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Hindgut plasticity in wallabies fed hay either unchopped or ground and pelleted: fiber is not the only factor

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Abstract

Phenotypic plasticity of the gastrointestinal tract is crucial for optimal food processing and nutrient balance in many vertebrate species. For mammalian herbivores, gut plasticity is typically correlated with the fiber content of forage; however, we show here that other factors such as ingesta particle size may effect profound phenotypic plasticity of the fermentative hind-gut in a medium-sized (10-kg body mass) marsupial herbivore, the red-necked wallaby (Macropus rufogriseus). When dietary fiber contents were comparable, red-necked wallabies that were fed a finely ground, pelleted hay for 60-72 d had hindguts that were some 28% heavier (empty wet mass) than those fed unchopped hay. The hindguts of pellet-fed wallabies contained more wet ingesta, which was also of a finer particle size, than those fed hay, indicating some separation of large- and small-particle fermentation between the foregut and the hindgut, respectively. Such a digestive strategy would benefit animals by allowing fermentation of a range of ingesta particle sizes that are expected for free-ranging animals faced with a spectrum of diet types and qualities. The heavier hindgut of pellet-fed wallabies was correlated with increased concentrations of short-chain fatty acids (SCFAs) in the fermentative hindgut (cecum and proximal colon) and particularly with increases in the molar proportions of n-butyric acid. The mechanisms facilitating gut plasticity in herbivorous mammals are uncertain, but we suggest that manipulating ingesta particle size rather than dietary fiber could provide a useful tool for evaluating causal explanations. In particular, altering ingesta particle size could help to distinguish possible direct processes (e.g., the favoring of smaller intestinal microbes and production of specific SCFAs) from indirect affects of feed structure (e.g., muscular hypertrophy to compensate for increased intakes and digesta bulk or the fermentation of mucus secreted to promote the flow of viscous, fine-particle material).

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Hindgut Plasticity in Wallabies Fed Hay either Unchopped or Ground and Pelleted: Fiber Is Not the Only Factor

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ABSTRACT

Phenotypic plasticity of the gastrointestinal tract is crucial for optimal food processing and nutrient balance in many vertebrate species. For mammalian herbivores, gut plasticity is typically correlated with the fiber content of forage; however, we show here that other factors such as ingesta particle size may effect profound phenotypic plasticity of the fermentative hindgut in a medium-sized (10-kg body mass) marsupial herbivore, the red-necked wallaby (*Macropus rufogriseus*). When dietary fiber contents were comparable, red-necked wallabies that were fed a finely ground, pelleted hay for 60–72 d had hindguts that were some 28% heavier (empty wet mass) than those fed unchopped hay. The hindguts of pellet-fed wallabies contained more wet ingesta, which was also of a finer particle size, than those fed hay, indicating some separation of large- and small-particle fermentation between the foregut and the hindgut, respectively. Such a digestive strategy would benefit animals by allowing fermentation of a range of ingesta particle sizes that are expected for free-ranging animals faced with a spectrum of diet types and qualities. The heavier hindgut of pellet-fed wallabies was correlated with increased concentrations of short-chain fatty acids (SCFAs) in the fermentative hindgut (cecum and proximal colon) and particularly with increases in the molar proportions of *n*-butyric acid. The mechanisms facilitating gut plasticity in herbivorous mammals are uncertain, but we suggest that manipulating ingesta particle size rather than dietary fiber could provide a useful tool for evaluating causal explanations. In particular, altering ingesta particle size could help to distinguish possible direct processes (e.g., the favoring of smaller intestinal microbes and production of specific SCFAs) from indirect affects of feed structure (e.g., muscular hypertrophy to compensate for increased intakes and digesta bulk or the fermentation of mucus secreted to promote the flow of viscous, fine-particle material).

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Introduction

Phenotypic plasticity (or flexibility; Piersma and Lindström 1997; Starck 2005) of the gastrointestinal tract is of particular interest because of the gut's role in resource acquisition and processing and the constraint trade-offs required to balance gut maintenance with nutrient extraction (Hume 2005). Remarkable plasticity of the gut has been observed in a wide variety of vertebrate species, including carnivores (e.g., Secor and Diamond 1998; Starck and Beese 2001; Starck 2005), omnivores (e.g., Derting 1996; Karasov and McWilliams 2005), and herbivores (e.g., Gross et al. 1985; Hammond and Wunder 1991) and under a range of circumstances, including long-distance migrations in birds (e.g., Piersma et al. 1999; Karasov et al. 2004), diet switching in birds (e.g., Starck 1999*a*; van Gils et al. 2003) and mammals (e.g., Gross et al. 1985; Hammond and Wunder 1991), and after arousal from hibernation (e.g., Hume et al. 2002; see also Carey 2005). In mammals at least, the gut is arguably the most energetically expensive organ system to maintain, contributing disproportionately to basal metabolism and whole-body protein turnover (Stevens and Hume 1995). It is not surprising, then, that some animals are able to up- or downregulate gut size and/or function, especially if they feed on intermittent or unreliable food sources (Starck 1999*b*). However, the mechanisms by which up- or downregulation of the gut occurs in mammals, or in vertebrates in general, are not fully understood (Starck 2005), although they are crucial for understanding the evolution of flexible traits (Piersma and Drent 2003).

