BANKWEST *LANDSCOPE* CONSERVATION VISA CARD TRUST FUND
GRANTS

Gilbert's Potoroo Recovery – Nutrient analysis of hypogeal fungi

Final report

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SUMMARY

The diet of Gilbert's potoroo, Australia's rarest mammal, has been shown to comprise over 90% of fungi throughout the year. Breeding has been irregular within a captive colony established in 1994. The captive diet used since 1994 consists essentially of fruit, vegetables, nuts and grains, although it has been supplemented by up to 10% of hypogeal fungi since 2001.

In order that the captive diet can be redesigned to approach more closely the nutrient content of the diet in the wild, nutrient analyses of hypogeal fungi was carried out. The results of those analyses are reported here.

The cover photo shows sporocarps of *Rhizopogon luteolus* (larger yellow "truffles") and *R. roseus* (smaller pinkish "truffles"). Photo by Linda Reinhold.

INTRODUCTION

Gilbert's potoroo (*Potorous gilbertii*) is Australia's rarest mammal, existing in only one population estimated to number less than 40 (Courtenay and Friend, 2004). Recovery of the species depends on increasing its numbers outside the surviving population, as the habitat available is fully occupied. In addition, the population does not have the capacity to support the removal of a group of 10 or more animals that would be needed to establish a second population. Efforts since the rediscovery of the species in 1994 have focussed on the production of sufficient animals for translocation through captive breeding (Start and Burbidge 1995; Courtenay *et al.* 1998).

A captive breeding colony was set up at Two Peoples Bay in 1994 using animals retained in captivity from the rediscovery. Altogether six adults and three wild-bred progeny were taken into captivity between December 1994 and June 1996. While breeding has occurred, only eight young have been born, and most of these were bred in the first three years of the colony. Breeding occurs routinely in the wild population, so it appears that the conditions of captivity have led to a depression in the breeding rate. The diet fed to captive animals was worked out in consultation with institutions keeping long-nosed potoroos (*P. tridactylus*). Numerous changes have been made since 1994, and the potoroos are now fed a low oxalate diet with the addition of 10% hypogeous fungi. Changes have occurred gradually, however, without an objective assessment of how closely the artificial diet approaches a wild diet. Parallel studies of diet in the wild are now available, and it is possible to carry out nutritional analyses of important fungi species, to come up with a better approximation of the wild diet.

Two studies of the diet of Gilbert's Potoroos in the wild have been carried out, using analysis of faecal samples. A preliminary study by Bougher (1998) was based on about 30 scats from 5 individual potoroos and indicated that Gilbert's potoroo is highly dependent on fungi, and predominantly hypogeous fungi (underground-fruiting fungi or "truffles"). Nguyen (2000) analysed 66 faecal samples from different animals or sampling times, to give seasonal perspective. He found 44 spore types, amongst which were ten that occurred in over 50 % of his scat samples. He identified these spore types as corresponding to the taxa shown in Table 1. *Mesophellia* sp. and *Hysterangium* sp. occurred in all 66 scats. *Elaphomyces* sp. occurred in 65 of the samples. Nguyen found that fungal material composed over 90% of material in the scats of Gilbert's potoroo. This makes Gilbert's potoroo at least as fungi-dependent as the long-footed potoroo (*Potorous longipes*) (Green *et al.* 1999), one of the most fungi-dependent mammals studied worldwide.

Bougher	Nguyen
NB1 *Elaphomyces*?	*Mesophellia* sp.
NB2 *Hysterangium* or *Mesophellia*	*Hysterangium* sp.
NB3 Unknown sp.	*Elaphomyces* sp.
NB4 *Hysterangium* sp.	Sp. 1
NB5 *Austrogauteria manjimupana* or *A. chlorospora*	*Descomyces* sp.
NB6 *Austrogauteria* sp.	*Thaxterogaster* or *Cortinarius*
NB7 *Descomyces* sp.	*Castoreum tasmanicum*
NB8 *Castoreum tasmanicum*	*Quadrispora* sp.
NB9 *Thaxterogaster* sp.	Sp. 14
NB10 *Quadrispora* sp.	Sp. 6

Table 1. Fungal spore types recorded commonly in Gilbert's potoroo faeces by Bougher (1998) and Nguyen (2000).

