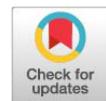




Faster Response to High-Fat Diet in Body Mass Regulation from Lower Altitude Population in *Eothenomys miletus* from Hengduan Mountain Regions



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ABSTRACT

Small mammals usually showed physiological and behavioral adaptations to cope with seasonal environmental changing, such as temperature, photoperiod, and food etc. To investigate the physiological and behavioral adaptation strategies in *Eothenomys miletus* of different areas from Hengduan mountain regions in response to high-fat diet, *E. miletus* in Jianchuan (JC) and Xianggelila (XGLL) were fed a high-fat (HF) diet for 4 weeks and returned to a low-fat (LF) diet for another 4 weeks, body mass, food intake, resting metabolic rate (RMR), activity behavior, serum leptin levels, hypothalamic neuropeptide expressions and body compositions were measured. The results showed that regions and HF diet affected body mass, food intake and RMR significantly, HF diet increased body mass in *E. miletus*, while regions had significant effect on activity behavior, but HF diet had not affect activity behavior. Regions and HF diet also showed remarkable effects on leptin and hypothalamus Neuropeptide Y (NPY) expression, and leptin positively correlated with body mass and negatively correlated with NPY expression. Moreover, *E. miletus* from two regions showed some physiological differences, such as RMR and food intake in XGLL were higher than that of JC, but body mass was lower than that of JC. Body mass reduced quickly in JC after re-feeding LF diet, while it decreased body mass gradually in XGLL. All of the results showed that body mass increased in *E. miletus* of two regions when faced with HF diet, which returned to the control levels after re-feeding LF diet, showing higher phenotypic plasticity. Leptin and NPY expression may play an important role in body mass regulation. Phenotypic differentiations in *E. miletus* of two regions may be closely related to food resources, altitude and temperature of Hengduan mountain regions.

INTRODUCTION

Efficiency of energy intake and expenditure were essential for survival in small mammals (Mitchell *et al.*, 2016; Hu *et al.*, 2018). Food quality was an important environmental factor, which affected survival and reproduction in the wild small mammals (Delciellos *et al.*, 2018). Previously studies showed that fat content was closely related to body fat mass accumulation, high-fat (HF) diet increased energy intake and body mass in animals (Yoo *et al.*, 2006). However, different animals had different body mass regulation for HF diet. For example, rats and

mice fed *ad libitum* or HF diet increased body mass significantly (Rothwell and Stock, 1988), but HF diet did not affect body mass in *Meriones shawi* (El-Bakry *et al.*, 1999). Therefore, it can be speculated that different species had different physiological adaptation strategies to cope with changes of food quality.

Leptin, is an adipostatic signal linking energy metabolism and food intake regulation (Flier and Maratos-Flier, 2017). It entered the brain through blood circulation and affected the neuropeptides expressions such as hypothalamus neuropeptide Y (NPY), agouti-related peptide (AgRP), pro-opiomelanocortin (POMC), cocaine and amphetamine regulated transcript peptide (CART), thus controlling food intake and energy balance in small mammals (Trayhurn and Bing, 2006). Recent studies showed that serum leptin levels were positively correlated

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Authors' Contribution

GX-n and JT carried out the studies of body mass, food intake and activity behavior. ZD carried out the studies of sex ratio, serum leptin levels, hypothalamus neuropeptide expressions and body compositions. ZW-I conceived of the study and participated in its design, coordination and drafted the manuscript.

Key words

Eothenomys miletus, High-fat diet, Serum leptin, Hypothalamic neuropeptide, Activity behavior

with body mass, and higher leptin levels inhibited animals' food intake (Zhao and Wang, 2009; Zhu et al., 2017). Therefore, leptin could inhibit NPY/AgRP pathway and stimulated POMC/CART pathway to regulate energy balance (Pérez-Maceira et al., 2016). Resting metabolic rate (RMR) refers to the minimum number of calories needed basic functions, including breathing and circulation (Splinter and Wilson, 2019), which is an important physiological indicator for field small mammals to respond to different environmental pressures (Zhang et al., 2019). Activity behavior of animals had great significance for adapting environmental changes (Kingsbury et al., 2019). Mammals usually increased the activity behavior under low temperature or food restriction. For example, food restriction increased activity significantly in *Mus musculus* (Blank and Desjardins, 1985). HF diet reduced activity behavior significantly in rats (Chen et al., 2014).

