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Adam J. Munn
University of Sydney, amunn@uow.edu.au

Peter Banks
University of New South Wales

Ian D. Hume
University of Sydney

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Digestive plasticity of the small intestine and the fermentative hindgut in a marsupial herbivore, the Tammar Wallaby (*Macropus eugenii*)

Adam J. Munn[1]*, Peter Banks[2] and Ian D. Hume[1]

[1]Institute of Wildlife Research, School of Biological Sciences, A08, University of Sydney NSW 2006, Australia

[2]School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia

*To whom correspondence should be directed

Abstract

We investigated the effects of a ground, pelleted diet versus natural forage on the gross morphology of the gastrointestinal tract of a medium sized (5 - 7 kg body mass) macropodid marsupial, the tammar wallaby (*Macropus eugenii*). The empty wet mass (g) of the small intestine of tammar wallabies maintained on a pelleted diet for six weeks was 22% heavier than that of animals maintained on natural forage, once body mass was taken into account by ANCOVA. Similarly, the body-mass adjusted length of the tammar wallaby caecum and proximal colon combined was 25% longer in animals maintained on a pelleted diet compared with those maintained on forage. Our data suggest that food particle size may be directly involved in controlling the size of the post-gastric alimentary tract in tammar wallabies, and thus in their diet choice and nutritional ecology. We conclude that the tammar wallaby is an excellent model for exploring the causes and consequences of digestive plasticity in macropodid marsupials.

Introduction

Digestive plasticity in response to changes in energy demand, food intake and/or food quality (digestibility) has been reported for numerous mammalian herbivores (Foley and Cork 1992). Perhaps the best known examples are from microtine rodents, particularly the voles (*Microtus* spp; Gross et al. 1985; Hammond and Wunder 1991). *Microtus* are small (< 50 g) strictly herbivorous rodents, many of which inhabit the arctic/tundra regions of the northern hemisphere where they face considerable variation in seasonable energy demands and diet quality. The ability to alter gut capacity in response to cold acclimation and/ or increased forage fibre content has significant advantages for maintaining digestible energy intakes in the face of environmental challenges (Hammond and Wunder 1991). A number of larger herbivores (> 5 kg) are also known to increase the length and volume of digestive compartments in response to increasing dietary fibre, including hindgut (e.g. rabbits) and foregut (e.g. ruminants) fermenting species. In wild rabbits (*Oryctolagus cuniculus*) seasonal increases in forage fibre content have been correlated with larger small intestinal volumes and longer small and large intestines (Sibly et al. 1990). In a wild ruminant, the white-tailed deer (*Odocoileus virginianus*), the caecum and colon were 32% and 25% longer, respectively, in winter than in autumn, but this was not correlated with diet quality (Weckerly 1989). Increases in the capacity of post-ruminal gut compartments have also been reported in relation to elevated energy demands for lactation in domestic cattle (Smith and Baldwin 1974). It is not known to what extent marsupial herbivores exhibit digestive plasticity, particularly in the kangaroos and wallabies (Family Macropodidae), but many species face considerable regional, seasonal and stochastic variations in diet quality (Lentle et al. 1998, 2004a,b). We therefore investigated the potential for digestive plasticity in a medium sized macropodid (5 – 10 kg), the tammar wallaby (*Macropus eugenii*).

Historically, tammar wallabies were abundant across much of south and south western Australia, but they are now restricted to a few offshore islands (Kangaroo Island, the Abrolhos Islands and Garden Island) and as an introduced herbivore in New Zealand's Rotorua district and Kawau Island (Hume 1999; Lentle et al. 1998). Like all macropodids, the bulk of fermentative digestion in tammar wallabies occurs in a large, colon-like forestomach, but surprisingly little is known about their natural diet (Hume 1999). They are considered grazers based on their dentition, but they do persist on browse in some areas and possess several features common to browsing hindgut fermenters (Lentle et al. 1998, 1999, 2004a,b; Hume 1999). Studies have shown that the hindgut (i.e. the caecum and/ or proximal colon) of tammar wallabies plays an important role in their overall digestive strategy (Lentle et al. 1998, 1999, 2004a), and high levels of short- chain fatty acids from microbial fermentation are found in these gut regions (Dellow and Hume 1982). Lentle et al. (1999) also found that ingesta particle size in wild tammar wallabies was more like that of the hindgut fermenting common brushtail possum (*Trichosurus vulpecula*) rather than the larger grazing euros (*M. robustus*). Ingesta particle size is a principal feature affecting food retention time, microbial fermentation and digestibility (Hume 1999). For example, in a study on small ponies (a colon fermenter), Drogoul et al. (2000) found that those fed ground, pelleted hay retained fluid and particle markers for longer than those fed chopped hay. The pelleted hay had a smaller mean particle size than the chopped hay and the longer retention time was attributed to finer particles being retained in the colon, but Drogoul et al. (2000) did not investigate possible changes in colon size. Thus, we have conducted an experiment to determine whether a ground, pelleted diet could affect the morphology of the gastrointestinal tract of tammar wallabies. If so, the tammar wallaby could prove an excellent model for investigating the mechanistic processes by which changes in gut size and function are controlled, processes which are still largely unknown.

