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Mycophagy by small mammals in the coniferous forests of North America: nutritional value of sporocarps of *Rhizopogon vinicolor*, a common hypogeous fungus

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Abstract We evaluated the nutritional value of sporocarps of *Rhizopogon vinicolor*, a common hypogeous fungus in the coniferous forests of North America, for two small mammal species: the Californian red-backed vole (*Clethrionomys californicus*) and the northern flying squirrel (*Glaucomys sabrinus*). Although the nitrogen concentration of sporocarps was high, much of it was in non-protein form or associated with cell walls, suggesting that it may be of low nutritional value or protected from mammalian digestive enzymes. Sporocarps also had high concentrations of cell wall constituents, indicating low availability of digestible energy. When fed a diet of this fungus alone in a controlled feeding experiment both mammal species lost a small amount of body mass. Digestibilities of dry matter, nitrogen, cell wall constituents and energy from sporocarps by both species were lower than the digestibilities of other food types by other similarly sized small mammals. Red-backed voles digested the various components of sporocarps at least as well as the flying squirrels, even though they were almost six-fold smaller in body mass. This observation supports the notion that red-backed voles, like other microtine rodents, have morphological and physiological adaptations of the digestive system that are postulated to permit greater digestion of fibrous diets than predicted on the basis of body size. Despite this, our results re-affirm previous conclusions that hypogeous fungi are only of moderate nutritional value for most small, hindgut-fermenting mammals. Future studies should focus on the importance of mixed-species of fungi in the diet of small mammalian mycophagists.

Key words Nutrition · Mycophagy · Fungi · Voles · Squirrels

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Introduction

In the temperate forests of North America, a wide range of small mammals eat the sporocarps (fruit-bodies) of hypogeous (underground fruiting) fungi (Fogel and Trappe 1978). These small mammals may be important components of healthy forest systems because dispersal of viable fungal spores in their feces helps establish mycorrhizae that benefit plant growth (Maser et al. 1978). This tripartite interrelationship, between plants, fungi and small mammals, is paralleled in South America (Perez Calvo et al. 1989), Australia (Claridge and May 1994) and Europe (Blaschke and Baumler 1989).

It is assumed that the benefit of this tripartite interrelationship for mammals is the contribution of fungi to their nutrition (Fogel and Trappe 1978; Maser et al. 1978). Sporocarps of hypogeous fungi generally contain high concentrations of nitrogen, minerals and vitamins (Fogel and Trappe 1978), leading some authors to conclude that they represent a nutritious food resource (Grönwall and Pehrson 1984). However, a substantial proportion of the energy and nitrogen in fungal sporocarps is resistant to digestion because it is associated with complex carbohydrates or is protected within the indigestible spores (Cork and Kenagy 1989a). Furthermore, much of the nitrogen in fungal sporocarps is in non-protein forms, such as ammonia, which are utilized inefficiently by many mammals (Cork and Kenagy 1989a).

Adding to the variation in chemical composition between species of fungi, the nutritional quality of hypogeous fungi might vary considerably among small mammals depending on digestive specializations (Cork and Kenagy 1989a, b; Claridge and Cork 1994). For example, small mammals in which microbial fermentation occurs primarily in an expanded forestomach, such as the rat-kangaroos (bettongs and potoroos) of Australia, digest fungal polysaccharides effectively. These animals extract a large proportion of cell wall nitrogen (Claridge and Cork 1994). Small foregut fermenters also should be capable of utilizing non-protein nitrogen efficiently because it is used to synthesize microbial protein which is digested in the hindstomach and intestine (Kinnear et al. 1979). In contrast, a simple-stomached animal like the golden-mantled ground squirrel (*Spermophilus saturatus*) appears to have limited abilities to digest fungal polysaccharides or extract nitrogen from cell walls and spores (Cork and Kenagy 1989a). The relative indigestibility of fungi for this species is postulated to limit the absolute amount that it can eat. Hypogeous fungi are of marginal nutritional quality for this species, but can be very important at times of the year when no alternative diet is available. However, other small hindgut fermenters might digest fungi more effectively and be capable of higher relative intakes. For example, colonic separation mechanisms that selectively retain bacteria and small particles of digesta in the cecum are thought to allow some microtine rodents (voles and lemmings) to maintain the high intake of food and rapid passage of digesta required for utilizing digestion-resistant diets while still allowing functional microbial fermentation in the hindgut (Hörnicke and Björnhag 1980; Björnhag 1987; Cork 1994).

