This is to certify that the

dissertation entitled

Nutritional Aspects of Insectivory

presented by

Mary Eleanor Allen

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Animal Science

Duane E. Ullrey
W. F. Magee
Major professor

Date 2/10/89

NUTRITIONAL ASPECTS OF INSECTIVORY

by

Mary Eleanor Allen

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1989

ABSTRACT

NUTRITIONAL ASPECTS OF INSECTIVORY

by

Mary Eleanor Allen

Insectivorous animals may be influenced by the nutrient composition of ingested prey items. Invertebrates were found to be highly variable in composition: 2 - 62% fat, 7 - 11% total nitrogen, 0.3-0.9% calcium (Ca), 0.3 -1.2% phosphorus (P) and 4.4 -7.5 kcal/g, on a dry matter basis (DMB). Although live prey such as crickets (Acheta domestica) are typically low in Ca, the levels of Ca were increased by feeding high Ca diets. Mature fox geckos (Hemidactylus garnoti) fed high-Ca (1.3%) crickets had significantly higher whole body Ca levels than did geckos fed low-Ca (0.23%) crickets, but dietary Ca had no effect on body composition of mature Cuban tree frogs (Osteopilus septentrionalis). Growth rate, feed intake, bone ash percentage and bone Ca percentage were greater in growing leopard geckos (Eublepharis macularius) fed high-Ca crickets (0.85% Ca) than in geckos fed low-Ca crickets. Vitamin D_3 content of crickets was also altered, and had an effect on bone Ca content independent of dietary Ca effects. However, juvenile day geckos (Phelsuma madagascariensis) experienced high mortality and bone demineralization when maintained on the same diets as the leopard geckos, suggesting that basking diurnal lizards may have different nutritional requirements. A three-month calcium balance trial

revealed that leopard geckos that had been previously depleted of Ca retained a very high percentage of ingested Ca (95%) during the first 2 months. Thereafter retention dropped to as low as 16% among animals on high-Ca diets (0.85% Ca), indicating repletion of body Ca stores. Digestibility of crickets was measured in the southern grasshopper mouse (*Onychomys longicaudus*,), the pygmy hedgehog tenrec (*Echinops telfairi*) and the musk shrew (*Suncus murinus*). Mice were selective, discarding cricket legs and heads, and had significantly higher DM (71.0%) and nitrogen (72.8%) digestibilities than either tenrecs or shrews. Tenrecs digested more chitin (19.8%) than did shrews (1.8%) or mice (12.0%). The digestible energy intake of tenrecs was extremely low (28.4 kcal/kg$^{0.75}$) by comparison to the shrews (177 kcal/kg$^{0.75}$) and mice (150 kcal/kg$^{0.75}$). This research confirms that insect nutrients affect the performance of insectivorous animals, but all species do not respond alike.

ACKNOWLEDGMENTS

The efforts reflected in this dissertation were possible only because of the help and support of many volunteers, friends, colleagues, and teachers. To Dr. Duane E. Ullrey, my committee chairman, I owe a special thanks for encouraging me to return to academia to pursue my doctoral degree. He generously provided logistical and financial support despite limited resources and was always willing to share his time although it, too, was often scarce. I will always be indebted to him for giving me the opportunity to help expand the developing field of zoo animal nutrition. I am deeply indebted to the other members of my committee. Dr. James Miller patiently helped me learn about insect cuticular structure and function so that I might better understand the meaning of insectivory. I also had to learn about insectivores. Dr. Donald Straney had the task of trying to teach an animal scientist how the "real" animals have adapted and evolved. Dr. Mel Yokoyama was genuinely interested and always enthusiastic about my research even though it was far afield from most Animal Science projects. I am deeply grateful to each of these individuals for their patience and commitment to this multi-disciplined project.

Most of this work would not have been possible without the financial support of the Friends of the National Zoo. FONZ provided research funds for 18 months which enabled me to complete the

earlier phases of this research. The Smithsonian Office of Fellowships and Grants awarded a pre-doctoral fellowship for one year in which I completed the remainder of the animal experimentation.

Most of the animal trials were conducted at the National Zoo. I am grateful to Dr. Dale Marcellini, Dr. Olav Oftedal and Dr. Devra Kleiman for their support, encouragement and constructive criticism. I am especially indebted to Michael Jakubasz for his patience with me and my many animals, and for his logistical help which was crucial to the success of my experiments.

The bulk of the laboratory analyses was conducted at Michigan State University, much of which was made easier because of the help of Ms. Phyllis Whetter. She provided invaluable assistance in the thousands of analyses required for this research. She willingly shared her expertise in analytical methods and generously gave of her time. I am also grateful to Dr. Pau Ku for teaching me sound laboratory techniques and always finding the time to answer my many questions.

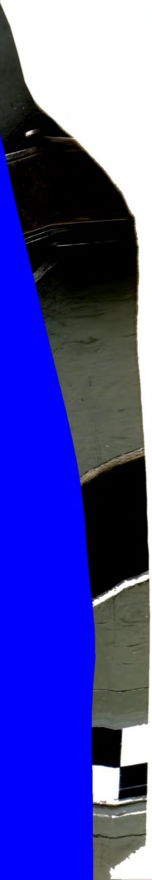
The chapters represent a series of experiments in which I obtained assistance from many sources:

Chapter 1. Drs. Thomas Kunz, James Malcolm and Olav Oftedal provided insect samples and allowed publication of the data. Dr. Rudy Rudran obtained support from the Smithsonian IESP program to allow me to travel to Venezuela where additional insects were collected.

Chapter 2. Karen Warnell and Gail Gullickson provided help in feeding and sampling the crickets. Miles Roberts, Department of Zoological Research, provided crickets from tarsier cages for analysis. Dr. U.V. Mostosky kindly allowed access to radiographic equipment at Michigan State University, and Dixie Middleton patiently helped in the unconventional task of cricket radiography. Dr. Mark Subramanyam, Zeigler Brothers, Inc., was very helpful in cricket diet formulation and manufacture. Dr. Lee Ann Hayek provided helpful comments regarding the statistical analyses. I thank Dr. Elsie Widdowson and Dr. Olav Oftedal for helping to inspire this research and for their valuable comments.

Chapter 3. At Michigan State University, Dr. John Shelle, Dr. Kristin Johnson, Karen Studer, Mark Strzelewicz and Lisa Haberstroh all gave willingly of their time and provided much needed assistance in the care and maintenance of the crickets, frogs and geckos. Dixie Middleton and Dr. U.V. Mostosky provided helpful comments and access to radiographic equipment. Dr. Susan Crissey and Dr. Larry Douglas kindly assisted in the statistical analysis of the data.

Chapter 4. I am especially indebted to Bela Demeter from whom I learned much about the care and management of insectivorous lizards. His support and friendship throughout my experiments with geckos is greatly appreciated. Others in the Department of Herpetology, especially Dr. Dale Marcellini and Michael Davenport were always enthusiastic and supportive. Diana Sexton and Sean O'Brien volunteered many hours of their time for feeding and cage cleaning.



Dr. Don Sweet, Armed Forces Institute of Pathology, generously allowed the use of radiographic equipment. Mr. Marty Bueno and Ms. Melina Villalobos, Food and Drug Administration, provided laboratory space and much technical advice and guidance for the vitamin D analyses. Dr. Robert Placious and Dr. Dan Polansky, U.S. Bureau of Standards, kindly allowed the use of dark room equipment. Dr. Ron Horst, Animal Disease Center, generously determined plasma vitamin D concentrations. Dr. Larry Douglas provided valuable comments concerning experimental design and statistical analyses. Dr. Susan Crissey willingly gave her time and expertise during data analysis.

Chapter 5. Bela Demeter coordinated the leopard gecko breeding schedule to provide me with the necessary animals for this experiment. David Edwards assisted during the many months of gecko care and feeding prior to the balance trial. Dr. Lee Ann Hayek assisted in the statistical analysis and data interpretation.

Chapter 6. I thank Dr. Elizabeth Horner who encouraged me to study grasshopper mice and who donated mice from her collection at Smith College for that purpose. Dr. Gil Dryden generously gave me musk shrews from his colony at Slippery Rock University. Miles Roberts permitted the use of hedgehog tenrecs from the research collection at the National Zoo. I learned much about the management of tenrecs from his staff, Frank Kohn, Angela Keppel and Mike Deal. Gail Gullickson assisted in various aspects of animal care and sample processing. I owe a special thanks to Tasha Belfiore who, over the course of many months, volunteered so much of her time during the

animal trials and later, during the laborious and time-consuming sample processing. Dave Baer provided some much-needed help when there seemed to be too many animals and not enough time.

Other friends and associates helped in the many different phases of this research. To Dr. William Rumpler, Dave Baer and Joni Bernard, I am grateful for advice, assistance and friendship. I am indebted to Allen and Baer Associates, Inc. for the use of computer equipment and supplies, and for covering some of the costs associated with preparation of the dissertation.

I owe a special thanks to my colleague, friend, and husband, Olav Oftedal. His patience, encouragement, assistance and advice have made a critical difference to my research efforts.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION: INSECTS AS FOOD

Insectivory

The term insectivory is commonly used to describe the consumption of a wide variety of invertebrate species, including arachnids, annelids, crustaceans and insects. Despite an increasing number of detailed behavioral and ecological studies of insectivorous species (cf. Eisenberg, 1981; Redford, 1987), the nutritional and metabolic consequences of eating insects and other invertebrates are little known.

Insectivory is prevalent among all classes of higher vertebrates. Many amphibians, reptiles, birds and mammals are obligate insectivores that do not normally consume other types of prey than invertebrates, and a large number of additional species are facultative insectivores that consume invertebrates as available or to supplement other food items. From a nutritional viewpoint this distinction is important as the obligate insectivore needs to obtain <u>all</u> required nutrients from invertebrates, but the facultative insectivore does not. However, there is some evidence that insectivorous species may supplement mineral intakes by consuming calcareous material or soil (cf. Robbins 1983).

Relatively little is known about the nutrient requirements of captive wildlife, and especially of insectivorous species. In order to manage insectivorous zoo species more successfully, a better

understanding of the composition of invertebrate prey is required. This is especially important both because many insectivorous zoo species must be fed insects, and because available prey is usually restricted to a few species.

Investigators have been interested in nutritional aspects of insects and insectivory for a variety of reasons. Ecologists and evolutionary biologists are primarily concerned about the interrelationships between insectivores and their prey, but have thought little about the nutrient composition of invertebrates except in regard to energy content. Certainly invertebrates have played an important role in vertebrate evolution. For example, the earliest mammals that evolved from therapsid reptiles in the Triassic were probably small (ca. 30 grams), nocturnal and insectivorous (Crompton, 1980). Among extant mammals, the Insectivora (8 families, 60 genera and 379 species; Nowak and Paradiso, 1983) is often considered "primitive" because many morphological characteristics retained by its members resemble those of the earliest mammals (cf. Eisenberg, 1980, 1982; Nowak and Paradiso, 1983; Crompton, 1980). The conservative, or plesiomorph, features shared by many members of the order include small eyes, presence of a cloaca, simple tooth structure, reduced or absent zygomatic arch and fusion, distally, of tibia and fibula. By contrast some mammalian insectivores exhibit highly specialized anatomic features associated with feeding on insects, especially social insects such as ants and termites (cf. Griffiths 1978, Montgomery 1985, Redford 1987).

understanding of the composition of invertebrate prey is required

both because many insectivorous zoo

available prey is usually

Animal nutritionists have been mostly concerned with the nutritional composition of invertebrates or invertebrate by-products (eg. shrimp waste meal, crab waste meal) especially in relation to their potential use as ingredients in feeds for domestic livestock. For example, a number of investigators have studied the value of insects as supplemental sources of protein for livestock, poultry and lab animals (Teotia and Miller, 1973; McInroy, 1971; Modzelewski and Culley, 1974; McHargue, 1917; Landry, et al., 1986; Finke, et al., 1985; DeFoliart, et al., 1982; Finke, et al., 1987). The impetus for such investigations has usually been the desire to develop least-cost feeds for the livestock industry. However, the efficient harvest of such resources represents a formidable challenge yet to be solved.

The nutrient composition of invertebrates is also of some interest to human nutritionists since insect-eating (also called entomophagy in the literature) occurs in indigenous cultures in many parts of the third world. In such cultures insects may be an important source of nutrients, since the average diets of people in many under-developed countries are considered to be protein and energy deficient by comparison to standards set by the Food and Agriculture Organization of the United Nations. Much of the existing, yet scant, information on insect composition comes from studies of insects commonly eaten by human populations (Taylor, 1975; Bodenheimer, 1951; Tihon, 1946; Oliveira, et al., 1976). It is difficult to apply these data to the study of insectivory in animals because the analyses were often performed on cooked (fried

Animal nutritionists have been mostly concerned with the

nutritional composition of invertebrates or invertebrate

shrimp waste meal, crab waste meal) especially in

ingredients in feeds for domestic

investigators have studied the

livestock.

or boiled) insects or on insects with inedible portions (i.e. parts not eaten by humans, such as legs, wings, heads or gastrointestinal tracts) removed.

Data on nutritional composition of invertebrates are especially important for management of animals in zoos. Although some insectivorous mammal species, such as shrews, anteaters and tenrecs, can be fed non-insect diets in captivity, this is not the case for species of amphibians, birds, reptiles and tarsiers that must have live prey to elicit a feeding response. Bats and birds that feed on flying insects may be virtually impossible to maintain in captivity due to the difficulty in supplying an adequate supply of flying prey. Since live insects may be the only food offered to some insectivorous species, nutritional deficiencies can easily arise if the nutrient levels in the live prey are imbalanced. In addition, insects are widely used in zoos for supplementing diets of amphibians, reptiles, birds and mammals, either with the intent of providing behavioral stimulation or as a supplemental source of nutrients. Many different types of invertebrates are used in the feeding of aquarium fishes, and are commonly considered essential for successful reproduction (Jahn, 1977; Masters, 1975; Jocher, 1966; Gannon, 1960).

Invertebrates used as feed in U.S. zoos are typically terrestrial. Prey species may be either cultured in the zoo or may be obtained from a commercial supplier, and are usually limited to crickets (Acheta domestica), mealworm larvae (Tenebrio molitor), earthworms (Lumbricus spp.), wax moth larvae (Galleria mellonella)

and fruit flies (*Drosophila spp.*). European and Australian zoos often raise a somewhat wider variety of insects for use as food, including various species of grasshoppers, flies, cockroaches and worms (Meaden, 1979; personal observation).

It is not uncommon for insectivorous reptiles to be fed only crickets. For animals whose natural diets probably include hundreds of invertebrate species, it is not surprising that problems have been identified that are presumed to be associated with the consumption of such a limited diet. Two of the commonly heard complaints about insects as food involve calcium and chitin. One of the problems is that the insects used as food for zoo animals appear to be poor sources of calcium (Allen and Oftedal, 1982; Zwart and Rulkens, 1979). It has also been stated that "some reptiles fed a diet of mealworms exclusively may develop intestinal impaction from the accumulation of the ring-like chitinous body parts of the larvae" (Frye, 1981).

The objectives of this chapter are to:

1. Examine data on the nutritional composition of insects and other invertebrates, including both published information and original results.
2. Discuss the implications of the findings relative to expected nutrient requirements of insectivorous species.

Insect Composition

Sources of Data and Methods of Analysis

The existing data on insect composition are of variable quality. The fact that many papers do not report specific

analytical methods makes interpretation of the values difficult. Papers oriented toward the nutritional value of insects for human consumption often are limited to processed material (eg. cleaned and cooked). Interpretation of data may also be complicated if the gastrointestinal contents of the invertebrates are included, but the diets being consumed by the invertebrates are not reported. For the purpose of this chapter, only data from entire, unprocessed insects (including gut contents) that were analyzed by known and acceptable laboratory methods were included.

Samples of live insects and other invertebrates were obtained for analysis from a variety of sources (Table 1). Some samples were taken from zoo collections of cultured insects, and some were from commercial suppliers. Samples were also collected in the wild by myself or by collaborators in California, the District of Columbia, Kenya, Maryland, New Hampshire, Nova Scotia, and Venezuela. Freeze-dried and frozen insects and invertebrates were also purchased from local aquarium supply stores.

The original data that are presented were analyzed in duplicate (sample sizes ranged from 0.1 to 5.0 grams) by the following analytical methods:

1. Dry matter - frozen or freeze-dried samples were dried to constant weight in either forced-air convection ovens or in vacuum ovens at 60^{o} or 100^{o} C, respectively. For frozen insects drying usually took 2 days.

2. Gross energy - oven-dried samples were completely combusted in a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline,

analytical methods makes interpretation of the values difficult. Papers oriented toward the nutritional value of insects for human consumption often are limited to processed material (eg. cleaned and

data may also be complicated if the

invertebrates are included, but the

for the

Table 1. Sampling information for invertebrates analyzed in the present study.

Common name	Samples analyzed	Order	Species (if known)	Stage	Type of Sample[1]	Source[2]	Habitat[3]
CRUSTACEANS							
'Ocean plankton'	1	?	?	?	com.,F	Taiwan	Mar
'White shrimp'	1	?	?	?	com.,D	Taiwan	Mar
'Brine shrimp'	1	?	?	?	com.,D	Taiwan	Mar
brine shrimp	1	Anostraca	*Artemia salina*	?	com.,F	CA (USA)	Mar
water flea	1	Diplostraca	*Daphnia* sp.	?	com.,D	USA	Aqu
krill	1	Euphausiacea	*Euphausia* sp.	?	com.,D	Japan	Mar
INSECTS							
junebug	1	Coleoptera	*Phyllophaga sp.*	larva	wild,L	NS (CAN)	Fos
mealworm	3	Coleoptera	*Tenebrio molitor*	larva	cult.,L	zoo	Ter
Haitian cockroach	1	Dictyoptera	*Blaberus discoidalus*	adult	cult.,L	zoo	Ter
American cockroach	3	Dictyoptera	*Periplaneta americana*	adult	wild,L	DC (USA)	Ter
mosquito	1	Diptera	*Culex* sp.?	larva	com.,D	USA	Aqu
mosquito	1	Diptera	*Culex* sp.	adult	wild,D	MD (USA)	Aqu
fruitfly	1	Diptera	*Drosophila melanogaster*	adult	cult,L	zoo	Vol
seaweed fly	1	Diptera	*Coelopa sp.*	adult	wild,L	CA (USA)	Vol
flies	3	Diptera	pooled aggregate	adult	wild,D	NH (USA)	Vol
midge (bloodworm)	2	Diptera	*Tendipes* sp.	larva	com.,F/D	USA	Aqu
midges	2	Diptera	pooled chironomids	adult	wild,D	NH (USA)	Vol
mayflies	1	Ephemeroptera	pooled aggregate	adult	wild,D	NH (USA)	Vol
unident. Hemipteran	1	Hemiptera	not identified	adult	wild,D	Venez.	Ter
Apoica wasp	3	Hymenoptera	*Apoica* sp.	adult	wild,D	Venez.	Vol
termite Hodotermes	1	Isoptera	*Hodotermes mossambicus*	worker	wild,D	Kenya	Ter
termite Nasutitermes	3	Isoptera	*Nasutitermes sp.*	adult[4]	wild,D	Venez.	Ter
European corn borer	1	Lepidoptera	*Ostrinia nubilalis*	larva	cult.,L	zoo	Ter
European corn borer	1	Lepidoptera	*Ostrinia nubilalis*	pupa	cult.,L	zoo	Ter
wax moth	1	Lepidoptera	*Galleria mellonella*	larva	cult.,L	zoo	Ter
house cricket	5	Orthoptera	*Acheta domestica*	adult	cult.,L	zoo	Ter

Table 1 (Cont'd.).