Material and methods

<u>Study site, animals and procedures</u>

This study was carried out at the University of New South Wales Cowan Field Station (151° 10'E, 33° 35'S) 50 km north of Sydney, NSW, in autumn (April-May) 2004. Ten tammar wallabies were sourced from excess captive stock at Cowan Field Station and Macquarie University Fauna Park, Sydney. At least two weeks prior to the commencement of the study, all female tammar wallabies were caught and any pouch young were removed. At the beginning of the study, all animals were weighed ($\pm$ 0.05 kg) and separated into two treatment groups. The first treatment group was maintained on natural forage diet and the second received a ground, pelleted diet of Kangaroo Cubes (hammer milled and steamed at ca. 100ºC; Doust and Rabbidge Pty Ltd, Sydney).

Animals offered the natural forage diet (2 males, 3 females) were maintained in a large open yard (ca. 100 m^2) that had been ungrazed for more than a year prior to the commencement of the study. The yard contained an open-sided shed for shelter and sufficient vegetation to support the animals throughout. The vegetation consisted mainly of native grasses (*Microlaena stipoides*, *Echinopogon caespitosus* and *Imperata cylindrica*), one introduced grass (*Serata gracilis*), four large *Angophora costata* (Sydney red gum trees) and patches of bracken fern (*Pteridium esculentum*). Water was available ad libitum. The animals were acclimated to the natural forage and yard for 14 days prior to experimentation.

Animals maintained on the ground, pelleted diet (1 male, 4 females) were housed in two small pens (5.5 m long x 5 wide x 3 m high; housing n = 3 animals in one cage and n = 2 in another) to prevent their access to natural forage. The pens were cleaned daily and each pen contained permanent shade shelter. Water and Kangaroo Cubes (pelleted diet) were available ad libitum. Animals were acclimated to the pelleted diet for three weeks,

during which fresh, chopped lucerne hay was also available, but the quantity was slowly

reduced and completely eliminated by 10 days before the beginning of the pellet-only trial.

During acclimation and throughout the trial animals were offered pellets and water ad

libitum.

Gut morphology

Animals were maintained on each diet treatment (natural forage or pellets) for at least 40

days (range: 40 - 46 days), after which they were euthanised by lethal intravenous injection

of sodium pentobarbitone (160 mg/kg) into the lateral tail vein or the hind-limb saphenous

vein. Immediately after death (cessation of heart beat), animals were dissected via a ventral

incision and the entire gasterointentinal tract (GIT) was removed and ligated at the junction

of each major section (see below) to prevent any mixing of compartmental digesta during

handling. The foregut (F) was tied at the oesophageal junction (the oesophagus was

excluded from analysis) and at the pyloric sphincter. The midgut, or small intestine (SI),

was tied at the illeocaecal junction before being dissected from the surrounding mesentery.

In the hindgut, the caecum plus proximal colon (C+PC) were considered as one

compartment because both sections are involved in fermentative digestion (Hume 1999).

The distal colon (DC) was distinguished from the proximal colon at the point of faecal

pellet formation (Dellow and Hume 1982) and was dissected distally at the rectum (i.e. the

distal colon was ligated and separated from the carcass at the rectum, immediately

proximal to the anal orifice). The entire GIT was further dissected to remove mesentery,

other connective tissue and fat, and was blotted dry with tissue paper before being weighed

($\pm$ 0.1 g). Each compartment was then separated, weighed ($\pm$ 0.1 g) and its length measured

($\pm$ 0.1 cm). Foregut length was measured from the oesophageal junction along the greater

curvature of the stomach to the pyloric sphincter. Each gut compartment was then emptied

of digesta and re-weighed to determine digesta load (g wet mass) and organ empty wet

mass (g).

