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DIGESTA RETENTION AND FIBRE DIGESTION IN BRUSHTAIL POSSUMS, RINGTAIL POSSUMS AND RABBITS

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Abstract—1. Mean retention times (MRTs) of fluid (marked with Co-EDTA), fine particles (mordanted with Yb) and large particles (mordanted with Cr) were measured in brushtail possums (*Trichosurus vulpecula*), ringtail possums (*Pseudocheirus peregrinus*) and laboratory rabbits fed semipurified diets.

2. In brushtail possums there were no significant differences in MRT among the three digesta markers.

3. In ringtail possums MRTs of the fluid and fine particle markers were approximately twice that of the large particle marker, indicative of selective retention of both fluid and fine particles in the caecum.

4. In the rabbit MRT of fine particles was also greater than that of large particles, again indicative of selective retention of fine particles in the caecum.

5. Fibre digestibility was greater in the rabbits than in the ringtail possums, and greater for neutral-detergent fibre (including agar) but less for acid-detergent fibre in the rabbits than in the brushtails. Differences in fibre digestibility between brushtails and rabbits were explained by differences in patterns of digesta flow. However, the higher digestibilities of fibre in the rabbits than in the ringtail possums could not be explained on a similar basis.

INTRODUCTION

Some small hindgut fermenters such as rabbits have a digesta separation mechanism in the colon (Hörnicke and Björnhag, 1980) which results in selective retention of fluid and fine particles in the caecum (Pickard and Stevens, 1972). This digestive strategy has several consequences: it maintains a high concentration of microorganism for maximal rates of fermentation in the caecum (Björnhag, 1987), it minimises losses of microbial protein in the faeces; and it results in the relatively rapid elimination of large, hard-to-digest particles from the hindgut, enabling the animal to maintain a higher level of feed intake than it would otherwise be able to.

In small hindgut fermenters, or caecum fermenters (Hume and Warner, 1980) that have a colon separation mechanism, selective retention of fluid and particles in the caecum is often coupled with coprophagy (ingestion of faeces) or caecotrophy (ingestion of high-nutrient faeces derived from caecal contents) (Hörnicke and Björnhag, 1980). This strategy enables the animal to derive nutritional benefit from the microbial cells produced in the caecum once they are digested in the stomach and small intestine.

Selective retention of fluid digesta markers has been demonstrated in the marsupial ringtail possum (*Pseudocheirus peregrinus*), which feeds largely on the foliage of *Eucalyptus* spp. (Chilcott and Hume, 1985), but not in the brushtail possum (*Trichosurus vulpecula*) (Foley and Hume, 1987). This marsupial also feeds on *Eucalyptus* foliage, but supplements this with leaves from other species of trees and shrubs, as well as with fruits, flowers and also herbs (Kerle, 1984). In contrast to the ringtail possum, the brushtail possum is difficult to maintain on a sole diet of *Eucalyptus* foliage (Foley and Hume, 1987).

Fibre digestibility in rabbits is low. Sakaguchi *et al.* (1987) have suggested that this is a consequence of the relatively rapid elimination of large digesta particles from the hindgut. If this is so, fibre digestibility should also be low in the ringtail possum, for similar reasons, but higher in the brushtail possum, since this folivore does not selectively retain fluid and fine particles. This paper describes an attempt to test this hypothesis by comparing fibre digestibility and mean retention times of digesta markers in rabbits, ringtail possums and brushtail possums fed a common diet.

MATERIALS AND METHODS

Animals and feeding

Four brushtail possums (mean body mass 2.6 kg), four ringtail possums (661 g) and four laboratory rabbits (2.9 kg) were used. The brushtail possums were trapped in wire-mesh box traps in residential areas, while the ringtails were caught by hand in natural bushland areas of Sydney. All animals were housed individually in stainless steel mesh metabolism cages, each 60 × 60 × 45 cm high, in an air-conditioned room. The possums were allowed at least three weeks to adjust to these conditions, during which time they were maintained on a diet of mixed fruit and vegetables (brushtails) or several species of *Eucalyptus* foliage (ringtails). Once accustomed to the experimental room, all animals were introduced to increasing proportions of a pelleted semipurified diet. Two diets, the composition of which is shown in Table 1, were used. Diet 1 was fed to the brushtail possums and the rabbits in the first period. The ringtail possums were withdrawn from this period because of unacceptably low feed intakes. Instead, they were offered a

number of different diets, of which that shown as Diet 2 in Table 1 was the most successful. Subsequently, this diet was fed to the ringtail possums and the rabbits in the second period. In both periods feed and water were freely available, and caecotrophy by the ringtail possums and rabbits was not prevented; brushtail possums are not coprophagous (Foley and Hume, 1987).

