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Effects of Tannic Acid Food on Energy Metabolism in Male *Eothenomys miletus*

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Authors' Contribution
WLZ conceived the study and participated in its design, coordination and drafted the manuscript. ML and DZ carried out the studies of body mass, food intake and other markers.

Key words
Eothenomys miletus, Tannic acid, Energy metabolism, Body mass, Energy intake.

ABSTRACT

Plant secondary metabolites affect the survival, reproduction, and distribution of herbivores. In order to test the effects of plant secondary metabolite on energy metabolism and thermogenesis, changes in resting metabolic rate (RMR), nonshivering thermogenesis (NST) and energy intake were measured in *Eothenomys miletus* fed diets containing 0, 3.3% and 6.6% tannic acid, respectively. The results showed that *E. miletus* fed the diets with 6.6% tannic acid increased RMR compared with control on day 14, and reduced the gross energy intake (GEI) and digestible energy intake (DEI). On day 28, *E. miletus* fed diets containing tannic acid showed no significant changes in RMR, GEI and DEI. The apparent digestibility and NST were kept stabled during the experiment. These results indicated that tannic acid can increase RMR and reduce energy intake within a short period and the changed physiological function returned to baseline for a long period. Tannic acid has no effect on body mass and no significant difference was detected in NST between pre- and the end of experiment in *E. miletus.* All of the results indicated that *E. miletus* can adjust their physiological functions to match the changes in food conditions.

INTRODUCTION

Tannins are the most widely distributed of a class of phenols, which varies from 1% to 5% generally in the leaf tissue of the plant (Cork and Foley, 1991). According to their chemical properties, they can be divided into the hydrolysable tannins and condensed tannins, tannic acid (TA) is one of the most common material of hydrolysable tannins (Cork *et al*., 1993). In the animals' digestive tract, TA can form complexes with proteins to inhibit microbial fermentation, thereby reducing the availability of food intake (Clausen *et al*., 1990). When TA is absorbed into the circulatory system, which can use as an uncoupler of oxidation and phosphorylation, resulting in increasing of oxygen consumption (Singleton and Kratzer, 1969). Animals in general through the liver of a variety of enzymes to oxidation and reduction of TA, and then excreted it (Watkins *et al*., 1987). TA had different concentrations in different plants, therefore, differences in the effects of TA on physiological function of mammals may reflect the differences in feeding habits among species, and also may reflect the ability of animals to adapt to environment (Foley *et al*., 1999). There are many studies about the effect of TA on energy metabolism in wild small mammals, but the conclusions were not consistent, such as *Micortus ochorgaster*, *M. pennsylvarnicus*, *M. oeconomus* and *Sciurus carolinenis* (Thomas *et al*., 1988; Meyer and Richardson, 1993; Chungmaccoubrey *et al*., 1997; Li *et al*., 2001).

Eothenomys miletus is an inherent species in Hengduan mountain region (Zhu *et al*., 2011). *E. miletus* feeding mainly with green plant leaves, buds and seeds, these foods contain tannin and other plant secondary metabolites. There were some studies about the physiological ecology reports in *E. miletus* (Zhu *et al*., 2010, 2012, 2014, 2017). But we know nothing about effects of TA on body mass regulation in *E. miletus*. Resting metabolic rate (RMR), nonshivering thermogenesis (NST) and energy intake were measured in *E. miletus* fed diets containing 0%, 3.3% and 6.6% tannic acid, respectively. We predicted that *E. miletus* may change its body mass, thermogenesis and energy intake according to the different concentrations of TA.