Ideally, in order to provide data to assist in the development of a suitable artificial diet, the fruiting bodies of these and other fungal species important in the potoroo's diet should be subjected to nutrient analysis. There are a number of other factors, however, that affect the practicality of targeting all of these species.

The usual method of locating and collecting underground fungi is by scratching in the litter and upper soil layers in likely places until the fungi are seen. This method is rather destructive, as it may require the raking over of large areas before significant numbers are found. As large quantities of material are required for some nutritional tests (e.g. Vitamins B_6, D and E, 10 g dry weight required per assay), the species chosen for the purposes of this work were those able to be found in large quantities and particularly in tree plantations, where the effect of raking to find underground fruiting bodies is unlikely to have a serious conservation impact.

AIM

To determine the concentrations of proximate constituents and important nutrients in fruiting bodies of selected hypogeous fungi eaten by Gilbert's potoroo. This will provide a basis for the refinement of a nutritionally appropriate artificial diet for the species in captivity.

METHODS

Selection of fungi to be analysed

The most important factor in this choice was importance in the potoroos' diet, followed by the availability of the particular species in the quantities required for analytical purposes. Within the resources available to the project, the only species found in the quantities needed by the labs were the following:

- *Elaphomyces* sp. and
- *Hysterangium* sp. (both important in the diet of potoroos at Two Peoples Bay),
- *Gellopellis purpurascens* (fungi with very large sporocarps found occasionally),
- *Protrubera canescens* (a patchily distributed fungus with a relatively large fruiting body that is easy to find due to its tendency to push up above the soil),
- *Rhizopogon luteolus* and
- *R. roseus* (two European fungi with which *Pinus radiata* seedlings are inoculated to improve growth).

Regular diet supplementation of the captive potoroo diet with hypogeal fungi was implemented in 2001 when ready supplies of these fungi were discovered locally by Linda Reinhold. Fungi used are those found readily in plantations near Albany, specifically the two *Rhizopogon* species, from *Pinus radiata* plantations and *Hysterangium* sp. in *Eucalyptus globulus* plantations. Extra sporocarps are collected during winter, dried and stored, to be used as a food supplement over summer when none are available from the field. This way approximately 10% by weight (or dry weight equivalent) of the captive potoroos' diet year-round comprises hypogeal fungi, collected without impact on natural bushland. The nutrient content of the *Rhizopogon* species was determined because of its use in the captive diet.

Selection of nutrients for analysis

In deciding on the analyses to be conducted, several experts were provided with a list of nutrients and asked to add or subtract items, with reasons. Those consulted were Dr Ralph Swan (Professor of Veterinary Science), Dr Kris Warren (veterinarian specialising in wildlife), and Mr Warren Potts (animal nutritionist), Dr Anne-Marie Horwitz (veterinarian) and Dr David Forshaw (veterinary pathologist).

Laboratories offering relevant analyses were contacted to determine the cost and the minimum quantities required for each test. This information was also used to decide on the final list of nutrients in order to gain maximum information for the funding available (analyses, laboratories, minimum quantities required and costs are shown in the Appendix).

The analyses selected were grouped in a nested hierarchy so that maximum information could be gained from each class of sample size. The following groups were used:

2.5 g dry wt available:

Total nitrogen
Nitrogen as nitrate
Moisture
Ash
ICP analysis (S, P, K, Na, Ca, Mg, Cu, Zn, Mn, Fe, B, Cl)

12 g dry wt available:

Tests above plus:
Crude fibre
Neutral detergent fibre
Carbohydrate (free sugars)
Fat

53 g dry wt available:

Test above plus:
Amino acids
Fatty acids
Oxalate
Vitamins A, B_6, C, D, E

All six truffle species were analysed at the 12 g level. Replicate samples of some species were submitted for the 2.5 g tests. *Elaphomyces* sp., *Hysterangium* sp. and *Rhizopogon luteolus* were available in sufficient quantity that they were submitted for the 53 g tests.

Proximate constituents

This term refers to the main dietary components i.e. protein, carbohydrate and fat. Water and ash content also need to be determined to provide a reference for the value of the food item. Protein content is determined by measuring the nitrogen content and multiplying by 6.25. Carbohydrate is usually determined by difference, that is, by measuring the content of protein, fat, water and ash, then assuming that the rest is carbohydrate. In determining the carbohydrate content of hypogeal fungi, however, we attempted to determine free sugars (simple sugars and soluble polysaccharides) by analysis, although problems were encountered (see below).