This study was performed to test the hypothesis that the specialized digestive system of microtine rodents allows effective digestion of the sporocarps of hypogeous fungi in comparison with species with a less specialized hindgut. The two species chosen as study animals were the Californian red-backed vole (*Clethrionomys californicus*) and the northern flying squirrel (*Glaucomys sabrinus*), both of which inhabit the coniferous forests of the Pacific Northwest United States. The red-backed vole is almost entirely dependent on fungi as a food resource (Ure and Maser 1982; Hayes et al. 1986) and the northern flying squirrel is a key mycophagist in many forest systems in North America (Maser et al. 1978). The digestive system of voles, including members of the genus *Clethrionomys*, features a large cecum and an expanded proximal colon arranged in a spiral in which a system of mucosal channels and folds separates bacteria from food residues and retains the former in the proximal colon and cecum (Björnhag 1994). The digestive tract of the northern flying squirrel resembles that of the golden mantled ground squirrel (Cork and Kenagy 1989b) and, being a sciurid, it would be expected to have at best a poorly developed colonic separation mechanism (Cork and Kenagy 1989b). We made this comparison across a six-fold difference in body mass (147 g versus 26 g) because no direct comparisons were possible between voles and comparably sized mycophagous rodents without colonic separation mechanisms. Our interpretation of the results, therefore, took into account the expected allometric scaling of food intake and digesta passage. The larger size of the less-specialized species was expected to decrease rather than increase our chances of falsely concluding differences due to gut specialization.

Materials and methods

Source and maintenance of animals

Red-backed voles were trapped in old-growth forest dominated by Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) in the Cascade Mountains at Fairview (43°22′11 N, 122°53′52 W), Honey Creek (43°19′20 N, 122°56′12 W) and Smith Springs (43°17′56 N, 122°55′40 W), Oregon. Flying squirrels were trapped in old-growth Douglas-fir at Miner Creek in the Coast Range Mountains near Roseburg, Oregon (43°27′15 E, 123°39′37 S; see Witt 1991 for details of site). Animals were held for a maximum of 3 weeks before being used in experiments, during which time they were fed a mixture of laboratory rodent chow, fresh vegetables, fruits and nuts. By the time of the experiments, the animals' food intake had become relatively constant and their body mass stabilized at 109 ± 2% (SE) (red-backed voles) and 103 ± 2% (flying squirrels) of capture mass (24 ± 1 g, and 143 ± 5 g respectively).

Choice, collection and preparation of hypogeous fungal sporocarps

We were able to collect sufficient sporocarps of one species of fungus, *Rhizopogon vinicolor*, to conduct our feeding experiment. *R. vinicolor* is among the more common and widespread spring-fruiting hypogeous fungi in the Pacific Northwest United States (Luoma et al. 1991). It is a significant component of the fungal diet of mycophagous rodents such as red-backed voles and flying squirrels at that time of the year (Maser et al. 1978; Hayes et al. 1986). Sporocarps of *R. vinicolor* were collected by raking the soil-litter interface around the base of Douglas-fir trees using a four-tined garden cultivator (Castellano et al. 1989), at a range of sites in Oregon and southern Washington from March through May 1994. As it was not possible to maintain a constant supply of fresh sporocarps, collections were frozen and stored at −20 °C until use. Previous studies had already established that freezing does not affect either the chemical composition or palatability of the fungi (Cork and Kenagy 1989a; Claridge and Cork 1994). We also collected small quantities of sporocarps of several other species of hypogeous fungi. These were chemically analyzed for general comparison with *R. vinicolor*.

