CAN OMNIVORES BE DIET SPECIALISTS?

ADAPTABILITY OF GUT FORM AND FUNCTION IN AN OMNIVOROUS LIZARD

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by

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ABSTRACT

CAN OMNIVORES BE DIET SPECIALISTS?

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Omnivory can be viewed as a Jack-of-all-trades diet strategy that allows some animals to exploit a variety of food items. However, the extent to which omnivores can adaptively regulate gut form and function in response to different diets has not been widely investigated. When reared on specialist diets, do the digestive systems of omnivores respond like those of specialists, or are they constrained by the Jack-of-all trades/master-of-none paradigm? I raised juvenile bearded dragons (Pogona vitticeps), a naturally omnivorous lizard, for ~135 d on either a diet of ground alfalfa (herbivore), crickets (carnivore), or a 50/50 mix of both (omnivore). I compared aspects of gut form (mass, surface area, histology) and function (pH, passage rate, apparent digestive efficiency, and rates and capacities of nutrient transport) and used rates of growth as a measure of whole-animal digestive performance. After accounting for body size there was no effect of diet on surface-area measurements of gut sections. Herbivores had
relatively lower small intestine mass than both carnivores and omnivores and lower large
intestine mass than carnivores, but not omnivores. Microscopy revealed that carnivores
had longer small intestine villi than either herbivores or omnivores. Lizards raised on the
herbivore diet exhibited higher rates of nutrient transport for proline and glucose than
carnivores, but similar rates as omnivores. Estimates of total nutrient uptake capacity did
not differ among diet treatments for either proline or glucose. The pH of the large
intestines of herbivores was higher than for carnivores, but neither of these treatments
differed from lizards raised on the omnivore diet. Omnivores grew faster than herbivores,
but not carnivores, and carnivores grew slightly faster than herbivores. Differences in
growth rate indicate a selective advantage to maintain an omnivorous diet during growth.
These results suggest that, on a proximate scale, these diet generalists may actually be
“specialist” of omnivory, inviting further consideration and study of other diet
generalists. Furthermore, the diet-specific changes in both gut form and function indicate
that the digestive system of *P. vitticeps* exhibits modest levels of plasticity, an ability that
may be important for ontogenetic shifts in diet as well as ultimate shifts toward herbivory
or carnivory.
Introduction

An animal's diet is a fundamental determinant of its morphology, physiology, and ecology and the digestive tract serves as the interface regulating which constituents of the diet are assimilated as energy and nutrients. For many animals, diet can vary in quantity and quality on a daily basis. Species that experience such variance have evolved the ability to regulate gut form and function to accommodate both predictable and stochastic changes in diet (Levey and Karasov, 1992; Nagy, 1977; Secor and Diamond, 1998; Starck, 1999). This type of phenotypic flexibility (sensu Piersma and Drent, 2003) allows animals to adjust to diet items that are novel or vary (e.g., seasonal diet shifts), thus maintaining energy and nutrient balance.

Although most animals are likely capable of regulating the form and function of their digestive tract, some exhibit substantially more flexibility than others. The most flexible are those animals that experience wide fluctuations in feeding frequency or diet quality (Sabat et al., 2005; Secor et al., 1994; Starck and Beese, 2001; Tracy and Diamond, 2005). For example, sit-and-wait or ambush-foraging snake species (e.g., most boids, pythonids, and viperids) exhibit dramatic reductions in both gut form and function during long periods of fasting. Soon after feeding, these reptiles exhibit factorial increases in gut size, intestinal nutrient absorption capacities, and digestive enzyme activity (Secor, 2005a; Secor and Diamond, 1998; Starck and Beese, 2001). Similarly, animals experiencing changes (daily or seasonal) in diet quality or quantity are capable of adaptively regulating their guts as well (Afik et al., 1995; Buddington, 1987; Buddington et al., 1987). In contrast, these abilities are rather limited in species with specialized diets
and those that feed at frequent intervals (Naya and Bozinovic, 2004; Naya et al., 2005; Sabat and Bozinovic, 1996). These interspecific findings suggest that there are predictable ecophysiological adaptations linking diet to digestion in vertebrates.

Although gut flexibility and digestive performance, induced by changes in food quality, have been widely studied in birds and mammals (e.g., Karasov, 1994; Levey and Karasov, 1989; McWhorter et al., 2006; McWilliams and Karasov, 1998; Piersma and Lindstrom, 1997; Sabat et al., 1998), comparatively little is known of these responses among squamate reptiles (lizards and snakes). Most of the studies involving squamate reptiles have focused on either the effects of diet on whole-animal performance (e.g., digestive efficiency and growth) or morphological and physiological characteristics of the gut (e.g., gross morphology or nutrient transport; Donoghue et al., 1998; van Marken Lichtenbelt, 1992; Waldschmidt et al., 1986). Indeed, few studies have attempted to correlate diet quality with morphological, physiological, and whole-animal performance in the same animals (but see Andres, 2005). Current knowledge of the affects of diet quality on morphology and physiology suggests that changes in one variable (e.g., gut size or nutrient uptake) are not necessarily accompanied by changes in the other. For example, when comparing the digestive capacities of carnivorous and herbivorous lizards (Crotaphytus bicinctores and Sauromalus ater, respectively) fed crickets (Gryllus) or dandelion flowers (Taraxacum), Ruppert (1980) found that, when force-fed insects, the herbivores digested the meal as well as the carnivores (likely a retention of an ancestral ability), whereas the carnivores were unable to digest the plants. These findings were correlated with apparent morphological limitations on digestive tract flexibility (i.e., no diet-induced change in gut size) for the carnivorous lizards, and perhaps physiological
constraints as well (Ruppert, 1980). In contrast, the herbivorous lizard *Dipsosaurus dorsalis* did not exhibit changes in gut morphology when fed insects vs. plants, despite physiological changes in intestinal uptake of an amino acid and a sugar (Andres, 2005; Karasov and Diamond, 1983). Thus, changes occurring at the subcellular level may not elicit concomitant changes in gross morphology. Unfortunately, both of the aforementioned studies were conducted on diet specialists—species considered to have a limited capacity to adaptively regulate their digestive tracts (Naya and Bozinovic, 2004; Sabat and Bozinovic, 1996; Sabat et al., 2005). A better model for understanding how gut regulation may affect fitness variables, such as digestive efficiency and growth rate, are animals with more catholic diets. In contrast to diet specialists, omnivores eat a variety of foods and, therefore, might be expected to exhibit the greatest measurable morphological and physiological responses to changes in diet quality. Examining the digestive and whole-animal costs and benefits of diet generalism may provide multiple benefits including: (1) providing new insights into how diet specialization evolves because specialized diets are generally expected to evolve from lineages with a greater diet breadth (Futuyma, 2001; Futuyma and Moreno, 1988) and (2) testing whether diet generalists conform to the Jack-of-all-trades paradigm.

