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The effect of silver birch (*Betula pendula*) powder on physiological performance of field voles (*Microtus agrestis*)

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Many northern arvicoline rodents consume bark of woody plants in times of high population densities. However, the significance of this behaviour to voles themselves is poorly known. We fed adult field voles (*Microtus agrestis*) for two weeks on diets containing 15% to 20% protein mixed with birch (*Betula pendula*) twig powder in concentrations of 0%, 10% or 50% on dry weight basis. Addition of birch powder increased consumption, but did not affect significantly the body mass of voles. With increasing amounts of birch powder the most pronounced effects were increased liver size and activation of two microsomal detoxication enzymes. The results confirm that birch twig is low quality food for the field vole. High proportion of woody forage in the diet may cause severe physiological stress and contribute to the sudden crashes of vole populations.

1. Introduction

Like most boreal arvicoline rodents, field vole (*M. agrestis* L.) is a generalist herbivore (Tahvanainen et al. 1991a), which feeds mainly on grasses and herbs throughout the year (Hansson 1971, Hansson & Larsson 1978). In winter, field vole is also known to cause damage on young deciduous and coniferous trees by gnawing the bark (Hansson 1988). Seedlings of economically valuable trees such as the silver birch (*B. pendula* Roth) can be severely and extensively damaged by vole feeding, especially during peak vole densities.

Generally, however, woody species offer low-quality forage to voles by containing high concentrations of plant secondary compounds and fibre (Bryant et al. 1991) and lower amounts of nutrients (e.g. protein) as compared to herbaceous species (Bucyanayandi et al. 1992). Secondary compounds can deter the feeding by herbivorous mammals, decrease growth and survival of individuals or affect reproduction (Berger et al. 1977, Jung & Batzli 1981, Lindroth & Batzli 1984, Batzli 1985, Lindroth et al. 1986).

The bark of *B. pendula* seedlings is rich in phenolics and terpenoids (Tahvanainen et al. 1991b), which apparently provide the plants with

some protection against voles and other mammalian herbivores (Bryant & Kuropat 1980, Bryant et al. 1991, Rousi et al. 1991). In this study we analyzed how the birch twig powder, added in the laboratory diet, affects the field voles. In a feeding experiment we measured food consumption, changes in body mass, size of liver and kidneys, and reactions of detoxification system. The detoxification capability was studied by measuring the activities of three hepatic microsomal enzymes.

2. Materials and methods

2.1. Experimental diets

Winter-dormant silver birch twigs, rich in resin droplets (see Tahvanainen et al. 1991b) were collected from about 5 year-old saplings nearby Joensuu, Eastern Finland. The twigs were cut into small pieces (5–10 mm) and dried in a drying chamber at 45°C for about 20 hours. The pieces were then ground, and the powder was used directly or it was frozen (–20°C) for later use. In the experiment, commercial laboratory chow containing about 20% of crude protein was used, and three different diets were prepared by adding 0% (control), 10% and 50% (d.w.) of birch powder. The protein concentration of the birch twig powder was 9%. The protein content of experimental diets was not measured, but was calculated as: 20% for the control, about 19% for the 10%-pellets, and about 15% for the 50%-pellets. The amount of total phenolics in the birch powder was 66.6±2.4 mg/g (d.w.), determined by the method of Singleton & Rossi (1965). In addition to phenolics the bark of birch contains high amounts of terpenoids such as papyriferic acid (Tahvanainen et al. 1991b).

2.2. Feeding trial

The field voles used in this experiment were captured in autumn 1987 at the Konnevesi research station, in Central Finland. They were kept in the laboratory for about 4 months before the experiment and were about 6–8 months old at the beginning of feeding. They were kept under natural light and the temperature was + 15 to + 17°C. Voles were caged individually and fed *ad libitum*. The voles were divided randomly into three groups and fed for two weeks with their respective diets: control pellets (n = 5), pellets containing 10% of birch powder (n = 5) and pellets containing 50% of birch powder (n = 7). Consumption was monitored daily and the body mass twice during the experiment. After two weeks feeding the voles were sacrificed by dislocating the neck. Liver and kidneys were removed and weighed, and the liver was stored in liquid nitrogen for later analyzes.