Feeding experiments (balance trials)

Feeding experiments lasted 10 days for voles and 11 days for flying squirrels, during which five adults of each species were fed ad libitum and housed individually in stainless steel metabolism chambers (265 × 203 × 165 mm). The floors of the cages were meshed to permit separate collection of feces and urine below the cage. The walls of the chambers were solid, which prevented animals from seeing one another. Throughout the experiment animals were kept under a 12:12 h light:dark photo regime and temperature of 22 ± 1 °C. The first 6 days of each feeding trial was an equilibration period in which the animals were fed a mixture of the fungus (*R. vinicolor*), nuts, fruits and seeds for 3 days and fungus alone for another 3 days. Food intake and excretion patterns had stabilized after this time for both species. Measurements of intake

and excretion were made for 5 consecutive days for flying squirrels but for only 4 days for red-backed voles because intake by some individuals became unstable after this time.

The 3-day equilibration period was considered sufficient to allow the contents of the gut to equilibrate with a fungal diet. Assuming first-order kinetics for dilution of a single mixing compartment ($y = Ae^{-kt}$, where y is concentration, A is the initial concentration, k is the proportional turnover rate and the reciprocal of mean retention time, and t is time), the time required for components of previous diets to decline to 1% of their equilibrium value (A) is expected to be 4.6 times mean retention time. This time was predicted to be 2.5 days and 3.0 days for voles and flying squirrels, respectively, based on mean retention times of 13.0 h and 15.5 h reported for markers of particulate digesta in voles (Hume et al. 1993) and degus (*Octodon degus*, a 177-g rodent) (Sakaguchi and Ohmura 1992).

Intakes of food and its chemical components by the animals in the feeding experiments were determined by providing known amounts of previously frozen sporocarps (thawed at 5 °C each morning before being offered to the animals) and collecting, drying, weighing and chemically analyzing refusals after 24 h. Sub-samples for chemical analysis were taken when fresh sporocarps were weighed into daily portions and frozen for storage. Prior to chemical analysis, these sub-samples were thawed in the same way as the food offered to the animals (Claridge and Cork 1994). Urine, feces and uneaten food were collected daily and stored at −20 °C until analyzed. Urine was collected into sufficient glacial acetic acid to maintain the pH below 3 to minimize bacterial activity. Each animal was weighed on the last day of the equilibration period and the last day of the collection period to determine changes in body mass during the trial.

Chemical analyses

To determine the concentration of cell contents in fungal fruiting bodies, it is necessary to rupture all cells. Most fungal cells rupture during grinding or chemical analysis, but the spores are resistant. Prior to chemical analysis, we attempted to rupture the fungal spores by vigorous shaking in the presence of glass beads (Cork and Kenagy 1989a) but this method was unsuccessful for the species in the present study.

All samples of fungi, including refusals, and excreta were analyzed for dry matter, gross energy, ash, cell wall constituents, total nitrogen, cell wall nitrogen, protein nitrogen and non-protein nitrogen, as described by Claridge and Cork (1994). Briefly, fungus, food and feces were dried at 50 °C to constant mass to estimate water content and then ground through a sieve of 1 mm mesh in a hammer mill. The concentration of cell wall constituents was determined by extraction of samples firstly with pepsin in citrate buffer at 45 °C for 24 h (Faichney and White 1983) to remove protein, and then with neutral detergent solution (Goering and Van Soest 1970), without sodium sulfite, for 3 h in tubes immersed in a boiling (98 °C) water bath to remove remaining cell contents.

Total nitrogen was determined in samples of fungus, including refusals, feces, cell walls and urine by micro-Kjeldahl digestion followed by distillation and auto-analysis of the resulting ammonia (Williams and Twine 1967). This form of chemical analysis probably slightly underestimates the total amount of nitrogen in the samples because it does not measure nitrate nitrogen.

Nitrogen in fungus, food and feces was partitioned as follows: (1) cell wall nitrogen was analyzed on the residue after extraction with pepsin and neutral detergent, (2) protein and cell wall nitrogen was analyzed by extraction of samples with 5% (mass/volume) trichloroacetic acid (to remove non-protein nitrogen), centrifugation, removal of the supernatant liquid and analysis of the residue by micro-Kjeldahl digestion (Faichney and White 1983), and (3) true digestibility of dietary nitrogen was estimated by assuming that nitrogen other than that trapped in undigested cell walls was completely digested (Mason and Palmer 1973). Thus, true digestibility = (nitrogen intake − cell wall nitrogen excreted in feces) × 100 ÷ nitrogen intake.