Eothenomys miletus belongs to the genus *Eothenomys* is an inherent species in Hengduan mountain regions. *E. miletus* was a nocturnal rodent, and its main food was fresh pulp plants and roots of grass. Hengduan Mountain regions is located the boundary between the Palaearctic region and the Oriental region (Zhu et al., 2017), which altitude and climate changes dramatically, and small mammals in different locations may have different physiological characteristics (Ren et al., 2020). Previous studies showed that changes of RMR and relative fatness in *E. miletus* from different regions were related to environmental temperature and food resources (Mu et al., 2015); temperature and food restriction had significant effects on physiological characteristics in *E. miletus* (Zhu et al., 2010, 2014). In the present study, we selected *E. miletus* from two areas (Jianchuan, JC and Xianggelila, XGLL) with significant phenotypic differentiation (Zhang et al., 2019). XGLL has higher altitude, lower annual average temperature (5.5°C), less crops and vegetations, so food conditions in winter were relatively poor; while JC has lower altitude, higher average annual temperature (13.9°C), higher vegetations and almost no snow cover, so food resources in winter were relatively better, so the present study was to investigate the effects of HF diet on physiological and behavioral plasticity, body mass, food intake, RMR, activity behavior, serum leptin levels, hypothalamic neuropeptide expressions and body compositions were measured. We hypothesized that *E. miletus* would respond to HF diet by changing body mass, serum leptin levels, activity behavior and adjusting the hypothalamic neuropeptides genes expressions. We predicted that *E. miletus* may change the physiological responses to regulate body mass, and there were regional differences in physiological changes in *E. miletus* in response to high-fat diet and refeeding.

MATERIALS AND METHODS

Samples

E. miletus were obtained from farmland in XGLL (99°83'E, 27°90'N, altitude 3321 m) and JC county (99°75'E, 26°43'N, altitude 2590 m) in January 2019. *E. miletus* were maintained at a room temperature of 25±1°C, under a photoperiod of 12L:12D (with lights on at 08:00), food (standard mice chow pellets; produced by Kunming Medical University, Kunming) and water were provided *ad libitum*. All pregnant, lactating or young individuals were excluded in the present study. HF diet used in the experiment was prepared from the standard mice chow pellets with about 18% soybean oil, and low-fat (LF) diet was the standard mice chow pellets, the main food components were shown in Table I. All animal procedures were compliance with the Animal Care and Use Committee of School of Life Science, Yunnan Normal University. This study was approved by the Committee (13-0901-011).

Table I. Composition of experimental diets.

Contents	Low-fat diet	High-fat diet
Crude fat (%)	6.2	21.4
Crude protein (%)	20.8	17.6
Neutral detergent fiber (%)	21.5	19.6
Acid detergent fiber (%)	12.5	10.6
Ash (%)	10.0	8.5
Caloric value (KJ/g)	17.5	19.7

Experiment 1

Effects of HF diet on body mass, food intake, RMR and activity behavior in *E. miletus*. Sixteen adult *E. miletus* of XGLL (n=8, ♀4 and ♂4) and JC (n=8, ♀4 and ♂4) were housed individually (were maintained at 12L: 12D (light on at 08:00am), 25±1°C, respectively), and kept for 1 weeks to familiarize with the environment. After the acclimatizing period, the animals of XGLL and JC were acclimated to HF diet for 28 days, and then re-feeding LF diet for another 28 days, animals were acclimated for 8 weeks. Food intake was calculated as the mass of food missing from the hopper, subtracting orts mixed in the bedding. Body mass, food intake, RMR and activity behavior were measured every day.

Experiment 2

Effects of HF diet on body mass, serum leptin levels, hypothalamic neuropeptide genes expression, body compositions and gastrointestinal tract in *E. miletus*. 43 adult weight-matched *E. miletus* from two regions (XGLL: n=21, ♀11 and ♂10; JC: n=22, ♀10 and ♂12) were

selected, which were maintained at 12L: 12D (light on at 08:00 am), 25±1°C, respectively. Animals of one region were randomly assigned to a control group, and a HF diet and refeeding LF diet group (HF-LF). Controls were fed LF diet for 8 weeks, while HF-LF group were fed HF diet for 4 weeks, then fed LF diet for a further 4 weeks. On day 28, animals were randomly selected from HF-LF group for the measurement of body mass, serum leptin levels, body composition, gastrointestinal tract mass and length, and hypothalamic neuropeptide genes expression. These measurements were taken again from the remaining animals of each group (Control group and HF-LF group) on day 56. All animals were sacrificed between 09:00 h and 11:00 h by decapitation, and animals were dissected to evaluate organ morphology. Blood was centrifuged at 4,000 rpm for 30 min after a 30 min interval. Blood serum was collected and stored at -75 °C prior to hormone measurement.

