Journal of Chemical Ecology, Vol. 26, No. 2, 2000

SELECTION FOR SMALL AMOUNTS OF HYDROLYSABLE TANNINS BY A CONCENTRATE-SELECTING MAMMALIAN HERBIVORE

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(Received February 2, 1999; accepted September 18, 1999)

Abstract—Although herbivores usually avoid plants with high concentrations of tannins, roe deer in natural habitats select tannin-rich plants. We tested the hypotheses that: (H1) roe do not seek tannins, rather they select for other (unmeasured) factors such as minerals or vitamins; and (H2) roe do select for tannins. Tame roe deer were offered a choice of food pellets to which hydrolyzable chestnut tannins had been added or control pellets. The daily consumption of pellets containing a high level of tannins (9.1%) was lower than consumption of control pellets. However, when offered medium level tannin pellets (3.8%), roe deer ate more tannin than control pellets. Moreover, the roe regulated their intake of these tannins closely. The regression of tannin intake versus total pellet intake across all the tests was linear and precise (r^2 = 0.94): intake was about 28 g of tannin per kilogram of pellets eaten. We therefore reject H1 and suggest that roe can monitor tannin concentrations in their food, and regulate their intake precisely. The results suggest that a certain level of tannins induces no costs or that they provide a nutritional benefit for roe deer.

Key Words—Tannin, diet, herbivore, roe deer.

INTRODUCTION

Tannins are a chemically diverse set of phenolic metabolites that occur widely in plants and have long been known to have antiherbivore properties. The mech-

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0098-0331/00/0200-0351$18.00/0

anisms involved are not fully understood, but they include erosive effects on the digestive mucosa, and if degraded in the gut, hydrolyzable tannins can have toxic effects (see Bernays et al., 1989 for a review). Hydrolyzable and condensed tannins can reduce the activity of digestive enzymes and, thus, the digestibility of foods, in particular, proteins, but this effect varies among animal species and tannin structures (Robbins et al., 1991). The literature suggests that mammalian herbivores typically avoid foods with high concentrations of phenolics, particularly tannins (see Waterman and Mole, 1994). This rule does not hold for the roe deer (*Capreolus capreolus*), which prefers plants with relatively high concentrations of phenolics (Duncan et al., 1998). This behavior, which may occur in other small ruminants, could be explained by selection for other associated factors (such as minerals or vitamins) in the high-phenolic plants.

Alternatively, ingestion of phenolics may not impose any cost on the roe deer if they are well adapted to these secondary metabolites. It has been shown recently that the saliva of roe deer contains a complex protein fraction that has a high binding affinity for tannin (Fickel et al., 1999), and tannin-binding salivary protein is known to prevent tannin metabolization and the reduction of protein digestibility in another cervid, the mule deer (Robbins et al., 1991). Further, roe deer have relatively large livers (Duncan et al., 1998), and this suggests that they are physiologically equipped to deal with tannins.

Tannin ingestion may have beneficial effects on mammalian herbivores because condensed and hydrolyzable tannins are important biological antioxidants. Hagerman et al. (1998) suggested that tannin antioxidant properties could protect proteins, carbohydrates, and lipids in the digestive tract from oxidative damage during digestion. Another beneficial effect could be the control of intestinal worm parasites (Hodgson et al., 1996). The effect most often described is the formation of tannin–protein complexes that can enhance protein assimilation by avoiding microbial breakdown of protein in the rumen. For example, Liu et al. (1998) and Terrill et al. (1992) tested the effect of small quantities of condensed tannin in the diet on ammonia concentrations in the rumen of lambs. They found that condensed tannin in the diet at a concentration of 0.5% dry matter (DM) could induce an increase in protein assimilation. However, this finding needs to be treated with caution since: (1) the protection of protein is not nutritionally beneficial unless high-quality protein is eaten or dietary protein levels are very high; and (2) the chemical reaction of protein with tannins is reversible only in part, and most of the protein is lost with feces as tannin–protein complexes.