Gross energy content was determined on dried samples of food, including refusals, feces and urine in an IKA-C700 T bomb calorimeter. From these measurements, a value was calculated for "available energy" as: energy intake − (fecal + urinary energy excretion). This differs from metabolizable energy (Van Soest 1982) in that we were unable to calculate energy lost as gas during digestion. Gross energy content was not measured for the fungal species not used in the feeding trial as it is not a useful indicator of nutritional value without measurements of its availability. Ash (mineral content) was determined by weighing samples before and after ignition at 550 °C for 3 h.

Calculations and statistical analyses

The null hypothesis that there was no difference in digestive ability between red-backed voles and flying squirrels was tested by comparing species means for individual measures of performance (e.g., intake, digestibility, balance or body mass change) using two-tailed t-tests. All data on digestibility or body mass change, which were calculated as a percentage of intake or original body mass respectively, were arcsine transformed prior to statistical comparison.

Results

Chemical composition of hypogeous fungal sporocarps

The sporocarps from nine species of hypogeous fungi were analyzed. A considerable range in total nitrogen concentration (6–26 $mg \cdot g^{-1}$) was evident (Table 1). With the exception of one species (*Leucogaster meridionales*), more than half of this nitrogen was in

Table 1 Chemical composition of sporocarps from different species of hypogeous fungi. Each value is the mean of duplicate analyses on a combined sample from several sporocarps. Data are expressed as gram per kg of dry matter, unless indicated otherwise. (*GAC Gautieria crispa* nom. ined., *GAM Gautieria monticola*, *HYC Hysterangium coriaceum*, *HYM Hymenogaster hiemalis* nom. ined., *LEM Leucogaster meridionales*, *LER Leucogaster rubescens*, *MET Melanogaster tuberiformis*, *RHT Rhizopogon truncatus*, *RHV Rhizopogon vinicolor*

Component	Species								
	GAC	GAM	HYC	HYM	LEM	LER	MET	RHT	RHV
Total nitrogen	16.9	10.6	23.5	25.8	19.2	6.1	13.9	12.0	10.5
(% protein nitrogen)	43.4	37.8	33.1	40.6	57.1	30.7	32.2	16.6	39.3
(% non-protein nitrogen)	21.2	13.0	21.2	24.1	19.2	36.4	33.6	25.8	26.4
(% cell wall nitrogen)	35.4	49.2	45.7	35.3	23.7	32.9	34.2	57.6	34.3
Cell-wall constituents ("fiber")	553	519	498	476	422	251	498	530	562
Gross energy (MJ/kg)									19.8
Water (g/kg fresh matter)	768	778	700	868	733	705	785	742	726

non-protein and cell wall form, suggesting poor nutritional quality. Because we were unable to rupture the spores of the fungi, cell wall nitrogen includes nitrogen trapped inside spores. Concentrations of cell wall constituents also varied among sporocarps from different species and was highest among sporocarps of *R. vinicolor*. Water content of sporocarps from nearly all species exceeded 70% of fresh mass, being highest in the soft-bodied *Hymenogaster hemialis* (86%) and lowest in the rubbery-bodied *Hysterangium coriaceum* (70%).

Intake and digestion of hypogeous fungal sporocarps

For a few measures of digestive performance, there were no statistically significant differences between the two species. Neither species was able to gain body mass on the diet of fungus alone (Table 2). The apparent digestibilities of dry matter, cell wall constituents and energy did not differ significantly between species, nor did the true digestibility of nitrogen (Table 2).