Common name	Samples analyzed	Order	Species (if known)	Stage	Type of Sample[1]	Source[2]	Habitat[3]
INSECTS							
lubber grasshopper	3	Orthoptera	*Romalea microptera*	juv.	cult.,L	zoo	Ter
grasshopper Venez.	1	Orthoptera	not identified	adult	wild,D	Venez.	Ter
stick insect	1	Phasmida	not identified	juv.	cult.,L	zoo	Ter
caddis flies	5	Trichoptera	pooled aggregate	adult	wild,D	NH (USA)	Vol
OLIGOCHAETES							
common earthworm	3	Haplotaxida	*Lumbricus terrestris*	adult	cult.,L	zoo	Fos
tubifex worm	1	Tubificida	*Tubifex* sp.	adult	com.,D	USA	Aqu

1 Abbreviations as follows: com. = purchased from a commercial supplier; cult.= sample from cultivated colony; wild = wild-caught; L = live when obtained; F.= frozen when obtained; D = dried when obtained.
2 Source indicates state and/or country in which invertebrate caught (for wild-caught) or processed (for commercial samples). zoo = obtained from zoo.
3 Habitat in which invertebrate is active. Mar = marine; Aqu = aquatic (fresh-water); Fos = fossorial (burrowing); Ter = terrestrial; Vol = volant (flying).
4 Both workers and soldiers collected from nest.

IL). To correct for the formation of nitric acid during combustion, the residue was titrated with 0.0725 N sodium carbonate.

3. Nitrogen - oven-dried samples were digested and assayed according to the Semiautomated Method (#7.025) (Williams, 1984).

4. Chitin - oven-dried samples were digested in acid detergent according to Goering and Van Soest (1970). Chitin was estimated gravimetrically as the acid-detergent residue after corrections for ash were made (see Chapter 6).

5. Fat - oven-dried samples were extracted in petroleum ether by either Goldfisch or Soxhlet methods.

6. Ash - freeze- or oven-dried samples were burned in a muffle furnace at 600^{o} C overnight and the inorganic fraction was determined gravimetrically.

7. Minerals - frozen, freeze- or oven-dried samples were digested in nitric and perchloric acids under a perchloric acid fume hood. Digests were analyzed for calcium, magnesium, sodium, potassium, iron, manganese, copper and zinc by flame atomic absorption spectrophotometry. Selenium was analyzed fluorometrically according to Whetter and Ullrey (1978). Phosphorus was analyzed colorimetrically according to Gomorri (1942).

Fat and Energy

Fat varies considerably depending on the species and on the developmental state of the invertebrate, with a range of 2-62% (dry matter basis), but most values fall in the narrower range of 5-17% (Table 2). Some larval forms appear to have particularly high fat concentrations, including two species frequently used in zoos,

mealworms and waxmoth larvae. On the other hand, the larvae of mosquitoes, midges and corn borers do not appear to be particularly high in fat content. Low fat levels are seen in invertebrates with a high proportion of mineral matter (ash), including crayfish and some termites (Table 2).

There are ecological and physiological reasons for developmental or seasonal changes in fat content in a given species (Chapman, 1982). Many holometabolous insects accumulate fat in the fat body during larval development so that this organ may represent as much as 33% of the wet weight of the larva at maturity. Fat will also accumulate in migratory insects to provide an energy store to be utilized during flight. In other insects the fat body reserves increase prior to diapause or other periods of quiescence (Chapman 1982). Redford and Dorea (1984) and Griffiths (1978) have noted that mammalian predators may focus attacks on ant and termite nests to coincide with a seasonal abundance of reproductive alates that are high in fat content. Fat provides almost twice as much energy as do protein and carbohydrate, and thus the energy content of invertebrates reflects fat content. Thus it is not surprising that mealworm larvae are rather high in energy content. Unless the developmental and reproductive state of the insect is accounted for the energy and fat values should not be considered representative of the species as a whole.

mealworms and waxmoth larvae. On the other hand, the larvae of mosquitoes, midges and corn borers do not appear to be particularly high in fat content. Low fat levels are seen in invertebrates with mineral matter (ash), including crayfish and

Table 2. Proximate Analyses of Invertebrates.[1]

Common name	DM %	EE %	TN %	ASH %	GE kcal/g	Source[2]
CRUSTACEANS						
'Ocean plankton'	9.0	7.5	8.75	14.8	--	ND
'White shrimp'	--	5.3	10.78	11.5	--	ND
'Brine shrimp'	--	9.4	7.17	19.6	--	ND
brine shrimp	11.0	7.0	7.73	11.3	--	ND
crayfish, Mexico	33.5	4.0	8.21	36.9	--	1
water flea	--	6.6	8.83	10.8	--	ND
krill	--	17.4	7.66	12.5	--	ND
INSECTS						
junebug larvae	59.2	--	6.80	--	--	ND
mealworm larvae	36.1	41.7	7.74	4.6	7.49	ND
	42.3	35.4	8.45	3.2	6.53	2
	--	34.9	8.06	3.2	--	3
Haitian cockroach	30.3	--	10.04	--	5.95	ND
American cockroach	33.3	--	10.16	5.6	5.52	ND
mosquito larvae	--	16.1	6.75	11.8	--	ND
house fly pupae	--	9.3	9.82	11.9	--	4
	--	16.1	10.51	5.5		5
fruitfly	29.6	12.6	11.22	4.5	5.12	ND
seaweed fly	31.5	--	11.14	--	--	ND
midge larvae	--	8.3	7.87	14.5	--	ND
termite Armitermes	26.2	--	3.64	42.0	--	6
termite Grigiotermes	33.7	1.5	2.99	59.9	--	6
termite Hodotermes	--	--	--	7.8	--	ND
termite Nasutitermes	--	--	9.83	--	4.53	ND
	24.7	3.0	7.77	10.0	--	6
Agave caterpillar	32.7	41.7	8.10	3.0	--	1
corn borer larvae	27.3	17.2	9.66	2.9	5.69	ND
corn borer pupae	28.0	17.0	10.27	2.6	5.6	ND
wax moth larvae	43.9	61.5	4.92	1.8	--	ND
house cricket	29.9	17.3	10.58	6.1	5.34	ND
	29.3	18.6	10.68	5.5	--	7
Mormon cricket	29.7	15.1	9.28	7.1	--	8
grasshopper	30.5	6.3	12.67	5.0	5.25	9
Mexican grasshopper	--	7.3	8.47	19.8	--	10
caddis flies	--	--	10.98	--	4.7	ND
OLIGOCHAETES						
common earthworm	17.4	7.2	10.39	10.3	4.71	ND
	11.7	6.0	9.82	7.4	--	7
	17.4	6.0	8.56	23.1	4.42	11
red earthworm	16.3	4.8	10.19	15.1	4.70	11
dung earthworm	16.4	4.4	9.82	15.7	4.73	11
	12.9	6.4	10.90	5.2	--	12
tubifex worms	--	15.1	7.38	6.9	--	ND

Table 2 (cont'd.).

[1] Dry matter is expressed as a percentage of fresh (live) weight; all other nutrients are expressed on a dry matter basis.

[2] Source refers to publication from which data were taken, as follows:

1. Cambarus sp., Aegiale hesperiaris -Massieu et al. 1951.
2. Tenebrio molitor - Jones et al. 1972.
3. Tenebrio molitor - Thompson and Grant 1968.
4. Musca domestica - Teotia and Miller 1974.
5. Musca domestica - Calvert et al. 1969.
6. Armitermes euamignathus, Grigiotermes metoecus, Nasutitermes sp. (Means for soldiers and workers) - Redford and Dorea 1984.
7. Lumbricus sp., Acheta domestica - Modzelewski and Culley 1974.
8. Anabrus simplex (Means for males and females) - DeFoliart et al. 1982.
9. Melanoplus femurrubrum - Bird et al. 1982.
10. Sphenarium - Massieu et al. 1959.
11. Lumbricus terrestris, Lumbricus rubellus, Eisenia sp. - French et al. 1957.
12. Eisenia foetida - McInroy 1971.

ND = new data (see Table 1 for species names and sampling details).

Nitrogen and Protein

The nitrogen content of the analyzed invertebrates (and from published reports) does not vary, from species to species, to the same degree that fat does (Table 2). Most species contain about 8 to 10% nitrogen (DMB), with lower values observed in cases where nitrogen content is diluted by high fat (eg. waxmoth and some other larvae) or high ash contents (eg. some crustaceans and termites). Protein is usually reported in published data, on the assumption that nitrogen can be converted to protein by multiplying by 6.25 (assuming 16% nitrogen in protein). On this basis most insects would be calculated to contain 50 - 65% protein (DMB). The protein content of foods is commonly determined by a method (Kjeldahl) that liberates organic nitrogen from the sample. In the case of invertebrates, nitrogen is liberated from other nitrogenous constituents as well as protein. Insects and invertebrates contain chitin (N-acetylglucosamine), a nitrogenous-containing compound (see below). In addition, many insects contain other sources of non-protein nitrogen, such as uric acid, which may be stored in the insect body (Chapman, 1982). The use of the 6.25 conversion factor will therefore lead to an overestimation of the protein content of invertebrates. Unless chitin nitrogen and other non- protein nitrogen of the invertebrate is known, it is not possible to obtain an accurate protein value from nitrogen content.

Ash

Ash represents the inorganic portion of animal or plant material. Some invertebrates have a remarkably high ash content,

which can be attributed either to a calcareous exoskeleton (eg. crustaceans) or to ingested soil minerals (eg. in geophagous termites) (Table 2). The 59.9% ash value for Grigiotermes worker termites is matched by a value of 61.0% ash in Orthognathotermes worker termites (Redford and Dorea, 1984); by contrast other species of termites with different food habits have much lower ash values (Table 2; Redford and Dorea, 1984). Inclusion of the shell also leads to high ash values (64-89%) in whole-body analyses of clams (Thompson and Sparks, 1978). Relatively high, but variable, ash concentrations are seen in earthworms, presumably due to variation in the amounts of soil in the gastro-intestinal tract at the time of sampling. It is intriguing that detritus-feeding and filter-feeding aquatic insects (eg. mosquito larvae, midge larvae) also tend to have ash values that are somewhat elevated (10-15%; Table 2).

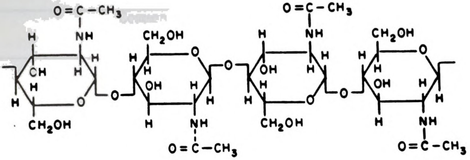
Chitin

Chitin, a structural, nitrogen-containing polysaccharide, is an integral part of invertebrate cuticle (Figure 1a). It is very similar in structure to cellulose (Figure 1b). It can be assayed by various methods including an enzymatic procedure (Richards, 1978), the Van Soest acid-detergent fiber method (White, 1981; Stelmock, et al., 1985), and the Welinder method (gravimetric determination after treatment with hot sodium hydroxide and filtration) (Welinder, 1974). When the acid-detergent fiber method is used, as in this study, a correction for the ash content (inorganic material from the insect body and from gut contents) is necessary.

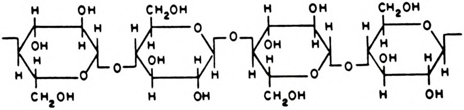
Most insects contain about 7-15% chitin (DMB) (Table 3). The which can be attributed either to a calcareous exoskeleton (eg. crustaceans) or to ingested soil minerals (eg. in geophagous termites) (Table 2). The 50.9% ash value for *Cryptotermes* worker

a value of 61.0% ash in *Orthognathotermes*

contrast other species



a. Chitin



b. Cellulose

Figure 1. a. Structural representation of chitin.
b. Structural representation of cellulose.

Table 3. Chitin content of invertebrates.[1]

Common name	'Chitin' %	Source[2]
CRUSTACEA		
crayfish	5.3	1
Crangon sand shrimp	5.8	2
Carcinus crab	8.3	2
INSECTS		
junebug larvae	9.4	ND
may beetle	16	2
mealworm larvae	5.3	ND
	4.4	1
	4.9	2
Haitian cockroach	11.2	ND
American cockroach	12.6	ND
fruitfly	27.0	ND
seaweed fly	13.7	ND
chironomid midge larvae	3.6	1
mayfly naiads	7.4	1
mayflies	8.1	2
Apoica wasp	11.4	ND
termite Hodotermes	27.0	ND
termite Nasutitermes	29.0	ND
corn borer larvae	13.1	ND
corn borer pupae	15.4	ND
dragonfly naiads	12.5	1
house cricket	9.1	ND
lubber grasshopper	9.7	ND
caddis flies	12.9	ND
OLIGOCHAETES		
Common earthworm	9.2	ND

[1] Chitin expressed on a dry matter basis.
[2] Source refers to publication from which data obtained.
1 = Windell (1967), using method of Richards (1951); no further identification of species given.
2 = cited by Richards (1951); no further identification of species given.
ND = new data, using ADF method (see text; see Table 1 for species names and sampling details).

somewhat lower values for mealworms and some crustaceans may be attributable to dilution by fat and ash, respectively. The chitin levels of the termites analyzed were remarkably high, but it is possible that the ADF fraction included lignin and cellulose in the gut contents of these animals. The analytical values reported for fruit flies and chironomid midge larvae seem somewhat aberrant, and should be replicated to confirm that they are correct.

Available data indicate that even with a chitin content of 15%, only a small part of the total nitrogen is represented by chitin. Assuming that chitin contains 7% nitrogen (Richards 1951), chitin nitrogen would represent 1% nitrogen on a dry matter basis. This is equivalent to about 6% 'protein' or about 10% of the total nitrogen in an insect. The degree to which animals may utilize chitin and the nutritional contribution chitin makes to some animals is discussed in Chapter 6.

Minerals

Invertebrates do not have internal, calcified skeletons as do vertebrates. As a consequence the calcium content of insects is low, relative to phosphorus, compared to animals with a bony, structural support. However, some invertebrates such as the crustaceans may have calcified cuticles, which also contain chitin and protein. Data on the mineral composition of invertebrates is presented Tables 4 and 5. Some, but not all, crustaceans have high calcium levels (3-10%). It is interesting that two products distributed commercially as brine shrimp are very different in calcium composition. Frozen brine shrimp were true _Artemia_ and were somewhat lower values for mealworms and some crustaceans may be attributable to dilution by fat and ash, respectively. The chitin levels of the termites analyzed were remarkably high, but it is

ADF fraction included lignin and cellulose in the

values reported for

Table 4. Analyses of the major minerals in invertebrates.[1]

Common name	Ca %	P %	Ca:P ratio	Mg %	Na %	K %	K:Na ratio	Data Source
CRUSTACEANS								
'Ocean plankton'	4.28	0.89	4.81	0.540	1.57	1.02	0.65	ND
'White shrimp'	5.46	0.81	6.74	0.440	0.50	0.22	0.44	ND
'Brine shrimp'	5.21	0.82	6.35	0.550	1.14	0.53	0.46	ND
brine shrimp	0.12	0.93	0.13	0.140	3.27	1.40	0.43	ND
crayfish, Mexico	9.70	1.26	7.70	--	--	--	--	1
water flea	0.10	1.17	0.09	0.160	0.98	0.99	1.01	ND
krill	2.53	0.83	3.05	0.480	2.18	0.31	0.14	ND
INSECTS								
mealworm larvae	0.07	0.60	0.11	0.115	0.06	0.71	11.05	ND
	0.62	0.54	1.15	--	0.09	0.85	9.44	2
(8%Ca)	1.01	0.76	1.33	--	--	--	--	3
American cockroach	0.57	0.74	0.77	0.153	0.61	1.57	2.57	ND
mosquito larvae	0.79	1.07	0.74	0.210	0.39	0.52	1.33	ND
mosquito	0.82	1.24	0.66	0.332	--	--	--	ND
house fly pupae	0.93	0.88	1.06	--	0.56	0.88	1.57	4
fruitfly	0.10	1.05	0.10	0.080	0.42	1.06	2.52	ND
seaweed fly	0.09	0.87	0.10	0.106	0.53	1.20	2.24	ND
flies	0.18	0.93	0.19	0.096	0.55	1.19	2.19	ND
midge larvae	0.41	0.96	0.43	0.195	0.89	0.66	0.74	ND
midges	0.19	0.99	0.19	0.112	0.51	1.20	2.34	ND
mayflies	0.17	1.08	0.15	0.170	--	--	--	ND
unident. Hemipteran	0.18	0.42	0.43	0.259	0.23	1.09	4.64	ND
Apoica wasp	0.11	0.33	0.34	0.068	0.15	0.89	5.76	ND
conifer sawfly larva	0.24	0.64	0.38	0.220	0.06	0.85	14.17	5
honey bee	0.15	--	--	0.177	0.02	0.74	40.43	6
termite Hodotermes	0.34	0.57	0.60	0.100	0.60	2.36	3.90	ND
termite Nasutitermes	0.30	0.38	0.79	0.231	0.42	0.95	2.28	ND
Agave caterpillar	0.43	0.43	1.00	--	--	--	--	1
corn borer larvae	0.23	0.64	0.36	0.120	--	--	--	ND
corn borer pupae	0.22	0.67	0.33	0.130	--	--	--	ND
wax moth larvae	0.03	0.39	0.08	0.055	0.04	0.52	14.56	ND
	0.07	0.26	0.27	--	--	--	--	7
spruce budworm moth	0.03	0.86	0.04	0.092	0.01	1.03	112.4	8
owlet moth	0.11	1.29	0.09	0.463	--	1.62	--	9
house cricket	0.18	0.86	0.21	0.105	0.50	1.27	2.55	ND
lubber grasshopper	0.31	0.72	0.43	--	--	--	--	ND
grasshopper Venez.	0.09	0.56	0.16	0.073	0.20	0.94	4.78	ND
grasshopper Canada	0.31	1.27	0.24	0.173	--	--	--	10
grasshopper Mexico	0.29	0.83	0.35	--	--	--	--	11
stick insect	0.30	0.96	0.31	--	--	--	--	ND
caddis flies	0.14	0.88	0.16	0.094	0.38	0.98	2.61	ND

Table 4 (cont'd.).

Common name	Ca %	P %	Ca:P ratio	Mg %	Na %	K %	K:Na ratio	Data Source
OLIGOCHAETES								
common earthworm	1.18	0.90	1.31	0.112	0.55	1.01	1.86	ND
tubifex worm	0.19	0.73	0.26	0.090	0.46	0.79	1.72	ND
dung earthworm	0.39	0.85	0.45	--	--	--	--	12

[1] All data expressed on a dry matter basis.
[2] Sources of data as follows:

1. Cambarus sp., Aegiale hesperiaris - Massieu et al. 1951
2. Tenebrio molitor - Jones et al. 1972.
3. Mealworms maintained on 8% calcium diet - M. Allen, pers. obs.
4. Musca domestica - Teotia and Miller 1974.
5. Neodiprion sertifer - Larsson and Tenow 1979.
6. Apis mellifera - Levy and Cromoy 1973.
7. Galleria mellonella - Strzelewicz et al. 198?.
8. Choristoneura fumiferana - Mattson et al. 1983.
9. Noctua pronuba - Bowden et al. 1984.
10. Melanoplus femurrubrum - Bird et al. 1982.
11. Sphenarium - Massieu et al. 1959.
12. Eisenia foetida - McInroy 1971.

ND = new data (see Table 1 for species names and sampling details).