MATERIALS AND METHODS

Samples

E. miletus were obtained from a laboratory colony,

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which were captured in a farmland (26°15′~26°45′N; 99°40′~99°55′E; altitude 2,590 m) in Jianchuan County, Yunnan province, 2010. *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 and water were provided *ad libitum*. 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). Young individuals were excluded in the present study. After 1 month stabilization, 30 male *E. miletus* were randomly divided into following three experimental regimes: 0%TA group (n=10), 3.3%TA group (n=10) and 6.6%TA group (n=10), which were fed with different concentration of TA food. According to the method of Lindorth and Batzli (1984), the concentrations of TA in food were set to 0%, 3.3% and 6.6% (produced by Kunming Medical University, Kunming). The calorific value of the prepared material block was maintained at a constant value (17.56 kJ/g for the 0%TA group, 17.52 kJ/g for the 3.3%TA group and 17.49 kJ/g for the 6.6%TA group). The experimental period was 28 days. Body mass was measured every week, all animals were sacrificed between 0900 and 1100 hours by decapitation after 28 day, determination of body composition. Before the experiment, body mass showed no significant differences among three groups (P>0.05).

Measurement of metabolic rates

Metabolic rates were measured by using an AD ML870 open respirometer (AD Instruments, Australia) at 25°C within the TNZ (thermal neutral zone) on day 0, 14 and 28, gas analysis were using a ML206 gas analysis instrument, the temperature was controlled by SPX-300 artificial climatic engine (±0.5°C), the metabolic chamber volume is 500ml, flow is 200 ml/min. The voles were stabilized in the metabolic chamber for at least 60 min prior to the RMR measurement, oxygen consumption was recorded for more than 120 min at 1 min intervals. Ten stable consecutive lowest readings were taken to calculate RMR (Zhu *et al*., 2010). The method used for calculating the metabolic rate is detailed in Hill (1972).

Nonshivering thermogenesis (NST) was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co., Ltd.) and measured at 25°C. Two consecutive highest recordings of oxygen consumption more than 60 min at each measurement were taken to calculate the NST (Zhu *et al*., 2011). The doses of NE were approximately 0.8-1.0 mg/kg according to dose-dependent response curves that were carried out before the experiment (Zhu *et al*., 2010).

Measurement of energy intake

Food intake was measured following Zhao and Cao (2009) on day 0, 14 and 28. Each animal was put in a metabolic cage (20×15×15cm^3), animals were fed a fixed quantity at a set time (9.5–10.5g, 11:00 am), body mass was recorded and residual food collected on next day. Residual food was dried in a vacuum dryer until the mass was invariable. Energy contents of the food and feces were determined by a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA). Gross energy intake (GEI), digestible energy intake (DEI) and digestibility were calculated according to Zhao *et al*. (2014) as follows:

GEI (kJ/d) = Dry matter intake (DMI, g/d) × gross energy content of food (kJ/g)

DEI (kJ/d) = GEI - [mass of feces (g/d) × gross energy content of feces (kJ/g)]

Digestibility = DEI /GEI

Morphology

Immediately after sacrifice on day 28, visceral organs including liver, brown adipose tissue (BAT), heart, lung, kidneys, spleen and gastrointestinal tract (stomach, small intestine, caecum, large intestine) were excised and weighed (±1 mg). The stomach and intestines were rinsed with saline to remove gut contents and then weighed. The remaining carcass and the excised organs were dried to constant mass in an oven at 60 °C (for at least 72 h), and then weighed again to obtain a dry mass.

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. Body mass, RMR, NST, DMI, GEI, DEI and digestibility among three groups were analyzed using one-way ANOVA analysis. Results are presented as means ± SEM and P < 0.05 was considered to be statistically significant.

RESULTS

RMR and NST

Before the experiment, RMR among three groups showed no significant differences ($F_{2,27}$=0.53, P>0.05). On day 14, RMR showed significant difference among three groups ($F_{2,27}$=3.59, P<0.01, Fig. 1), RMR in 3.3%TA and 6.6TA% group were 19.68% and 20.91% higher than that of 0%TA group. But on day 28, no significant differences were found among three groups ($F_{2,27}$=0.61, P>0.05, Fig. 1). Before the experiment, NST among three groups showed no significant differences ($F_{2,27}$=0.47, P>0.05), which also showed no differences significantly on day

14 ($F_{2,27}$=0.65, P>0.05). Although NST in 3.3%TA and 6.6TA% group were higher than that of 0%TA group on day 28, no significant differences were found among three groups ($F_{2,27}$=0.98, P>0.05, Fig. 2A).

