Effect of Tannic Acid on the Growth and Survival of Root Voles (Microtus oeconomus)

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Abstract: The objective of this study was to investigate the effects of tannic acid on the growth and survival of small mammalian herbivores. Measurements were conducted with weaned root voles fed with 3 % or 6 % tannic acid and 10 % or 20 % protein in their diets. The results indicated that the effect of tannic acid on growth rate of weaned root voles was greater when given a lower protein diet than a higher protein diet. After 20 d, with 10 % protein diets, mean growth rates of the weaned voles fed with 3 % or 6 % tannic acid were - 0.135 g/d and - 0.25 g/d, respectively. When given 20 % protein diet, mean growth rates of weaned root voles fed with 3 % and 6 % tannic acid for 20 d were 0.134 g/d and - 0.116 g/d, respectively. Food utilization efficiencies of the voles fed with 3 % and 6 % tannic acid diets were significantly lower than that of the control diet at the level of 10 % protein. When given the 20 % protein diet, food utilization efficiencies of weaned voles fed with 6 % tannic acid were significantly lower than that of the voles fed with 3 % tannic acid diet or the control diet with the 10 % protein diets, the average survival days of the weaned voles fed with 3 % and 6 % tannic acid diets decreased 26.23 % and 49.36 % compared to controls at the end of trial period, respectively. With 20 % protein diets, the average survival of weaned voles given 6 % tannic acid diet decreased 39.41 % compared to controls at the end of trial period, although weaned voles given 3 % tannic acid had a slight decrease of average survival days. The results of this study suggested that tannins could substantially affect the individual performance of weaned root voles.

Key words: Tannic acid; Growth rate; Food utilization efficiencies; Survivability; Root vole (*Microtus oeconomus*)

单宁酸对根田鼠断乳幼体生长和存活的影响

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摘要: 在食物中含 10 %和 20 %蛋白质条件下, 测定了单宁酸对根田鼠幼体在断乳后 20 d 内的生长和存活的影响。结果表明, 食物中含蛋白质为 10 %的条件下, 摄食含 3 %和 6 %单宁酸食物的试验个体生长速率分别为 - 0.135 g/d 和 - 0.25g/d, 食物利用效率均显著低于对照组, 断乳后 20 d 内平均存活天数较对照组分别下降 26.23 % 和 49.36 %。在食物蛋白质为 20 %的条件下, 摄食含 3 %和 6 %单宁酸食物的试验个体生长速率分别为 0.134 g/d 和 - 0.116 g/d, 摄食 6 %单宁酸食物的试验个体食物利用效率显著低于摄食 3 %单宁酸食物的试验个体和对照组个体, 断乳后 20 d 内的平均存活天数较对照组下降 39.41 %, 摄食 3 %单宁酸食物的试验个体较对照组略有下降, 但不显著。上述结果验证了单宁酸能显著影响植食性小哺乳动物生长和存活的假设。

关键词: 单宁酸; 生长速率; 食物利用效率; 存活率; 根田鼠

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Years of co-evolution between plants and herbivores have resulted in a diverse array of plant defense and herbivore counter defenses. Tannins, a diverse group of polar, high molecular weight, polyphenolic compounds

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found in many vascular plants, especially woody perennials, that are characterized by their ability to interact with protein[1]. A variety of experiments indicate that foraging patterns and growth rates of insects respond to the concentrations of tannins present in plants[2,3]. Numerous studies also have found plant tannins affect many aspects of the consumer's physiology and metabolism including dietary intake, dietary protein availability, digestive enzyme activity, post absorptive metabolism[4-6] and growth[7,8].

Although Jung et al.[7] and Lindroth et al.[8] investigated the effect of plant secondary compounds on the growth in microtine rodents, the particular dietary levels of digestible energy, crude protein and fiber were not given at the same level in experimental diets. West et al.[9] showed that concentrations of diet nutrients influenced the digestibility of protein and neutral detergent fiber, and affected the growth of lactating cows. Lindrioh et al.[8] wetted and heated the tannin containing diets during the study on the effect of tannins on the growth of meadow voles (*Microtus pennsylvanicus*). Price et al.[5] postulated that when water was added to the grain, tannin formed insoluble complexes with grain proteins, heating enhanced this process. Dietz et al.[10] found that the diet containing 6% tannin prepared by moistening and heating a mixture of lab chow had significantly higher rates of mortality in meadow voles than the diet with 6% tanning added directly to dry chow. Thus, tannin became chemically unextractible and protein became nutritional unavailable.

It is necessary to investigate the effect of tannic acid on the growth and survival of mammalian herbivores at the same level of other nutrients, and tannic acid is directly mixed with other ingredients without wetting and heating.

Root voles (*Microtus oeconomous*) are dietary generalists[11,12] that encounter a variety of defensive compounds in plants, but are unlikely to ingest appreciable amounts of plant secondary compounds. It is well established that the root vole is a tannin consuming mammalian herbivore.

