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Digestive performance and digesta passage in the omnivorous greater bilby, *Macrotis lagotis* (Marsupialia: Peramelidae)

Abstract Omnivores such as the greater bilby (*Macrotis lagotis*) consume a variety of dietary items and often are faced with large changes in the nutrient composition of their food. This paper explores the basis for the dietary flexibility of the bilby by comparing digestive performance and digesta retention patterns of captive bilbies fed either an insect diet (mealworm larvae) or a plant diet (mixed seeds). Mean retention times (MRTs) of particle and solute markers in the gastrointestinal tract did not differ significantly between the two diets, but MRT of the particle marker was significantly longer than that of the solute marker on both the mealworm (particle: 23.5 h; solute: 17.9 h) and mixed seed (particle 33.0 h; solute: 30.2 h) diets. Lack of selective retention of solutes and small particles in the bilby gastrointestinal tract probably restricts them to relatively low-fibre diets, such as those based on seeds rather than leaves and stems of plants. It was observed radiographically that the major sites of digesta retention were the caecum, proximal colon and distal colon, and thus the hindgut is probably the principal site of microbial fermentation. The mealworms were more digestible than the mixed seeds, but digestible energy intake (mealworm: 939 kJ · kg$^{-0.75}$ · d^{-1}; mixed seed: 629 kJ · kg$^{-0.75}$ · d^{-1}) was high enough for maintenance of body mass and positive nitrogen balance on both diets. Thus, although bilbies may be limited in their ability to utilize high-fibre diets by a lack of selective retention of solutes and small particles in their hindgut, their digestive strategy is flexible enough to accommodate at least some diets of both animal and plant origin. Such a strategy should benefit an animal inhabiting environments in which food resources are unpredictable in their relative abundance.

Key words Omnivore · Digestibility · Mean retention time · Radiography · Bilby · *Macrotis lagotis*

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Introduction

The greater bilby (*Macrotis lagotis*) is a small (800–2500 g) omnivorous marsupial in the family Peramelidae. Prior to the arrival of Europeans the bilby was distributed throughout 70% of Australia (Southgate 1990a). Although its distribution was centred on arid land, much of this former range included more temperate regions. Since the arrival of Europeans, bilby numbers have significantly decreased and its distribution has contracted into the driest and least fertile parts of its former range (Southgate 1990a).

Arid-zone animals face the problem of acquiring food which satisfies their metabolic requirements from an environment low in several nutrients, including nitrogen (Hume 1999). Nutrient acquisition problems are exacerbated in small animals (< 3 kg) because of their relatively high mass-specific metabolic requirements and small absolute gastrointestinal tract size (Foley and Cork 1992). Small mammals must therefore either eat more food or eat food with a higher concentration of digestible energy than larger animals. Small mammalian insectivores or carnivores mitigate this problem by eating generally highly digestible food. Small mammalian omnivores eat both animal and plant materials, but may be restricted to plant parts that are low in fibre content, such as flowers, fruits, seeds or nectar.

In small mammalian herbivores the conflicting problems of fast passage of food and relatively slow microbial fermentation of plant fibre are alleviated by one or more of four different digestive strategies (Björnhag 1987): (1) only the readily digestible parts of plants are consumed; (2) metabolic rates are lower than

predicted for their body size; (3) selective retention of solutes and fine particles that are easily digested in the caecum (or both the caecum and proximal colon), while passage of larger particles of fibrous material of low digestibility is facilitated; and (4) selective retention of microbes in the caecum which promotes more complete fermentation. The colonic separation mechanism in the third and fourth strategies improves digestive efficiency by minimizing the residence time of indigestible material and simultaneously maximizing the concentration of digestible nutrients and microbes in the hindgut.

Omnivores generally select higher quality food than herbivores, and their digestive tract morphology and processes are usually simpler, as seen in myomorph rodents (Björnhag 1987). Nevertheless, a colonic separation mechanism has been reported in two species of marsupial omnivores with relatively high fibre intakes, the long-nosed bandicoot, *Perameles nasuta* (Moyle et al. 1995) and the northern brown bandicoot, *Isoodon macrourus* (McClelland et al. 1999).

The question of whether selective retention of solutes in the caecum is a general feature of peramelid marsupials is addressed in this paper by examination of digestive performance and digesta retention patterns in the bilby. An examination of the digestive tract of a bilby showed that the stomach and small intestine were simple and the caecum, although only moderate in size, was an obvious feature of the hindgut (Hume 1999). Given the bilby's omnivorous diet (Southgate 1990b), small body mass (< 3 kg) and seasonally low-nutrient environment, it is possible that its digestive efficiency is improved by a hindgut separation mechanism. Moyle et al. (1995), in their examination of digestive performance in captive long-nosed bandicoots (*P. nasuta*), concluded that retention of plant material in the caecum and proximal colon and the preferential retention of solutes in the caecum were key contributing factors in the ability of bandicoots to switch between insect and plant foods, and therefore to exploit nutritionally unpredictable environments. In this paper we explore this possibility by comparing the digestive performance and digesta retention patterns of captive bilbies fed a seed diet with those fed a diet consisting solely of insects.

Materials and methods

Morphometrics of the digestive tract

As a result of accidental road kills, two sub-adult bilbies (body mass 657 g and 365 g) were available for examination of the gross morphology of the digestive tract. These animals were fresh kills and were immediately frozen until dissection. After the complete tract had been removed from the animal and cleared of mesenteric attachments, the digesta in each segment, i.e. the stomach, small intestine, caecum, proximal colon and distal colon, were measured by weighing the segments full and then emptied of contents. The junction between caecum and proximal colon is defined by the opening of the ileum into the hindgut. The junction between proximal and distal colon was taken to be the point at which segmentation of digesta first occurred. The lengths of each segment

were also measured. To determine the particle size distribution in each segment, further samples of digesta (approximately 1 g) were removed and washed through a series of sieves (mesh sizes: 1.0, 0.5, 0.25, 0.125, 0.075 and 0.045 mm), and each fraction dried at 80 °C to constant weight.

Animals

Twelve animals (five female, seven male) were selected from a captive colony at the Charleville District Office of the Queensland Parks and Wildlife Service, Queensland, Australia. Due to insufficient numbers of adult bilbies, the experimental procedures were repeated when more adults became available. The first experiment was conducted in November 1997, with three female and three male bilbies. Two females and four males were studied in the second experiment, conducted in June 1998.

Animals were held in individual stainless steel mesh metabolism cages (60 cm × 60 cm × 45 cm high) in a darkened temperature-regulated room at approximately 27 °C [within the thermoneutral zone of the bilby (Kinnear and Shield (1975)]. Each cage contained a wooden nest box and the floor of the cages was covered with soft rubber matting except during faecal collection periods. Animals were weighed weekly at 1700 hours.