The goals of my study were to characterize diet-induced responses to growth rates, digestive efficiency, and a suite of morphological and physiological variables associated with digestion in a growing omnivorous lizard. Specifically, I fed groups of juvenile bearded dragons (*Pogona vitticeps*) either (1) crickets (hereafter “carnivores”), (2) alfalfa-based rabbit chow (“herbivores”), or (3) a 50/50 mix of both (“omnivores”).
Based on the findings of similar studies (Buddington, 1987; Buddington et al., 1987; Levey and Karasov, 1989; Naya et al., 2005; Stevens and Hume, 1995; Troyer, 1984), I predicted how the lizards would respond to the diet treatments (Table 1). *Pogona vitticeps* are omnivorous in nature (Badham, 1971). As such, lizards raised on the omnivore diet were expected to exhibit little phenotypic change as they were reared on a diet (albeit somewhat artificial) that was not dramatically different from what they would experience in nature. In contrast, the lizards reared on the carnivore and herbivore diets were expected to respond to their treatments as would a natural carnivore or herbivore specialist. At the whole-organism level (measured as digestive efficiency and growth rate), I reasoned that lizards raised on the carnivore diet should digest meals more efficiently and grow fastest, followed, in succession, by lizards on the omnivore and herbivore treatments. Carnivores were expected to have the highest values because animal tissue is generally more readily digestible and has more protein than plant tissue or a mix of both (Table 2), which contain (in whole or in part) structural carbohydrates that require fermentation for digestion (Zimmerman and Tracy, 1989). Following the feeding and growth trials, a subset of lizards from each diet treatment was subjected to a series of morphological and physiological measures in an attempt to determine the mechanism(s) underlying the expected differences in digestive efficiency and growth. Here, I hypothesized that the lizards would exhibit substantial variance in digestive tract plasticity in both form and function in response to each diet. In particular, I expected the lizards fed the carnivore and herbivore diets to respond similarly to other diet specialists (Table 1). I hypothesized that the lizards raised on the carnivore diet would have smaller/shorter digestive tracts, faster transit rates of digesta, and higher rates of amino
acid uptake; as reported for other carnivorous lizards (Buddington, 1987; Guard, 1980; Zimmerman and Tracy, 1989). In contrast, lizards on the herbivore treatment were expected to have larger/longer digestive tracts, slower transit rates of digesta, and higher rates of D-glucose uptake because of the higher concentration of sugars in the herbivore diet.

If bearded dragons reared on a carnivore or herbivore diet are able to regulate their digestive tract in the manner of natural diet specialists (while maintaining energy balance and positive growth rates), this would indicate substantial gut plasticity and may illustrate how diet specialization evolves. But should these naturally omnivorous lizards prove incapable of diet-induced gut regulation, than the commonly held view that diet generalists are Jacks-of-all-trades must be reexamined. In other words, omnivory might be better viewed as an alternative form of diet specialization and departures from a mixed diet may have negative consequences on common proxies of fitness (i.e., digestion, energy balance, growth rate, etc.).
Materials and Methods

Experimental Animals and Husbandry

Captive-bred juvenile (~5-6 wk old) bearded dragons (*Pogona vitticeps*) were purchased from several sources and toe clipped for permanent identification. Initial body lengths (snout-vent length, SVL) averaged 9.1±2.2 cm and mass averaged 22.1±14.7 g (mean±SE). Prior to experiments (~30 d) lizards were housed in small groups (*N*=6; to stimulate feeding) in glass aquaria (20x36x28 cm) and maintained on a 14 L:10 D photoperiod provided by fluorescent UV lights and a 60-W heat lamp. The aquaria were lined with ~4 cm sand and had inverted clay pots for shelter and basking sites. Lizards were offered live crickets (*Acheta domesticus*) and the omnivore diet (see below) daily. Water was provided ad libitum.

Experimental Diets

Lizards were randomly assigned to one of three diet treatments (initially *N*=12 per treatment): (1) hydrated ground crickets (hereafter, the “carnivore” diet), (2) hydrated ground alfalfa (“herbivore”), and (3) a 50:50 mix (by mass) of the carnivore and herbivore diets (“omnivore”). The carnivore diet consisted of adult crickets (*Acheta domesticus*) that were frozen (ca. -17°C), dried to constant mass (105–110°C), ground in a coffee mill, and passed through a 1x1-mm screen. The herbivore diet consisted of ground rabbit chow (Extreme Animal Nutrition, Ontario, CA), which is primarily alfalfa. According to the manufacturer, this diet consists of 16% protein, 3.9% fat, and 14.6% crude fiber (Table 2). Diets were hydrated 1:3 (food:water) by mass with distilled water.
immediately before feeding. Calcium and vitamins were added to the diets weekly to ensure the lizards were receiving vital nutrients. Water was available ad libitum. Initially, most of the lizards did not feed voluntarily on their experimental diets. During this diet-acclimation period (25–111 d, mean=62 d) the lizards were also offered live crickets. By the time the lizards reached 26 g, most were feeding voluntarily on their experimental diets. However, seven lizards did not fully acclimate to their assigned diet, resulting in a reduction in sample sizes: N=9 carnivore, 9 herbivore, and 11 omnivore. The mean initial body mass of those lizards used for the study (carnivore=37.4±8.2 g, herbivore=39.3±6.2 g, omnivore=37.7±6.4 g) did not differ among treatments (ANOVA: $F_{2,28}=0.017$; $P=0.983$).

_Passage Rate and Digestive Efficiency_

Passage rate, digestive efficiency, and intake rate were determined concurrently over a 32-d period for lizards fed each of the three experimental diets. During this time lizards were housed individually in ventilated plastic containers (30.0×16.5×9.0 cm), which were lined with a plastic grate to reduce contact with feces. Containers were placed in environmental chambers with a 14 L:10 D photoperiod and temperature programmed for 33°C during photophase and 30°C during scotophase (±1°C). Containers were randomly assigned to a new shelf position in one of the two chambers each day to ameliorate chamber effects.

