Role of Tannic Acid on Digestion by Red Squirrels

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Introduction

The effects of dietary phenolics (or tannins) have been of considerable interest to nutritionists and ecologists because they apparently reduce nutrient availability and subsequently affect metabolism. Phenolics form both reversible and irreversible chemical complexes with proteins and, to a lesser extent, soluble carbohydrates (46). Formation of these complexes in the gut is potentially deleterious to digestion and/or assimilation regardless of whether the phenolics complex with macromolecules of dietary (31) or endogenous origin (26). Additionally, phenolic complex formation may greatly alter gastric and intestinal secretions (9,12,22,25), and nutrient assimilation may be hindered by the action of phenolics on intestinal epithelium (27). Some phenolics may themselves be hydrolized by digestive enzymes or gut microflora and absorbed (24). Circulating phenolics have been implicated with a wide variety of metabolic dysfunctions including interruption of general protein and lipid metabolism (39), changes in cholestrol metabolism (49), hepatic malfunctions (36), interaction with thiamine (40), alterations in the metabolism of methionine and choline (3), and impairment of reproductive performance (7,38).

Phenolic content has been correlated with reduced palatability to a variety of herbivorous species (11,13,30,34,35), probably as a result of their astringent characteristics when in contact with oral epithelium or subsequent metabolic effects. However, interpretation of herbivore-plant interaction may be significantly complicated by the observation that in some cases phenolics promoted efficient digestion by favorably altering gut pH when acidosis existed. The potential for ruminal or cecal acidosis is increased when grains represent a large portion of the diet (48). In fact, some ungulates may even select bark containing high concentrations of phenolics to control rumen fermentation (Prins—personal communication 1977).

Knowledge of animal adaptations to plant defenses to herbivory are generally lacking. Although some research has been concerned with the response of ruminants to plant secondary compounds (8,15,16,22,25,29), almost nothing is known about the response of wild monogastric herbivores to the ingestion of phenolics. Most nutritional studies of squirrels and other small herbivorous mammals have concentrated on food habits and energy contributions of foods. Certainly the viability of squirrel populations is dependent upon nutritional parameters since their breeding rate depends on the extent of fall mast crop (2,5,19,32,33,37,45). Presumably the biological quality of the crop is an important determinant as well. Forage selectivity and physiological considerations of squirrels apparently involve dynamic interelationships of mineral assimilation (14,43) and energy metabolism (17,21,28,42). Ofcarcik et al. (35) inferred that there exists an inverse relationship of phenolic content and feeding preferences of squirrels. However, no study has

been conducted on any herbivore for the purpose of quantitatively elucidating the digestive effects of phenolics. The multitide of changes in pH and substrate availability in the gut complicate the precise definition of phenolic influence since no quantitative methodologies assure accurate determination of phenolic content of both feed and feces. Such problematic considerations can, however, be avoided if purified diets are utilized to which phenolic content is added in known quantities. This study seeks to determine the quantitative effects of a common phenolic (tannic acid) on the digestive response of captive red squirrels (*Tamiasciurus hudsonicus*) consuming different phenolic concentrations in otherwise equivalent artificial diets.

Methods

Mature male squirrels were live trapped in St. Joseph Co., Indiana and maintained in individual wire cages (45x45x35 cm). Squirrels were fed a balanced rodent ration from Bio-Serv. Inc. (18.50% protein, 5.05% fat, 2.99% ash) for a minimum of 10 days prior to the initiation of the experimental trials. Feed and water were available *ad libitum* throughout the study and photoperiod was determined by natural lighting.

Test feeds consisted of the complete and balanced rodent ration (Bio-Serv) to which 0.00%, 0.50%, 0.75%, 1.00%, 2.00%, and 5.00% tannic acid were added to the test rations replacing undigestible fiber. The feeding trials lasted 7 days for each of the 6 diets. Six different squirrels were fed diets in sequence from tannic acid-free rations through the 5.00% tannic acid diet. Days 1 through 3 were allowed for the squirrels to adjust to the experimental diets. Daily feed consumption did not shift significantly within trial periods for individual squirrels even though feed was available *ad libitum*. Days 4 through 7 served as the collection period when feces was saved for digestibility determinations. Uncontaminated feed and feces samples were dried at 80C for 24 hours to determine dry matter content, then ground to pass a 20 mesh screen in a Wiley mill and stored in airtight containers for later analysis. Lipid content of feed and feces was determined by standard Soxhlet extraction procedures using purified ether as the solvent. Results of the feeding trials were statistically analyzed by one way analysis of variance and, if significant, by Duncan's Multiple Range Test. The 0.05 level of significance was applied.