Digesta markers

Fluid digesta were marked with Co-EDTA. Fine and large digesta particles were mordanted with Yb and Cr respectively. These particles were prepared from the cell-wall constituents (CWC) of hammer-milled oaten hay, following the methods of Van Soest and Wine (1967). The dried CWC were then washed through a set of 0.6, 0.3 and 0.075 mm mesh screens. Particles retained on the 0.3 mm screen were used as large particles, and those which passed through the 0.075 mm screen were used as fine particles. Cr–CWC and Yb–CWC mordants were prepared by the methods of Udén *et al.* (1980) and Ellis and Beever (1984) respectively.

Collection procedures

Each period consisted of a 7–10 day pre-collection period which commenced only after feed intakes had stabilised on one of the two diets, followed by a 14 day collection period. The collection periods commenced with the oral administration of the three digesta markers at 15.00 hr. The animals were fed between 15.00 hr and 16.00 hr each day. Faeces were then collected from all animals every 2 hr for the first 24 hr after dosing, every 6 hr for the next 24 hr and every 8 hr for the next 5 days, and then for the possums daily for the next 7 days. Feed consumption and total faecal output of each animal was recorded daily.

Table 1. Composition of experimental diet (g/kg)

	Diet 1*	Diet 2†
Ingredients		
Alfalfa meal	444	200
Oat rolled	333	600
Agar	133	—
Corn starch	—	100
Sugar	89	100
Analysis		
Dry matter	895	913
Organic matter (g/kgDM)	852(952)	887(972)
NDF (g/kgDM)	356(398)	291(319)
ADF (g/kgDM)	143(160)	100(110)
Crude protein (g/kgDM)‡	115(128)	97(106)

*Fed to Brushtail possums and rabbits. †Fed to Ringtail possums and rabbits. ‡Nitrogen × 6.25.

Analytical methods

Feed, pooled faecal examples and pooled feed residues from each animal were oven-dried at 60°C, then ground and analysed for dry matter, ash and total nitrogen (AOAC, 1980) and for neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) by methods described by Van Soest and Wine (1967) and Van Soest (1963) respectively. Organic matter was determined by subtracting ash from dry matter.

For determination of Cr, Yb and Co, individual faecal samples were dried and ashed at 550°C for 6 hr in a muffle furnace. The ashed samples were treated according to the methods described by Williams *et al.* (1962), and analysed for Cr, Yb and Co using an inductive coupled plasma quantometer and atomic absorption spectrophotometer.

Calculations

Single exponential regression equations were fitted statistically to the time-course decline in faecal concentrations of Cr and Yb in all animals, and of Co in the possums; excretion curves for Co, the fluid digesta marker, in the rabbits were too irregular for regression equations to be fitted. The turnover time of each marker was estimated from the decline in faecal concentration of the marker by the function (Brandt and Thacker, 1958):

$$Y = Y_0 \cdot e^{-kt}$$

where Y is the concentration of marker in the faeces at time t, Y_0 is a constant depending on the level of marker given, k is the rate-constant and t is the time interval (hr) after dosing with the marker. Turnover time was calculated as the reciprocal of the rate-constant (k) of the exponential curve fitted to the time-course excretion of each marker after faecal marker concentration had reached a maximum. Total mean retention time (MRT) in the gastrointestinal tract was calculated as the sum of turnover time and transit time, which was taken as the time of first appearance of the marker in the faeces after dosing. Differences between mean values were evaluated statistically by Student's t test (Snedecor and Cochran, 1967).

RESULTS

Retention of digesta markers (Table 2)

Except in the brushtail possums, markers were still detectable in the faeces 14 days after dosing. Thus the direct calculation of MRT by the method of Blaxter *et al.* (1956) could not be used. Instead, the method of Brandt and Thacker (1958) was used for all three

Table 2. Measures of retention of digesta markers in the brushtail possum, ringtail possum and laboratory rabbit (mean ± SD).