Table 5. Analyses of trace mineral levels in invertebrates.[1]

Common name	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Se ppm	Source[2]
CRUSTACEANS						
'Ocean plankton'	225	68	82	8.0	0.73	ND
'White shrimp'	198	39	55	19.0	1.73	ND
'Brine shrimp'	1335	25	55	33.0	1.21	ND
brine shrimp	402	11	62	40.0	0.69	ND
water flea	3049	39	250	73.0	1.46	ND
krill	59	66	41	4.0	1.30	ND
INSECTS						
mealworm larvae	115	13	193	7.4	0.54	ND
American cockroach	5081	62	226	31.6	0.55	ND
mosquito larvae	3057	57	281	93.0	0.57	ND
mosquito	616	76	1057	70.4	--	ND
house fly pupae	465	34	275	370	--	1
fruitfly	138	18	171	39.0	0.07	ND
seaweed fly	409	15	92	6.2	0.10	ND
flies	1099	26	182	45.6	--	ND
midge larvae	4723	44	115	62.5	0.37	ND
midges	1360	29	218	18.5	1.05	ND
mayflies	447	23	144	6.4	--	ND
unident. Hemipteran	559	43	250	56.2	--	ND
Apoica wasp	774	72	80	186.0	1.69	ND
honey bee	58	12	--	--	--	2
termite Hodotermes	1562	30	206	31.4	0.24	ND
termite Nasutitermes	510	61	333	85.7	0.60	ND
corn borer larvae	289	24	90	18.0	0.31	ND
corn borer pupae	269	20	98	16.0	0.20	ND
wax moth larvae	44	6	43	2.5	0.66	ND
spruce budworm moth	81	15	115	5.0	--	3
owlet moth	140	59	401	42.0	--	4
house cricket	230	21	217	50.0	0.49	ND
grasshopper, Venez.	166	64	131	13.0	--	ND
grasshopper, Canada	331	50	200	25.1	--	5
caddis flies	390	36	122	47.0	0.68	ND
OLIGOCHAETES						
common earthworm	1786	13	359	70.5	2.15	ND
tubifex worm	1702	108	190	30.0	2.16	ND

[1] All data expressed on a dry matter basis.
[2] Sources of data as follows:
1. Musca domestica - Teotia and Miller 1974.
2. Apis mellifera - Levy and Cromoy 1973.
3. Choristoneura fumiferana - Mattson et al. 1983.
4. Noctua pronuba - Bowden et al. 1984.
5. Melanoplus femurrubrum - Bird et al. 1982.

ND = new data (see Table 1 for species and sampling details).

rather low in calcium content, but the freeze-dried shrimp from Taiwan are apparently oceanic crustaceans that are high in calcium.

Most insects contain relatively little calcium (about 0.1-0.4% DMB; Table 4) and have very low calcium:phosphorus ratios (about 0.1 to 0.4 Ca:P). Higher calcium values are found in mosquitoes and house fly pupae (0.8-0.9%) but not in other dipterans examined. Apparently, some fly pupae are known to accumulate calcium (M. Finke, pers. communication). The relatively high calcium level (0.6%) reported by Jones, et al. (1972) for mealworm larvae appears anomalous unless compared to data on mealworms fed a high calcium (8% Ca) diet (Table 4). Gut contents can clearly affect the whole-body calcium content of invertebrates (see Chapter 2), and may explain some of the diversity in calcium levels in Table 4.

Sodium and potassium concentrations in invertebrates are presented in Table 4, as well as the ratio of potassium to sodium. Sodium content of marine species appears to be higher than that of non-marine species. Conversely, the potassium content of non-marine insects is usually higher than the sodium content, although there are some exceptions (Mattson and Scriber, 1987). It has been suggested that this may be due to the fact that potassium is more important, for phytophagous insects, as a blood cation than is sodium, which is the important blood cation in mammals (Mattson and Scriber, 1987).

Trace mineral levels for invertebrates are presented in Table 5. Although the iron contents of most insects are in the range of 100 - 1000 ppm (DMB), some insects appear to contain much higher

levels. Soil feeders (such as earthworms and tubifex worms) and aquatic detritus feeders (water fleas, mosquito larvae, midge larvae) appear to have especially high iron concentrations (Table 5). Zinc, copper, manganese and selenium levels are also presented in Table 5. Both crustaceans and oligochaetes appear to have relatively high selenium levels.

Problems of Special Interest Related to this Thesis

A. Calcium

Captive insectivorous animals may develop osteodystrophic signs when fed diets of insects unsupplemented with calcium (see Chapter 4). This is presumably due to the low calcium content and inverse calcium to phosphorus ratio of insects used as food for zoo animals.

The inorganic matrix of the vertebrate skeleton is comprised primarily of calcium and phosphorus. The bones and teeth contain about 99% of the calcium and 80% of the phosphorus (Maynard, et al., 1979). Calcium represents approximately 1 to 2% of total body weight in vertebrates (2 to 6% on a dry matter basis), and the skeleton acts as both a structural support and as a reservoir of minerals (NAS, 1980). Bone is an extremely labile tissue, with mobilization and deposition of minerals occurring in response to physiological demands (NAS, 1980). The dietary requirements of domestic animals for calcium vary depending on dietary phosphorus, vitamin D status, and physiological state and are difficult to define in absolute terms (Maynard, et al., 1979). When the ratio of calcium to phosphorus is between 1.5:1 and 2:1 the requirements of domestic animals usually fall between 0.2 and 1.2% of the diet, but levels. Soil feeders (such as earthworms and tubifex worms) and

mosquito larvae, midge

ums (Table

higher dietary concentrations are needed by laying hens (cf. NRC, 1982; 1984a; 1984b; 1986).

Homeostasis of calcium is regulated by a number of hormones, including calcitonin, prolactin and parathyroid (PTH) hormone. Vitamin D, now considered to be a hormone, is also essential in promoting adequate skeletal growth and function. The active form of the vitamin is believed to act on the intestine to promote increased absorption of dietary calcium. Resorption of bone mineral is also regulated by vitamin D and PTH; PTH also regulates excretion and resorption of calcium and phosphorus by the kidney (Miller and Norman, 1984).

Most animals and pre-industrial age humans probably satisfied their requirement for this vitamin (hormone) by exposure to the sun, specifically ultraviolet light in the range of about 295 to 315 nanometers (Loomis, 1970). A precursor to vitamin D_3, 7-dehydrocholesterol, is converted to previtamin D_3 in the epidermis by the action of ultraviolet light. Previtamin D_3 undergoes a thermal conversion to vitamin D_3 at 37^{o} C. Hydroxylation of this compound occurs first in the liver at the 25 position and subsequently in the kidney by the action of 25-hydroxyvitamin-D-1-hydroxylase. Similar conversions in the liver and kidney occur when ergosterol, the plant form of vitamin D (D_2), is ingested.

With the confinement of animals in barns and cages and humans in nursing homes, dietary sources may be critical in satisfying vitamin D requirements (Toss, et al., 1982; Maynard, et al., 1979). However, it is believed that some zoo animals require a source of higher dietary concentrations are needed by laying hens (cf. NRC, ultraviolet light of the appropriate wavelengths (295 to 315 nm) (Townsend and Cole, 1985). Many species of basking lizards are typically exposed to an artificial source of UV in zoos. It is not known whether this 'requirement' for UV exists only in the face of low dietary vitamin D.

The dietary requirements of animals for vitamin D vary depending on factors such as exposure to natural light, calcium and phosphorus content of the diet, color and density of body covering and physiological state. Dietary vitamin D requirements for domestic animals are between 200 to 1,200 IU/kg of diet (NAS, 1987). Domestic poultry apparently utilize vitamin D_3 better than D_2 (Miller and Norman, 1984). With respect to the ability of animals to bind vitamin D_2 or D_3 by plasma proteins, Hay and Watson (1977) have shown that, in those species studied, reptiles and birds bound vitamin D_3 more efficiently. Perhaps because of the phylogenetic proximity of birds and reptiles, and in light of such studies (Hay and Watson, 1977), reptiles are thought to require vitamin D_3 (Frye, 1981). Renal 25-hydroxycholecalciferol-1-hydroxylase activity has been demonstrated in a number of species in the classes Reptilia and Amphibia (Henry and Norman, 1975). However, Robertson (1975) and Schlumberger and Burk (1953) have demonstrated that some insectivorous frogs (*Rana pipiens* and *Xenopus laevis*) respond to both vitamin D_2 and vitamin D_3.

It is not known how insectivorous animals satisfy their need for calcium. Wild, birds have been observed consuming calcareous material and it is suggested that such behavior is linked to the ultraviolet light of the appropriate wavelengths (295 to 310 nm) (Townsend and Cole, 1985). Many species of basking lizards are typically exposed to an artificial source of UV in zoos. It is not

for UV exists only in the face of

need for calcium in preparation for egg-laying (cf. Robbins, 1983). Joshua and Mueller (1979) have suggested that domestic poultry may have a 'calcium appetite' correlated with the demands of egg shell formation.

It is generally believed that captive animals do not possess nutritional wisdom with respect to selecting appropriately balanced diets. In the wild, feeding behavior, or more specifically, the food choices animals make, are inextricably bound with ecological, physiological and evolutionary factors. Animals do not make correct choices because they "know" what nutrients they need on any given day. Rather, it is believed that, through natural selection, those animals which selected foods that met nutrient requirements were the ones that were successful.

One explanation for the apparently adequate intake of calcium by insectivorous animals in the wild is that they may preferentially select calcium-rich foods when there is a need for calcium, such as for egg-laying, lactation or growth. There may be an innate, physiological response which elicits specific behaviors, perhaps only at certain times. The presence of gastroliths in the intestinal tract of reptiles has long been known. It has been suggested that such mineral material, including small rocks and pebbles, may serve to mechanically disrupt the chitin in the cuticle of consumed invertebrates thereby rendering the chitin more accessible to digestive enzymes (Skoczylas, 1978). Phelsuma madagascariensis (giant day gecko) have been observed eating pebbles and mineral material contained in the substrate on which they are

kept (Digney and Tytle, 1982; Demeter, 1976). These authors suggest that this behavior may be related to a need for calcium, rather than a function of reducing the insect cuticle. Digney and Tytle (1982) observed that geckos ingesting sand or gravel were often animals that had recently laid eggs or exhibited abnormal skeletal development. It is a common practice to make available to Phelsuma small containers of calcium carbonate, which the geckos have been observed to consume (B. Demeter, National Zoological Park, personal communication). It is not known whether this behavior is typical of behavior in the wild or is an artifact of captivity. Studies which correlate the consumption of such calcareous material with egg laying or growth are needed.

Another explanation is that calcium requirements of insectivorous species may be different from those of domestic animals. Are there differences in ability to absorb calcium at the level of the gut? Calcium absorption can vary markedly in domestic animals, depending on the source of calcium and the physiological state of the animal. Young, milk-fed animals apparently absorb more calcium than do older animals of the same species (Roy, 1980).

A third possibility is that, while insects may typically be poor sources of calcium, at certain times their gastrointestinal tracts may contain sufficient amounts of calcium to satisfy requirements of the insectivorous predator. Bilby and Widdowson (1971) suggested that the calcium required for growth in nestling thrushes and blackbirds may have been provided by the gut contents of the invertebrates fed to them by their parents.

B. Digestibility of Insects - Chitin

The extent to which chitin is digested and presumably utilized by an insectivorous predator is not known. Relatively few studies have been conducted comparing the digestibility of insects among different mammalian species (see Chapter 6). The distribution of refractory compounds in insect bodies may be important in determining the nutrient availability of ingested insects or insect parts. In this regard, analogies exist between the diets of herbivorous and insectivorous mammals. Plants may contain tannins, lignins and other polyphenolic plant defenses that deter consumption by vertebrates and invertebrates because the compounds are toxic or unpalatable for the consumer (Swain, 1979). Although the primary function of tannins in plants may be to convey protection to the plant from fungal and bacterial attack, tannins bind with and precipitate plant proteins and digestive enzymes following ingestion by consumers, rendering the ingested plant material less digestible. For example, tannins and other phenolic compounds reduce the protein (nitrogen) digestibility of forages eaten by ruminants (Van Soest, 1982).

Insect cuticle consists largely of protein and chitin (Muzzarelli, 1977). Protein is always found associated with cuticular chitin to which it is thought to be covalently bound (Chapman, 1982). In insects and some other invertebrates the outer part of the cuticle, called the exocuticle, is tanned or sclerotized with phenolic compounds as a means of providing structural support and protection. The inner, untanned part of the cuticle is referred

to as the endocuticle. The protein-chitin complexes of the exocuticle can be tanned in two different ways. Quinone tanning results from proteins being linked directly to the aromatic ring of N-acetyldopamine quinone, causing the cuticle to darken as sclerotization proceeds (Chapman, 1982). Beta-sclerotization involves the binding of proteins to the beta carbon of N-acetyldopamine and results in a colorless cuticle. In either case, the resultant sclerotized protein-chitin compound is highly resistant to enzymatic or chemical attack, so much so that during moulting, insects digest and resorb the undifferentiated cuticle but not the sclerotized parts (Chapman, 1982).

The degree of tanning in insect cuticle depends on the species of insect, its developmental state and the body part. Mandibles, and portions of the legs, head and wings are usually more heavily sclerotized (Chapman, 1982). Presumably, the chitin and protein in the untanned part of the cuticle, the endocuticle, is potentially available to a predator, as it is available to the insect itself during moulting. Insects are able to digest and 'recycle' this undifferentiated endocuticle through the action of epidermal chitinases on the endocuticle (Chapman, 1982). For a predator consuming an insect, the nitrogen and chitin that is part of this tanned protein-chitin complex is presumably unavailable, whereas the nitrogen and chitin in the endocuticle may be potentially available. It is thus important to understand the physio-chemical properties of insect integument, especially when evaluating the potential feed value of an insect to a predator.

to as the endocuticle. The protein-chitin complexes of the

exocutic different ways. Quinone tanning

aromatic ring of

Zoologists often suggest that certain mammals 'cannot digest chitin', since insect parts are present in mammalian stool. As explained above, the sclerotized portion is only one part of the cuticle, and it probably is indigestible. It may be the sclerotized cuticle that is reportedly seen in the stool of insectivorous animals. Recently it has been suggested that fecal analysis may be an unreliable method for determining prey items of insectivores, especially if soft-bodied prey are consumed (Dickman and Huang, 1988; Kunz and Whitaker, 1983). Even though scat analysis is a useful tool for zoological research, it may be biased by variation in the extent of sclerotization among insect prey items.

I address these issues in the following five chapters. An initial objective was to determine if the calcium composition of crickets (Acheta domestica) could be increased by feeding crickets high-calcium diets (Chapter 2). I then studied the effect of dietary calcium on the whole body calcium content of frogs and geckos (Chapter 3). I also examined the effect of two dietary calcium levels and two vitamin D_3 levels in immature geckos of a diurnal species and a nocturnal species, by assessing differences in bone composition and growth (Chapter 4). I attempted to better define the calcium requirement of an insectivorous lizard (Chapter 5). I conducted a calcium balance trial using young leopard geckos fed four different dietary calcium levels. In Chapter 6, I report the results of an experiment with three species of small mammals fed crickets. The objective of this study was to determine the extent to which insectivorous mammals digest the nutrients in insects.

List of References

Allen, M.E. and Oftedal, O.T. 1982. Calcium and phosphorus levels in live prey. NE Regional Proceedings American Association of Zoological Parks and Aquariums. Toronto, Ontario, Canada. Pp. 120-128.

Bilby, L.W. and Widdowson, E.M. 1971. Chemical composition of growth in nestling blackbirds and thrushes. British Journal of Nutrition 25:127-134.

Bird, D.M., Ho, S. and Macdonald, D.P. 1982. Nutritive values of three common prey items of the American kestrel. Comparative Biochemistry and Physiology A 73:513-515.

Bodenheimer, F.S. 1951. Insects as Human Food. W. Junk. The Hague, Netherlands.

Bowden, J., Digby, P.G. and Sherlock, P.L. 1984. Studies of elemental composition of insects as a biological marker in insects. I. The influence of soil type and host-plant on elemental composition of Noctua pronuba (L.). (Lepidoptera: Noctuidae). Bulletin of Entomological Research 74:207-225.

Calvert, C.C., Martin, R.D. and Morgan, N.O. 1969. House fly pupae as food for poultry. Journal of Economic Entomology 62:939-939.

Chapman, R.F. 1982. The Insects, Structure and Function. Third Edition. Harvard University Press, Cambridge, MA.

Crompton, A.W. 1980. Biology of the earliest mammals. In: K. Schmidt-Nielson, L. Bolis, C. Taylor, eds., Comparative Physiology: Primitive Mammals, Cambidge University Press, Cambridge, U.K.

DeFoliart, G.R., Finke, M.D. and Sunde, M.L. 1982. Potential value of the Mormon cricket (Orthoptera: Tettigoniidae) harvested as a high-protein feed for poultry. Journal of Economic Entomology 75:848-852.

Demeter, B. 1976. Observations on the care, breeding and behavior of the giant day gecko. International Zoo Yearbook 16:130-133.

Dickman, C.R. and Huang, C. 1988. The reliability of fecal analysis as a method for determining the diet of insectivorous mammals. Journal of Mammalogy 69:108-113.

Digney, T. and Tytle, T. 1982. Captive maintenance and propagation of the lizard genus Phelsuma. Proceedings of the 6th Annual Reptile Symposium on Captive Propagation and Husbandry. Washington, D.C.

Eisenberg, J.F. 1981. The Mammalian Radiations. University of Chicago Press, Chicago, IL.

Eisenberg, J.F. 1980. Biological strategies of living conservative mammals. In: K. Schmidt-Nielson, L. Bolis, C. Taylor, eds., Comparative Physiology: Primitive Mammals, Cambidge University Press, Cambridge, U.K.

Finke, M.D., DeFoliart, G.R. and Benevenga, N.J. 1987. Use of a four-parameter logistic model to evaluate the protein quality of mixtures of Mormon cricket meal and corn gluten meal in rats. Journal of Nutrition 117:1740-1750.

Finke, M.D., Sunde, M.L. and DeFoliart, G.R. 1985. An evaluation of the protein quality of Mormon crickets (Anabrus simplex Haldeman) when used as a high protein feedstuff for poultry. Poultry Science 64:708-712.

French, C.E., Liscinsky, S.A. and Miller, D.R. 1957. Nutrient composition of earthworms. Journal of Wildlife Management 21:348.

Frye, F.L. 1981. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Veterinary Medicine Publishing Compan, Edwardsville, KS.

Gannon, R. 1960. Live Foods for Aquarium Fishes. T.F.H. Publications, Neptune, NJ.

Goering, J.K. and Van Soest, P.J. 1975. Forage fiber analyses: apparatus, reagents, procedures and some applications. Agriculture Handbook 8, USDA, Washington, D.C.

Gomorri, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. Journal of Laboratory Clinical Medicine 27:955-960.

Griffiths, M. 1978. The Biology of the Monotremes. Academic Press, New York, NY.

Hay, A.W.M. and Watson, G. 1977. Vitamin D_2 in vertebrate evolution. Comparative Biochemistry and Physiology 56B:375-380.

Henry, H. and Norman, A.W. 1975. Presence of renal 25-hydorxyvitamin -D-1-hydroxylase in species of all vertebrate classes. Comparative Biochemistry and Physiology 50B:431-434.

Jahn, J. 1977. Lebendfutter. Lehrmeister-Bucheri Nr. 17. Albrecht Philler Verlag, Minden, West Germany.

Jocher, W. 1973. Live Foods for the Aquarium and Terrarium. T.H.F. Publications, Neptune, NJ.

Jones, L.D., Cooper, R.W. and Harding, R.S. 1972. Composition of mealworm Tenebrio molitor larvae. Journal of Zoo Animal Medicine 3:34-39.

Joshua, I.G. and Mueller, W.J. 1979. The development of a specific appetite for calcium in growing broiler chicks. British Poultry Science 20:481-490.

Kunz, T.H. and Whitaker, J.O. 1983. An evaluation of fecal analysis for determining food habits of insectivorous bats. Canadian Journal of Zoology 61:1317-1321.

Landry, S.V., DeFoliart, G.R. and Sunde, M.L. 1986. Larval protein quality of six species of Lepidoptera (Saturniidae, Sphingidae, Noctuidae). Journal of Economic Entomology 79:600-604.

Larsson, S. and Tenow, O. 1979. Utilization of dry matter and bioelements in larvae of Neodiprion sertifer Geoffr. (Hym. Diprionidae) feeding on Scots pine (Pinus sylvestris L.). Oecologia (Berlin)43:157-172.

Levy, R. and Cromroy, H.L. 1973. Concentrations of some major and trace elements in forty-one species of adult and immature insects by atomic absorption spectroscopy. Annals of the Entomological Society of America 66:523-526.

Loomis, W.F. 1970. Rickets. Scientific American 223(6):77-91.

Massieu, G., Guzman, J., Cravioto, R.O. and Calvo, J. 1951. Nutritive value of some primitive Mexican foods. Journal of the American Dietetic Association 27:212-214.