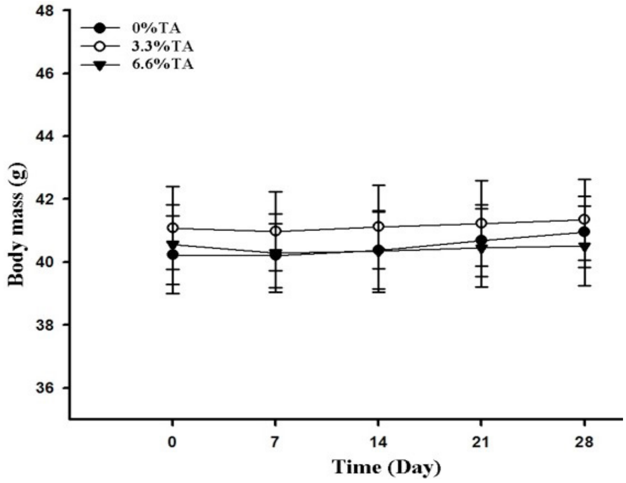

Fig. 1. Effects of tannic acid food on body mass in *Eothenomys miletus*. Error bars represent SE.

Body mass

Before the experiment, body mass among three groups showed no significant differences ($F_{2,27}$=0.12, P>0.05). During the acclimation, body mass among three groups also had no significant changes (0%TA group: $F_{2,27}$=0.34, P>0.05; 3.3%TA group: $F_{2,27}$=0.26, P>0.05; 6.6TA% group: $F_{2,27}$=0.15, P>0.05, Fig. 2B).

Energy intake

Before the experiment, DMI, GEI and DEI among three groups showed no significant differences (DMI : $F_{2,27}$=0.14, P>0.05; GEI: $F_{2,27}$=0.53, P>0.05; DEI: $F_{2,27}$=1.35, P>0.05, Table I). On day 14, DMI and GEI in 6.6%TA group were significant lower than that of 0%TA group ($F_{2,27}$=4.85, P<0.01), DEI in 3.3%TA and 6.6TA% group were lower than that of 0%TA group ($F_{2,27}$=9.24, P<0.01). But on day 28, DMI, GEI and DEI among three groups showed no significant differences (DMI: $F_{2,27}$=0.98, P>0.05; GEI: $F_{2,27}$=1.03, P>0.05; DEI: $F_{2,27}$=1.23, P>0.05, Table I). Digestibility among three groups showed no significant differences (Day 0: $F_{2,27}$=0.56, P>0.05; Day 14: $F_{2,27}$=0.48, P>0.05; Day 28: $F_{2,27}$=0.45, P>0.05, Table I).

Body composition

On day 28, wet mass of small intestine and caecum among three groups showed significant differences (small intestine: $F_{2,27}$=3.25, P<0.05; caecum: $F_{2,27}$=2.98, P<0.05, Table II), other masses showed no significant differences (P>0.05).