Among mammals, gut plasticity has been extensively studied in small herbivorous rodents, which increase gut size in response to low-quality diets or when faced with thermal challenges that drive increased food intakes (e.g., Gross et al. 1985; Hammond and Wunder 1991). For herbivorous mammals, diet quality is typically related to the level of lignified cellulose and hemicellulose in plant cell walls (fiber; Robbins 1993); that is, the higher the fiber content, the lower the quality. Mammalian herbivores cannot autoenzymatically break down plant fiber;

instead, they rely on its fermentation by intestinal microbes to yield short-chain fatty acids (SCFAs) that are absorbed and metabolized by the host (Stevens and Hume 1995). Some SCFAs stimulate gut-cell proliferation (Sakata 1995) and have been correlated with changes in organ mass, particularly of the hindgut (e.g., Hume et al. 2002). Consequently, studies of herbivore gut plasticity usually focus on the fiber content of whole diets, but other factors such as plant-cell contents (e.g., proteins, soluble sugars) and/or feed particle size might be involved. Indeed, the importance of the size and structure of food particles postmastication is commonly overlooked. Mastication is influenced by leaf biomechanical properties—often correlated with plant fiber contents—that in turn influence the size and shape of ingested particles (Perez-Barberia and Gordon 1998; Read and Sanson 2003; Sanson 2006). Ingesta particle size is particularly important for herbivorous mammals because it affects food passage rates and accessibility by microbes, impacting fermentation and subsequent SCFA production (Bjorndal et al. 1990).

We show here that a medium-sized (10–24-kg) marsupial herbivore, the red-necked wallaby (*Macropus rufogriseus*), exhibits phenotypic plasticity of the hindgut when fed one of two diets of similar fiber contents, demonstrating that factors other than diet fiber are involved. In particular, we suggest that digesta particle size is important for wallaby hindgut plasticity, which is correlated with changes in the concentration of cecal and proximal-colonic SCFAs, particularly of *n*-butyrate, and may have profound consequences for animals feeding on mixed diets in a nutritionally heterogeneous environment.

Material and Methods

This study was conducted at the University of New South Wales Cowan Field Station (15°10′E, 33°35′S), Sydney, Australia, in winter (June–August) 2006. Fifteen adult, nonreproductive female red-necked wallabies were reared in captivity on a diet of whole-sward (unchopped) lucerne hay (*Medicago sativa*), ground and pelleted lucerne hay (Young Stock Feeds, Young, New South Wales), and pasture (mainly native grasses). The pelleted diet was lucerne based but also contained some concentrates. The specific ingredients of the pellets were unknown because they were commercial-in-confidence (Young Stock Feeds, Young, New South Wales), but there was little difference in the fiber contents of the unchopped and the pelleted diets (Table 1). Two weeks before experimentation animals were weighed (initial mass) and restricted to a diet of pellets that were free of coccidiostat additives. Thereafter, unchopped lucerne hay was gradually introduced to the diets of eight of the wallabies until pellets were completely eliminated after 10 d. The remaining seven wallabies continued on diets of pellets only. Water and feed were available ad lib. throughout.

After 60–72 d, gastrointestinal tracts were examined for contents and morphology. Wallabies were collected between 0600 and 0700 h, transferred to our laboratory, and euthanized by intracardiac injection of sodium pentobarbitone (160 mg kg^{-1} body mass). Carcasses were weighed, dissected via ventral incision, and the gastrointestinal tract (Fig. 1) was removed and immediately ligated at the junction of each major section, minimizing mixing between compartments. The gastrointestinal tract was then cleaned of mesentery, connective tissue, and fat, weighed, and sectioned into its component parts (Fig. 1). The foregut was separated cranially at the esophageal junction (cardiac sphincter) and caudally from the small intestine at the pyloric sphincter. The small intestine was separated caudally at the ileocecal junction. The cecum was separated from the caudal proximal colon. The proximal colon and the distal colon were examined as one compartment (PC+DC) and dissected distally at the rectum. Organ wet mass and length were recorded from full organs. Foregut length was measured from the esophageal junction, along the greater curvature of the stomach, to the pyloric sphincter. Organs were then opened by making a small incision, and the digesta pH was measured (Activon pH meter, AS-211M; Biolab, Victoria, Australia). Grab samples of ingesta were taken from the sacciform forestomach, adjacent to the cardiac sphincter (Fig. 1), and from the cecum, proximal colon, and distal colon (fecal pellets). Organs were subsequently emptied of contents, rinsed clean (except for the small intestine, which was digitally palpated to remove contents), blotted dry, and reweighed before being dried at 70°C to constant mass.