Massieu, G., Cravioto, R.O., Cravioto, O.Y. and Figueroa, F. 1959. Nuevos datos sobre el valor nutritivo de algunos insectos comestibles Mexicanos. Annales Societe Biologica, Pernambuco 16:91-104.

Masters, C.O. 1975. Encyclopedia of Live Foods. T.F.H. Publications, Neptune City, NJ.

Mattson, W.J. and Scriber, J.M. 1987. Nutritional ecology of insect folivores of woody plants: Nitrogen, water, fiber and mineral considerations. In: F. Slansky and J.G. Rodriguez, eds., Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates. John Wiley and Sons, New York, NY.

Mattson, W.J., Slocum, S.S. and Koller, C.N. 1983. Spruce budworm performance in relation to foliar chemistry of its host plants. In: Proceedings Forest Defoliator - Host Interactions: A comparison between gypsy moth and spruce budworms. USDA Forestry Service General Technical Report. NE-85. Forest Ecperiment Station, Hamden, CT.

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G. 1979. Animal Nutrition, McGraw Hill, New York, NY.

McHargue, J.S. 1917. A study of the proteins of certain insects with reference ot their value as food for poultry. Journal of Agricultural Research 10:633-637.

McInroy, D.M. 1971. Evaluation of the earthworm, Eisinia foetida, as food for man and domestic animals. Feedstuffs (Feb. 20):37,46.

Meaden, F. 1979. A Manual of European Bird Keeping. Blandford Press, Poole, Dorset, U.K.

Miller, B.E. and Norman, A.W. 1984. Vitamin D. In: L.J. Machlin, ed. Handbook of Vitamins. Marcel Dekker, Inc., New York, NY.

Modzelewski, E.H. and Culley, D.D. 1974. Growth responses of the bullfrog, Rana catesbeiana fed various live foods. Herpetologica 30:396-405.

Montgomery, G.G., 1985. Movements, foraging and food habits of the four extant species of Neotropical Vermilinguas (Mammalia: Myrmecophagidae) In: G.G. Mongomery, ed., Ecology and Evolution of Sloths, Anteaters and Armadillos (Mammalia, Xenarthra = Edentata), Smithsonian Institution Press, Washington, D.C.

Muzzarelli, R.A.A. 1977. Chitin. Permagon Press, Oxford, U.K.

NAS (National Academy of Sciences) 1987. Vitamin Tolerances of Domestic Animals. National Academy Press, Washington, DC.

Nowak, R.M. and Paradiso, J.L., (eds.) 1983. Walker's Mammals of the World, Vol. 1. The Johns Hopkins University Press, Baltimore, MD.

NRC (National Research Council). 1986. Nutrient Requirements of Cats. National Academy Press, Washington, D.C.

NRC (National Research Council). 1984a. Nutrient Requirements of Beef Cattle. National Academy Press, Washington, D.C.

NRC (National Research Council). 1984b. Nutrient Requirements of Poultry. National Academy Press, Washington, D.C.

NRC (National Research Council). 1982. Nutrient Requirements of Mink and Foxes. National Academy Press, Washington, D.C.

NRC (National Research Council). 1979. Nutrient Requirements of Swine. National Academy Press, Washington, D.C.

NAS (National Academy of Sciences) 1980. Mineral Tolerances of Domestic Animals. National Academy Press, Washington, DC.

Oliveira, J.F., Passos de Carvalho, S.J., Brunode Sousa, R.F. and Simao, M.M. 1976. The nutritional value of four species of insects consumed in Angola. Ecology of Food and Nutrition 5:91-97.

Redford, K.H. 1987. Ants and termites as food. In: H.H. Genoways, ed. Current Mammalogy, Vol. 1., Plenum Press, New York, NY.

Redford, K.H. and Dorea, J.G. 1984. The nutritional value of invertebrates with emphasis on ants and termites as food for mammals. Journal of Zoology, London 203:385-395.

Richards, A.G. 1951. The Integument of Arthropods. University of Minnesota Press, Minneapolis, MN.

Richards, A.G. 1978. The chemistry of insect cuticle. In: M. Rockstein, ed., Biochemistry of Insects, Academic Press, New York, NY.

Robbins, C.T. 1983. Wildlife Feeding and Nutrition. Academic Press, New York, NY.

Robertson, D.R., 1975. Effects of the ultimobranchial and parathyroid glands and vitamins D_2 D_3 and dihydrotachysterol$_2$ on blood calcium and intestinal calcium transport in the frog. Endocrinology 96:934-940.

Roy, J.H.B. 1980. The Calf. Butterworth and Company, Ltd., London, U.K.

Schlumberger, H.G. and Burk, D.H. 1953. Comparative study of the reaction to injury II. Hypervitaminosis D in the frog with special reference to the lime sacs. Archives of Pathology 56(2):103-124.

Skoczylas, R. 1978. Physiology of the digestive system. In: C. Gans, ed., Biology of the Reptilia Volume 8, Physiology B. Academic Press, London, UK.

Stelmock, R.L., Husby, F.M. and Brundage, A.L. 1985. Application of Van Soest acid detergent fiber method for analysis of shellfish chitin. Journal of Dairy Science 68:1502-1506.

Strzelewicz, M.A., Ullrey, D.E., Schafer, S.F. and Bacon, J.P. 1985. Feeding insectivores: increasing the calcium content of wax moth (Galleria mellonella) larvae. Journal of Zoo Animal Medicine 16:25-31.

Swain, T. 1979. Tannins and Lignins. In: G.A. Rosenthal and D.H. Janzen, eds. Herbivores, Their Interaction with Secondary Plant Metabolites. Academic Press, New York, NY.

Taylor, R.L. 1975. Butterflies in My Stomach. Woodbridge Press Publishing Co., Santa Barbara, CA.

Teotia, J.S. and Miller, B.F. 1974. Nutritive content of house fly pupae and manure residue. British Poultry Science 15:177-182.

Tihon, L. 1946. A propos des termites au point de vue alimentaire. Bulletin Agricole du Congo Belge 37:865-868.

Thompson, C.M. and Sparks, R.E. 1978. Comparative nutritional value of a native fingernail clam and the introduced Asiatic clam. Journal of Wildlife Management 42:391-396.

Thompson, R.D. and Grant, C.V. 1968. Nutritive value of two laboratory diets for starlings. Laboratory Animal Care 18:75-79.

Toss, G., Andersson, R., Diffey, B., Fall, B.A., Larko, O. and Larsson, L. 1982. UV-irradiation or oral vitamin D for the prevention of vitamin D deficiency in the elderly? In: Vitamin D, Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism. Walter de Gruyter and Co., Berlin.

Townsend, C.R. and Cole, C.J. 1985. Additional notes on requirements of captive whiptail lizards (Cnemidophorus), with emphasis on ultraviolet light. Zoo Biology 4:49-55.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O and B Books, Corvallis, OR.

Welinder, B.S. 1974. The crustacean cuticle - I. Studies on the composition of the cuticle. Comparative Biochemistry and Physiology 47A:779-787.

Whetter, P.A. and Ullrey, D.E. 1978. Improved fluorometric method for determining selenium. Journal of Official Analytical Chemists 61:927-931.

White, R.L., 1981. Ruminant utilization of chitin and other components of Tanner crab meal. M.S. Thesis, University of Alaska, Fairbanks, AL.

Williams, S. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA.

Windell, J.T. 1967. Rates of digestion in fishes. In: S.D. Gerking, ed., The Biological Basis of Freshwater Fish Production. Blackwell Scientific Publications, Oxford, U.K.

Zwart, P and Rulkens, R.J. 1979. Improving the calcium content of mealworms. International Zoo Yearbook 19:254-255.

Whetter, P.A. and Ullrey, D.E. 1978. Improved fluorometric method for determining selenium. Journal of Official Analytical Chemists 61:927-931.

White, R.L., 1981. Ruminant utilization of chitin and other components of Tanner crab meal. M.S. Thesis, University of Alaska, Fairbanks, AL.

Methods of Analysis. Association of Official Arlington, VA.

2. DIETARY MANIPULATION OF THE CALCIUM CONTENT OF FEED CRICKETS

Introduction

Crickets (<u>Acheta domestica</u>) and mealworm larvae (<u>Tenebrio molitor</u>) are the two most commonly used species of insect prey in U.S. zoos. They are fed to insectivorous and omnivorous mammals, birds, reptiles and amphibians. Some captive animals, such as frogs, geckos, flycatchers and tarsiers, may be obligately insectivorous, so that insects or invertebrates constitute their sole source of nutrients. There has been little research on the suitability of crickets and mealworm larvae as the complete diet for zoo animals. Limited information on nutrient composition indicates that these insects are poor sources of calcium, have high concentrations of nitrogen (protein) and, in the case of mealworm larvae, contain high concentrations of fat (Allen and Oftedal, 1982; Goulet et al., 1978; Jones et al., 1972; Modzelewski and Culley, 1974; Thompson and Grant, 1968; Zwart and Rulkens, 1979).

Metabolic bone disease is seen in a number of species of zoo animals (Fowler, 1986) and is usually the result of dietary imbalances of calcium, phosphorus and/or vitamin D. Zoo animals fed a diet solely of crickets or mealworm larvae may show signs of poorly mineralized bones (Allen et al., 1986; Modzelewski and Culley, 1974). In an attempt to avoid this problem, zoo personnel frequently supplement insects with a calcium source. For example,

calcium carbonate powder or vitamin and mineral preparations may be dusted on insects just prior to feeding. Although dusting insects with a calcium source can increase calcium concentrations (Allen and Oftedal, 1982) the effect will depend on the calcium content of the supplement as well as the amount that is adhering at the time the insect is eaten. If a cricket is not consumed within a short period of time, it may use its appendages to groom the dust from its body. It is not uncommon for crickets to be eaten hours after they have been placed in a zoo cage, at which time little if any of the supplement may remain on the crickets.

Another approach is to use insects that have been previously maintained on high-calcium feed materials (Allen and Oftedal, 1982; Zwart and Rulkens, 1979). Bilby and Widdowson (1971) suggested that it was the calcium content in the guts of invertebrates which provided sufficient calcium for nestling blackbirds and thrushes. In a preliminary study I demonstrated that both the calcium content and the calcium to phosphorus (Ca:P) ratio of crickets could be altered by manipulation of the calcium content of the food eaten by the crickets (Allen and Oftedal, 1982). The changes in mineral levels are probably due to changes in the composition of gastrointestinal contents. The present study was designed to confirm these results and to define the quantitative relationship between diet composition and resultant cricket composition. I am particularly interested in establishing:

1. the level of dietary calcium that is necessary to produce a cricket with a Ca:P ratio of approximately 1:1.

2. the length of time that crickets need to be maintained on experimental diets in order to effect this change in calcium concentrations.
3. the site at which calcium is accumulated in crickets fed high calcium diets.

Materials and methods

A two-way analysis of variance design (dietary treatment, treatment duration) was used to determine the effect of dietary calcium concentration on the whole-body calcium concentration of adult crickets.

Crickets were fed 6 different diets (3 replicate groups per dietary treatment) and sampled at sequential time intervals. A basal high-calcium (8% Ca) diet (Table 6), that had been developed and tested in cooperation with Zeigler Bros., Inc. (Gardners, PA), was modified to produce a series of experimental diets containing 2, 4, 6, 8, 10 and 12% calcium. Calcium levels in the diets were manipulated by replacing ground corn with varying amounts of limestone (calcium carbonate) and were manufactured by Zeigler Bros., Inc. Diets were ground to pass through a 2.4 mm mesh screen. Samples of each diet were saved for subsequent analysis.

The experimental cages consisted of white plastic bins (approximately 30 cm wide x 20 cm high x 40 cm long) with securely-fitting lids. The lids included openings (20 x 30 cm) that were covered with metal screening to allow ventilation and illumination for the crickets. The crickets were exposed to a 12:12 photoperiod,

Table 6. Ingredients used in the formulation of a cricket (8% calcium) diet.

Ingredient	% by weight
Corn, ground	8.3
Alfalfa, dehydrated	10.0
Soybean meal,48% CP	28.7
Wheat, ground	27.0
Calcium carbonate	20.0
Dicalcium phosphate	2.0
Salt, granular	0.5
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Soy oil	3.0

[1]The vitamin premix contained the following nutrients per gram: 28,000 IU vitamin A, 2,800 IU vitamin D^3, 132 IU vitamin E, 0.6 mg vitamin K, 6.0 ug vitamin B^{12}, 7.1 mg vitamin B^1, 2.0 mg riboflavin, 35.6 mg niacin, 9.5 mg pantothenic acid, 2.0 mg pyridoxine, 1.5 mg folic acid, 99 ug biotin, 190 mg choline.

[2]The mineral premix contained the following nutrients per gram: 144 mg calcium, 0.04 mg phosphorus, 4.3 mg magnesium, 0.60 mg potassium, 84.2 mg iron, 83.3 mg zinc, 81.1 mg copper, 119 mg manganese, 0.08 mg selenium, 0.32 mg iodine.

and ambient temperature ranged from 26^{o} to 29^{o} C. Six to eight pieces of cardboard egg carton, cut into 8 cm square pieces, were placed in each cricket bin to provide surfaces for climbing and resting. In each bin, feed was presented in a 23 cm (diameter) metal pan with 2.5 cm high sides which was placed on the bottom of the cage. Every 24 hours, feed was replaced and pans were cleaned. Distilled, deionized water was presented in a 15 cm (diameter) plastic petri dish lined with paper towel. The water supply of each bin was contained in a 250 ml Berzelius beaker inverted over the petri dish. The flow rate of water was controlled by placing a 3 mm wide rubber band under the lip of the beaker. The rubber band served as a wick, directing the water into the petri dish.

Adult crickets were purchased from a commercial supplier (Jiminy Cricket, Richmond, VA). An allotment of approximately 500 grams of crickets (mean weight = 0.32 grams $\pm$0.015 SE, n=30) was placed into each of the 18 bins. Initial (0 time) cricket samples of 50 grams were taken from each bin prior to introduction of the feed pans. Each dietary treatment was randomly assigned to three bins. The crickets had free access to the feed pans and were observed feeding within 1 hour of food presentation. Subsequent cricket samples of approximately 50 grams were removed from each of the bins at 12, 24, 48, 72, 96 and 120 hours after introduction of the feed pans. The crickets were placed in plastic bags and immediately frozen at -10 ^{o}C.

Duplicate 5-10 g samples of crickets were removed from each of the bags and weighed to the nearest 0.001 g into tared 250 ml

Phillips beakers. The samples were digested by nitric (10 ml) and perchloric (3 ml) acids under a perchloric acid fume hood. Calcium determinations were performed on the wet ash, with duplicate readings per sample, by flame atomic absorption spectrophotometry. Phosphorus was measured colorimetrically, with duplicate readings per sample, according to Gomorri (1942). Dry matter was determined on duplicate 5-10 g samples by drying to constant weight in a vacuum oven at 100 ^{o}C for two days. Feeds were assayed for dry matter, calcium and phosphorus by these same methods.

Sites of calcium accumulation were identified by radiography of frozen crickets (approximately 25 crickets per dietary treatment at 72 hours) with a Hewlett Packard Faxitron X-Ray unit. Crickets were exposed for 0.2 minutes at 2 ma and 35 kvp using Kodak NMB-1 film.

Data were analyzed using a PC SAS (SAS Institute, Cary, NC) statistical program for two-way analysis of variance (ANOVA) A probability level of 0.05 was chosen for determining statistical significance of observed differences. Means were compared using the Least Significant Difference procedure (LSD) controlling the comparisonwise error rate at 0.05. More conservative means comparison tests were also employed but did not substantially alter the conclusions and hence are not reported.

Results

The assayed calcium concentrations of the experimental diets are presented in Table 7. While there was some deviation from target calcium levels, the analyses approximate the expected range of calcium levels (2.9 to 11.4% Ca). The deviations may stem from

Phillips beakers. The samples were digested by nitric (10 ml) and perchloric (3 ml) acids under a perchloric acid fume hood. Calcium determinations [illegible] performed on the wet ash, with duplicate [illegible] absorption spectrophotometry.

Table 7. Calcium and phosphorus concentrations of cricket diets[1]

Diet	%Ca	%P
2%	2.92	0.76
4%	5.59	0.78
6%	7.69	0.70
8%	8.95	0.66
10%	10.57	0.72
12%	11.44	0.70

[1]Values are based on duplicate analyses and are expressed on a dry matter basis.

some settling and segregation of ingredients during shipping, causing sampling error. Since diets were mixed prior to placement in feed pans, deviation of offered feed from target calcium levels is apt to be less than the analyses in Table 7 indicate. For the sake of convenience I will refer to diets by their target calcium levels. The phosphorus (P) concentrations of all diets ranged from 0.66 to 0.78% P.

Both duration of treatment and type of dietary treatment had significant effects on calcium content and on Ca:P ratio of crickets (Figures 2 and 3; Table 8). The significant interaction of dietary treatment and duration of treatment indicates that the change in calcium content over time differed among treatment groups. There were no significant dietary treatment, treatment duration or interaction effects for phosphorus.

Table 8. Analysis of variance of dry matter and mineral levels in crickets fed experimental diets for 0 to 120 hours.

		DM		Ca		P		Ca:P	
	df	F	prob.	F	prob.	F	prob.	F	prob.
Dietary trt	5	2.60	.0309	34.13	.0001	0.24	.9442	21.09	.0001
Duration	6	8.27	.0001	24.33	.0001	0.58	.7419	24.33	.0001
Interaction	30	0.74	.8264	1.93	.0100	0.61	.9379	1.40	.1100

F = F Value
df = degrees of freedom
prob. = probability
Interaction = Dietary treatment x treatment duration

The Effect of Treatment Duration

Table 9 presents the means for dry matter (DM), calcium (Ca), phosphorus (P) and Ca:P ratio as functions of the duration of treatment. The length of time crickets were maintained on diets appeared to have an effect on dry matter content. In general, crickets fed for longer periods appeared to be lower in dry matter content.

Calcium concentration in crickets also differed according to duration of treatment. Crickets maintained for 48 to 120 hours had higher calcium levels than did those fed for 12 to 24 hours. Phosphorus concentrations were not affected by treatment duration. Differences among Ca:P ratio means were similar to those among calcium means; the ratio improved with duration of treatment from 0 to 48 hours; thereafter mean Ca:P ratio did not change significantly. I conclude that crickets must be fed on

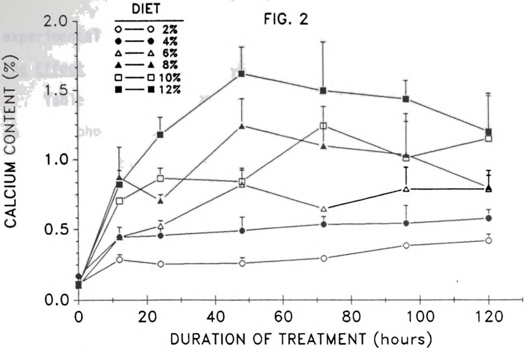


Figure 2. Effect of dietary calcium level on the calcium content of adult crickets.

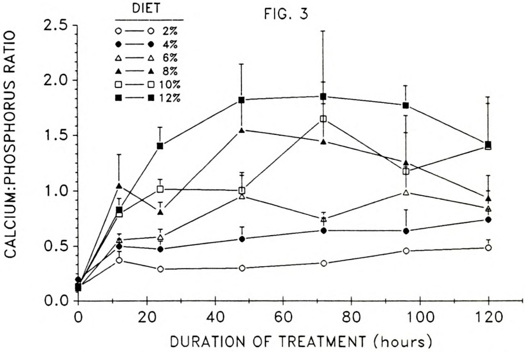


Figure 3. Effect of dietary calcium on the calcium:phosphorus ratio of adult crickets.

experimental diets for 48 hours to reach stable Ca:P levels.