Experimental diets

The bilbies were offered the experimental diets immediately after entry into the metabolism cages. The two diets chosen were live mealworm (*Tenebrio molitor*) larvae and a commercial bird seed mix. These diets were used to mimic the invertebrate and seed components of their diet in the wild (Gibson 1999). Their compositions are shown in Table 1. Mealworms were cultured in bran and, to enhance storage life, they were kept in a refrigerator. The use of smooth, round-sided, plastic feeding bowls prevented the escape of mealworms after placement in cages. The mixed seed diet was supplemented with three drops (approximately 0.5 ml) of a multi-vitamin mix (Pentavite, Roche, New South Wales) in the drinking water daily. Animals were fed at 1900 h each day and both food and water were provided ad libitum. Animals were offered 60–90 g (as fed) of mealworms and 80–130 g (air dry, as fed) of seeds daily. The amount offered varied because some individuals consistently spilled food from their food containers.

Six animals (three female, three male) were offered the mealworm diet and six animals (two female, four male) the mixed seed diet.

Rate of digesta passage

After a 14-day adjustment period, the rate of passage of two digesta markers was measured on the two diets. Prior to feeding, all 12

Table 1 Chemical composition of the two experimental diets offered to bilbies (expressed as % of dry matter)

	Diet	
	Mealworm	Mixed seeds
Dry matter (% of wet weight)	41.1	92.7
Organic matter	92.6	96.7
Total ash	7.4	3.3
Neutral detergent fibre (NDF)	11.3	15.6
Acid detergent fibre (ADF)	6.3	10.4
Lignin	5.3	6.5
Total nitrogen	8.0	2.1
Crude lipid	38.4	5.9
Gross energy (kJ · g DM^{-1})	28.2	18.9

animals were given a pulse dose of two inert markers in 10 g of commercial canned dog food (PAL puppy). The two markers were cobalt-ethylenediaminetetra-acetic acid (Co-EDTA), which remains in solution (Udén et al. 1980), and chromium mordanted onto cell wall constituents (Cr-CWC) prepared from ground oaten (*Avena sativa*) hay (Udén et al. 1980). The cell walls were prepared using the neutral detergent fibre (NDF) technique of Van Soest and Wine (1967), then washed through a set of Endecott (London, UK) sieves. The particles that passed through a 1.2 mm sieve but were retained on a 0.6 mm sieve were retained for mordanting.

Eight of the 12 bilbies ate their dose within 60 min. The remaining four were force-fed (within 90 min) by injecting the dose into the back of their mouths using a modified 5 ml syringe. The doses of Cr-CWC and Co-EDTA were 0.6 g · kg^{-1} and 0.3 g · kg^{-1} respectively for the first trial and 0.3 g · kg^{-1} for both markers in the second trial.

After ingestion of the markers, animals were offered their experimental diet. Faecal collection trays were checked and any faeces present collected 2-hourly for the first 24 h, then 4-hourly for the next 24 h, 6-hourly for the next 48 h, followed by 12-hourly for the next 48 h. The time of defecation was taken to be the midpoint of each inspection interval. To determine the absorption of markers from the digestive tract and subsequent excretion through the kidneys, urine was also collected and bulked for each animal. Faeces and urine were stored at −20 °C until analysis.

Transit time of each marker was taken as the midpoint of the interval in which that marker first appeared in the faeces (Warner 1981). Mean retention time (MRT) of both markers was calculated by the formula:

$$\text{MRT}(h) = \frac{\sum_{i=1}^{n} M_i t_i}{\sum_{i=1}^{n} M_i}$$

as recommended by Warner (1981), where M_i is the amount of marker excreted in the *i*th defecation at time t_i after dosing, and n is the last collection interval in which marker could be detected.

Sites of digesta retention

Two female and two male bilbies from the first trial were used to investigate sites of digesta retention along the digestive tract by radiography. One female and one male were continued on the mixed seed diet and the remaining two on the mealworm diet. The radiographic marker used was barium sulphate (David Craig, Rocklea, Queensland, Australia); a non-specific bulk digesta marker (Hume and Carlisle 1985). A 2 ml dose of 30 g barium sulphate per 15 ml water was injected into the back of the mouth of each animal using a modified 5 ml syringe, followed by an additional 1–2 ml of water to wash down the barium sulphate suspension. Once the dose was ingested, animals were offered their usual diets.

Left lateral and dorsoventral projections were taken with a Toshiba X-ray machine (model DXB-0324CS) at 52 kV, 100 mA, 92 cm focal-film distance and 0.02 mAs/s exposure on Fuji Medical X-ray film (Super HR-G30; 24 × 30 cm). The first set of radiographs was taken at 21:30 h before dosing, then approximately 10–15 min, 20 min, 30 min, 1, 1.5, 2.5, 4, 6, 9, 12, 18, 24, 36, 49, 59 (3 animals) and 73 h (1 animal) after administration of barium sulphate. Animals were restrained by hand using lead-lined gloves. Between radiographs, animals were either returned to portable wooden nest boxes (short intervals) or to their metabolism cages (longer intervals).

Food intake and digestibility

With one exception, the same animals used in the measurement of rates of digesta passage were used to measure food intake and digestibility on the two experimental diets. Faeces, food residues and urine were collected daily (each morning) over a period of 7 days. Faeces and food residues were bulked for each animal and stored at −20 °C until analysis. Urine was collected in bottles containing 5 ml acetic acid which kept the pH of the urine below 3.0, thus preventing the loss of nitrogen as ammonia and discouraging bacterial growth (Moyle et al. 1995). At the end of the collection period the total volume of urine from each animal was recorded and then stored at −20 °C until analysis. Samples of the two diets were collected daily, bulked and similarly stored. Food intakes were determined by subtracting the mass of food residues from the mass offered, all on a dry matter basis.

Chemical analysis

For marker analysis, collected faeces were thawed to room temperature, dried to constant weight at 50 °C over 2–3 days, then crushed by hand. A 0.25–0.30 g sub-sample was digested with 5 ml nitric acid and 1 ml hydrogen peroxide in a high pressure microwave oven (Milestone, Italy). The digests were transferred to 100 ml (trial 1) or 50 ml (trial 2) volumetric flasks, made up with distilled water, and then filtered (Whatman Number 1 filter paper). Sub-samples (5 ml) of urine were similarly digested. The concentrations of Co and Cr in the digests were determined by flame atomic absorption spectroscopy (Varian model AA/400P).