Table 1: Contents of whole diets offered to red-necked wallabies

Content	Pelleted Hay	Unchopped Hay
DM (% air-dry mass)	90.2 ± .6	90.5 ± .7
Ash content (% DM)	22.6 ± .1	12.0 ± .2
Energy (kJ g OM^{-1})	16.9 ± .03	17.1 ± .1
Protein (% OM)	6.9 ± 1.0	12.9 ± .2
NDF (% OM)	38.7 ± 2.4	34.0 ± 1.4
ADF (% OM)	12.4 ± .3	17.9 ± .3

Note. DM = dry matter; OM = organic matter; NDF = neutral-detergent fiber; ADF = acid-detergent fiber.

At dissection, subsamples of digesta from the tubiform forestomach, cecum, and proximal colon (Fig. 1) were filtered through clean muslin, and at least 3 mL of fluid was collected, acidified 3 : 1 (v : v) with 6% H_3PO_4, and immediately frozen (−8°C) in preparation for analysis of SCFAs. The concentrations (mmol mL^{-1}) of SCFAs (acetic, propionic, *i*-butyric, *n*-butyric, *i*-valeric, and *n*-valeric acids) were determined by gas chromatography (Analytical Services, Land and Food Sciences, University of Queensland, St. Lucia, Queensland, Australia). Insufficient fluid could be expressed from the cecum of some animals ($n = 5$ pellet-fed individuals and $n = 4$ hay-fed individuals). Fluid extrusion was particularly difficult from the cecal digesta of pellet-fed animals; we therefore supplemented our results for cecal SCFAs on this diet with data from three large males that were part of a separate study but that were exposed to the same diet and experimental procedures.

Whole diets were dried at 60°C to constant mass. Subsamples (0.5 g) of dry matter (DM) were ashed in triplicate at 500°C to determine organic matter (OM; DM − ash). Subsamples (1.5 g) of DM were pulverized to a consistent particle size (<25

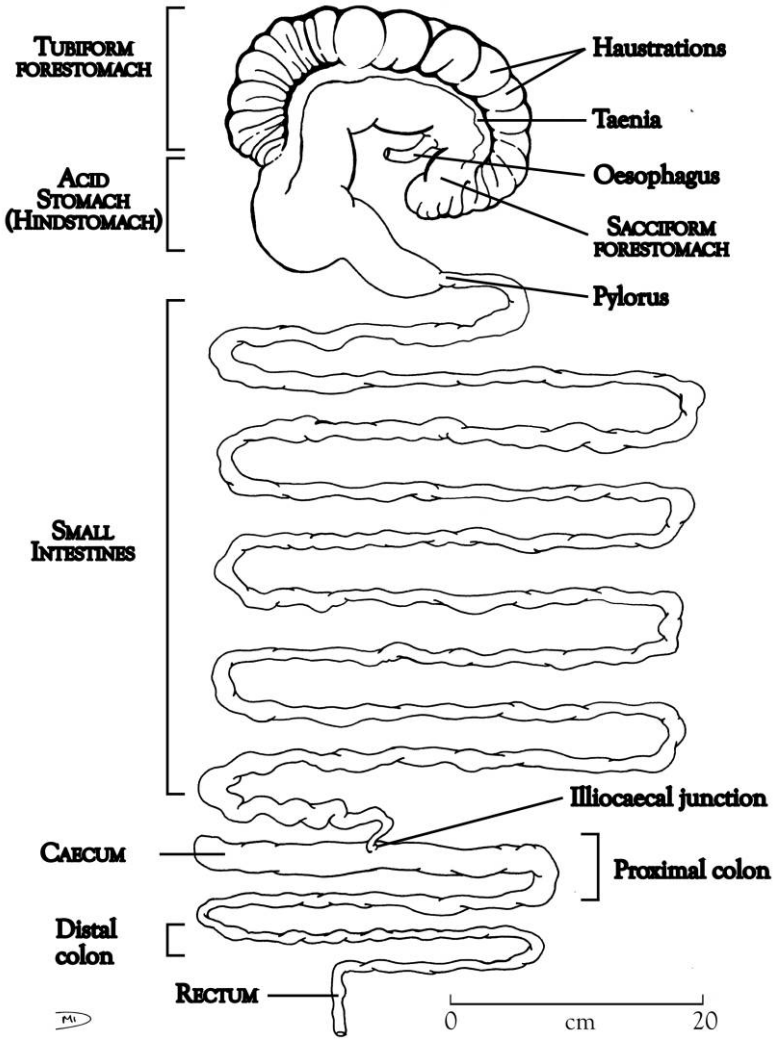

Figure 1. Gastrointestinal tract of the red-necked wallaby (*Macropus rufogriseus*).

μm; Retsch MM301, MEP Instruments), and their energy (using bomb calorimetry), protein (Clissold et al. 2006), and fiber (neutral-detergent fiber [NDF] or acid-detergent fiber [ADF]; Van Soest et al. 1991) contents were measured in triplicate.

Particle size distributions for ingesta from each organ were determined by wet sieving (Barboza 1993) through a series of Endicott screens, trapping particles at apertures of 1,000, 500, 250, 125, 75, and 45 μm. Material passing through the finest screen, the eluate, was determined by difference.