In this study, we measured experimentally the daily intakes by captive roe deer of pelleted food mixed either with hydrolyzable tannins (at two different concentrations, 3.8% and 9.1%) or control pellets with no tannins, in order to test two hypotheses: H1: roe deer do not seek tannins in their feeding (they prefer phenolic-rich plants simply because they have high concentrations of other unmeasured nutrients). If true, roe should prefer food without tannin rather than identical food with added tannin in order to avoid the costs of detoxification and/or of salivary protein production. H2: roe deer prefer tannin-containing foods. Since there can be costs involved in eating tannins, especially when these are toxic, we expect under H2 that roe will not maximize their intake of tannin.

METHODS AND MATERIALS

Pellets. We offered the deer standard pellets for goats (Alicoop Proxima, 19.5% crude protein) mixed either with hydrolyzable pyrogallic tannins from chestnut wood (*Castanea sativa*) (Raoul Duvall and Co., Le Havre, France) or with water. In the plants of the surrounding forest, the content of polymerized phenolics that can potentially bind to proteins varied between 0.1 and 6% DM (data in Tixier et al., 1997). The tannins were offered at two levels of dry matter, 3.8 and 9.1%. These were medium to high values compared with the levels of total polyphenolics in forest plants in our study area.

Tannic pellets were made by pouring a solution of the tannins onto the pellets in order to obtain a level of 3.8 or 9.1% tannin and then drying them at 60°C. This treatment changed the structure of the pellets, making them more powdery; the control pellets were therefore soaked with the same volume of water as the tannic pellets and dried in the same manner.

Animals. It is known that the capacity of an herbivore to use tannin-containing foods depends to some extent on the animal's own experience with antiherbivore defenses; goats appear to be more willing to feed on tannin-containing food if they have experienced this type of food previously (Distel and Provenza, 1991). Roe deer familiar with tannin-containing plants may be more prepared to eat tannin-rich pellets than individuals living in grassland pens without access to high tannin plants. We, therefore, used animals living in grassland pens as well as animals in seminatural conditions with free access to woodland vegetation.

In order to provide the deer with visual clues for the two types of pellets, we used food bowls of different colors for the tannic and control pellets. In preliminary tests, the animals ate standard pellets willingly from bowls of both colors, and we reversed the colors between successive tests. We used four groups of deer, two living in pens on a woodland edge and two in grassland pens in December 1997 and April and August 1998 (Table 1). The same quantities of tannic and control pellets were offered simultaneously. For pens 1 and 3, the control pellets were provided in white bowls and the tannic ones in blue bowls; for pens 2 and 4 the colors were reversed. The white and blue bowls were positioned alternately in a line, protected from rain and sun, and their positions were reversed each day. After 24 hr, each bowl was weighed and refilled with a standard amount of tannic or water pellets. For the analysis, we ignored the

TABLE 1. ANIMAL GROUPS USED FOR MEASUREMENT OF DAILY CONSUMPTION OF TANNIC AND CONTROL PELLETS

Habitat	Animals using feeding bowls	Month	Tannin levels (%)	Range of pellet intake: Tannic (g/ind/day)	Range of pellet intake: Control (g/ind/day)	Duration (days)
Grassland pen 1	2 nonpregnant ♀, 2 pregnant ♀, 1 adult ♂	Dec	9.1	94–157	274–382	5
	1 pregnant ♀, 1 lactating ♀	Apr	9.1	283–643	723–1065	5
Grassland pen 2	1 pregnant ♀, 3 nonpregnant ♀, 1 adult ♂, 6 juveniles	Apr	9.1	176–270	432–497	6
	1 pregnant ♀, 3 nonpregnant ♀, 1 adult ♂, 5 juveniles	Aug	3.8	254–398	144–230	7
Woodland pen 3	5 pregnant ♀, 1 adult ♂	Dec	9.1	37–211	79–194	5
	5 pregnant ♀, 1 adult ♂	Apr	9.1	79–338	365–493	5
Woodland pen 4	1 pregnant ♀, 2 nonpregnant ♀, 1 adult ♂	Apr	9.1	69–281	353–694	8
	1 lactating ♀, 2 fawns, 2 nonlactating ♀, 1 adult ♂	Aug	3.8	207–500	63–388	15

results for the few days in which the animals finished the pellets of one type, so there was no longer a choice. The results are expressed as the proportion of pellets eaten that were tannic ones, and as the amount of tannin eaten (grams per individual per day).