Oxalate

Oxalate content was measured because several of the captive potoroos have died from kidney failure through renal oxalosis. Crystals of calcium oxalate accumulate in the renal tubules, damaging them and eventually causing kidney failure. There are several possible causes of renal oxalosis, including high oxalate concentrations in the diet. While all food items known to contain more than minimal levels of oxalate have been eliminated from the captive diet, there was no information about levels in hypogeal fungi.

RESULTS

Proximate constituents

The analysis of free sugars was abandoned due to gelling at the extraction stage. Although accurate values were not obtained for these sugars, the extracts obtained were run on the HPLC, showing that glucose was present in *Gelopellis*, *Protrubera*, *Elaphomyces*, and *Rhizopogon roseus*, while *R. luteolus* contained both fructose and glucose. The attempt to determine carbohydrate accurately was therefore not successful, as only simple sugars and soluble polysaccharides were measured accurately. Carbohydrate content should therefore be determined by difference, so long as accurate values can be obtained for the other proximate constituents.

The relative proportions of water, ash, protein, carbohydrate, and fat in fruiting bodies of each fungus are shown in Figure 1.

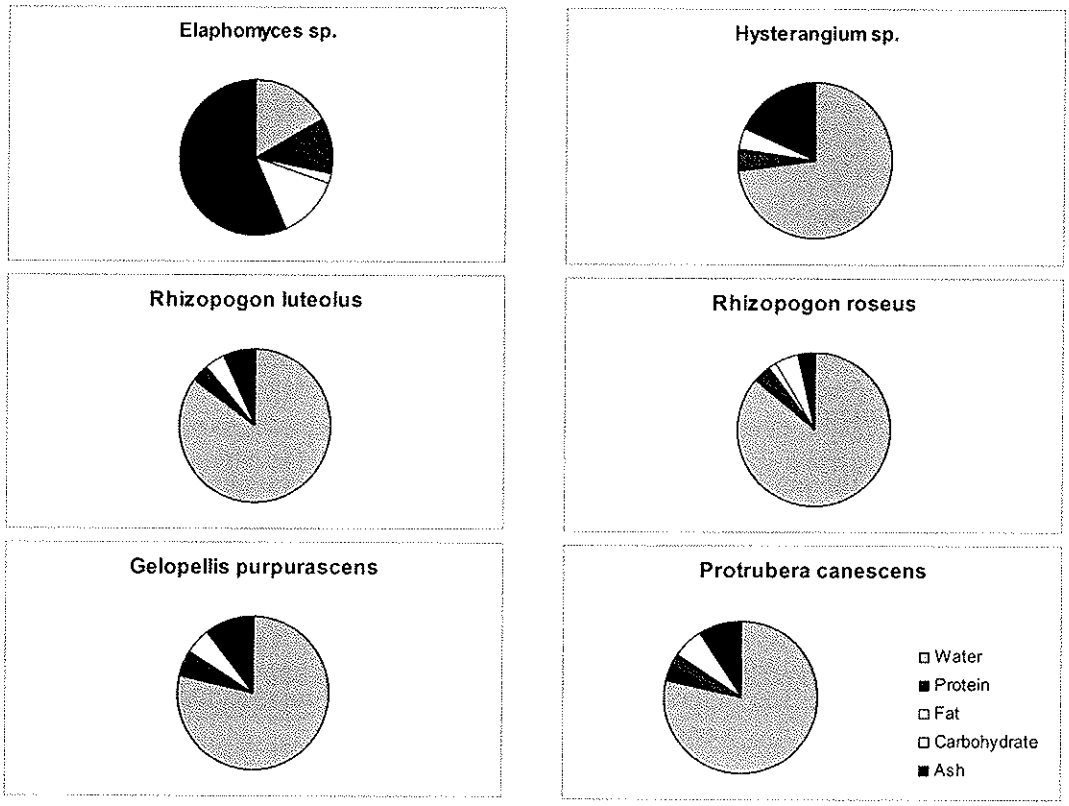
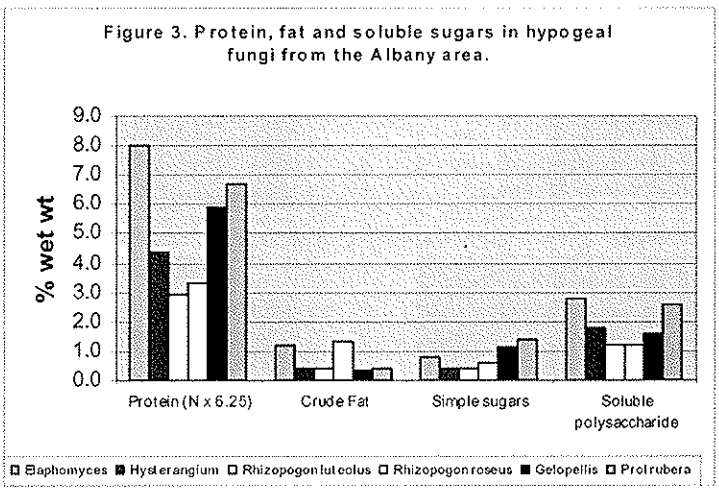


