



Effects of dietary tannic acid on the growth, hepatic gene expression, and antioxidant enzyme activity in Brandt's voles (*Microtus brandti*)



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ABSTRACT

This study was designed to investigate the physiological and biochemical responses of Brandt's voles to the persistent presence of dietary tannic acid. The diet for animals in the experimental group was supplemented with 3% dietary tannic acid for 5 weeks. The control group received a commercial lab chow. No significant differences were detected in body weight, organ (heart, kidney, and liver) weights, and organ parameters between animals from two groups. However, voles in the experimental group had significantly higher daily food intake, increased contents of proline and histidine in saliva and feces after protein hydrolysis, and elevated hepatic expression of transferrin than the control. Our results suggested the existence of adaptive strategies developed in Brandt's voles to overcome the adverse effects of dietary tannic acid. (1) Food consumption was increased to satisfy their nutritional demands. (2) The secretion of tannic-acid-binding salivary proteins was promoted. (3) The absorption of iron was enhanced. These alterations contributed to neutralize the negative effects of tannic acid and maintain body mass in animals supplemented with tannic acid. As the result of the consumption of tannic acid, hepatic expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase was significantly decreased, while the overall potential of the antioxidant system, characterized by increased hepatic enzymatic activities of catalase and glutathione peroxidase, was enhanced. Our results also implied the involvement of tannic acid in the regulation of lipid metabolism and oxidative stress in voles.

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1. Introduction

Tannic acid (TA), one member of hydrolyzed tannins abundant in a wide variety of plants, is a model compound representative of polyphenols with high molecular weight that occur mainly in plant-derived feeds, food grains, and fruits. Ingestion of TA is known to cause various types of effects in animals. Having the ability to form complexes with proteins, starch, and digestive enzymes, TA is often considered to be an anti-nutritional factor for many animals (Chung et al., 1998) and has adverse effects of reducing food efficiency and growth rate (Mansoori and Acamovic, 2007; Lee et al., 2010). On the other hand, its beneficial biological effects were also observed. It has been reported to possess antioxidant (Wu et al., 2004; Andrade et al., 2005; Gülçin et al., 2010; Bouki et al., 2013), anti-mutagenic (Ferguson, 2001; Chen

and Chung, 2000), and anti-carcinogenic (Nepka et al., 1999; Tikoo et al., 2011) properties. It is also used as an effective antioxidant in food industry (Maqsood et al., 2012).

Brandt's voles (*Microtus brandti*) are non-hibernating herbivores that mainly inhabit the grasslands of Inner Mongolia of China, the Republic of Mongolia, and the region of Beigaer Lake in Russia, where winter lasts for more than 5 months (Li and Wang, 2005). The Brandt's vole is one of the smallest (40–50 g) strictly herbivorous mammals and feeds on both monocotyledons and dicotyledons. Despite its small size, the Brandt's vole consumes about 40 g fresh grass per day. Under cold conditions, the amount of food it consumes daily can even exceed its body mass (Zhang and Wang, 1998). It is a dominant pest rodent in North China. During the past decade, much work has been done to investigate the role of cold acclimation on energy metabolism and the regulation of body mass in Brandt's voles (Li et al., 2001; Li and Wang 2005; Zhang and Wang, 2006, 2007; Zhang et al., 2009; Li et al., 2010; Zhang et al., 2015). Whereas available information about the adaptive changes in morphology and physiology in response to nutritional factors, which are critical for their survival, are limited in Brandt's voles. Several researchers studied the influence of dietary fiber on the food intake and digestibility in Brandt's voles. Pei et al. (2001) reported the digestive responses of Brandt's voles to an increased content of dietary fiber

Abbreviations: TA, Tannic acid; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; Trf, Transferrin; SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase.

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in terms of the digestive performance, gut morphology, and rate of passage of digesta. Their results indicated that Brandt's voles could compensate the poor-quality diet physiologically by the means of increasing food intake. Similarly, increased food intake and decreased digestibility were observed in Brandt's voles fed with high-fiber diet (Song and Wang, 2006; Zhao and Wang, 2007). It has been reported that TA constitutes the main phenolics in the Brandt's vole's daily food, accounting for 5% of the dry food (Li et al., 2007). However, little is documented about the physiological modifications that occur as a consequence of TA consumption in Brandt's voles.

As is known, TA is one of the most common anti-nutritional factors in food associated with poor utilization of protein. It has also been recognized as the inhibitor of non-heme-iron absorption (Kalganekar and Lönnnerdal, 2008; Lee et al., 2010; Jin et al., 2009; Lavin et al., 2010; Andrews et al., 2014; Hurrell and Egli, 2010; Jaramillo et al., 2015). We assumed that in order to avoid the possible deficiency of nutrients (protein and iron, especially), the Brandt's voles must develop corresponding mechanisms in their daily interactions with dietary TA to counteract its adverse effects. In this study, we investigated the influences that 3% dietary TA (which is within the average content of TA in Brandt's voles' natural foods) might have on voles in terms of (1) average daily food intake, (2) the profile of amino acids in saliva and feces after protein hydrolysis, (3) hepatic expression of HMGR (3-hydroxy-3-methylglutaryl coenzyme A reductase, the major rate-limiting enzyme for *de novo* cholesterol synthesis in the liver) and Trf (transferrin, produced especially by hepatocytes and critical for iron uptake), (4) activities of antioxidant enzymes represented by SOD (superoxide dismutase), CAT (catalase), and GPx (glutathione peroxidase). In this way, we try to explain how the Brandt's voles manage to maintain their normal growth status in the continuous presence of dietary TA.

