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EFFECTS OF DIET COMPOSITION AND AMBIENT TEMPERATURE ON DIGESTIVE FUNCTION AND BIOENERGETICS OF THE GREEN IGUANA (Iguana iguana)

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DAVID JONATHAN BAER

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EFFECTS OF DIET COMPOSITION AND AMBIENT TEMPERATURE ON DIGESTIVE FUNCTION AND BIOENERGETICS OF THE GREEN IGUANA (*Iguana iguana*)

By

David Jonathan Baer

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ABSTRACT

EFFECTS OF DIET COMPOSITION AND AMBIENT TEMPERATURE
ON DIGESTIVE FUNCTION AND BIOENERGETICS
OF THE GREEN IGUANA (*Iguana iguana*)

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The green iguana (*Iguana iguana*) is a folivorous ectotherm that has been used as a source of food for humans in Central and South America. Intensive production of captive iguanas may help re-establish native populations that have declined in the recent past. A component of captive management is the development of diets that support rapid growth and reproduction. Two studies were conducted with individually-housed, *ad libitum*-fed, captive iguanas to determine the relationship between diet composition, temperature, digestive function, and bioenergetics. During the first study, 21 iguanas were fed 3 diets (Latin-square crossover design) that contained 3 levels of dietary fiber (19%, 24%, and 27% of dry matter as neutral detergent fiber (NDF)). Mean (±SEM) daily dry matter (DM) and metabolizable energy (ME) intake (6.99, 7.57, 6.86 ± 0.62 g/d/kg body weight (BW); and 22.2±2.1, 23.2±2.2, 19.1±2.1 kcal/d/kg BW) were not different between the 3 diets, and mean daily

growth rate (GR) (2.22 and 2.35 [±0.21] g/d) was not different between the low and medium level fiber diets, respectively. Mean daily GR was lower ($p<0.0037$) when the iguanas were fed the high fiber diet (1.42 g/d). Metabolizability of DM (65.8, 62.2, 57.8 [±1.4%]), and energy (70.7, 66.4, 62.3 [±1.2%]) were lowest on the high fiber diet ($p<0.0011$ for DM, $p<0.0001$ for energy). NDF digestibility followed a similar pattern, (47.1, 42.5, 38.5 [± 1.8%]). During the second study, 12 iguanas were housed (crossover design) at 2 ambient temperatures (28 C and 35 C). Daily DM intake (3.5, 12.7 [±.5] g/d), GR (0.6, 3.4 [±.3] g/d), and energy expenditure [EE, determined by respiratory exchange (RE: 5.5, 14.3 [±.9] kcal/d) and doubly-labelled water (DLW: 11.3±3.3, 33.4±3.0 kcal/d)] were lower for iguanas housed at 28 C than 35 C. Nutrient metabolizability, and the efficiency of energy deposition above maintenance (k_g) were not different between the iguanas housed at the different temperatures. Mean EE_{dlw} (24.0±3.6 kcal/d) was higher ($p<0.001$) than EE_{re} (10.0±.9 kcal/d), and EE_{dlw} may overestimate EE. These studies demonstrate that in captivity, herbivorous lizards will consume manufactured diets ad libitum, and digestion and bioenergetic studies can be conducted under controlled conditions. A diet containing less than 27% NDF, and an ambient temperature of 35 C may improve growth rate of captive iguanas, but ambient temperature does not affect nutrient metabolizability.

To my dearest Charlotte,

thank you for your steadfast support

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INTRODUCTION

The green iguana (Iguana iguana) has been captured and eaten by some peoples of Central and South American countries since ancient times (Werner, 1986; Fitch et al., 1982). Hunted animals are either eaten by compesinos (local farmers) or taken to city markets where they are sold. Both the meat and the eggs (a delicacy) from gravid female iguanas are consumed (Fitch et al., 1982). These products can be an important source of food (e.g., protein), especially for the rural compesinos (Werner, 1986). Unfortunately, populations of green iguana have diminished over the past few years (Fitch and Henderson, 1977; Fitch et al., 1982; Werner, 1986). Some populations of the green iguana have become extinct (Fitch and Henderson, 1977), and the species has been declared endangered in most countries where it is hunted (Fuller and Swift, 1985).

There are at least two reasons for the decline in population size: 1) habitat destruction by humans, and 2) unmanaged hunting or overhunting. Slash and burn agricultural practices destroy iguana habitat (Stoney, 1987). Regrowth of the habitat often takes many years, and the new habitat may not be sufficient to support populations of iguanas. The meat and eggs from hunted animals that are

sold in city markets generally costs more per unit weight than fish, poultry, pork or beef, but consumers are apparently willing to pay the additional price. Some cultures claim a medicinal value (e.g., cure for impotence) of the meat of iguanas or stew made from the meat (Fitch et al., 1982). Villa (1968) estimated that 150,000 animals were eaten annually in Nicaragua. Gravid females burrow in ground nests to lay eggs. These slow-moving females are especially easy prey, and their capture is particularly devastating to future population growth.

In 1983, the Smithsonian Tropical Research Institute in Panama undertook a project to develop an economically feasible iguana management project based on the principles of sustainable agriculture (Werner, 1986; Gradwohl and Greenberg, 1988). In the late 1980s, the project moved from Panama to Costa Rica and the Fundación Pro Iguana Verde was established to continue the project. This project involves raising iguanas in captivity and releasing them into appropriate habitats. In release areas where habitats have been previously destroyed, new and ecologically appropriate habitats would be established by reforestation (Stoney, 1987). Concomitant with the release of captive-raised animals into reforested areas are educational programs designed to teach compesinos the basics of wildlife and habitat management (Chapin, 1986). The goals of the iguana management project are: 1) to develop methods for the rational exploitation of a renewable natural resource, 2) to

promote the conservation of an endangered species, and 3) to improve habitat by developing an incentive for preservation or reforestation (Werner, 1986, 1989).

Vital to the success of this intensive animal production project is an understanding of the nutritional requirements of the iguana. Appropriately formulated diets should improve growth rate and animal health, and minimize food costs. The results of initial nutritional studies in Panama indicate that such is the case, and increased growth rate of hatchlings and yearlings in captivity has been noted (D. Werner, O. Oftedal, M. Allen, unpublished data, summarized by Allen et al., 1991). However, these studies were preliminary, and few data are available on the relationship between diet composition and ambient temperature on the bioenergetics, nutrient metabolizability, and growth rate of ectotherms, especially those that are herbivorous. Thus, more rigorous scientific experimentation is required.

The goals of the research described here were 1) to determine the role of dietary plant fiber in modulating energy intake, growth rate, and nutrient metabolizability, 2) to determine the effect of ambient temperature on nutrient intake, growth rate, and nutrient metabolizability, and 3) to estimate the maintenance energy requirements and efficiency of energy utilization above maintenance of the green iguana.

REVIEW OF THE LITERATURE

Natural History

The green iguana (Iguana iguana) is primarily an arboreal species over most of its home range. While egg laying and tree-to-tree movements occur on or across the ground, feeding occurs primarily in trees (Rand, 1978). Iguana iguana inhabits Neotropical forests ranging from southern Mexico to northern South America, although some populations are insular (Etheridge, 1982; Taylor, 1956). Adults can weigh up to 6 kg, reaching a total length of 169 cm and a snout-vent length (SVL) of 51 cm (Swanson, 1950). Females lay one clutch annually in a deep ground burrow (Cohn, 1989; Fitch and Henderson, 1977). Clutch size is dependent on several factors, including female body size. Larger females produce more eggs. Therefore, by increasing growth rate of females, egg production may be improved. Clutch size of wild females ranges between 20-70 eggs, and females can begin laying eggs in their third year of life (Dugan, 1982; Wiewandt, 1982). Egg laying occurs between late January and April, and hatching occurs between late April and July. Seasonal and geographic variations

influence the timing of egg laying and hatching (Rand and Greene, 1982; Harris, 1982; Fitch and Henderson, 1977; Swanson, 1950).

Natural predators of the green iguana include birds (e.g., hawks), mammals, other lizards (e.g., the basilisk) and snakes (e.g., boas) (Van Devender, 1982; Greene et al., 1978). Mortality of wild hatchling iguanas can be as high as 95% (Cohn, 1989; Harris, 1982; Van Devender, 1982; Rand and Dugan, 1980; Rand and Robinson, 1969). This high mortality rate is an important impetus for captive rearing. Animals raised in captivity and released at a larger size may be better able to escape predators. One tactic iguanas use to avoid detection by predators is to become immobile when disturbed (Greene et al., 1978; Moberly, 1968a). If disturbed further, iguanas can escape from predators by climbing, running, or swimming away (Swanson, 1950). Prime iguana habitats are trees with branches that overhang a river or stream. When disturbed, the animals drop into the water, submerge, and swim away. Moberly (1968a) reported that animals can drop into the water from a height of 18 m. It is difficult to determine how far the animals can swim submerged because it is almost impossible to locate them in the brush when they surface. Arendt (1986) observed one iguana swimming submerged for approximately 20 m. Furthermore, iguanas can remain submerged for extended periods. Moberly (1968b) conducted diving studies with nine iguanas and reported that they voluntarily remained

submerged for 50-270 min (mean: 82 min). This escape behavior is possible, in part, because of a high capacity for anaerobic metabolism (Moberly, 1968b).

Natural Diet and Herbivory in Lizards

It is difficult to determine the natural diet of the green iguana, in part because of the limited time that is spent feeding. Moberly (1968a) estimated that green iguanas were inactive 90% of the time. The limited amount of time spent feeding, their inactivity, and the difficulty in observing animals in the field has restricted the amount of information available regarding the natural diet and feeding behavior. Anecdotal reports of field researchers have provided some information (Swanson, 1950; Hirth, 1963; Henderson, 1974), but a recent report on Iguana iguana feeding habits from islands and from nearby mainland Panama has provided some of the first quantitative data on the natural diet (Rand et al., 1990). Focal animal observations were made on Iguana iguana for 378 hr (Rand et al., 1990). Approximately 145 min of the 378 hr of observations were spent feeding (0.6% of the time). Stomach samples of 31 animals were analyzed and found to contain primarily leaves from trees and vines. Stomachs from more than half the animals contained leaves from only one plant species. Other major components of the diet were blossoms, buds, and

fruits.

There are some sporadic reports of individual iguanas eating atypical feedstuffs such as animal or insect matter (Hirth, 1963; Arendt, 1986; Loftin and Tyson, 1965). These observations appear to be isolated occurrences, but they have led to confusion about the green iguana's feeding strategy. Iguana iguana currently are considered strictly herbivorous and primarily folivorous (Rand et al., 1990; Troyer, 1984a,b; Iverson, 1982; Van Devender, 1982). Iverson (1982) defined truly herbivorous reptiles as those species whose diet consists only of vegetation throughout the year. Based on this definition, herbivory in extant reptiles occurs primarily in the families of Agamidae and Iguanidae (only three other extant herbivorous lizard species exist outside these two families) (Zimmerman and Tracy, 1989). Thus, of the 3,751 species of lizards (Halliday and Adler, 1986), herbivory is limited to approximately 50 species (Pough, 1973; Iverson, 1982; Zimmerman and Tracy, 1989), and folivory is apparently limited to the green iguana (Rand, 1978).

Several hypotheses have been set forth to explain why herbivory has not had a greater radiation among lizards. One hypothesis suggests that herbivorous reptiles are at a higher predation risk as a consequence of the need to forage (Szarski, 1962). Ostrom (1963) and Sokol (1967) suggested that changes in the morphology of the jaw prevented the evolution of an efficient grinding system to masticate plant

tissue and initiate plant digestion. Other hypotheses relate to body size. Herbivory is a feeding strategy found more frequently in larger lizards (Pough, 1973). Therefore, Pough (1973) suggested that smaller lizards have a higher weight-specific energy requirement than larger lizards even though total energy requirements are greater for larger lizards. Thus, a relatively low energy dense herbivorous diet may restrict energy intake too severely for smaller species. A higher weight-specific energy requirement may explain the size-related ontogenetic shift in diet from carnivory or insectivory to herbivory of some species (Rand, 1978) but does not provide an energetic explanation for the herbivorous diet apparently consumed by hatchling green iguanas (Van Devender, 1982). Hypotheses explaining the radiation of herbivory in lizards have not been rigorously tested, partially due to the lack of information on fiber digestion and energy requirements of herbivorous lizards.

Although young iguana were originally thought to be insectivorous (Swanson, 1950; Hirth, 1963; Henderson, 1974), it is now generally accepted that the green iguana is herbivorous, beginning immediately post-hatching (Van Devender, 1982; Iverson, 1982; Rand, 1978; Troyer, 1984a,b). Hatchling weight (approximately 10 g across several incubation conditions [Werner, 1988]) relative to adult body mass (perhaps 5000 g) is approximately 0.1-0.3%. Herbivory in mammals does not become a viable feeding strategy until

the digestive system is functional. This occurs peri-weaning. At this point, mammalian young are approximately 30% of adult weight (Derrickson, 1986; Troyer, 1984b). The extraordinary 100-fold difference between the timing of the onset of herbivory in mammals and in the green iguana is further evidence for the unique adaptations of the green iguana to its ecological niche.

Adaptations to Herbivory

The digestion of plant fiber by Iguana iguana requires behavioral, physiological, and morphological specializations and symbiotic microbial populations. As with all herbivorous species, mechanisms for the acquisition of fermentative microbial populations, an ability to tear and ingest leaves, a capacious organ for fermentation, and a mechanism for sufficient digesta retention are all important factors.

Adaptations to Herbivory: Behavioral Mechanisms

Dispersal behavior of hatchlings emerging from the nest site and social organization of Iguana iguana have been associated with the acquisition of microbial populations (Troyer, 1984c; 1984d). In mammals, there is close contact between young and adults, especially the mother. In

reptiles, parental care is limited; therefore, there have to be other behavioral mechanisms for the acquisition of microbial populations from the adults. Consumption of older conspecific feces by hatchlings has been reported (Troyer, 1982, Troyer, 1984c), and it is thought that fecal consumption is essential to establish microbial populations in the hindgut. Hatchlings must disperse from their nest site to have contact with the adults and access to the adult feces since adult feces are not found in nests (Troyer, 1982). The social contact between hatchlings and older conspecifics is limited to a short time period since hatchlings and adults generally occupy different ecological niches (Troyer, 1984c). Troyer (1982, 1984c) suggested that the dispersal pattern of hatchlings is influenced by their need to contact older conspecifics for the acquisition of microbial populations. However, fecal consumption by hatchlings does not increase bacterial concentrations in the hindgut and may increase growth rate only slightly (Troyer, 1982, 1984c).

Geophagy has been reported in Iguana iguana (Troyer, 1984c; Sokol, 1971). Troyer (1984c) suggested that this behavior is critical for the acquisition of microbial populations, especially in hatchlings from 0-3 weeks of age. Hatchlings offered soil had a higher concentration of bacteria in their hindgut and a slightly greater growth rate than control iguanas. The significance of Troyer's (1982, 1984c) findings regarding fecal and soil consumption are not

clear since the methods of sample collection and bacterial counting were not described. There is further confusion because the species of bacteria in the hindgut were not identified.

Adaptations to Herbivory: Dentition

Insectivorous lizards can select prey that are small enough to be swallowed whole (Throckmorton, 1976). Folivorous species would have a difficult time swallowing many of the items they ingest (e.g., large leaves). Iguana iguana possess teeth that allow them to crop leaves, using head movements around the atlantoocipital joint to assist in cropping. The teeth are pleurodont (attached to the side of the jaw), isodont (of equal length), and multi-cusped. There are 15-20 cusps on the anterior and posterior sides of the teeth, creating a very sharp serrated edge. The number of teeth ranges from 19-32 per jaw; older animals with larger skulls typically have more teeth (Throckmorton, 1976; Montanucci, 1968; Ray, 1965). Tooth replacement occurs in a wave pattern, starting with the teeth in the back of the mouth and proceeding to the anterior of the jaw (Kline and Cullum, 1984). In young animals, there are six replacement waves annually, with four teeth added per wave (Kline and Cullum, 1984, 1985). The fleshy tongue assists in manipulating the food in the oral cavity, but no mastication occurs (Throckmorton, 1976).

Adaptations to Herbivory: Digestive Organs and Digesta Retention

A capacious digestive organ and digesta retention are essential for microbial processing and the fermentative extraction of energy from plant fiber. The primary site of fermentation in Iguana iguana is the hindgut. The relative capacity (defined as the percentage of the wet digesta mass of the organ compared to empty body mass) of the stomach and small intestine is higher in the iguana than in mammalian herbivores (8.7% versus 2.6-3.2%, respectively), and the pH of the stomach is very low (pH=1.5) (Troyer, 1984a; Parra, 1978). Although there appears to be some disappearance of hemicellulose in the stomach, this disappearance may be a result of hemicellulose hydrolysis as a consequence of the high acid concentration (Troyer, 1984a). As might be expected, the concentration of volatile fatty acids (VFAs) is lowest in the stomach and highest in the hindgut (Troyer, 1984a). It is also unlikely that typical anaerobic microbes would be able to survive at the low pH of the stomach. The relative capacity of the hindgut (large intestine and cecum/colon) is 11.8%. In large nonruminants (horse, capybara) the relative capacity of the large intestine, cecum and colon combined is 11.4%, and in small nonruminants the relative capacity of the hindgut (large intestine, cecum, colon) is 4.7% (Parra, 1978; Troyer, 1984a). The relative size of the iguana hindgut is comparable to that of

mammalian nonruminant herbivores.

Some authors have described the hindgut of Iguana as consisting of a cecum and colon separated by a valve or mucosal folds (Parsons and Cameron, 1977; Guard, 1980), although histological evidence has not been provided. Iverson (1980, 1982) did not distinguish between these two compartments and described the hindgut as a colon containing two types of transverse valves (Iverson, 1980, 1982). Presumably, these valves increase the absorptive surface of the hindgut, and are important in regulating digesta retention. The presence and number of each type of valve is species-specific (Iverson, 1980; 1982). One type of valve, the circular valve, contains a sphincter muscle in some species. The circular valves, if present, are always proximal to the other type of valve, the semilunar valve. Iguana iguana has one circular valve and four to six semilunar valves (Iverson, 1980). The circular valve can occlude the lumen of the colon by 61-97% (n=20 animals) and the semilunar valves can occlude the lumen by 14-54% (Iverson, 1980). There are no significant ontogenetic changes with respect to the number of valves (Iverson, 1980). This observation provides some evidence that morphological adaptations for digesta retention, an important component of herbivory, are present in hatchlings.

Mean transit time of digesta is reported to be 3.1 days for hatchlings (n=7), 3.6 days for juveniles (n=5), and 5.5 days for adults (n=4) (Troyer, 1984b). These passage rates

were determined using glass beads or surveyor's tape as markers. The diet during these passage studies was leaves of Lonchocarpus pentaphyllus. There was no apparent effect of age of the leaf on mean transit time. However, the leaves were force-fed. The effect of force-feeding on digesta passage and nutrient metabolizability or digestibility in iguanas is unknown. Digesta are retained in the iguana foregut for less time and in the hindgut for more time than predicted based on relative weight or length of the specific organs (Troyer, 1984b).

Passage time for other lizard species has been calculated by several methods (e.g., mean time, peak appearance, 25%, 50% or 90% disappearance, transit time), using different markers with animals fed a variety of diets (all force-fed) as summarized by Zimmerman and Tracy (1989). The range of mean retention times in six herbivorous species is 3.1 days (Iguana iguana) to 5.5 days (Iguana iguana and Sauromalus obesus (chuckwalla)).

Adaptations to Herbivory: Volatile Fatty Acids and Microbial Populations

In vitro rates of volatile fatty acid production have been measured in two male iguanas (Iguana iguana). Hindgut contents were removed from the anesthetized males and placed in plastic bags (the type of plastic was not reported) that were flushed with oxygen-free carbon dioxide. Hindgut digesta subsamples were withdrawn from the bags at 15 min

intervals for 45 min. For the two animals, the rates of production (μmoles/hr) of acetate were 184 and 168, of propionate 68.4 and 11.6, and of butyrate 231 and 218. These values are equivalent to 0.185 and 0.153 kcal of metabolizable energy/hr (McBee and McBee, 1982). This amount of energy is estimated to be 30-38% of the required amount necessary for the animals to sustain themselves under field conditions (McBee and McBee, 1982; Nagy, 1982a). Concentrations of VFAs in the Iguana iguana hindgut (from 3 animals) are comparable to the concentrations of VFAs in the rumen, and higher than in the hindgut of several species of other nonruminants (Troyer, 1984a; Parra, 1978). These measurements provide some evidence that fermentation of fiber occurs in the hindgut, and fermentation of fiber may be an important source of energy. Nevertheless, the data represent a limited sample size.

