the digestive tract and increasing energy intake (Yang and Shao, 2011). The energy capacity of the digestive tract to accommodate and process food is one of the key factors limiting animal energy balance. In this experiment, when facing high-fat and high-sugar foods, the length of the large intestine, small intestine, and cecum increased except for the stomach. By increasing the length of the digestive tract and thus increasing the retention time of food, the digestive and absorption capacity also increases, thus ensuring energy balance even in low intake situations.

In addition to altering the length of the digestive tract, another approach is to alter the structure and tissue of the digestive tract wall, which can be measured by the weight of the digestive tract tissue (Woodall, 2009). The weight of the contents in the digestive tract is one of the important indicators for measuring the daily food intake of animals (Green and Millar, 1987). The total digestive tract contents and contents of high-fat diet fed high-altitude *A. chevrieri* were significantly reduced (Gao *et al*., 2013). Low-quality food significantly increases the content weight of the cecum in *Microtus chrogaster* (Gross *et al*., 1985). The stomach is the place where animals temporarily store food and digest and absorb it (Green and Millar, 1987). Due to the increased weight of animals, there is an increased risk of predation. Therefore, increasing the capacity of the stomach can lead to a one-time intake of more food, thereby reducing foraging time and reducing the risk of predation (Perrin and Curtis, 1980; Li *et al*., 2003). The stomach content and weight of *E. milletus*, fed on high-energy food were significantly higher than those of the control group. Due to the increase in body mass, their movement and foraging behaviors were hindered. Therefore, a larger stomach can effectively reduce the frequency of foraging outside and avoid the risk of arrest. The cecum is the main site for digesting and absorbing cellulose, which can reflect changes in food quality (Schieck and Millar, 1985). Due to the low-fiber content in the high-sugar and high-fat feed fed to the experimental group, there was no significant difference in cecum weight compared to the control group. The small intestine is an important site for digestion and absorption, and its changes best reflect the energy demand (Wang *et al*., 1995). The small intestine was an important part for digestion and absorption, and changes in the small intestine can best reflect the demand for energy (Wang *et al*., 1995). After 28 days of high-sugar and high-fat feeding, the small intestine weight of the experimental group was significantly higher than that of the control group, indicating that when faced with different food qualities, *E. milletus* will regulate the morphology and energy balance of the digestive tract to adapt to the environment. The large intestine is the main part that absorbs nutrients after fermentation and decomposition by the cecum and colon, and its size is closely related to food quality (Liu t al., 2007). Similarly, high-fat and high-sugar also adapt to high-quality food by up regulating the content weight of the large intestine, and the increase in the weight of the large intestine also synergistically increases the body mass of *E. milletus*.

CONCLUSION

In conclusion, animals undergo a series of physiological and ecological adjustments to maintain energy balance and survive and reproduce in different environments. Through this experiment, it was found that when faced with high-quality food, the body mass of *E. milletus* increased, and similar trends were observed in stomach weight, small intestine weight, and large intestine weight. However, it had no effect on resting metabolic rate and food intake. This indicated that the *E. milletus* maintain energy balance by increasing and digestive tract weight, suggesting that the *E. milletus* can enhance its adaptability to the environment by changing body mass and physiological indicators under different quality food conditions.

DECLARATIONS

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IRB approval

This study was approved by the Committee from

School of Life Sciences, Yunnan Normal University (13-0901-011).

Ethical statement

All animal procedures were within the rules of Animals Care and Use Committee of School of Life Sciences, Yunnan Normal University.

Statement of conflict of interest

The authors have declared no conflict of interest.

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