Measurement of RMR, food intake and activity behavior

Body mass, RMR, food intake and activity behavior were measured using the metabolic system (BXY-R, Sable Systems). *E. miletus* were acclimated to calorimetry cages prior to 30 min the study and data collection (Zhu et al., 2010).

Measurement of morphology, serum leptin levels and hypothalamic neuropeptide gene expression

Measurements of morphology was details in Zhang et al. (2018). Serum leptin levels were determined by radioimmunoassay (RIA) with the 125I Multi-species Kit (Millipore), and leptin values were determined in a single RIA; the lowest level of leptin that can be detected by this assay is 1.0 ng/mL when using a 100-µL sample size (instructions for Multi-species Kit). The inter- and intra-assay variabilities for leptin RIA were 3.6% and 8.7%, respectively. Measurements of hypothalamic neuropeptide gene expression was details in Ren et al. (2020).

Statistical analysis

Data were analyzed using the software package SPSS 15.0. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. Since no gender effects were found on almost all measured parameters, data from females and males were combined. Differences in body mass, food intake, RMR, activity behavior, serum leptin levels and hypothalamic neuropeptide genes expression were analyzed by two-way ANOVA, and differences in body compositions and gastrointestinal tract were analyzed by two-way ANCOVA with body mass as a covariate, followed by Tukey's post hoc test. Results are presented as means ± SE, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass, food intake, RMR and activity behavior

Region and HF diet had significant effects on body mass (Region: $F_{1,907} = 200.61$, $P < 0.01$; HF: $F_{1,907} = 29.36$, $P < 0.01$), but the interaction had no effect ($F_{1,907} = 0.60$, $P > 0.05$, Fig. 1). Region and HF diet affect food intake significantly in *E. miletus* (Region: $F_{1,907} = 212.57$, $P < 0.01$; HF: $F_{1,907} = 54.56$, $P < 0.01$), but the interaction showed no significant effect ($F_{1,907} = 0.08$, $P > 0.05$, Fig. 2). The influence of region on activity behavior of *E. miletus* was extremely significant ($F_{1,907} = 273.18$, $P < 0.01$), but no effect of high-fat diet on activity behavior ($F_{1,907} = 0.01$, $P > 0.05$), and the interaction was also had no effect ($F_{1,907} = 0.07$, $P > 0.05$, Fig. 3). Region, HF diet and the interaction had remarkable effects on RMR in *E. miletus* (Region: $F_{1,907} = 116.44$, $P < 0.01$; HF: $F_{1,907} = 16.45$, $P < 0.01$; Interaction: $F_{1,907} = 15.09$, $P < 0.01$, Fig. 4).

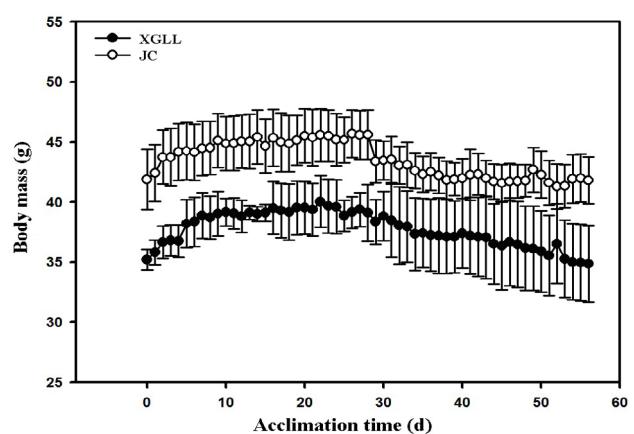


Fig. 1. Effects of high-fat diet on body mass in *Eothenomys miletus*.