2. Material and methods

2.1. Animals

Thirty-two 4-week-old Brandt's voles, weighing 23.6 ± 1.39 g, were used in the present study. They came from a breeding colony of Brandt's voles maintained at the College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, Jiangsu Province, China. Founder animals in the colony were originally captured in the wild in Xilinhaote League, Inner Mongolia Autonomous Region of China. Animals were kept individually in wire-bottomed cages under a 12-h light/dark cycle and controlled temperature (22–25 °C) with a relative humidity of 40–60%. Drinking water and commercial chow pellets (containing no tannic acid) for growing and breeding rabbits were provided *ad libitum*. The nutrient contents of the food were as follows: crude protein $\geq 15\%$, crude fat $\geq 4\%$, crude fiber 10–15%, ash $\leq 8\%$, calcium 1.0–1.8%, and phosphorus 0.6–1.2%. All experimental procedures were performed following the "Guide for Care and Use of Laboratory Animal" of the Comparative Medical Centre of Yangzhou University (a registered facility for supervising experiments on laboratory animals), and in accordance with the local Animal Care and Use Committee.

2.2. Animal grouping and sample collection

Brandt's voles were divided randomly into two groups (control group and TA-treated group, respectively) with sixteen animals in each group, half male and half female. Voles in the control group were fed with commercial lab chow, obtained from the Comparative Medical Centre of Yangzhou University. Animals in the TA-treated group were fed with a diet containing 3% TA (molecular weight 1701.2), which was purchased as dry power from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. TA was added to the standard diet at the expense of the whole diet. All the voles were monitored for general health and weighed individually each day. Food intake was recorded daily for calculation of average food intake by each vole. The treatments lasted

for continuous 5 weeks. On the 36th experimental day, feces uncontaminated by urine were collected and whole saliva of individual vole was obtained. Saliva production was induced with an intraperitoneal injection of pilocarpine (Sigma, 5 mg per kg body weight), dissolved in 1 mL 9% (m/v) sodium chloride, as described by Muenzer et al. (1979). Saliva was collected directly from the mouth with a micropipette. Both the feces and saliva samples were frozen immediately at -80 °C and used for amino acid analysis. Saliva samples were also separated on 10% SDS-Tris-glycine polyacrylamide gels to get a general profile of saliva proteins. Animals were sacrificed after being anesthetized with 50 mg sodium pentobarbital per kg body weight. Body weight, heart, kidney (both sides), and liver weight were recorded. Liver tissue was cut into pieces, snap-frozen in liquid nitrogen for subsequent analyses.

2.3. Determination of amino acid

Tannin-binding salivary proteins, mainly proline-rich proteins and histatins (rich in histidine), have been reported for many species of rodents and lagomorphs, such as mouse, root vole, rabbit, mountain hare, and North American pika (Shimada 2006). These salivary proteins were reported to be one of the important means by which herbivores minimize the effects of dietary tannins. The presence or absence of these proline- and histidine-rich salivary proteins in the Brandt's vole has not been documented. In this study, the composition of total amino acids in feces and saliva samples was analyzed using an amino acid analyzer (L-8900, Hitachi, Japan) according to the Chinese Standard GB/T 5009.124-2003 (2004). Briefly, samples were hydrolyzed in 6 N HCl solution at 110 °C for 22 h after 10 min of nitrogen blowing in vacuum-sealed glass tubes. For each tube, 1 mL hydrolysate was passed through a 0.22 μ m membrane filter and aliquoted into two vials. From each vial, 20 μ L of the hydrolysates was injected using an auto-sampler. Mixed standard amino acids were analyzed before sampling. The amino acids were identified and quantified by comparing peak profiles of the tested samples with profiles of standard amino acids.

2.4. PCR amplification for HMGR and Trf gene in the liver tissue

Protein sequences for HMGR and Trf from various species were obtained from the EMBL database at <http://srs.ebi.ac.uk/>. After alignment of the protein sequences with the ClustalW 2 program (<http://www.clustal.org/>), consensus-degenerate PCR primers were designed according to the CODEHOP strategy (<http://blocks.fhcrc.org/codehop.html>). All the PCR-related reagents and commercial kits used in this assay were obtained from Takara Biotech Co., Ltd., Dalian, China.

In brief, total RNA was isolated from the liver tissue with Trizol reagent and complementary DNA (cDNA) was synthesized from 2 μ g of total RNA from each sample using the cDNA Synthesis Kit according to the manufacturer's instructions. A touchdown PCR was then executed under the following conditions: a pre-denaturation step of 5 min at 95 °C, followed by 15 cycles of touch down PCR (30 s at 95 °C, then an initial annealing temperature of 61 °C with a temperature decrement of 1 °C following each cycle and treated for 2 min at 72 °C thereafter). From the 16th cycle, the annealing temperature of 47 °C was kept fixed for the next 15 cycles, ending with an extension period of 10 min at 72 °C. The PCR products with expected fragment length were ligated into pGEM-T vector (Promega, Madison, USA) and transformed to *E. coli* Cells (TOP10 strains). Sequencing of the positive clones was performed on an ABI 3130 DNA Sequencer (Applied Biosystem). 3'RACE (rapid amplification of cDNA ends) was also performed for HMGR gene by using a commercial 3'-Full RACE Core Set Ver.2.0 kit. These derived sequences for HMGR gene and Trf gene were used to design the real-time primers using Primer 3.0 software. All primers were synthesized by Sangon Biotech Co., Ltd., Shanghai, China. The complete sequences of primers used in this study were listed in Table 1.

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