Direct microscopic counts (clump-counts) of formalin preserved bacteria from hindgut contents of Iguana iguana (n=11) yielded concentrations of $30x10^9$ clumps of bacterial cells per gram of digesta. Colony counts from roll tubes in anaerobic conditions yielded 3.3 to 23.5 $x10^9$ bacterial cells per gram (McBee and McBee, 1982). It is interesting to note that the dominant bacteria in three animals were Clostridium species, and in eight animals the dominant bacteria were Leuconostoc species (McBee and McBee, 1982). Lachnospira species (McBee and McBee, 1982) and Lampropedia merismopediodes (Troyer, 1982) were also identified. No

bacterial species from the genera Bacteroides, Fusobacterium, or Ruminoccocus have been cultured from hindgut contents (McBee and McBee, 1982). Ciliated protozoa have been observed in some samples (Troyer, 1982) but not in others (McBee and McBee, 1982).

The hindgut of some iguanine lizards (e.g., Cyclura species) contains large populations of nematode worms (Oxyuridae and Atractidae families). A single, apparently healthy adult Cyclura carinata (ground iguana) had in excess of 15,000 worms in the colon (Iverson, 1979). This large infestation has not been observed in the gastrointestinal tracts of carnivorous or omnivorous lizards. Therefore, there has been speculation that these nematodes are not necessarily parasitic but may be commensalistic or mutualistic (Iverson, 1982). Iverson (1982) proposes that nematodes may aid in: 1) the mechanical breakdown or mixing of digesta, 2) producing useful waste products (e.g., vitamins, cellulase, VFAs), or 3) controlling the composition or number of other gastrointestinal tract microbes. McBee and McBee (1982) reported that nematodes were not present in remarkable numbers in the gastrointestinal tract contents they obtained from 11 animals. Thus, the role of nematodes in the gastrointestinal tract remains unclear.

Temperature and Digestion

Ectothermic species regulate body temperature primarily by absorption of heat from the environment (Pough and Gans, 1982), but physiological mechanisms may be important (Bartholomew, 1982). Behavioral responses to changes in environmental temperature are particularly important in maintaining the mean activity temperature. This temperature is defined as the arithmetic mean of body temperature at which a free-ranging animal engages in its ordinary routine (Pough and Gans, 1982). Many physiological processes are temperature sensitive, and some of these physiological changes can indeed help maintain a relatively stable body temperature. Temperature influences metabolic rate (Moberly, 1968a,b), ventilation rate (Giordano and Jackson, 1973), heart rate and cardiac output (Tucker, 1965; Baker and White, 1970), peripheral blood flow (Baker et al., 1972), pattern of glycogen utilization (Stolk, 1960), acid-base balance (Wood and Moberly, 1970), and embryonic development (Licht and Moberly, 1965).

The mean body (cloacal) temperature of adult Iguana iguana was reported to be 36.1 C (range 34.0-37.2 C) when mean ambient temperature was 29.6 C (range 29.0-32.0 C) (Hirth, 1963). McGinnis and Brown (1966) reported a mean cloacal temperature for 14 adults and 5 juveniles of 36.1 C (31.0-39.0 C) when mean ambient temperature was 32.1 C (29.0-33.5 C). Moberly (1968a) reported a mean cloacal

temperature of 33.8 C (26.0-40.4 C) but did not report the ambient temperatures. Based on these data, the self-selected body temperature is probably between 33 C and 36 C. This temperature is defined as the body temperature maintained by an ectotherm in a temperature gradient, including temperatures above and below the mean selected temperature, under controlled laboratory conditions (Pough and Gans, 1982). Panting begins when cloacal temperature reaches 42 C (McGinnis and Brown, 1966). The approximate lethal temperature is 46-47 C, and animals begin to move to shade when cloacal temperatures are between 41 C and 44 C (McGinnis and Brown, 1966).

Of particular interest is the effect of temperature on digestion. It has long been thought that reptiles must increase body temperature in order to digest their food efficiently (Huey, 1982). Unfortunately, results have been conflicting. In a study with Dipsosaurus dorsalis (desert iguana), gross energy metabolizability increased with increasing temperature (Harlow et al., 1976). However, Zimmerman and Tracy (1989) measured gross energy metabolizability in the same species and reported that it is unaffected by temperature. In both studies, metabolizability was determined at 33, 37 and 41 C, and animals were force-fed. Zimmerman and Tracy estimated the amounts to be force-fed based on the calculated field metabolic rate at each temperature. Harlow's group fed the same amount of food at each temperature. Therefore, Harlow

et al. may have grossly overfed their animals, especially at the lower temperature. Zimmerman and Tracy suggested that the overfeeding of the lizards may have resulted in a decreased metabolizability in Harlow's studies. In a study with green iguanas, temperature was manipulated by setting light cycles for either 4 hr or 8 hr of light per day (Troyer, 1987). Iguanas housed with lights on for 8 hr maintained higher cloacal temperatures, for a longer period of time, than the iguanas housed with lights on for 4 hr. Animals housed under the 8 hr cycle had higher dry matter metabolizability coefficients than the other group (Troyer, 1987). These animals were also force-fed, and the diet consisted of Lonchocarpus leaves. Differences in metabolizability may have been related to differences in temperatures but the results were confounded by differences in light:dark cycle.

The degree of nutrient metabolizability is influenced both by the rate of digesta passage and the rate of digestion (Van Soest, 1982). Retention time (measured using ^{51}Cr-labelled fiber) was shorter for Sauromalus obesus (chuckwalla), a herbivorous species, housed at 36 C than for lizards housed at 32 C (Zimmerman and Tracy, 1989). Zimmerman and Tracy also concluded that rate of digestion is slower at lower temperatures. However, Troyer (1987) reported that there was no significant difference in mean transit time at two temperatures. One might expect digestibility to be thermally stable since temperature

changes should influence both rate of passage and rate of digestion in a similar manner. Troyer (1987) speculated that fluctuations in body temperature may affect fermentative microbes, perhaps by affecting the number or the species of microbes present. Small sample sizes, force-feeding, the use of nutritionally incomplete diets, and short or no adaptation periods may all contribute to the apparent confusion about the effect of temperature on digestion.

Bioenergetics

The energetics of reptiles has received much attention since the late 1800s (reviewed by Benedict, 1932). There have been many publications on energy expenditure (average daily, resting, field metabolic rates, etc.) in reptiles since the early work. Several publications have summarized these studies (Bennett and Dawson, 1976; Bennett, 1982; Congdon et al., 1983; Andrews and Pough, 1985). Data on energetics have been used to help explain activity patterns, allometric relationships, feeding strategies, and other ecological relationships. There has been no attempt to develop a comprehensive system to describe energy utilization and flow in reptiles. Such a system can be critical for comparing animal performance and the value of different feeds (Lofgreen and Garrett, 1968; Moe and

Tyrrell, 1973).

There are several problems associated with measuring energy expenditure in reptiles (McDonald, 1976). Some of these problems relate to changes in energy expenditure as a function of ambient temperature, but temperature can be easily adjusted under controlled conditions. Other problems relate to the methods of measurement. Indirect methods of calorimetry are commonly used to measure energy expenditure. These methods include open- or closed-respiratory exchange systems, and doubly-labelled water turnover. There are inherent problems with measurement of energy expenditure based on the turnover of doubly-labelled water ($^2H_2{}^{18}O$) (Lifson et al., 1955; Nagy, 1980; Nagy and Costa, 1980). However, the $^2H_2{}^{18}O$ technique is very useful, especially in long-term studies with free-ranging animals. Oxygen consumption has been measured by respiratory exchange under a variety of conditions, and in some studies, energy expenditure has been calculated based on carbon dioxide production. Measuring CO_2 production is an attractive method because it is relatively simple, but it is also inaccurate. Energy equivalents of oxygen consumed or carbon dioxide produced vary according to the substrate being oxidized. Measurement of energy expenditure of reptiles based on CO_2 production has additional drawbacks. Reptiles can have variable respiratory quotients (RQs) that can be below 0.7. Two possible explanations for the unusually low RQs sometimes observed in reptiles are: 1) the retention of

CO_2 as bicarbonate during gastric acid secretion and the urinary excretion of bicarbonate, and 2) the nasal secretion of bicarbonate from nasal salt glands (McDonald, 1976; Schmidt-Nielson et al., 1963). The errors associated with CO_2 production are greater when measurements are made over short periods of time. Because of these problems, it is common to report results of energetic experiments directly as the rate of oxygen consumption rather than to assign energy equivalents. Another problem related to measuring energy expenditure indirectly is that many species, including Iguana iguana, have a relatively large capacity for anaerobic metabolism (Moberly, 1968a,b). Thus, short-term measurements may not provide an accurate estimate of aerobic metabolism.

Field metabolic rate (FMR) is a measurement of average daily energy expenditure. FMR has been measured in nine iguanid species, and it is related to body mass as described by the relationship FMR (kcal/d) = 0.0535body weight $(g)^{0.80}$ (Nagy, 1982a). This relationship is based on CO_2 production measured using doubly-labelled water (Lifson et al., 1955). Respiratory quotients (RQ) are used to calculate energy expenditure from carbon dioxide production, but the RQs used for the FMR relationship have been measured in only one herbivorous lizard (Sauromalus obesus, desert iguana) (RQ=0.93) (Nagy and Shoemaker, 1975) and one insectivorous lizard (species not identified) (RQ=0.75) (Nagy, 1982). An additional problem with this allometric relationship is the

relatively narrow weight range of the nine species used (0.5 g to 1,481 g; only three species were greater than 100 g and there is only one observation over 1,000 g). In addition, most of the nine species are desert-adapted animals (Troyer, 1983). Nevertheless, this equation is an important step in understanding allometric energy relationships in lizards.

Moberly (1968a,b) measured O_2 consumption directly in Iguana iguana but used animals in a limited size range (370-1220 g), and the animals were physically restrained. The relationship between body size and energy expenditure is important because of its influence on feeding strategy. Pough (1973) emphasized that smaller animals have higher weight-specific energy requirements. This fact may have an impact on the evolution of herbivory in lizards since most herbivorous species are large. However, even 10 g Iguana iguana hatchlings are herbivorous (Iverson, 1982). Therefore, Iguana iguana hatchlings may need to be more selective feeders than adults to ensure that their diet can be easily fermented and is high in digestible energy (Troyer, 1987).

Mammalian arboreal folivores generally have atypically low metabolic rates (McNab, 1986). Iguana iguana does not appear to have an unusually low metabolic rate even though it is an arboreal folivore (Andrews and Pough, 1985). This analysis is based upon actual oxygen consumption, but the data were not collected under standardized conditions (e.g., animals were physically restrained) (Moberly, 1968a).

Digestive Physiology and Function

The digestive physiology differences between comparably-sized endotherms and ectotherms occur at several levels, including differences in organ weight, small intestine length and surface area, and *in vitro* substrate uptake capacity. Liver, kidney, heart and brain are smaller (by weight) in *Amphibolurus nuchalis* (body weight = 34 g) than in *Mus musculus* (body weight = 29 g) (Else and Hulbert, 1981). In addition, the length of the small intestine is approximately half as long in ectotherms (*Dipsosaurus dorsalis*, *Sauromalus obesus*) than in endotherms (*M. musculus*, *Neotoma lepida*) (Karasov and Diamond, 1985; Ferraris et al., 1989). Furthermore, the total surface area of the small intestine is smaller in ectotherms than in endotherms. This difference in surface area may be important in explaining the slower rate observed in ectotherms than in endotherms of carrier-mediated transport of D-glucose and L-proline (Karasov et al., 1985; Karasov and Diamond, 1985; Ferraris et al., 1989). Ectotherms also have lower mitochondrial volume density, and smaller cristae and inner mitochondrial membrane surface areas than endotherms (Else and Hulbert, 1981).

Metabolizability of nutrients has not been rigorously studied in reptiles. Limited amounts of daily food consumption and long rates of digesta passage make metabolizability studies difficult to conduct and

time-consuming. Digestion of dry matter, energy, and fiber has been determined in several species of lizards and summarized by Zimmerman and Tracy (1989). There have been some technical problems associated with the measurement of digestibility of these nutrients. In most studies performed to date, animals have been force-fed. In many studies, animals have been fed nutritionally incomplete diets. For instance, some diets used to measure digestibility have been sweet potato tubers (Throckmorton, 1973), Opuntia fruit (Christian et al., 1984), or dandelions (Ruppert, 1980). A commercially available rabbit diet has been used in some studies (Harlow et al., 1976; Zimmerman and Tracy, 1989). Dry matter metabolizability ranges from 45-86% (Karasov et al., 1986; Voorhees, 1981), gross energy metabolizability ranges from 48-86% (Christian et al., 1984; Throckmorton, 1973) and neutral detergent fiber (NDF) digestibility ranges from 21-81% (Karasov et al., 1986; Voorhees, 1981). These values should be regarded as preliminary because of the potential problems associated with force-feeding, inadequate adaptation to diet, and potentially inappropriate diets.

Troyer (1984a) measured dry matter and gross energy metabolizability of Lonchocarpus pentaphyllus leaves at different stages of maturities in hatchling (n=9), juvenile (n=10) and adult (n=4) Iguana iguana. The animals were force-fed and there was no indication of an adaptation period to the diet. Dry matter digestibility ranged from 46-53% and NDF digestibility ranged from 38-57%. There was

no effect of animal age (hatchling vs juvenile vs adult) on digestibility.

EFFECT OF DIETARY FIBER ON DAILY INTAKE, RATE OF GROWTH, AND NUTRIENT AND ENERGY METABOLIZABILITY

INTRODUCTION

The green iguana (Iguana iguana) is a herbivorous lizard. This feeding strategy is unusual in extant lizards, and it occurs primarily in two families. Furthermore, of the 3,751 lizard species (Halliday and Adler, 1986), the green iguana is probably the only truly folivorous lizard (Rand, 1978). Thus, its ability to digest plant fiber is interesting from an ecological and evolutionary perspective. Aside from its unusual feeding strategy, the green iguana has also been an important source of nutrients, particularly protein, for some of the people in Central and South America. People in these regions have hunted and consumed this species for many years. Overhunting and habitat destruction have decimated local populations and have prompted the development of new techniques for captive management and farming of this species.

Reptilian herbivores may be different from mammalian herbivores in many respects, although it appears that the process of fiber digestion is similar. Anaerobic microbial species have been identified from the hindgut of the green

iguana (McBee and McBee, 1982), including cellulolytic species. Volatile fatty acid production rates have been measured in samples of digesta taken from the hindgut (Troyer, 1984a; McBee and McBee, 1982). Furthermore, morphological adaptations in the hindgut provide a mechanism for digesta retention for fermentation (Iverson, 1980, 1982).

While there may be similarities in fiber digestion, there are many metabolic and feeding differences between herbivorous mammals and reptiles. Some of these differences include metabolic rate, thermoregulatory mechanisms, comminution patterns, and digestive capacity. Metabolic rates are lower in reptiles and thermoregulation is achieved primarily by behavioral responses to ambient conditions. Comminution patterns are different in reptilians as a consequence of jaw structure. In addition, digestive capacity may be lower in reptilian herbivores than in comparably-sized mammalian herbivores (Karasov et al., 1985; Ferraris et al., 1989).

Diet metabolizability and digestibility has been determined in some species of reptilian herbivores but methodological problems with feeding may have biased previously reported data. Diet formulation for captive green iguanas, and its impact on animal performance are key issues in developing diets for captive management of this important source of nutrients for people of Central and South America. The goal of this research project was to

determine if established methods of assessing diets, such as digestion and metabolism trials, could be applied to the green iguana. In addition, the relationships between plant fiber intake and nutrient digestibility, metabolizability, and growth rate are not known. Therefore, this study was designed to determine the effect of three different levels of dietary plant fiber on dry matter intake, fiber digestibility, dry matter and energy metabolizability, and growth rate of captive green iguanas fed a manufactured diet.

MATERIALS AND METHODS

Twenty-one individually-housed green iguanas (Iguana iguana) were used to determine the effect of dietary fiber on nutrient intake, digestibility, metabolizability, and growth rate. The animals were hatched in Panama as part of a captive breeding program and brought to the National Zoological Park (Washington, DC) for this study. The iguanas were approximately 2.5 yr old at the beginning of this study. The study began in May, 1988 and ended in April, 1989. Many of the animals were infested with protozoa or other flagellate parasites (observed either directly or by flotation techniques) and all animals were treated orally with ivermectin (MSD Agvet, Rahway, NJ) twice prior to the start of the study. Animals were housed in cages with plywood sides and backs, and hardware cloth tops and floors. There was a shelf in each cage. The cage was approximately 66 cm wide, 53 cm high, and 51 cm long. Overhead fluorescent lights were on a 12:12 L:D cycle, and suspended approximately 0.5 m over each cage was an infrared light to provide heat. A sheet metal tray was placed under the hardware cloth bottom of each cage to collect excreta. All procedures used in this study were approved by the Animal Welfare Committee of the National Zoological Park.

This study was designed as a Latin-square crossover

study. Three levels of dietary fiber were fed to each iguana. The iguanas were blocked into homogeneous groups based on body weight (a total of 7 blocks) and randomly assigned to one of three diets, and fed each diet in a random order. The iguanas consumed the diet for approximately 12 wk, and then they were gradually switched to a different diet (over a 7 to 10 d period). Thus, each iguana received each one of the three diets during the three different periods.

The diets were meal-type diets that the iguanas readily consumed without the need for force-feeding. Diets were purchased from a commercial supplier (Zeigler Bros. Inc., Gardners, PA) and stored in a freezer for the length of the study. Two batches of diet were purchased. One batch was used for periods 1 and 2 and the second batch was used for period 3. Nutrient intakes were calculated based on diet composition of each batch in order to control for small changes in nutrient concentrations between the two batches. The three diets were similar in chemical composition except for the concentrations of plant fiber. Plant fiber levels were manipulated by altering the relative amount of corn, soybean meal and alfalfa meal. Small adjustments were made in the limestone concentration of the diets to maintain an appropriate calcium-to-phosphorus ratio (Table 1).

Table 1. Ingredient composition of three diets with three levels of dietary fiber (% of total).

Ingredient	Low fiber	Medium fiber	High fiber
Corn, ground	27.7	16.7	5.7
Soybean meal, 48.5% CP	36.5	33.0	29.5
Alfalfa meal, dehy, 17% CP	10.0	25.0	40.0
Wheat bran	15.0	15.0	15.0
Soybean oil	3.0	3.0	3.0
Meat and bone meal	2.0	2.0	2.0
Molasses, cane	2.0	2.0	2.0
Limestone	2.0	1.5	1.0
Calcium phosphate, dibasic	0.8	0.8	0.8
Vitamin premix[1]	0.4	0.4	0.4
Salt	0.4	0.4	0.4
Mineral premix[2]	0.2	0.2	0.2

[1]Formulated to contain 4,000,000 IU vitamin A/kg, 600,000 IU vitamin D/kg, 48,000 IU vitamin E/kg, 1,200 ppm vitamin B_1, 2,000 ppm vitamin B_2, 2,400 ppm vitamin B_6, 36,000 niacin, 9,600 pantothenic acid, 100 ppm biotin, 1,600 ppm folic acid, 6 ppm vitamin B_{12}, 600,000 ppm choline chloride.

[2]Formulated to contain 40,000 ppm iron, 4,000 ppm cooper, 40,000 ppm zinc, 52,000 ppm manganese, 160 ppm iodine, 80 ppm selenium.

Animals were weighed, and cloacal temperature and snout-vent length were measured at the beginning and end of each period. Animals were weighed on an electronic balance. Snout-vent length was measured by placing the snout of the iguana flush with the end of a board and marking the location of the cloaca. Cloacal temperature was measured using an electronic thermometer, immediately after the iguanas were removed from their cages to avoid a temperature rise associated with agitation due to handling. The thermocouple was lubricated with KY jelly and placed at least 6 cm into the cloaca. The temperature was recorded after approximately 1 min.

Iguanas were offered fresh diet three times per week (Monday, Wednesday and Friday), usually in the morning. Enough diet was offered to ensure that diet was always available. Amounts offered were increased as the iguanas grew, and their intake increased. Food consumption was determined by weighing the amount of diet offered and reweighing the uneaten portion 24 or 48 hr later. Any diet that had spilled was collected and reweighed. The amount of diet consumed was determined as the difference between the amount offered and the amount reweighed.

Metabolism trials were started after the iguanas had consumed the diet for approximately 8 wk. To determine the starting and ending points of excreta collection, iguanas were pulse-dosed with 0.15% brilliant blue (FD&C #1, Allied Chemical Corp., Morristown, NJ) in their diet for 1 day.

Collections started when the marker appeared in the feces. Fecal and urinary excreta were collected together and pooled for an individual over the collection period, which lasted approximately 4 wk. The samples were collected into pre-tared containers. During the collection periods, fresh diet was offered on a daily basis. Samples of at least 5 kg of diet were collected and stored in a freezer for subsequent nutrient and energy analyses.