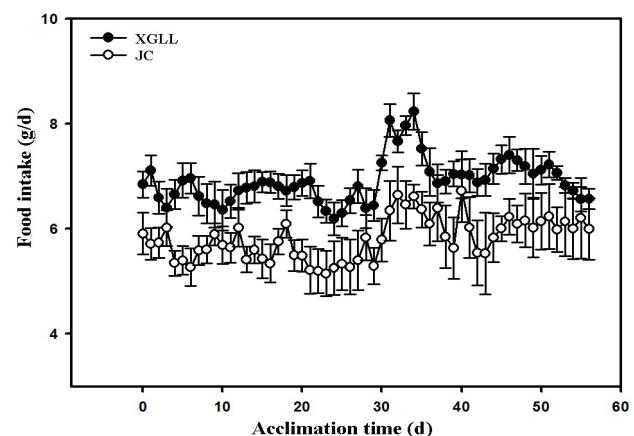


Fig. 2. Effects of high-fat diet on food intake in *Eothenomys miletus*.

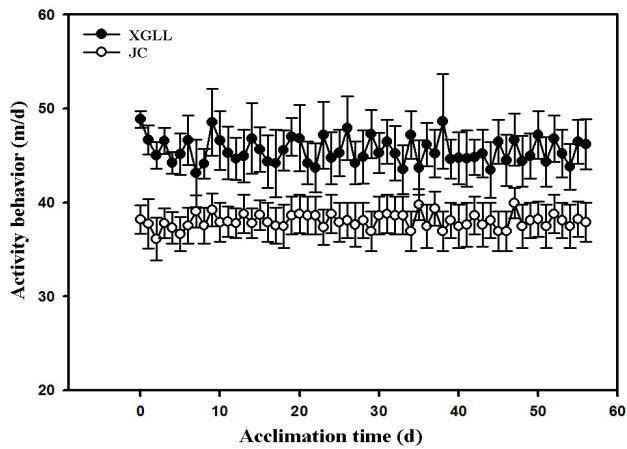


Fig. 3. Effects of high-fat diet on activity behavior in *Eothenomys miletus*.

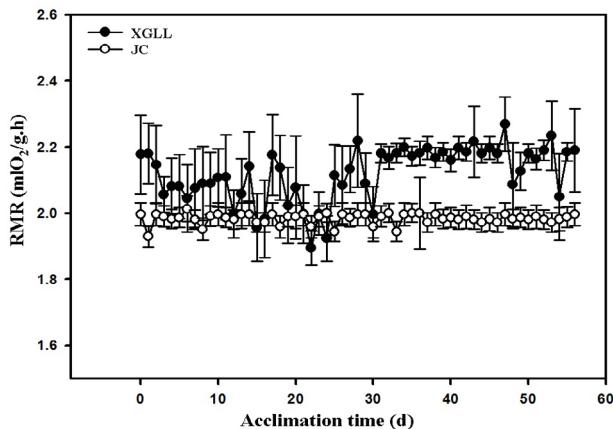


Fig. 4. Effects of high-fat diet on RMR in *Eothenomys miletus*.

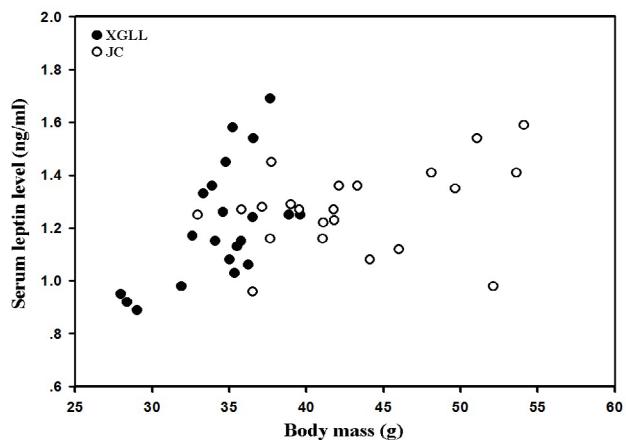


Fig. 5. Correlation between serum leptin levels and body mass in *Eothenomys miletus*.