To determine dry matter content, each bulk faecal, food residue and food sample was thawed to room temperature, weighed, then dried in an oven to constant weight at 50 °C. For chemical analyses, these dried samples were ground through a 1 mm screen in a laboratory mill, except for the mealworms which were ground in a coffee grinder. To determine organic matter content, representative samples (1–2 g) of faeces, food and food residues were ashed in a muffle furnace for 3 h at 550 °C. Further samples (0.5–1.0 g dry matter) were analysed for NDF by the procedure of Goering and Van Soest (1970), with the following modifications: the relatively tannin-free diets did not require the inclusion of sodium sulphite (Hanley et al. 1992) and samples were pre-treated with 50 µl of heat-stable α-amylase to remove starch (Van Soest et al. 1991). Decalin was also omitted as suggested by Moyle et al. (1995). Acid-detergent fibre (ADF) content of the two diets offered was determined using the method of Van Soest (1963). The ADF residues were sequentially analysed for lignin content according to Van Soest (1963). The total nitrogen content of faeces, food, food residues and urine was determined by the method of Ivan et al. (1974); a semi-micro Kjeldahl method using a selenium catalyst and approximately 0.2 g of dry sample and 200 µl of urine. The determination of the crude lipid content of faeces, food and food residues was based on the extraction procedure of Bligh and Dyer (1959) and Atkinson et al. (1972). The lipid was extracted with a mixture of chloroform: methanol:water in the proportions of 1.0:1.0:0.9. The gross energy content of faeces, food, food residues and urine was determined in a Gallenkamp adiabatic bomb calorimeter, using benzoic acid as the standard. All samples (0.5–1.0 g) were compressed into the base of a crucible prior to combustion. Because faecal samples could not be effectively compressed, they were first compressed as much as possible then wetted with a few drops of distilled water, and dried in an oven (50 °C) overnight. This prevented the sample blowing out of the crucible after ignition and thus ensured complete combustion. Urine samples (20 ml) were weighed, then freeze-dried to a powdery texture (4–5 days); approximately 1 g of the freeze-dried sample was then lightly compressed into the base of a crucible.

Feed equivalent fill

Feed equivalent fill (FEF), which is a relative measure of gastrointestinal tract capacity (G. J. Faichney, personal communication), was calculated as:

$$\text{FEF (g DM} \cdot \text{kg}^{-1}) = (\text{DMI} \times \text{PMRT})/24$$

where DMI is dry matter intake (g · kg^{-1} · day^{-1}) and PMRT is the mean retention time (h) of the particle marker (Cr-CWC in this case).

Statistical analysis

Initially, the distribution of all data was examined for normality by constructing probability plots (Gnanadesikan 1977) and frequency histograms (with a normality curve superimposed) (SYSTAT 1992). Where appropriate, variables were logarithmically transformed or, if the values were expressed as proportions, arcsine transformed prior to statistical analysis. Independent samples t-tests were used to examine differences in both the transit times and the MRT of each marker (Co-EDTA and Cr-CWC) between the two diets (seed and mealworm) (SYSTAT 1992). Differences in transit times and MRT between markers within each diet were tested by paired t-tests (SYSTAT 1992).

Analysis of covariance (ANCOVA) was used to examine differences in intakes (expressed as $g \cdot day^{-1}$) and digestibilities between the two experimental diets with initial body mass as the covariate. Studentized residuals were plotted against estimated values (cell means) to check for homogeneity of variances (SYSTAT 1992). Heterogeneity of slopes was examined by a preliminary general linear model to test the significance of the treatment (diet) by covariate (body mass) interaction (SYSTAT 1992). If the data fitted the requirements of parametric analysis, independent samples t-tests were used to examine differences in intakes (expressed as mass-specific units allometrically scaled to 0.75) between the two experimental diets, otherwise the non-parametric Mann-Whitney U test was used (SYSTAT 1992). $P < 0.05$ was used to define statistical significance.

Results

Morphology and morphometrics of the digestive tract

The complete digestive tract of one of the bilbies (365 g) is shown in Fig. 1 and the capacities and lengths of the regions of the digestive tracts from both animals are given in Table 2. Overall, the digestive tract morphology of both bilbies was similar.

The stomach was proportionally small (14–16% total tract contents) and the small intestine was relatively long and capacious (65–71% total length, 24–25% total tract contents), and both were unremarkable. The caecum was the smallest segment of the digestive tract, in both length (1.8–2.3% total length) and capacity (3.2–3.3% total tract contents). Although the proximal colon was short (3.7–4.2% total length), its capacity was relatively large (27–29% total tract contents). The distal colon was only 26–41% of the length of the small intestine but it had the largest capacity of all the segments (29–30% total tract contents). Together the three segments of the hindgut (caecum, proximal colon and distal colon) made up 60–63% of total tract contents.

The smallest particle size (< 75 μm) constituted the largest proportion of digesta in all regions of the digestive tract of both animals (Fig. 2). No other consistent trends were apparent, and there was no difference in the proportions of large and small particles between the caecum and distal colon. If a colonic separation mechanism was operative it would be most likely located in the proximal colon, and would be reflected in differences in proportions of large and small particles in the caecum and distal colon, the two sections adjacent to but on opposite sides of the proximal colon.
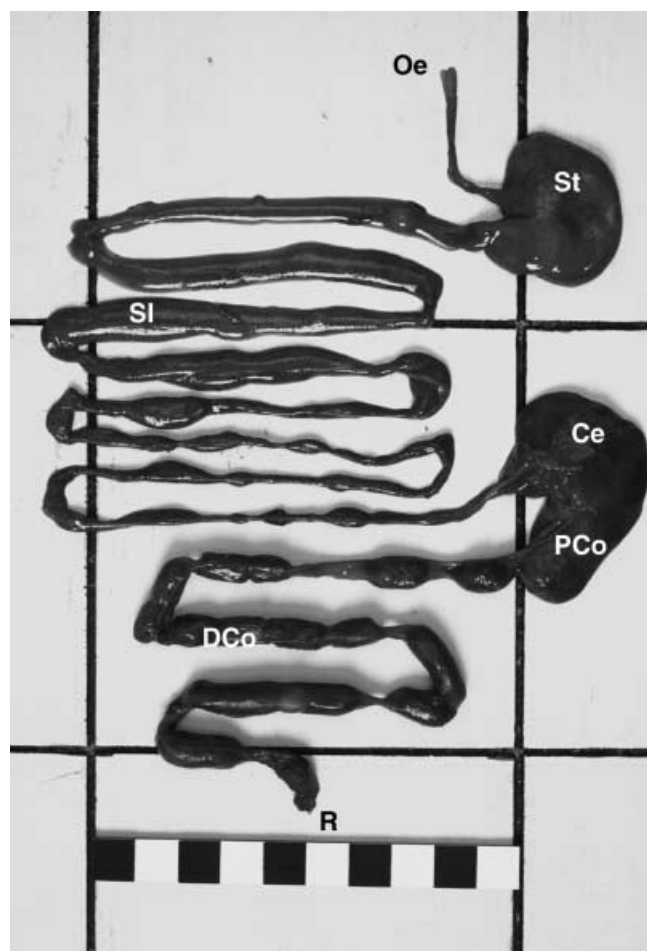

Fig. 1 The gastrointestinal tract of *Macrotis lagotis* (365 g) *Oe:* oesophagus, *St:* stomach, *SI:* small intestine, *Ce:* caecum, *PCo:* proximal colon, *DCo:* distal colon, *R:* rectum