Figure 2. Percentage content of water, protein, fat, carbohydrate (simple sugars and soluble polysaccharides only) and ash in six species of hypogeal fungi from the Albany area.

The relative percentages of fat, protein, simple sugars and soluble polysaccharides are shown in Figure 3.



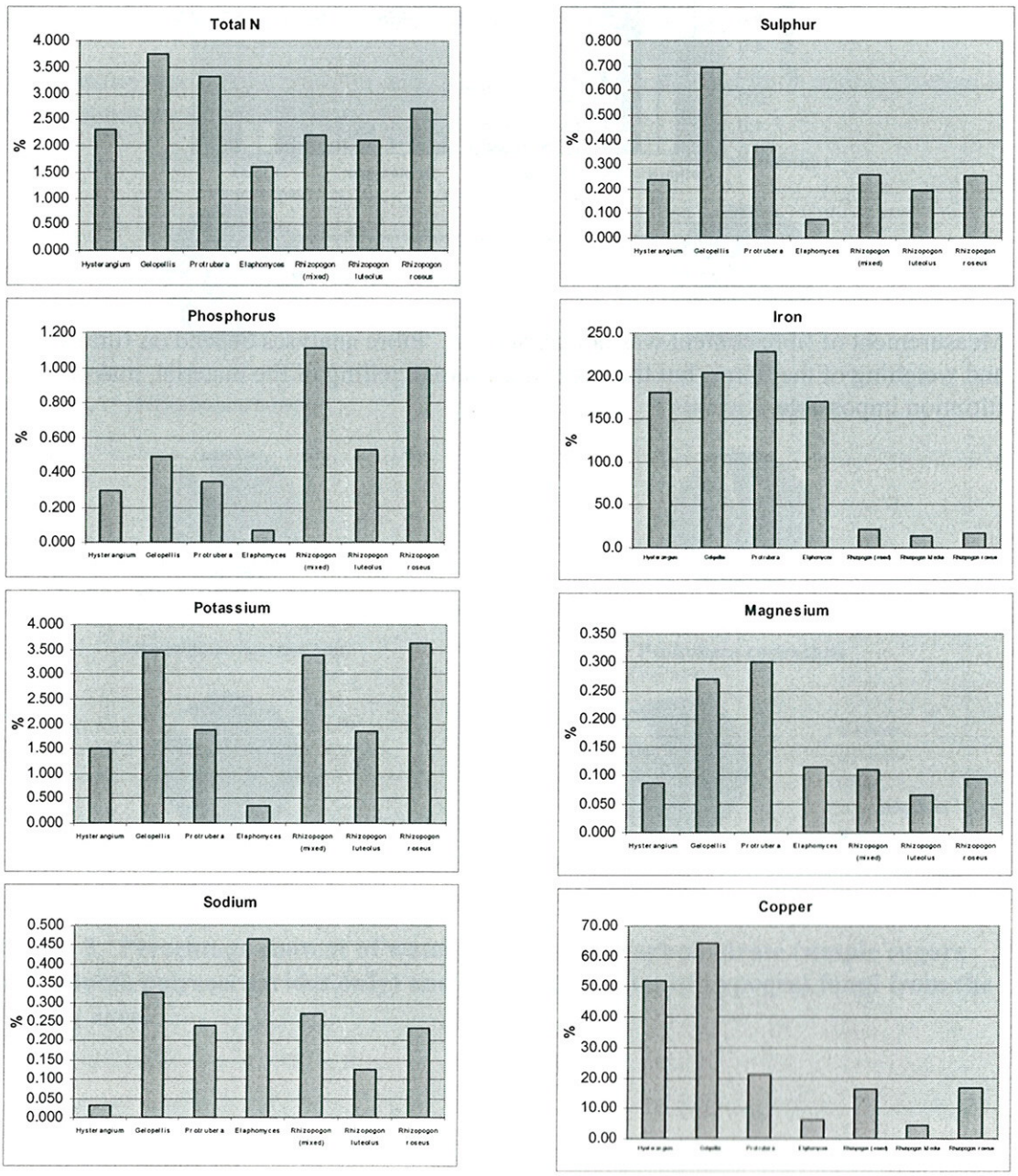
Fibre

Measurement of fibre content was not carried out. Fibre analyses depend on filtration and weighing of the fibres, but the extraction caused gelling of the material, making filtration impossible.

ICP analysis

This process gives concentrations of various metals and other ions. The variability in content of these elements amongst the fungal species is shown in Figure 4a and 4b.

