




Research Article

Preliminary study on the immunological function in *Eothenomys miletus* from Hengduan mountain region

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Abstract: The immune functions of animals play an important role in their own survival, enabling animals to resist the invasion of foreign bodies and maintain the homeostasis of their internal environment. *Eothenomys miletus* is a typical representative of mammals in the Hengduan mountains of China, in order to preliminarily explore the immunological function of *E. miletus*, the present study took 12 *E. miletus* as the research object, 6 for experimental group, injected Phytohemagglutinin (PHA), 6 for the control group, injected with Phosphate-Buffered Saline (PBS), to determine the degree of swelling of the posterior foot pads, the wet organ mass and the body composition. The results showed that the PHA response decreased with the prolongation of time in *E. miletus*, and the 6 hours after injection reached the maximum, reaching 0.89, which gradually decreased after 6 h. And the difference in wet organ mass and body composition between the control group and the experimental group was not obvious. In conclusion, injection of PHA makes immune system respond to the interference of adverse conditions and maintain its normal survival in *E. miletus*. The current study can provide a theoretical basis for the subsequent study of the immunological function of *E. miletus* in Hengduan mountains under different conditions.

Keywords: *Eothenomys miletus*; cellular immunity; phytohaemagglutinin (PHA) response; Hengduan mountains

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1. Introduction

The immune function of an animal refers to the function of its immune system in responding accordingly to foreign pathogens or foreign bodies to protect its homeostasis from being destroyed. The immune system protects animals from harmful factors from the outside world and removes foreign bodies from within, playing an important role in ensuring the survival of animals (Zhang et al., 2017). Whether the immune function is normal is crucial for the healthy survival of the animal body, it can reflect the animal's viability to a certain extent, and is an important influencing factor in the suitability of the animal (Sheldon et al., 1996). There are many factors that affect animal immunity, and studies have shown that vitamin A can affect the immunity of animals (Roy et al., 2019), not only in innate immunomodulation, but also in adaptive immunomodulation (Kyle et al., 2018). Hypothermia inhibits the cellular immunity of third-line hamsters (Shen et al., 2021). With the prolongation of fasting time, the phytohaemagglutinin response (PHA) response of long-clawed gerbils was gradually sup-

pressed, and the fasting time had a significant impact on the immune function of long-clawed gerbils (Xu et al., 2015), and different components of the immune system also showed different seasonal variation patterns, and the cellular immunity of female haddial hamsters was lowest in summer (Xu et al., 2021).

Eothenomys miletus is endemic to China and is a typical representative of mammals in The Hengduan Mountains of China. With the increase of altitude, the temperature decreases, precipitation increases, with the characteristics of dry and wet seasons, small annual temperature difference, large daily temperature difference, three-dimensional climate and other characteristics (Wang et al., 2015), so that the physiological indicators of the *E. miletus* have certain differences, for its individual, organ, hormone level and energy countermeasures and environmental adaptation under various conditions Have a relatively in-depth study(Zhu et al., 2016; Zhang et al., 2018; Zhu et al., 2008), but in the study of immunological function is relatively small, this study takes the *E. mletus* as the experimental object, by injecting PHA, The degree of swelling, organ freshness and weight of body components in the posterior foot pads were measured, and their immune function was preliminarily explored.

2 Materials and methods

2.1 Materials

The experimental animals were all adult *Eothenomys miletus* in the non-breeding stage, which were captured in the farmland and bushes near Chenggong District, Kunming City, Yunnan Province in 2021 (102 ° 45 ' E, 24 ° 42 ' N), brought back to the animal room of Yunnan Normal University for single cage rearing (26 cm× 16 cm×15 cm), free food and drinking water, photoperiod of 12L:12D, and began experiments after the surrounding animals stabilized. All animal procedures were within the rules of Animals Care and Use Committee of School of Life Science, Yunnan Normal University. This study was approved by the Committee (13-0901-011).

2.2 Methods

2.2.1 Detection of cellular immunity (PHA response).

PHA can be used as an important indicator of cell-mediated immunity (Zhang et al., 2011), and this study detected the cellular immune response of by injecting pha implant PHA into the posterior left foot pad. The specific operation method is as follows: the thickness of the left posterior foot pad of the large *E. miletus* is measured with a digital electronic micrometer, and each *E. miletus* is measured 6 times, and the data is recorded and the average is taken as the thickness of the front foot pad for injection. Wipe and sterilize the rear left foot pad of the *E. miletus* with 75% alcohol, and then inject 0.03 mL of PHA solution (sterile phosphate buffer (PBS) containing 0.1 mg PHA with a PH value of 7.4) at the left rear foot pad, and the control group injects only PBS buffer. The thickness of the left posterior foot pad of the giant *E. miletus* was measured at 6h, 12h, 24h, 48h and 72h after injection, 6 times at each time point, and finally the average value was taken as the experimental data. The formula for the PHA reaction is: (post-injection foot pad thickness - injection prefoot pad thickness) / injection prefoot pad thickness (Xu et al., 2010; Bellocq et al., 2006)