	Diet 1					
	Brushtail possum ($n = 4$)			Rabbit ($n = 4$)		
	1/k	TT	MRT	1/k	TT	MRT
Fluid (Co)	30.6 ± 13.6	5.8 ± 0.5	36.3 ± 13.1	—	9.0 ± 6.7	—
Particles						
Fine (Yb)	33.9 ± 13.4	5.8 ± 0.5	39.6 ± 13.0	40.2 ± 7.3*	9.0 ± 6.7	49.2 ± 10.8
Large (Cr)	27.1 ± 13.8	5.8 ± 0.5	32.8 ± 13.3	29.3 ± 5.0	9.0 ± 6.7	38.3 ± 11.0
	Diet 2					
	Ringtail possum ($n = 4$)			Rabbit ($n = 4$)		
	1/k	TT	MRT	1/k	TT	MRT
Fluid (Co)	203.2 ± 61.8	6.5 ± 0.6	209.7 ± 62.3	—	6.5 ± 1.9	—
Particles						
Fine (Yb)	241.8 ± 122.5	6.5 ± 0.6	248.3 ± 123.0	190.0 ± 104.8†	6.5 ± 1.9	196.5 ± 106.2†
Large (Cr)	106.3 ± 68.1	6.0 ± 0.8	112.3 ± 68.5	84.0 ± 38.7†	5.8 ± 1.7	90.5 ± 40.3†

k is a rate-constant which is the dilution rate(/hr) of the marker in the digestive tract. TT (transit time) is the time-interval between feeding and first appearance of the marker in the faeces. MRT is the sum of 1/k and TT. *Significantly different from Cr ($P < 0.05$). †Significantly different from Diet 1 ($P < 0.05$).

species. However, the lack of a significant regression relationship between faecal marker concentration and time after dosing in the rabbits precluded the calculation of turnover times and thus MRT for the fluid marker (Co-EDTA) in the rabbits on both diets.

In the brushtail possum there were no significant differences in turnover time ($1/k$), transit time or MRT among the three digesta markers. In the ringtail possum, turnover times and MRTs for both the fluid (Co) and fine particle (Yb) markers were approximately twice those of the large particle marker (Cr), but between-animal variability was such that the difference was not statistically significant.

In the rabbits on Diet 2, turnover times and MRTs for the fine particle marker (Yb) were also twice those of the large particle marker (Cr), but again the difference was not statistically significant. On Diet 1 however, the difference for turnover time was significant ($P < 0.05$), even though the magnitude of the difference was less; this was related to smaller between-animal variability. Turnover times and MRTs were both lower ($P < 0.05$) on Diet 1 than on Diet 2.

Feed intake and digestibility (Table 3)

All animals gained weight over the 14-day collection periods. On Diet 1, the brushtail possums consumed less dry matter than the rabbits ($P < 0.05$). Apparent digestibilities of dry matter, organic matter and crude protein were very similar between the two species, but the brushtails digested significantly less ($P < 0.05$) NDF but more ($P < 0.10$) ADF than did the rabbits.

In the rabbits, dry matter intake on Diet 2 was similar to that on Diet 1, but apparent digestibilities of dry matter and organic matter, and digestibilities of NDF and ADF were significantly greater ($P < 0.01$). Apparent digestibility of crude protein was similar on the two diets.

On Diet 2, the ringtail possums consumed less dry matter per day than did the rabbits ($P < 0.01$). However, on a metabolic body weight basis, the 31% lower dry matter intake by the ringtails than the rabbits was not significant ($0.10 < P < 0.20$). Apparent digestibilities of dry matter and organic matter were similar between the two species, but that of crude protein was greater ($P < 0.01$) in the ringtail possums. In contrast, digestibilities of both NDF and ADF were greater ($P < 0.05$) in the rabbits.

Table 3. Body weight, weight gain, feed intake and digestibilities of feed in the brushtail possum, ringtail possum and laboratory rabbit (mean ± SD)