The objectives of this study were to investigate the effects of tannic acid on the growth and survival of root voles. We designed our experiments to test the hypothesis that tannic acid in concentrations similar to those found in nature would reduce the growth of weaned root voles.

1 MATERIALS AND METHODS

Experimental voles came from a root vole colony maintained at the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, Xining, Qinghai, China. Founders for the colony were caught at the Haibei Alpine Eco-System Research Station of the Chinese Academy of Sciences in May, 1998. The voles were housed in plastic cages with hardwood chip bedding and cotton nesting material in the lab with the controlled temperature (20-22℃) and Photo-period (14L:10D). The animals were maintained on rabbit chow and water ad libitum and provided with carrots daily.

Experimental diets were prepared with tannic acid (Tannin Corporation, Peabody, MA, USA). The tannic acid powder was mixed directly with other ingredients at concentration of 0%, 3%, and 6% tannic acid of total dry matter of the diet, and made the mixture into pellets with pellet machines. Dietary level of tannic acid selected in this study spanned the normal range of phenolics in plants eaten by microtine species[8,13].

To detect the presence of a protein-tannic interaction, the levels of proteins in the experimental diets were at 10% or 20%. The crude fiber, digestible energy and minerals of diets were at the similar levels to minimize the effects of those nutrients (Table 1).

Table 1 The ingredients and nutritive value of experimental diets

Ingredients	Food 1	Food 2	Food 3	Food 4	Food 5	Food 6
Corn (%)	39.0	36.0	32.0	11.0	0.0	0.0
Wheat bran (%)	0.0	0.0	0.0	2.0	0.0	0.0
Soybean (%)	0.0	0.5	10.0	26.0	29.0	29.0
Hay powder (%)	60.5	60.0	60.0	60.5	58.5	55.5
Minerals (%)	0.5	0.5	0.5	0.5	0.5	0.5
Tannic acid (%)	0.0	3.0	6.0	0.0	3.0	6.0
Starch (%)	0.0	0.0	0.0	0.0	9.0	9.0
CP%	10.0	10.0	10.0	20.0	20.0	20.0
DE (KJ/g)	2.19	2.20	2.15	2.14	2.16	2.18
CF (%)	18.19	18.20	18.60	18.70	19.0	18.90

CP: Crude protein; CF: Crude fiber; DE: Digestible energy

Experimental animals were weaned at 20 d old. Thirty weaned voles were then randomly assigned to one of the six diets and housed in plastic cages, siblings were given with different experimental diets. This procedure eliminated founding genotypic and maternal effects. During a three days of acclimation period, the animals were fed with a transition diet of rabbit pellets. Each experimental animal received 10.0 g diet once daily for 20 d. The remaining food, including spillage was weighted each day to calculate intake and the body mass of each vole was recorded daily. Food utilization efficiency (g total growth/g total food intake) was calculated as the change in body mass during the trial period divided by the total food consumed, growth rate (g/d) was calculated as the change in body mass during the trial period divided by 20 d, and average survival was calculated as the total survival of the animals in each group divided by numbers of experimental animals in each group.

1.1 Statistics analyses

A two-way ANOVA was used to test whether tannic acid or protein caused a difference of growth rate, survivability, food utilization efficiencies in root voles followed by LSD test with Systat computer package. The $P < 0.05$ level was considered to be significantly different.

2 RESULTS

Analysis of variance of growth rate of root voles showed that the main effect of tannic acid was significantly different ($F = 3.349$, $df = 2, 24$, $P < 0.05$), and the main effect of protein and their interaction were significantly different ($F = 3.081$, $df = 1, 29$, $P < 0.05$; $F = 3.257$, $df = 2, 24$, $P < 0.05$) (Fig. 1). The effect of tannic acid on growth rate of weaned root voles was greater when given with the lower protein diet (10%) than with the higher protein diet (20%). With 10% protein diets for 20 d, mean growth rates of the voles fed with 3% and 6% tannic acid were 0.135 g/d and 0.25 g/d, respectively (Fig. 1). With 20% protein diets, mean growth rates of the weaned root voles fed with 3% and 6% tannic acid for 20 d were 0.134 g/d. and 0.116 g/d, respectively.

As in growth, with 10% protein diets, food utilization efficiencies of the voles fed with 3% or 6% tannic acid diets were significantly lower than that of the control diet (Fig. 2). Food utilization efficiencies did not decrease as much when given with the 20% protein diet. With 20% protein and 6% tannic acid diets, food utilization efficiencies of voles were significantly lower than that of the voles fed with 20% protein and 3% tannic acid diet or the control diet (Fig. 2).