2.2.2 Body composition and organs

The rat is weighed and the neck is severed, the brain is removed intact and weighed, and then dissected. The heart, liver, spleen, lungs and kidneys are completely removed, the adhesion connective tissue is carefully removed, and after dipping dry on the filter paper, weigh the fresh weight (accuracy is 0.1 mg). Isolate the stomach, small intestine, large intestine and cecum, carefully remove the mesenteric membrane and other connective tissues of each organ, flatten to the maximum length, do not stretch, and then measure the length of each part (accuracy of 1 mm), each organ of the digestive tract is dried on the filter paper, placed on aluminum foil paper, weigh the weight of the contents; then use dissection scissors to longitudinally cut the organs, rinse the contents with normal saline, dry them with filter paper, and weigh the weight of the contents (Wang D H et al.,2000). The separated brown adipose tissue, subcutaneous fat, peritoneal fat, intestinal fat, and retroperitoneal fat are the total fat weight of the *E. miletus*, and the subcutaneous fat weight, peritoneal fat weight, intestinal fat weight, retroperitoneal fat weight, and total fat weight are divided by the final weight, that is, the fat content of each part (Xu et al., 2017). The whole process is well documented with experimental data.

2.3 Statistical Methods

All experimental data were processed using spss26.0 software analysis package, and the data were statistically processed with one-way ANOVA, and the results were expressed as mean \pm standard deviation (mean \pm SE), of which $P < 0.05$ was significantly different and $P < 0.01$ was very significant.

3 results

3.1 PHA reaction

The difference in PHA responses between the experimental group and the control group in the *E. miletus* reached a very significant difference at the 6th hour ($F=15.214$, $P=0.006 < 0.01$), and the difference in PHA reactions between the two groups at the 24th hour was not significant ($F=4.302$, $P=0.077$). The rats in the experimental group reached the maximum response to PHA at the 6th hour, and the difference in PHA response between the 6th hour and the 12th hour reached a very significant ($F=12.490$, $P=0.002 < 0.01$), and the PHA response gradually decreased after the 6th hour. The rats in the control group reacted only for 6 hours after PBS injection, and there was basically no response after 6 hours (**Fig 1**).

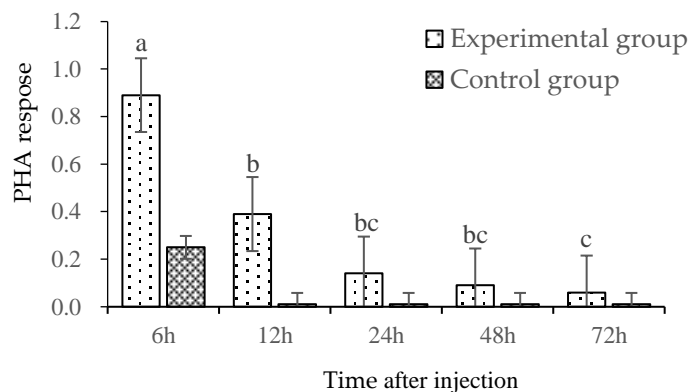


Fig 1 PHA response in *Eothenomys miletus*

3.2 Body weight and fat content

There was no significant difference in the initial weight of the rats before injection, and there was no significant change in the body weight of the rats 72 hours after injection of PHA or PBS ($F=0.225$, $P=0.648$; $F=1.110$, $P=0.333$). After 72 hours of injection of PHA and PBS in the experimental group and control group, there were no significant differences in the final weight, carcass weight, subcutaneous fat weight and content, peritoneal fat weight and content, mesenteric fat weight and content, and retroperitoneal fat weight and content between the two groups. ($P>0.05$, Table 1)

Table1 Body composition of *Eothenomys miletus*

parameters	experimental	control	Statistical value
sample size	6	6	P
Initial body mass(g)	44.61±4.36	45.15±2.88	0.925
Final body mass(g)	43.25±4.91	43.15±2.06	0.986
Wet carcass mass(g)	27.29±2.30	31.25±1.90	0.242
Subcutaneous fat (g)	2.44±0.82	1.25±0.43	0.277
Subcutaneous fat content(%)	0.051±0.013	0.030±0.011	0.278
Perigonadal fat(g)	0.66±0.22	0.39±0.09	0.342
Perigonadal fat content(%)	0.014±0.004	0.009±0.003	0.302
Mesenteric fat(g)	0.40±0.15	0.2±0.04	0.28
Mesenteric fat content(%)	0.008±0.003	0.005±0.001	0.278
Retroperitoneal fat(g)	0.20±0.03	0.15±0.07	0.501
Retroperitoneal fat content(%)	0.005±0.001	0.003±0.001	0.347
Total body fat(g)	3.942±1.131	2.245±0.671	0.268
Total body fat content(%)	0.085±0.016	0.053±0.017	0.232

3.3 Body composition and gastrointestinal morphology

After 72 hours of injection of PHA and PBS, there were no significant differences in the morphology of brown adipose tissue, brain, heart, liver, spleen, kidney, lungs, and digestive tract in the control group and the experimental group of *E. miletus* ($P>0.05$, table 2).