Statistical Analysis. We performed a one-way ANOVA to test the effect of the habitat type on the amount of tannic pellets eaten and on the total amount of pellets eaten. To compare the daily consumption of tannic and water pellets, we used the Wilcoxon signed-rank test for paired data with the same variables. To study the amount of tannin ingested, we tested the parameters of the regression of the amount of tannin eaten on the total pellet intake per day and per individual by using analysis of covariance with habitat type (grassland or forest) and tannin levels (medium or high) as factors.

TABLE 2. DAILY CONSUMPTION BY GROUPS OF ROE DEER OFFERED CHOICE OF CONTROL AND TANNIC PELLETS AND SIGNIFICANCE OF DIFFERENCES

Tannin level	Mean consumption: Control pellets (g/individual/day, SD)	Tannic pellets (g/individual/day, SD)	Wilcoxon signed-rank test	Total intake (g/individual/day)
Medium (3.8%)				
Overall $N = 22$ days	196 (63)	384 (79)	0.0001	580
Grassland $N = 7$	183 (27)	357 (50)	0.0001	540
Woodland $N = 15$	202 (89)	396 (88)	0.0015	598
High (9.1%)				
Overall $N = 29$ days	488 (236)	217 (137)	0.0001	705
Grassland $N = 13$	585 (264)	278 (163)	0.0002	863
Woodland $N = 16$	408 (182)	168 (90)	0.0001	576

RESULTS

The deer ate more medium tannin pellets than control ones, and the difference was significant in both grassland and forest habitats (Table 2). The deer ate more control than high tannin pellets (Table 2), and the difference was again significant in both grassland and forest habitats. Had the animals eaten the pellets without reference to the tannin content, their diets would have contained 50% tannic pellets and 50% control pellets on average. Deer in the woodland ate less tannin per day than those in grassland (15.24 g/day and 21.24 g/day, respectively; $F_{1,49} = 4.84$, $P = 0.033$, $R^2 = 0.09$).

The regression of the amount of tannin eaten versus the total amount of pellets eaten was significant (Figure 1). Tannin level (3.8 vs. 9.1%) had no effect on this relationship (covariance analysis with habitat type and tannin level as a factor: $F_{1,23} = 4.01$, $P = 0.051$ and $F_{1,2} = 2.57$, $P = 0.11$, respectively). The double and triple interactions (amount of pellets eaten × habitat type × tannin level) were not significant: $F_{1,7} < 0.21$, $P > 0.65$. The intercept was not different from zero ($T = -1.59$, $P = 0.12$), and the regression with an intercept equal to zero (Figure 1) was: tannin intake = 0.0277 total pellet intake; $P = 0.0001$, $F_{1,50} = 723.15$, $R^2 = 0.935$.

This analysis shows that the animals did not maximize their tannin intake, but regulated it at about 28 g of tannin per kilogram of pellets eaten.

DISCUSSION

These roe deer ate more medium (3.8%) tannin pellets than control pellets on a daily basis, and fewer high (9.1%) tannin pellets. These results show that