<u>The Effect of Diet Composition</u>

Table 10 presents the means and standard errors for dry matter, calcium, phosphorus and Ca:P of crickets fed diets ranging from 2% to 12% Ca for the time periods 48 to 120 hours. Crickets that had fed for only 12 or 24 hours were excluded as they had yet to reach stable calcium and Ca:P levels. For this data set, analysis of variance revealed no significant differences attributable to duration of treatment for dry matter (probability (P) = .394), calcium (P = .923), phosphorus (P = .864) or Ca:P ratio (P = .823). Diet had no significant effect on either dry matter (P = .319) or phosphorus (P = .995).

In general, as dietary calcium increased, the concentration of calcium in crickets increased (Table 10). Crickets that received 12% Ca diets for 48 to 120 hours had a mean calcium value of 1.44% which was significantly higher than that of any other group. Crickets fed 10 and 8% Ca diets had means of 1.06 and 1.04% Ca respectively which were significantly lower than the 12% group but higher than the 6, 4 or 2% Ca crickets. Phosphorus concentrations did not differ among groups (range 0.85-0.87% P). The Ca:P ratio of the 12% Ca group (1.72:1) was significantly higher than that of any other treatment group (Table 10). There was no difference in the ratio between the 10 and 8% Ca groups (1.31:1 vs. 1.30:1), but the 6, 4 and 2% Ca groups had significantly lower ratios.

Table 9. Comparison of the dry matter (%) and mineral contents[1] of crickets in relation to the duration of time that diets were fed.

		DM		Ca		P		Ca:P	
Time	N	Mean	± SE	Mean	± SE	Mean[2]	± SE	Mean	± SE
0	18	32.033^{A}	0.272	0.120^{A}	0.006	0.8745	0.017	0.138^{A}	0.00
12	18	30.694^{B}	0.417	0.597^{B}	0.063	0.8750	0.027	0.685^{B}	0.07
24	18	29.739^{BC}	0.345	0.665^{B}	0.076	0.8931	0.022	0.764^{BC}	0.09
48	18	29.867^{BC}	0.498	0.879^{C}	0.118	0.8643	0.022	1.032^{CD}	0.14
72	18	29.294^{C}	0.268	0.886^{C}	0.118	0.8357	0.024	1.114^{D}	0.17
96	18	28.911^{C}	0.270	0.868^{C}	0.106	0.8572	0.022	1.049^{D}	0.13
120	18	29.517^{C}	0.417	0.824^{C}	0.093	0.8544	0.020	0.970^{D}	0.12

SE = Standard error

[1]Expressed as a percent of dry matter.
[2]Since P showed no significant diet effect by ANOVA, means were not compared by the LSD procedure.

Means in a column with different superscripts are significantly different at the 0.05 probability level (LSD Test).

Table 10. Comparison of the dry matter (%) and mineral contents[1] of crickets fed experimental diets[2] varying in calcium concentration.

		DM		Ca		P		Ca:P	
Diet	N	Mean[3]	± SE	Mean	± SE	Mean[3]	± SE	Mean	± SE
2%	12	30.242	0.452	0.340^{A}	0.024	0.859	0.019	0.344^{A}	0.029
4%	12	29.600	0.510	0.539^{AB}	0.039	0.850	0.024	0.649^{AB}	0.059
6%	12	28.625	0.460	0.761^{B}	0.051	0.853	0.022	0.883^{B}	0.081
8%	12	29.458	0.469	1.044^{C}	0.100	0.840	0.031	1.296^{C}	0.153
10%	12	29.333	0.470	1.062^{C}	0.106	0.852	0.035	1.307^{C}	0.160
12%	12	29.125	0.364	1.439^{D}	0.115	0.865	0.031	1.716^{D}	0.176

[1]Expressed as a percent of dry matter.
[2]Based on treatment durations of 48 to 120 hours.
[3]Since DM and P showed no significant diet effect by ANOVA, means were not compared by the LSD procedure.

Means in a column with different superscripts are significantly different at the 0.05 probability level (LSD test).

Radiographic Evaluation

Radiography revealed that radiopaque material accumulated in the gastrointestinal tracts (GIT) of crickets fed high calcium diets. The GIT of most crickets fed diets containing 8, 10 or 12% Ca appeared as well-defined structures (Figure 4). Crickets fed 2, 4 or 6% Ca diets had less radiodense material in the GIT, and in most cases it was impossible to visualize the GIT (Figure 5).

Discussion and conclusions

This study has demonstrated that the calcium concentration of adult crickets can be effectively increased by feeding diets containing high levels of calcium if fed for a sufficient period of time. The optimal diet to use for feeding crickets will depend on the desired level of calcium (and desired Ca:P ratio) and the length of time the crickets are to be maintained on the diet. While the calcium requirements of insectivorous animals are unknown, a dietary Ca:P ratio of 1:1 to 2:1 is the usual recommendation for birds and mammals (NRC, 1986; Scott et al., 1982). Higher levels may be desirable for birds producing large numbers of eggs (Scott et al., 1982). It appears that crickets must be fed a diet containing at least 8% calcium and that it should be fed for at least 48 hours to achieve a Ca:P ratio of 1:1 or higher.

The diets used in this study were formulated to produce changes in whole-body calcium levels of the insects, not to provide optimal nutrient levels for cricket growth or reproduction. Very high concentrations of dietary calcium were achieved by inclusion of large amounts (up to 30%) of limestone. As it may be difficult for

Figure 4. Radiograph of crickets fed 12% calcium diet for 72 hours.

The gastrointestinal tract is outlined as a radiopaque structure along the midline of the cricket. The radiopacity is presumably due to calcium contained in the gut.

Figure 5. Radiograph of crickets fed 2% calcium diet for 72 hours.
The gastrointestinal tract is poorly defined.

crickets to obtain sufficient energy and nutrients from such diets, I do not recommend that high calcium diets be fed to crickets for prolonged periods of time. It is also questionable whether very young crickets (e.g., "pinheads") will survive for very long on such diets. Studies are needed on the effect of high dietary calcium on growth and survival of young crickets.

A source of clean water may be an important factor in consumption of food by crickets, especially with diets high in dry matter and containing such high levels of limestone. Crickets offered cut pieces of orange and apple in addition to an 8% Ca diet appear to feed on the fruit more frequently than on the 8% Ca diet (M. Allen, personal observation). The common zoo practice of offering fruit and vegetables to crickets as a supplemental source of water and food may undermine the benefits of diets high in calcium. The crickets in this study only had access to the experimental diets and water.

The physical form of the diet may also be important to food consumption by crickets. In a preliminary study I determined that crickets eat twice as much of either 2% or 8% calcium diets when ground than when presented in pelleted form (3/16 inch pellets). The effects of factors such as water source, diet form, temperature and cricket size on food intake of crickets needs further investigation.

As illustrated in Figure 4, the ingested calcium becomes concentrated in the digestive tract. Thus the extent of GIT fill in the crickets may be an important factor in determining whole-body crickets to obtain sufficient energy and nutrients from such diets. I do not recommend that high calcium diets be fed to crickets for prolonged periods of time. It is also questionable whether very young crickets (e.g., "pinheads") will survive for very long on such high dietary calcium

calcium content. GIT fill is apt to be influenced by a number of behavioral and physiological factors, including pattern of feeding, transit time of ingesta and fecal excretion. GIT capacity may also differ among diverse species of insects, so the relationship of dietary calcium and calcium in the bodies of insects may be species-specific.

Crickets dusted with a calcium source are apt to provide little calcium for a predator if consumed many hours later. Similarly, crickets fed high calcium diets prior to introduction into the cages of predators will eventually excrete calcium from the GIT. One solution for this problem has been investigated at the National Zoo. Feed pans containing an 8% Ca cricket diet are placed in enclosures housing tarsiers (Tarsius bancanus) as part of routine husbandry. Crickets are introduced into the enclosures periodically, and consume the high calcium diet which the tarsiers ignore. Random samples of crickets caught at various locations in these enclosures had mean CA:P ratios of 1.64:1 $\pm$0.26 SE (n=10). Thus, although the tarsiers forage periodically rather than consume food at discrete meal times, the ingested crickets contain appropriate Ca:P ratios.

In the final analysis the best method for cricket supplementation must be judged by effects on growth, reproduction and health of the insectivorous animals. Allen et al. (1986) examined the effects of feeding hatchling leopard geckos (Eublepharis macularius) with crickets that had been kept on either low (1.3% Ca) or high (8.9% Ca) calcium diets. After seven months geckos fed high calcium crickets had significantly greater bone ash

(61.0% ash) and bone calcium (21.6% of dry fat-free bone) than did geckos receiving low calcium crickets (27.7% ash and 17.8% Ca). Bone integrity, as evaluated radiographically and histologically, was abnormal in geckos fed low calcium crickets compared to geckos fed high calcium crickets (Allen et al., 1986; M. Allen, unpubl.). Weight gains were also significantly greater in geckos fed high calcium crickets. Similar studies are needed with other insectivorous species.

It is not clear whether crickets fed on high calcium diets provide sufficient quantities of all nutrients required by insectivorous species. Nutrients such as trace minerals, amino acids and vitamins could still be limiting, but knowledge of the nutrient composition of insects and invertebrates is very incomplete. An insectivorous predator in its natural habitat normally selects from a wide variety of invertebrate species. Zoos that maintain insectivorous animals have an obligation to expand the variety of invertebrate prey used in feeding programs as well as to investigate ways to improve further the nutrient concentrations of the few insect species presently available.

List of References

Allen, M.E., and Oftedal, O.T. 1982. Calcium and phosphorus levels in live prey. NE Regional Proceedings, American Association of Zoological Parks and Aquariums, Toronto, Ont. Pp. 120-128.

Allen, M.E., Crissey, S.D. and Demeter, B.J. 1986. The effect of diet on growth and bone development in the leopard gecko (Abstr.). Annual Proceedings, American Association of Zoo Veterinarians. Chicago, IL. Pp. 44-45.

Bilby, L.W. and Widdowson, E.M. 1971. Chemical composition of growth in nestling blackbirds and thrushes. British Journal of Nutrition 25:127-134.

Fowler, M.E. (ed.). 1986. Zoo and Wild Animal Medicine, 2nd ed. W.B. Saunders Co. Philadelphia.

Gomorri, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. Journal of Laboratory and Clinical Medicine 27:955-960.

Goulet, G., Mullier, P., Sinave, P. and Brisson, G.J. 1978. Nutritional evaluation of dried Tenebrio molitor larvae in the rat. Nutrition Reports International 18:11-15.

Jones, L.D., Cooper, R.W. and Harding, R.S. 1972. Composition of mealworm Tenebrio molitor larvae. Journal of Zoo Animal Medicine 3:34-41.

Modzelewski, E.H., and Culley, D.D. 1974. Growth responses of the bullfrog Rana catesbeiana fed various live foods. Herpetologica 30:396-405.

NRC (National Research Council). 1986. Nutrient requirements of cats. National Academy Press, Washington, DC.

Scott, M.L., Nesheim, M.C. and Young, R.J. 1982. Nutrition of the Chicken. M.L. Scott and Assoc., Ithaca, NY

Thompson, R.D., and Grant, C.V. 1968. Nutritive value of two laboratory diets for starlings. Laboratory Animal Care 18:75-79.

Zwart, P. and Rulkens, R.J. 1979. Improving the calcium content of mealworms. International Zoo Yearbook 19:254-255.

3. THE EFFECT OF DIETARY CALCIUM CONCENTRATION ON MINERAL COMPOSITION OF FOX GECKOS AND CUBAN TREE FROGS

Introduction

Animals that eat insects may be facultatively or obligately insectivorous. For those that are compelled to consume insects and other invertebrates without mineralized skeletons, this feeding strategy may present some challenges for the mineral, particularly calcium, homeostatic mechanisms of the predator. The insect-eater in its natural habitat may obtain the required complement of minerals in a number of ways: by diversifying its food selection to include high-mineral invertebrate species, by ingesting soil along with the insect, as does the anteater for example, or by ingesting insects that feed on mineral-rich substrates, as suggested by Bilby and Widdowson (1971).

Certainly there are documented examples of unusual characteristics of the calcium homeostatic mechanisms of some reptiles and amphibians. For example, some species possess well-developed endolymphatic sacs that may be filled with calcium carbonate. These structures are sometimes visible externally as paired white structures in the ventral neck region in some lizards, particularly gekkonid lizards. The sacs are continuous with the endolymphatic system of the inner ear and were originally thought to play a role in hearing (Ruth 1918). In amphibians (e.g.,ranid and hylid frogs) they are sometimes

referred to as paravertebral lime sacs. They may be found as bilateral sacs adjacent to, but not continuous with, the vertebral column. Dacke (1979) and others (Robertson, 1969, 1971; Schlumberger and Burk, 1953) have demonstrated that a number of substances, namely calcium, vitamin D_3, parathyroid hormone and calcitonin, seem to influence the developmental state of these calcium carbonate deposits. It is also believed that the lime sacs play a role in the regulation of acid-base balance in amphibians by supplying carbonate in response to respiratory acidosis (Simkiss, 1968). Ruth (1918) was the first to provide a detailed description of the structure of the endolymphatic sac in several species of Philippine geckos. He noted that calcium-containing material was most evident in pregnant female geckos, and indicated that the endolymphatic sacs might play a role in eggshell formation. It has been suggested (Simkiss, 1967) that these deposits might respond to an ovarian hormone and function much as medullary bone functions in birds.

Some captive insectivorous animals develop osteomalacia and depressed reproductive performance as a result of calcium-deficient or calcium-imbalanced diets (Chapter 4). Certain species of geckos appear to be particularly prone to the stress imposed by a low calcium diet (R. Montali, D. Marcellini, National Zoological Park, personal communication).

The present study was designed to investigate the effects of two dietary calcium levels on mature Cuban tree frogs (_Osteopilus septentrionalis_) (formerly classified as _Hyla_) and fox geckos

(Hemidactylus garnoti, a parthenogenic species, sometimes referred to as the Indo-Pacific gecko). These species were selected because they are strictly insectivorous, they are likely to have endolymphatic or paravertebral lime sacs, and they are readily available from south-west Florida where introduced populations thrive. Dietary calcium concentration of insect prey (crickets) was manipulated in this study by feeding the crickets on rations of either high or low calcium content. When fed to crickets, an 8% calcium diet has been shown to increase the calcium to phosphorus ratio to at least 1:1 (Allen and Oftedal, 1982; Chapter 2). The objectives of the study were:

1. To determine if these species exhibit clinical or radiographic signs of osteomalacia when maintained on low-calcium (unsupplemented) insect prey.
2. To determine if dietary calcium content has an effect on total body content of calcium and phosphorus in these species.
3. To demonstrate by radiography the response of endolymphatic/paravertebral structures to dietary calcium levels.

Materials and methods

Crickets

Crickets (Acheta domestica) of approximately 7 to 10 mm in length were obtained from a commercial supplier (Flukers Cricket Farm, Baton Rouge, LA). In order to produce crickets

Table 11. **Ingredient formulation and chemical composition of experimental cricket diets.**

Ingredient	Low Calcium Diet (%)	High Calcium Diet (%)
Corn	28.6	6.7
Alfalfa meal	9.7	9.7
Soybean meal	23.7	27.8
Wheat	29.1	29.1
$CaCO_3$	1.65	19.4
Mono-dical Phos	1.8	1.9
Salt	0.4	0.4
Corn oil	2.9	2.9
MSU VTM premix[1]	1.6	1.6
Se 90 premix[2]	0.11	0.11
Vit E premix[3]	0.12	0.12
Vit A premix[4]	0.17	0.17
Vit D3 premix[5]	0.17	0.17
Calculated Analysis		
Crude protein, %	20.1	20.1
Calcium, %	1.2	8.2
Phosphorus, %	0.7	0.7
Vitamin A, IU/kg	61,500	61,500
Vitamin D3, IU/kg	7,200	7,200
Vitamin E, IU/kg	330	330

[1] 136,364 IU vitamin A/kg; 27,273 IU vitamin D3/kg
[2] 200 mg Se/kg
[3] 56,818 IU vitamin E/kg
[4] 30,000 IU vitamin A/g
[5] 3,000 IU vitamin D3/g

varying in calcium content, two bins of crickets were established in which either a low (1.2%) calcium diet or a high (8%) calcium diet were fed (Table 11). The crickets had free access to water, and pieces of cardboard egg carton were provided for shelter and climbing. Crickets were maintained on the diets for at least 48 hours prior to use as feed for geckos and frogs (Chapter 2). Crickets were sampled and frozen at periodic intervals for subsequent chemical analysis.

Geckos

Thirty adult fox geckos (ave. weight 2.7g) were purchased in late July 1982 from a commercial supplier (Herpetofauna, Ft. Myers, FL). The geckos were caught approximately two weeks prior to air shipment. As this species is parthenogenic, all animals were females. Upon arrival in East Lansing they were individually housed in 4-liter glass jars with vented tops. They were provided with paper towels for shelter and with sponges that were moistened daily to maintain humidity. The geckos were misted daily with distilled, deionized water and fed live crickets on an ad libitum basis three times per week. The jars were thoroughly cleaned at two-week intervals. As one animal was dead on arrival and several others seemed weak, a four-week acclimation period was allowed prior to initiation of the study. The geckos were fed high-calcium crickets during this period, but a large number of geckos (16 of 29 or 55%) died. Five of these were randomly selected as representing pre-treatment animals and were frozen for later analyses (to provide baseline calcium and phosphorus varying in calcium content, two bins of crickets were established in which either a low (1.2%) calcium diet or a high (8%) calcium diet were fed (Table 11). The crickets had free access to water, and pieces of cardboard egg carton were provided for shelter and climbing. Crickets were maintained on the diets for at least 48 hours prior to use as [illegible] levels). The remaining animals were randomly assigned either to a high-calcium (HiCa) group (n=7) or to a low-calcium (LoCa) group (n=6), and were fed crickets from the high-calcium and low-calcium bins, respectively. The experimental geckos were maintained on the treatments for seven months. Cool-white fluorescent lighting provided 14 hours light and 10 hours dark, and room temperature ranged from 20 to 26^{o} C. Geckos were weighed to the nearest 0.001 g before and after the experiment. An electrically heated pad (pig warmer) placed directly under the glass jars provided a thermal gradient of 22-30^{o} C in each jar.

Tree frogs

Twenty-six adult Cuban tree frogs were purchased in late July 1982 from a commercial supplier (Herpetofauna, Ft. Myers, FL). The tree frogs were caught approximately ten days prior to air shipment. Sixteen were males (ave. weight 7.5g) and ten were females (ave. weight 26.9 g). Upon arrival in East Lansing, each frog was housed individually in a 4-liter glass jar with a vented top. Approximately 150cc distilled, deionized water was placed in each jar along with small branches and crumpled paper towels that provided shelter.

The jars were thoroughly cleaned twice weekly. The animals were fed live crickets on an ad libitum basis three times a week. After an initial two-week acclimation period during which the frogs were fed high-calcium crickets, four tree frogs (two males and two females) were randomly selected as representatives of the pre-treatment animals, and were sacrificed by rapid freezing.

levels). The remaining animals were randomly assigned either to a high-calcium (HiCa) group (n=7) or to a low-calcium (LoCa) group (n=6), and were fed crickets from the high-calcium and low-calcium bins respectively. The experimental geckos were

The remaining frogs were randomly assigned to either a high-calcium (HiCa) group (n=7 males, 5 females) or a low-calcium (LoCa) group (n=6 males, 4 females). The frogs were maintained on the dietary treatments for seven months. The lighting schedule was 14:10 light:dark under cool-white, fluorescent lights. Room temperature ranged from 20 to 26^{o} C. Carbon dioxide levels were measured in six frog jars, which contained frogs, using a carbon dioxide analyzer (Bendix Gastec Pump). Carbon dioxide was 0.05% in all jars, including those containing fresh, distilled, deionized water and water that had been in the jars two days. The frogs were weighed to the nearest 0.001 g before and after the experiment.