All statistical tests were performed using Minitab for Windows 12.1 (Minitab, State College, PA) and JMP for Windows (JMP 5.1.2, SAS Institute, Cary, NC). Means are reported $\pm$ SE. Initial and final body masses, organ morphologies and contents, and ingesta particle size distributions within each organ were examined using generalized linear models with diet as a factor. Cecum length data were $\log_{10}$ transformed to achieve normal distribution. Wet-content masses (g) for the foregut, cecum, and PC+DC, the molar proportions for some SCFAs, and stomach acid pH values could not be made normally distributed by transformation and as such were examined using nonparametric, two-tailed Wilcoxon/Kruskal-Wallis tests. Particle size distributions (%) and molar SCFA proportions (%) were arcsine transformed for analysis.

Results

Diets

There was little difference in the energy or fiber contents of the whole diets; pellets had slightly more NDF but less ADF than unchopped hay (Table 1). However, the pelleted diet contained more ash and less protein than did the unchopped lucerne hay.

Body Mass and Gastrointestinal Morphology

Wallaby body mass was not different between diet treatments, and all animals maintained body mass throughout (Table 2). We found no evidence for gastrointestinal parasitism or other

Table 2: Body mass and gastrointestinal morphology of wallabies fed pellets or hay

Parameter	Diet Treatment		Diet Effect	
	Pelleted Hay ($n = 7$)	Unchopped Hay ($n = 8$)	F Ratio or Z[a]	P
Body mass:				
Initial (kg)	11.0 ± .7	10.2 ± .7	.73	.409
Final (kg)	10.8 ± .6	10.2 ± .6	.51	.486
Gastrointestinal tract:				
Empty wet mass (g)	378.8 ± 18.3	359.6 ± 17.0	.59	.455
Empty dry mass (g)	64.9 ± 5.0	64.0 ± 2.6	.03	.861
Length (mm)	6,576.4 ± 303.8	6,434.8 ± 129.9	.20	.661
Contents (g)	821.8 ± 91.9	948.3 ± 64.9	1.32	.272
Forestomach:				
Empty wet mass (g)	207.2 ± 10.4	201.1 ± 10.2	.18	.683
Empty dry mass (g)	33.9 ± 3.3	32.4 ± 1.4	.20	.661
Length (mm)	728.4 ± 41.2	812.3 ± 22.3	3.44	.086
Contents (g)	556.4 ± 88.7	779.2 ± 52.6	−2.03[a]	**.037**
Small intestine:				
Empty wet mass (g)	87.8 ± 4.6	92.5 ± 5.3	.45	.517
Empty dry mass (g)	16.3 ± 1.3	16.0 ± 1.1	.04	.849
Length (mm)	4,529 ± 218	4,459 ± 91	.10	.762
Contents (g)	66.0 ± 12.6	68.2 ± 7.9	.02	.884
Cecum:				
Empty wet mass (g)	10.3 ± .6	8.7 ± .7	2.98	.108
Empty dry mass (g)	2.2 ± .34	1.8 ± .16	1.74	.211
Length (mm)	142.9 ± 13.5	112.3 ± 4.2	5.52	**.035**
Contents (g)	28.8 ± 5.5	14.7 ± 1.8	2.14[a]	**.032**
PC+DC:				
Empty wet mass (g)	73.6 ± 5.5	57.4 ± 3.7	6.31	**.026**
Empty dry mass (g)	12.5 ± 1.2	13.8 ± 1.6	.49	.495
Length (mm)	1,177 ± 49	1,051 ± 42	3.82	.073
Contents (g)	160.6 ± 24.2	86.3 ± 10.2	1.91[a]	.056

Note. PC+DC = proximal and distal colon. Bolded values are significant.

[a] Two-sample Wilcoxon/Kruskal-Wallis ranks test.

gross pathologies on carcass dissection. There was no difference in the empty wet mass of the foregut from pellet- or hay-fed wallabies (Table 2), but that of hay-fed animals contained more wet contents ($P = 0.04$). Pellet-fed wallabies had average PC+DC empty wet masses that were some 28% greater than those of hay-fed wallabies ($P = 0.03$; Table 2). Pellet-fed animals tended to contain more wet contents in their PC+DC ($P = 0.06$), which also tended to be longer ($P = 0.07$) than those of hay-fed animals. The cecum of pellet-fed animals was almost 20% longer ($P = 0.04$; Table 2) and contained around twice as much wet content as that of hay-fed animals ($P = 0.03$).

Particle Size Distributions

Hay-fed kangaroos carried significantly more particles that were larger than 1,000 μm in their forestomach, proximal colon, and feces (Fig. 2) compared with those who were fed pellets. Conversely, the forestomach of pellet-fed wallabies carried more fine particles than those animals fed unchopped hay; that is, significantly more particulates from the foregut of pellet-fed wallabies were trapped on the 500-, 250-, 125-, and 75-μm sieves compared with those from wallabies fed unchopped hay. Furthermore, digesta from the cecum, proximal colon, and feces of pellet-fed animals contained a greater proportion of particles that were between 500 and 1,000 μm than those fed unchopped hay. There were no significant differences in the proportion of very fine particles (<75 μm) or eluate in the digesta from any organ or in feces between pellet- and hay-fed wallabies (Fig. 2).