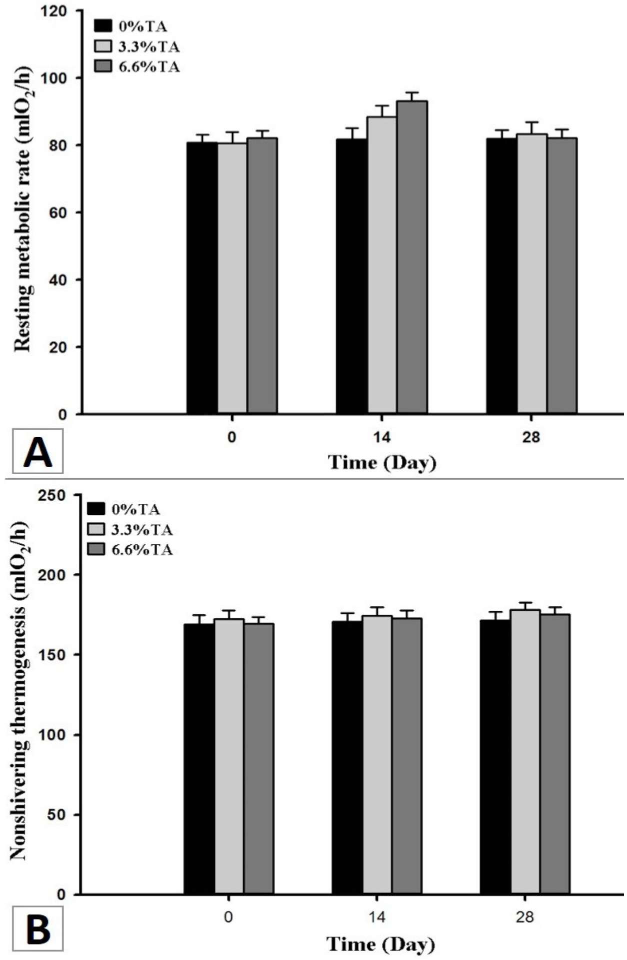

Fig. 2. Effects of tannic acid food on resting metabolic rate (A) and nonshivering thermogenesis (B) in *Eothenomys miletus*. Error bars represent SE.

DISCUSSION

RMR and NST

In the present study, it showed that TA diet can lead to increase in metabolic rate of *E. miletus*. *Microtus pennsylvarnicus* fed with 6% gallic acid diet, RMR were 13.6% and 22.6% higher than that of the controls on day 10 and 21 (Thomas *et al*., 1988). Under 10% or 20% protein food conditions, *Microtus oeconomus* fed with 3% or 6% TA diet on day 5, 10 and 20, its BMR increased significantly (Li *et al*., 2001). But for *Phyllotis darwini* and *Octodon degus*, when they fed with 4% TA diet, their RMR showed no significant differences during the acclimation (Bozinovic *et al*., 1997), similar results was found in *Micortus ochorgaster* (Meyer and Richardson, 1993). The inconsistency caused by the above results may be closely related with the concentration of TA and their mechanism. Thomas *et al*. (1988) pointed out that there were mainly two possible reasons about the increase of energy expenditure caused by plant secondary metabolism: first reason is the process of the detoxification of secondary metabolites in mammals; second reason is the process of repairing the damage caused by secondary metabolites. Because most plant secondary metabolites are complex, which were difficult to determine the number of absorption, excretion rate and pathways of the mammals. Iason and Palo (1991) studied the effect of phenolic compounds on energy budget in sheep, the results showed that the intake of phenolic compounds in free feeding does not cause the energy expenditure increased, but when the dose exceeded the free feeding range, energy expenditure increased significantly. Therefore, they pointed out that the increase in energy expenditure is not caused by the detoxification process, which may be due to the uncoupling of oxidative phosphorylation. In the present study, RMR in 6.6%TA group was significant higher than that of the 0%TA group on day 14, which suggested that within the concentration range of free feeding, the energy expended in the detoxification of plant secondary metabolites may be smaller in *E. miletus*. On day 28, there was no significant difference in RMR among three groups, suggesting that *E. miletus* can change its physiological function to adapt to the external environment of food change in a short period of time, at the same time, this result also showed

Table I.- Effects of tannic acid on the DMI, GEI, DEI and digestibility in *Eothenomys miletus.*