Passage rates were estimated to examine the effects of diet on retention time and to determine the time interval for apparent digestive efficiency calculations (see below) using a modification of the method described by (Bjorndal et al., 1991). On day one, fine
strips (4×1 mm; approximately four times the particle size of the ground diets) of blue plastic flagging tape were mixed in with each lizard’s diet (10 strips per lizard). Lizards were force-fed this meal and subsequent meals lacking plastic markers every other day for 32 d via oral gavage in an amount sufficient to maintain individual body mass (±10%). Because some mixing of food is expected to occur in the gut (Sakaguchi, 2003), resulting in longer retention of some of the flagging strips, and because some flags were not retrieved even after 32 d, passage rate was estimated as the time (d) it took a lizard to pass three of the 10 flags.

Apparent digestive efficiency (ADE) was estimated for 29 lizards over the same 32-d period as the passage-rate analysis. The amount ingested was determined by recording the lizard’s mass before and after force-feeding. Feces used to estimate ADE and passage rate were collected daily, separated from the uric acid pellet, placed in paper envelopes, dried to constant mass at 55°C, weighed (±0.001 g), and crushed with a pestle and mortar. A dissecting scope (40x) was used to check for the presence of flagging tape. Apparent digestive efficiency (ADE) was calculated as:

\[
\text{ADE} = \frac{(\text{mass of food ingested} - \text{mass of feces})}{\text{mass of food ingested}},
\]

with all components as dry mass. I used the mean estimated passage rate (d) for each diet treatment (carnivores=12 d, herbivores=8 d, omnivores=10 d; see Results) to calculate ADE. Although herbivores actually had a slightly shorter passage rate (6.9±1.1 d), I used an 8-d passage rate (within the range of the SE) for calculations so more sample days could be included. A mean ADE was calculated for each diet treatment based on the mean of all point estimates recorded for a particular individual within a treatment group.
Daily intake rate was calculated as the difference between the mass of each lizard before and after force-feeding over the same period that ADE and passage rate were determined.

**Gut Morphology and pH**

Diet-induced differences in gut morphology were assessed upon completion of the feeding trials as changes in (1) gut dry mass, (2) gut surface area, (3) intestine length, and (4) the thickness of the mucosal and muscularis/serosal layers. Lizards were killed by severing their spinal cord at the base of the skull. Measurements were taken of the naturally outstretched length of each digestive tract immediately after the gut was removed and rinsed of its contents using reptilian Ringer's solution (137 mM NaCl, 2.7 mM KCl, 1.4 mM CaCl₂, 11.9 mM NaHCO₃, 0.5 mM MgCl₂, 0.4 mM NaH₂PO₄, and 2.8 mM glucose). The hindguts were subsequently dried to constant mass in a drying oven (70°C). Because the fresh small intestine was used to gather other morphological (villi/serosa-muscularis length) and physiological (nutrient absorption) data, I had to estimate its dry mass. I did this by using the equation for the relationship between wet and dry mass for the stomach ($r^2=0.95$) and large intestine ($r^2=0.95$). I assumed the small intestine had the same wet/dry mass relationship as these other gut sections. In support of this assumption, the wet versus dry mass relationship did not differ between the stomach and the large intestine ($F_{1, 46}=2.507; P=0.121$). Thus, I used the equation derived from the linear regression of the combined stomach and large intestine mass data (wet vs. dry) to estimate the dry mass of the small intestine, where: $\text{mass}_{\text{dry}}=0.64+0.452*\text{mass}_{\text{wet}}$. Gut surface area was measured using digital-imaging analysis of whole, naturally outstretched sections of the digestive tract prior to flushing the gut contents. Images were analyzed
with NIH image 1.63f (http://rsb.info.nih.gov/nih-image). Surface-area measurements were taken for the small intestine (pyloric sphincter to the ileo-cecal junction) and large intestine (ileo-cecal junction to the cecal-colonic junction). Total surface area was estimated as the sum of the surface area of the two gut sections. The mucosal and muscularis/serosal layers were prepared for light microscopy by fixing 1-cm sleeves of the proximal small intestine in 10% neutral-buffered formalin, embedding in paraffin, sectioning into 6-μm slices, and staining with hematoxylin and eosin (Goodland et al., 1991). Measurements of each layer (mucosa, muscularis/serosal) were taken with a light microscope (400x) and video camera (Motic Image v. 1.3) linked to a computer, and analyzed via NIH image 1.63f. Mucosa thickness was measured as the length from the tip of a villus to the smooth muscle layer and the muscularis/serosal layer was measured from the smooth muscle layer to the distal margin of the tissue (Secor, 2005b). Ten measurements were taken for each tissue and the mean was used as the datum for that individual. Because the everted sleeve method has been reported to cause damage to the mucosal surface of the intestine in some bird species, resulting in unreliable measurements of uptake rates (Starck et al., 2000; Stein and Williams, 2003), slides were prepared to check for tissue damage.

The pH of the digestive tract was recorded from contents in the small intestine (proximal, middle, and distal), and large intestine with EMD colorpHast* pH strips (pH range 0–14; EMD Chemicals, Gibbstown, NJ). I used the mean of the pH readings as judged by two or three persons to account for variation in investigator color perception (Andres, 2005).
Nutrient Absorption and Uptake Capacity

I compared estimates of intestinal uptake rates of a sugar (D-glucose) and an amino acid (L-proline) across diet treatments using the everted sleeve technique (Karasov and Diamond, 1983; Secor et al., 1994). From each lizard the small intestine was removed and weighed, cut into thirds, and its contents were placed into a weighing boat. Other organs were removed, weighed, and dried to constant mass at 55°C (Table 3).

Each third of the small intestine was weighed, everted, and divided into 1-cm segments. Sleeves were mounted on a metal rod with two grooves spaced 10 mm apart and secured to the rod at the grooves with surgical thread. Sleeves were first incubated in reptile Ringer’s solution at 30°C for 5 min. Sleeves were next incubated for 2 min at 30°C in stirred reptile Ringer’s containing an unlabeled and radio-labeled nutrient (L-proline, at 50 mmol and labeled with \(^3\)H, or D-glucose at 20 mmol and labeled with \(^{14}\)C) and an adherent fluid marker labeled with a different radioisotope ([\(^{14}\)C] polyethylene glycol; [\(^{14}\)C] PEG, or L-[\(^3\)H] glucose, respectively). Thereafter, the sleeves were weighed wet then solubilized by incubating them in 0.1 ml distilled water and 1 ml TS-1 tissue solubilizer (Research Products, Mount Prospect, IL) overnight at 55°C. After cooling to room temperature, 10 ml of scintillation cocktail (Econofluor, PerkinElmer, Wellesley, MA) was added and the radioactivity of the solubilized sections was measured using a liquid scintillation spectrometer (Beckman LS 235, Fullerton, CA) for ~15 min. Radioactivity was used to determine the nutrient uptake of each section by measuring the difference between the radioactivity of each sleeve and that of the nutrient solution. The adherent fluid markers ([\(^{14}\)C] PEG and L-[\(^3\)H] glucose) were used to corrected for the amount of radiolabeled L-proline and D-glucose, respectively adherent to the sleeves.
With these corrections, rates of total L-proline uptake (passive and carrier-mediated) and carrier-mediated D-glucose uptake were quantified as nm of nutrient transported per min per mg of sleeve wet mass. Uptake capacity, an indication of overall intestinal capacity to transport nutrients, was estimated as the summed products of nutrient uptake rates (nmol min\(^{-1}\) mg\(^{-1}\)) multiplied by intestine mass (mg) for each of the three small intestine sections (Karasov and Diamond, 1983; Secor et al., 1994).