Table 2 Capacities and lengths of regions of the digestive tracts of two bilbies. *A*, 365 g body mass; *B*, 657 g

Tract region	Length (cm)		Contents (g)	
	A	B	A	B
Stomach	3.7 (3.3)	4.8 (3.6)	3.0 (14.2)	6.5 (16.2)
Small intestine	74.0 (64.9)	94.0 (71.3)	5.1 (23.9)	10.0 (24.9)
Caecum	2.1 (1.8)	3.0 (2.3)	0.7 (3.3)	1.3 (3.2)
Proximal colon	4.2 (3.7)	5.5 (4.2)	6.1 (28.6)	10.9 (27.1)
Distal colon	30.0 (26.3)	24.5 (18.6)	6.4 (30.0)	11.5 (28.6)

Percentage of total digestive tract are in parentheses

Rate of digesta passage

There were no significant differences in the transit times or the MRTs of either marker between the two diets (Table 3). Likewise, the difference in transit times between the particle marker (Cr-CWC) and solute marker (Co-EDTA) on both the mixed seed and mealworm diets was not significant (Table 3). However, the particle MRT was longer than the solute MRT on both diets (mealworm, $P < 0.01$; mixed seed, $P < 0.05$) (Table 3).

Fig. 2 Distribution of particle sizes in the digesta for each digestive tract region in two bilbies (**a** and **b**)
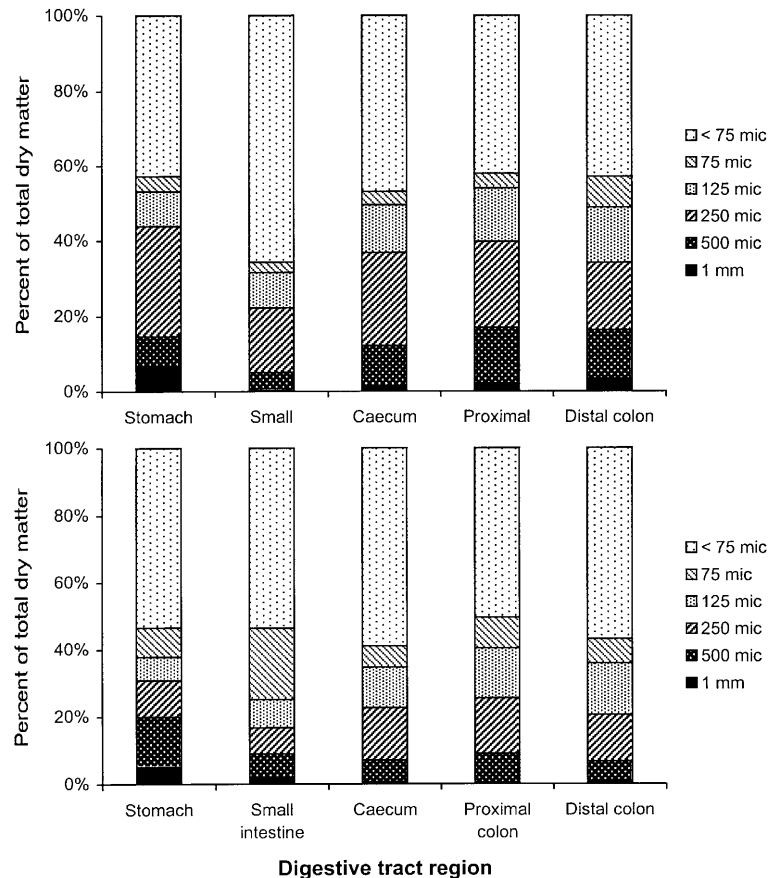

Table 3 Transit times and mean retention times (MRT) (mean ± S.E.) of two digesta markers in bilbies fed two experimental diets. Co-EDTA represents solutes and small particles (< 75 μm) in digesta; Cr-CWC represents large particles (300–600 μm) (*n* sample size; *n.s.* not significant)

	Diet		Significance of difference between diets
	Mealworm (*n* = 6)	Seed (*n* = 6)	
Transit time (h)			
Co-EDTA	11.2 ± 4.2	14.2 ± 3.6	n.s.
Cr-CWC	11.2 ± 4.2	12.5 ± 4.6	n.s.
Significance of difference between markers	n.s.	n.s.	
MRT (h)			
Co-EDTA	17.9 ± 5.0	30.2 ± 5.2	n.s.
Cr-CWC	23.5 ± 4.6	33.0 ± 5.0	n.s.
Significance of difference between markers	**	*	

*$P < 0.05$, **$P < 0.01$

Co-EDTA was detected in the urine, but only in small amounts (0.9 ± 0.2% of the dose). This small amount of Co-EDTA may have been absorbed from the gut or was the result of contamination of the urine by faeces on the collection screen (Moyle et al. 1995).

Radiography

The best representative radiographs (one from each diet) taken at 10–15 min, 30–35 min, 6 h, and 36 h after dose administration are shown in Figs. 3–6, respectively.