Table 2 Wet organ mass of *Eothenomys miletus*

parameters	experimental	control	Statistical summary
sample size	6	6	P
BAT(g)	0.25±0.04	0.26±0.13	0.933
Brain(g)	0.60±0.06	0.72±0.03	0.191
Heart(g)	0.28±0.04	0.27±0.02	0.860
Liver(g)	2.86±0.85	1.89±0.13	0.350
Spleen(g)	0.05±0.01	0.06±0.01	0.232
Kidneys(g)	0.36±0.03	0.35±0.02	0.309
Lungs(g)	0.30±0.02	0.32±0.02	0.444
Stomach with contents (g)	1.41±0.27	1.46±0.30	0.912
Stomach (g)	0.39±0.04	0.46±0.07	0.402
Stomach length (g)	2.28±0.14	2.25±0.10	0.874

Small intestine with contents(g)	1.57±0.16	1.59±0.19	0.937
Small intestine(g)	0.84±0.11	0.81±0.11	0.828
Small intestine length(cm)	36.98±1.88	35.71±1.28	0.614
Large intestine with contents(g)	0.68±0.10	0.59±0.13	0.629
Large intestine (g)	0.40±0.04	0.42±0.07	0.842
Large intestine length (cm)	17.74±0.88	16.23±1.55	0.400
Caecum with contents (g)	1.66±0.28	1.51±0.10	0.669
Caecum (g)	0.42±0.04	0.39±0.06	0.667
Caecum length (cm)	10.66±1.06	9.25±0.90	0.361

4 Discussions and Conclusions

In terms of cell-mediated immunity, phytoagglutinins (PHA) are a widely used indicator that reflects the response to immune challenges by the degree of thickening of foot pads or skin tissue at the injection site (Zhu et al., 2008). Generally, the response is greatest at the 6th h after injection of PHA and can be used as a cellular immunity of experimental animals (Xu et al., 2015). From the experimental results, it was found that the rat had a significant response to the injection of PHA, and the response reached the maximum at 6 hours, which was 0.89, and gradually decreased with the prolongation of time. Through statistical analysis, it was found that the difference in PHA response between the experimental group and the control group at the 24th hour was not significant, and the measurement of the time point after 24 hours of injection of plant lectins could be considered in the future study of the immunological function of the rat.

Although there is no obvious difference in body composition and organ fresh weight between the experimental group and the control group in this study, we can see that the liver, colon length and cecum length of the experimental group of *E. miletus* may respond to PHA, and the average weight is relatively higher than that of the control group. Diet restriction has been found to significantly reduce perirenal fat, retroperitoneal fat, and overall lipid weight, as well as humoral immunity in hamsters, and lower energy storage may not be sufficient to sustain expensive physiological processes, including the energy needed for the immune response (Xu et al., 2015). Female long-clawed gerbil PHA responses are inhibited from 2 days after fasting and gradually decrease with prolonged fasting (Xu et al., 2015). The overall lipid weight of three-line hamsters decreased significantly at low temperatures and inhibited the cellular immunity of third-line hamsters (Shen et al., 2021). Fasting had a significant effect on the weight of contents in the total digestive tract of *E. miletus* (Gao et al., 2013). The tendency of the digestive tract of the *E. miletus* is the longest in winter and the shortest in summer, showing seasonal variations. (Zhu et al., 2009) Therefore, in the future research on the immunological function of the giant *E. miletus* in the Hengduan Mountainous Area, it is necessary to consider the physical and chemical state of the body of the giant *E. miletus* and different domestication conditions.

In conclusion, the injection of PHA makes the immune system of *E. miletus* respond to the interference of adverse conditions and maintain its normal survival. PHA response decreased with the prolongation of time in *E. miletus*, and the 6 hours after injection reached the maximum, reaching 0.89, which gradually decreased after 6 h. But the difference in wet organ mass and body composition between the control group and the experimental group was not obvious. This experiment is only a preliminary study of the immunological function of the *E. miletus*, and the

immunological function of the large *E. miletus* in different areas of Hengduan Mountain and various domestication conditions needs to be further explored in the future.

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