Because of the unexpected finding of sexual difference in body composition (see Results), an additional group of wild-caught Cuban tree frogs was purchased from the same supplier in August 1984. These animals had been captured within one week of the date of shipping. On receipt in East Lansing, these animals were immediately sacrificed by quick freezing for subsequent compositional analysis. These frogs will be referred to as wild-caught (WC) frogs in this paper.

Radiography.

The HiCa and LoCa geckos and tree frogs were radiographed at approximately six-week intervals. Industrex (Kodak) M-2 high contrast film was exposed in a Faxitron (Hewlett-Packard) x-ray unit. Exposures were made at 40 kv (10 ma) for geckos, 45 kv (10 ma) for small frogs and 50 kv (10ma) for large frogs. Exposures

were of 0.3 minute duration. The animals were restrained in vented plastic bags so that they would be sufficiently immobile for radiography. Femur densities were evaluated from the radiographs with a densitometer (X-Rite model 301, Grand Rapids, MI).

Chemical analysis

Cricket samples, geckos and tree frogs were killed by rapid freezing at -20^{o} C and were freeze-dried for either 3 days (crickets) or 6 days (geckos and frogs). The freeze-dried geckos and frogs were quartered to increase surface area. The crickets, geckos and frogs were further dried to constant weight in a vacuum oven at 100^{o} C. The dried material was pre-digested overnight in nitric acid, subsampled and further digested with hot nitric and perchloric acids. Calcium was determined on duplicate subsamples of the digests by flame atomic absorption spectrophotometry. Phosphorus was measured in duplicate by the colorimetric method of Gomorri (1942).

Statistical Analysis

Body weight, dry matter, calcium, phosphorus, and calcium to phosphorus ratio were compared among geckos by Bonferroni T tests. In the frog diet study, these measures were analyzed, with sex and dietary treatment as the main effects, using two-way analysis of variance (PC SAS; SAS Institute, Cary, NC) on a Zenith 183 microcomputer. Means were compared with Bonferroni T tests. A 0.05 probability level was selected to determine significant differences.

were of 0.3 minute duration. The animals were restrained in vented plastic bags so that they would be sufficiently immobile for radiography. Femur densities were evaluated from the radiographs with densitometer (X-Rite model 301, Grand Rapids,

Results

Cricket composition

The high and low calcium diets fed to crickets produced crickets that contained markedly different calcium levels (Table 12). Dry matter and phosphorus levels were similar between the two treatments. The calcium to phosphorus ratios were 1.42:1 and 0.28:1 for high-calcium and low-calcium crickets, respectively. The low-calcium crickets were similar in composition to crickets that had been maintained on a stock diet of monkey biscuit (Ralston Purina, St. Louis, MO) (Table 12).

Table 12. Dry matter, calcium and phosphorus concentration[1] (mean ±SE) of crickets.

Item	High calcium crickets (n=4)	Low calcium crickets (n=5)	Stock crickets (n=7)
Dry matter (%)	26.70 ±0.70	26.00 ±1.02	26.53 ±1.75
Calcium (%)	1.26 ±0.35	0.23 ±0.04	0.20 ±0.04
Phosphorus (%)	0.89 ±0.03	0.82 ±0.04	0.93 ±0.04

[1]Calcium and phosphorus expressed as a percentage of dry matter.

Gecko Dietary Experiment

The geckos apparently did not adapt well to capture, shipment and relocation. In addition to the 16 that died during the adaptation period, one animal died after 35 days on the HiCa treatment and two died on the LoCa treatment (at 68 and 140

days). The HiCa animal was excluded from chemical and statistical analyses as it had been on the diet for only one month, but due to small sample size it was decided to include the two LoCa animals that died before the end of the study. All other animals lived until they were killed after 212 days on trial. The geckos were apparently sexually mature, as indicated by the fact that three animals laid eggs during the study, one in the HiCa group and two in the LoCa group (including the animal which died at 140 days, 6 weeks after laying two eggs). There was no significant weight change in either experimental group.

The mean calcium concentration (DMB) of geckos maintained on high-calcium crickets was 4.02% ±0.10 (SE). This was significantly higher ($P < 0.025$, t-test) than 3.43% ±0.24 (SE), the mean for the low-calcium geckos (Table 13). The five geckos

Table 13. Body weight and composition[1] of pre-treatment geckos and geckos fed high-calcium or low calcium crickets.

Item	Pre Trt (n=5)	Hi Ca (n=6)	Lo Ca (n=5)
Wt (g)	2.30^{A} ±0.14[2]	2.81^{A} ±0.17	2.60^{A} ±0.23
DM (%)	25.31^{A} ±0.93	29.44^{A} ±1.22	28.79^{A} ±1.90
Ca (%)	5.22^{A} ±0.18	4.02^{B} ±0.10	3.43^{C} ±0.24
P (%)	2.18^{A} ±0.18	1.40^{B} ±0.06	1.47^{B} ±0.05
Ca:P	2.44^{A} ±0.15	2.89^{B} ±0.10	2.34^{A} ±0.16

[1]Calcium and phosphorus expressed as a percentage of dry matter.
[2]Means (±SE) in a row with the same superscript are not significantly different at the 0.05 probability level (T tests).

selected to provide pre-treatment baseline data had a mean calcium level (DMB) of 5.22% $\pm$0.18 (SE) which was significantly higher than the means of either the low ($P < 0.025$, t-test) or the high ($P < 0.05$, t-test) calcium groups. Dry matter and phosphorus concentrations did not differ between the HiCa and LoCa groups, but both groups had lower phosphorus concentration than did the pre-treatment group. The HiCa group had a higher Ca:P ratio (2.89) than either the LoCa (2.34) or the pre-treatment (2.44) groups.

There were no quantifiable bone density differences, based on radiographic results, between high- or low-calcium treated geckos. Radiographs of two animals are shown in Figures 6 and 7. Although the endolymphatic sacs seemed to be somewhat more pronounced in radiographs of geckos in the HiCa group, there was great inter- and intra-animal variation. Endolymphatic sacs developed and regressed from one to three times in each gecko over the course of the trial. However in those geckos that produced eggs, the endolymphatic sacs were well-developed and clearly visible six to eight days prior to egg laying (Figure 8). They were observed to be much reduced in size when viewed within 24 hours after egg deposition.

Tree Frog Dietary Experiment

Dietary treatment did not appear to affect whole body composition of the tree frogs (Tables 14, 15). The significant effect of treatment on dry matter content reflects a difference between the pre-treatment group on the one hand and the HiCa and selected to provide pre-treatment baseline data had a mean calcium level (DM) of 5.22%±0.18 (SE) which was significantly higher than the means of either the low (P < 0.025, t-test) or the high (P < 0.05, t-test) calcium groups. Dry matter and phosphorus concentrations did not differ between the HiCa and LoCa groups, but both groups had lower phosphorus concentration

Figure 6. Radiograph of fox gecko fed high calcium crickets. The endolymphatic sacs appear as paired, white structures on either side of the cervical vertebrae.

Figure 7. Radiograph of fox gecko fed low calcium crickets. The endolymphatic sacs are not prominent.

Figure 8. Radiograph of fox gecko, with egg, fed low calcium crickets. An egg is forming in the oviduct, and the endolymphatic sacs are visible.

Table 14. Body weight and composition[1] of pre-treatment frogs and frogs fed high-calcium or low-calcium crickets (mean ±SE).

Item	Pre Trt		Hi Ca		Lo Ca	
	Male (n=2)	Female (n=2)	Male (n=6)	Female (n=4)	Male (n=7)	Female (n=5)
Wt (g)	8.05 ±1.06	33.57 ±5.03	7.42 ±0.51	32.57 ±1.90	9.28 ±1.01	30.65 ±1.98
DM (%)	21.99 ±0.81	25.43 ±0.34	24.70 ±1.01	28.74 ±0.56	26.06 ±0.69	28.92 ±0.73
Ca (%)	5.90 ±0.02	3.34 ±0.45	4.60 ±0.28	3.13 ±0.28	4.45 ±0.25	3.82 ±0.37
P (%)	2.19 ±0.18	1.84 ±0.09	1.87 ±0.12	1.52 ±0.13	1.66 ±0.08	1.83 ±0.16
Ca:P	2.71 ±0.22	1.80 ±0.16	2.47 ±0.04	2.05 ±0.05	2.71 ±0.18	2.10 ±0.11

[1]Calcium and phosphorus expressed as a percentage of dry matter.

Table 15. Analysis of variance of tree frogs (including pre-treatment frogs) with comparison of means, by sex.

	ANOVA						Comparisons of Means by sex[1]	
	Trt df=2		Sex df=1		Trt x Sex df=2			
Item	F	Prob.	F	Prob.	F	Prob.	Male (n=15)	Female (n=11)
Wt	0.10	0.9029	261.55	0.0001	1.06	0.3667	8.37^{A}	31.88^{B}
DM	6.44	0.0069	18.25	0.0004	0.28	0.7602	24.98^{A}	28.22^{B}
Ca	1.80	0.1917	27.22	0.0001	3.25	0.0601	4.70^{A}	3.48^{B}
P	2.09	0.1502	2.10	0.1632	2.90	0.0773	1.81^{A}	1.72^{A}
Ca:P	0.75	0.4874	24.33	0.0001	0.99	0.3883	2.61^{A}	2.03^{B}

[1]Data are pooled across treatments, as there was no significant treatment effect. Means in a row with the same superscript are not significantly different at the 0.05 probability level (T tests).

LoCa groups on the other, rather than a difference due to dietary calcium level. The mean calcium concentration (DMB) of the sexes combined was 4.20% ±0.19 (SE) in the HiCa group, 3.97% ±0.20 (SE) in the LoCa group, and 4.62% ±0.34 (SE) in the pre-treatment group, but these means are influenced by different numbers of males and females in the different groups (Table 14).

Sex had a highly significant effect on all measures except phosphorus concentration (Table 15). Females were several-fold larger than males, and were significantly higher in dry matter concentration but lower in calcium concentration (Tables 14, 15). The mean calcium level of male tree frogs in the combined pre-treatment, LoCa and HiCa groups was 4.70% ±0.20 (SE), as compared to a combined mean value for females of 3.48% ± 0.20 (SE) (Table 15). Some of the difference between female and male frogs may be related to reproductive state. All female frogs had egg-filled abdominal cavities at the end of the study, and these eggs were included in the whole body analyses.

No differences in bone density were evident among the frogs, as measured by densitometry of the radiographs. Although many species of frogs have been reported to have paravertebral lime sacs (Dacke, 1979), these could not be visualized radiographically in any of the frogs in this study (Figure 9).

Wild-caught Tree Frogs

A series of wild-caught frogs were analyzed to investigate the nature of the sexual difference in body composition noted above. Although there were only 5 males in this series, the 14

Figure 9. Radiograph of Cuban tree frog fed high calcium crickets.

females represented a wide range of body size (9.2-35.3 g) and were quite variable in fat content (2.6-8.2% DMB). Regression of fat content (DMB) on body weight for both sexes (Figure 10) revealed a significant positive trend (Fat% = 0.1333*wt + 2.4049, r^2 = 0.472, P < 0.002). Dry matter content was also positively related to weight (DM% = 0.1547*wt + 23.9075, r^2 = 0.444, P < 0.002) so the relationship between fat content on a live weight basis and body weight was especially pronounced. Calcium, phosphorus and calcium:phosphorus ratio were not significantly related to body weight, however.

When all male frogs were compared to all female frogs, there were significant differences in dry matter, but not in fat, calcium, phosphorus, Ca:P ratio, calcium as a percent of fat-free dry mass, or phosphorus as a percent of fat-free dry mass (Table 16). For comparison to the data from the tree frog dietary experiment, it is more appropriate to exclude immature females, however. If females less than 25 g were excluded, a significant sexual difference was observed in dry matter, fat and Ca:P ratio, but not in calcium or phosphorus, whether expressed on a dry matter basis or as a percentage of fat-free dry mass (Table 16).

Discussion

An effect of dietary treatment on body calcium concentration was observed in geckos. Diet had an effect on the calcium to phosphorus ratio, which was greater in the HiCa geckos than in the LoCa geckos. However, absolute calcium and phosphorus levels, expressed as a percentage of dry matter, declined in both

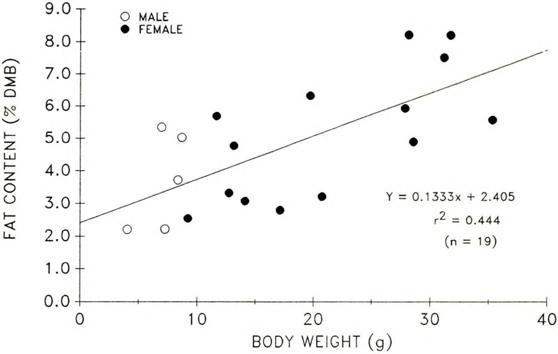


Figure 10. The relationship between body weight and fat content of wild-caught tree frogs.

Table 16. Body composition[1] (mean ±SE) of wild-caught tree frogs.

Item	Male (n = 5)	Female[2] All (n = 14)	Female[2] > 25 g only (n = 6)
Weight (g)	7.05 ±0.82	21.52* ±2.34	30.48* ±1.18
Dry matter (%)	24.80 ±0.82	27.31* ±0.57	28.64* ±0.61
Fat (%)	3.70 ±0.67	5.15^{NS}±0.53	6.72* ±0.59
Calcium (%)	5.09 ±0.43	4.68^{NS}±0.22	4.71^{NS}±0.19
Phosphorus (%)	2.49 ±0.24	2.60^{NS}±0.11	2.79^{NS}±0.14
Calcium:Phosphorus	2.05 ±0.09	1.83^{NS}±0.12	1.70* ±0.04
Calcium (%) Fat-free, dry[3]	5.28 ±0.43	4.93^{NS}±0.22	5.05^{NS}±0.19
Phosphorus (%) Fat-free, dry[3]	2.59 ±0.23	2.74^{NS}±0.12	2.99^{NS}±0.14

[1]Nutrients expressed as a percentage of dry matter.
[2]Columns refer to all females analyzed or to the subset of females greater than 25 grams. Statistical comparison to males (T tests) indicated by: * = significant at the 0.05 probability level; NS = not significant.
[3]Calcium and phosphorus expressed on a fat-free, dry matter basis.

treatments. One explanation may be that the relative proportions of skeleton, muscle and fat differed before and after seven months of captivity. Although this was not measured directly, the animals that were analyzed as pre-treatment geckos had died of unknown causes and appeared thin. Part of the explanation may also be related to mineral loss associated with reproductive effort. Egg production is extremely costly to calcium stores (Table 17). While there were substantial differences in the amount of calcium in gecko eggs, in the three instances where egg calcium content could be compared to total body calcium of the females that produced them, egg calcium represented an amount equivalent to 42 to 103% of the amount in the females.

The endolymphatic sacs are believed to be important to calcium homeostasis in reptiles although their specific function is still unknown (Dacke, 1979). In this study the endolymphatic sacs in Hemidactylus were observed to be very variable. Some of the geckos laid eggs, as noted above. A radiograph of one gecko revealed an apparently well-formed egg in the peritoneal cavity, but that egg was never observed in the gecko jar. Another gecko produced an egg, but twelve hours later the egg had disappeared. As the container was tightly closed, it was assumed that the egg had been ingested by the gecko, since captive geckos have been observed eating egg shell fragments (B. Demeter, National Zoological Park, personal communication). The observation that the endolymphatic sacs of the geckos seemed to be reduced in size after egg formation or egg laying, would support the idea that calcium was mobilized for egg

Table 17. Comparison of the calcium and phosphorus content of eggs to that in the whole body of the geckos that produced them.[1]

	Body			Eggs					
Gecko #	Dry wt. (mg)	Ca (mg)	P (mg)	No. eggs	Dry wt. (mg)	Ca (mg)	P (mg)	Ratio eggs:gecko Ca	Ratio eggs:gecko P
3	897	39.47	12.38	2	203	16.73	1.93	0.424	0.156
10	695	23.14	11.12	2	215	23.87	2.28	1.032	0.205
17	363	15.03	5.70	3	150	8.27	1.46	0.552	0.256

[1]Eggs laid 84 to 108 days after beginning of experiment. Geckos were analyzed after 140 (# 17, LoCa treatment) or 212 days (# 3, HiCa treatment; # 10, LoCa treatment).

shell formation.

Endolymphatic sacs are not present in all species of geckos. They have not been found in two species of Hemidactylus in the subfamily Gekkoninae (Ruth, 1918) or in Colenyx species in the subfamily Eublepharinae (Kluge, 1962). If these structures do, indeed, serve a function in supplying calcium for reproductive effort, it is not clear why they are seen in some egg-laying lizards but not in others. This suggests that generalizations about lizard reproductive physiology must be made with caution.

In the tree frogs there was no effect on whole body calcium attributable to dietary treatment. All females produced eggs, but since the females were not given access to the males, the eggs were not laid. Eggs were included in whole body analyses. There were large sex differences in the calcium content of treatment frogs, but not in the wild-caught frogs. Initially, it was thought that the sex difference might be due to differences in fat content. Fat content of wild-caught mature female frogs was greater than that of wild-caught male frogs, but there was no difference in calcium content (as a percentage of dry matter) between wild-caught male and female frogs. Gender differences in calcium content have been reported in the rat (Spray and Widdowson, 1950). Female rats at 120 days had a 25% higher calcium concentration than did males of the same age. The difference disappeared during pregnancy and lactation.

By comparison to wild-caught frogs, it appears that all frogs in the diet study lost about 0.1 gram of calcium

(approximately 25% of body calcium content) over the seven-month period, even when fed a HiCa diet. Zwarenstein and Shapiro (1933) reported differences in serum calcium between male and female Xenopus toads, and observed that, over a twelve-month period of captivity, serum calcium declined in females, but not in males. They hypothesized that the decline in serum calcium may have been due to parathyroid gland atrophy. Many environmental and hormonal factors (e.g., lunar cycles, parathyroid and gonadal hormones) are known to influence the marked fluctuations that are seen in serum calcium in amphibians (Dacke, 1979). In addition, Simkiss (1968) noted that calcium excretion increased in frogs (Rana temporaria) kept in deionized water, as in the present study. The calcium loss was exacerbated after induced acidosis. In the present study, it was determined that the frog jars were appropriately ventilated, with little carbon dioxide accumulation. Future dietary calcium studies with frogs should include assessment of body calcium stores when frogs are kept in water varying in ionic calcium content, since calcium uptake and loss through transpiration are important components of calcium homeostasis in most anuran amphibians (Dacke, 1979).

The use of densitometry measurements to assess bone integrity was not instructive. There were no osteodystrophic signs, nor was there radiographic evidence of bone demineralization. In cases of osteoporosis it is recognized that the organic matrix of bone must be fairly well depleted, perhaps as much as thirty percent, before conventional radiographic

methods can detect reduced density (Jubb and Kennedy, 1970). Although there was a dietary effect on whole body calcium in the geckos, the reduction in skeletal stores was not significant enough to be quantitated by bone density measurements.

In conclusion, mature fox geckos and tree frogs appeared to deplete body calcium during the course of this study. In fox geckos the depletion was less severe when high calcium (1.2%) crickets were fed, suggesting that greater amounts of calcium were being retained. Given the large percentage of body calcium that is lost when eggs are laid, it is possible that 1.2% calcium is not sufficient for Hemidactylus lizards producing eggs. Poultry producing large numbers of eggs may require more than 3% dietary calcium (NRC, 1984). However, it is hard to imagine that insects in the wild ever contain such high levels of calcium. In the present study it was not possible to control the timing and numbers of eggs laid. Further research is needed on the calcium demands of egg production in reptiles.

It was surprising that the calcium content of mature tree frogs appeared to be unaffected by dietary calcium level. There was no evidence that the disparity in calcium content between male and female frogs observed in this study had an adverse effect on the female frogs, and indeed all female frogs were replete with eggs when they were killed. The lower calcium concentration in the females may not be normal, however, as indicated by the data on wild-caught frogs. Perhaps the deionized water or other environmental conditions in this study

caused a disruption in female calcium homeostasis. Research on the calcium requirements of frogs will need to take into account such environmental factors as temperature, photoperiod and chemical constituents in water, as these might influence calcium absorption and excretion.

caused a disruption in female calcium homeostasis. Research on the calcium requirements of frogs will need to take into account such environmental factors as temperature, photoperiod and chemical constituents in water, as these might influence calcium

List of References

Allen, M.E. and Oftedal, O.T. 1982. Calcium and phosphorus levels in live prey. Northeast Regional Proceedings, American Association of Zoological Parks and Aquariums. Toronto, Ontario. Pp. 120-128.