Figure 4a. Concentrations of total N, P, K, S, Na, Fe, Mg and Cu in hypogeal fungi from the Albany area.



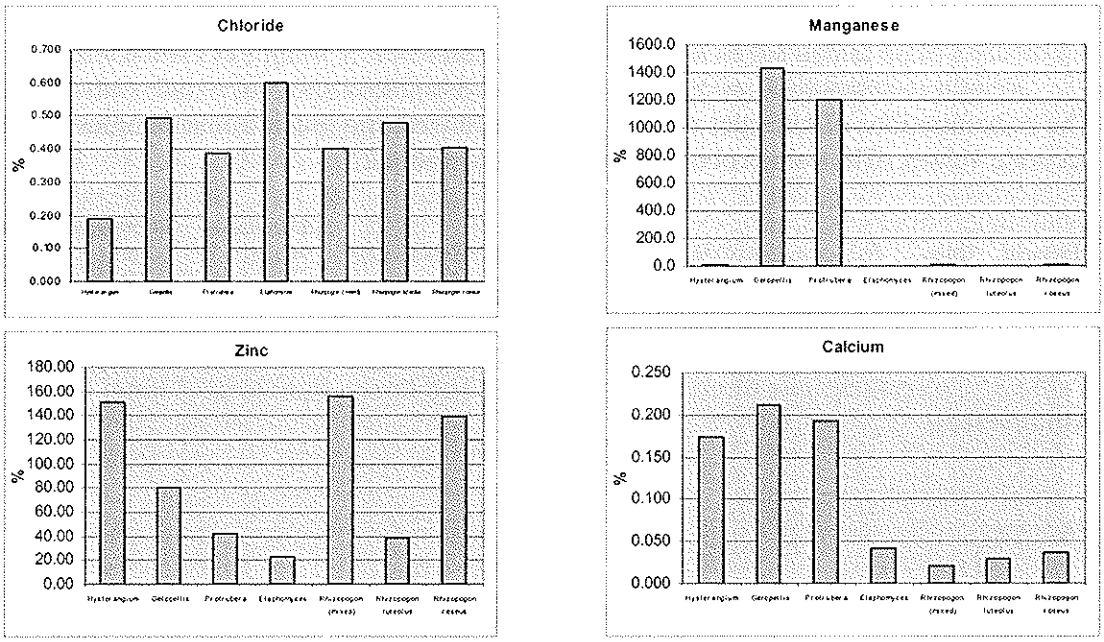


Figure 4b. Concentrations of Cl, Zn, Mn and Ca in hypogeal fungi from the Albany area.

The concentrations of nitrogen as nitrate and boron were too low in some samples to get accurate values. The values obtained are shown in Table 2.

Element	Units	*Hysterangium*	*Gelopellis*	*Protrubera*	*Elaphomyces*	*Rhizopogon (mixed)*	*Rhizopogon luteolus*	*Rhizopogon roseus*
NO_3 N	mg/Kg	44.3	50.8	74.5	34.6	95.0	<40	50.70
Bo	mg/Kg	3.0	6.6	1.9	8.83	0.7	<1.0	<1.0

Table 2. Concentrations of nitrogen as nitrate and boron in hypogeal fungi from the Albany area.