	Day 0	Day 14	Day 28
0%TA group			
DMI (g/d)	5.45±0.45	5.94±0.39	5.31±0.32
GEI (kJ/d)	95.71±6.65	104.31±7.36	93.24±5.32
DEI (kJ/d)	67.51±4.35	73.25±3.21	64.35±3.16
Digestibility (%)	70.54±4.36	70.23±3.21	69.02±3.32
3.3%TA group			
DMI (g/d)	5.24±0.41	5.01±0.33	5.12±0.29
GEI (kJ/d)	91.81±6.58	87.78±5.52	89.70±5.13
DEI (kJ/d)	63.23±3.87	60.15±3.32	61.59±2.89
Digestibility (%)	68.87±3.12	68.52±2.69	68.66±2.13
6.6%TA group			
DMI (g/d)	4.98±0.26	4.88±0.25	4.91±0.22
GEI (kJ/d)	87.11±6.32	85.35±6.89	85.88±6.59
DEI (kJ/d)	59.32±2.32	57.21±2.11	58.39±2.03
Digestibility (%)	68.09±2.21	67.02±1.26	67.99±1.69

Table II.- Effects of tannic acid on body composition in *Eothenomys miletus.*

Parameters	0%TA group	3.3%TA group	6.6%TA group
Heart wet mass (g)	0.232±0.014	0.229±0.008	0.225±0.007
Heart dry mass (g)	0.065±0.003	0.064±0.002	0.063±0.002
Lungs wet mass (g)	0.302±0.021	0.306±0.019	0.298±0.012
Lungs dry mass (g)	0.068±0.005	0.065±0.004	0.062±0.004
Liver wet mass (g)	1.624±0.125	1.615±0.142	1.602±0.129
Liver dry mass (g)	0.395±0.012	0.394±0.013	0.389±0.009
BAT wet mass (g)	0.201±0.005	0.198±0.004	0.202±0.003
BAT dry mass (g)	0.053±0.002	0.051±0.001	0.049±0.002
Kidney wet mass (g)	0.187±0.006	0.182±0.003	0.178±0.006
Kidney dry mass (g)	0.038±0.003	0.039±0.004	0.036±0.002
Spleen wet mass (g)	0.019±0.002	0.018±0.002	0.018±0.001
Spleen dry mass (g)	0.004±0.001	0.004±0.001	0.003±0.001
Stomach wet mass (g)	0.412±0.021	0.406±0.019	0.402±0.017
Stomach dry mass (g)	0.095±0.006	0.096±0.005	0.097±0.003
Small intestine wet mass (g)	0.665±0.054	0.756±0.049	0.769±0.052
Small intestine dry mass (g)	0.031±0.003	0.029±0.002	0.031±0.004
Caecum wet mass (g)	0.426±0.021	0.458±0.023	0.489±0.028
Caecum dry mass (g)	0.043±0.003	0.041±0.002	0.044±0.003
Large intestine wet mass (g)	0.328±0.012	0.325±0.009	0.321±0.006
Large intestine dry mass (g)	0.045±0.003	0.042±0.002	0.44±0.003

that lower energy cost in the process of detoxification in *E. miletus*. NST is an important way to maintain the body temperature of small mammals, which is mainly produced in brown adipose tissue (BAT) (Zhu *et al*., 2010). TA as an uncoupler of oxidation and phosphorylation may increase the levels of NST (Chen *et al*., 2005), but in our results, TA had no significant effect on NST and BAT mass, which need further study.

Energy intake

On day 14, DMI, GEI and DEI in TA groups were significantly lower than that of the 0%TA group, and on day 28, all of the above indexes showed no significant differences compared with the 0%TA group. Besides, digestibility showed no changes throughout the experimental period. There are some studies about the inhibitory effect of TA in the feeding habits of mammals, for example, in *Lepus timidus* and *L. europaeus* fed with TA diet, food intake and protein digestibility decreased significantly, but the digestibility was not affected by TA (Iason and Palo, 1991), similar results were found in *Micortus ochorgaster* (Meyer and Richardson, 1993) and *Sciurus carolinenis* (Chungmaccoubrey *et al*., 1997). At the same time, TA can also interact with the protein and fiber in food, thus affecting food intake and digestibility (Li *et al*., 2001). TA may affect the animal's digestive function through a variety of ways: 1) combined with protein, fiber and starch in food closely; 2) combined with beneficial bacteria in the appendix, inhibiting the growth of other bacteria; 3) combined with a variety of enzymes in the stomach, inhibiting the decomposition of food ingredients, digestion, *etc*. (Foley *et al*., 1999). In the present study, TA did not decrease the digestibility in *E. miletus*, but decreased food intake, suggesting that tannic acid mainly affects the foraging behavior in *E. miletus*.