Diet 1	Brushtail possum ($n = 4$)	Rabit ($n = 4$)
Body weight (kg)		
Initial	2.50 ± 0.38	2.78 ± 0.21
Gain (14 days)	0.06 ± 0.15	0.09 ± 0.12
Intake of DM		
g/day	40.0 ± 14.1*	67.6 ± 6.4
g/kgBW ^{075}/day	20.1 ± 6.4*	30.6 ± 4.0
Digestibility (%)		
Dry matter	67.0 ± 2.5	65.2 ± 4.0
Organic matter	67.3 ± 2.6	65.2 ± 4.0
NDF	42.7 ± 9.6*	60.5 ± 4.7
ADF	36.1 ± 5.6	25.8 ± 8.2
Crude protein	67.6 ± 0.1	68.2 ± 2.7
Diet 2	**Ringtail possum ($n = 4$)**	**Rabbit ($n = 4$)**
Body weight (kg)		
Initial	0.628 ± 0.114	3.10 ± 0.11
Gain (14 days)	0.022 ± 0.025	0.03 ± 0.15
Intake of DM		
g/day	13.1 ± 3.5†	62.4 ± 18.5
g/kgBW ^{075}/day	18.5 ± 3.3	26.8 ± 8.3
Digestibility (%)		
Dry matter	86.0 ± 1.2	88.0 ± 2.5‡
Organic matter	86.3 ± 1.0	88.4 ± 2.5‡
NDF	69.4 ± 2.6*	82.7 ± 3.6‡
ADF	34.8 ± 5.5*	57.0 ± 9.2‡
Crude protein	85.7 ± 1.3†	64.2 ± 9.8

*† Significantly different from the rabbit ($P < 0.05$, $P < 0.01$, respectively).
‡Significantly different from Diet 1 ($P < 0.01$).

DISCUSSION

Direct comparisons between the two marsupial species in this study were limited by the refusal of the ringtail possums to eat Diet 1. Diet 2 was lower in fibre content than Diet 1 and higher in starch and sugar. The form of the fibre also differed between the two diets; more of the dietary fibre of Diet 2 than of Diet 1 was in the form of rolled oats, and less as alfalfa. In addition, agar, included only in Diet 1, functions in the gut as soluble dietary fibre, and is almost completely recovered in the NDF fraction (Sakaguchi, unpublished).

These differences in composition help to explain the higher digestibilities in the rabbits of dry matter, organic matter, NDF and ADF on Diet 2 than on Diet 1. However, the main explanation probably lies in the longer MRTs of both fine (4 times) and large (2.4 times) particles on Diet 2, allowing more time for digestion. Despite the much longer MRTs on Diet 2, the selective retention of fine relative to large particles seen on Diet 1 was maintained.

Digestibility of ADF in the brushtail possums was higher than that in the rabbits on the same diet. However, NDF digestibility was significantly higher in the rabbits than in the brushtails. If agar behaves as soluble fibre in the gastointestinal tracts, this component of Diet 1 may be expected to be selectively retained in the rabbit caecum along with fluid and fine particles, and consequently be extensively degraded by microbial enzymes. Brushtail possums have been shown to lack any selective retention of fluid or fine particles in the caecum (Wallard and Hume, 1981; Foley and Hume, 1987). These differences in hindgut function between brushtails and rabbits therefore provide an explanation for the different digestibilities of ADF and NDF. The caecum of the guinea pig functions much like the caecum of the brushtail possum, with no selective retention of digesta; guinea pigs also digest more fibre (both ADF and NDF) than rabbits on a 50% alfalfa diet (Sakaguchi *et al.*, 1987).

In contrast to the brushtails, in the ringtail possums the digestibilities of both NDF and ADF were lower than in the rabbits. The reason for the difference in fibre digestibility between the rabbits and ringtails is not clear. MRTs of the fine particle marker in both species were approximately twice those of the large particle marker (Table 2), and of the same magnitude in the two species.

In the ringtail possums the MRTs of the fluid and fine particle markers were similar, indicating that fine digesta particles, as well as bacteria, are selectively retained in the caecum as suggested by patterns of distribution of fine and large particles in the digestive tract of ringtail possums maintained on *Eucalyptus* foliage (Chilcott and Hume, 1985). Further, the differential between the fluid and large particle markers in the present experiment was similar to that reported by Chilcott and Hume (1985) between fluid and particulate markers, even though MRTs on the semipurified diet used here were about three times those on *Eucalyptus* foliage. These long MRTs are related to lower dry matter intakes in the present study (19 vs 35 g/kg$^{0.75}$/d). Thus the selective retention mechanism is effective across a wide range of feed intakes and on non-foliage as well as foliage diets.

Sakaguchi *et al.* (1987) found that the extent of fibre digestion in four small hindgut fermenters, or caecum fermenters (Hume and Warner, 1980) fed a common diet was related more closely to the turnover time of large particles in the caecum than to their MRT in the whole digestive tract. That is, those species which were more effective in selectively retaining fluid and fine particles in the caecum showed the lowest fibre digestibilities. This is because most of the fibre is contained in the large particles, which are eliminated from the hindgut relatively rapidly. This trend appears to hold for the rabbit–brushtail possum comparison; the rabbits, with selective digesta retention, had ADF digestibilties ten percentage units below those of the brushtails (Table 3). The reason for the opposing trend with NDF has been discussed above. However, the rabbit–ringtail possum comparison does not lend itself to similar analysis, since both species are effective selective digesta retainers (Table 2), yet there were significant differences in digestibility of both ADF and NDF between them.