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Vitamins

Vitamin assays require significant quantities of material (10 g dry weight for B_6, C and D). Results of these assays are shown in Table 3. Significant departures in vitamin content are shown by *Rhizopogon luteolus* (high in B6) and *Hysterangium* (high in D and E).

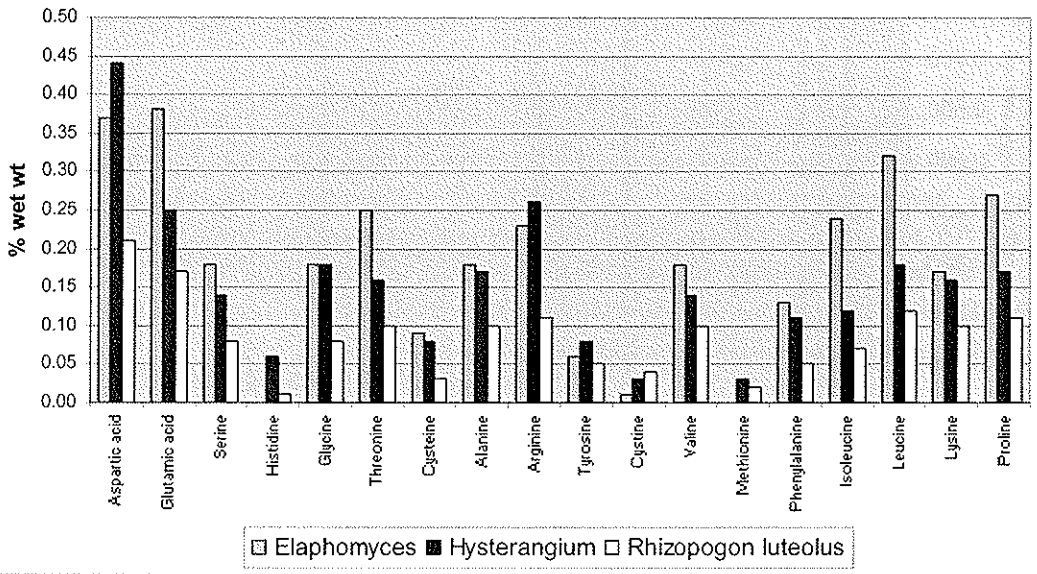
Nutrient	Units	Elaphomyces	Hysterangium	Rhizopogon luteolus
Vitamin A	mg/kg ar	0.00	0.00	0.00
Vitamin B_6	mg/100g ar	0.02	0.12	0.95
Vitamin C	mg/100g ar	7	6	8
Vitamin D	IU/g ar	54	1050	116
Vitamin E	mg/kg ar	2.2	7.1	0

Table 3. Results of vitamin analyses of sporocarps of three hypogeal fungal species.

Amino acid profile

These are shown in Figure 5. The relative concentrations of the amino acids generally follow the relative proportions of protein in the three species: *Elaphomyces* highest, *Hysterangium* next, *Rhizopogon luteolus* lowest.

Figure 5. Amino acids in hypogeal fungi



Fatty acid profile

The fatty acid profiles of the six fungi analysed are shown in Figures 5 and 6.

Figure 6. Fatty acids in hypogeal fungi (not C18:1)