Serum leptin levels, hypothalamus neuropeptide expressions and body compositions

There were significant differences in the effect of region and HF diet on serum leptin levels in *E. miletus* (Region: $F_{1,36}=9.03$, $P<0.01$; HF: $F_{2,36}=13.64$, $P<0.01$), but the interaction showed no significant difference ($F_{2,36}=0.68$, $P>0.05$). Effect of region on NPY expression in *E. miletus* was significant ($F_{1,36}=8.28$, $P<0.01$), and HF diet also affect NPY expression significantly ($F_{2,36}=4.85$, $P<0.05$), but the interaction effect was not significant difference ($F_{2,36}=2.31$, $P>0.05$). However, the region and diet had no effects on the expression levels of the other three hypothalamic neuropeptides ($P>0.05$, Table II). There was a significant positive correlation between serum leptin levels and body mass ($r=0.40$, $P<0.01$, Fig. 5), and a significant negative correlation between serum leptin levels and NPY expression ($r=-0.58$, $P<0.01$), but no correlation with the expression of three other hypothalamic neuropeptides ($P>0.05$). Region had significant influence on cecum length ($F_{1,36}=4.76$, $P<0.05$), large intestine mass with content ($F_{1,36}=10.71$, $P<0.01$), small intestine mass with no content ($F_{1,36}=6.01$, $P<0.05$), cecum mass with no content ($F_{1,36}=7.71$, $P<0.01$). Region and HF diet had no effects on the remaining indicators (Table II).

DISCUSSION

Phenotypic plasticity is the ability of an organism to change its phenotype in response to changes in the environment, such as temperature and photoperiod (Miner et al., 2005). Food quality was also an important factor affecting animals' phenotypic changes (Gao et al., 2013). HF diet increased body mass and body fat mass significantly in mice (Wang et al., 2008), but it has no significant effect on body mass in *Cricetulus barabensis* (Shi et al., 2017). Changes in organ mass and digestive tract morphology play important roles in energy balance (Hume, 2002). The present results showed that body mass in *E. miletus* in JC was larger than that of in XGLL, but total digestive tract in XGLL area was longer and lighter than that in JC area, which may be related to the different food resources in the two regions. During the HF diet acclimation, body mass increased in *E. miletus* in the two areas, and then returned to the control level on day 56, showing higher phenotypic plasticity. However, body mass changes in the two areas were different after re-feeding LF diet. Body mass loss in JC was significant, which might be related to smaller changes of food intake after re-feeding LF diet. Body mass in XGLL decreased gradually after re-feeding, which was due to the increasing of food intake, suggesting that *E. miletus* in XGLL was more sensitive to changes in food quality. Moreover, the initial food intake in XGLL was

Table II. Effects of high-fat food on body mass, serum leptin levels, hypothalamic neuropeptide expressions and body compositions in *Eothenomys miletus* from different regions.

Parameters	Xianggelila			Jianchuan		
	Control group (n=6)	HF group (n=8)	HF-LF group (n=7)	Control group (n=6)	HF group (n=8)	HF-LF group (n=7)
Body mass(g)	33.68±1.88	35.71±0.66	33.59±0.98	41.98±1.73	44.99±2.80	41.81±1.93
Serum leptin levels(ng/ml)	1.08±0.05	1.40±0.07	1.11±0.05	1.20±0.05	1.41±0.04	1.19±0.05
NPY(RU)	1.21±0.06	0.99±0.03	1.16±0.04	1.00±0.05	0.95±0.03	0.97±0.05
AgRP(RU)	1.15±0.06	0.98±0.03	1.09±0.03	1.00±0.05	0.99±0.06	0.99±0.04
CART(RU)	0.93±0.03	1.02±0.07	0.89±0.02	1.00±0.03	1.03±0.04	0.97±0.02
POMC(RU)	0.90±0.06	0.91±0.08	0.89±0.07	1.00±0.05	1.02±0.07	1.00±0.05
Heart weight(g)	0.18±0.02	0.25±0.02	0.21±0.02	0.23±0.02	0.25±0.02	0.24±0.01
Liver weight(g)	2.11±0.16	2.00±0.12	1.92±0.07	2.28±0.22	2.11±0.30	2.05±0.14
Spleen weight(g)	0.06±0.001	0.09±0.008	0.06±0.006	0.09±0.017	0.08±0.008	0.09±0.012
Lung weight(g)	0.22±0.02	0.25±0.02	0.26±0.02	0.25±0.01	0.23±0.01	0.27±0.03
Kidney weight(g)	0.39±0.02	0.47±0.06	0.45±0.03	0.43±0.02	0.46±0.05	0.43±0.06
Stomach length (cm)	2.77±0.29	2.08±0.20	2.59±0.25	2.05±0.13	2.68±0.10	2.16±0.19
Small intestine length(cm)	37.75±1.69	36.30±1.73	36.12±1.94	36.85±2.46	35.77±1.30	37.03±1.60
Large intestine length(cm)	18.70±1.31	20.51±1.16	19.00±1.47	18.85±0.76	16.82±1.02	18.14±0.53
Cecum length(cm)	9.03±1.62	9.84±0.85	10.85±0.70	8.42±0.42	8.21±0.61	9.62±0.85
Stomach mass with content(g)	0.72±0.10	0.65±0.09	0.76±0.11	0.81±0.18	0.98±0.09	0.67±0.04
Small intestine mass with content(g)	1.78±0.14	1.73±0.09	1.57±0.10	1.68±0.07	1.93±0.19	1.35±0.06
Large intestine mass with content (g)	0.54±0.12	0.62±0.05	0.63±0.07	0.52±0.07	0.44±0.04	0.46±0.06
Cecum mass with content (g)	1.64±0.23	1.80±0.11	1.99±0.14	1.85±0.19	2.01±0.33	1.87±0.24
Stomach mass with no content(g)	0.36±0.05	0.49±0.03	0.38±0.04	0.30±0.01	0.35±0.03	0.35±0.04
Small intestine mass with no content(g)	0.45±0.05	0.69±0.04	0.57±0.04	0.65±0.03	0.93±0.13	0.75±0.14
Large intestine mass with no content(g)	0.29±0.03	0.33±0.05	0.29±0.05	0.32±0.02	0.33±0.02	0.35±0.05
Cecum mass with no content (g)	0.36±0.08	0.49±0.03	0.38±0.03	0.50±0.03	0.65±0.07	0.61±0.05