The refusal of the ringtail possums to eat the semipurified diet accepted by the brushtail possums precluded direct testing of the hypothesis that, as found in eutherian caecum fermenters by Sakaguchi *et al.* (1987), in the possums fibre digestibility should be inversely related to the extent to which fluid and fine particles are selectively retained in the caeum. Nevertheless, the results obtained confirm earlier observations that the ringtail possum selectively retains fluid digesta (Chilcott and Hume, 1985), and demonstrate that fine particles are selectively retained along with fluid digesta.

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REFERENCES

Association of Official Analytical Chemists (1980) Official Methods of Analysis. 13th Ed. Ass. Off. Anal. Chemists: Washington, D. C.

Björnhag G. (1987) Comparative aspects of digestion in the hindgut of mammals. The colonic separation mechanism (CSM) (a Review). *Dtsch. tierarztl. Wschr.* **94,** 33–36.

Blaxter K. L., Graham N. Mc. and Wainman F. W. (1956) Some observations on the digestibility of food by sheep, and on related problems. *Br. J. Nutr.* **10,** 69–91.

Brandt C. S. and Thacker E. J. (1958) A concept of rate of food passage through the gastrointestinal tract. *J. Anim. Sci* **17,** 218–223.

Chilcott, M. J. and Hume I. D. (1985) Coprophagy and selective retention of fluid digesta: Their role in the nutrition of the common ringtail possum, *Pseudocheirus peregrinus*. *Aust. J. Zool.* **33,** 1–15.

Ellis W. C. and Beever D. E. (1984) Methods for binding rare earths to specific feed particles. In *Techniques in Particle Size. Analysis of Feed and Digesta in Ruminants.* (Edited by Kennedy P. M.), pp. 154–165. Can. Soc. Anim. Sci. Occasional Publ. No: 1, Edmonton.

Foley W. J. and Hume I. D. (1987) Passage of digesta markers in two species of arboreal folivorous marsupials —the greater glider (*Petauroides volans*) and the brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol.* **B157,** 67–76.

Hörnicke H. and Björnhag G. (1980) Coprophagy and related strategies for digesta utilization. In *Digestive Physiology and Metabolism in Ruminants.* (Edited by Ruckebusch Y. and Thivend P.), pp. 707–730. MTP Press, Lancaster.

Hume I. D. and Warner A. C. I. (1980) Evolution of microbial digestion in mammals. In *Digestive Physiology and Metabolism in Ruminants.* (Edited by Ruckebusch Y. and Thivend P.), pp. 665–684. MTP Press, Lancaster.

Kerle J. A. (1984) *Variation in the ecology of* Trichosurus: *its adaptive significance. In Possums and gliders.* (Edited by Smith A. P. and Hume I. D.), pp. 115–128. Australian Mammal Society, Sydney.

Pickard D. W. and Stevens C. E. (1972) Digesta flow through the rabbit large intestine. *Am. J. Physiol.* **222,** 1161–1166.

Sakaguchi E., Itoh H., Uchida S. and Horigome T. (1987) Comparison of fibre digestion and digesta retention time between rabbits, guinea-pigs, rats and hamsters. *Br. J. Nutr.* **58,** 149–158.

Snedecor G. W. and Cochran W. G. (1967) *Statistical Methods.* 6th Ed. Iowa State University Press, Ames.

Udén P., Colucci P. E. and Van Soest P. J. (1980) Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. *J. Sci. Food Agric.* **31,** 625–632.

Van Soest P. J. (1963) The use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and liguin. *J. Ass. Off. Agric. Chem.* **46,** 829–835.

Van Soest P. J. and Wine R. H. (1967) Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. Ass. Off. Agric. Chem.* **50,** 50–55.

Wallard G. A. and Hume I. D. (1981) Digestion and digesta passage in the brushtail possum, *Trichosums vulpecula* (Kerr). *Aust J. Zool.* **29,** 157–166.

Williams C. H., David D. J. and Iisuraa O. (1962) The determination of chromic oxide in faeces samples by atomic absorption, spectrophotometry. *J. Agric. Sci.* **59,** 381–385.