The contrast medium moved more quickly through the stomach of mealworm-fed animals than seed-fed animals (Fig. 3); by 30–35 min, almost all of the barium sulphate had emptied from the stomach into the small intestine (Fig. 4a), whereas in the seed-fed animals, the bulk of the barium sulphate remained in the stomach (Fig. 4b) until between 1 and 1.5 h when it had moved through to the small intestine. Marked digesta in the mealworm-fed animals also reached the hindgut earlier (by 2–5 h) than in the seed-fed animals. Passage of barium sulphate through the caecum and proximal colon was similar on both diets (Fig. 5). However, the rate of clearance from the distal colon and rectum tended to be slower in mealworm-fed than seed-fed animals (Fig. 6). In the mealworm-fed animals, almost all of the barium sulphate was eliminated by 60 h in one animal, and 73 h in the other. In contrast, the digestive tract of the seed-fed animals was clear of all barium sulphate by 58 h in one animal, and 49 h in the other.
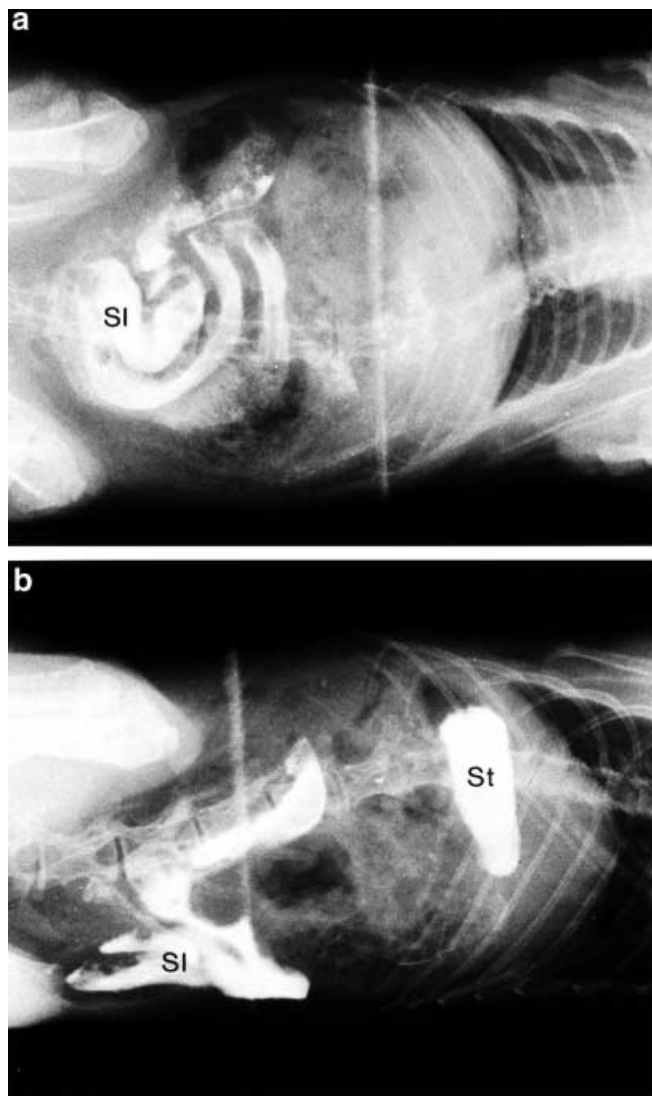
Fig. 3 Left lateral recumbent radiograph of bilbies (**a**) maintained on mealworms, and (**b**) maintained on seeds, 10–15 min after dosing with barium sulphate. *St* stomach, *SI* small intestine
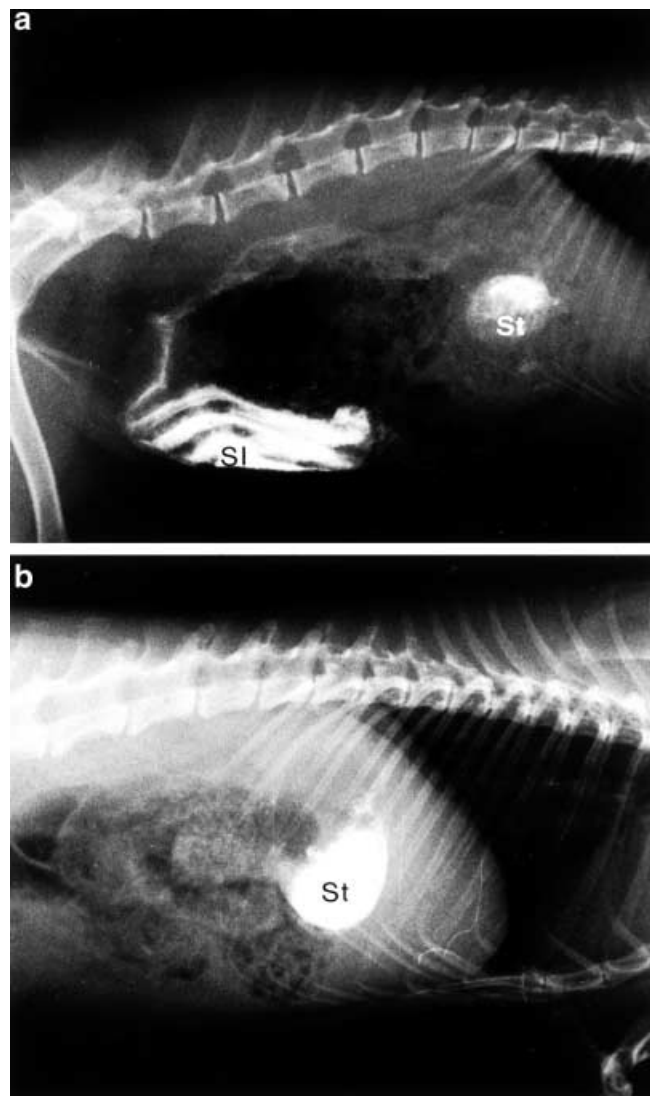

Fig. 4 Dorsoventral recumbent radiograph of bilbies (**a**) maintained on mealworms, and (**b**) maintained on seeds, 30–35 min after dosing with barium sulphate. *St* stomach, *SI* small intestine

Food intake and digestibility

The dry matter content of the seed diet was more than twice that of the mealworm diet (Table 1). On a dry matter basis, mealworms were much higher in total nitrogen and crude lipid contents than the seeds, and ash and gross energy contents were 55% and 33% higher than the seeds respectively. In contrast, the seeds were higher in fibre contents (NDF and ADF) than the mealworms. The NDF of plants consists chiefly of the structural carbohydrates cellulose, hemicellulose and lignin. Chitin (in the cuticle of mealworms) is also a structural carbohydrate (Stevens and Hume 1995), and is included in NDF in the Goering and Van Soest (1970) procedure.

Animals on the mealworm diet gained significantly more body mass than animals on the mixed seed diet ($P < 0.01$) (Table 4). Intakes and digestibilites of the two experimental diets are shown in Tables 4 and 5. Although dry matter intake on a metabolic body mass basis was similar on the two diets, the higher lipid, nitrogen and gross energy contents of the mealworms (Table 1) resulted in higher intakes of these dietary components (lipid and nitrogen: $P < 0.01$; gross energy: $P < 0.05$) on the mealworm diet. Mealworm-fed animals also digested a greater proportion of ingested dry matter ($P < 0.001$), lipid ($P < 0.01$), nitrogen ($P < 0.001$) and gross energy ($P < 0.001$) than animals on the mixed seed diet.

Organic matter intake on a metabolic body mass basis did not differ between the two diets, but apparent digestibility of organic matter was higher on the mealworm diet ($P < 0.001$).

Despite a similar intake of digestible dry matter on both diets, the intake of digestible energy was higher on mealworms than on seeds ($P < 0.01$). The metabolis-
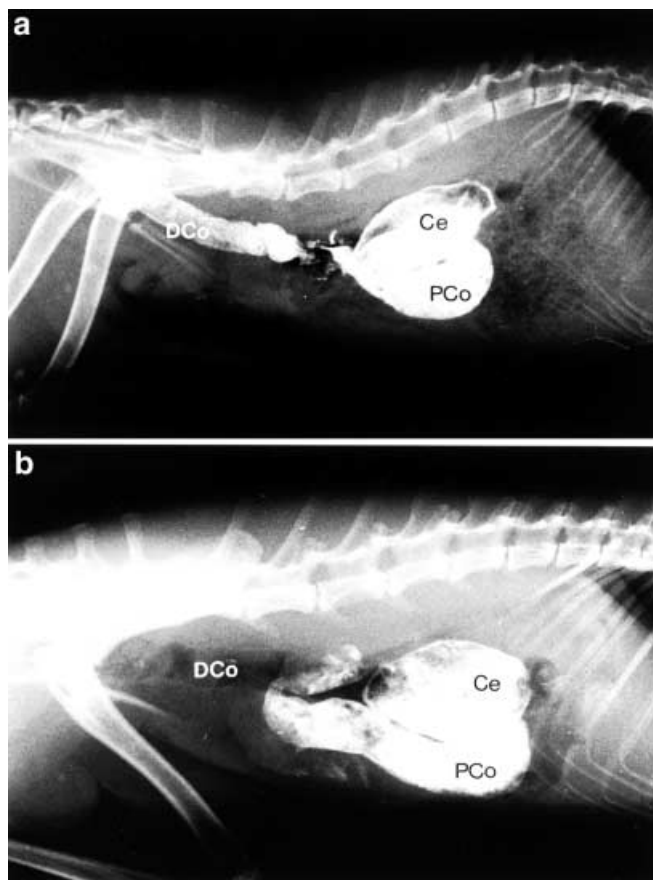
Fig. 5 Left lateral recumbent radiograph of bilbies (**a**) maintained on mealworms, and (**b**) maintained on seeds, 6 h after dosing with barium sulphate. *Ce* caecum, *PCo* proximal colon, *DCo* distal colon