Excreta samples were freeze-dried and immediately reweighed. The samples were then ground in a Wiley mill fitted with a 2-mm screen. Subsamples were dried in a forced-air convection oven at 105 C to determine dry matter content of the previously freeze-dried samples. The corrected dry matter was determined as the product of the freeze-dried weight and the weight of the oven-dried subsamples. Diets and excreta were analyzed for ash, neutral detergent fiber, acid detergent fiber, acid lignin, and gross energy. Diets were also analyzed for crude fat and minerals. Crude fat was determined by methylene chloride extraction (FES 80, CEM Corp., Matthews, NC). Minerals were analyzed by a commercial laboratory (New York Dairy Herd Improvement Corp., Ithaca, NY). Ash was determined by combustion at 650 C. Gross energy was determined by adiabatic bomb calorimetry (Parr Instrument Co., Moline, IL). The residue remaining after combustion of the subsample in the bomb was titrated with 0.0725 N sodium carbonate to correct for the heat of formation of nitric

acid and sulfuric acid. Fiber fractions were determined by the methods of Van Soest (Robertson and Van Soest, 1981) using a Fibertec system (System M, Tecator AB, Höganäs, Sweden). Heat-stable α-amylase (Sigma Chemical Co., St. Louis, MO, catalog # A-3306, 200 µl/sample) and protease (Sigma P-3910, 5 mg/sample) were used to facilitate filtration during fiber analysis. Neutral detergent fiber (NDF) samples were preincubated with α-amylase for 1 hr at 60 C and then incubated with the protease for 1 hr at 80 C prior to refluxing with the neutral detergent. Cellulose was calculated as the difference between acid detergent fiber (ADF) and acid lignin (AL) and hemicellulose was calculated as the difference between NDF and ADF.

The excreta was comprised of a combination of fecal and cloacal wastes. The apparent digestibility of fiber fractions could be calculated since there were no quantitatively significant fiber components excreted in non-fecal wastes. However, for other nutrients and energy, metabolizability coefficients were calculated since the excreta contained a non-fecal source of these nutrients and energy. Metabolizable energy intakes were determined as the difference between gross energy intake and gross energy of the excreta. No correction was made for energy lost in skin through ecdysis. Nutrient intakes were calculated on a daily basis to correct for differences in the length of the collection periods for different iguanas. Metabolizability and digestibility coefficients were calculated using the

direct method (without internal or external markers) since total excreta collections were feasible (Schneider and Flatt, 1975). Apparent metabolizability or digestibility coefficients were calculated as:

$$\frac{\textit{nutrient intake (g or kcal)} - \textit{nutrient excreted (g or kcal)}}{\textit{nutrient intake (g or kcal)}} 100$$

Mean daily growth rate was determined as the difference between final and initial weight, divided by the number of days between weighing.

Data were analyzed with PC-SAS version 6.03 (SAS Institute Inc., Cary, NC). The data were first analyzed for normality (PROC UNIVARIATE) and subsequently analyzed for homogeneity of variance. Variance was analyzed by Spearman correlation analysis of the absolute values of the residuals and predicted values, and by graphical analysis of the plot of the residuals and predicted values. If these two assumptions of analysis of variance were met (normality and homoscedasticity of variance), analysis of variance was used to determine the effects of diets (PROC GLM). The statistical model included square, iguana(square), period(square), and diet. Least-squares means (LSMEANS) and probability of the difference of the least-squares means (PDIFF) were used to determine diet effects. Gross energy of feces from one animal during one period (diet 2) was not determined (insufficient sample size) so metabolizable energy content and energy metabolizability was not determined for that individual.

RESULTS

Mean cloacal temperature of the iguanas used in this study was 31.8 ± 0.2 C (n=48). Chemical composition of the three diets is presented in Tables 2 and 3. Mean initial body weight of iguanas is presented in Table 4. Although the iguanas were randomly assigned to a sequence of diets, at the beginning of each period, mean body weight of iguanas on the medium fiber diet (606.3 g) was lower than mean body weight when the iguanas started the low fiber diet (540.1 g, $p<0.0425$). Mean initial body weight of iguanas fed the high fiber diet was 601.4 g. Mean initial body weights were not different between iguanas when fed the high fiber diet and medium fiber diet, or when fed the high fiber and low fiber diets.

Mean final body weight was lower when iguanas were fed the high fiber diet (750.1 g) than when they were fed the medium fiber diet (861.2 g, $p<0.0041$). Mean final body weight was also lower when iguanas were fed the low fiber diet (769.8 g) than when they were fed the medium fiber diet ($p<0.0155$) (Table 4).

Rate of body weight gain was lowest when iguana were fed the high fiber diet (1.42 g/d). This rate was lower

Table 2. Nutrient composition of three diets with different levels of dietary fiber.[1]

Nutrient	Low fiber		Medium fiber		High fiber	
	Mean	SE	Mean	SE	Mean	SE
Dry matter (%)	91.4	0.8	91.7	0.6	92.0	0.6
Crude protein (%)[2]	29.3	0.4	28.7	0.4	29.1	0.3
Crude fat (%)	6.2	0.1	6.4	0.2	5.8	0.2
Crude fiber (%)[2]	5.9	0.1	9.3	0.5	12.4	0.7
Acid detergent fiber (ADF) (%)	11.0	0.2	16.0	0.3	19.6	0.6
Neutral detergent fiber (NDF) (%)	18.6	0.6	23.6	0.6	27.3	0.7
Lignin (%)	2.5	0.1	4.5	0.1	6.5	0.8
Cellulose (%)[3]	8.5		11.5		13.1	
Hemicellulose (%)[4]	7.6		7.6		7.6	
Ash (%)	8.5	0.1	8.8	0.1	9.5	0.1
Gross energy (kcal/g)	4.51	0.02	4.52	0.01	4.51	0.03

[1] Mean of 2-6 analyses.

[2] Analyzed by commercial laboratory.

[3] Calculated as difference between ADF and lignin.

[4] Calculated as difference between NDF and ADF.

Table 3. Mineral composition of three diets with different levels of dietary fiber.[1,2]

Nutrient	Low fiber		Medium fiber		High fiber	
	Mean	SE	Mean	SE	Mean	SE
Calcium (%)	2.02	0.21	2.06	0.06	1.96	0.20
Phosphorous (%)	0.87	0.04	0.83	0.04	0.82	0.05
Magnesium (%)	0.30	0.01	0.31	0.02	0.33	0.00
Potassium (%)	1.61	0.03	1.80	0.01	2.05	0.05
Sodium (%)	0.20	0.02	0.22	0.02	0.20	0.01
Iron (ppm)	471	16	553	40	615	4
Zinc (ppm)	253	5	278	12	267	16
Copper (ppm)	33	2	35	3	35	2
Manganese (ppm)	293	2	327	23	317	25
Molybdenum (ppm)	2.9	0.4	3.9	0.4	3.7	0.1
Selenium (ppm)	0.38	0.00	0.41	0.03	0.43	0.02

[1] Mean of 2 analyses

[2] Analyzed by commercial laboratory

Table 4. Body weight, rate of gain, and feed efficiency of iguanas fed different levels of dietary fiber (N=63 observations).

Variable	Units	Mean			SEM	Probability		
		Low fiber	Medium fiber	High fiber		Low fiber vs medium fiber	Medium fiber vs high fiber	Low fiber vs high fiber
Initial body weight	g	540.1	606.3	601.4	21.9	0.0425	0.8760	0.0589
Final body weight	g	769.8	861.2	750.1	25.0	0.0155	0.0041	0.5813
Rate of gain	g/d	2.22	2.35	1.42	0.21	0.6638	0.0037	0.0106
Feed efficiency	g feed/ g gain	3.58	3.93	6.37	0.75	0.7468	0.0286	0.0137

($p<0.0037$) than when the iguanas were fed the medium fiber diet (2.35 g/d) or when they were fed the low fiber diet (2.22 g/d, $p<0.0106$). There was no difference in rate of body weight gain when the iguanas were fed the medium and low fiber diets (Table 4).

Nutrient and energy intakes are presented in Table 5. The minimum length of the excreta collection period was 7 d and the maximum length was 25 d. As a consequence of the difference in the length of the collection periods, these data are presented on a daily basis. Daily dry matter intake was also measured during the entire length of the three periods as well as during the shorter excreta collection period. The first period lasted between 88 and 99 d (mean 90 d), the second period lasted between 88 and 116 d (mean 104 d), and the third period lasted between 110 and 131 d (mean 122 d). Daily dry matter intake was not different between the three diets. During the complete period, the overall mean daily dry matter intake was 9.71 g/d/kg BW. During the excreta collection period, the overall mean daily dry matter intake was also not different between the three diets. The range in daily dry matter intake was from 1.08 to 15.98 g/d/kg BW and the overall mean daily dry matter intake during the fecal collection period was 7.14 g/d/kg BW. Likewise, daily organic matter, gross energy and metabolizable energy intakes were not different between the three diets. NDF, ADF, hemicellulose, and

Table 5. Nutrient and energy intake of iguanas fed different levels of dietary fiber (N=63 observations).

Variable	Units	Mean			SEM	Probability		
		Low fiber	Medium fiber	High fiber		Low fiber vs medium fiber	Medium fiber vs high fiber	Low fiber vs high fiber
Dry matter intake	g/d/kg bw	6.99	7.57	6.86	0.62	0.5178	0.4256	0.8789
	% bw/day	0.70	0.76	0.69	0.06	0.5178	0.4256	0.8789
Organic matter intake	g/d/kg bw	6.40	6.90	6.21	0.56	0.5389	0.3938	0.8089
NDF intake	g/d/kg bw	1.31	1.80	1.93	0.14	0.0219	0.5180	0.0047
ADF intake	g/d/kg bw	0.86	1.23	1.32	0.10	0.0180	0.5159	0.0037
Cellulose intake	g/d/kg bw	0.68	0.87	0.84	0.07	0.0675	0.7188	0.1346
Hemicellulose intake	g/d/kg bw	0.46	0.58	0.61	0.06	0.1493	0.6674	0.0658
Metabolizable energy intake	kcal/d/kg bw	22.22	23.17[1]	19.13	2.11	0.7609	0.2012	0.3112
	kcal/g dry matter	3.20	3.00[2]	2.81	0.05	0.0148	0.0237	0.0001
Gross energy intake	kcal/d/kg bw	29.09	32.08	28.01	2.61	0.4251	0.2800	0.7719

[1]SEM ± 2.23, N=62 observations.
[2]SEM ± 0.06, N=62 observations.

cellulose intakes were not different between the medium and high fiber diets but NDF and ADF intakes were lower on the low fiber diet than the high fiber diet, and lower on the low fiber diet than the medium fiber diet. On the otherhand, cellulose and hemicellulose intakes were not different between the three diets.

Nutrient and energy metabolizabilities, and fiber digestibilities are presented in Table 6. In general, metabolizability of nutrients and energy decreased with increasing concentrations of dietary fiber. Dry matter, organic matter and gross energy metabolizability were highest on the low fiber diet and lowest on the high fiber diet. Mean dry matter digestibility was 65.8% on the low fiber diet and 57.8% on the high fiber diet. Mean dry matter digestibility of the medium fiber diet was 62.2%. Dry matter metabolizability was not different between the medium and low fiber diets ($p<0.0680$). Organic matter metabolizability was 69.6%, 65.7%, and 60.7% for the low, medium, and high fiber diets, respectively. Gross energy metabolizability was 70.7%, 66.4%, and 62.3% for the low, medium, and high fiber diets, respectively.

Neutral detergent and acid detergent fiber digestibilities were not different between the medium and high fiber or between the low and medium fiber diets. However, NDF and ADF digestibilities were higher on the low fiber diet than the high fiber diet. Mean NDF digestibility of the low fiber diet was 47.1%, and was 38.5% on the high

fiber diet ($p<0.0026$). Mean ADF digestibility was 40.7% on the low fiber diet and 27.8% on the high fiber diet ($p<0.0103$). Cellulose digestibility was higher ($p<0.0266$) on the low fiber diet (44.4%) than on the medium fiber diet (34.4%), and was higher ($p<0.0003$) on the low fiber diet than the high fiber diet (21.5%). However, cellulose digestibility was not different between the low and medium fiber diets. Hemicellulose digestibility was not different between the medium and high fiber diets (58.5% and 58.6%, respectively) but was lower on the low fiber diet (27.5%) ($p<0.0106$ and $p<0.0108$, respectively).

Table 6. Nutrient and energy metabolizability of diets with different concentrations of dietary fiber when fed to iguanas (N=63 observations).

Variable	Units	Mean			SEM	Probability		
		Low fiber	Medium fiber	High fiber		Low fiber vs medium fiber	Medium fiber vs high fiber	Low fiber vs high fiber
Dry matter metabolizability	%	65.8	62.2	57.8	1.4	0.0680	0.0283	0.0003
Organic matter metabolizability	%	69.6	65.7	60.7	1.2	0.0355	0.0087	0.0001
Energy metabolizability	%	70.7	66.4[1]	62.3	1.2	0.0173	0.0244	0.0001
Neutral detergent fiber digestibility	%	47.1	42.5	38.5	1.8	0.0891	0.1316	0.0026
Acid detergent fiber digestibility	%	40.7	34.9	27.8	3.3	0.2299	0.1367	0.0103
Cellulose digestibility	%	44.4	34.4	21.5	3.9	0.0816	0.0266	0.0003
Hemicellulose digestibility	%	27.5	58.5	58.6	8.0	0.0108	0.9917	0.0106

[1]SEM ± 1.2, N=62 observations.

DISCUSSION

The digestive tract morphology of the green iguana is well suited to support a symbiotic population of microbes for fiber digestion and to retain digesta for fermentation. In fact, the extent to which fiber digestion occurs has been measured in the green iguanas as well as in several other herbivorous reptilian species (Troyer, 1984a,b; Zimmerman and Tracy, 1989). All species in the subfamily Iguaninae, except one, possess two types of valves in the colon: 1) the circular valve, and 2) the semilunar valves (Iverson, 1980; 1982). The number and types of valves present is species specific (Iverson, 1980). It is thought that the function of these valves is to control digesta retention. The green iguana can retain digesta in the hindgut for fermentation as assessed by VFA production and fiber fraction disappearance (Troyer, 1984a). In fact, digesta is retained in the hindgut longer than expected (X^2 test) based on the relative volume and length of the hindgut as compared to the foregut and small intestine (Troyer, 1984a).

Several theoretical models have been suggested to explain and describe the relationship between the two competing processes of 1) rate of digesta passage, and 2) rate of digestion (especially fermentation), and how these two processes may interact and influence daily intake and

animal performance. Most of these models have been developed for mammalian herbivores and have not been applied to non-mammalian species. The physical form of the diet is one important factor in determining physiological responses (e.g., food intake, nutrient digestibility, rate of digesta passage) to dietary manipulations. The relationship between particle size and voluntary feed intake has been known for many years (Minson, 1963) and continues to be of interest (Weston and Kennedy, 1984). The relationship between changes in dietary plant fiber intake and nutrient intake, metabolizability and growth rate has not been studied in reptilian herbivores.

In this study, the iguanas were blocked by body weight and randomly assigned to a sequence of three levels of dietary fiber. Nevertheless, mean initial body weights were different between iguanas at the start of each of the three periods. The most extreme difference in mean initial body weight (66 g) was between iguanas fed the low fiber and the medium fiber diets. As a consequence of this difference, nutrient and energy intakes were adjusted to a per unit body weight (kg) basis for more appropriate comparisons between iguanas fed different diets.

Daily dry matter, organic matter, gross energy, and metabolizable energy intakes were not affected by changes in the concentration of dietary fiber. However, there were differences in the apparent metabolizability of these nutrients. Increased concentrations of dietary fiber

decreased metabolizability of these nutrients. These diets were formulated to contain different amounts of NDF and ADF, but the ratio of these two fiber fractions was similar between all three diets. Thus, the difference between NDF and ADF (the hemicellulose) was not different between the three diets. The difference in cellulose concentration was not the same for all diets, but the magnitude of increase from low to high fiber diets was not as great as that for NDF or ADF. Thus, there was no difference in cellulose and hemicellulose intake between the three diets. On the other hand, NDF and ADF intakes were different between the high and low fiber diets and the medium and low fiber diets, but intakes were not different between the high and medium fiber diets. The absence of a difference in NDF and ADF intake between the medium and high fiber diets may be related to a slight decrease in daily dry matter intake from the medium to the high fiber diet even though this change in daily dry matter intake was not significant. The increase in fiber concentration did not offset the decrease in intake, so daily fiber intakes were not different.

Fiber fraction digestibility (NDF, ADF, hemicellulose, and cellulose) were affected by fiber concentration. In general, fiber fraction digestibility increased with a decrease in NDF and ADF concentration. However, fiber fraction digestibility was not different between the medium and high fiber diets (except for cellulose), nor between the low and medium fiber diets (except for hemicellulose).

Fiber digestibility of the medium fiber diet was medial to that of the low and high fiber diets. However, the pattern of hemicellulose digestibility was different. Hemicellulose digestibility was not different between the medium and high fiber diets but was much lower on the low fiber diet than on the medium and high fiber diets. This difference may be related to analytical problems in determining NDF and ADF, especially in feces, and because hemicellulose is determined as a difference. Small errors in NDF and ADF analyses may result in a relatively large error in estimates of hemicellulose content (Van Soest, 1982; McAllan and Griffith, 1984). Furthermore, the endogenous fecal component may include compounds that are measured as part of the ADF fraction, and these compounds may be proportionally greater in the excreta from iguanas fed the low fiber diet than the other diets.

Differences in fiber digestion between the diets, especially the large difference between the low and high fiber diets, may be related to non-selective retention of feed particles in herbivorous lizards (Foley et al., 1987). Thus, all feed particles would have a similar rate of passage with little or no compensation for changes in diet composition. There is evidence that even fine particles can be selectively retained in the hindgut of mammalian fermenters, such as the rabbit (Warner, 1981) and in the ringtail possum (Pseudocheirus peregrinus) (Chilcott and Hume, 1985). Foley et al. (1987) reported that selective

retention of fine particles in the hindgut of Uromastyx aegyptius (a herbivorous Agamid lizard) did not occur, but the method of determining particulate retention was not described. If there is limited or no selective retention of particles, then the higher concentration of fiber fed to the iguanas on the high fiber diet in this study may have been more refractory to microbial attack. Microbial attachment requires time (Akin, 1986). Without additional retention time relative to the lower fiber diets, fiber digestion may be expected to be lower on the high fiber diet, as was observed (Table 6). Furthermore, if there was a change in digesta retention, it would be likely that daily dry matter intake would change, and no change in daily dry matter intake was detected (Table 5).

Another possible explanation for the observed decrease in fiber digestibility on the high fiber diet is related to the lignification index (percent lignin in the ADF) of the three diets. This index shows a strong negative relationship with NDF digestibility in vivo, presumably due to lignin's ability to resist microbial attack (Deinum, 1971; Van Soest, 1982; Jones and Wilson, 1987). The lignification index of the three diets was approximately 23%, 28%, and 33%, for the low, medium and high fiber diets, respectively (Table 2). The higher lignification index of the high fiber diet would suggest that this diet would have the lowest NDF digestibility, and that the low fiber diet would have the highest NDF digestibility. These predictions

are supported by the data collected from these green iguanas (Table 5).

Although dry matter intake and metabolizable energy intake were similar for all diets, energy metabolizability was different between all three diets, and it was lowest on the high fiber diet. Thus, the ME density (kcal/g dry matter consumed) was different in all three diets. The ME density was lowest on the high fiber diet and highest on the low fiber diet. Furthermore, the rate of daily gain was lowest, and the ratio of feed-to-gain (feed efficiency) was highest on the high fiber diet. There were no differences between the daily rate of gain and feed efficiency between the medium and low fiber diet but there were differences between the high fiber diet and the low and medium fiber diets.

Mammalian hindgut fermenters and ruminants generally respond to a decrease in digestible energy (DE) density of ground diets by increasing food intake. Thus, total energy intake and gain can remain constant. Rabbits can compensate with increased intake for decreased DE density from 3.51 to 2.08 kcal/kg DM to maintain a constant growth rate (Spreadbury and Davidson, 1978). Similarly, horses can compensate with increased intake for decreased DE density from 3.39 to 2.61 kcal/kg DM and maintain a constant growth rate (Laut et al., 1985). Foregut fermenters respond to changes in energy density in a similar manner (Montgomery and Baumgardt, 1965). The response to changes in DE density

from long or coarse forages is opposite to that of ground diets. This has been documented in ruminants (Minson, 1982), foregut marsupial fermenters (McIntosh, 1966; Forbes and Tribe, 1970), as well as in hindgut fermenters (e.g., horses) (Darlington and Hershberger, 1968).

The iguanas in this study were fed a meal-type diet that consisted of relatively small particles. There was little change in daily dry matter intake of the iguanas in this study in response to a change in energy density (in this case ME). However, the energy density change may not have been severe enough to alter daily dry matter intake. The change in ME density was only from 2.74 kcal/g dry matter to 2.39 kcal/g dry matter. This difference is smaller than the ranges that elicited a response in mammalian herbivores.

As a consequence of the relatively slow rate of digesta passage and large amount of individual variability (e.g., 1-8 d for the brilliant blue marker to appear in the feces), it was not possible to quantitate the rate of digesta flow of the iguanas in this study. Based on the observation that the brilliant blue was usually excreted as a single bolus on a single day, there was little evidence for much mixing or selective retention of the digesta. There was a clear and clean demarcation of the marked feces (e.g., those feces that were blue/green) with other feces, even when unmarked feces were excreted at the same time. This observation may provide additional support for Foley's conclusion based on

his observation in Uromastyx (Foley et al., 1987). However, the observation of little mixing or selective retention in the iguana may be a function of the type of diet fed, and selective retention may be more important when the more natural diet, such as fruits, flowers, and leaves, are consumed.