For other measures of performance, however, differences between species were either statistically significant at $P < 0.05$ or were apparent as trends indicated by statistical probabilities only slightly higher than 0.05 (Table 2). Voles lost proportionally more body mass than ground squirrels. Mass loss by voles was small but significantly different from zero ($P < 0.05$), while mass change was not significantly different from zero for flying squirrels ($P > 0.05$). Nitrogen balance tended to be higher, when adjusted for metabolic body mass ($kg^{3/4}$), in voles than ground squirrels ($P = 0.063$). Nitrogen balance in voles differed significantly from zero ($P < 0.05$) but that in flying squirrels did not. Intakes of both total energy and available energy per unit of metabolic body mass in voles were 1.5 times those in ground squirrels ($P = 0.068$ and $P = 0.066$ respectively). Intakes of total and apparently digestible nitrogen, and cell wall constituents were significantly higher for voles ($P < 0.05$), and intakes of dry matter and digestible energy showed a similar trend ($P = 0.053$ and $P = 0.054$ respectively). Red-backed voles maintained higher apparent digestibilities of nitrogen than flying squirrels (i.e., they maintained proportionately lower losses of fecal nitrogen in relation to dietary intake). Neither fecal nor urinary nitrogen output per unit of metabolic body mass differed significantly between the two species ($P > 0.05$).

Discussion

Relative performance of red-backed voles

Despite being nearly six-fold smaller in body mass than flying squirrels, and hence having a much smaller gut

Table 2 Intake and digestion of the sporocarps of *Rhizopogon vinicolor* by *Clethrionomys californicus* and *Glaucomys sabrinus* in the feeding trials. Values are mean ± SE. Probabilities are for a two-tailed Student's *t*-test between the two mammal species

Attribute	*C. californicus*	*G. sabrinus*	Probability
Initial body mass of animals (g)	26 ± 1	147 ± 6	7.3×10^{-9}
Mass change (% per day)	−2.1 ± 0.4	−0.5 ± 0.3	0.014
Number of animals	5	5	
Days for which collections were made	4	5	
Dry matter			
Intake ($g \cdot kg^{-1} \cdot day^{-1}$)	102.2 ± 12.1	48.6 ± 3.4	0.003
($g \cdot kg^{-3/4} \cdot day^{-1}$)	40.8 ± 4.4	30.0 ± 1.8	0.053
Apparent digestibility (%)	67.1 ± 2.7	65.9 ± 1.3	0.657
Total nitrogen			
Intake ($g \cdot kg^{-1} \cdot day^{-1}$)	1.41 ± 0.46	0.61 ± 0.05	0.003
($g \cdot kg^{-3/4} \cdot day^{-1}$)	0.56 ± 0.07	0.37 ± 0.03	0.033
Apparent digestibility (%)	34.8 ± 7.8	11.4 ± 2.7	0.022
True digestibility (%)	74.1 ± 3.8	70.2 ± 1.0	0.300
Apparently digestible intake ($g \cdot kg^{-1} \cdot day^{-1}$)	0.52 ± 0.14	0.07 ± 0.02	0.012
($g \cdot kg^{-3/4} \cdot day^{-1}$)	0.21 ± 0.05	0.05 ± 0.01	0.018
Nitrogen balance ($g \cdot kg^{-1} \cdot day^{-1}$)	0.32 ± 0.15	−0.012 ± 0.022	0.066
($g \cdot kg^{-3/4} \cdot day^{-1}$)	0.13 ± 0.06	−0.008 ± 0.014	0.063
Cell wall constituents			
Intake ($g \cdot kg^{-1} \cdot day^{-1}$)	62.9 ± 8.3	26.9 ± 2.4	0.003
($g \cdot kg^{-3/4} \cdot day^{-1}$)	25.1 ± 3.1	16.6 ± 1.3	0.033
Digestibility (%)	71.6 ± 2.9	66.7 ± 2.3	0.212
Energy			
Intake ($MJ \cdot kg^{-1} \cdot day^{-1}$)	2.137 ± 0.341	0.922 ± 0.079	0.009
($MJ \cdot kg^{-3/4} \cdot day^{-1}$)	0.853 ± 0.127	0.569 ± 0.044	0.068
Apparent digestibility (%)	65.4 ± 2.4	61.7 ± 1.5	0.211
Digestible intake ($MJ \cdot kg^{-1} \cdot day^{-1}$)	1.392 ± 0.223	0.572 ± 0.058	0.008
($MJ \cdot kg^{-3/4} \cdot day^{-1}$)	0.555 ± 0.084	0.352 ± 0.033	0.054
Available intake ($MJ \cdot kg^{-1} \cdot day^{-1}$)	1.337 ± 0.221	0.559 ± 0.054	0.009
($MJ \cdot kg^{-3/4} \cdot day^{-1}$)	0.534 ± 0.083	0.345 ± 0.031	0.066