2.3. Chemical analyses

The microsomal fraction of hepatic tissue was prepared as described in Burke & Mayer (1974), with slight modifications. Hepatic tissue was homogenized in 4 vol of 0.25 M saccharose and the microsomal pellets were resuspended in a volume of 0.25 M saccharose, 0.060 M Tris buffer, pH 7.4 (HCl), equivalent to that of the original sample weight. The EROD (ethoxyresorufin-O-dealkylase) and PROD (pentoxyresorufin-O-dealkylase) activities were measured as described in Lubet et al. (1985). The UDP-GT (UDP-glucuronosyltransferase) activity of microsomes was measured by the method of Hänninen (1968), using p-nitrophenol as substrate. The protein content of hepatic microsomes was measured by the Lowry method (Lowry 1951).

The analysis of variance (ANOVA) was used in the statistical analysis of the data. To remove the effect of variation in the size of experimental animals, the initial body mass of voles was used as a covariate.

3. Results

The diet had a significant effect ($F_{2,14} = 35.3$, $P = 0.000$) on the intake of food (Fig. 1). Addition of birch powder increased consumption slightly in 10%-treatment and even more in 50%-treatment. However, the diets did not have any significant effect ($F_{2,14} = 0.1$, $P = 0.865$) on final body mass of voles (control 26.3 ± 5.6 g, 10%-treatment 25.8 ± 6.6 g and 50%-treatment 24.8 ± 4.7 g). No

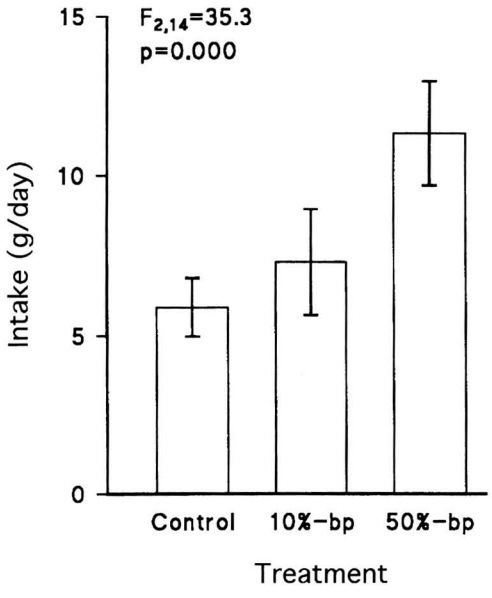

Fig. 1. Food consumption of field voles (g/day, mean±95% confidence limits). Treatments: control = no birch powder addition, 10%-bp = 10% (d.w.) birch powder added, and 50%-bp = 50% (d.w.) birch powder added to the diet.

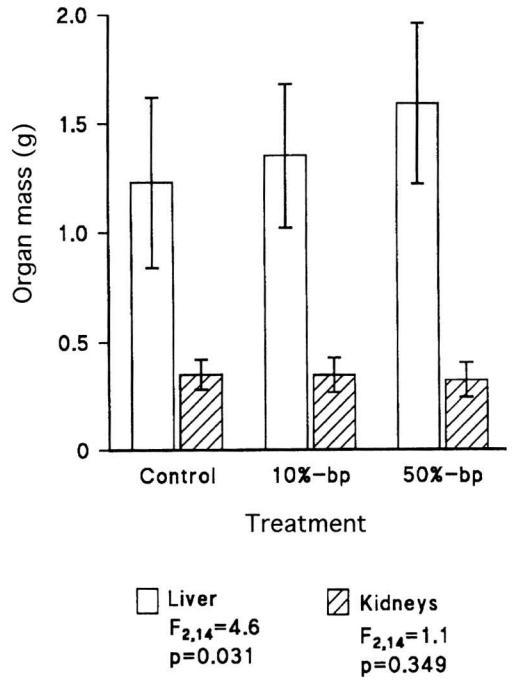

Fig. 2. Masses of liver and kidneys (mean±95% confidence limits). Treatments as in Fig. 1.

correlation was found between food intake and body mass ($r = 0.245$, $P = 0.343$).

The absolute mass of liver increased when birch powder was added to the diet ($F_{2,14} = 4.6$, $P = 0.031$; Fig. 2). The weight of kidneys was not affected ($F_{2,14} = 1.1$, $P = 0.349$). The diets had no effect on hepatic microsomal protein content ($F_{2,14} = 0.3$, $P = 0.761$).