Statistical analysis

Small sample sizes because of quarantine restrictions prevented the use of an orthogonal

design with regard to sex and body mass. Thus we compared the length (cm), the mass of

gut contents (digesta wet-mass; g) from each compartment and organ empty wet-mass (g)

between diet treatments using ANCOVA, with initial body mass as the covariate (Zar

1999). Assumptions for ANCOVA were tested using the Kolmogorov-Smirnov test for

normality ($\alpha = 0.05$) and Levene's test for homogeneity of variances ($\alpha = 0.05$).

Results

The initial and final mean body mass (kg) of tammar wallabies on each diet (natural forage

vs pellets) were significantly different (Table 1). Animals in each treatment lost body mass

throughout (7.3 – 8.6% of initial mass), but there was no significant difference in the

proportion of body mass loss between treatments. Body mass loss was significantly related

to initial body mass on each diet (Table 1). Initial body mass (kg) was also a significant

covariate for the empty wet mass (g) of the entire GIT, F, SI and DC, but not for the C+PC.

However, when the C+CP and DC were examined as a single component, initial body mass

was a significant covariate for the entire hindgut wet mass (Table 1). Initial body mass (kg)

was a significant covariate for the length (cm) of the GIT, F, SI and the C+PC, but not the

DC (Table 1). Initial body mass was a significant covariate for the length of the entire

hindgut (C+CP and DC) Table 1.

There were no significant differences between the treatments with respect to digesta

wet mass (g) from the entire GIT, F, SI, C+PC or DC, or the from entire hindgut when

considered as one compartment (Table 1). There was no significant co-variation of digesta wet mass with initial body mass for any gut compartment (Table 1).

When differences in initial body mass were taken into account by ANCOVA, diet was found to have a significant effect on the gross morphology of the SI and the C+PC. The least squares adjusted means for empty wet mass (g) of the SI were 22% greater for wallabies offered the pelleted diet than those offered natural forage, though there was no difference in component length (Table 1). The least squares adjusted means for the C+PC length (cm) were 25% greater in tammar wallabies offered the pelleted diet than in those offered natural forage, but we found no significant difference in empty wet mass (g) of this compartment (Table 1). When considered as one compartment, the body mass adjusted length (cm) of the entire hindgut (C+CP+DC) was 17% longer in the tammars offered the pelleted diet compared with those offered natural forage, but this difference was not statistically significant, with P = 0.10 (Table 1).

Discussion

The gross anatomical dimensions of the tammar wallaby alimentary tract in our study were comparable to those reported by Dellow and Hume (1982) and Lentle et al. (1998). This is the first study to relate plasticity in the size/length of gut segments in a macropodid marsupial to diet. Osawa and Woodall (1990) found that swamp wallabies (*M. bicolor*) feeding on higher fibre forage had shorter intestinal villi and a larger caecum than those feeding on low-fibre forage. Similarly, Lentle et al. (2004a) found that tammar wallabies from poorer nutritional habitats had a longer small intestine and longer caecum than those from areas with abundant, high-quality forage. However, neither Osawa and Woodall (1990) nor Lentle et al. (2004a) could distinguish probable dietary effects from seasonal or other local phenomena. Seasonal fluctuations in available forage and body mass have been observed in tammar wallabies on Kangaroo Island (Inns 1980 after Hume

1999). The magnitude of the body mass changes seen in Kangaroo Island tammars ranged between 16% and 21%, greater than the 7 - 9% loss (% initial mass) observed here (Table 1). On Kangaroo Island, the cyclical pattern of body-mass loss by tammars wallabies begins around January and continues until June (Hume 1999, see page 294). Our experiment was conducted through April and May (autumn) and the body mass changes observed reflect those expected in the field. Importantly, our animals lost comparable proportions of body mass regardless of diet treatment, indicating that the observed differences in the small intestinal mass (g) and caecal plus proximal colon length (cm) were related to some dietary property, though seasonal factors could certainly influence the magnitude of the plasticity observed. Notably, two females in the natural forage treatment carried very small pouch-young (< 3 g) by the end of the experiment, but these young were each less than 20 days old and would have had a negligible impact on the mother's food requirements (see Green et al. 1988) and were therefore unlikely to have affected digestive tract morphology.