Bilby, L.W. and Widdowson, E.M. 1971. Chemical composition of growth in nestling blackbirds and thrushes. British Journal of Nutrition 25:127-134.

Dacke, C.G. 1979. Calcium Regulation in Sub-Mammalian Vertebrates. Academic Press, London.

Gomorri, G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. Journal of Laboratory Clinical Medicine 27:955-960.

Jubb, K.V. and Kennedy, P.C. 1970. Pathology of Domestic Animals, Vol. 1, 2nd edition. Academic Press, New York.

Kluge, A.G. 1962. Comparative osteology of the Eublepharid lizard Genus Coleonyx Gray. Journal of Morphology 110: 299-332.

NRC (National Research Council). 1984. Nutrient Requirements of Poultry. National Academy of Science, Washington, D.C.

Robertson, D.R. 1969. The ultimobranchial body of Rana pipiens X. Effect of glandular extirpation on fracture healing. Journal of Experimental Zoology 172:425-442.

Robertson, D.R. 1971. Cytological and physiological activity of the ultimobranchial glands in the premetamorphic anuran Rana catesbeiana. General and Comparative Endocrinology 16:329-341.

Robertson, D.R. 1975. Effects of the ultimobranchial and parathyroid glands and vitamins D2, D3 and dihydrotachysterol 2 in blood calcium and intestinal calcium transport in the frog. Endocrinology 96:934-940.

Ruth, E.S. 1918. A study of the calcium glands in the Philippine house lizard. Philippine Journal of Science 13 B:311-318.

Schlumberger, A.G. and Burk, D.H. 1953. Comparative study of the reaction to injury. II. Hypervitaminosis D in the frog with special reference to the lime sacs. Archives of Pathology 56:103-124.

Simkiss, K. 1967. Calcium in Reproductive Physiology. Chapman and Hall, London, U.K.

Simkiss, K. 1968. Calcium and carbonate metabolism in the frog (Rana temporaria) during respiratory acidosis. American Journal of Physiology 214:627-634.

Spray, C.M. and Widdowson, E.M. 1950. The effect of growth and development on the composition of mammals. British Journal of Nutrition 4: 332-353.

Zwarenstein, H. and Shapiro, H.A. 1933. Metabolic changes associated with endocrine activity and the reproductive cycle in Xenopus laevis III. Changes in the calcium content of the serum associated with captivity and the normal reproductive cycle. Journal of Experimental Biology 10:372-378.

4. THE EFFECTS OF DIETARY CALCIUM AND VITAMIN D ON INTAKE, GROWTH AND BONE DEVELOPMENT IN YOUNG GECKOS (*EUBLEPHARIS MACULARIUS* AND *PHELSUMA MADAGASCARIENSIS*)

Introduction

The causes of rickets and osteomalacia in vertebrates are well known, yet these conditions are still seen in zoo animals fed inappropriate diets. Poorly formed bone is reported in many species of reptiles, birds and mammals fed unbalanced rations with respect to calcium, phosphorus and /or vitamin D (Nichols et al., 1983; Frye, 1982; Fowler, 1986). Insects have been shown to be poor sources of calcium and have inverse calcium to phosphorus ratios (Zwart, 1980; see Chapter 2). Although it hasn't been demonstrated experimentally, it is widely believed among zoo personnel that insectivorous species, if fed diets of crickets or mealworm larvae unsupplemented with calcium, will develop signs of osteomalacia (Frye, 1982; D. Marcellini, National Zoological Park, personal communication). While the etiology of nutritional bone disease is known and its incidence is often related to human ignorance of food composition, the factors responsible for its occurrence can be complicated. This is especially true for animals, such as insectivorous reptiles, whose dietary requirements for calcium, phosphorus and vitamin D are entirely unknown.

The quantitative nutrient requirements of most reptiles are virtually unknown. Some nutritional studies have been conducted

with crocodilians, largely as a consequence of the need to improve husbandry conditions for farmed alligators (Lance et al., 1983; Elsey and Lance, 1983; Coulson and Hernandez, 1965). Clinical case reports exist which describe the apparent effects of dietary factors on bone development in rachitic animals. However, with the exception of a few studies of calcium in reptiles (Kass, et al., 1982; Anderson and Capen, 1976a,b) no systematic experiments have been conduced which specifically characterize the effect of different dietary calcium and vitamin D levels on insectivorous lizards using quantitative measures. Since there is little economic incentive to study reptiles, they usually receive little attention from nutritionists.

There have been some studies of the effect of calcium intake on reptile health. Anderson and Capen (1976a,b) have examined the effects of different dietary calcium and phosphorus levels on bone morphology, growth and blood chemistry of the green iguana. Kass et al. (1982) studied the effects of 3 dietary calcium levels on growth, feed intake and shell and bone characteristics of the red-eared slider turtle. In both of these studies, it was demonstrated that these reptiles have growth and tissue responses similar to those of mammals and birds; both absolute concentrations and the ratio of calcium and phosphorus were important. In addition, Packard et al., (1984), Packard and Packard (1984), Dacke (1979) and Simkiss (1967) have investigated various aspects of calcium metabolism in reptiles and found the regulatory systems to be similar to those of mammals and birds.

An additional complicating factor in maintaining reptiles is that some species appear to require a source of ultraviolet light (B. Demeter, National Zoological Park, personal communication; Townsend and Cole, 1984), presumably to allow the photobiogenesis of vitamin D. In zoos, this usually means an artificial source of ultraviolet light is provided. Digney and Tytle (1982) have reported success in large breeding colonies of Phelsuma madagascariensis, if maintained under Vita-Lite (Duro Test, No. Bergen, NJ). Other species of reptiles have also been successfully maintained using Vita-Lites or fluorescent blacklights (McCrystal and Behler, 1982; B. Demeter, National Zoological Park, personal communication; Laszlo, 1969).

The species selected for the present study were the leopard gecko (Eublepharis macularius) and the giant day gecko (Phelsuma madagascariensis). The leopard gecko is considered relatively easy to keep in captivity. It will reproduce successfully on a diet of crickets, supplemented with calcium, and typically is not maintained under ultraviolet lights. The leopard gecko is considered to be nocturnal and does not bask. The day gecko is a basking species which is more difficult to keep in captivity. It is commonly fed crickets, supplemented with a calcium source. Most captive Phelsuma occasionally receive preparations that, typically, contain honey and multivitamin/mineral supplements (Demeter, 1976; Digney and Tytle, 1982. The Phelsuma spp. are believed to be difficult if not impossible to maintain unless a source of UV light is provided.

An additional complicating factor in maintaining reptiles is that some species appear to require a source of ultraviolet light (B. Demeter, National Zoological Park, personal communication; Townsend and Cole, 1984), presumably to allow the photobiogenesis of vitamin D. In zoos, this usually means an artificial source of

The objectives of this study are:

1. To evaluate the effect of providing a dietary source of vitamin D to a diurnal gecko (Phelsuma) without the use of an artificial UV light source.
2. To determine the effect of dietary calcium and vitamin D on growth and feed intake in a nocturnal and a diurnal gecko.
3. To characterize any changes in bone composition by radiographic evaluation and chemical analysis.

MATERIALS AND METHODS

Experimental Design

A two by two factorial design was employed to test the effects of 2 dietary levels of calcium and 2 dietary levels of vitamin D on growth, food intake, bone composition and skeletal integrity in the leopard gecko and the giant day gecko. In addition, a control group was also maintained for each species for comparison to animals in the experimental treatment groups.

Feed Crickets

Crickets fed to geckos for the first four months were purchased from a commercial supplier (Walker's Cricket Farm, Little Rock, AK). Due in inconsistencies in the amounts of the weekly cricket shipments, crickets for the last four months were obtained from another supplier (Jiminy Cricket, Richmond, VA). Crickets were maintained as described in Chapter 2. Four bins were established, with each bin receiving one of four experimental diets (Table 18), manufactured by Zeigler Brothers (Gardners, PA), that were

Table 18. Ingredient formulation and nutrient composition of experimental cricket diets.

Ingredient	Experimental Diets[1] 1 (%)	2 (%)	3 (%)	4 (%)
Corn	31.1	31.1	8.3	8.3
Alfalfa meal	10.0	10.0	10.0	10.0
Soybean meal	24.4	24.4	28.7	28.7
Wheat	27.0	27.0	27.0	27.0
CaCO3	1.6	1.6	20.0	20.0
Mono-dical Phos	1.9	1.9	2.0	2.0
Salt	0.5	0.5	0.5	0.5
Soy oil	3.0	3.0	3.0	3.0
Vitamin premix[2]	0.25	--	0.25	--
Vitamin premix[3]	--	0.25	--	0.25
Mineral premix[4]	0.25	0.25	0.25	0.25
Calculated Analysis:				
Crude protein, %	19.95	19.95	19.89	19.89
Calcium, %	1.17	1.17	8.18	8.18
Phosphorus, %	0.76	0.76	0.75	0.75
Vitamin A, IU/kg	70,154	70,154	70,154	70,154
Vitamin D3, IU/kg	--	7,004	--	7,004
Vitamin E, IU/kg	330	330	330	330
Laboratory Analysis:				
Calcium (%), DMB	1.49	1.48	7.73	8.29
Phosphorus (%) DMB	0.77	0.72	0.65	0.67

[1]Diets are as follows: Diet 1, low calcium, no vitamin D; Diet 2, low calcium, high vitamin D; Diet 3, high calcium, no vitamin D; Diet 4, high calcium, high vitamin D.

[2]The vitamin premix used in diets 1 and 3 contained no source of vitamin D^3 but was otherwise identical to the premix listed below.

[3]The vitamin premix contained the following nutrients per gram: 28,000 IU vitamin A, 2,800 IU vitamin D^3, 132 IU vitamin E, 0.6 mg vitamin K, 6.0 ug vitamin B^{12}, 7.1 mg vitamin B^1, 2.0 mg riboflavin, 35.6 mg niacin, 9.5 mg pantothenic acid, 2.0 mg pyridoxine, 1.5 mg folic acid, 99 ug biotin, 190 mg choline.

[4]The mineral premix contained the following nutrients per gram: 144 mg calcium, 0.04 mg phosphorus, 4.3 mg magnesium, 0.60 mg potassium, 84.2 mg iron, 83.3 mg zinc, 81.1 mg copper, 119 mg manganese, 0.08 mg selenium, 0.32 mg iodine.

formulated to contain (dry matter basis):

Diet 1 - 1.4% calcium and 0 IU vitamin D_3/kg

Diet 2 - 1.4% calcium and 7,000 IU vitamin D^3/kg

Diet 3 - 8.0% calcium and 0 IU vitamin D^3/kg

Diet 4 - 8.0% calcium and 7,000 IU vitamin D^3/kg

Crickets were maintained on these diets for 48-72 hours before being fed to geckos.

A fifth bin contained crickets to be fed to geckos in a control group. These crickets were fed an avian maintenance diet (Table 19) (Zeigler Bros., Gardners, PA.) but were otherwise maintained as were the crickets fed the experimental diets. When these crickets were to be fed to control geckos they were 'dusted' with a vitamin and mineral supplement (Table 19) (Pervinal, Thayer Laboratories, New York, NY), as is common practice in reptile husbandry. This was accomplished by shaking the crickets in a 30 ml polypropylene bottle containing approximately 4 grams of the supplement. Once they were visibly coated with the supplement, they were immediately weighed and fed to geckos assigned to the control group.

Leopard Geckos

Fifteen leopard geckos that hatched at the National Zoological Park between August 15 and September 18, 1983 were used in the study. Initially, when geckos were small (from 2.7-4.1 g at hatching to 8.8-13.7 g at 4-5 months) 'half-grown' crickets (approximately 9 mm long) were used (average weight 0.11 g). Thereafter, adult crickets (average weight 0.36 g) were used. From hatching until assignment to treatment groups on September 19, they

Table 19. Ingredient formulation and nutrient standards of Avian Maintenance Diet and composition of Pervinal.

Avian Maintenance Diet		Pervinal[1]	
Ingredient	(%)	Nutrient Composition (DMB):	
Corn	62.73	Vitamin A, IU/g	520.0
Barley or wheat	20.00	Vitamin D_2, IU/g	104.0
Soybean meal, 48.5% CP	10.00	Vitamin E, ppm	502.0
Alfalfa meal, 17% CP	2.50	Thiamin, ppm	134.0
Fish meal, 65% CP	2.00	Riboflavin, ppm	266.0
Dicalcium Phosphate	1.50	Niacin, ppm	1,100.0
Limestone	0.50	Pyridoxine, ppm	26.0
Salt	0.25	Calcium, %	4.5
DL-methionine	0.02	Phosphorus, %	4.2
Vitamin and mineral		Magnesium, %	0.292
premixes [2]	0.50	Potassium, %	2.08
		Sodium, %	1.68
Calculated Analysis:		Iron, ppm	720.0
		Zinc, ppm	110.0
Crude protein, %	12.5	Copper, ppm	110.0
Calcium, %	0.8	Manganese, ppm	72.0
Phosphorus, %	0.6	Iodine, ppm	2.0

[1]Provided from label.
[2]Premixes provide the following nutrient levels to the finished product (as per specifications, National Zoological Park): vitamin A, 6,000 IU/kg; vitamin D3, 500 IU/kg; vitamin E, 120 IU/kg; vitamin K, 1.0 ppm; thiamin, 3 ppm; riboflavin, 4 ppm; niacin, 60 ppm; pyridoxine, 5 ppm; pantothenic acid, 20 ppm; folic acid, 1.5 ppm; biotin, 0.25 ppm; vitamin B_{12}, 0.015 ppm; choline, 1000 ppm; iron, 40 ppm; copper, 4 ppm; zinc, 25 ppm; manganese, 25 ppm; iodine, 0.4 ppm; selenium, 0.2 ppm.

were fed control crickets (see above).

Four experimental treatments and a control treatment were provided to the geckos by using crickets fed five different diets. In addition the control crickets were supplemented by 'dusting'. It was the original intention that four geckos would be randomly assigned to each of the four treatment groups and to the control group. Due to late hatching or failure to hatch of some eggs, only three animals could be included in each treatment group, but four animals were assigned to the control group. Prior to the initiation of the experiment, the geckos were fed dusted crickets (as described above). An adaptation period was initiated on September 19 using crickets maintained on the experimental diets, and after an initial weight was obtained for all animals on October 6 the experiment was considered to have begun. At this date the post-hatching age of the geckos ranged from 18 to 48 days.

Geckos were fed live crickets, ad libitum, twice per week between 11:00 and 14:00 hours. Crickets were weighed, placed in the gecko cages for 3 hours, and then uneaten crickets were removed and weighed. Cricket consumption was calculated by weight for each gecko. Geckos were weighed and measured (snout to vent length, SVL) every three to four weeks. The experiment was conducted for 255 days and concluded on May 31, 1984.

From hatching, and during the experiment, each gecko was individually housed in a plastic box with a tightly fitting lid. Boxes were approximately 35 cm L X 12.5 cm W X 19 cm H. Air holes had been provided by melting about 25 small holes (0.5 cm) in each

plastic lid, and along the top edge of the sides of the box, using a soldering iron. Distilled, deionized water was provided at all times in a plastic jar lid 5 cm in diameter and approximately 10 mm high. A green plastic leaf (approximately 8 X 14 cm) was provided in each cage for hiding and climbing. Boxes were lined with one layer of paper towel. Cages were thoroughly cleaned twice per week. Geckos were misted with water daily. Artificial fluorescent lights, mounted in ceiling fixtures, were on for 10 hours per day. Geckos were also exposed to natural light through glass skylights in the room so that day length was somewhat longer than 10 hours during the last two months of the experiment (April and May). Geckos were misted with water daily to maintain humidity within each box.

Ambient temperature was 27 to 29^{o} C. In mid-April, six months after the start of the experiment, the central heat was shut down in the building and room temperature dropped to 24 to 26^{o} C.

Day Geckos

Fifteen day geckos were used in this study, which was also designed as a 2 X 2 factorial design, with an additional control group. Twelve geckos hatched at the National Zoo and three were obtained from a private breeder. Five animals that hatched between June 26 and July 15, 1983 were randomly assigned to the five diets on September 19. Weights ranged from 2.22 to 2.58 grams. Ten additional geckos that hatched between September 16 and November 6 were randomly assigned to diets on November 11. Weights ranged from 0.85 to 2.28 grams. Until assignment to treatment groups, all geckos were fed control crickets as described above. The study was plastic lid, and along the top edge of the sides of the box, using a soldering iron. Distilled, deionized water was provided at all times in a plastic jar lid 5 cm in diameter and approximately 10 mm high. A green plastic leaf (approximately 8 X 14 cm) was provided in each cage for hiding and climbing. Boxes were lined with one conducted for 202-255 days (depending on start date) and was concluded on May 31, 1984.

Day geckos were individually housed in glass jars, approximately 23 cm H and 18 cm in diameter, and located in the same, off-exhibit, holding area as were the leopard geckos. Metal lids containing 10-12 holes, 1 cm in diameter, fit loosely on the jars. The lids were fitted with metal screening to prevent geckos or crickets from escaping. A single layer of paper towel on the jar bottom and a green plastic leaf (approximately 8 X 14 cm) were provided in each jar. Jars were thoroughly cleaned twice per week. Geckos were misted with water daily.

From hatching until assignment to treatment groups, all day geckos were maintained under a 4 foot, 2 bulb, fluorescent fixture that contained one black light (GE BL-40, General Electric, Schenectady, NY) and one broad spectrum fluorescent bulb (Chroma-50, General Electric). The bulbs were approximately 20 cm from the top of each jar and were on for 10 hours per day. The jars containing the day geckos were placed over a heat tape which resulted in temperatures of 29 to 31^{o} C at the jar bottom, even after ambient temperatures dropped in mid-April (as noted above). After geckos were assigned to treatment groups, only those receiving the control diet were maintained under the black light. Otherwise the experimental conditions were identical among treatment groups. The feeding schedule and protocol were the same as for the leopard geckos.

Tissue Processing

All geckos were injected intraperitoneally with heparin (600 units/kg body weight) approximately 12 hours before they were killed. They were sedated by administration of gas anesthetic (Halothane) until complete anesthesia was achieved. Body weights and lengths were recorded. A ventral incision was made longitudinally into the thoracic cavity, exposing the heart. Unsuccessful attempts were made to obtain blood by cardiac puncture. Geckos were killed by gas anesthesia and, after prepping the neck region with ethanol, exsanguinated via a ventral incision made horizontally across the neck but not severing the vertebral column. Geckos were positioned over 12 ml heparinized test tubes and blood was collected. Tubes containing the whole blood were capped, placed in an ice bath (5^{o} C) and placed in the dark. Blood was centrifuged and plasma was collected and frozen. Plasma was shipped (24 hours) on dry ice to the National Animal Disease Center, Ames Iowa, for vitamin D analyses. 25-(OH) D_3 and 1,25-(OH) D_3 were determined on plasma according to Horst (1984). Due to extremely small blood volumes, plasma was pooled within treatment groups to obtain a large enough sample (0.3 to 0.75 ml) for vitamin D analyses. Visceral organs were removed and examined, and any grossly unusual features were noted. The eviscerated carcasses were placed in plastic bags and temporarily held at 5^{o} C in an insulated box. Immediately after necropsy the refrigerated carcasses were taken to the Armed Forces Institute of Pathology, Department of Orthopedic Pathology, to be radiographed. Gecko carcasses were radiographed using a Faxitron X-

ray unit, and films (Kodak Industrex, Ready-Pak M-2, Rochester, NY) were exposed at 40 kvp and 2 ma for 0.3 minutes. Exposed films were refrigerated for subsequent development. Films were processed at the U.S. Bureau of Standards, Radiation Physics Laboratory dark room using industrial film developer (Kodak GBX) and fixer (Kodak GBX).