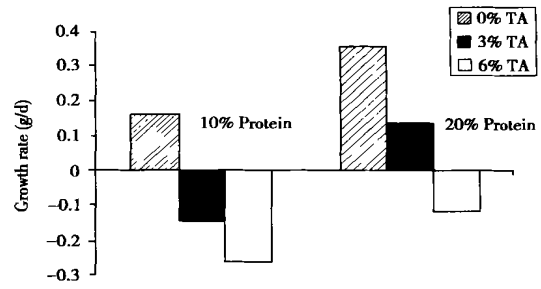

Fig. 1 Growth rates of weaned root voles fed with various tannic acid diets

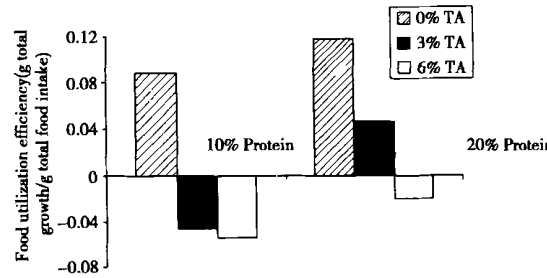

Fig. 2 Food utilization efficiency of weaned root voles fed with various tannic acid diets

Analysis of the variance of average survival days of root vole indicated that the main effect of tannic acid and main effect of protein and their interaction was significantly different ($F = 22.372$, $df = 2, 24$, $P < 0.01$; $F = 13.267$, $df = 1, 29$; $P < 0.05$; $F = 14.879$, $df = 2, 24$, $P < 0.05$). Tannic acid in diet also had a significant effect on the average survival days of weaned root voles. The average survival days of the voles fed 3% and 6% tannic acid diets decreased 26.23% and 49.36% compared to controls at 10% protein at the end of trial period, respectively (Fig. 3). On the high-protein diet, average of survival of weaned voles given 6% tannic acid diet decreased 39.41% compared to controls at 20% protein at the end of trial period, although

weaned voles given 3 % tannic acid showed a slight decrease of average days (Fig. 3) .

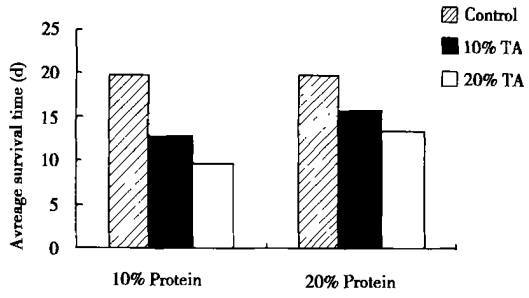

Fig. 3 Average survival time of weaned root voles fed with various tannic acid diets

3 DISCUSSION

3.1 Growth rate and food utilization efficiency

Tannins are considered to be the most widespread and possibly the most important defensive secondary compounds in plants[13,14] , and are frequently ingested by generalist herbivores. The responses of mammalian herbivores to the plant secondary compounds are different due to their gastrointestinal anatomy and items of diets , feeding niche , physiological ability to deal with plant secondary compounds. It has been suggested that tannins could bind with protein , and form insoluble complex in vitro[15,16] . Feeding trials also indicated that tannins inhibited the protein digestibility and suppressed the growth and reproduction of mammalian herbivores[16-18] . This study indicated that tannic acid significantly depressed the growth rate and food utilization efficiencies of weaned root voles at a level of 10 % protein (Fig. 1 and 2). While at 20 % protein level , growth rates and food utilization efficiency of weaned root voles fed with 3 % tannic acid diet were unaffected by tannic acid. This agrees with the results of Lindroth et al.[8] , who showed that tannic acid greatly inhibited the growth rate of prairie voles (*Microtus ochrogaster*). It is possible that the growth depression of weaned voles observed in tannin feeding trials resulted from the intake suppression and the protein digestibility. Voles have enlarged ceca , cecal microbes metabolize a variety of plant secondary compounds and addition of phenolics in the diet of a herbivore often induces a shift in the activity and composition of its microbial communi-ty[19] . Tannins may also interact with digestive enzymes and reduce their activities.

Weanling survival is a crucial process in the life history of mammalian herbivores. The weanling 's food was changed to solids containing fiber and defensive compounds from milk and the weanling 's regulation of body temperature depends on thermogensis instead of depending on mother 's thermogensis. It is sensitive to the environmental factors , and food quality that can substantially affect the growth and survival of weanings[20] . We found that root voles fed with diet containing 6 % tannic acid had significantly lower survivability than those fed with diets containing 3 % tannic acid or no tannic acid. This suggested that the upper limit of tolerance to tannin in root vole depends on its contents and also the protein in the diets. The results suggested that tannic acid or their metabolites were toxic to mammals. It is possible that the loss in body mass observed in this study resulted from the use of fat stores to provide energy for detoxification , and the depletion of these stored fat may have stimulated increase in the consumption to sustain detoxification processes. When the rate of absorbed tannins excesses the rate of detoxification , tannins reach toxic levels , leading to the death of the animals , and this may result in oscillatory instability in the population of voles.

This study strongly supports the hypothesis that tannins can substantially affect the individual performance of root voles. There is growing evidence that the detoxification of absorbed tannins influence the energy for growth and the fat deposit.

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