Nutrient and energy digestibility has been measured by others in herbivorous lizards, including the green iguana. These data are summarized in Table 7 and are adapted from Zimmerman and Tracy (1989). In many of these studies, the animals were force-fed (Ruppert, 1980; Zimmerman and Tracy, 1989; Troyer, 1984a,b, 1987; Karasov et al., 1986). In several studies, the lizards were fed only one item, such as sweet potato tubers, dandelion leaves, or Lonchocarpus leaves (Throckmorton, 1973; Ruppert, 1980; Troyer, 1984a, 1987). In the study reported by Throckmorton (1973), it appears that the lizards were fed only sweet potato tubers, and they were fed sweet potato tubers for at least four months prior to the start of measurements. In other studies, adaptation periods for these herbivorous species were as short as four days (Ruppert, 1980). Adaptation periods as short as four days may be inappropriate since many of these lizard species have mean retention times as long as 7 days (Zimmerman and Tracy, 1989). The effects of: 1) force-feeding, 2) monotypic diets, or 3) short adaptation period may result in data that are difficult to interpret. Dry matter metabolizability from previous studies ranged

from 45% to 86%, with an overall mean of 60% (Table 7). The dry matter metabolizability from the current study ranged from 58% to 68%, depending on the level of dietary fiber (Table 6). Energy metabolizability of diets fed to lizards in previous studies ranged from 48% to 83%, with an overall mean of 65%. Gross energy metabolizability of diets fed to iguanas in the current study ranged from 62% to 71%. Neutral detergent fiber digestibility from previous studies ranged from 21% to 81%, with an overall mean of 44%. Neutral detergent fiber digestibility in the current study ranged from 38% to 47%. Thus, despite potential methodological problems with previous studies, the overall mean from these studies agrees well with data collected from iguanas fed ad libitum, a nutritionally complete diet, and adapted to the diet for six to eight weeks prior to excreta collections.

Unfortunately, dry matter intake from several of the previous studies has not been reported. In several instances, investigators that force-fed their lizards fed the lizards an amount based on the estimated energy expenditure from an equation published by Nagy (1982a) (Ruppert, 1980; Troyer, 1987). Dry matter intake is not reported in other studies where lizards were fed ad libitum (Throckmorton, 1973; Christian et al., 1984).

The ability of green iguanas to digest fiber from these diets was comparable to that of other mammalian herbivores, including ruminants (Parra, 1978). Of course the absolute

amount of fiber digested on a daily basis was relatively low and the growth rate was relatively slow. However, the feed efficiency of these iguanas was comparable to that of domesticated species used for productive purposes (NRC 1984a,b, 1985, 1988). Again, the absolute rate of gain was relatively slow compared to production animals.

The data from these iguana provide important baseline data on intake, growth rate, fiber digestibility, and dry matter and energy metabolizability. This information is critical to formulation of diets for captive management and production. Daily intake and growth rate of iguanas when fed the medium fiber diet (24% NDF, dry matter basis) was not different from that when the same iguanas were fed the low fiber diet (19% NDF, dry matter basis). When the same iguanas were fed the high fiber diet (27% NDF, dry matter basis), daily dry matter intake did not change but growth rate and digestibility were depressed. However, these data are based on diets that may not reflect the diet of free-ranging green iguanas. Differences in the physical form of the diet consumed by free-ranging iguanas (e.g., large pieces of unmasticated leaves) may have a large impact on intake, growth rate, and digestibility, even with leaves of comparable nutrient composition. It is interesting to note that dry matter and neutral detergent fiber digestibilities of fresh picked Lonchocarpus leaves (47% NDF, dry matter basis) were similar to those of the diets used in the current studies (Troyer, 1984a, 1987) even though the NDF

content of the leaves was higher than the diets used in this study.

Table 7. Metabolizability of dry matter and energy and digestibility of neutral detergent fiber (NDF) in diets fed to herbivorous lizards.

Species	Temperature (C)	Diet	Metabolizability/Digestibility (%)		
			Dry matter	Energy	NDF
Conolophus subcristatus[1]	unknown	Opuntia fruit		48	40
Ctenosaura pectinata[2]	24-42	Sweet potato tuber		86	
Dipsosaurus dorsalis[3]	41	Rabbit diet		69	
Dipsosaurus dorsalis[3]	37	Rabbit diet		63	
Dipsosaurus dorsalis[3]	33	Rabbit diet		54	
Dipsosaurus dorsalis[4]	33	Rabbit diet	45		
Dipsosaurus dorsalis[5]	41	Rabbit diet	61	61	37
Dipsosaurus dorsalis[5]	37	Rabbit diet	63	61	40
Dipsosaurus dorsalis[5]	33	Rabbit diet	60	57	36
Iguana iguana[6]	31-36	Lonchocarpus pentaphyllus	53		46
Iguana iguana[7]	37 (8 hr)	Lonchocarpus pentaphyllus	56		
Iguana iguana[7]	34 (4 hr)	Lonchocarpus pentaphyllus	49		

Table 7. Metabolizability of dry matter and energy and digestibility of neutral detergent fiber (NDF) in diets fed to herbivorous lizards (continued).

Species	Temperature (C)	Diet	Metabolizability/Digestibility (%)		
			Dry matter	Energy	NDF
Sauromalus obesus[4]	37	Alfalfa pellets	47		21
Sauromalus obesus[8]	37	Dandelion leaves		65	
Sauromalus obesus[8]	20-37	Dandelion leaves		67	
Sauromalus obesus[5]	36	Rabbit diet	67		50
Sauromalus obesus[5]	32	Rabbit chow	65		49
Sauromalus obesus[5]	28	Rabbit chow	70		
Sauromalus varius[9]	unknown	Carrots, lettuce, yams, sunflower seedlings, dandelions	86	83	81

[1] Christian et al., 1984.
[2] Throckmorton, 1973.
[3] Harlow et al., 1976.
[4] Karasov et al., 1986.
[5] Zimmerman and Tracy, 1989.
[6] Troyer, 1984a.
[7] Troyer, 1987.
[8] Ruppert, 1980.
[9] Voorhees, 1981.

EFFECT OF AMBIENT TEMPERATURE ON DIGESTIVE FUNCTION, RATE OF GROWTH, AND BIOENERGETICS

INTRODUCTION

Ambient temperature is an important modulator of physiological and behavioral responses that occur in ectotherms, such as the green iguana (Iguana iguana). Temperature affects many diverse physiological functions, ranging from arterial oxygen concentration to water flux, but the effects of temperature on digestive physiology and bioenergetics are not well understood. Herbivory is relatively rare in reptiles, so many studies of digestive function have been performed using insectivorous or carnivorous reptiles. Moreover, the evolution of herbivory in lizards, and the ecology of herbivorous reptiles may be related to constraints imposed by ambient temperature. In addition, manipulation of temperature in captivity may be a technique that can improve management and propagation. Therefore, the relationship between temperature, herbivory, and an ectotherm's ability to extract energy from plant tissue has theoretical and practical significance.

Previous nutritional research on herbivorous lizards has been plagued with several problems. A serious problem

with many published studies is that the herbivorous lizards were force-fed (Zimmerman and Tracy, 1989; Troyer, 1987; Harlow et al., 1976). Force-feeding was necessitated by the fact that many captive lizards would not eat ad libitum. However, force-feeding may alter digestive processes and create difficulty with data interpretation. In fact, published data on the effect of temperature on fiber digestibility, and dry matter and energy metabolizability, are contradictory (Troyer, 1987; Zimmerman and Tracy, 1989, Harlow et al., 1976; Karasov and Diamond, 1985). The extent of nutrient and energy digestibility or metabolizability is a function of two competing processes, the rate of digesta flow, and the rate of digestion or fermentation (Van Soest, 1982). Changes in ambient temperature may affect both of these processes and have little net effect on apparent digestibility or metabolizability. The use of digested nutrients, and the partitioning of energy between maintenance functions and growth may also be modulated by temperature in ectotherms.

Whereas the effects of temperature on metabolic rate are well established in many reptilian species, bioenergetics are not well understood. The primary measure in many reptilian studies is the metabolic rate, usually the rate of carbon dioxide production or oxygen consumption (Bennett, 1976). The relationship between intake energy, energy expenditure, and the efficiency of use of energy above maintenance for growth is unknown. Manipulating

growth rate and maximizing efficiency of tissue deposition are of great importance for successful captive management and production of the green iguana as a source of food for humans in Central and South America.

Different methodologies are often necessary to study energy metabolism in the field as compared to a controlled laboratory environment. The doubly-labelled water (DLW) method is well suited for field studies. The principle of the DLW method is the measurement of the difference in flux between ^{2}H excreted as $^{2}H_2O$, and ^{18}O excreted as both $C^{18}O_2$ and $H_2{}^{18}O$ (Lifson et al., 1955). Once the isotopes are administered, the animal has to be recaptured only once to determine the average rate of carbon dioxide production. Respiratory exchange calorimetry is well suited for controlled studies in the laboratory. This method permits the direct measurement of carbon dioxide production and oxygen consumption. Few studies of lizards comparing these methods have been published (Congdon et al., 1978).

The objectives of this research were to determine the effect of two different ambient temperatures on nutrient intake, growth rate, nutrient metabolizability and bioenergetics, including the rate of energy expenditure and the efficiency of use of energy above maintenance for tissue deposition, in the green iguana. Furthermore, the study was designed to compare energy expenditure measured by the DLW method and by respiratory exchange indirect calorimetry. The iguanas were housed at two different ambient

temperatures, 35 C and 28 C. The first temperature, 35 C, was selected because it is near the iguana's self-selected body temperature (Moberly, 1968a). Since death can occur at temperatures above 40 C, a temperature lower than 35 C was selected as the second temperature. During tests at 25 C the iguanas did not eat, but they did eat at 28 C. Therefore, 28 C was selected as the second temperature.

MATERIALS AND METHODS

Animals and housing

Twelve individually-housed green iguanas (Iguana iguana) were used in this study. Two groups of six iguanas each were randomly assigned to either 28 C or 35 C ambient temperature. Each group was equally balanced for sex, as determined by femoral pore size. After the first period (54 d), the ambient temperatures were switched, and a second period (37 d for iguanas at 35 C, and 39 d for the iguanas at 28 C) was completed. The iguanas were hatched in Costa Rica as part of a captive breeding program (Fundación Pro Iguana Verde). The iguanas were approximately 6 months old in September, 1989, when they were brought to the Smithsonian Institution's National Zoological Park (Washington, DC), where these studies were conducted. All procedures were approved by the Animal Welfare Committee of the National Zoological Park.

The iguanas were acclimated to environmental chambers (Environmental Specialties, Inc., Raleigh, NC) for approximately 8 months prior to the beginning of this study. During the acclimation process, the iguanas were not individually housed but were housed as two groups in two environmental chambers. During this time period, the

iguanas were treated orally with ivermectin (MSD Agvet, Rahway, NJ) for external and internal parasites. Approximately 6 months prior to the start of data collection, the iguanas were moved to individual cages that were located inside the environmental chambers. The cages were approximately 66 cm wide, 53 cm high, and 51 cm long.

One environmental chamber was maintained at 28 C and the other at 35 C. Both chambers were maintained at 60% relative humidity. The chambers provided a stable temperature (± 0.1 C), and relative humidity (± 1.0%). Overhead fluorescent-type bulbs (Chroma-50®, General Electric Co.) were used for incidental lighting with a 12:12 light:dark cycle throughout the acclimation process and study. These bulbs may have also provided appropriate wavelengths for the bio-conversion of vitamin D_3 precursors to the active forms of vitamin D_3 (Allen, 1988).

Diet and measurement of food intake

The ingredients and chemical composition of the meal-type diet are presented in Tables 1, 2, and 3. The medium-fiber diet was used for this study. The diet was manufactured commercially (Zeigler Bros. Inc., Gardners, PA). Fresh diet was stored in a freezer for the entire length of the study. Only one manufactured lot of feed was used. Iguanas were fed in ceramic crocks three times each

week (Sunday, Tuesday, and Thursday). On these days, the diet remaining in the cage from the previous feeding (the orts) was collected, weighed, and discarded. Fresh diet was weighed into a ceramic crock to the nearest 0.1 g. An amount of new diet was provided in excess of current intake to ensure that there would be diet remaining the next time the crock was removed and remeasured. Any diet that spilled into the cage was collected. If an iguana had defecated in the feed crock, food intake was estimated using the mean food intake for the period immediately preceding and following that day's feeding. A subsample of approximately 30 g of fresh diet was subsampled from the manufactured lot of diet each time the animals were fed. These subsamples were composited by weight, and separately for each period. Fresh water was provided in ceramic crocks, and the amount of water was monitored each day to ensure fresh water was available ad libitum.

Total excreta and skin collections

During periods of excreta (fecal and cloacal waste combined) and skin collections for the balance and digestion trials, the excreta and skin were collected in separate, pre-tared containers. These containers were stored in a freezer until needed for nutritional analyses. Collections of excreta and skin were made from the metal tray located

beneath the hardware cloth floor of the cage and from the cage surface. One day each week (Sunday), all cages, trays, ceramic crocks, and environmental control chambers were scrubbed with appropriate cleaning solutions.

To determine the starting and stopping point for the collection of excreta samples for balance measurements, the iguanas were fed, once at the beginning and once at the end of the collection period, a measured amount of diet that had been mixed with brilliant blue (0.15% w/w, FD&C #1, Allied Chemical Corp., Morristown, NJ). When the feces appeared as a green or blue color, the excreta collections started. At the end of the collection period, the iguanas were again fed a measured amount of marked diet, and when the feces appeared green or blue, the collections stopped. Skin collections started and stopped at the same time as the excreta collections.

Indirect calorimetry

Indirect calorimetry was performed on all animals by respiratory exchange, and by the doubly-labelled water (DLW) method. Respiratory exchange calorimetry measurements were performed for three consecutive days at the beginning and end of each of the two periods. However, during the first period, the iguanas housed at 35 C were measured only twice at the beginning of the period, and three times at the end

of the period. The mean of the six (or five) measurements was used in subsequent calculations and statistical analyses. These measurements were performed in closed-system calorimeters. Doubly-labelled water was administered at the beginning of each period, after respiratory exchange measurements were completed.

Indirect calorimetry: respiratory exchange

Six closed-system calorimeters were constructed for respiratory exchange calorimetry by covering the inside of plywood shells with polyester resin-coated fiberglass (Figure 1). Following at least three coats of polyester resin on the fiberglass, the interiors of the calorimeters were painted with epoxy-based paint. Mean chamber volume was 0.304 m^3, based on measured linear dimensions. High density urethane foam (0.64 cm thick x 2.54 cm wide) was used to form a gasket between the fiberglass box and a 0.64 cm thick plexiglass top. The top was fastened to the box with 14 bolts to create an airtight seal. A normally-closed quick-connect sampling port (Swagelok Co., Solon, OH), and a mixing fan were mounted on the inside of the plexiglass tops. The mixing fan (1840 l/min capacity) was used to circulate the air within the chamber prior to gas sample collection. Rubber matting was placed on the floor of the calorimeter to prevent slipping when the iguanas walked

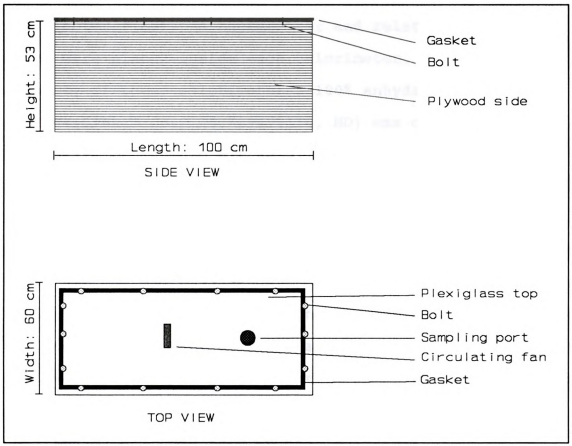

Figure 1. Schematic drawing of closed-circuit respiratory calorimeter.

across the floor. A piece of wood was also placed in each calorimeter to provide a climbing surface for the iguanas. When the iguanas were in the calorimeter, a piece of burlap was placed over the top of the chamber to minimize excitatory visual cues. Separate ceramic dishes containing a preweighed amount of diet and water were placed on the calorimeter floor. A thermometer and relative humidity meter were placed inside each calorimeter. To test the accuracy of these calorimeters, 100% anhydrous ethanol (Warner-Graham Co., Cockeysville, MD) was combusted. Initially, background air samples were collected. Small alcohol lamps were filled and weighed, placed into the calorimeter, and ignited. The calorimeter top was bolted in place and the ethanol was allowed to burn for approximately 1 hr. At the end of the hour, the mixing fan was turned on (which blew out the lamp) and the air was allowed to mix for at least 5 min. Subsequently, an air sample was pumped out of the calorimeter with a diaphragm pump, through anhydrous calcium sulfate (Drierite®, 4 mesh, W.A. Hammond Drierite Co., Xenia, OH), and into an 86-l polyvinyl fluoride bag (Tedlar®, E.I. du Pont De Nemours & Co., Wilmington, DE). The calorimeter top was then opened and the alcohol lamp removed, and reweighed. The amount of ethanol combusted was determined as the difference in weight. Recovery of oxygen and carbon dioxide was measured and compared to the theoretical values based on 3 moles of oxygen and 2 moles of

carbon dioxide produced per mole of ethanol combusted:

$$CH_3CH_2OH + 3O_2 \rightarrow 2CO_2 + 3H_2O.$$

Prior to being placed in a calorimeter for respiratory exchange measurements, the iguanas were weighed. The iguanas were placed in a calorimeter with the plexiglass top in place but not secured for approximately 36 hr. After 36 hr, the mixing fan was started and allowed to run for at least five minutes. The plexiglass tops then were closed and secured with bolts, and initial temperature and relative humidity were recorded. Based on initial temperature and relative humidity, initial dry air volume of the chamber was calculated. After approximately 24 hr, the mixing fan was started, and the air in the chamber was mixed for at least 5 min. A gas sample was then pumped out of the calorimeter, through the Drierite®, and into a Tedlar® bag. Normally-closed valves were used on the calorimeter top and on the gas collection bag. The tops were then opened, and the fans were left on to introduce fresh air into the calorimeters. Prior to being resealed for the next 24-hr period, a background gas sample was collected from inside the chamber.

The following day, the gases were pumped out of the Tedlar® bag and analyzed. Oxygen was analyzed by paramagnetic deflection using a Beckman Industrial (LaHabra, CA) Model 755A analyzer. Carbon dioxide was analyzed by infrared spectroscopy using a Beckman Industrial Model 880 non-dispersive infrared analyzer. These analyzers were

calibrated using two tanks of mixed gases whose concentrations were determined after calibrating the analyzers with appropriate gas standards from the National Institute of Standards and Technology (Gaithersburg, MD). Hydrogen gas concentration was determined using a Quintron Microlyzer Model 12 analyzer (Quintron, Inc., Milwaukee, WI). Gas permeability of the Tedlar® bag was tested by filling the bags, and analyzing the gas composition of the bag over a 48-hr period. No changes in gas composition were detected.

Respiratory exchange carbon dioxide production rate (pCO_{2re}, l/d) and oxygen consumption rate (pO_2 l/d) were calculated as:

$$pCO_{2re} = \frac{[CO_2]_{final} - [CO_2]_{initial} \ (chamber\ volume\ (l))}{elapsed\ time\ (\text{min})},$$

and

$$pO_2 = \frac{[O_2]_{final} - [O_2]_{initial} \ (chamber\ volume\ (l))}{elapsed\ time\ (\text{min})}.$$

These rates were converted to standard temperature and pressure, and expressed on a 24-hr basis. Energy expenditure from respiratory exchange (EE_{re}, kcal/day) was calculated as (Weir, 1949):

$$EE_{re} = 3.941pO_2 + 1.106pCO_2.$$

Respiratory quotient was calculated as:

$$RQ = \frac{pCO_2\,(l/d)}{pO_2\,(l/d)}.$$

Indirect calorimetry: doubly-labelled water

In addition to respiratory exchange, energy expenditure was measured by the doubly-labelled water technique. Stable isotopes of oxygen ($H_2{}^{18}O$) and hydrogen (^2H_2O) were administered to the iguanas at the beginning of each period, immediately after the respiratory exchange calorimetry was completed.