Results and Discussion

Average daily consumption rates were highest for the control diet and lowest for the 0.75% tannic acid feed (Table 1). Differences in consumption rates, ex-

TABLE 1. *Mean (± Standard Deviation) daily consumption rates of red squirrels feeding on diets with variable tannic acid content.*

	Consumption			
	g-feed/day		per 100g BW/day	
Diet	$\bar{X}$	± SD	$\bar{X}$	± SD
0.00% Tannic Acid	16.29	1.01	8.82	0.23
0.50% Tannic Acid	12.15	0.93	6.58	0.67
0.75% Tannic Acid	11.23	0.57	6.08	0.59
1.00% Tannic Acid	12.48	1.12	6.76	0.67
2.00% Tannic Acid	12.17	0.87	6.60	0.48
5.00% Tannic Acid	12.75	1.02	7.45	0.93

pressed either as per squirrel or per 100 g body weight, among diets were insignificant except for the control diet which was significantly higher than each tannic acid feed. Although consumption data for red squirrels is lacking in the literature, these red squirrels consumed even the least preferred ration at a rate from 31.7% to 63.7% higher than gray or fox squirrels consuming native forages and commercial rations (data recalculated from:6,14,17,42) on a per body weight basis. Since consumption rates for boreal squirrels are apparently linked directly with metabolized nutrient efficiency (14), the high feed consumption observed in this study could be expected for an artificially balanced ration. Additionally, the increased metabolic rate for the smaller red squirrel could result in at least a portion of this increased overall consumption rate. The observed reduction in relative consumption of feeds containing any concentration of tannic acid is at least partially consistent with the inverse relationship of tannin content and food preferences by fox squirrels described by Ofcarcik et al (35). Conversely Smith and Follmer (44) concluded that tannin content is not an important factor in food preferences by squirrels. The data presented in this study suggests that intake is negatively affected by tannic acid content at any level, however, increased dietary concentration of this particular phenolic is not matched by a proportional (or even more extensive) reduction in intake. The suggestion by these data that reduced intake represents a qualitative rather than quantitative phenomenon must be carefully tempered by the fact that the inclusion of only one phenolic compound in the diet of this study may considerably underestimate the complexity of relationships observed with native forages.

Dry matter digestibility of the control ration averaged 92.57% and ranged from 91.44% to 94.49% (Figure 1). The affect of tannic acid on apparent dry matter digestibility was insignificant for diets containing 0.75, 1.00, 2.00, and 5.00% tannic acid, but was significantly reduced for the 0.50% tannic acid which averaged 90.57% dry matter digestibility. These data are contrary to the pattern that increased phenolic content is equated with reduced digestibility as found in a variety of other herbivores including avians (31), rodents (49), and ungulates (29). That digestibility was most affected in the 0.50% tannic acid ration is likely a result of the sequential feeding schedule in which it was the first phenolic containing diet offered. Apparently, red squirrels require an extended adaptation period (greater than the 3 day pretrial period) to adjust to the ingested tannic acid. Subsequent consumption of tannic acid is more easily tolerated since its adverse effects on digestion are clearly neutralized. Cecal digestors (including red squirrels) possess an active array of gut microflora which may respond to dietary phenolics. Rumen microorganisms are known to respond in terms of relative species abundance to dietary tannin content and exhibit no apparent deleterious effects upon the host by tannins comprising up to 2.5% of dietary intake (4). This adaptation by these red squirrels could also involve, in whole or in part, adjustments in the enzymatic activity and/or secretion rate by the squirrels.

The increased apparent dry matter digestibility (although not statistically significant) in the 0.75% and 5.00% diets over the control diet (Figure 1) suggests that tannic acid itself may be digestible since it replaced, as a percentage, undigestible fiber in the rations. Although the extent of tannic acid digestibility cannot be determined by this study, if indeed it is assimilated by red squirrels, there exists a potential for extensive histological and metabolic pathology. Circulating tannic acid has been demonstrated to precipitate acute hepato-toxicity in rats (36), reduced fundicity and result in renal abnormalities (24).