[a] Available energy intake = ingested energy − total faecal energy − total urine energy

capacity in relation to food requirements (Demment and Van Soest 1985; Cork 1994), red-backed voles equalled or exceeded the performance of flying squirrels in several respects. Red-backed voles maintained a substantially higher intake (in relation to metabolic body mass) and apparent digestibility of nitrogen than flying squirrels (Table 2), indicating a greater effectiveness at reducing losses of endogenous nitrogen in feces. This is an expected benefit of the colonic separation mechanism that selectively retains bacteria in the cecum of voles and reduces the concentration of nitrogen in feces (Sperber et al. 1983; Björnhag 1987). This colonic separation mechanism has been confirmed in two species of lemmings, *Lemmus lemmus* (Sperber et al. 1983) and *Myopus schisticolor* (Björnhag 1994), three species of voles, *Arvicola terrestris*, *Microtus agrestis* and *Clethrionomys glareolus* (Björnhag 1994), and rats, *Rattus norvegicus* (Sperber et al. 1983). It is postulated that all myomorph rodents possess the mucosal folds in the proximal colon necessary to achieve selective retention of bacteria but the voles and lemmings achieve by far the strongest retention, associated with a characteristic spiral construction of the proximal colon (Sperber et al. 1983; Björnhag 1987, 1994). There has been one report of a sciurid rodent (a ground squirrel) producing two types of feces (Hörnicke and Björnhag 1980), which suggests a colonic separation mechanism. However, the absence of other such reports and the simple construction of the hindgut in sciurids suggest that selective retention of bacteria in flying squirrels would be, at best, weak compared with that in voles.

Although we did not observe reingestion of feces in these experiments, it could have contributed to the difference in fecal nitrogen losses between voles and flying squirrels. Other species of microtine rodents are reported to practice coprophagy, but not cecotrophy (production of special feces formed from cecal contents, and ingestion of these directly from the anus), in captivity (Björnhag and Sjöblom 1977; Kenagy and Hoyt 1980). Because the cages had gridded floors, it is unlikely that they practiced coprophagy in these experiments. Sciurids appear to practice coprophagy less frequently than other rodents (Kenagy and Hoyt 1980). There is only one report of a ground squirrel practicing cecotrophy (Hörnicke and Björnhag 1980). Thus, it is possible that flying squirrels can reduce nitrogen losses by this route. If the flying squirrels in our experiment were practicing cecotrophy, it was not as effective at reducing fecal nitrogen losses as the colonic separation mechanism, possibly combined with coprophagy, in the voles.

In addition to their impressive performance with respect to nitrogen, red-backed voles were able to digest dry matter, cell wall constituents and energy from the fungal diet at least as efficiently as flying squirrels. This parity with flying squirrels was achieved despite the presumed shorter digesta retention times associated with smaller body size. The voles may have been assisted by the selective retention of bacteria in the hindgut, perhaps because it assists fiber digestion. The complex dentition of voles and the finer grinding of food achieved by very small species such as voles may also have been important (Vorontsov 1967; Hume et al. 1993). An alternative explanation is that both species achieved the maximum digestibility of the diet. This is unlikely as fungi do not contain a cell-wall polymer comparable with lignin, which limits the potential digestibility of other cell wall constituents in plants.

Digestive performances of red-backed voles and flying squirrels will differ between species of fungus due to differences in chemical composition, but the fact that the voles performed as well as a much larger species of hindgut-fermenter probably applies across diets. For example, digestibilities of dry matter (60%) and energy (52%) in golden-mantled ground squirrels eating *Elaphomyces granulatus* were similar to or lower than those for red-backed voles in our study (Cork and Kenagy 1989a). The golden-mantled ground squirrel has a gut morphology similar to that in flying squirrels (Cork and Kenagy 1989b) but is one-and-a-half times as large. The hypogeous fungus *E. granulatus* has a similar gross energy concentration and higher nitrogen and lower cell wall concentrations than *R. vinicolor* (Cork and Kenagy 1989a).