**Growth Rate**

Growth rates were recorded over a period of 61-141 d (mean = 135 d). This range results from individual variance in date of complete diet acclimation, but did not differ among diet treatment groups \((F_{2,28}=0.61, P=0.55)\). During this period lizards were housed individually in glass aquaria and voluntarily fed their respective diet as described above. Measurements of snout-vent length (SVL; ±0.5 mm) and body mass (±0.1 g) were taken every other week. Growth rates were calculated for each individual by plotting SVL as a function of days since the trial began and then fitting a regression line through the data. The slope of the line for each lizard provided the mean growth per day. Mean rates of growth were calculated for each diet treatment based on the means of individual growth rates within each treatment group.

**Statistical Analysis**

Analyses were conducted using JMP v. 5.0.1.2 (SAS-Institute, 2002). Passage rates, total nutrient uptake capacity, organ mass, and gut mass and surface area were compared across diets via ANCOVA with body mass (g) as the covariate. Differences
among treatments in ADE, rates of nutrient transport, gut pH, and growth rates were analyzed via ANOVA. If a particular dependent variable was determined to vary significantly with diet in a full model (ANOVA or ANCOVA), I used a pairwise Tukey HSD test (SAS Institute, 2002) to detect statistical differences among the three diet treatments.
Results

Passage Rate and Digestive Efficiency

As noted above, eight lizards did not pass all 10 flags used to estimate passage rate, and four lizards passed no flags at all. Consequently, sample sizes were reduced to 5 carnivores, 8 herbivores, and 8 omnivores. The lack of flag passage was not related to body size (logistic regression: $X^2=2.705$, $P=0.100$) or diet ($F_{2,28}=2.631$, $P=0.092$). Passed flags had no sign of damage from chemical digestion (as viewed at 400×), suggesting that the unrecovered flags had lodged along the digestive tract rather than decomposed. However, because the contents of the digestive tract were removed from the gut immediately following excision, I could not determine where the obstruction(s) occurred. Those lizards that did pass flags usually did so over a period of 1-3 following the passage of the first flag. Among these, mean passage rate of three flags across all diet treatments was 9.0±0.7 d. An ANCOVA, accounting for body size, revealed significant differences in passage rate among the three diet treatments ($F_{2,20}=4.172$, $P=0.034$; Table 4). The carnivores retained a food bolus for longer (12.4±1.3 d), than did both the omnivores (9.5±1.1 d) and the herbivores (6.9±1.1 d). Apparent digestive efficiency was highest in carnivores (89±1%), which was significantly higher than the ADE estimated for the herbivores (81±2%), but not omnivores (85±1%) ($F_{2,26}=3.721$, $P=0.039$; Table 4).

Gut Morphology and pH

After accounting for body mass, there was no difference across diets in the surface area of either the small or large intestine, or the length of the small intestine.
(Table 5). However, hindgut length was significantly different among diet treatments ($F_{2, 23}=4.464, P=0.023$) with longer large intestines recorded for the herbivores (7.23 cm) than for the carnivores (3.82 cm), but herbivores were not different from the omnivores (5.92 cm) (Table 5). Gut mass also differed among diet treatments ($F_{2,23}=9.007, P=0.002$) with the estimated mass of the small intestine of herbivores (0.15 g) less than those of both carnivores (0.23 g) and omnivores (0.25 g) (Table 5). The dry mass of the large intestine was also lower for herbivores (0.12 g) than for carnivores (0.13 g), but not for omnivores (0.15 g) ($F_{2,23}=6.147, P=0.009$; Table 5). Diet treatments did not have an effect on pH in the small intestine ($F_{2,23}=0.486, P=0.622$; Table 5), but pH did vary in the large intestine ($F_{2,22}=7.394, P=0.004$). The large intestines of carnivores were more acidic (6.6±0.3) than the herbivores (8.3±0.3), but carnivores were not different from the omnivores (7.4±0.3).

Microscopic (400×) analysis of the preserved segments of the small intestine (post-inversion) revealed no significant tissue damage to either the mucosal or muscularis/serosal layers. Mucosal thickness of the intestine, largely a function of villus length, was thicker in carnivores than in omnivores or herbivores ($F_{2,18}=12.45, P=0.001$; Table 6). Likewise, the muscularis/serosal layer was thicker in carnivores than in omnivores, but carnivores were not different from herbivores ($F_{2,18}=3.911, P=0.045$; Table 6).

**Nutrient Absorption and Uptake Capacity**

Rates of nutrient absorption were highest for omnivores and herbivores and lowest for carnivores for both proline ($F_{2,23}=3.763, P=0.041$) and glucose ($F_{2,23}=5.075$,
After accounting for body mass, there were no differences attributable to diet in total gut uptake capacity of glucose ($F_{2, 23}=2.013, P=0.161$) or proline ($F_{2, 23}=1.640, P=0.220$; Fig. 1).

_Growth Rate_

An ANOVA revealed differences in growth rates among diet treatments ($F_{2, 28}=6.546, P=0.005$) with omnivores growing 1.8 times faster ($0.32\pm0.02 \text{ mm*}d^{-1}$) than herbivores ($0.20\pm0.02 \text{ mm*}d^{-1}$), but neither of these treatments was different from carnivores ($0.25\pm0.03 \text{ mm*}d^{-1}$) (Fig. 2). At the conclusion of the growth trials the final body size (SVL) of lizards did not differ among diet treatments ($F_{2, 28}=2.149, P=0.137$).
Discussion

This study was conducted to test the phenotypic flexibility of the guts of growing omnivorous lizards with the assumption that the data would provide a test of the Jack-of-all-trades paradigm for diet generalists and perhaps insight into the evolution of diet specialization. The data indicate that growing *Pogona vitticeps* have a relatively moderate ability to regulate digestive tract form and function in response to differences in diet. However, many of the changes that occurred were contrary to those predicted, and appeared to be mechanisms that allowed for subsistence but not growth. The following sections discuss and interpret how each of the independently measured variables might have been affected by the diet treatments.