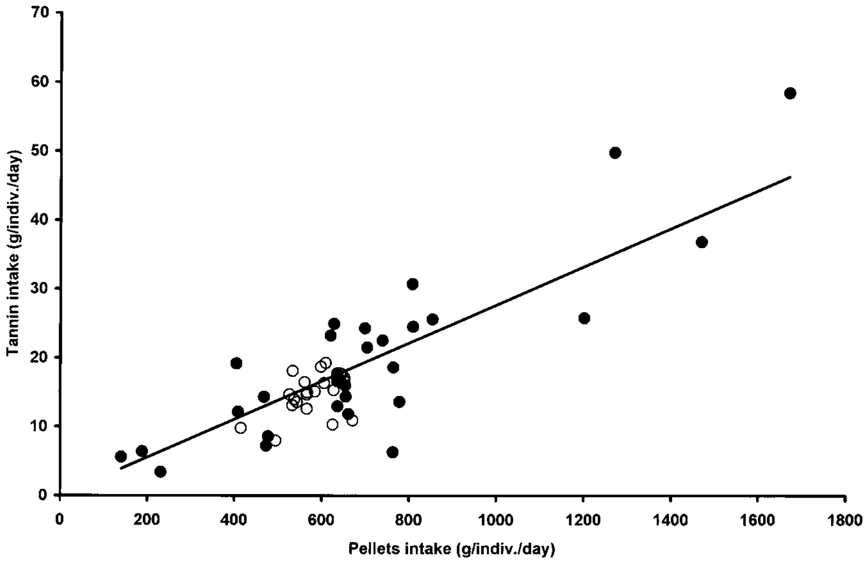

FIG. 1. The regression of the amount of tannin eaten versus the total amount of pellets eaten by groups of roe deer offered the choice between tannic (medium level, 3.8%: open circles; high level 9.1%: solid circle) and control pellets. The equation was tannin intake = 0.0277 total pellet intake; $R^2 = 0.935$, $P < 0.01$.

roe are able to recognize tannins in their food. We reject H1 since roe ate more medium tannin than control pellets. The roe ate 540–860 g/individual/day of pellets, which is about the total intake of natural foods recorded in other studies (e.g., 450–800 g DM; Drozdz, 1979). Although we did not measure the amount of natural food they ate, we consider that the pellets provided the majority of the food eaten. We, therefore, do not expect that seasonal effects on tannin consumption would affect the results substantially. However, the medium tannin pellets were offered in August only; it would be interesting to repeat the experiment in other seasons.

Roe deer adjusted their food choice to obtain a diet containing about 28 g of tannin per kilogram of pellets eaten, whatever the concentrations offered. Tixier et al. (1997) found that roe deer in a forest-edge environment selected for plants with relatively high levels of total polymerized phenolics and for a variety of other correlated phenolic types (short-chain, soluble, and protein-binding ones). The experiment described here suggests that this result was not fortuitous since the animals had a pattern of food selection that resulted in their eating a rather constant amount of hydrolyzable tannins per kilogram of artificial pellets. We did not measure how much tannin in the natural plants the animals in the woodland pens ate, so we cannot calculate the total amounts of tannin eaten. Nonetheless, we accept H2 since: (1) roe deer select tannic foods under some circumstances (when the tannin concentrations are not high); and (2) they appear to regulate the amount of tannin consumed in relation to the amounts in the food eaten.

We know of no similar results in the literature; however, in other trials involving mammalian herbivores feeding on tannins, animals were not given the choice between tannin-free and tannic foods because trials were conducted in order to assess the effects of tannins on protein digestibility (Robbins et al., 1987) or on ruminal digestive processes (Aharoni et al., 1998) or to explore physiological mechanisms used to counteract tannins (Austin et al., 1989; Robbins et al., 1991; Hagerman and Robbins, 1993).

These results imply that eating food with 4% tannins has no cost and may even provide a benefit(s). If roe deer normally eat a certain amount of tannic plants, it may be that physiological mechanisms of defense need to be maintained and that this is achieved by an intake of about 30 g/kg pellets. The tannin intake of these roe deer did not exceed 35 g/kg pellets, which suggests that above this threshold the costs of eating tannin exceed the benefit.

Other potential beneficial effects of dietary tannins have been proposed (protection of proteins from oxidative damage, enhancement of protein assimilation; see Introduction). We have no evidence that any of these accrue to these deer, and further work is required to test such hypotheses before the function of selection for tannin-rich plants by roe deer can be understood.

The diet of roe deer in the wild is diverse (Duncan et al., 1998), and this may allow them to reduce the toxic effects of the tannins they eat by consuming only low levels of the different types they encounter. In this way, they could obtain the advantages of tannin ingestion while minimizing the costs of the ingestion of large quantities of a single type. Moreover, mixing the types of tannins ingested could have other advantages, through synergistic (Waterman and Mole, 1994) or complementary actions. Squirrels have been shown to use artificial diets with different secondary metabolites (oxalate and tannic acid) in a complementary manner (Schmidt et al., 1998).

Acknowledgments—Ann Hagerman gave us useful advice on tannins, Nadine Guillon provided skilled assistance in the experiments, Hervé Fritz provided perceptive ideas at the analysis stage, and Joerns Fickel, Clare McArthur, and two anonymous referees made helpful comments on a previous version of this paper.

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