Gastrointestinal pH and SCFAs

There were no significant differences in the pH or total concentration of SCFAs in the tubiform forestomach of pellet- or hay-fed wallabies. However, there were differences in the molar proportions of SCFAs. In particular, forestomach digesta from pellet-fed wallabies contained less acetate but more propionate than that from hay-fed animals (Table 3).

The hindstomach (or gastric pouch; Fig. 1) of pellet-fed kan-

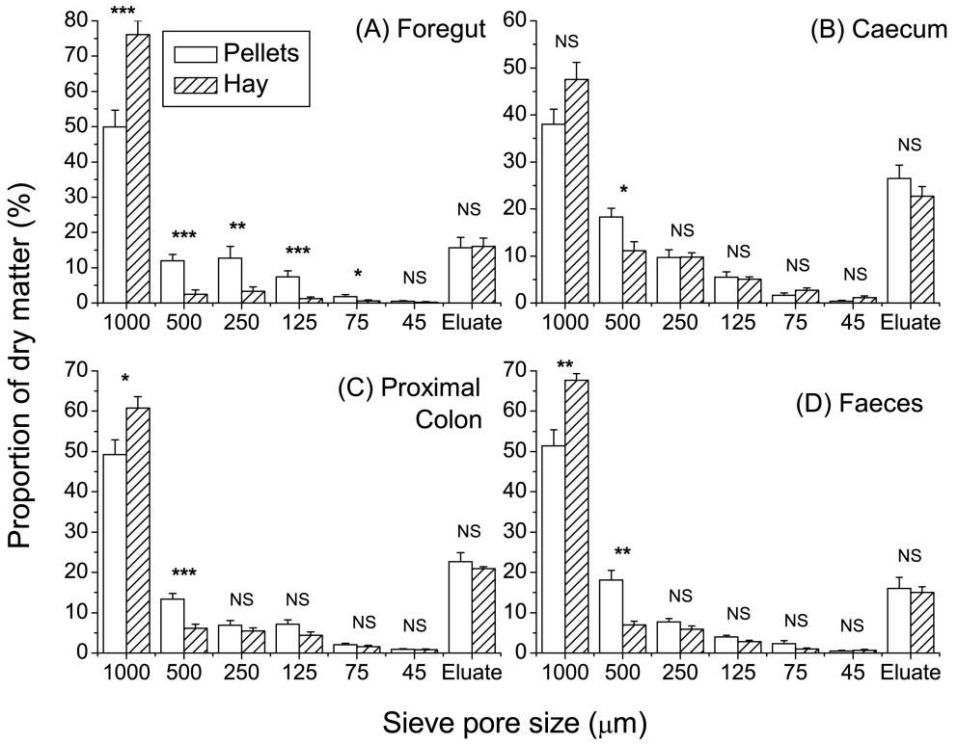

Figure 2. Distribution of ingesta particles (% dry matter) retained on a series of Endicott sieves from the forestomach (*A*), cecum (*B*), proximal colon (*C*), and feces (*D*) of red-necked wallabies fed pelleted or unchopped lucerne hay. Data are means (± SEM); asterisks denote significant differences between diets for each sieve pore size: one asterisk, $P \leq 0.05$; two asterisks, $P \leq 0.01$; three asterisks, $P \leq 0.001$; NS = not significant.

garoos was significantly less acidic, and their cecum was more acidic, than those of kangaroos fed unchopped hay (Table 4). This was correlated with pellet-fed kangaroos having significantly greater concentrations of SCFAs in the cecum (by 1.8 times; Table 3). Similarly, the proximal colon of pellet-fed wallabies tended to be more acidic than that of hay-fed animals ($P = 0.06$), and it also contained significantly higher concentrations of SCFAs (~1.4 times). Overall, as proportions of total SCFAs, digesta from the cecum ($P < 0.03$) and the proximal colon ($P < 0.007$) of pellet-fed animals contained significantly more *n*-butyrate than those of animals fed unchopped hay, but there were no differences in the proportions of acetate or propionate (Table 4). Of the branch-chain SCFA, *i*-butyrate was higher in the proximal colon ($P < 0.001$) of the animals fed the unchopped hay, and *i*-valerate was higher in both the cecum ($P = 0.021$) and the proximal colon ($P = 0.023$). The SCFA *n*-valerate was also higher in the cecum ($P = 0.009$) of the animals fed the unchopped hay.

Discussion

Historically, the kangaroo forestomach has been compared with the rumen reticulum of foregut-fermenting ungulates. However, in form and function, the tubiform forestomach of kangaroos is more like the hindgut of the colon-fermenting horse (Fig. 1; Stevens and Hume 1995; Hume 1999). The kangaroo forestomach is typified by numerous haustrations that likely provide elastic support for physical flexibility in content loads, as was observed here (Table 2), probably facilitating reserve gut capacity under different nutritional circumstances (e.g., Munn and Dawson 2006; Munn et al. 2006). In this study, we found that there was profound phenotypic plasticity of the hindgut, mainly in the proximal and distal colon but probably also in the cecum.