The most pronounced effects of the diets were seen in the detoxification system of voles (Fig. 3). The activity of two dealkylase enzymes (EROD and PROD) nearly doubled when high amount (50%) of birch powder was added to the forage (EROD: $F_{2,14} = 10.0$, $P = 0.002$, PROD: $F_{2,14} = 8.3$, $P = 0.004$). On the other hand, there was no significant effect on UDP-GT activity ($F_{2,14} = 0.5$, $P = 0.593$; Fig. 4).

4. Discussion

Our results show that the addition of birch in forage significantly increased the daily intake of food by field voles (Fig. 1), even though voles clearly reject birch bark when offered along with more favoured food items (Harju & Simola, unpubl. results). Usually the ingestion of secondary compounds has been reported to have no effect or to reduce the food intake of voles (Glick & Joslyn 1970, Jung & Batzli 1981, Lindroth & Batzli 1984, Batzli 1985, Lindroth et al. 1986, Meyer & Richardson 1993).

Since the birch powder contains high amounts of phenolic substances and terpenoids (Tahvanainen et al. 1991b), it is probable that these compounds reduce the quality of forage to voles. On the other hand, the addition of greater amount (50%) of birch twig powder reduced the crude protein content of pellets from 20% to about 15%. Since voles appear to adjust their intake of forage to meet their requirements for nutrients (Shenk et al. 1970, Batzli & Cole 1979, Batzli 1985), it would seem possible that the observed increase in intake could be partly caused by reduction in protein content. However, in our experiment the addition of 50% of birch twig powder in the diet decreased the protein content

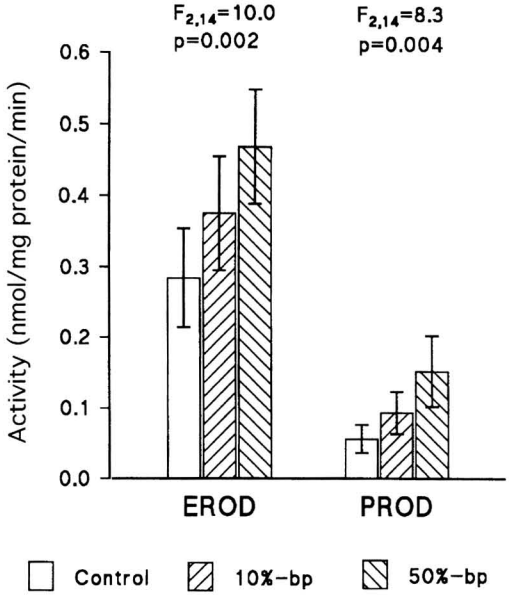

Fig. 3. Activity of ethoxyresorufin-O-dealkylase (EROD) and penthoxyresorufin-O-dealkylase (PROD), values presented as mean ± 95% confidence limits. Treatments as in Fig. 1.

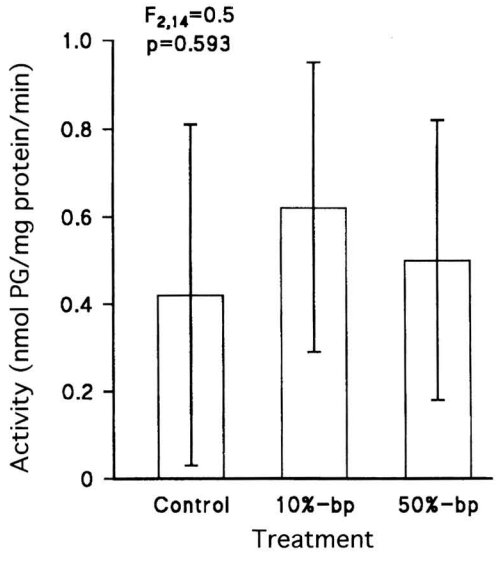

Fig. 4. Activity of UDP-glucuronosyltransferase (mean ± 95% confidence limits). Treatments as in Fig. 1.

of pellets only to 15%, which is still considered optimal protein level for arvicoline rodents (Lindroth & Batzli 1984). Thus the dramatic increase in food intake due to the addition of birch powder (Fig. 1) must have been to a large extent a compensatory reaction to the negative physiological effects of secondary compounds present in birch material. This is supported also by the fact that although voles in 50%-group consumed twice as much food as the control animals, they did not gain weight.