Nutritional value of hypogeous fungi

Our chemical assays on a range of species of hypogeous fungi collected in parts of the Pacific Northwest United States are consistent with earlier studies showing that a high proportion of the nitrogen in sporocarps can be present in non-protein compounds or bound in cell walls and spores (Cork and Kenagy 1989a; Claridge and Cork 1994). Nitrogen in cell walls has been shown to be poorly digested by hindgut fermenters such as the golden-mantled ground squirrel, and that in spores appears to be largely, if not totally, unavailable to most small mammals (Cork and Kenagy 1989a; Claridge and May 1994). Consistent with these conclusions, red-backed voles and flying squirrels, which are also hindgut-fermenters, achieved true digestibilities of nitrogen in the range 70–74%; considerably less than the 80–96% recorded for other small mammals feeding on a variety of plant materials (Van Soest 1982; Cork and Kenagy 1989a). The high concentration of cell wall constituents in *R. vinicolor* (57%), together with the low digestibility of nitrogen, accounts for the relatively low digestibilities of energy (62–65%) achieved by both species when fed this fungus. These values compare unfavorably with those recorded for ground squirrels (96%) feeding on nuts and seeds (Cork and Kenagy 1989a), but are nonetheless much higher than those recorded for voles and lemmings (34–58%) fed monocotyledonous and sedge plant material (Karasov 1982).

We are unable to make clear comparisons between the present study and previous work on foregut fermenting rat-kangaroos (*Potorous tridactylus*) because of differences in diet. We would expect the foregut-

fermenter to be better able to extract and digest energy and nitrogen from fungi than the small hindgut fermenters, not only because of its digestive adaptation but also its larger size (850–1000 g body mass). Consistent with this prediction, the rat-kangaroos digested more than 80% of the dietary dry matter and 75% of the energy in diets of the hypogeous fungi *Mesophellia glauca* and *Rhizopogon luteolus* (Claridge and Cork 1994). However, these values are partly due to *M. glauca* and *R. luteolus* having higher concentrations of nitrogen, a lower proportion of dietary nitrogen associated with cell walls, and lower concentrations of cell wall constituents than the *R. vinicolor* used in the present study.

Study limitations and future research directions

In this study we were able to assess the nutritional value of sporocarps of only one fungal species. In nature, many more species of fungi are regularly sought by red-backed voles and flying squirrels, both within and between different seasons (Maser et al. 1978; Ure and Maser 1982; Hayes et al. 1986). Although we used one of the more common and abundant fungal species, we still had to collect it from several sites over a wide geographic area. For less common species, or for species that do not fruit abundantly, even intensive and extensive sampling may not yield enough sporocarps to conduct feeding trials such as those described here. Moreover, there are no laboratory methods by which sporocarps can be grown in quantity, despite the fact that some species quickly produce vegetative (mycorrhizal) structures on the roots of plant seedlings from spore inoculum (Massicote et al. 1994). Therefore, resolving the relative nutritional values of other species of fungi for small mammals may be difficult.

The importance of a mixed-species fungal diet for mycophagous mammals requires investigation. Our studies to date have used one species of fungus at a time and we have examined digestion of only the quantitatively major dietary components. In all of these studies it was apparent that the animals would only tolerate a single species diet for a limited time. Whether this was due to palatability effects or to a need to balance requirements for vitamins, minerals and other quantitatively minor dietary constituents by eating a mixed diet is not known, but both factors are likely to be important. Studies using mixed diets would better mimic the natural situation, in which these animals feed on a diversity of fungi (Maser et al. 1978; Ure and Maser 1982). While technically and logistically demanding, such studies, along with direct comparisons between mammal species, are necessary to improve understanding of the constraints and limitations on mycophagy among small mammals.

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