Body mass and body composition

Change of body mass is closely related to its energy metabolism. The maintenance of body mass depends on the balance of energy intake and energy consumption, mammals that reduce food intake and digestibility, whereas increase energy consumption can lead to decline of body mass (Boon *et al*., 1997). When *E. miletus* fed with TA diet, it decreased food intake and increased RMR, although body mass in 6.6%TA group reduced, but the difference in body mass among three groups was not obvious, suggesting that *E. miletus* may maintain energy balance by reducing other energy expenditure, such as reducing foraging activities. The morphology and structure of animals' digestive tract can directly influence its energy efficiency (Derting and Begue, 1993). In the present study, wet mass of small intestine and caecum among three groups showed significant differences, which were heavier in 6.6%TA group, which may be a compensatory digestive strategies under lower food quality. Bozinovic and Novoa (1997) also found that tannic acid can increase the masses of digestive tract in *Octodon degus*.

CONCLUSION

TA diet can increase RMR of *E. miletus* in a short period of time, and reduced energy intake and digestible energy intake. With the extension of time, the physiological function can return to the control level in *E. miletus*.

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Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCE

Boon, P., Visser, H. and Daan, S., 1997. Effect of photoperiod on body mass, and daily energy intake and energy expenditure in young rats. *Physiol. Behav.*, **62**: 913-919. https://doi.org/10.1016/S0031-9384(97)00271-0

Bozinovic, F. and Novoa, F.F., 1997. Metabolic costs of rodents feeding on plant chemical defenses: a comparison between an herbivore and an omnivore. *Comp. Biochem. Physiol.*, **117**: 511-514. https://doi.org/10.1016/S0300-9629(96)00409-4

Bozinovic, F., Novoa, F.F. and Sabat, P., 1997. Feeding and digesting fiber and tannins by an herbivorous rodent, *Octodon degus*, (rodentia: caviomorpha). *Comp. Biochem. Physiol. Part A Physiol.*, **118**: 625-630. https://doi.org/10.1016/S0300-9629(96)00480-X

Chen, J.F., Zhong, W.Q. and Wang, D.H., 2005. Effects of tannic acid food on energy metabolism in Brandt's voles (*Lasiopodomys brandtii*). *Acta Theriol. Sin.*, **25**: 326-332.

Chungmaccoubrey, A.L., Hagerman, A.E. and Kirkpatrick, R.L., 1997. Effects of tannins on digestion and detoxification activity in gray squirrels (*Sciurus carolinensis*). *Physiol. Zool.*, **70**: 270-277. https://doi.org/10.1086/639595

Clausen, T.P., Provenza, F.D., Burritt, E.A., Reichardt, P.B. and Bryant, J.P., 1990. Ecological implications of condensed tannin structure: A case study. *J. chem. Ecol.*, **16**: 2381-2392. https://doi.org/10.1007/BF01017463

Cork, S.J. and Foley, W.L., 1991. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperature and tropical forests. In: *Plant defense against mammalian herbivory* (eds. R.T. Palo and C.T. Robbins). CRC Press, Boca Raton, FL, pp. 133-166.

Cork, S.J., Humw, I.D. and Dawson, T.J., 1993. Digestion and metabolism of natural foliar diet (*Eucalyptus punctala*) by an arboreal marsupial, the koala (*Phascolarctos cinereus*). *J. Comp. Physiol.*, **152**: 443-451.

Derting, T.L. and Bogue, B.A., 1993. Responses of the gut to moderate energy demands in a small herbivore (*Microtus pennsylvanicus*). *J. Mammal.*, **74**: 59-68. https://doi.org/10.2307/1381905

Foley, W.J., Iason, G.R. and Mcarthur, C., 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores - How far have we come in 25 years? In: *Nutrition ecology of herbivores* (eds. H.G. Jung and G.C. Hahey). American Society of Animal Science, Savoy, pp. 130-209.