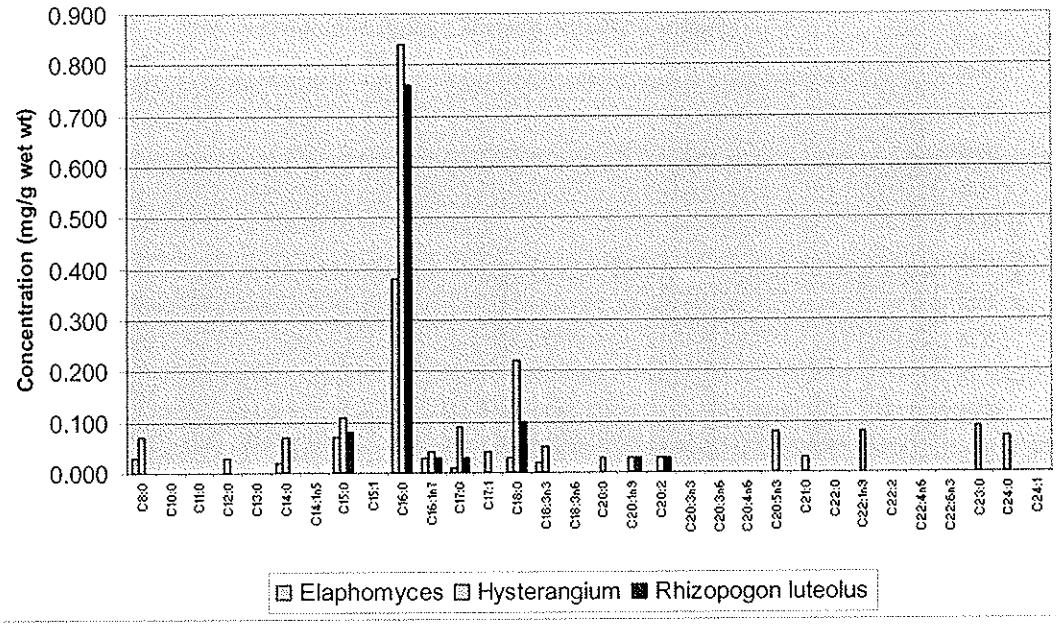
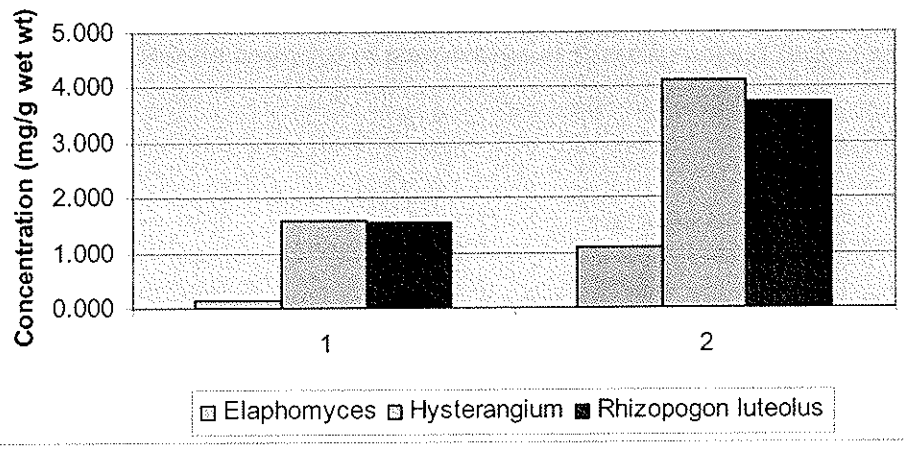


Figure 7. C18:1 fatty acids in hypogeal fungi



Oxalate

Oxalate values obtained were extremely low, only registering in one out of three samples. This was *Elaphomyces* sp., which returned a value of 0.02% dry weight, well below levels likely to cause kidney problems.

Selenium

While it was intended that selenium content would be determined, the laboratory did not return results for this element.

DISCUSSION

Determinations of a selection of nutrients of hypogeal fungi demonstrate that the concentrations of many of these differ very widely from one species to another. Even water content is highly disparate, varying from 12 % in one species to almost 90% in another. Given this finding, and the fact that only two of the species analysed were amongst the six species most frequently found in potoroo scats, it will be necessary to repeat these analyses for several more of the important species in order to gain a useful estimate of the nutrient intake of wild potoroos.

Table 1 shows species deemed most important in the diet of Gilbert's potoroo in the only studies carried out so far (Bougher 1998; Nguyen 2000). Some of these species may be available in quantity in blue gum plantations near Albany. Lu *et al.* (1999) documented fungi in blue gum plantations of 1-8 year-old blue gum plantations in the Manjimup-Northcliffe area and found that species richness increased with plantation age. Hypogeal fungi were found only in older plantations and included *Descomyces albellus* and *Descomyces* sp., *Hydnangium carneum* (also found by Nguyen in potoroo scats at Two Peoples Bay), *Hysterangium* sp. and a hypogeous species of *Scleroderma.*

In the meantime, comparison of the current diet used in the captive colony with the results of the current investigation will be carried out. The potoroos eat most hypogeous fungi presented to them, but strongly prefer *Hysterangium* sp., found in local blue gum plantations. In the first instance, this species will be used as a model against which to redesign the captive diet.