Prior to being infused with the isotopes, a baseline blood sample of 8-10 ml was taken by venipuncture on the ventral side of the tail, approximately 5 cm posterior to the cloaca, while the iguanas were manually restrained. A 12-cc syringe fitted with a 22-g x 1" needle was used for all blood collection. Samples of blood were placed in lithium heparinized tubes and gently mixed. The tubes were spun for 15 min in a refrigerated centrifuge at 2,000 RCF (3,000 rpm). The serum was collected and frozen (-20 C) in a sealed cryovial (#5000-0050, Nalge Co., Rochester, NY) until needed for isotope analyses. Isotopes were infused by gavage using a #12 stomach tube inserted into the stomach through a plastic syringe casing. The syringe casing was used to protect the gavage tube from being punctured by the

iguana's teeth, and it was wrapped with duct tape to minimize damage to the iguana's oral cavity. Deuterium oxide (^2H_2O, 99.9%, Cambridge Isotope Laboratories, Woburn, MA) and ^{18}oxygen ($H_2{}^{18}O$, 10.0% atom percent excess, (APE), Isotec, Miamisburg, OH) were mixed together in the ratio of 0.602 g ^2H_2O/g solution and 0.398 g $H_2{}^{18}O$/g solution. Approximately 5 g/kg body weight of the isotope solution was administered. The amount of isotope administered was quantitated by taring an empty syringe on an electronic balance, filling the tared syringe with the isotope solution, and reweighing the syringe. This weight was recorded to the nearest 0.01 g as the weight of the administered dose. When the isotope solution was administered, the time was recorded. After isotope administration and before the syringe and gavage tube were removed from the iguana's mouth, they were rinsed with 3-4 ml of water. Blood samples (8-10 ml) were drawn at day 2, day 11, day 21, day 34 and day 55 post-dosing during period 1, and on day 2, day 10, day 20, and day 41 during period 2. Each time a blood sample was taken, the time of sampling was recorded, and the iguana's weight, snout-vent length, and cloacal temperature were measured.

$H_2{}^{18}O$ APE of the plasma was analyzed by isotope-ratio mass spectroscopy by a commercial laboratory (Metabolic Solutions, Inc., Acton, MA). Previous analysis of $H_2{}^{18}O$ standards by this laboratory indicated that the results were accurate and precise (Seale et al., 1993). The ^2H_2O

concentration was determined by infrared (IR) spectroscopy (Miran 1FF, Foxboro Co., Foxboro, MA). Prior to analysis, subsamples of plasma (approximately 3 ml) were vacuum sublimed (Stansell and Mojica, 1968; Byers, 1979). An autosampler was used to deliver each sample to the spectrometer. The autosampler mixed the sample 1:1 with a BRIJ® solution to prevent the formation of air bubbles. The BRIJ® solution contained 1 ml octanol and 4 ml BRIJ® made to a total volume of 4 l with distilled water (Sigma Chemical Co., St. Louis, MO). The IR spectrometer was fitted with a temperature-controlled (20 C), calcium fluoride flow-through cell. The dc voltage output from the spectrometer was measured by an integrator (Model 4270, Spectra-Physics, San Jose, CA) and converted to peak height. Ten standards ranging from 0.01% to 0.25% $^{2}H_2O$ (v/v) were analyzed, and plasma sample concentration was determined by inverse regression analysis (Gill, 1978). Linear regression of the standard concentrations and their peak height produced a r^2 >0.999. Each sample was read in duplicate, and a set of 10 standards was read 8 times during each day's run. Approximately 60 samples and 80 standards were measured during each day's run.

^{18}Oxygen elimination rates (k_o, 1/d) and ^{2}hydrogen elimination rates (k_H, 1/d) were determined by least squares linear regression of the natural log of isotope concentration and elapsed time from isotope administration using PC-SAS (PROC REG) (Figure 2). The zero time

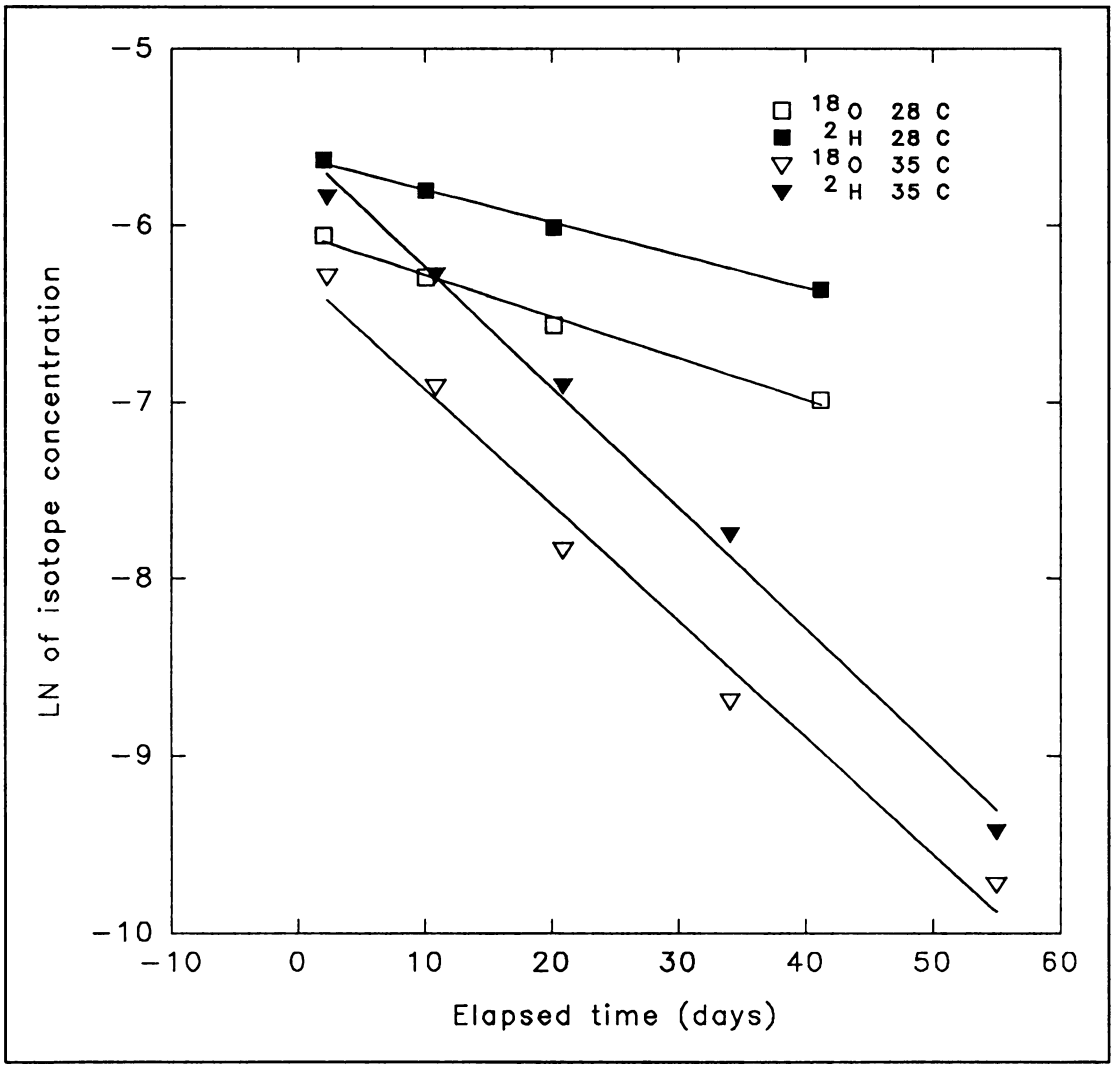

Figure 2. Typical regression lines for ^{18}O and ^{2}H turnover for one iguana at two different ambient temperatures.

intercepts were used to determine ^{2}H pool size (N_D, kg), and ^{18}O pool size (N_O, kg) at the time of dose administration.

For the DLW method, rate of carbon dioxide production (pCO_{2dlw}, l/d) was calculated based on the equation derived by Schoeller et al. (1986a) as:

$$pCO_{2dlw} = \left(\frac{N_0}{2.078}\right)\ (1.01k_O - 1.04k_H)\ - 0.0246r_{Gf}$$

where r_{Gf} is the rate of water loss from fractionating gaseous routes, and is estimated as:

$$r_{Gf} = 1.05N_O(k_O - k_H)\ .$$

Energy expenditure (EE_{dlw}, kcal/d) was calculated as:

$$EE_{dlw} = 3.941\left(\frac{pCO_{2dlw}}{RQ}\right)\ + 1.106pCO_{2dlw}$$

where RQ is the respiratory quotient (pCO_2/pO_2) measured from respiratory calorimetry.

Water flux (pH_2O, ml/d) was calculated as:

$$pH_2O = \frac{N_O k_H}{\left[1-(1-f_1)\frac{(2.3pCO_{2re})}{N_O k_H}\right]}$$

where f_1 = 0.941, and is the fractionation constant for the

exchange of $^2H_2O(gas)/^2H_2O(liquid)$ (Seale et al., 1989; Schoeller et al., 1986a).

Lean body mass (LBM, kg) was calculated as:

$$LBM = \frac{N_o}{0.732}$$

and body fat (%) was calculated as:

$$body\ fat = \frac{body\ weight - lean\ body\ mass}{body\ weight}\ 100.$$

No correction was made to LBM of body fat to correct for the weight of the gastrointestinal tract contents.

During isotope solution administration, the isotope dose was regurgitated by two iguanas in the first period (both housed at 28 C), and by one iguana in the second period (housed at 35 C). Therefore, isotope administration could not be quantitated for these iguanas. Doubly-labelled water data (energy expenditure, body composition) from those three iguanas were not used in subsequent analyses, but data on isotope elimination rates were used.

Sample preparation and analyses

Samples of feed, excreta, and skin were kept frozen (-20 C) until the time of sample preparation for analyses. Excreta samples were defrosted in a refrigerator overnight and then mixed with water in a Waring blender. The samples were homogenized for approximately 5 min. The entire contents of the blender were quantitatively transferred into a pre-tared plastic container that was then frozen, and subsequently freeze-dried. Immediately after freeze-drying, the pre-tared containers were reweighed. The difference in weight was the uncorrected dry weight of the excreta. The samples were then crushed with a mortar and pestle to produce a homogeneous powder. Subsamples of this powder were used for subsequent analysis for dry matter (DM), ash, carbon (C), nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid lignin (AL), and gross energy content. Skin samples were freeze-dried in a pre-tared container. The samples were then reweighed and ground in a small Wiley mill fitted with a 2 mm screen. Skin samples were analyzed for nitrogen, carbon, and gross energy content. Feed samples were analyzed for the same nutrients as the excreta.

Dry matter was determined in a vacuum drying oven at 55 C for 6 hr. Absolute dry matter content of the excreta was determined by correcting the freeze-dried excreta weight with the dry matter determined by vacuum oven drying.

Nitrogen and carbon were determined by combustion using approximately 100-mg subsamples (CHN 600, Leco Corp., St. Joseph, MI). Crude fat was determined by methylene chloride extraction (FES 80, CEM Corp., Matthews, NC). Gross energy content was determined by isoperibol dynamic bomb calorimetry (Parr Instrument Co., Moline, IL). Gross energy of the sample was corrected for acid formation by sodium carbonate titration. Ash was determined by combustion at 600 C for 6 hr. Fiber fractions were determined by the methods of Van Soest (Robertson and Van Soest, 1981) using a Fibertec system (System M, Tecator AB, Höganäs, Sweden). Heat-stable α-amylase (Sigma A-3306, 200 µl/sample) and protease (Sigma P-3910, 5 mg/sample) were used to facilitate filtration during fiber analysis. Neutral detergent fiber (NDF) samples were pre-incubated with α-amylase for 1 hr at 60 C and then with the protease for 1 hr at 80 C prior to refluxing with the neutral detergent. Cellulose was calculated as the difference between acid detergent fiber (ADF) and acid lignin (AL). Hemicellulose was calculated as the difference between NDF and ADF. Organic matter was calculated as the difference between dry matter and ash.

Calculations and statistical analyses

A preliminary study was conducted to determine if the iguanas were able to feed selectively on the meal-type diet.

Five collections of offered diet and orts were collected at five different times during a 5-wk preliminary period. The five diet samples were pooled. The diet and ort samples were analyzed for dry matter, ash, fat, nitrogen, carbon, and organic matter. Protein was calculated from nitrogen (Nx6.25) and percent total carbohydrate was calculated as the difference between 100% and the sum of fat, protein, and ash, expressed on a dry matter basis. Multivariate statistics (Hotteling's T-test) were used to determine if the composition of the offered feed (organic matter, protein, carbon, fat, ash, and total carbohydrate) was statistically different from the orts collected during each of the 5 weeks (Gill, 1978). On one occasion, one iguana consumed all of the offered diet; therefore, there were no orts.

Nutrient intakes were calculated as the product of nutrient concentration in the diet and daily dry matter intake. Since it was not possible to separate cloacal and fecal excreta, apparent metabolizability of dry matter and gross energy were calculated using the direct method (Schneider and Flatt, 1975). There is no quantitatively significant component of fiber excreted in the urinary wastes; therefore, apparent digestibilities were calculated by the direct method for NDF, ADF, cellulose, and hemicellulose. Average daily growth rate was determined as the difference between final weight and initial weight divided by the number of days on study. Metabolizable

energy intake (ME_i) was calculated as the difference between intake energy (IE) and energy lost in excreta (FE+UE) and skin (SE).

Nitrogen and carbon balance were calculated as the difference between intake and combined urinary and fecal excretion and skin ecdysis. Carbon balance was then corrected for carbon lost as carbon dioxide based on carbon dioxide production from respiratory exchange calorimetry measurements (pCO_{2re}).

Retained energy (RE) was calculated by three different methods. For all three methods, RE was expressed on a daily basis, and as a function of metabolic body size (body weight$^{0.75}$). By the first method, RE_{re} was determined as the difference between ME_i and EE measured by respiratory exchange (EE_{re}). Since food intake was depressed by approximately 50% in the calorimeters, RE_{re} was adjusted to correct for the decrease in IE. The depression in food intake may be a consequence of the movement of the iguanas from one environmental chamber to another for the calorimetry measurements, or due to a change in housing conditions during the calorimetric measurements. The correction was based on the difference between mean daily intake during the balance period, and intake when the iguanas were in the calorimeter. The amount of metabolizable energy associated with the decreased food intake was determined as the product of the difference in IE, and the measured ME density of the diet (3.18 kcal/g dry

matter). Fifteen percent of this difference (representing the heat increment) was added to the measured EE_{re}. This correction represents an estimate of the heat increment associated with the ingestion and digestion of food (Blaxter, 1989). By the second method, RE_{dlw} was determined as the difference between ME_i and the energy expenditure measured by the DLW method (EE_{dlw}). By the third method, RE_{CNbal} was calculated based on the sum of energy retained as fat and as protein based on C and N balance (Brouwer, 1965). The following assumptions were made: that the mean N and C concentration of protein deposited was 16.0% and 52.0%, respectively, and the mean gross energy content of the protein deposited was 5.7 kcal/g. Furthermore, the mean C concentration of fat deposited was 76.7%, and the mean gross energy concentration of the fat deposited was 9.5 kcal/g. In addition, it is assumed that there are no changes in the size of the carbohydrate stores. Retained energy was calculated as the sum of deposited protein energy and fat energy.

The apparent partial efficiency of use of metabolizable energy for growth (k_g) was determined for each temperature by the regression of RE_{re} and ME_i (Van Es et al., 1984). The slopes and intercepts of the two regression lines were compared to determine the effect of temperature (Gill, 1978). The metabolizable energy requirement for maintenance (ME_m) was calculated from the parameters of the regression lines of RE_{re} and ME_i for iguanas at each temperature, when

RE_{re} was equal to zero (Van Es et al., 1984). Two iguanas were in negative energy balance (both housed at 28 C). Data from these iguanas were excluded in the calculation of k_g and ME_m.

Statistical analyses were conducted using PC-SAS version 6.04 (SAS Institute Inc., Cary, NC). Tests of the assumptions of analysis of variance (ANOVA) were completed for normality (PROC UNIVARIATE) and homogeneity of variance. Homogeneity of variance was tested by plotting of residuals and predicted values, and by the calculation of Spearman correlation coefficients of the residuals and predicted values (PROC CORR). No violations of the assumptions of ANOVA were detected. Analysis of variance (PROC GLM), least-squares means (LSMEANS), and probability of the differences between least-squares means (PDIFF) procedures were used to test the effects of ambient temperature and sex. The ANOVA model included terms for sex, iguana(sex), period, temperature, and the temperature by sex interaction. Data were also analyzed using analysis of covariance (ANCOVA) with initial body weight as the covariate. There was no improvement in the coefficient of variation by ANCOVA so ANOVA was used for subsequent analyses. Tukey's studentized range test (PROC MEANS) was used to compare RE_{re}, RE_{dlw}, and RE_{CNbal}. Paired t-tests were used to determine differences between pCO_{2re} and pCO_{2dlw}, and between EE_{re} and EE_{dlw}.

RESULTS

Crude protein, total carbohydrate, crude fat, ash, carbon, and organic matter composition of the offered diet and the 99% confidence intervals (CI) of orts composition are presented in Table 8. The nutrient concentration of the offered diet is within the 99% CI of the orts. Thus, there was no difference for these nutrients between the offered diet and the orts, for any of the 5 times that ort samples were collected and analyzed. Therefore, there is no evidence that the iguanas were able to select for these specific nutrients from the offered diet; and, it is possible to determine nutrient intake as the product of nutrient concentration and the difference between the offered and uneaten portion, provided that all of the uneaten portion is collected.

Recoveries of carbon dioxide and oxygen were measured four times in each of the six calorimeters during the course of the study (Table 9). The mean amount of ethanol combusted was 3.48 g. Carbon dioxide and oxygen recoveries were used to test the accuracy of the calorimeters, and recoveries were calculated based on the amount of ethanol

Table 8. Diet composition of offered diet and 99% confidence intervals of orts composition (dry matter basis).

Diet or Orts	Date orts collected	N	Crude protein (%)	Total carbohydrate (%)	Crude fat (%)	Ash (%)	Carbon (%)	Organic matter (%)
Diet		5	27.5	57.9	5.54	9.14	43.6	90.9
Orts	02/03/91	12	25.6-28.8	56.1-59.8	5.51-5.92	8.54-9.81	42.6-45.2	90.2-91.5
Orts	02/10/91	12	24.8-31.6	54.3-60.5	5.42-6.14	7.54-9.74	38.4-47.7	90.3-92.5
Orts	02/17/91	11	24.4-32.6	53.0-61.3	5.25-6.21	7.18-10.15	38.5-47.8	89.9-92.8
Orts	02/24/91	12	26.0-29.8	55.0-60.0	5.53-6.46	7.64-9.64	41.0-44.8	90.4-92.4
Orts	03/03/91	12	26.5-29.6	55.4-59.3	5.17-6.60	7.87-9.64	42.1-44.9	90.3-92.1

combusted and the amounts of carbon dioxide produced and oxygen consumed relative to the predicted values. Predicted values were based on the stoichiometric relationship:

$$CH_3CH_2OH + 3O_2 \rightarrow 2CO_2 + 3H_2O.$$

The mean amount of carbon dioxide recovered was 96%, and ranged from 88% to 101%. The mean amount of oxygen recovered was 97%, and ranged from 84% to 102%. The mean respiratory quotient (RQ) was 0.66, and ranged from 0.64 to 0.76 (Table 9). The predicted RQ was 0.66 (each mole of ethanol combusted uses three moles of oxygen and produces two moles of carbon dioxide). On occasions when recoveries were low, repairs were made to the calorimeters prior to use for metabolic measurements of the iguanas. These repairs generally consisted of replacing the top gasket or resealing the top seams.

Due to differences in the length of the two balance periods (54 d for period 1, and 37-39 d for period 2), intake and growth data are presented on a daily basis. All variables were tested to determine differences between males and females, and there were no differences between the sexes for any variable. For the variables analyzed, there were no differences between the two periods, except for initial and final body weight. For all variables, the variances of the two treatment groups were not different.

Table 9. Carbon dioxide (CO_2) production, oxygen (O_2) consumption, and gas recoveries from ethanol combustion trials.

Variable	Mean [1]	SEM	Minimum	Maximum
Ethanol combusted (g)	3.48	0.90	2.56	5.65
Final CO_2 concentration (% of dry gas)	1.25	0.33	0.84	2.05
Final O_2 concentration (% of dry gas)	19.24	0.49	18.04	19.86
CO_2 produced (l, STP)	3.25	0.84	2.19	5.14
O_2 consumed (l, STP)	4.96	1.38	8.06	3.12
Respiratory quotient (RQ)	0.66	0.03	0.64	0.76
Recovery of CO_2 (%)	96.03	3.13	87.86	101.89
Recovery of O_2 (%)	97.24	4.65	83.51	101.84

[1] Mean of 24 combustion trials.

Thus, there is only one standard error for the mean (SEM). However, for some variables related to stable isotope kinetics, the sample sizes were not balanced since some iguanas regurgitated their dose. Therefore, each mean has a different SEM. All means presented in the following tables are the least-squares means ± standard error of the mean (SEM), except in Table 16.