*Passage Rate and Digestive Efficiency*

Lizards reared on the carnivore diet had longer passage rates than did the omnivores or herbivores (Table 4). Because I did not measure passage rate through the different gut regions (i.e., small vs. large intestine), I assumed that passage rate was a function of the length of a particular gut region. Although these passage-rate findings were not predicted (Table 1) and contrast with data on strictly herbivorous or carnivorous reptile species (reviewed by Zimmerman and Tracy, 1989), my data correspond well with intraspecific studies in which diet quality was varied for other reptile, bird, and mammal species (Bjorndal, 1989; Hailey et al., 1998; Karasov and Hume, 1997; Spencer et al., 1998; Troyer, 1984b; van Marken Lichtenbelt, 1992). Variation in retention times may be attributable to an “ileal brake,” which inhibits gut motility by duodenal receptors.
responding to high concentrations of amino acids and fats (Karasov and Hume, 1997). Several lines of evidence support the ileal brake hypothesis: (1) the carnivore diet was higher in energy and protein than the other diets (Table 2); (2) lizards fed the carnivore diet had longer retention times than did those fed the herbivore diet; (3) aside from mass, the digestive tracts were statistically indistinguishable in gross morphology across diet treatments; and (4) intake rates needed to maintain body mass did not differ across diet treatments. Thus, the higher energy concentration of the carnivore diet may have triggered an antiperistaltic response, which ultimately slowed the rate of food passage in lizards fed the carnivore diet. This could be verified by quantifying the diet dependence of peristaltic contractions of the small intestine using either ultrasound or via direct examination of excised guts.

The higher digestive efficiency experienced by lizards fed the carnivore diet relative to those fed the herbivore diet was expected given the differences in diet quality (Table 2) and composition (animal vs. plant). Studies of omnivorous turtles have found nonadditive interactions in mixed diets of plants and animals (Bjorndal, 1991; Bouchard and Bjorndal, 2006). In these studies both positive and negative nonadditive effects were reported and appeared to correlate with both diet quality and composition. Omnivores (mixed diet) in this study had ADEs that were statistically indistinguishable from ADEs for carnivores and herbivores, but the mean ADE for omnivores was most similar to that recorded for the carnivores (Table 4). This suggests that lizards fed the omnivore diet digestively benefited from positive (albeit small) nonadditive effects of the mixed diet.

Despite having the lowest digestive efficiency among the three diet treatments, when these naturally omnivorous lizards were fed the herbivore diet, they achieved ADE
values that are comparable to those reported for strictly herbivorous reptiles (Bjorndal and Bolton, 1990; Throckmorton, 1980; Troyer, 1984a; van Marken Lichtenbelt, 1992; Zimmerman and Tracy, 1989). However, because the herbivore diet was relatively low in crude fiber, high in protein (Table 2), and of relatively small particle size (<1×1 mm), these values are likely overestimates of the ability of juvenile *Pogona vitticeps* to digest the plants that they would encounter in nature (see also Bjorndal and Bolton 1990). Nevertheless, this finding suggests that juvenile bearded dragons are likely able to thrive on a diet of high-quality plant tissues.

**Gut Morphology and pH**

Among vertebrates, herbivores generally have relatively longer small intestines and more capacious hindguts compared to nonherbivores (Bjorndal, 1985; Espinoza, 2002; Guard, 1980; Herrel et al., 2004; Iverson, 1980, 1982; King, 1996; Stevens and Hume, 1995). Theoretically, a longer and more voluminous gut should slow passage rate, thereby enhancing digestive efficiency of low-quality diets (Sibly, 1981). However, I detected no such trends in small intestine size among diet treatments. In fact, the relative mass of the small intestine of the herbivores was lower (yet similar in length and surface area) than that recorded for lizards fed the carnivore and omnivore diets (Table 4), a finding that contrasts with both my prediction and other interspecific studies (reviewed by Guard, 1980). Because the herbivore diet had the lowest energy content, the lower mass of herbivore small intestines relative to those from the other diet treatments and the lack of a diet-induced size increase (length, surface area, or mucosa) suggests that gut
size was downregulated to conserve energy that would otherwise be allocated to an energetically costly organ (Cant et al., 1996; Schmidt-Nielsen, 1984).

Lizards fed the herbivore diet had longer large intestines than those fed the carnivore diet, but similar large intestine size to lizards fed the omnivore diet (Table 4). Yet again, the mass of the herbivore large intestine was the lowest among the diet treatments and surface area did not differ among diet treatments. If the relative length of the large intestine was longer without a concomitant increase in relative mass, then this gut region must have sacrificed mass to either stretching or because of atrophy (Starck and Beese, 2001). This was also born out in the histological data, which showed the mucosal layers of herbivores to be less thick than carnivores, but not omnivores. The longer large intestine was expected as hindgut-fermenting herbivorous vertebrates have more voluminous large intestines than do carnivores (Bjorndal, 1997; Dearing, 1993; Herrel et al., 2004; Iverson, 1980, 1982; Mackie et al., 2004; Stevens and Hume, 1995; Troyer, 1984a). Generally, longer guts translate into longer passage times (Sibly, 1981), yet despite having large intestines that were 1.9 times longer (yet not as voluminous; Table 4), the herbivores in my study had a passage rate 1.7 times faster than the carnivores. This longer large intestine, coupled with a more basic hindgut pH (Table 5), and the presence of a large number of nematodes in the colon (pers. observ.) are conditions associated with the presence of fermentative microsymbionts (Andres, 2005; Bjorndal, 1997; Iverson, 1980, 1982; Troyer, 1991), which herbivorous vertebrates rely on to digest plant fiber. These cellulolytic microbes (primarily bacteria and protozoa) digest cellulose into volatile fatty acids (VFAs), which are passively absorbed by the hindgut and can provide a substantial portion of the host’s energy requirements.
(Bjorndal, 1979; Bjorndal, 1991; Mackie et al., 2004; McBee and McBee, 1982; Pryor and Bjorndal, 2005; Troyer, 1991). Although such adaptations have not been widely reported in omnivorous vertebrates, like *Pogona vitticeps*, they are expected precursors for lineages in the process of evolving herbivory (Andres, 2005).