higher than that of JC, which mainly because the environment temperature was lower and food resources were poor in winter of XGLL, when food was sufficient, food intake would be increased.

Behavioral regulation is an important adaptive strategy for wild small mammals to cope with environmental changes, especially for uncertainty of food resources (Zhao *et al.*, 2009). Rats reduced their foraging behavior by a HF diet (Chen *et al.*, 2014). In the current research, activity behavior only varied between regions, which was related to their habitat temperature and food conditions. There was no significant change in activity behavior form two regions, indicating that *E. miletus* did not need to increase activity behavior when food quality is good. But activity behavior in XGLL fluctuated larger, probably because *E. miletus* in XGLL were more sensitive to food resources in winter. RMR is often used to study the

adaptation of small mammals to extreme environments (Li and Huang, 1994). LF diet increased RMR in mammals (Camp *et al.*, 2018), while HF diet has no significant effect on RMR and body compositions in *Labrador* (Yoo *et al.*, 2006). In the present study, higher RMR in XGLL was related to lower temperature and cold temperature. RMR in JC remained unchanged under acclimation, which was consistent with its activity behavior. RMR in XGLL was more volatile, one reason is that it may be related to the fluctuation of their activity behavior, the other reason is that the environment in XGLL is more volatile in winter.

Leptin plays an important role in the regulation of body mass and energy metabolism (Abelenda *et al.*, 2003). In the present study, serum leptin levels were positively correlated with body mass, supporting the hypothesis that leptin could be used as a lipid signalling molecule (Schneider *et al.*, 2000). Moreover, there was a significant difference

in leptin levels between two regions. Serum leptin levels in JC was higher than that of in XGLL, which was related to the higher body mass in JC. Hypothalamus controls food intake and energy expenditure mainly by regulating the expression of two types of neuropeptides (food promoters: NPY, AgRP; food inhibition: POMC, CART), which plays an important role in body mass regulation (Trayhurn and Bing, 2006). Previous studies have shown that high-quality food increased NPY expression significantly (Kaga et al., 2001). However, other studies have shown that HF diet has no effect on POMC expression (Marco et al., 2013). In our study, HF diet decreased NPY expression significantly, and NPY showed a significant negative correlation with leptin levels. But HF diet had no significant effect on the other three neuropeptides expressions, suggesting that leptin and NPY expression may play an important role in body mass regulation in *E. miretus*.

In conclusion, HF diet increased body mass in *E. miretus*, which returned to the control level when re-feeding LF diet. Leptin and NPY play important roles in the regulation of body mass and food intake. There were differences in body mass regulation patterns in *E. miretus* from different regions when they were exposed to HF diet, which may be related to different environmental conditions in Hengduan mountain regions.

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Statement of conflict of interest

The authors have declared no conflict of interests.

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