Measurement of cloacal temperature confirmed that the iguana's temperature was near its ambient temperature. Initial body weight (BW), final BW, snout-vent length, metabolic body size ($BW^{0.75}$), and body composition of the iguanas are presented in Table 10. Mean body weight was 942.4 g (ranging from 606 to 1393 g) and 882.5 g (ranging from 500 to 2082 g) (± 38.9 g) for iguanas housed at 28 C and 35 C, respectively. Mean metabolic weights were 0.97 and 0.99 $kg^{0.75}$ for iguanas housed at 28 C and 35 C, respectively. Initial snout-vent lengths (SVL) were 26.7 cm and 26.5 cm (± 0.30) for the iguanas housed at 28 C and 35 C, respectively. There was no difference in body weight or SVL between the two groups at the beginning of each of the periods. Body weight at the end of the period was lower for the iguanas housed at 28 C than at 35 C, as was daily rate of gain. Mean daily rate of gain was 0.57 g/d and 3.40 g/d (±0.25) for the iguanas housed at 28 C and 35 C, respectively.

Table 10. Physical characteristics and daily rate of gain of iguanas housed at different ambient temperatures.

Variable	Units	Mean 28 C (N=24)		Mean 35 C (N=24)		P
Initial body weight[1]	g	942.4		882.5		0.3043
Final body weight[2]	g	1009.4		1137.5		0.0093
Rate of gain[3]	g/d	0.57		3.40		0.0001
Snout-vent length[4]	cm	26.7		26.5		0.7584
Mean metabolic body weight[5]	$kg^{0.75}$	0.97		0.99		0.4927
		Mean 28 C (N=21)	SEM	Mean 35 C (N=21)	SEM	P
Lean body mass	g	756.8	30.0	757.1	26.9	0.9950
Body fat	%	14.8	1.0	16.2	0.9	0.3820

[1]SEM ± 38.9.

[2]SEM ± 27.5.

[3]SEM ± 0.25.

[4]SEM ± 0.30.

[4]SEM ± 0.02.

Body composition was determined based on the kinetics of ^{18}O and its total water pool size at the time of isotope dose administration (zero time intercept). Body fat was 14.8 ± 1.0 and 16.2 ± 0.9% of total weight at the beginning of each period, for iguanas housed at 28 C and 35 C, respectively. There was no difference in body composition between the two groups at the beginning of each of the balance periods.

Nutrient intakes and metabolizabilities (or digestibilities) are presented in Table 11, and nitrogen (N) and carbon (C) intakes are presented in Table 12. Nutrient intakes were lower for iguanas housed at 28 C than for iguanas housed at 35 C ($p<0.0001$); however, there were no differences in nutrient metabolizability or digestibility between the two groups of iguanas. The difference in nutrient intakes between iguanas housed at the two temperatures was approximately 3 to 4 fold.

Dry matter intake is expressed on an absolute basis (g/d), and on a body weight basis (% of body weight/d). Mean daily dry matter intake (±SEM) was approximately four times greater ($p<0.0001$) for iguanas housed at 35 C than for iguanas housed at 28 C (12.70 and 3.51 ± 0.51 g/d, respectively). Daily dry matter intake ranged from 1.36 to 9.48 g/d for iguanas housed at 28 C, and from 8.55 to 20.74 g/d for iguanas housed at 35 C. Daily dry matter intake as a percent of body weight was also less for iguanas housed at

28 C than for iguanas housed at 35 C (0.35 and 1.33 ± 0.05% of body weight/d, respectively). Apparent dry matter metabolizability was not different between the two groups (67.0 and 67.2 ± 1.1%). Mean daily metabolizable energy intake (ME_i) was lower ($p<0.0001$) for iguanas housed at 28 C than for iguanas housed at 35 C (11.38 and 41.75 kcal/d ± 1.99 kcal/d, respectively). Gross energy metabolizability was 71.0 and 70.9 ± 0.9% for the iguanas housed at 28 C and 35 C, respectively, and it was not different between the two temperatures. The mean dietary ME density (kcal/g dry matter) was 3.18 ± 0.04 kcal/g dry matter, and was not different between the two ambient temperatures. Fiber digestibility was not different between the two ambient temperatures.

As with the other nutrients, nitrogen and carbon intakes (Table 12) were significantly greater at the higher temperature ($p<0.0001$, 0.16 vs 0.58 ± 0.03 g N/d, and 1.5 vs 2.54 ± 0.14 g C/d for 28 and 35 C, respectively). Furthermore, nitrogen and carbon balances were significantly lower for iguanas housed at 28 C than for iguanas housed at 35 C ($p<0.0001$, 0.07 vs 0.24 ± 0.02 g N/d and 0.30 vs 1.96 ± 0.14 g C/d for iguanas housed at 28 and 35 C, respectively).

These temperature-dependent differences in C and N balance correspond to a significantly lower deposition of

Table 11. Nutrient and energy intake and apparent metabolizability by iguanas housed at different ambient temperatures (N=24).

Variable	Intake					Metabolizability (%)[1]			
	Units	Mean 28 C	Mean 35 C	SEM	P	Mean 28 C	Mean 35 C	SEM	P
Dry matter (DM)	% of BW/d	0.35	1.33	0.05	0.0001	67.0	67.2	1.1	0.8926
	g/d	3.51	12.70	0.51	0.0001				
Organic matter	g/d	3.17	11.84	0.57	0.0001	69.6	70.0	1.0	0.7474
Metabolizable energy	kcal/d	11.38	41.75	1.99	0.0001	71.0	70.9	0.8	0.9654
	kcal/g DM	3.18	3.18	0.04	0.9642				
Neutral detergent fiber	g/d	0.71	2.65	0.13	0.0001	39.2	42.3	1.6	0.2071
Acid detergent fiber	g/d	0.40	1.49	0.07	0.0001	22.3	27.6	3.1	0.2479
Cellulose	g/d	0.30	1.05	0.05	0.0001	21.8	28.1	3.2	0.1954
Hemicellulose	g/d	0.31	1.16	0.06	0.0001	61.1	61.3	1.9	0.9429

[1] Digestibility for fiber fractions.

Table 12. Nitrogen and carbon intake and balance, and protein and fat gain of iguanas housed at different ambient temperatures (N=24).

Variable	Units	Mean 28 C	Mean 35 C	SEM	P
Nitrogen intake	g/d	0.16	0.58	0.03	0.0001
Nitrogen balance	g/d	0.07	0.24	0.02	0.0001
Carbon intake	g/d	1.50	2.54	0.26	0.0001
Carbon balance	g/d	0.30	1.96	0.14	0.0001
Protein gain	g/d	0.43	1.42	0.06	0.0001
	kcal/d	2.48	8.07	0.32	0.0001
Fat gain	g/d	0.09	1.58	0.15	0.0001
	kcal/d	0.85	14.89	1.42	0.0001

protein and fat for iguanas housed at 28 C as compared to those housed at 35 C ($p<0.0001$). Rate of protein (Nx6.25) deposition was 0.43 and 1.42 ± 0.06 g/d for iguanas housed at 28 C and 35 C, respectively. The amount of protein deposited corresponds to 2.48 and 8.07 ± 0.32 kcal deposited as protein/d for iguanas housed at 28 C and 35 C, respectively. Fat deposition was 0.09 and 1.58 ± 0.15 g/d for iguanas housed at 28 and 35 C, respectively. The amount of fat deposited corresponds to 0.85 and 14.89 ± 1.42 kcal/d for iguanas housed at 28 C and 35 C, respectively.

Isotope kinetics are presented in Table 13. Enough isotope was administered to the iguanas that regurgitated their isotope dose to determine turnover, and to calculate isotope half life. Therefore, the mean isotope half life from all iguanas is presented in Table 13. However, since the amount of isotope administered was not quantitatively known in the iguanas that regurgitated the dose, pool size, carbon dioxide production rate, water production rate, and energy expenditure were not calculated. Therefore, these means have a different SEM as a consequence of the unequal replication.

The half lives of ^{2}H and ^{18}O were approximately three times longer for iguanas housed at 28 C than for those housed at 35 C ($p<0.0001$). The mean ^{2}H half life for iguanas housed at 28 C was 32.25 ± 1.37 d, and ranged from 18.70 to 52.96 d. The mean ^{2}H half life for iguanas housed

at 35 C was 11.11 ± 1.37 d, and ranged from 5.26 to 13.53 d. As expected, the mean ^{18}O half life was less than the ^{2}H half life. Mean ^{18}O half life for iguanas housed at 28 C was 24.51 ± 0.91 d, and the mean ^{18}O half life at 35 C was 8.52 ± 0.91 d. The range in ^{18}O half life was 14.38 to 37.36 d and 4.52 to 11.43 d for iguanas housed at 28 C and 35 C, respectively.

Ambient temperature did not affect the estimates of total body water pool size (TBW, l) when measured by ^{2}H ($p<0.5413$) or ^{18}O ($p<0.9951$). Mean TBW pool size measured by ^{2}H zero time intercept was 0.692 ± 0.02 and 0.667 ± 0.025 l for iguanas housed at 28 C and 35 C ambient temperatures, respectively. Mean TBW pool size measured by ^{18}O zero time intercept was 0.554 ± 0.022 and 0.554 ± 0.020 l for iguanas housed at 28 C and 35 C ambient temperatures, respectively. TBW pool size based on ^{2}H kinetics was approximately 20% to 25% greater than the pool size based on ^{18}O kinetics. The ratio of TBW pool size determined by the two isotopes (N_D/N_O) was 1.25 ± 0.01 and 1.20 ± 0.01 for iguanas housed at 28 C and 35 C, respectively. This ratio was independent of ambient temperature ($p<0.0616$). Water flux (pH_2O, ml/d) was less for iguanas housed at 28 C (10.19 ± 4.94) than for iguanas housed at 35 C (46.73 ± 4.42) (Table 13).

The rate of carbon dioxide production (pCO_{2dlw}, l/d),

Table 13. Doubly-labelled water kinetics and pool sizes of iguanas housed at different ambient temperatures.

Variable	Units	Mean 28 C (N=24)		Mean 35 C (N=24)		P
^{2}H half life[1]	d	32.25		11.11		0.0001
^{18}O half life[2]	d	24.51		8.52		0.0001
		Mean 28 C (N=21)	SEM	Mean 35 C (N=21)	SEM	P
^{2}H pool size (N_D)	l	0.692	0.028	0.667	0.025	0.5413
^{18}O pool size (N_O)	l	0.554	0.022	0.554	0.020	0.9951
Ratio of N_D/N_O		1.25	0.01	1.20	0.01	0.0616
Carbon dioxide production rate (pCO_{2dlw})	l/d	1.92	0.56	5.31	0.50	0.0049
Water production rate (pH_2O)	ml/d	10.19	4.94	46.73	4.42	0.0019
Energy expenditure (EE_{dlw})	kcal/d	11.34	3.33	33.35	2.99	0.0033

[1] SEM ± 1.37.

[2] SEM ± 0.91.

measured by doubly-labelled water turnover, was lower for iguanas housed at 28 C than for iguanas housed at 35 C ($p<0.0049$) (Table 13). Mean pCO_{2dlw} was 1.92 ± 0.56 and 5.31 ± 0.50 l/d for iguanas housed at 28 C and 35 C, respectively. Using the RQ measured during respiratory calorimetry, and the energetic equivalents of carbon dioxide and oxygen (Weir, 1949), mean daily energy expenditure (EE_{dlw}) was approximately one-third lower ($p<0.0033$) for iguanas housed at 28 C (11.34 ± 3.33 kcal/d) than for those housed at 35 C (33.35 ± 2.99 kcal/d).

Rates of carbon dioxide production and oxygen consumption from respiratory exchange calorimetry are presented in Table 14. These rates are based on the mean of all six measurements (except for period 1 when the iguanas housed at 35 C were measured 5 times) from each iguana. Rate of carbon dioxide production and oxygen consumption was approximately 2 to 3 times greater for iguanas housed at the higher temperature ($p<0.0001$). Mean rate of carbon dioxide production was 1.43 and 3.49 ± 0.19 l/d, and mean rate of oxygen consumption was 1.79 and 4.60 ± 0.29 l/d for iguanas housed at 28 C and 35 C, respectively. These rates of gas consumption and production correspond to RQs that are higher at 28 C than at 35 C ($p<0.0252$). RQs were 0.80 and 0.77 (±0.01) for iguanas housed at 28 C and 35 C, respectively. Based on the caloric equivalents of carbon dioxide and

Table 14. Respiratory exchange calorimetry gas production rates, and energy expenditure of iguanas housed at different ambient temperatures (N=24).

Variable	Units[1]	Mean 28 C	Mean 35 C	SEM	p
Carbon dioxide production rate (pCO_{2re})	l STP/d	1.43	3.49	0.19	0.0001
Oxygen consumption rate (pO_2)	l STP/d	1.79	4.60	0.29	0.0001
Hydrogen gas production rate (pH_2)	ml STP/d	4.46	12.59	2.04	0.0201
Respiratory quotient (RQ)		0.80	0.77	0.01	0.0252
Energy expenditure (EE_{re})	kcal/d	5.48	14.26	0.93	0.0001
	kcal/mbw/d^2	5.84	14.19	0.40	0.0001

[1]STP = Standard temperature and pressure.

[2]mbw = metabolic body weight.

oxygen, energy expenditure (EE_{re}, kcal/d) was 2 to 3 times greater ($p<0.0001$) for iguanas housed at 35 C than when the same iguanas were housed at 28 C. Mean rate of daily energy expenditure was 5.48 and 14.26 kcal/d (±0.93) for iguanas housed at 28 C and 35 C, respectively.

Rate of hydrogen gas (H_2) production (Table 14) was higher for the iguanas housed at 35 C (12.59 ml/d) than for the iguanas housed at 28 C (4.46 ml/d) ($p<0.0201$).

Retained energy (kcal/mbw/d) was calculated by three different methods, and the least-squares means are presented in Table 15. RE_{re} and RE_{CNbal} (kcal/mbw/d) were lower for iguanas housed at 28 C than for those housed at 35 C ($p<0.0001$). However, there was no difference in RE_{dlw} for iguanas at housed at 28 C or 35 C. The SEM for RE_{dlw} was higher than the SEM for RE_{re} and RE_{CNbal} (Table 15).

The three means (RE_{re}, RE_{dlw}, and RE_{CNbal}) were compared using Tukey's test (Table 16). There was no difference between the overall mean (independent of temperature) of RE_{re} and RE_{CNbal}, but RE_{dlw} was lower than RE_{re} and RE_{CNbal} ($p<0.05$).

The ratio of pCO_{2dlw} to pCO_{2re}, and the ratio of EE_{dlw} to EE_{re} are presented in Table 15. There was no effect of temperature on the magnitude of the ratio of the rate of carbon dioxide production or energy expenditure between the DLW method and respiratory exchange calorimetry (Table 15). The mean ratio of carbon dioxide production rate

Table 15. Retained energy, and ratios of CO_2 production and energy expenditure measured by respiratory exchange and doubly-labelled water (DLW) of iguanas housed at different ambient temperatures.

Variable[1]	Units[2]	Mean 28 C[3]		Mean 35 C[3]		P
Retained energy from CN balance (RE_{CNbal})[4]	kcal/mbw/d	2.71		23.19		0.0001
Retained energy from respiratory exchange (RE_{re})[5]	kcal/mbw/d	4.52		23.87		0.0001
		Mean 28 C[6]	SEM	Mean 35 C[6]	SEM	P
Retained energy from DLW technique (RE_{dlw})	kcal/mbw/d	-1.28	4.49	8.57	4.02	0.1688
Ratio of pCO_{2dlw}/pCO_{2re}		1.60	0.21	1.53	0.19	0.8123
Ratio energy expenditure (EE_{dlw}/EE_{re})		2.39	0.31	2.19	0.28	0.6724

[1] See text for description of methods used to calculate retained energy.

[2] mbw = metabolic body weight.

[3] N=24.

[4] SEM ± 2.02.

[5] SEM ± 1.75.

[6] N=21.

Table 16. Carbon dioxide production, energy expenditure, and retained energy determined by different methods.

Variable	Units[4]	Method					
		Doubly-labelled water (DLW, N=21)		Respiratory exchange (RE, N=24)		Carbon - nitrogen balance (CNbal, N=24)	
		Mean	SEM	Mean	SEM	Mean	SEM
Carbon dioxide production rate[1]	l/d	3.807	0.529	2.464	0.237		
Energy expenditure[2]	kcal/mbw/d	24.03	3.64	10.01	0.93		
Retained energy[3]	kcal/mbw/d	3.43	3.11	14.19	2.29	12.95	2.57

[1] Paired t-test ($p<0.01$).

[2] Paired t-test ($p<0.001$).

[3] Tukey's HSD (DLW vs RE and CNbal, $p<0.05$; RE vs CNbal, not significant).

[4] mbw = metabolic body weight.

(pCO_{2dlw}/pCO_{2re}) was 1.60 ± 0.21 for iguanas housed at 28 C and 1.53 ± 0.19 for iguanas housed at 35 C. The mean ratio of energy expenditure rate (EE_{dlw}/EE_{re}) was 2.39 ± 0.31 and 2.19 ± 0.28 for iguanas housed at 28 C and 35 C, respectively.

A paired t-test was used to determine that the overall mean (independent of temperature) of pCO_{2dlw} was higher than the overall mean of pCO_{2re} ($p<0.01$) (Table 16). The overall mean of EE_{dlw} was also higher than the overall mean of EE_{re} ($p<0.001$) (Table 16).

The apparent efficiency of use of metabolizable energy above maintenance (k_g) was not different for the iguanas housed at the two ambient temperatures (Figure 3) but the intercepts were different ($p<0.001$). The k_g ($n=10$) for iguanas housed at 28 C was 0.79 ± 0.09, and the k_g ($n=12$) for iguanas housed at 35 C was 0.69 ± 0.08. The calculated ME_m was 5.82 kcal/mbw/d and 8.82 kcal/mbw/d for the iguanas housed at 28 C and 35 C, respectively.

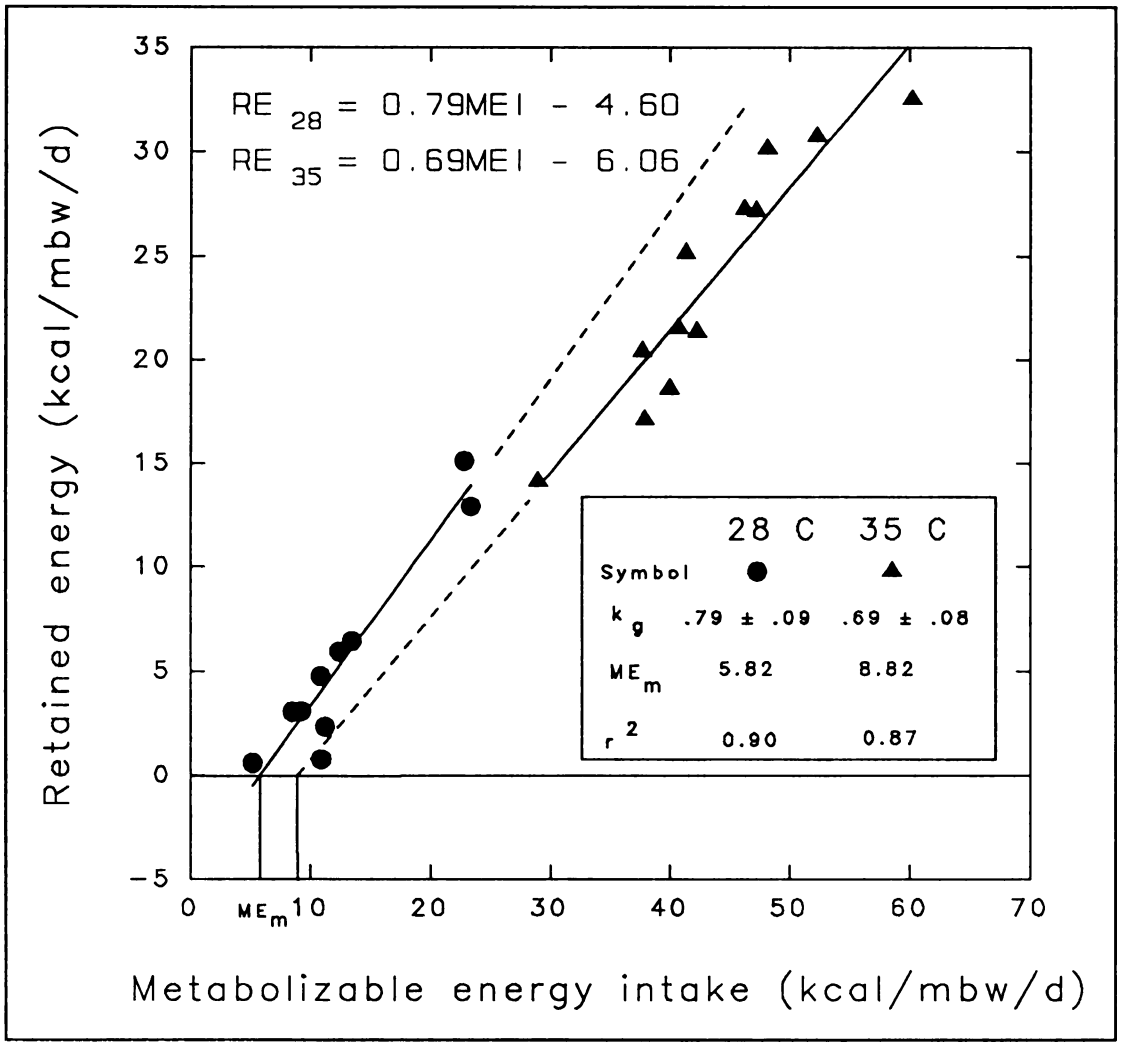

Figure 3. Regression of retained energy and metabolizable energy intake of iguanas housed at different ambient temperatures (see text for description of symbols).