The between-treatment differences in hindgut morphology that we observed were not related to diet fiber contents, as the pelleted and unchopped hay diets had comparable fiber levels (Table 1). The main dietary differences between the unchopped and the pelleted hay were in their contents of ash and protein (Table 1). However, ash content is unlikely to have influenced gut plasticity as it is largely made up of minerals (Stevens and Hume 1995), which are not known to affect gut size. On the other hand, the higher protein content of the unchopped hay might be expected to favor microbial activity, which would presumably increase gut hypertrophy via SCFAs, but we found the opposite to be the case: wallabies fed the lower-protein but fine-particle-size pelleted hay had larger hindguts. Moreover, plant proteins are usually rapidly digested in the wallaby forestomach (Dellow and Hume 1982; Hume 1999) and are unlikely to have any impact on the hindgut directly. Furthermore, in the few cases where protein has been implicated in gut plasticity, the effect has been opposite to that observed here. For example, higher dietary protein concentrations were associated with larger gut sizes in locusts (Raubenheimer and Bassil 2007) and increased ileal mucosal masses in rats (Spector et al. 1977).

Table 3: Total concentrations and molar proportions of SCFAs from wallabies fed pellets or hay

	Diet Treatment		Diet Effect	
Location, Parameter	Pelleted Hay	Unchopped Hay	*F* Ratio or *Z*[a]	*P*
Tubiform foregut	$n = 6$	$n = 8$		
SCFA (mmol L^{-1})	105.3 ± 18.1	129.4 ± 10.9	1.61	.229
Acetate (%)	54.1 ± 1.5	66.2 ± 1.3	43.76	**<.001**
Propionate (%)	28.3 ± 2.1	19.4 ± .5	2.78[a]	**.006**
i-butyrate (%)	.9 ± .3	1.3 ± .2	1.82	.209
n-butyrate (%)	13.6 ± 2.3	8.7 ± .8	1.23[a]	.220
i-valerate (%)	.8 ± .2	1.6 ± .3	5.28	**.040**
n-valerate (%)	2.4 ± .3	2.9 ± .2	1.69	.229
Cecum	$n = 4$[b]	$n = 4$		
SCFA (mmol L^{-1})	142.2 ± 8.3	77.4 ± 5.4	42.58	**.001**
Acetate (%)	67.2 ± 6.9	77.7 ± .2	1.01[a]	.312
Propionate (%)	17.9 ± 4.6	13.5 ± .3	−.433[a]	.665
i-butyrate (%)	1.2 ± .3	1.9 ± .2	3.73	.108
n-butyrate (%)	13.0 ± 2.6	4.0 ± .2	−2.165[a]	**.030**
i-valerate (%)	.3 ± .0	1.5 ± .1	2.165[a]	**.021**
n-valerate (%)	.5 ± .2	1.4 ± .1	16.18	**.009**
Proximal colon	$n = 5$	$n = 8$		
SCFA (mmol L^{-1})	123.3 ± 8.9	86.6 ± 10.0	7.49	**.020**
Acetate (%)	69.8 ± 3.7	75.6 ± 1.2	2.18	.201
Propionate (%)	17.6 ± 3.5	13.7 ± .5	.95[a]	.340
i-butyrate (%)	.2 ± .1	1.5 ± .1	56.78	**<.001**
n-butyrate (%)	10.8 ± 1.3	4.8 ± .3	2.71[a]	**.007**
i-valerate (%)	.8 ± .5	3.1 ± .8	−2.27[a]	**.023**
n-valerate (%)	.7 ± .2	.7 ± .2	4.36	.100

Note. Bolded values are significant.

[a] Two-sample Wilcoxon/Kruskal-Wallis ranks test.

[b] One female, three males.

Similarly, soluble sugars are rapidly fermented in the wallaby foregut (Dellow and Hume 1982) and were unlikely to have affected hindgut plasticity. After these considerations, ingesta/digesta particle size becomes the likely candidate affecting the hindgut plasticity we observed in the pellet-fed wallabies. Notably, Munn et al. (2006) reported similar plasticity in another macropodid, the tammar wallaby (*Macropus eugenii*), when they compared animals fed natural forage versus pelleted hay. These authors suggested a potential for ingesta particle sizes to affect hindgut plasticity, and we have provided further support for that suggestion.