Fig. 6 Left lateral recumbent radiograph of bilbies (**a**) maintained on mealworms, and (**b**) maintained on seeds, 36 h after dosing with barium sulphate. *Ce* caecum, *PCo* proximal colon, *DCo* distal colon, *Re* rectum
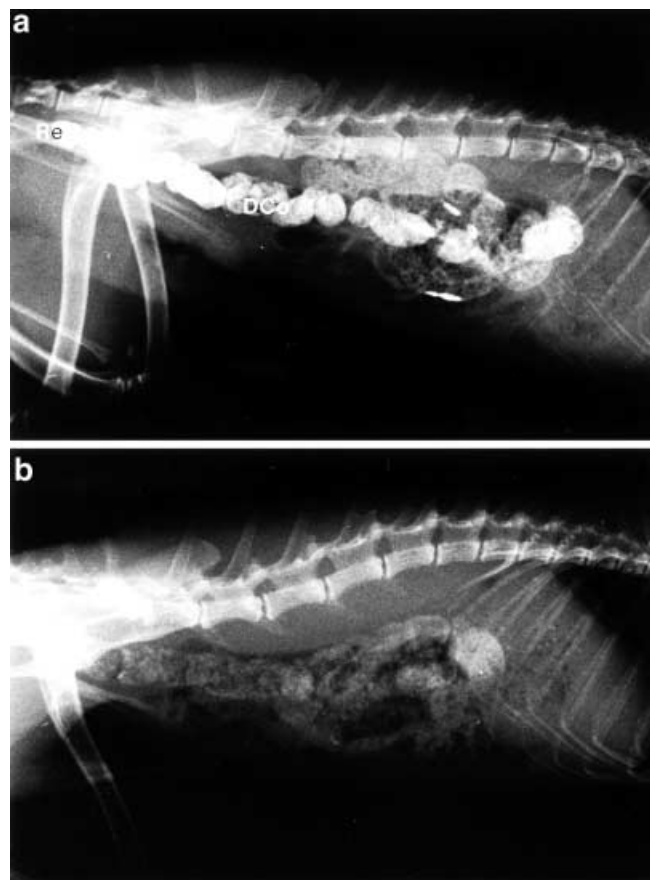
ability of gross energy did not differ between the two diets, but the intake of metabolisable energy (digestible energy intake minus urinary energy output) on mealworms tended to be higher than on seeds ($P = 0.059$). The proportion of digestible energy that was metabolisable was 77% and 79% for the mealworm and mixed seed diets respectively.

Animals fed seeds had higher intakes of NDF than those fed mealworms ($P < 0.05$), but the apparent digestibility of NDF was lower on the mixed seed diet ($P < 0.001$).

Although faecal and urinary nitrogen losses were higher on mealworms ($P < 0.01$), the higher intake of nitrogen resulted in a stronger positive nitrogen balance on the mealworm diet ($P < 0.01$).

Feed equivalent fill

The mean FEF, or gut fill, of 36.5 ± 9.3 g DM · kg^{-1} and 50.4 ± 5.8 g DM · kg^{-1} for animals on the mealworm diet and mixed seed diet, respectively, did not differ significantly, despite a 27% difference between the two diets.

Discussion

The relatively uncomplicated morphology of the bilby gastrointestinal tract is consistent with an omnivorous trophic strategy (Hume 1999). The simple stomach but relatively large caecum-proximal colon are indicative of a hindgut fermenter. The stomach, small intestine and distal colon of the bilby are similar in appearance to those of the northern brown bandicoot, *Isoodon macrourus* (Tedman 1990; McClelland et al. 1999) and the long-nosed bandicoot, *Perameles nasuta* (Moyle 1992). However, the form of the caecum clearly differs from the other two species, being short and bulbous compared with the tubular and elongated caecum of *P. nasuta* and *I. macrourus* (Hume 1999; Moyle 1992; McClelland et al. 1999). Its capacity as a proportion of the total tract is also smaller than in the other two peramelid marsupials. The capacity of the proximal colon was greater in the sub-adult bilbies than in the other two species, and the distal colon, the main region of net water absorption (Stevens and Hume 1995), was longer. Although based on only two animals, these differences in hindgut morphology and capacity between our bilbies and *P. nasuta* and *I. macrourus* might suggest a different digestive

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Table 4 Body mass, and intakes and digestibilities (mean ± S.E.) of dry matter, organic matter, fibre and crude lipid in bilbies (*NDF* Neutral detergent fibre, *n* sample size, *n.s.* not significant)

	Diet		*P*
	Mealworm (*n* = 7)	Seed (*n* = 5)	
Body mass			
Mean (g)	934 ± 83	1062 ± 121	n.s.
Change (g · day^{-1})	+10 ± 2	+1 ± 1	**
Dry matter			
Intake (g · day^{-1})	28.5 ± 2.2	39.1 ± 1.5	*
Intake (g · kg$^{-0.75}$ · day^{-1})	34.9 ± 2.5	39.4 ± 3.5	n.s.
Apparent digestibility (%)	93.2 ± 0.3	84.4 ± 0.9	***
Intake digestible DM (g · day^{-1})	26.6 ± 2.0	33.0 ± 1.5	n.s.
Intake digestible DM (g · kg$^{-0.75}$ · day^{-1})	32.4 ± 2.2	33.4 ± 3.3	n.s.
Organic matter			
Intake (g · day^{-1})	26.4 ± 2.1	38.9 ± 1.8	*
Intake (g · kg$^{-0.75}$ · day^{-1})	32.2 ± 2.3	39.3 ± 3.9	n.s.
Apparent digestibility (%)	93.4 ± 0.3	87.0 ± 0.9	***
Fibre			
NDF[a] intake (g · day^{-1})	3.2 ± 0.3	6.2 ± 0.3	***
NDF intake (g · kg$^{-0.75}$ · day^{-1})	3.9 ± 0.3	6.3 ± 0.8	*
NDF digestibility (%)	79.2 ± 0.9	43.3 ± 4.8	***
Crude lipid			
Intake (g · day^{-1})	11.1 ± 0.8	2.3 ± 0.1	***
Intake (g · kg$^{-0.75}$ · day^{-1})	13.6 ± 0.9	2.4 ± 0.3	**
Apparent digestibility (%)	98.0 ± 0.1	87.7 ± 1.1	**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 5 Intakes, digestibilities and balance (mean ± S.E.) of energy and nitrogen in bilbies [*GE* gross energy, *bDE* digestible energy, *cME* metabolisable energy (= DE – urinary energy), *n* sample size, *n.s.* not significant]