DISCUSSION

The relationship between ambient temperature and digestive physiology of herbivorous lizards has been used as a theoretical framework to explain several evolutionary and ecological phenomena, including the evolution of large body size (Pough, 1973), and the geographic distribution of herbivorous lizards (Zimmerman and Tracy, 1989; Rand, 1978; Szarski, 1962). A central component of these various evolutionary and ecological theories is the effect of temperature on rate of digestion (especially fermentation), rate of digesta passage, and digestive efficiency, which usually refers to metabolizability or digestibility. In the Western Hemisphere, many herbivorous species of lizards are distributed near the tropics. There is little species radiation from the hot climate near the equator. Herbivorous species not found near the tropics are found in other hot environments. For example, Dipsosaurus dorsalis and Sauromalus obesus are two herbivorous lizard species that are found at high latitudes, but they are most widely distributed in the warmest environments of temperate North America (the Sonoran and Mojave deserts). Zimmerman and Tracy (1989) discussed the relationship between geographical distribution of herbivorous lizards and temperature, and

stated that ' . . . herbivory may have more stringent environmental constraints on its effectiveness compared with constraints imposed by carnivory.' Others suggested that warmer temperatures are necessary for improving digestion, and that the limited radiation of herbivory may be related to temperature constraints. Rand (1978) stated ' . . . the possibility of putrefaction or fermentation proceeding faster than digestion at low temperatures is a real risk and zoo keepers report that this is a cause of death in tropical herbivorous reptiles kept in temperate areas.' Many herbivorous lizards are relatively large (Pough, 1973), and increased body mass may be a thermoregulatory strategy (Spotila et al., 1973). Thus, the increased body size may help stabilize the thermal conditions for the symbionts of the gastrointestinal tract (Skoczylas, 1978). Skoczylas (1978) stated that ' . . . this [improved thermal conditions] may affect the degree to which plant food is utilized and may determine the evolutionary success of the species.'

All of these theories related to the interplay between environment and digestive physiology, yet this relationship is poorly understood. These relationships are important to consider. For instance, understanding the effect of temperature on digestion may provide insight on the evolutionary aspects of reptilian herbivory, or may help explain the limited geographic distribution of reptilian herbivores. Moreover, ambient temperature is an

environmental factor that can be manipulated in captivity. Appropriate alterations in temperature that improve rate of growth and reproductive success in captivity may be important tools for captive management of reptiles.

Intake

Daily dry matter and nutrient intakes of iguanas used in this study were three to four times greater at 35 C than at 28 C, yet there were no differences in nutrient or energy metabolizability between the iguanas housed at the two temperatures. Gut fill is finite since it is limited by physical constraints of gastrointestinal tract volume. Since gut fill can limit daily dry matter intake, there must be an increase in rate of digestion, rate of passage, or both in order to account for the observed increase in daily dry matter intake.

Rate of digestion

There is some evidence from carnivorous reptiles that the rate of gastric digestion may increase with increasing temperature (Coulson and Hernandez, 1983; Parmenter, 1981; Greenwald and Kantner, 1979; Diefenbach, 1975a,b; Skoczylas, 1970). Since all of these studies have been conducted with

carnivorous species, it is unknown if microbial fermentation in herbivorous lizards would follow a similar pattern. In ruminants, digestibility decreases in cold-stressed animals, and there is also a decrease in microbial activity (Kennedy and Milligan, 1978).

Rate of hydrogen gas production may be a good indicator of microbial fermentation in hindgut fermenters (Marthinsen and Fleming, 1982). Hydrogen gas production rate was lower in the iguanas housed at 28 C than those housed at 35 C. Thus, it is possible that there may have been a decreased rate of digestion or fermentation in the iguanas housed at 28 C as compared to the iguanas housed at 35 C.

Rate of digesta passage

Concomitant with a change in rate of digestion there may also be a change in rate of passage. An effect of ambient temperature on rate of passage has been reported for several species of reptiles, including herbivorous lizards. Rate of passage was not quantitated for the iguanas used in this study. However, it was observed that the iguanas housed at 28 C generally required 1-2 d additional days for the brilliant blue marked feces to appear. The iguanas in this study were maintained at a constant temperature for the entire period with no diurnal changes. Troyer (1987) manipulated ambient temperature by exposing iguanas (Iguana

iguana) to incandescent bulbs for 4 hr or 8 hr. These bulbs were suspended directly over the cages. Iguanas exposed to the lights for 8 hr maintained higher cloacal temperatures for a longer period of time than iguanas exposed to the lights for 4 hr. Under both conditions, there was a diurnal fluctuation in temperature that may better mimic iguanas in natural conditions than does a constant ambient temperature. Mean transit time of 2 mm glass beads was not different between these two groups of iguanas (80 hr vs 75 hr; Troyer, 1987). However, these iguanas were force-fed. Marker appearance (^{51}Cr-mordanted fiber) in the feces of Sauromalus obesus increased by approximately 1 day, from 4.7 d to 5.5 d, for force-fed chuckwallas housed at 36 C and 32 C, respectively (Zimmerman and Tracy, 1989). A review of data from other lizard species (Zimmerman and Tracy, 1989) revealed that increased ambient temperature increased the rate of digesta passage (Anolis carolinensis, Windell and Sarokon, 1976; Uta stansburiana, Waldschmidt et al., 1986; Cnemidophorus tigris, Gerrhonotus multicarinatus, Sceloporus occidentalis, Harwood, 1979).

Nutrient and energy metabolizability

If there is an increase in rate of digestion with an increase in temperature, it may be offset by an increase in rate of digesta passage at the higher temperature. The net

effect of these two competing processes may result in little or no difference in digestibility, and probably metabolizability, at different ambient temperatures. This hypothesis would be supported by the data collected from the iguanas in the present study, and by Karasov and Diamond (1985) in studies with Sauromalus obesus. A similar conclusion was reached by Zimmerman and Tracy (1989) in studies with Dipsosaurus dorsalis. Conversely, Troyer (1987) reported that iguanas maintained under incandescent lights for 8 hr rather than 4 hr had a higher 'digestive efficiency' (56% vs 49% energy metabolizability). Furthermore, Harlow et al. (1976) reported that energy metabolizability in Dipsosaurus dorsalis was 54%, 63%, and 70% for lizards housed at 33, 37, and 41 C, respectively, and that these differences in metabolizability were significantly different. In all previous studies, lizards were force-fed, and this may have confounded the results. In some cases (Harlow et al., 1976), lizards at the lower temperature (33 C) may have been overfed by as much as 330% of their estimated energy requirement for maintenance (Zimmerman and Tracy, 1989). This degree of overfeeding may dramatically increase fecal output and lead to an underestimation of metabolizability. Based on data from this study, using ad libitum-fed iguanas, it appears that nutrient and energy metabolizabilities were not influenced by differences in ambient temperature. However, the increased nutrient and energy intake at the higher

temperatures may increase the amount of nutrients and energy supplied to the iguanas at a given metabolizability. While there may be an increased amount of energy available to iguanas at a higher temperature, there may also be an increase in the energy requirement for maintenance.

Bioenergetics

Energy metabolism has been studied extensively in reptiles for many years (reviewed by Benedict, 1932; Bennett and Dawson, 1976; Bennett, 1982). Studies have included the measurement of metabolic rate, energy metabolizability, and the influence of environmental factors that may influence energy metabolism, such as temperature. Measurements of metabolic rate have been performed in the field using the doubly-labelled water technique, and in controlled environments using respiratory exchange. Only one published study has simultaneously compared these two techniques in lizards (Congdon et al., 1978). With most studies of reptilian energetics, metabolic rate is reported as the rate of oxygen consumption or carbon dioxide production. There has been little emphasis on the conversion of data collected from respiratory gas exchange to energetic equivalents. Therefore, it has not been possible to study the bioenergetics of reptiles. Few studies have integrated the aspects of energy intake and metabolizability, and the

partition of energy between expenditure and storage. No studies have been reported that establish the effect of temperature on the efficiency of use of energy for growth.

The effect of ambient temperature on energy expenditure and food intake is different for reptiles (Coulson and Hernandez, 1983) than for mammals and birds (Blaxter, 1989) (Figure 4). Endotherms have a thermoneutral zone (TNZ). Body temperature is maintained in the TNZ by behavioral mechanisms, and temperature changes within the TNZ do not change energy expenditure. Below and above the TNZ, body temperature is maintained by physiological mechanisms, and energy expenditure increases (Blaxter, 1989, NRC, 1981). The response to changes in ambient temperature is much different for ectotherms than endotherms. As temperature decreases from the preferred body temperature, energy expenditure in ectotherms decreases. As temperature increases, energy expenditure increases, but the increase is a non-linear function of temperature (Coulson and Hernandez, 1983). Food intake of endotherms increases below the TNZ and decreases above the TNZ. Food intake of ectotherms increases as temperature increases, and the increase in food intake may be necessary to support the non-linear increase in energy expenditure.

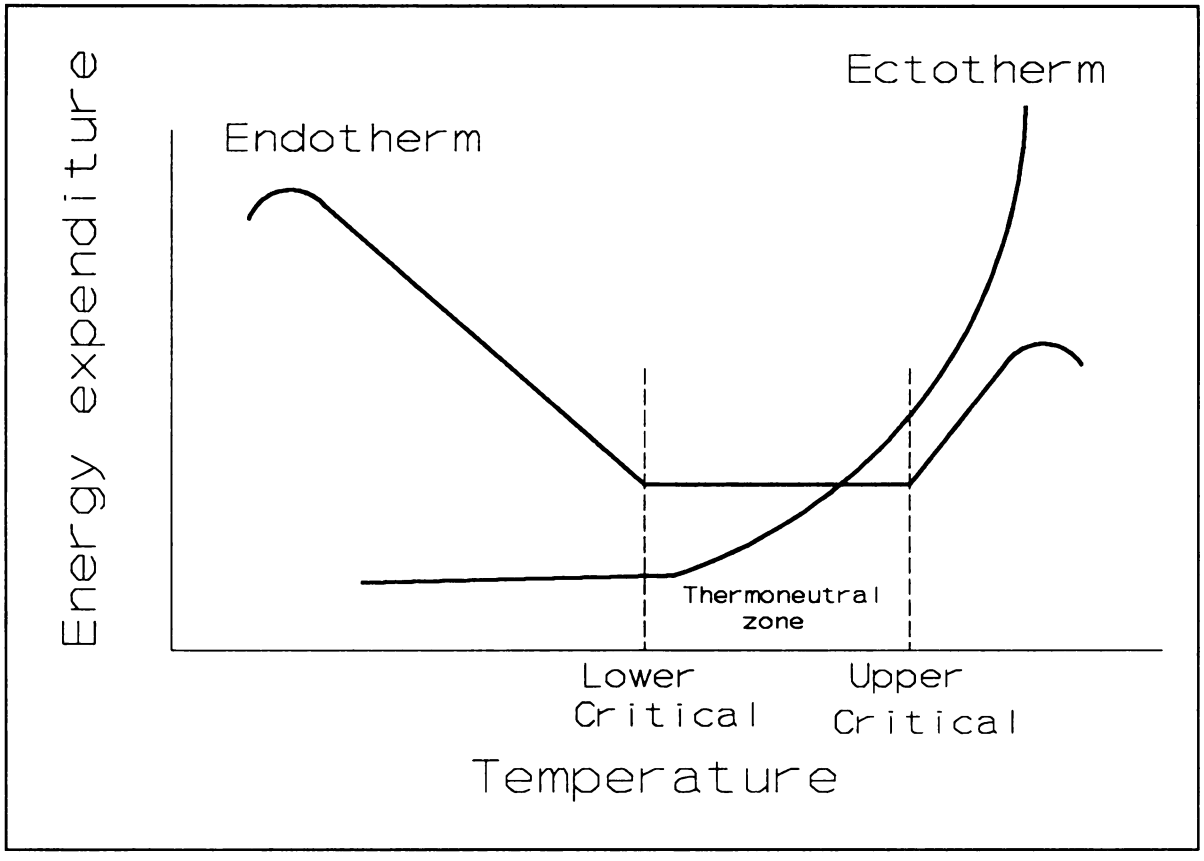

Figure 4. Relationship between energy expenditure and ambient temperature for endotherms and ectotherms.

Comparison of respiratory exchange and doubly-labelled water

In this study, energy expenditure was measured using two methods of indirect calorimetry: 1) respiratory exchange (EE_{re}), and 2) doubly-labelled water (EE_{dlw}). In general, these two methods agree very well under controlled conditions (Seale et al., 1993). In field studies, EE_{dlw} may be higher than EE_{re} as a consequence of the increased activity of free-ranging animals in their environment. For the iguanas used in this study, the magnitude of the difference between EE_{re} and EE_{dlw} was large. The measurements of pCO_{2dlw} and EE_{dlw} were 1.5 and 2 times greater than pCO_{2re} and EE_{re}. These differences may be related to: 1) actual differences in energy expenditure under the different measurement conditions, 2) problems associated with measuring respiratory exchange, or 3) violations of underlying assumptions, especially for the DLW method.

Analysis of respiratory exchange calorimetry

Potential problems with respiratory exchange measurements with the iguanas used in this study may be a consequence of the need to move the iguanas to a different environmental chamber (but with the same ambient conditions) for the measurements, and a decrease in mean dry matter intake when they were in the calorimeters. In addition, it

was not possible to collect and measure excreted urinary nitrogen from the calorimeters. Inability to collect this source of N may result in errors when converting gas production to caloric equivalents; however, the error would be small (Seale et al., 1989; Farrell, 1974). Based on the recoveries of oxygen and carbon dioxide from ethanol combustion, it appeared that these closed-circuit calorimeters were well suited for measurement of respiratory exchange. They were relatively inexpensive to construct, and if sealed properly, they provided accurate and precise measurements.

Analysis of the doubly-labelled water method

The assumptions and problems with the doubly-labelled water method have been discussed by several authors (Lifson, and McClintock, 1966; Nagy, 1980; Speakman, 1990; Seale et al., 1989; Nagy and Costa, 1980; Speakman and Racey, 1986). Some assumptions of the DLW method are difficult or impossible to control, such as the assumption that water and CO_2 fluxes are constant. Thus, the isotope turnover rates and the calculation of rate of CO_2 production is an average measurement over the length of the particular study (Nagy, 1980). Other assumptions of the DLW method were minimized in this study, such as changes in background isotope concentrations due to changes in diet or water source

(Coward, 1991; Roberts et al., 1988; Klein et al., 1984). For example, the iguanas in this study were offered diet from the same manufactured lot and drinking water from the same source throughout both periods. Thus, changes in background isotope concentrations are unlikely. Furthermore, with the large rate of air turnover in the environmental chambers, it is unlikely that labelled CO_2 could reenter the iguanas via pulmonary tissue exchange.

There were other potential problems associated with the DLW technique applied to these iguanas. The problems may include: 1) change in total body water pool size, 2) unknown fractionation constants and the effect of temperature on fractionation constants, 3) the loss of isotopes in forms other than CO_2 and H_2O, and 4) unknown RQ during the DLW measurement period. The RQ is necessary to establish the relationship between pCO_2 and pO_2, and to convert the rate of pCO_2 to a rate of energy expenditure. Each of these potential problems of the DLW method may have affected the estimation of energy expenditure in these iguanas, and may help explain the relatively large difference between EE_{re} and EE_{dlw}. Furthermore, there was a large difference in body water pool size when it was determined by ^2H_2O space compared to $H_2{}^{18}O$ space (N_D/N_O). The difference in ^2H_2O and $H_2{}^{18}O$ pool size, and the discrepancy between EE_{re} and EE_{dlw} may be a consequence of violating different assumptions of the DLW method. Four of these assumptions will be considered in detail.

First, the equation used to calculate CO_2 production is based on the assumption that the body water pool size is constant (Nagy, 1980). With these iguanas, body water pool size may have increased as a consequence of the increase in body size (Table 10). Furthermore, repeated blood sampling may also result in an apparent increase in body water pool size. However, for the iguana with the greatest weight gain, who also had approximately 48 ml of blood drawn, the increase in water pool size would be less than 50%, and this increase would result in an error of less than 5% in calculation of CO_2 production rate (Nagy, 1980). The ratio of pCO_{2dlw}/pCO_{2re} is much greater than 5%. Furthermore, this ratio was not different for the few iguanas that did not change body weight, and presumably had little change in body water pool size over the DLW measurement period. Thus, it is unlikely that changes in body water pool size would explain the large difference between EE_{re} and EE_{dlw}.

Second, the isotopic fractionation constants used to calculate pCO_2 may not be valid for these lizards. Fractionation constants are used to correct for potential changes in isotopic concentrations that may occur for H_2O and CO_2 during changes in state from liquid to vapor. The three constants are (Schoeller et al., 1986a,b):

1) $f_1 = 0.941$ for $^2H_2O(gas)/^2H_2O(liquid)$

2) $f_2 = 0.992$ for $H_2{}^{18}O(gas)/H_2{}^{18}O(liquid)$

3) $f_3 = 1.039$ for $C^{18}O_2(gas)/H_2{}^{18}O(liquid)$.

The fractionation constants may be species-specific and temperature dependent (Nagy, 1980; Nagy and Costa, 1980). In some situations, potential errors associated with isotopic fractionation (especially f_1 and f_2) will negate each other, and produce little additional error in the calculation of pCO_2 (Nagy, 1980). For instance, if temperature affects both H and O fractionation to the same extent, then the difference between H and O turnover will not be affected (Lifson and McClintock, 1966; Nagy, 1980). There was no effect of temperature on the ratio of pCO_{2dlw}/pCO_{2re} in this study. This observation suggests that ambient temperature had little effect on isotopic fractionation. Furthermore, the extent of the error associated with isotopic fractionation, especially f_1 and f_2, may be minimized in these iguanas since insensible water loss is very limited in iguanas. Transcutaneous water loss in humans is approximately 5 times greater than in iguanas (Schoeller et al., 1986a,b; Bentley and Schmidt-Nielsen, 1966; Mautz, 1982), and this difference in water loss may be related to a lipid barrier in reptilian epidermis (Roberts and Lillywhite, 1980; Lillywhite and Maderson, 1982).

An incorrect value for the fractionation constant f_3 [$C^{18}O_2$ (gas)/$H_2{}^{18}O$ (liquid)] may introduce an error that could explain the high flux of ^{18}O observed in these iguanas. This fractionation can occur if the enzyme carbonic anhydrase can discriminate between ^{16}O and ^{18}O. If f_3 is higher than previously measured (Schoeller et al., 1986b),

then ^{18}O flux may be increased. Isotopic discrimination between ^{18}O and ^{16}O is known to occur in plants (Lou and Sternberg, 1992; Bricout, 1979). Isotopic discrimination in the green iguana could be associated with its own carbonic anhydrase enzyme, or with enzymes associated with the symbiotic microbes in the gastrointestinal tract.

Third, violations of the assumption that ^{2}H and ^{18}O isotopes are lost from the body only as H_2O and CO_2 can lead to an error in the calculation of pCO_2 (Haggarty et al., 1991). Several sets of reported data suggest that hydrogen can be sequestered in several forms in the body. Hydrogen but not oxygen can exchange with the non-water pool during fatty acid synthesis, and during peptide bond formation (Haggarty et al., 1991; Humphrey and Davis, 1974). This sequestration can result in an overestimation of total body water pool, and an overestimation of water flux (Culebras et al., 1977; Nagy and Costa, 1980). Consequently, pCO_2 will be underestimated (Haggarty et al., 1991). The overestimation of water flux and the underestimation of CO_2 flux will be dependent on the relative size, and the relative rate of exchange of the ^{2}H between the water pool and the non-water pool. The exchange and sequestration of ^{2}H in the non-water pool is generally used to account for the larger body water pool size that is usually observed when measured by ^{2}H as compared to ^{18}O (N_D/N_O). However, the difference in these pools is usually no greater than 10% (Schoeller et al., 1980, 1986a). In these iguanas the

difference in pool size was 20% to 25%. This larger difference may be a function of greater ^{2}H exchange into a relatively large non-water pool in the green iguana than has been observed in other species.