Our results support suggestions that hindgut fermentation may be more important in macropodids than previously thought, and particularly for smaller species (Dellow and Hume 1982; Lentle et al. 1998, 1999, 2004a). Moreover, the plasticity of the tammar wallaby hindgut may be especially important in the face of variations in food quality. What regulates these changes in gut morphology in response to diet is unknown, but we suggest that food particle size may be involved. Our data provide some support for Lentle et al.'s (2004a) predictions that a higher intestinal load of smaller particles would increase digesta viscosity, thereby disrupting optimal flow and ultimately reducing digestibility and/or absorbability. Lentle et al. (2004a) argue that, to compensate for the lower digestibility, animals should increase the length of the small intestine. Our data on SI length were highly variable on natural forage, but overall wet tissue mass was greater in the tammars on the ground, pelleted diet (Table 1). Furthermore, Lentle et al. (1999) and Lentle et al. (2003) have shown that food comminution and ingesta particle size are important factors affecting

the feeding ecology of wild tammar wallabies. We suggest that these factors could also have a significant bearing on the morphological plasticity of the tammar wallaby mid- and hind-gut, but further investigation is needed. We did not analyse the chemical content of the diets used here, but this could also be involved in regulating gut morphology (Foley and Cork 1992). Finally, neither Lentle et al. (2004a) nor Osawa and Woodall (1990) could provide a biological time frame for their observed differences in intestinal morphology between macropodids feeding on high- or low-quality diets, but we have shown that these changes can occur in as little as six weeks, making the tammar wallaby an excellent model for investigating the regulation of digestive tract morphology in marsupial herbivores.

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Table 1: Body mass and gut parameters for Tammar wallabies fed ground, pelleted *vs* natural forage diets. GIT = Gastrointestinal tract; C+CP = Caecum and proximal colon; DC = Distal colon). All data are mean ± 1 SD. [†] Values in parenthesis are least squares means adjusted for body mass.

	Pellets (n=5)	Forage (n=5)	P-values Diet	Body Mass Covariate
Body mass				
Initial (kg)	6.4 ± 0.9	4.9 ± 1.1	**0.03**	-
Final (kg)	5.9 ± 0.7	4.4 ± 0.8	**0.01**	-
Change (g)	470 ± 284	420 ± 309	0.21	**0.03**
GIT				
Mass (g, empty)	237.1 ± 34.1	188.8 ± 24.6	0.66	**0.001**
Mass contents (g)	544.0 ± 117.9	373.0 ± 92.1	0.35	0.15
Length (cm)	603.8 ± 11.7	533.2 ± 62.2	0.52	**0.02**
Foregut				
Mass (g, empty)	122.0 ± 19.2	108.0 ± 12.1	0.27	**0.002**
Mass contents (g)	392.6 ± 121.1	293.1 ± 96	0.85	0.21
Length (cm)	64.2 ± 8.2	61.8 ± 4.3	0.13	**0.01**
Small Intestine				
Mass (g, empty)	65.9 ± 10.4 (59.8 ± 2.7)[†]	42.9 ± 7.1 (49.0 ± 2.7)[†]	**0.04**	**0.004**
Mass contents (g)	35.6 ± 10.6	27.8 ± 11.6	0.14	0.27
Length (cm)	410.1 ± 9.6	363.9 ± 42.8	0.47	**0.04**
C+CP				
Mass (g, empty)	28.3 ± 4.7	21.9 ± 3.0	0.31	0.20
Mass contents (g)	89.3 ± 16.0	52.0 ± 26.7	0.28	0.21
Length (cm)	51.6 ± 6.6 (48.5 ± 2.5)[†]	35.4 ± 4.6 (38.6 ± 2.5)[†]	**0.04**	**0.04**
DC				
Mass (g, empty)	20.8 ± 3.0	16.0 ± 4.6	0.96	**0.01**
Mass contents (g)	40.0 ± 11.5	25.7 ± 15.8	0.71	0.23
Length (cm)	78.0 ± 5.0	72.1 ± 16.5	0.96	0.39

Hindgut (C+CP+DC)				
Mass (g; empty)	49.2 ± 6.9	37.9 ± 7.1	0.48	**0.03**
Mass contents (g)	129.3 ± 21.9	77.6 ± 42.1	0.37	0.18
Length (cm)	100.8 ± 12.0 (93.7 ± 4.3)[†]	73.3 ± 11.4 (80.4 ± 4.3)[†]	**0.10**	**0.02**