	Diet		*P*
	Mealworm (*n* = 7)	Seed (*n* = 5)	
Energy			
Intake GE[a] (kJ · day^{-1})	812.7 ± 62.4	744.7 ± 24.6	n.s.
Intake GE (kJ · kg$^{-0.75}$ · day^{-1})	994.3 ± 70.6	751.4 ± 67.8	*
Apparent digestibility of GE (%)	94.5 ± 0.3	83.4 ± 1.0	***
Intake DE[b] (kJ · day^{-1})	767.1 ± 57.3	621.7 ± 25.3	*
Intake DE (kJ · kg$^{-0.75}$ · day^{-1})	938.8 ± 65.3	628.8 ± 62.2	**
Metabolisability of GE (%)	72.5 ± 3.1	66.3 ± 5.5	n.s.
Intake ME[c] (kJ · day^{-1})	592.0 ± 55.5	494.3 ± 46.4	n.s.
Intake ME (kJ · kg$^{-0.75}$ · day^{-1})	725.6 ± 70.0	502.5 ± 75.1	n.s.
ME:DE (%)	76.7 ± 3.2	79.3 ± 6.2	n.s.
Nitrogen			
Intake (g · day^{-1})	2.3 ± 0.2	0.8 ± 0.0	***
Intake (g · kg$^{-0.75}$ · day^{-1})	2.8 ± 0.2	0.8 ± 0.1	**
Faecal loss (g · kg$^{-0.75}$ · day^{-1})	0.2 ± 0.0	0.1 ± 0.0	**
Apparent digestibility (%)	93.0 ± 0.3	86.1 ± 0.6	***
Urinary loss (g · kg$^{-0.75}$ · day^{-1})	1.6 ± 0.1	0.4 ± 0.0	***
Balance (g · kg$^{-0.75}$ · day^{-1})	+1.0 ± 0.1	+0.3 ± 0.1	**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

strategy in the bilby. A long distal colon is characteristic of arid-zone herbivores (Hume 1999) and, presumably, omnivores such as the bilby, as well. Diet had no statistically significant effect on the transit times or MRTs of either marker. However, the MRT of the particle marker (Cr-CWC) was longer than the solute marker (Co-EDTA) on both diets. Thus, in contrast to long-nosed and northern brown bandicoots (Moyle et al. 1995; McClelland et al. 1999), the bilbies did not selectively retain solutes but, instead, retained relatively large particles longer than solutes. The absence of selective retention of solutes and fine particles in the bilbies was supported by the lack of any difference in particle size

distribution along the hindgut (Fig. 2), whereas in northern brown bandicoots the proportion of fine (very small) particles was higher in the caecum than the distal colon (McClelland et al. 1999). Selective retention of solutes and fine particles in the caecum is advantageous to small animals consuming high-fibre diets. This is because larger particles are eliminated relatively rapidly, thereby allowing higher rates of food intake and, at the same time, a more concentrated digestive effort on solutes and fine, potentially more fermentable particles (Björnhag 1987). That the bilbies did not utilize this strategy suggests that they need to consume relatively low-fibre diets. The largest plant component of the

Table 6 Mean retention times (MRT) of solute and particle markers in captive bandicoots fed plant and insect diets

Species	Diet	MRT (h)		Reference
		Solutes	Particles	
Bilby	Mealworm	17.9	23.5	This study
Bilby	Seed	30.2	33.0	This study
Northern brown bandicoot	Mealworm[a]	30.4	24.7	McClelland et al. (1999)
Northern brown bandicoot	Lucerne[a]	27.4	10.0	McClelland et al. (1999)
Long-nosed bandicoot	Mealworm	23.6	11.2	Moyle et al. (1995)
Long-nosed bandicoot	Sweet potato	33.1	27.0	Moyle et al. (1995)

[a] Combined with wombaroo (Glen Osmond, South Australia); small carnivore mix

bilby's diet is seeds (Gibson 1999). Seeds generally have a much lower fibre content than the vegetative parts of plants, and thus are usually more easily digested (Hammond 1993). An absence of selective solute retention was also observed in three species of sciurid rodents (Hume et al. 1993) in contrast to voles. Sciurid rodents select greater proportions of more highly digestible seeds, fruits and influorescences and less grass than do voles.

Although there was no significant difference between diets, there was a tendency for longer retention of both the solute (41% longer) and particle (29% longer) marker on the mixed seed as compared to the mealworm diet. This result is consistent with the higher indigestible residue content (%NDF content × %NDF indigestibility) of the mixed seed diet (8.8% vs 2.4%). Intake of a less digestible diet can lead to increased gut fill and increased MRTs, which was likely the case in the present study. Gut fill was 27% higher in bilbies on the mixed seed diet than those on the mealworm diet, although this difference was not significant.

Similar studies on two captive bandicoots, *P. nasuta* (Moyle et al. 1995) and *I. macrourus* (McClelland et al. 1999), showed that the pattern of digesta passage in these two species differed from that in the bilbies. Both bandicoots exhibited selective retention of solutes and fine particles in the hindgut (Table 6). This pattern was consistent across both mealworm and sweet potato diets in *P. nasuta* but was only observed in *I. macrourus* fed lucerne (as opposed to mealworms) (Table 6). It is difficult to directly compare the results obtained from the three species when different sources of plant fibre were used. Even so, selective retention of the particle marker rather than the solute marker in the bilbies supports the notion of a digestive strategy that differs from that of the other two peramelid species.

MRT reflects the net effect of differential passage and retention in all segments of the gut (Moyle et al. 1995), but segments where digesta are retained cannot be determined using the procedures employed in this study. Radiographic analysis of the flow of barium sulphate as a contrast medium and given as a pulse dose was therefore used in an attempt to identify major sites of digesta retention in the digestive system of bilbies. Although similar MRTs of digesta were recorded for animals on both diets, retention of contrast medium within some regions of the digestive tract differed between diets.