It is difficult to interpret the functional (adaptive) significance of the hindgut plasticity that we observed in pellet-fed wallabies. In other herbivores, low-quality foods apparently induce compensatory increases in gut size (Gross et al. 1985; Hammond and Wunder 1991), helping to maintain digestible intakes. However, lower-quality (high-fiber) diets are normally associated with larger, not smaller, ingesta particle sizes, at least in ruminants (Grenet 1989; van Bruchem et al.1991) and swine (Robertson et al. 1992). This makes it difficult to reconcile the apparent particle size–induced plasticity that we observed with possible adaptive significance for utilizing poor-quality diets. Of note, the protein and energy contents of both the pelleted and the unchopped lucerne hays were in excess of those required for nitrogen and energy balances, respectively, in similarly sized macropodid marsupials (see Hume 1999). Therefore, it is unlikely that the pellet-fed wallabies' larger hindguts were a compensatory response to diet contents per se. However, pelleted diets fed ad lib. are known to have lower apparent digestibilities (dry matter and fiber) than whole swards in some herbivores (e.g., see Drogoul et al. 2000 and references therein), including in kangaroos (Freudenberger and Hume 1992). This is probably because pelleted foods have low energy costs for oral processing and as such afford higher dry matter intakes (DMIs), forcing shorter mean retention times (MRTs) normally associated with lower digestive efficiencies (Robbins 1993). However, we do not believe that the increased hindgut capacity of our pellet-fed wallabies acted to compensate for a faster food passage or a lower DM digestibility, as these were likely balanced by higher DMIs (Freudenberger and Hume 1992; Hume 2005). Instead, the larger hindgut capacity of the pellet-fed wallabies suggests a mechanism that allows the macropodid digestive system to efficiently utilize a range of food particle sizes.

In macropodids, there is a marked separation of small and

Table 4: Gastrointestinal pH of wallabies fed pellets or hay

	Diet Treatment		Diet Effect	
Location	Pelleted Hay (n = 7)	Unchopped Hay (n = 8)	*F* Ratio or *Z*[a]	*P*
Tubiform forestomach	6.02 ± .19	6.25 ± .12	1.014	.337
Hindstomach (gastric pouch)	4.14 ± .49	2.42 ± .15	2.257[a]	**.024**
Small intestine	7.10 ± .16	7.24 ± .15	.4089	.534
Cecum	6.41 ± .12	6.94 ± .13	8.646	**.012**
Proximal colon	6.70 ± .11	7.01 ± .10	4.285	.059
Distal colon (feces)	6.72 ± .19	7.07 ± .10	2.729	.133

Note. Bolded values are significant.
[a] Two-sample Wilcoxon/Kruskal-Wallis ranks test.

large particles in the foregut, with smaller particles and solutes exiting the tubiform forestomach faster than larger particles (Dellow 1982; Munn and Dawson 2006). Thus, as intakes of small particles increase, such as in animals receiving pelleted diets (Freudenberger and Hume 1992) or high-quality forage (inferred from ruminants and swine; Grenet 1989; van Bruchem et al. 1991; Robertson et al. 1992), more bulk flow of potentially digestible material would pass to the lower gut. Our data support this suggestion. Pellet-fed red-necked wallabies had lower levels of fermentation in the foregut, as indicated by lower SCFA concentrations, compared with those fed unchopped hay. Conversely, cecal and proximal colonic fermentation was higher in the pellet-fed wallabies (i.e., higher SCFA concentrations; Table 3). Very few studies consider mixed diets in herbivores or how food particles of different sizes might be processed in the gut. For free-ranging animals, being able to ferment both larger and smaller particles simultaneously could substantially broaden their foraging options. Red-necked wallabies are classified as grazers according to their dentition (Sanson 1989), but they are known to consume up to 30% nongrass items (Sprent and McArthur 2002). Interestingly, the particle size distributions of foregut ingesta from free-ranging red-necked wallabies (Sprent and McArthur 2002) were comparable with those from pellet-fed animals in our study, and each contained more than 45% of particles that were less than 600 μm in size. Therefore, fermentation of differently sized particles in different parts of the gut could simultaneously help to maximize nutrient gain on a mixed diet, that is, via hindgut fermentation of fine particles normally associated with high-quality diets, while lower-quality forages might be fermented mainly in the foregut. However, this hypothesis would need to be tested on animals fed a mixed diet of different fracture properties to yield variations in ingesta particle sizes after oral processing.

In addition to spatial variation in available diets, free-ranging herbivores are expected to experience temporal variation in forage types and qualities, particularly seasonally. As such, wallabies might benefit from hindgut atrophy via reduced energy/nutrient costs during dry seasons, when mainly poor-quality food is available. How rapidly the wallaby hindgut can respond to changes in diet is unknown, but some birds can increase the size of their gizzard over 4–8 d (Starck 1999*a*). If comparable time frames could be achieved for the hindgut plasticity of red-necked wallabies, this would optimize nutrient gains from a nutritionally heterogeneous landscape. However, experiments to determine time frames for gut size changes in wallabies and other herbivorous mammals are critically lacking (Starck 2005).

Plasticity of the red-necked wallaby hindgut was suggestive of changes in cell size (hypertrophy) rather than cell number (hyperplasia) because there were no differences in organ dry masses (Table 2). Similar hypertrophy has been reported in quail gizzards (Starck 1999*a*) and in the ceca of Mongolian gerbils (Lui and Wang 2007) and laboratory rats (Rémésy et al. 1992) in response to high-fiber diets. Nonetheless, it is conceivable that whole-organ hypertrophy could be related to changes in the number of smaller or larger cells, with little change in dry mass; histological studies are needed to clarify the cellular basis for the organ-mass changes we observed. More important, it is uncertain how feed particle size may be involved with the hindgut hypertrophy, but SCFAs are known to affect whole-organ trophic responses of the gut in other species (Goodlad et al. 1989; Sakata 1995; McCullough et al. 1998). For example, SCFAs are associated with hyperplasic stimulation of cell proliferation (Goodlad et al. 1989), but they can also increase total cell counts in the gut by inhibiting apoptosis (Mentschel et al. 2001). However, little is known of the basis for SCFA-mediated changes in cell size. Our data suggest that the greater load of fine-particle ingesta in pellet-fed wallabies had some affect on their hindgut microbiome, indicated by differences in their SCFA concentrations and profiles, compared with those wallabies that were fed hay (Table 3).