**Nutrient Absorption and Uptake Capacity**

Glucose absorption was 1.6 times higher for herbivores relative to carnivores and 1.7 times higher for omnivores relative to carnivores. These differences are generally consistent with my predictions (Table 1), and follow from studies of fishes, of which herbivores and omnivores tend to have greater uptake rates of glucose than carnivores (Buddington, 1987; Buddington et al., 1987). Karasov (1989) proposed that when confronted with higher-than-typical levels of a non-essential dietary nutrient, animals will respond in one or both of the following ways: optimizing uptake (digestive modulation hypothesis) and/or maintaining osmotic balance (Karasov, 1992). According to the adaptive modulation hypothesis, if an animal is fed a diet rich in carbohydrates (e.g., herbivores in this study), its gut should respond by increasing glucose uptake, thereby increasing energy derived from dietary sugars. This hypothesis is supported as well for lizards fed the carnivore diet (low in carbohydrates), which had low rates of glucose absorption. Because dietary glucose was lower for carnivores and most of their energetic needs may have been met via paracellular transport (Chediack et al., 2005; McWhorter et al., 2006), synthesizing and maintaining a similar number or density of glucose transporters as the lizards fed the herbivore and omnivore diets would be costly and without added benefit.
An alternative, albeit nonexclusive hypothesis may also explain the higher glucose uptake by lizards fed the herbivore and omnivore diets. Insufficient absorption of dietary nutrients can cause diarrhea in some mammals (Sunshine and Kretchmer, 1964) and may explain why some birds avoid certain sugars (Afik et al., 1995). In response to their high-carbohydrate diets, herbivores and omnivores in this study may have increased glucose uptake to maintain osmotic balance in the small intestine, thereby reducing the chances of developing potentially life-threatening diarrhea. Both nutrient acquisition and osmoregulation are critically important to survival, so it is not possible to conclude which hypothesis is better supported in this study.

Consistent with my results for glucose absorption, rates of proline uptake were also much higher (1.8 times) in herbivores than in carnivores. Although these results do not support either hypothesis cited above for glucose uptake (or my initial predictions), they are consistent with findings by other investigators. Herbivorous desert iguanas (Dipsosaurus dorsalis) had greater uptake rates of L-proline when fed alfalfa pellets than when fed mealworms (larval Tenebrio molitor) (Karasov and Diamond, 1983). Similar results were reported by Andres (2005) for D. dorsalis, which she fed crickets, alfalfa pellets, or an equal mix of the two. A comparable study of marine intertidal prickleback fishes (Stichaeidae) showed that, when fed a low-lipid diet, herbivorous species had higher levels of lipase than carnivorous species, suggesting an adaptive response of the gut for fat assimilation as well (German et al., 2004). Collectively, previous studies and data from my investigation suggest that vertebrate guts increase the assimilation of essential nutrients (e.g., amino acids or lipids) when they are in short supply, and decrease their uptake when there is a dietary surplus. Both strategies appear to be geared
toward achieving energetic efficiency in digestion and assimilation.

Uptake capacity, which is a function of absorption rate and small intestine mass, estimates how much nutrient can be absorbed by the small intestine per minute. Thus the observed lack of treatment effects for uptake capacities can be explained by a lack of difference among treatments in either absorption rate or mass. For example, carnivores had relatively heavy small intestines, yet low rates of uptake for both proline and glucose (Table 5; Fig. 1). In contrast, the small intestines of herbivores weighed less, yet had high uptake rates for both nutrients (Table 5; Fig. 1). Finally, omnivores had intermediate rates of uptake and gut mass, which translated into intermediate levels of uptake capacity. These results, in accord with mass-specific rates of nutrient absorption, suggest that *Pogona vitticeps* can up- or downregulate nutrient transporters in response to the availability of specific nutrients in the diet.

The results of the nutrient transport study suggest that two strategies were adopted by the growing lizards: skimming and retention. Qualitatively, each of these strategies corresponds well with the rate of food passage and the rate at which nutrients were absorbed. For example, lizards raised on the herbivore diet apparently adopted a skimming strategy in which their moderately high nutrient uptake rates matched the rapid rate at which food was passing through their guts. Perhaps by moving food quickly through the small intestine, digesta could ferment longer in the large intestine, resulting in high nutrient uptake in the form of VFAs. In contrast, carnivores apparently retained food in the small intestine for much longer, wherein most of the available nutrients were further hydrolized and absorbed before the digesta reached the large intestine. Despite adopting apparently different digestive strategies, nutrient uptake capacity did not differ.
among the three diet treatments, suggesting that the guts of these lizards adaptively responded to differences in nutrient and energy demand and availability.

Most studies of nutrient absorption in the small intestine of vertebrates have focused on adaptive regulation in response to bouts of feeding and fasting (reviewed in Secor, 2005). Relatively fewer studies of vertebrates have examined the uptake of nutrients in response to differences in diet from either intra- or interspecific perspective (Buddington, 1987; Buddington et al., 1987; Karasov and Diamond, 1983; Levey and Karasov, 1989, 1992). Of the few studies that have examined nutrient absorption in response to differences in diet quality in reptiles (Andres, 2005; Karasov, 1989) only a single species (*Dipsosaurus dorsalis*) was used, indicating a need for more comparative research in this area.

**Growth Rate**

Diet treatment had a significant effect on growth rates of juvenile *Pogona vitticeps*. Omnivores grew significantly faster (1.6 times) than the herbivores, and somewhat faster (1.3 times; \( P=0.096 \)) than the carnivores. These results most likely reflect differences in nutrient content of the diet and assimilation efficiency rather than diet energy and intake (see discussion of passage rate above). Thus, the digestive and growth performance of *P. vitticeps* is not consistent the paradigm of a “Jack-of-all trades” feeding strategy. If my findings supported this hypothesis, I would have found equal performance across all diet treatments. Instead, this omnivorous lizard appears to behave as a specialist of omnivory. My finding that growth was not maximized for lizards fed the diet with the highest protein concentration (carnivore) is somewhat unexpected.
Numerous studies have examined the effects of diet quality on intake and growth in reptiles (e.g., Andrews, 1982; Avery et al., 1993; Baer et al., 1997). A common finding is that growth rates are positively correlated with protein concentrations up to ~25% (Avery et al., 1993; Bouchard and Bjorndal, 2006; Donoghue et al., 1998). The results of my study parallel the findings of these studies and suggest that there may also be a maximum concentration of protein beyond which digestive performance diminishes in non-carnivorous reptiles like *P. vitticeps*. For some vertebrates, a high-protein diet will increase the activity of renal and liver enzymes responsible for the catabolism of protein (Koutsos et al., 2001). Increased protein catabolism may lead to higher levels of plasma uric acid, which may be indicative of renal dysfunction and gout (Donoghue, 2006). I did not measure activity levels of liver and kidney enzymes; however, the larger size of these organs in the carnivores (Table 3) may be indicative of heightened metabolic activity, which would divert energy from growth to maintenance. Thus the lower rates of growth for lizards fed the carnivore diet (Fig. 4) may have resulted from the high protein load, which posed a metabolic challenge for the liver and kidneys.