Further investigation of the fungi important in the diet of Gilbert's potoroo is warranted. In particular, it will be important to link spore types with fungal fruiting bodies, as this will provide a greater understanding of the ecological requirements of the fungi and thus of the potoroos that depend on them.

COSTS

The costs of this project, without including salaries, are shown below.

Supplier	Item	Cost
AgWA	Amino acids, fatty acids, oxalate, vitamins A, B_6 and E	$300.44
CSBP	Moisture, ash, total N, P, K, S, Na, Ca, Mg, Cl, Cu, Zn, Mn, Fe, NO_3 N, Bo	$231.00
CSBP	Ash, total N, P, K, S, Na, Ca, Mg, Cl, Cu, Zn, Mn, Fe, NO_3 N, Bo	$170.50
DTS	Vitamin C & D	$985.71
CALM	Vehicle running 1910 km @ $0.55	$1,050.00
Total		**$2,737.65**

ACKNOWLEDGEMENTS

I am very grateful to Linda Reinhold, Department of Conservation and Land Management, Albany, who laboured long and diligently to collect all the hypogeal fungi used in this study. I would also like to thank Dr Teresa Lebel, Melbourne Botanic Gardens, who carried out the initial identifications of fungi. I am grateful to Professor Ralph Swan and Dr Kris Warren of Murdoch University, Warren Potts of Glen Forrest Stockfeeds, Dr Anne-Marie Horwitz, Lockyer Avenue Veterinary Hospital and Dr David Forshaw, Agriculture WA, for their assistance in selection of analyses. Thanks are also due to Dr Jeremy Allen, Agriculture WA, David Harris, Chemistry Centre WA and Dr Geof Proudfoot, CSBP Futurefarm Soil Laboratories, for their interest and assistance in handling the sample analyses.

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APPENDIX

Nutrient analyses carried out

ANALYSIS	Lab	Dry wt needed (g)	Cumulative wt (g)	Cost sample 1	Cost sample 2-10	Species/dry wt on hand (g)
*Protein**	CSBP	0.2	0.2	$30.00	$30.00	
Fat	CCWA	2.0	2.2	$50.00	$45.00	
Moisture	CSBP	2.0	4.2	$8.00	$8.00	
& Ash (same sample)	CSBP					
*Chloride**	CSBP	0.2	4.4			
Crude fibre	CCWA	2.0	6.4	$100.00	$80.00	
Neutral detergent fibre	CCWA	1.0	7.4	$70.00	$35.00	
Acid detergent fibre	CCWA	1.0	8.4	$70.00	$35.00	
Carbohydrate (free sugars)	CCWA	5.0	13.4	$110.00	$110.00	Protrubera canescens 15g Gellopellis purpurascens 15+ g
Vitamin A & E	AgAHL	1.0	14.4	$30.00	$30.00	
Vitamin B6	AGAL	10.0	24.4	$163.00	$163.00	
Vitamin C	DTS	10.0	34.4	$130.00	$130.00	
Vitamin D3	DTS	10.0	44.4	$180.00	$180.00	
Selenium	AgAHL	1.0	45.4	$34.00	$34.00	
Oxalate	AgAHL	5.0	50.4	$34.00	$34.00	
Amino acid profile	AgAHL	1.0	51.4	$150.00	$150.00	
Fatty acid profile	AgAHL	2.0	53.4	$52.00	$52.00	
ICP analysis includes:*	CSBP	0.2	53.6			Hysterangium 55g Rhizopogon luteolus 70g Elaphomyces sp. 102g
Calcium						
Magnesium						
Potassium						
Sodium						
Phosphorus						
Sulphur						
Copper						
Manganese						
Zinc						
Iron						
Boron						

*Protein, Chloride, ICP analysis all for $30.

AgAHL: Agriculture WA Animal Health Laboratories, Perth
CSBP: CSBP Futurefarm labs, Perth
CCWA: Chemistry Centre, Perth
AGAL: Melbourne via AgAHL
DTS: Dairy Technical Services, Melbourne