Predictably, lipid digestibility was high for the artificial rations fed in this study (Figure 1). Statistical deviation among diets was observed, but only in the

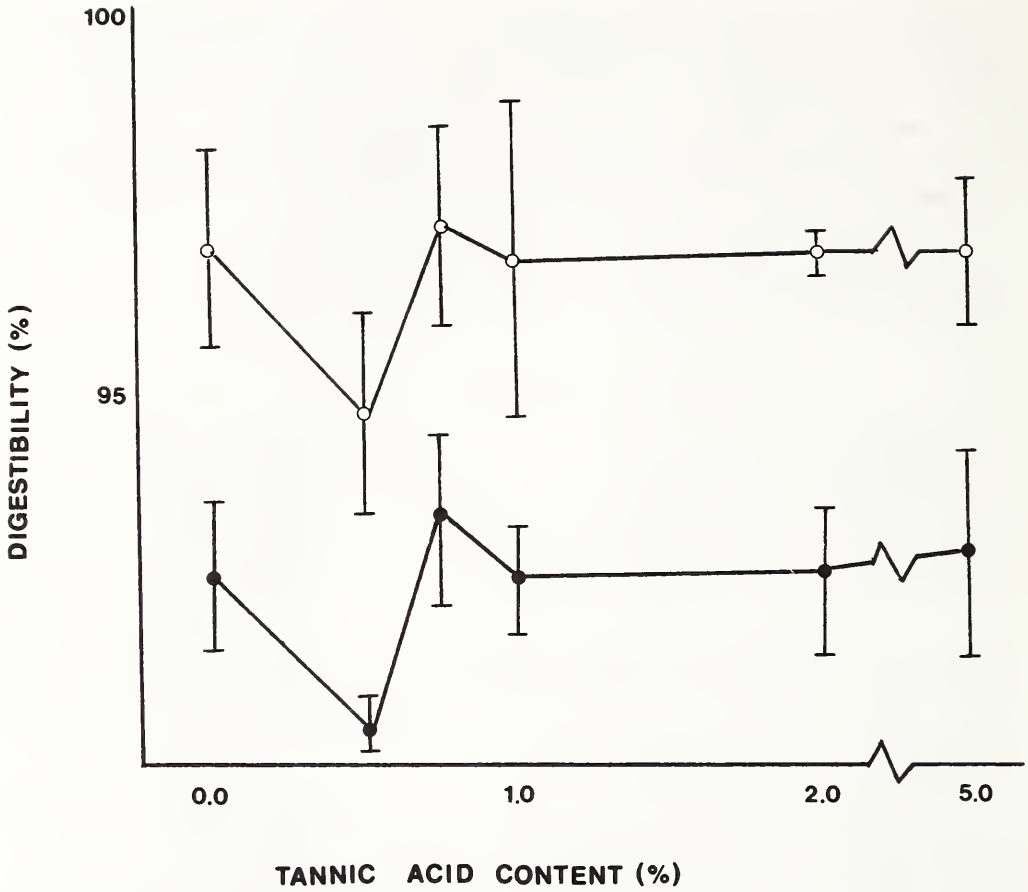

FIGURE 1. *Dry matter (closed circles) and lipid (open circles) digestibility by red squirrels on artificial diets with variable tannic acid content. Bars indicate ± 1 standard deviation.*

0.50% tannic acid diet, as was the case in dry matter digestibility. This too, indicates that red squirrels or their gut floras require an extended adaptation period to physiologically adjust to dietary tannic acid. This is especially noteworthy because the effects of phenolics on dietary lipid digestion have been generally ignored, probably because phenolics are not known to form complexes with nonpolar entities while complexes are readily formed with proteins and carbohydrates (24). That phenolics reduce protein and dry matter digestibility has been well documented, however, the actual site(s) of phenolic involvement is unknown. Due to the multitude of potential binding substrates available in the digestive system, phenolics could complex with proteins and carbohydrates from dietary, secretory, or epithelial sources. The observed effect on lipid digestibility during the adaptation to tannic acid would indicate the extensive involvement of complex formations with endogenous sources since complexes with dietary fats is unlikely. Fecal microflora of these squirrels was apparently altered by dietary tannic acid (10). However, alterations of gastric and intestinal secretions or changes in absorptive epithelium may be more important since lipid digestion is generally attributed to the foregut of cecal digestors (20).

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