Few data have been reported on the sequestration of oxygen, and the loss of oxygen from sources other than CO_2. Oxygen can exchange with the bicarbonate pool through the action of carbonic anhydrase (Nagy, 1980; Haggarty and McGaw, 1988). In fact, this is the metabolic basis of the DLW method (Lifson et al., 1949). However, there may be a disproportionate loss of ^{18}O since HCO_3^- or CO_3^{2-} may contain a disproportionate amount of ^{18}O relative to H_2O or CO_2 (Nagy, 1980). In reptiles, the excretion of sodium, potassium, and ammonium bicarbonate salts may be important in regulating acid-base balance (Minnich, 1972, 1982; Schmidt-Nielson et al., 1963; Coulson and Hernandez, 1983), and the bicarbonate pool size may be larger in reptiles than in mammals (Bickler, 1981; Thorson, 1968). The error associated with ^{18}O exchange with the bicarbonate pool is estimated to be only 0.1% in humans (Haggarty and McGaw, 1988), and is much less than the error associated with ^{2}H exchange (approximately 4-10%). In reptiles, the large bicarbonate pool, and the excretion of bicarbonate as salts through the nasal gland may increase the turnover rate of ^{18}O. This increase in ^{18}O flux could result in an overestimation of the difference of the turnover rates of ^{18}O and ^{2}H, and an overestimation of CO_2 flux. Thus, a large

bicarbonate pool that has a high flux in these iguanas may help to explain the magnitude of the observed difference between pCO_{2re} and pCO_{2dlw}. Furthermore, in reptiles, the concentration of plasma bicarbonate is temperature independent (Howell and Rahn, 1976), and in these iguanas, the discrepancy between pCO_{2re} and pCO_{2dlw} was also temperature independent. Overestimation of ^{18}O flux may be exacerbated by isotopic discrimination between ^{16}O and ^{18}O and high rates of bicarbonate excretion.

Fourth, the DLW method provides an estimation of pCO_2 and there may be errors in using RQ to convert from rate of carbon dioxide production to the rate of energy expenditure. The energy equivalent of CO_2 can vary, depending on the combination of substrates being oxidized. The RQ (pCO_2/pO_2) will reflect the mix of substrates being oxidized. Thus, it is important to have an estimate of the RQ, or preferably, to have measured RQ by respiratory exchange from animals under similar metabolic conditions. For the iguanas in this study, the RQ measured by respiratory exchange was used to determine pO_2 from the pCO_2 measured by the differential turnover of the DLW. The overall mean RQ of 0.78 was used (Table 14). In general, the RQ of reptiles is lower than the RQ of ureotelic species. This difference is a consequence of the lower RQ associated with uric acid formation from protein oxidation as opposed to urea formation (Brafield and Llewellyn, 1982). Even if the RQ of the iguanas during the DLW measurement period was 1.0, EE_{dlw}

would be approximately 17% less, and would still be more than 100% greater than EE_{re}. The use of RQ is important for converting the rate of CO_2 production to EE. Ignoring the RQ and estimating EE based solely on pCO_2 can lead to errors as high as 20% (Blaxter, 1989). However, errors in RQ alone could not explain the large difference between EE_{re} and EE_{dlw} in these iguanas.

There may have been systematic errors that have led to an overestimation of EE_{dlw} or the iguanas may have been much more active outside of the respiratory calorimeters. Calculation of RE by different methods may provide some insight into which set of data (EE_{dlw} or EE_{re}) provides a more accurate estimate of EE.

Comparison of RE calculated by different methods

In order to make comparisons between RE calculated by different methods, it is first necessary to assign caloric equivalents to pCO_2 and pO_2 in order to calculate EE. Nagy (1982a) has used a caloric equivalent of 5.19 kcal/l CO_2 from DLW experiments to calculate energy expenditure. This energy equivalence of CO_2 was based on an estimated RQ of 0.93 for herbivorous lizards. The RQ was estimated based on the composition of the diet consumed by Sauromalus obesus, but it has been used for all herbivorous lizards. The energy equivalents used in this study were those suggested

by Weir (1949). However, in this study there was no correction for protein oxidation based on urate excretion. For the iguanas in this study, it was not possible to separate N excreted in feces from non-fecal sources (urates). The correction for nitrogen excretion in birds, who also excrete nitrogen as uric acid, is only 0.28 kcal/g N. Urate N excretion of Sauromalus obesus may represent as much as 90% of total N excretion (Nagy and Shoemaker, 1975). Based on this assumption, the correction for urate nitrogen excretion from the iguanas in this study would be less than 0.5% of EE_{re}.

RE was determined by three methods: 1) the difference between ME_i and EE_{re} (corrected for decreased dry matter intake in the calorimeter) (RE_{re}), 2) the difference between ME_i and EE_{dlw} (RE_{dlw}), and 3) the sum of energy stored as fat and protein, determined by the balance of carbon and nitrogen (RE_{CNbal}). RE_{dlw} and RE_{re} were calculated as a difference whereas RE_{CNbal} was calculated as the sum of energy stored as fat and protein. Therefore, RE_{dlw} and RE_{re} are independent of RE_{CNbal}. RE_{dlw} is significantly lower than RE_{re} and RE_{CNbal}, but RE_{re} and RE_{CNbal} are not different. Furthermore, there are several iguanas that had negative values of RE_{dlw} but had positive growth rates. Given the similarity between RE_{re} and RE_{CNbal}, and the negative values of RE_{dlw} of some growing iguanas, these data would suggest that EE_{dlw} may be an overestimate of actual energy expenditure.

Assessment of the difference between respiratory exchange and doubly-labelled water methodologies

pCO_2 and EE measured by the DLW method are greater than when measured by respiratory exchange. In addition, RE_{re} and RE_{CNbal} were not different from each other but RE_{dlw} is much lower than the RE calculated by the other two methods. Furthermore, several iguanas had negative values of RE_{dlw}, even though those iguanas were growing. Thus, it appears that the DLW method overestimated EE in these iguanas.

There are at least two inconsistencies that are apparent from the DLW method: 1) there is a large discrepancy between water pool size measured by ^2H and ^{18}O at their zero time intercepts, and 2) pCO_2 is high relative to respiratory exchange. Body composition estimates based on ^2H pool size at zero time intercept result in estimates of body fat that are negative whereas body composition based on ^{18}O pool size at zero time intercept results in estimates of body fat that may be reasonable. Thus, it appears that the ^2H pool size at the zero time intercept may have been overestimated. There may be a relatively large pool of ^2H in a form other than H_2O that is not being accounted for when measuring from the blood pool. Water flux, a function of ^2H flux, was somewhat lower than predicted for free-ranging tropical reptiles (Nagy, 1982b), but this difference may be related to the relatively dry diet that the iguanas were fed in this study. Based on these observations, the measurement of ^2H flux may be valid, but the ^2H zero time

intercept may be high.

Therefore, the overestimation of pCO_2 may be a consequence of a high rate of ^{18}O flux, perhaps from a disproportionate excretion of ^{18}O-labelled bicarbonate. The only reported direct comparison between respiratory exchange and DLW in lizards (Sceloporus species) was done with four animals for 7.5 days. DLW overestimated pCO_2 measured by respiratory exchange by only 3.2% in these lizards. Therefore, the DLW method may be valid under other conditions and for other species. Species-specificity may be a function of species-specific metabolism of bicarbonate that is reported to occur in reptiles (Howell and Rahn, 1976).

In these iguanas, there may have been more than one assumption of the DLW method that was violated, and the violations seem to be temperature independent. Indirect calorimetry data from respiratory exchange may provide a better understanding of energy metabolism for the iguanas in this study.

Comparison of EE_{re} to other respiratory exchange data

pO_2 and pCO_{2re} of these iguanas is similar to other data reported for green iguanas (Table 17). The similarity of these data to previously reported data helps confirm the suitability of the closed-circuit calorimeters used in this

study to measure EE, and the accuracy of EE_{re}. The similarity between published data and pCO_{2re} also supports the suggestion that pCO_{2re} is a more accurate estimate of actual pCO_2 than pCO_{2dlw}, in these iguanas.

Comparison of EE_{re} to predicted field metabolic rate

Nagy (1982a) developed a regression equation to predict field metabolic rate (FMR) of iguanid lizards as a function of body weight. The DLW method was used in nine iguanid species that ranged in weight from 0.5 g to 1,481 g. Data from Iguana iguana were not available. The range in body weight may appear large, but almost 90% of the observations were from species whose body weight was from 1.6 g to 167 g, and only one observation was from a species with a body weight of over 1 kg. EE_{re} from the iguanas used in this study was compared with predicted FMR using Nagy's equation (Figure 5). The EE_{re} of the iguanas housed at 35 C was not different from the predicted FMR (paired t-test). The mean difference was 0.33 ± 0.48 kcal/d. However, EE_{re} of the iguanas housed at 28 C was lower than predicted FMR ($p<0.001$). The mean difference of the predicted FMR and actual EE_{re} was -8.78 ± 0.93 kcal/d. Field metabolic rate is the energy expenditure of free-ranging animals in their

Table 17. Resting oxygen consumption and carbon dioxide production of Iguana iguana at different ambient temperatures.

Reference	N	Weight range (kg)	Temperature (C)	pO_2 (ml/g/hr)	pCO_2 (ml/g/hr)
1	15	0.4-1.2	15	0.022	
2	15	0.4-1.2	20	0.040	
3	12	0.6-1.9	28	0.089	0.071
2	15	0.4-1.2	30	0.081	
2	15	0.4-1.2	35	0.150	
4	4	0.7-1.0	35	0.183	
3	12	0.5-2.5	35	0.194	0.150
5	3	0.6-1.5	35	0.26	0.20
1	15	0.4-1.2	40	0.165	

1 Moberly, 1964.

2 Moberly, 1968a.

3 This study, measurements from respiratory exchange.

4 Gleeson et al., 1980.

5 Gleeson and Bennett, 1982.

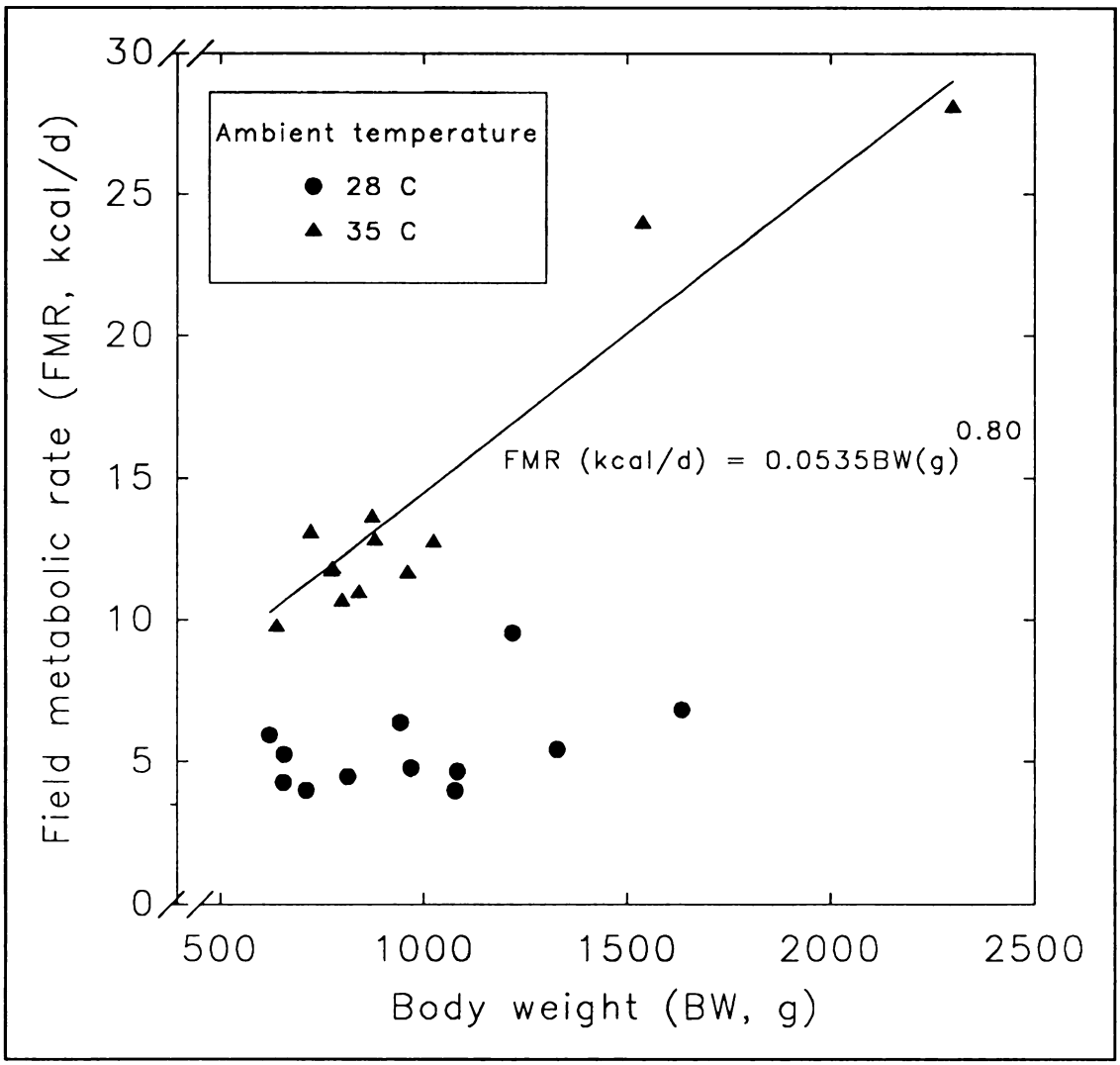

Figure 5. Comparison of predicted field metabolic rate (FMR, Nagy, 1982a) with actual energy expenditure of iguanas housed at different ambient temperatures.

normal habitat. Presumably, in the field, reptiles can self-select ambient temperatures. Therefore, the FMR of reptiles should be a function of their self-selected body temperature range. The self-selected body temperature of Iquana iquana is thought to be near 35 C. Thus, the iguanas housed near their self-selected body temperature range had predicted FMRs that were not different from EE_{re}. Furthermore, the predicted FMR of iguanas housed below their self-selected body temperature overestimated measured EE_{re}. Therefore, this prediction equation may be valid for iguanid lizards over 1000 g, and in their self-selected body temperature range.

Field metabolic rate was not different from the EE_{re}, yet FMR includes energy expenditure associated with some activities that may have been limited for the captive iguanas used in this study. For instance, some behaviors of free-ranging iguanas were not exhibited in these captive iguanas. Examples of these behaviors include foraging for leaves, escaping predators, and climbing to the forest canopy. Restricted activity in respiratory calorimeters may result in differences between FMR and EE measured under controlled conditions (Weathers et al., 1984). These differences are likely to be a function of a species activity pattern; and, the error may be species specific. The similarity of FMR and EE_{re} in this study may reflect the rather limited activity of iguanas in the wild (Moberly, 1968a), and their relative inactivity in captivity. Thus,

the energy expenditure associated with activity in the field may not be different from energy expenditure associated with activity under the captive conditions experienced by these iguanas, although the actual activities may be different.

Maintenance energy requirement

The relationship between RE and ME_i can be used to determine the energy requirement for maintenance (ME_m) using regression methods. In general, calculation of ME_m by linear regression of RE and ME_i does not take into account the non-linearity of energy storage above maintenance that has been reported in many species, and assumes that there are no measurement errors in ME_i (Van Es, 1972). Even with all of the apparent problems, the regression technique may be accurate. In human subjects, the ME_m was not different when determined by the use of regression coefficients as compared to maintaining the subjects near maintenance and correcting for small changes in energy balance (Van Es et al., 1984). ME_m was calculated to be 5.82 and 8.82 kcal/d for iguanas housed at 28 and 35 C, respectively. There are many factors that can affect ME_m. Some of these factors are weight, age, diet composition, and rate of growth (Blaxter, 1989). The difference in ME_m between the two groups of iguanas may be a function of temperature or may be a function of other variables that could confound the measure,

such as rate of growth. Other methods for estimating ME_m were not possible with the current experimental design. The regression method used to estimate ME_m of these iguanas may be prone to many errors, but these data provide a framework for future experimentation.

Use of energy above maintenance for growth

No other studies have attempted to determine the apparent partial efficiency of use of metabolizable energy for tissue deposition above maintenance (k_g) for reptiles. This measure is often determined by maintaining an individual animal at two levels of food intake, one near weight maintenance and one above weight maintenance (Blaxter, 1989). This method was not possible under the current design, but the estimates from this study may provide a set of initial data for further validation.

The k_g may be function of level of intake in relation to the maintenance requirement (defined as RE = 0), and this relationship may be curvilinear. However, there was no curvilinear component detected by least-squares regression for these iguanas at either temperature. Furthermore, for these iguanas, there was no effect of temperature on the efficiency of tissue deposition above maintenance. Thus, at both temperatures, energy consumed above maintenance was used for growth with similar efficiency. These efficiencies

are based on assumptions that were made to correct for the decrease in food intake during the calorimetry measurements. These efficiencies may be different for a different set of assumptions, but the parallel relationship between the two efficiencies would not necessarily change.

Summary

Daily food intake of male and female Iguana iguana housed at 35 C was greater than for the iguanas housed at 28 C. However, the fiber fraction digestibility, and the dry matter and energy metabolizability of the diet consumed was not affected by ambient temperature. Even though metabolic rate and maintenance requirements may have been higher at 35 C than at 28 C, daily rate of gain was also greater at 35 C than at 28 C. Thus, the increase in food intake may have been proportionally greater than the increase in ME_m. However, metabolizable energy consumed above maintenance was stored with similar efficiency at both temperatures. Maintaining iguanas at an ambient temperature that includes their self-selected body temperature may promote growth and improve reproductive success in captivity.

CONCLUSIONS

Diet composition and ambient temperature affect the digestive function and bioenergetics of the green iguana. This study confirmed that dietary fiber is digested in the gastrointestinal tract of the green iguana. The range in concentration of dietary fiber used in this study did not affect daily dry matter intake but did affect nutrient and energy metabolizability, and growth rate. To maximize growth rate, a diet that contains less than 27% neutral detergent fiber (% of the dry matter) is recommended. The fiber study demonstrated that herbivorous lizards will eat a manufactured diet, ad libitum, in captivity, and that rigorous metabolizability and growth trials can be conducted using standard techniques.

Nutrient and energy metabolizability were not affected by the ambient temperature to which these iguanas were exposed, although these temperatures affected energy expenditure, daily dry matter intake, and growth rate. However, with increasing temperature, the increase in energy intake was greater than the increase in energy expenditure. Since metabolizability was constant, greater amounts of nutrients and energy were available for growth. Ambient temperature did not affect the efficiency with which

metabolizable energy consumed above maintenance was used for growth.

The agreement was poor between energy expenditure measured by respiratory exchange and the doubly-labelled water method. The rate of energy expenditure measured by the doubly-labelled water method was approximately twice the rate measured by respiratory exchange. Analysis of retained energy computed from each method of measuring energy expenditure, and comparison of the rate of oxygen consumption to previously published data from a number of sources, suggests that the discrepancy may be related to errors in the doubly-labelled water method used in these iguanas. The overestimates of the rate of carbon dioxide production when using the doubly-labelled water method may be related to a violation of the assumptions on which the method is based. It is also likely that more than one violation may have occurred. Mathematical adjustments that improved the ratio of N_D/N_O led to an even larger difference between pCO_{2dlw} and pCO_{2re}. Thus, a single, systematic error, is unlikely the cause of the discrepancy between respiratory calorimetry and the DLW method.

Energy expenditure measured by respiratory exchange of iguanas, in closed-circuit calorimeters, was accurate and economical. A minimal amount of equipment was necessary for these measurements, and measurements on several iguanas could be made at one time, in a relatively small space.

The physiological responses of the iguanas used in

these studies are a function of the diets that were fed and the environment in which the iguanas were housed. These diets and the environment are much different from those experienced by free-ranging iguanas in the tropics. These diets consisted of relatively fine particles. Wild iguanas would normally consume large pieces of leaves, fruits or flowers. This difference in particle size may influence digestive physiology. Iguanas in the wild also are likely to experience diurnal and seasonal changes in temperature, rainfall, and food availability. The iguanas used in these studies were maintained in a stable environment. Thus, the interpretation of the data from these studies must take these differences into account. While the conditions used in these studies may be different from those experienced by free-ranging iguanas, they are similar to environmental conditions in zoos that maintain this species. Thus, the data may be applicable to systems of captive management.

The data collected from these studies provides a framework for future research with the green iguana. Fiber intake of free-ranging iguanas may be much higher than the highest level of fiber used in these studies. There is still little known about hindgut fermentation in this species of iguana. In the wild, iguanas undoubtedly encounter secondary plant compounds that may have wide ranging effects, including effects on digestive processes and food selection. Furthermore, only two ambient temperatures were used in this study. Physiological

responses to changes in temperature are probably non-linear, and studies of the response to more temperatures will be necessary to better understand their effects. A primary goal of the captive management plan for the green iguana in Central America is intensive production. Defining the nutritional requirements of this species is likely to be important for successful intensive production.

Folivory in lizards is rare, and the green iguana has been able to exploit this ecological niche. This species has the potential to provide a source of nutrients to humans in Central America. The intensive production of iguanas may encourage an alternative agriculture that is less destructive than traditional slash-and-burn techniques, and the overgrazing associated with intensive beef cattle production. Thus, reintroduction of captive-raised iguanas into appropriately restored habitat has the potential to provide a source of human food, and a monetary return, without the adverse ecological impact of the more exploitive and environmentally harmful practices currently in use in some areas of the tropics.

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