In their diet, generalist herbivores consume a diverse array of potentially dangerous plant metabolites (Jung & Batzli 1981, Bergeron & Jodoin 1985). Herbivores have, however, physiological and biochemical mechanisms to detoxify the secondary metabolites. In our experiment, animals in the 50%-group had larger livers than control animals, suggesting that the capacity to handle the potentially toxic components of birch powder has increased. On the other hand, the excretion capacity (i.e. size of kidneys) was not affected. It has been shown earlier that the size of liver and the kidneys may change in response to plant toxins (Jung & Batzli 1981, Bergeron & Jodoin 1982, 1984, Lindroth & Batzli 1984, Siess et al. 1988) either by hypertrophy or by hypotrophy.

The detoxification capacity of herbivores can be increased also by the induction of detoxifying enzymes in the liver. Both oxidative (phase I) and synthetic/conjugative (phase II) enzymes are reported to respond to plant secondary metabolites (Brattsten et al. 1977, Chadha & Madyastha 1984, Alldrick et al. 1988, Siess et al. 1988). Our results show that ingestion of birch powder activated markedly the detoxification system of field voles. The activities of oxidative enzymes EROD and PROD were nearly two-fold in the 50%-group as compared to control animals. The total detoxification capacity of liver, including the increase in the size of liver, was increased by about 3 to 4-fold in the 50%-group compared to control. Following such an induction of the detoxification system the herbivore would be less susceptible to dietary poisoning (Brattsten et al. 1977). The induction of a conjugation enzyme (UDP-GT) observed e.g. with flavone addition in the diet of

laboratory rats (Siess et al. 1988) was not found in our experiment.

In conclusion, our results show that field voles are chemically stressed when forced to feed on a diet containing birch twigs. Increased intake of forage together with negligible or no weight gain by animals suggests lowering of food quality. This could have ecological significance in times of high population densities and shortage of high-quality food. The risk of tree seedlings to be heavily browsed increases and the voles become subjected to deleterious, food-based physiological stress which may be one of the several reasons resulting in dramatic population crashes of northern vole populations.

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References

Alldrick, A. J., Lake, B. G., Mallett, A. K. & Rowland, I. R. 1988: Modulation of hepatic-phase I and gut-microflora xenobiotic metabolizing enzymes by dietary flavonoids. — In: Cody, V., Middleton, E., Jr. & Harborne, J. B. (eds.), Plant flavonoids in biology and medicine II: biochemical, cellular, and medicinal properties: 139–142. A. R. Liss, Inc., New York.

Batzli, G. O. 1985: Nutrition. — In: Tamarin, R. H. (ed.), Biology of New World microtus: 779–811. Special Publication of the American Society of Mammalogists.

Batzli, G. O. & Cole, R. F. 1979: Nutritional ecology of microtine rodents: digestibility of forage. — J. Mamm. 60:740–750.

Berger, P. J., Sanders, E. H., Gardner, P. D. & Negus, N. C. 1977: Phenolic plant compounds functioning as reproductive inhibitors in Microtus montanus. — Science 195:575–577.

Bergeron, J.-M. & Jodoin, L. 1982: Effets des composés secondaires des plantes sur le poids corporel et le poids de certains organes de la souris de laboratoire. — Can. J. Zool. 60:1855–1866.

— 1984: Impact de 12 plantes communes de millieu ouvert sur les variations de poids corporel et de poids d'organes internes du campagnol des champs (Microtus pennsylvanicus). — Can. J. Zool. 62:50–57.

— 1985: Fiabilité des mesures de poids et d'examens histopathologiques dans les études d'intoxication du campagnol des champs (Microtus pennsylvanicus). — Can. J. Zool. 63:804–810.

Bryant, J. P. & Kuropat, P. J. 1980: Selection of winter forage by subarctic browsing vertebrates: the role of plant chemistry. — Ann. Rev. Ecol. Syst. 11:261–285.

Bryant, J. P., Provenza, F. D., Pastor, J., Reichardt, P. B., Clausen, T. P. & du Toit, J. 1991: Interactions between woody plants and browsing mammals mediated by secondary metabolites. — Ann. Rev. Ecol. Syst. 22:431–446.