*Implications for Diet Evolution*

Because evolution typically takes place over many generations, the process is difficult to study in long-lived animals (Grant and Grant, 2006; Kennington et al., 2003) such as *Pogona vitticeps*. However, studies of evolution at the proximate scale may provide evidence for current selective pressures or elucidate causal mechanisms of past selective events (e.g., Sinervo, 1993; Sinervo and Licht, 1991a,b; Sinervo et al., 1992).
As such, studies of digestive performance and growth rate are important because they may identify proximate advantages and constraints of potential evolutionary shifts in diet.

Using growth rate as a proxy for fitness, my data suggest a selective advantage for juvenile *Pogona vitticeps* to avoid diet specialization (i.e., strict herbivory or insectivory). These data also show that juvenile bearded dragons have a modest ability to adaptively regulate certain aspects of their digestive system in response to differences in diet quality. The fact that these lizards did not perform best (considering measures reported here) on a solely insect or plant diet suggests that the morphological and/or physiological performance capacity of the digestive system was exceeded by these diets. Interestingly, the apparent limiting step, which would seemingly preclude wholesale exploitation of a strictly insect or plant diet, appears to be different for lizards fed the carnivore and herbivore diets. Carnivory is the ancestral condition for vertebrates and thus requires relatively little morphological specialization of the digestive tract, whereas herbivory usually requires a suite of specializations, which partly explains the paucity of herbivorous reptiles (Bjorndal, 1997; Espinoza et al., 2004; Iverson, 1980, 1982; Karasov and Hume, 1997; King, 1996; Stevens and Hume, 1995). The apparent limiting factor for lizards fed the carnivore diet was the ability to process the high concentration of protein, which may have taxed their renal system, thereby reducing rates of growth either by lowering the energy allocated to growth or perhaps via protein toxicity. In contrast, lizards fed the herbivore diet showed a physiological ability to assimilate their plant diet, but were apparently outperformed by omnivores because their gut morphology (i.e., short small intestine and simple large intestine) was not suitably specialized for strict
herbivory. For the herbivores, gut atrophy may have been a physiological response to reduce the energy used by an organ system that is expensive to maintain.

The results of this study are relevant to the discussion of how major evolutionary shifts occur (reviewed by Huey et al., 2003). One paradigm suggests that major evolutionary shifts, such as changes in diet (e.g., insectivory → omnivory → herbivory), evolve in a specific sequence: first behavior, followed by physiology, and finally morphology (Huey et al., 2003). The ability of individuals to behaviorally make a diet shift, and for selection to subsequently favor individuals that gradually improve their performance on specialized diets (e.g., herbivory) via heritable changes to their physiology and morphology, may be constrained by limits (i.e., adaptive valleys) occurring in any one of these categories at any stage in the process. Behavioral, physiological, and morphological limits may be manifested as speed bumps, roadblocks, or cul de sacs on the road to herbivory, and may explain why some lineages evolve herbivory readily, whereas others are likely evolutionarily precluded from venturing into this diet niche (Espinoza et al., 2004). My results support these predictions but also suggest that the evolutionary barriers to diet specialization may evolve in tandem, with each character simultaneously “driving” and being “driven” to a more specialized form or function in a manner consistent with the hypotheses of symmorphosis (Weibel, 2000) and correlated progression (Kemp, 2007).

More research is needed to determine whether the results of my study are attributable to developmental plasticity or phenotypic flexibility (Piersma and Drent, 2003). A fixed response to differences in diet early in ontogeny (e.g., strict herbivory or carnivory) would be maladaptive to lizards like Pogona vitticeps, which undergo
ontogenetic shifts in diet (i.e., omnivory → herbivory). Therefore, it is unlikely that the
diet-induced responses of digestive tracts noted here are fixed. However, despite
differences in growth rate, lizards were capable of assimilating their respective diets and
gaining mass, evidence that they would continue to grow and eventually reach sexual
maturity. Consequently, future research might examine long-term fitness (survival, age at
first reproduction, clutch size, etc.) to further assess the costs and benefits of diet
specialization. Additionally, it would be interesting to compare these results with those
from similar experiments involving closely related diet specialists. A recent synthesis of
the diet contents for more than 400 species of lizards found that none included 51–70%
plant matter in their diets (Cooper and Vitt, 2002). This rather large gap in what was
previously assumed to represent an uninterrupted continuum of diet selection across
lizard taxa suggests that the switch to herbivory (defined as >70% plant matter; Espinoza
et al., 2004; Stayton, 2005) requires an evolutionary commitment in terms of behavior,
physiology, and morphology (Espinoza, 2002; Espinoza et al., 2004; Stayton, 2005).
Evidence from this study, and another that examined the role of diet switching on
digestion and the endosymbiotic bacterial communities of the digestive tracts of
Dipsosaurus dorsalis (Andres, 2005), support this idea. A phylogenetic comparison with
closely related diet specialists would elucidate where along the diet spectrum P. vitticeps
lies, and may provide more clues about the ultimate constraints associated with diet
specialization as well as insight into how and why diet specialization evolves.


APPENDIX

Table 1. Predictions for measured morphological and physiological variables of *Pogona vitticeps* reared on one of three diets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Carnivore</th>
<th>Omnivore</th>
<th>Herbivore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut size</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Transit rate</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Villus length</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Liver size</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Kidney size</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>L-proline uptake</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>D-glucose uptake</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Values indicate order of placement with 1 being first or highest and 3 being last or lowest. Note: lizards fed the omnivore diet were not expected to change appreciably because their experimental diet was similar to their natural diet.
Table 2. Nutritional values and energy content (dry-matter basis) of the three experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Crude Protein (%)</th>
<th>Crude Fat (%)</th>
<th>Crude Fiber (%)</th>
<th>Crude Ash (%)</th>
<th>Crude Ca&lt;sup&gt;2+&lt;/sup&gt; (%)</th>
<th>Crude P (%)</th>
<th>Energy (kJ/g&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnivore (<em>Acheta domesticus</em>&lt;sup&gt;1&lt;/sup&gt;)</td>
<td>54</td>
<td>31.5</td>
<td>19.7</td>
<td>3.9</td>
<td>0.2</td>
<td>1.4</td>
<td>25.8</td>
</tr>
<tr>
<td>Herbivore (rabbit chow&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>16</td>
<td>3.9</td>
<td>14.6</td>
<td>0.9</td>
<td>1.2</td>
<td>0.6</td>
<td>17.9</td>
</tr>
<tr>
<td>Omnivore&lt;sup&gt;3&lt;/sup&gt;</td>
<td>35</td>
<td>17.7</td>
<td>17.2</td>
<td>2.4</td>
<td>0.7</td>
<td>1.0</td>
<td>22.8</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean reported by Donoghue (2006).