Hill, R.W., 1972. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. appl. Physiol.*, **33**: 261-263.

Li, J.N., Liu, J.K. and Tao, S.L., 2001. Responsive patterns of root voles to tannic acid. *Acta Theriol. Sin.*, **21**: 116-121.

Lindroth, R.L. and Batzli, G.O., 1984. Plant phenolics as chemical defenses: effects of natural phenolics on survival and growth of prairie voles (*Microtus ochrogaster*). *J. chem. Ecol.*, **10**: 229-244. https://doi.org/10.1007/BF00987851

Iason, G.R. and Palo, R.T., 1991. Effects of birch phenolics on a grazing and a browsing mammal: a comparison of hares. *J. chem. Ecol.*, **17**: 1733-1743. https://doi.org/10.1007/BF00993725

Meyer, M.W. and Richardson, C., 1993. The effects of chronic tannic acid intake on prairie vole (*Microtus ochrogaster*) reproduction. *J. chem. Ecol.*, **19**: 1577-1585. https://doi.org/10.1007/BF00984898

Singleton, V.L. and Kratzer, F.H., 1969. Toxicity and related physiological activity of phenolic substances of plant origin. *J. Agric. Fd. Chem.*, **17**: 497-512. https://doi.org/10.1021/jf60163a004

Thomas, D.W., Samson, C. and Bergeron, J.M., 1988. Metabolic costs associated with the ingestion of plant phenolics by *Microtus pennsylvanicus*. *J. Mammal.*, **69**: 512-515. https://doi.org/10.2307/1381342

Watkins, J., Smith, G.S. and Hallford, D.M., 1987. Characterization of xenobiotic biotransformation in hepatic, renal and gut tissues of cattle and sheep. *J. Anim. Sci.*, **65**: 186-195. https://doi.org/10.2527/jas1987.651186x

Zhao, Z.J. and Cao, J., 2009. Effect of fur removal on the thermal conductance and energy budget in lactating Swiss mice. *J. exp. Biol.*, **212**: 2541-2549. https://doi.org/10.1242/jeb.029603

Zhao, Z.J., Chi, Q.S. and Cao, J., 2014. Seasonal changes of body mass and energy budget in Striped hamsters: The role of leptin. *Physiol. biochem. Zool.*, **87**: 245–256. https://doi.org/10.1086/674974

Zhu, W.L., Cai, J.H., Lian, X., Wang, Z.K. 2010. Adaptive character of metabolism in *Eothenomys miletus* in Hengduan Mountains region during cold acclimation. *J. Therm. Biol.*, **35**:417-421. https://doi.org/10.1016/j.jtherbio.2010.09.002

Zhu, W.L., Cai, J.H., Lian, X. and Wang, Z.K., 2011. Effects of photoperiod on energy intake, thermogenesis and body mass in *Eothenomys miletus* in Hengduan Mountain region. *J. Therm. Biol.*, **36**: 380-385. https://doi.org/10.1016/j.jtherbio.2011.06.014

Zhu, W.L., Yang, S.C. and Wang, Z.K., 2012. Adaptive characters of energy metabolism, thermogenesis and body mass in *Eothenomys miletus* during cold exposure and rewarming. *Anim. Biol.*, **62**: 263-276. https://doi.org/10.1163/157075611X618200

Zhu, W.L., Zhang, H., Zhang, L., Yu, T.T. and Wang, Z.K., 2014. Thermogenic properties of Yunnan red-backed voles (*Eothenomys miletus*) from the Hengduan mountain region. *Anim. Biol.*, **64**: 59-73. https://doi.org/10.1163/15707563-00002430

Zhu, W.L. and Yang, G. 2017. Role of hypothalamic neuropeptides genes expression on body mass regulation under different photoperiods in Eothenomys miletus. *Pakistan J. Zool.*, **49**: 647-654.