The concentrations and molar proportions of SCFAs from the tubiform forestomach and hindgut of red-necked wallabies fed unchopped hay (Table 3) were similar to those previously reported for this species when individuals were fed chopped lucerne hay (Hume 1977). Together, these data highlight the significance of the wallaby hindgut (cecum and proximal colon) as a fermentation site (Dellow and Hume 1982; Lentle et al. 1998, 2004). Notably, the acid hindstomach of pellet-fed wallabies was less acidic than that of hay-fed animals, but this must be viewed with caution. Digesta from the foregut of pellet-fed animals was considerably wetter and more fluid than that from hay-fed animals (A. Munn, personal observation), and there may have been contamination of the acid hindstomach from tubiform digesta during handling (Fig. 1).

The higher proportions of propionate in the forestomach digesta from pellet-fed wallabies (Table 3) are indicative of greater fermentation of soluble carbohydrates (Annison 1954; Hume 1977) compared with those from hay-fed wallabies. On the other hand, hay-fed animals had higher proportions of acetate in their forestomach, which is indicative of the fermentation of more structural carbohydrates (i.e., fiber; Annison 1954; Hume 1977). Unchopped lucerne hay contained more ADF (mainly cellulose and lignin) than did pellets (Table 1), which may have contributed to their higher proportions of forestomach acetate with this diet. More important, there were no significant differences in the molar proportions of either acetate or propionate in the cecal or proximal colonic digesta from pellet- or hay-fed wallabies, indicating that the substrate loads for structural and nonstructural carbohydrates were similar. The major difference in the SCFA profile of the cecum and proximal colon of wallabies fed pellets compared with those fed hay was that they had greater proportions of *n*-butyrate (Table 3). Butyrate is involved in the trophic responses of the gastrointestinal tracts of numerous mammal species, including humans (Sakata 1987, 1995; Hume et al. 2002), and we have now shown that it may also be involved in hindgut plasticity in a marsupial herbivore. How feed particle size is involved with butyrate production is uncertain, but it must involve the intestinal microbiome in some way. Smaller food particles, for example, may favor smaller microbes, allowing them to competitively adhere to substrates and to produce specific SCFAs (Bjorndal et al. 1990). Such a mechanism would represent a direct effect of the physicochemical features of the ingesta, but there may also be indirect factors at play. For example, higher loads of fine particles increase digesta viscosity (Lentle et al. 2004, 2005), which, along with some SCFAs, stimulates mucus production (McCullough et al. 1998; Shimotoyodome et al. 2000; Piel et al. 2005). Mucus aids the flow of digesta through the small intestine and colon, but it would also present the hindgut with a source of readily fermentable sugars (e.g., the glycoprotein coat), the main fermentative by-product of which is *n*-butyrate (Stevens and Hume 1995). Moreover, the greater load of fine particles in the hindgut of pellet-fed wallabies was suggestive of particle retention mechanisms, in which mucus plays a key role. For example, in the colonic separation mechanisms of small hindgut fermenters, mucus assists the retrograde movement of digesta, trapping and moving fine particles and bacteria from the colon to the cecum (Sakaguchi 2003; Takahashi and Sakaguchi 2006). However, our data are correlative, and causal mechanisms linking hypertrophy with digesta particle sizes, SCFAs, and/or viscosity are likely to be complex. Cecal hypertrophy in rats, for example, has been linked to fine-particle bulk rather than SCFAs (Rémésy et al. 1992; Wyatt et al. 1998) and to increased digesta viscosity, even when fermentable contents were similar (Elsenhans and Caspary 2000). These data suggest that physical factors (e.g., viscosity or increased digesta bulk) might simply lead to increased smooth-muscle hypertrophy in the hindgut wall, which is necessary to support and/or promote the flow of viscous, bulky material through the digestive tract.

Conclusions

Gut plasticity in herbivorous mammals has often been associated with dietary fiber content (Gross et al. 1985; Hammond and Wunder 1991), but our data indicate that phenotypic plasticity of the wallaby hindgut can be dissociated from these hard-to-digest fractions. Teasing apart the functional and mechanistic basis for this plasticity is difficult, and our data are relevant for understanding that some animals increase gut capacity when diet quality is unchanged, such as when increased energy demands drive higher food intakes under cold acclimation (e.g., Gross et al. 1985; Hammond and Wunder 1991) or during lactation (e.g., Hammond and Diamond 1992; Derting 1996; Hammond and Kristan 2000). Presumably, increasing gut capacity under these circumstances helps to accommodate increased food intakes while maintaining MRTs to maintain digestive efficiency and digestible intake (Hammond and Wunder 1991; Hume 2005).

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