<sup>2</sup>Extreme Animal Nutrition, Ontario, CA.

<sup>3</sup>Average of carnivore and herbivore diets.
Table 3. Body and organ mass (g, mean±SE) for *Pogona vitticeps* reared on one of three diets (N = 8/treatment).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Carnivore</th>
<th>Omnivore</th>
<th>Herbivore</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>71.5±15.1</td>
<td>94.1±11.2</td>
<td>63.1±7.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Heart</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.04±0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Lung</td>
<td>0.07±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>0.45±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.14±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Similar superscripts within a row denote comparisons that were not significant (P>0.05) among the three diet treatments as determined via Tukey HSD test.

1 All measures are dry mass except body mass, which was taken just before the animals were killed.
Table 4. Passage rate, digestive efficiency, and intake rate (mean±SE) of *Pogona vitticeps* reared on one of three diets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Carnivore</th>
<th>Omnivore</th>
<th>Herbivore</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passage rate (d)</td>
<td>21</td>
<td>12.4±1.7\textsuperscript{a}</td>
<td>9.5±0.9\textsuperscript{a,b}</td>
<td>6.9±1.0\textsuperscript{b}</td>
<td>4.172</td>
<td>0.034</td>
</tr>
<tr>
<td>Digestive efficiency (%)</td>
<td>27</td>
<td>90±1\textsuperscript{a}</td>
<td>88±1\textsuperscript{a,b}</td>
<td>84±2\textsuperscript{b}</td>
<td>3.721</td>
<td>0.039</td>
</tr>
<tr>
<td>Intake rate (dry g d\textsuperscript{-1})</td>
<td>27</td>
<td>1.8±0.3\textsuperscript{a}</td>
<td>2.3±0.3\textsuperscript{a}</td>
<td>1.8±0.3\textsuperscript{b}</td>
<td>0.260</td>
<td>0.773</td>
</tr>
</tbody>
</table>

Similar superscripts within a row denote comparisons that were not significant (*P*>0.05) among the three diet treatments as determined via Tukey HSD test.
Table 5. Gut size\(^1\) and pH (mean±SE) for *Pogona vitticeps* (*N*=8/treatment) reared on one of three diets (C = carnivore; O = omnivore; H = herbivore).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Small intestine</th>
<th>Large intestine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>O</td>
<td>H</td>
</tr>
<tr>
<td>Length (wet, cm)</td>
<td>22.3±1.9</td>
<td>25.3±1.6</td>
<td>22.1±0.7</td>
</tr>
<tr>
<td>Surface area (wet, cm(^2))</td>
<td>9.3±1.1</td>
<td>10.0±1.0</td>
<td>7.9±1.0</td>
</tr>
<tr>
<td>Mass (dry, g)</td>
<td>0.2±0.6(^a)</td>
<td>0.3±0.0(^a)</td>
<td>0.1±0.1(^b)</td>
</tr>
<tr>
<td>pH</td>
<td>8.3±0.3</td>
<td>8.5±0.2</td>
<td>8.5±0.1</td>
</tr>
</tbody>
</table>

Similar superscripts within a row denote comparisons that were not significant (*P*>0.05) among the three diet treatments as determined via Tukey HSD test.

\(^1\)Corresponding body lengths (cm) were as follows: carnivores: 13.0±0.7; omnivores: 14.0±0.6; herbivores: 12.1±0.7.
Table 6. Thickness (mean±SE) of mucosal and muscularis/serosal layers of the small intestine of *Pogona vitticeps* reared on one of three diets.

<table>
<thead>
<tr>
<th></th>
<th>Carnivore</th>
<th>Omnivore</th>
<th>Herbivore</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N=6)</td>
<td>(N=8)</td>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucosa (µm)</td>
<td>809±119^a</td>
<td>586±52^b</td>
<td>462±15^b</td>
<td>12.453</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Muscularis/serosal (µm)</td>
<td>217±23^a</td>
<td>152±28^b</td>
<td>196±20^a,b</td>
<td>3.911</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Similar superscripts within a row denote comparisons that were not significant (P>0.05) among the three diet treatments as determined via Tukey HSD test.
Figure 1. Rates (mean±SE) of nutrient uptake (A and B) and uptake capacities (C and D) for captive *Pogona vitticeps* (*N*=8/treatment) raised on one of three diets for an amino acid (proline) and a sugar (glucose). Uptake rates were lowest in carnivores and higher in omnivores and herbivores for both proline (A) and glucose (B), but uptake capacities did not differ among diets.

Figure 2. Growth rates (mean±SE) of captive *Pogona vitticeps* raised on one of three diets. Lizards raised on the omnivore diet grew faster (0.32±0.02 mm day$^{-1}$; *N*=11) than did those fed the herbivore diet (0.20±0.02 mm day$^{-1}$; *N*=9), but similarly to those raised on the carnivore diet (0.25±0.03 mm day$^{-1}$; *N*=9).
Fig. 1.

**Proline**

<table>
<thead>
<tr>
<th></th>
<th>Carnivore</th>
<th>Omnivore</th>
<th>Herbivore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Specific Uptake (nmol min⁻¹ mg⁻¹)</td>
<td><img src="chart1.png" alt="Chart" /></td>
<td><img src="chart2.png" alt="Chart" /></td>
<td><img src="chart3.png" alt="Chart" /></td>
</tr>
</tbody>
</table>

**Glucose**

<table>
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<tr>
<th></th>
<th>Carnivore</th>
<th>Omnivore</th>
<th>Herbivore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Specific Uptake (nmol min⁻¹ mg⁻¹)</td>
<td><img src="chart4.png" alt="Chart" /></td>
<td><img src="chart5.png" alt="Chart" /></td>
<td><img src="chart6.png" alt="Chart" /></td>
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Fig. 2.