Brattsten, L. B., Wilkinson, C. F. & Eisner, T. 1977: Herbivore-plant interactions: Mixed-function oxidases and secondary plant substances. — Science 196:1349–1352.

Bucyanayandi, J.-D., Bergeron, J.-M., Soucie, J., Thomas, D. W. & Jean, Y. 1992: Differences in nutritional quality between herbaceous plants and bark of conifers as winter food for the vole Microtus pennsylvanicus. — J. Appl. Ecol. 29:371–377.

Burke, M. D. & Mayer, R. T. 1974: Ethoxyresorufin: direct fluorometric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. — Drug Metab. Dispos. 2:583–588.

Chadha, A. & Madyastha, K. M. 1984: Metabolism of geraniol and linalool in the rat and effects on liver and lung microsomal enzymes. — Xenobiotica 14:365–374.

Glick, Z. & Joslyn, M. A. 1970: Food intake depression and other metabolic effects of tannic acid in the rat. — J. Nutrition 100:509–515.

Hansson, L. 1971: Habitat, food and population dynamics of the field vole Microtus agrestis (L.) in south Sweden. — Viltrevy 8:267–373.

— 1988: Grazing impact by small rodents in a steep cyclicity gradient. — Oikos 51:31–42.

Hansson, L. & Larsson, T.-B. 1978: Vole diet on experimentally managed reforestation areas in northern Sweden. — Holarct. Ecol. 1:16–26.

Hänninen, O. 1968: On the metabolic regulation in the glucuronic acid pathway in the rat tissues. — Ann. Acad. Sci. Fenn. A2 142:1–96.

Jung, H.-J. G. & Batzli, G. O. 1981: Nutritional ecology of microtine rodents: effects of plant extracts on the growth of arctic microtines. — J. Mamm. 62:286–292.

Lindroth, R. L. & Batzli, G. O. 1984: Plant phenolics as chemical defences: Effects of natural phenolics on survival and growth of prairie voles (Microtus ochrogaster). — J. Chem. Ecol. 10:229–244.

Lindroth, R. L., Batzli, G. O. & Avildsen, S. I. 1986: Lespedeza phenolics and Penstemon Alkaloids: Effects on Digestion Efficiencies and Growth of Voles. — J. Chem. Ecol. 12:713–728.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. 1951: Protein measurement with the Folin phenol reagent. — J. Biol. Chem. 193:265–275.

Lubet, R. A., Mayer, R. T., Cameron, J. W., Nims, R. W., Burke, D. M., Wolff, T. & Guengerich, F. P. 1985: Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) p-450 by phenobarbital and other xenobiotics in the rat. — Arc. Biochem. Biophys. 238:43–48.

Meyer, M. W. & Richardson, C. 1993: The effects of chronic tannic acid intake on prairie vole (Microtus ochrogaster) reproduction. — J. Chem. Ecol. 19:1577–1585.

Rousi, M., Tahvanainen, J. & Uotila, I. 1991: A mechanism of resistance to hare browsing in winter-dormant European white birch (Betula pendula). — Am. Nat. 137:64–82.

Shenk, J. S., Elliot, F. C. & Thomas, J. W. 1970: Meadow vole nutrition studies with semisynthetic diets. — J. Nutrition 100:1437–1446.

Siess, M-H., Brouard, C., Vernevaut, M-F. & Suschetet, M. 1988: Effects of feeding quercetin and flavone on hepatic drug metabolizing enzymes of rat. — In: Cody, V., Middleton, E., Jr. & Harborne, J. B. (eds.), Plant flavonoids in biology and medicine II: biochemical, cellular, and medicinal properties: 147–150. A. R. Liss, Inc., New York.

Singleton, V. L. & Rossi, J. A., Jr. 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. — Am. J. Enol. Viticult. 16:144–158.

Tahvanainen, J., Niemelä, P. & Henttonen, H. 1991a: Chemical aspects of herbivory in boreal forest – feeding by small rodents, hares and cervids. — In: Palo, R. T. & Robbins, C. T. (eds.), Plant defenses against mammalian herbivory: 115–131. CRC Press, Boca Raton.

Tahvanainen, J., Julkunen-Tiitto, R., Rousi, M. & Reichardt, P. B. 1991: Chemical determinants of resistance in winter-dormant seedlings of European white birch (Betula pendula) to browsing by the mountain hare. — Chemoecology 2:49–54.