Passage of contrast medium through the stomach was much faster than through the hindgut, irrespective of diet, but the difference in passage rates between these two regions was greater in mealworm-fed than in seed-fed animals. Rapid passage through the stomach was also recorded in the bandicoots *P. nasuta* (Moyle 1992) and *I. macrourus* (McClelland et al. 1999). Passage of contrast medium through the small intestine was also rapid compared to the hindgut. Relatively slow passage of contrast medium through the caecum and proximal colon was observed in animals on both diets, but passage through the distal colon tended to be faster in seed-fed than in mealworm-fed animals. The presence of contrast medium in the rectum of one mealworm-fed animal for 45 h also indicated digesta retention within this region of the hindgut.

Therefore, the major sites of digesta retention were the caecum, proximal colon and distal colon, irrespective of diet. Digesta retention in the hindgut increases digestive efficiency by allowing increased time for microbial fermentation. The main differences between animals on the two diets were longer retention of contrast medium in the stomach of seed-fed animals but longer retention in the distal colon of mealworm-fed animals. The former observation is probably explained by the higher fibre content of seeds and hence a higher proportion of large particles compared to the mealworm diet. Universally, particles empty from the vertebrate stomach more slowly than fluid (Stevens and Hume 1995). Shorter retention of digesta in the distal colon of animals on the mixed seed diet may also be due to the higher fibre content of seeds and thus more indigestible residues on this diet. Increased digesta bulk has been shown to increase colonic motility in a wide range of mammalian species (Stevens and Hume 1995). Increased motility of the distal colon on a higher-fibre diet would tend to minimize gut fill.

Moyle et al. (1995) also radiographically recorded longer retention of contrast medium in the distal colon and rectum of long-nosed bandicoots fed mealworms than in animals fed a plant (sweet potato) diet but, in their study, animals on the plant diet retained contrast medium longer in the caecum and proximal colon, consistent with the selective retention of solutes in these animals. The major sites of retention of contrast medium in the northern brown bandicoot (*I. macrourus*) were radiographically determined to be the caecum, proximal

Table 7 Maintenance energy requirements (MER) of captive marsupials; digestible energy basis

Species	Body mass (kg)	MER ($kJ \cdot kg^{-0.75} \cdot d^{-1}$)	Reference
Carnivore			
Sarcophilus harrisii (Tasmanian devil)	3.8	545	Green and Eberhard (1979)
Dasyurus viverrinus (Eastern quoll)	1.3	545	Green and Eberhard (1979)
Omnivore			
Macrotis lagotis (Bilby)	1.1	629	This study
Perameles nasuta (long-nosed bandicoot)	0.8	511	Moyle et al. (1995)
Petaurus breviceps (Sugar glider)	0.2	686	Smith and Green (1987)
Herbivore			
Lagorchestes hirsutus (Rufous hare-wallaby)	1.2	326	Bridie et al. (1994)
Macropus rufus (Red kangaroo)	30.0	456	Hume (1974)
M. robustus erubescens (Euro)	27.0	414	Hume (1974)
Vombatus ursinus (Common wombat)	27.9	140	Barboza et al. (1993)
Lasiorhinus latifrons (Hairy-nosed wombat)	23.1	140	Barboza et al. (1993)

colon, distal colon and rectum on diets of lucerne and mealworms (McClelland et al. 1999). It may be concluded that, in all three species of bandicoots, there is extensive digesta retention in the hindgut, regardless of diet, but patterns of retention may differ among the species, reflecting different patterns of selective digesta retention, possibly related to the smaller caecum but larger proximal colon of bilbies relative to the bandicoots *I. macrourus* and *P. nasuta*.

The superior quality of the mealworms suggested by the chemical compositions of the mixed seed and mealworm diets was confirmed by the digestive performance of captive bilbies on the two diets. Although dry matter and organic matter intakes adjusted for metabolic body mass did not differ between diets, the higher lipid content of the mealworms meant that intakes of gross energy were higher on the mealworm diet. Differences between the two diets were even greater on a digestible intake basis, as the apparent digestibilities of dry matter, organic matter, lipid and energy were all higher on the mealworm diet.

Because the fibre (NDF) content of the mixed seeds was 28% higher than that of the mealworms, and given the similar dry matter intakes, the higher intake of fibre on the seed compared to the mealworm diet was expected. It is difficult to compare NDF digestibilities in this study because of the chemical differences between the NDF of the mealworms (mainly chitin) and the mixed seeds (mainly cellulose, hemicellulose and lignin). Although chitinase and chitobiase activities in bilbies have not been measured, it is possible that the ability of bilbies to digest chitin outweighs their ability to digest plant fibre. Similarly, it is difficult to compare the ability of the bilby to digest plant fibre with that of the bandicoots *I. macrourus* and *P. nasuta* because of the different sources of plant fibre used (grass seeds, lucerne and sweet potato); the higher NDF digestibility in *P. nasuta* (65%; Moyle et al. 1995) than in *I. macrourus* (40%; McClelland et al. 1999) and the bilbies (43%) may simply reflect the less lignified nature of sweet potato fibre.

That animals on the mealworm diet gained body mass suggests that the amount of digestible energy ingested more than satisfied their daily energy needs for maintenance. In contrast, the mean body mass of the seed-fed animals essentially remained constant throughout the digestion experiments, and if we assume that body composition did not change, digestible energy intake can be used as an estimate of their captive daily maintenance requirement. Table 7 compares the maintenance energy requirement (MER) of the bilby with other captive marsupials. The MER of the bilby falls within the range of carnivorous and omnivorous species, but is higher than all the herbivores listed.

That the bilbies maintained positive nitrogen balance, regardless of diet, indicates that they were also able to satisfy their nitrogen requirements from both diets. The higher faecal and urinary losses of nitrogen by mealworm-fed animals than seed-fed animals reflects the approximately three-times greater nitrogen intake of the mealworm-fed animals and the excretion of excess absorbed nitrogen not required for maintenance; thus daily urinary nitrogen outputs were four-fold greater on the mealworm diet.

We did not measure any parameters of microbial activity, but the retention of digesta in the caecum and colon suggests that the bilby is a hindgut fermenter. However, unlike many other small hindgut fermenters, the bilby's small and uncomplicated caecum may be less important than the colon as a site of digesta retention and microbial fermentation. The level of fibre in the diet (i.e. seed vs mealworm) had no significant effect on the MRTs of the two digesta markers, but there was a tendency for a longer retention of both the solute and particle marker on the mixed seed diet, which was probably the result of an increase in gut-fill on this diet. Bilbies did not selectively retain the solute marker, but retained the particle marker longer instead. Therefore, free-living bilbies are likely to be restricted to relatively low-fibre diets. This is consistent with the observation of Gibson (1999) that the bilby's diet is composed largely of invertebrates and seeds. Digestive performance was

superior on the mealworm diet, but intake of either mealworms or seeds was high enough for maintenance of body mass and positive nitrogen balance. Therefore, although restricted to relatively low-fibre diets, bilbies are able to utilize diets of different nutritional value from animal and plant origins, indicating a flexible digestive strategy. Such a strategy must benefit an animal inhabiting environments in which food resources are unpredictable in their relative abundance.

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