Carcasses were returned to the National Zoo, and skeletal tissues from each gecko were removed for chemical evaluation. The skull was bisected sagittally and the left half was removed for chemical analysis. These tissues were frozen at -10° C.

The frozen tissue was freeze-dried for 48 hours to facilitate removal of soft tissue adhering to bone. Since soft tissue was still persistently attached to bone, bones were further processed by boiling in deionized, distilled water for 2 hours. Bones were meticulously cleaned of all visible soft tissue. Individual skull bones were wrapped in ashless filter paper and ether extracted in a Soxhlet apparatus for four hours. Filter paper was removed and the fat-free bones were transferred into pre-ashed and weighed ashing crucibles. The crucibles and bones were weighed to the nearest 0.0001 g, placed in a muffle furnace and ashed at 600° C for 6 hours. When sufficiently cool, crucibles were equilibrated to room temperature in desiccators. Ash content of bone was determined gravimetrically by weighing to the nearest 0.0001 g. The ashed bone was solubilized in a known amount of 6 N hydrochloric acid. Calcium was determined on solubilized ash by flame atomic absorption spectrophotometry. Calcium as a percent of dry, fat-free bone and as a percent of ash were calculated.

ray unit, and films (Kodak Industrex, Ready-Pak M-2, Rochester, NY) were exposed at 40 kvp and 2 ma for 0.3 minutes. Exposed films were refrigerated for subsequent development. Films were processed at the U.S. Bureau of Standards, Radiation Physics Laboratory dark room using industrial film developer (Kodak GBX) and fixer (Kodak GBX).

Crickets from each treatment were sampled (20 g) every two-weeks and frozen (-10^{o} C) for subsequent analysis for dry matter, calcium and phosphorus. All cricket samples were freeze-dried and refrozen for subsequent analysis. The dry matter content of crickets was determined on duplicate samples (5-10 g) by drying to constant weight in a vacuum oven at 100^{o} C for approximately two days. For calcium and phosphorus analyses, duplicate 5-10 gram samples of crickets were weighed to the nearest 0.001 g into tared 250 ml Phillips beakers. The crickets were digested by nitric (10 ml) and perchloric (3 ml) acids under a perchloric acid fume hood. Calcium determinations were performed on the wet ash by flame atomic absorption spectrophotometry with duplicate readings per sample. Phosphorus was measured colorimetrically, according to Gomorri (1942), with duplicate readings per sample.

To confirm levels of vitamin D in crickets, vitamin D analyses were performed on two samples of crickets by high pressure liquid chromatography (HPLC) according to Horwitz (1980, #43.068, 43.070, 43.071; 1982, # 43.C03, #43.C07, # 43.C08). Modifications to these methods were made after consultation with the Nutrient Surveillance Laboratory at the Food and Drug Administration (M. Bueno, personal communication). Approximately 8 grams each, from Diet 1 and Diet 4 crickets, were saponified with ethyl alcohol, potassium hydroxide and ascorbic acid for one hour over a steam bath. Round-bottomed flasks were fitted with air condensers for refluxing. Saponified material was transferred to separatory funnels and extraction was carried out with pentane. After extraction and repeated agitation

with water, the pentane fraction was collected into a beaker after filtration through glass wool and anhydrous sodium sulfate. Extracted material was dried over steam and resuspended in pet ether. To remove tocopherols and carotenes, the sample was passed through a phosphate treated alumina column, which earlier had been tested for efficiency according to Horwitz (1980) (#43.070). The sample was further purified by passing through a polyethylene glycol column with isooctane. The fraction containing vitamin D was dried under nitrogen and resuspended in hexane. Vitamin D was determined by HPLC (Waters, Milford, MA). The sample was injected onto a stainless steel, 10 u, silica analytical column using 2% isopropyl alcohol in hexane.

Statistical Analysis

Statistical analyses were conducted using the general linear model program of PC SAS (SAS Institute, Cary, NC) on a Zenith 183 personal computer. The increases in gecko weight and length with time were fitted to both linear and quadratic regression models. Regression coefficients for growth and bone composition data were analyzed by two-way analysis of variance (calcium x vitamin D) and mean values for treatment groups were compared by T tests. If analyses involved 4 or more T test comparisons, the Bonferroni correction was applied to reduce the experimentwise error rate. Data on intake was analyzed by repeated measures analysis of variance which tested both between-subjects (treatment) and within-subject (time) effects. The assumption of equality of variance and covariance matrices was tested by a sphericity test, and the Huynh-

Feldt Epsilon adjustment was applied to the degrees of freedom. In all statistical analyses a probability level of 0.05 was selected to determine significance of observed differences.

RESULTS

Cricket Composition

The composition of crickets used to feed geckos was manipulated by feeding diets that differed in calcium content. By analysis, the crickets fed high-calcium diets (Treatments 3 and 4) contained 0.84 to 0.86% calcium (DMB), while crickets fed low-calcium diets contained 0.20% calcium (Table 20). Control crickets (Diet 5) had a calcium concentration of 1.23%. Samples of crickets from Treatments 1 and 4 were analyzed by high-performance liquid chromatography and were found to contain 319 IU/kg and 720 IU/kg vitamin D on a dry matter basis, respectively. Due to logistical problems in conducting the assays it was not possible to analyze cricket samples from treatments 2 and 3. These preliminary data on vitamin D content suggest that varying the level of vitamin D in the cricket diet had an effect on the vitamin D levels in the crickets although the small numbers of analyses do not permit this hypothesis to be tested statistically.

Phelsuma Experiment

Dramatic mortality occurred in all experimental groups of day geckos and no mortality occurred in the control group. Among day geckos fed the experimental diets, only one animal (gecko # 9 in treatment 2) lived for the duration of the experiment, and the age at which the other animals died did not appear to be related to a

Table 20. Composition (mean ±SE) of crickets fed to geckos[1]

	Diet[2]				
	1	2	3	4	5
DM (%)	28.46 ±1.22	28.74 ±0.98	28.01 ±1.05	28.20 ±0.78	30.99 ±0.68
Ca (%)	0.201 ±0.008	0.201 ±0.010	0.840 ±0.035	0.861 ±0.035	1.230 ±0.190
P (%)	0.764 ±0.035	0.790 ±0.030	0.805 ±0.022	0.829 ±0.033	0.966 ±0.054

[1]Based on sample sizes of 5 for dry matter, 10 for calcium and 10 for phosphorus. Calcium and phosphorus expressed as a percentage of dry matter.

[2]Diets are as follows: Diet 1, low calcium, no D; Diet 2, low calcium, high D; Diet 3, high calcium, no D; Diet 4, high calcium, high D; Diet 5, Control.

specific dietary treatment (Figure 11). All of the experimental day geckos grew poorly, and bones, including mandible and maxilla, were found to be soft and pliable at necropsy. By contrast the three animals in the control group appeared robust and healthy throughout the experiment. The snout to vent lengths of the control geckos were typical of normal growth in this species (Demeter, 1976). At the end of the study, the single surviving gecko fed diet 2 (gecko #9) had a snout to vent length (55 mm) that was about 8 mm less than expected for a gecko of this age, and its weight (4.07 g) was about one third of that of the control geckos. Radiographs of the control day geckos revealed well-mineralized bone and no evidence of pathology. Endlolymphatic sacs were pronounced in one of the three control geckos. Radiographically the bones of gecko # 9 were similar in appearance with respect to mineralization to those of the control geckos, although the skeleton was much smaller. The endolymphatic sacs were visible but small. Due to the high mortality rates, further comparisons among treatment groups and to the Eublepharis were not warranted.

Eublepharis Experiment

Growth

The weights and snout to vent lengths (SVL) of the leopard geckos are presented by treatment group in Figures 12 and 13. All animals gained weight, and were from 3 to 7 times heavier at the end of the study than at the beginning. There were also substantial increases in SVL. In order to describe the pattern of growth, the data were fitted to both linear and quadratic regression models.

specific dietary treatment (Figure 13). All of the experimental day geckos grew poorly, and bones, including mandible and maxilla, were found to be soft and pliable at necropsy. By contrast the three animals in the control group appeared robust and healthy throughout the experiment. The snout to vent lengths of the control geckos were typical of normal growth in this species (Gehrter, 1976). At

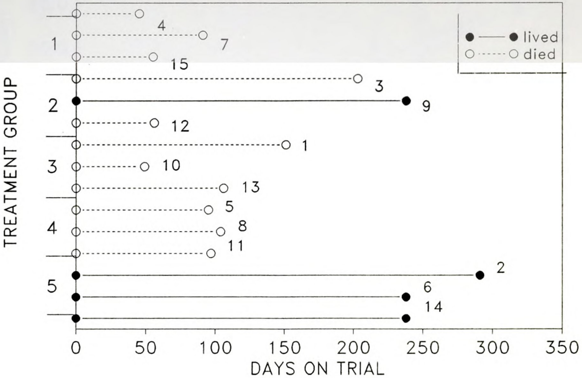


Figure 11. Mortality of day geckos.

Figure 12. Body weights of leopard geckos.

Figure 13. Snout to vent lengths of leopard geckos.

FIG. 12

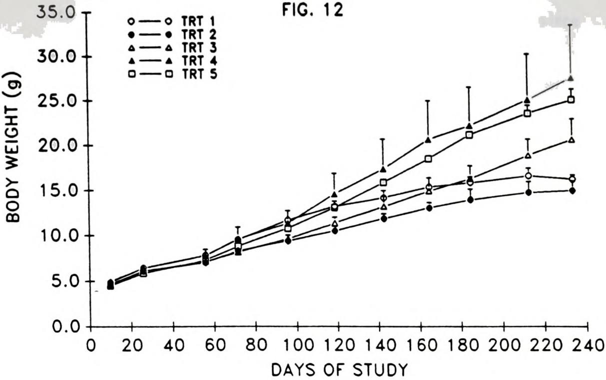
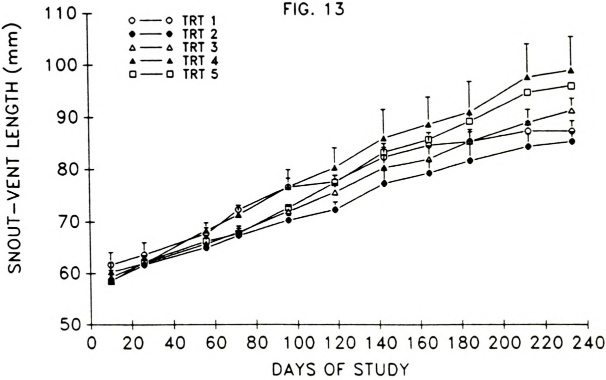

TRT 1
TRT 2
TRT 3
TRT 4
TRT 5
BODY WEIGHT (g)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0 20 40 60 80 100 120 140 160 180 200 220 240
DAYS OF STUDY

FIG. 13


TRT 1
TRT 2
TRT 3
TRT 4
TRT 5
SNOUT-VENT LENGTH (mm)
110
100
90
80
70
60
50
0 20 40 60 80 100 120 140 160 180 200 220 240
DAYS OF STUDY

The slope of the linear regression indicated that weight gain over the course of the study varied from 0.040 to 0.144 grams per day, with a mean for all animals of 0.076 $\pm$ 0.0074 (SE). However, since nine geckos (out of 16) had significant quadratic terms for body weight, and seven (out of 16) had significant quadratic terms for snout-vent length, the quadratic model was used as a more accurate description of the growth patterns that were observed. The quadratic equations for weight for each animal are presented in Table 21. The quadratic equations explained 97% or more of the observed variation of all animals except one (Table 21).

The effect of dietary calcium and vitamin D on growth was examined by analysis of variance of the linear and quadratic terms of the quadratic regression models (Table 22). Calcium had a significant effect on the linear and quadratic terms for weight but dietary vitamin D did not. However, there was a significant calcium and D interaction for the linear term. Animals in the high-calcium groups (3 and 4) all had positive quadratic terms, indicating that weight gain tended to increase with time. By contrast, animals in the low-calcium groups had negative quadratic terms, indicating that weight gain tended to decrease with time (Table 21). With respect to SVL, neither the linear or quadratic terms revealed significant effects of dietary calcium or vitamin D. It appears that leopard geckos are able to continue linear growth (as measured by SVL, i.e. vertebral column and cranium length) even in the face of low-calcium diets that do not support high weight gains.

The slope of the linear regression indicated that weight gain over the course of the study varied 14% grams with a

Table 21. Quadratic regression equations for leopard gecko growth (weight) by animal[1].

Animal	Intercept (a)	Linear (b)	Quadratic (c)	R^2
	Regression terms			
DIET 1:				
4	3.504497	0.092209	-0.000147	0.991
9	3.487313	0.093212	-0.000179	0.987
16	1.420345	0.133474	-0.000280	0.978
DIET 2:				
5	3.836668	0.061672	-0.000084	0.973
6	3.381066	0.060132	-0.000055	0.995
13	3.675743	0.072316	-0.000058	0.989
DIET 3:				
1	3.728413	0.045271	0.000069	0.996
8	4.046855	0.047935	0.000170	0.997
14	4.211263	0.045480	0.000055	0.995
DIET 4:				
3	3.040951	0.105201	0.000153	0.988
10	4.094078	0.046547	0.000023	0.993
12	2.840785	0.068375	0.000196	0.995
DIET 5:				
2	4.013450	0.050146	0.000147	0.881
7	0.936598	0.106015	-0.000057	0.970
15	5.010599	0.055021	0.000159	0.998
18	2.556736	0.058827	0.000155	0.988

[1]Based on the model: weight = $a + bx + cx^2$ where x = days of dietary treatment.

Table 22. **Analysis of variance (2 x 2 factorial) of quadratic regression parameters for leopard gecko growth.**

Source[1]	Linear F	Linear Prob.	Quadratic F	Quadratic Prob.
Weight:				
Calcium	5.36	0.0493	42.05	0.0002
Vitamin D	0.42	0.5336	4.65	0.0633
Interaction	9.58	0.0148	2.13	0.1822
SVL:				
Calcium	0.03	0.8717	2.87	0.1286
Vitamin D	0.15	0.7108	1.15	0.3143
Interaction	2.74	0.1368	2.07	0.1886

[1]Source of variation.

Since there were no significant vitamin D effects on growth, the animals in the control group were compared to the pooled data for animals on either low-calcium (1 and 2) or high-calcium (3 and 4) diets. The linear term for weight of the control geckos was not significantly different from that of either the low calcium or high calcium geckos. However, weight change as measured by the quadratic term was significantly greater for the control and high calcium geckos (diets 2 and 4 combined) than for low calcium geckos (t-tests, $P < 0.05$). There were no treatment differences in growth of SVL in comparisons between the control geckos and low and high calcium geckos, as assessed by the linear and quadratic terms of the regression model.

Feed Intake

Feed intake was measured each day that animals were fed, i.e. twice per week. The average intake of each animal per feeding day was calculated for consecutive, one-month (30 day) periods. These data are presented by treatment group in Figure 14. There was large variation in intake over the course of the experiment, both between animals and with time on the study. Average daily feed intakes (grams of live crickets) for the entire experiment were also calculated for each treatment group and these are as follows: 0.202, 0.161, 0.282, 0.343 and 0.309 for treatments 1, 2, 3, 4 and 5, respectively.

Intake data for the experimental animals were analyzed as a 2 x 2 factorial model with repeated measures analysis of variance, with calcium and vitamin D as the main effects and with the 8 monthly periods as the time effect (Table 23). Intake per feeding day was also expressed as a percentage of body weight, using predicted weights at the mid-point of each month derived from the quadratic regression equations in Table 21 (Figure 15).

Repeated measures ANOVA revealed that dietary calcium had a significant effect on both feed intake and intake as a percentage of body weight, but dietary vitamin D had no effect (Table 23). There was no significant interaction between calcium and vitamin D for either intake or intake as a percentage of weight. The time effect was highly significant ($P < 0.0001$) for both measures, and had a significant interaction with the calcium effect. Interactions between time and vitamin D effects were also indicated for intake,

Figure 14. Feed intake of leopard geckos.

Figure 15. Feed intake as a percentage of body weight of leopard geckos.

FIG. 14

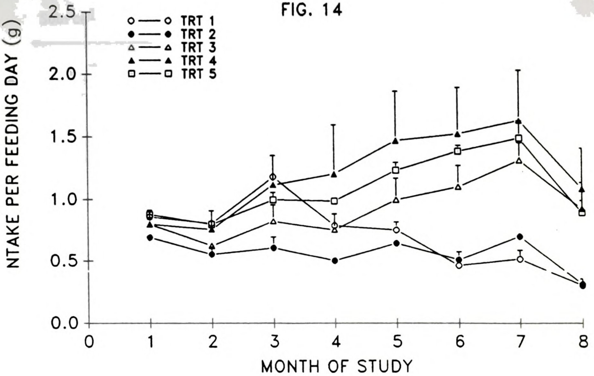

TRT 1
TRT 2
TRT 3
TRT 4
TRT 5
NTAKE PER FEEDING DAY (g)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
MONTH OF STUDY

FIG. 15

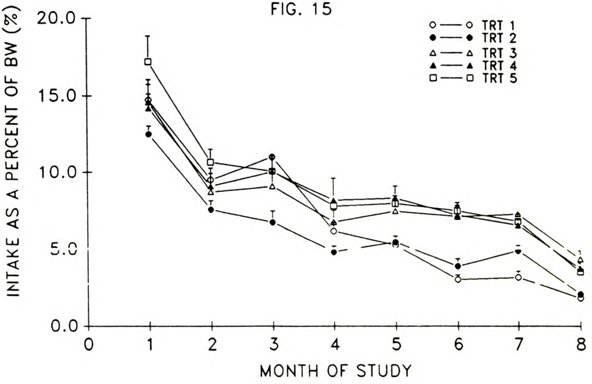

TRT 1
TRT 2
TRT 3
TRT 4
TRT 5
INTAKE AS A PERCENT OF BW (%)
20.0
15.0
10.0
5.0
0.0
0
1
2
3
4
5
6
7
8
MONTH OF STUDY

Table 23. Repeated measures analysis of variance of leopard gecko intake and intake as a percent of body weight (2 x 2 factorial)[1].

Source[1]	df	Intake F	Intake Prob.	Intake as % BW F	Intake as % BW Prob.
Calcium[3]	1	7.03	0.0292	14.77	0.0049
Vitamin D[3]	1	0.19	0.6727	0.37	0.5587
Ca * D Int.[3]	1	1.78	0.2185	1.21	0.3031
Time[4]	7	10.31	0.0001	133.17	0.0001
Time * Ca Int.[4]	7	18.11	0.0001	4.21	0.0015
Time * D Int[4]	7	3.08	0.0276	2.21	0.0554
Time * Ca * D Int.[4]	7	2.76	0.0416	4.48	0.0010

[1]Includes diets 1, 2, 3 and 4.
[2]Source of variation.
[3]Treatment = between subjects effects. Int. = interaction.
[4]Time and interactions with time are within subject effects to which univariate tests were applied. The probability levels reflect the Huynh-Feldt Epsilon adjustment of the degrees of freedom.

but not for intake as a percentage of weight (Table 23).

The pattern of intake of geckos in low-calcium (Treatments 1 and 2) and high-calcium groups (Treatments 3 and 4) were compared to control geckos by t-tests at each time period (Table 24). The data were pooled across vitamin D treatments as vitamin D had no significant effect on intake (Table 23). Over the first four months there were no significant differences in intake, but thereafter the geckos fed low-calcium diets had significantly lower intakes than did geckos fed either the high-calcium or control diets. In the last four months intake by high-calcium and control geckos did not differ. The decline in intake across all treatment groups towards

Table 24. Comparison of means of gecko intake[1] and intake as a percent of body weight[2] by treatment and time (mean ±SE).

Month:	Diet: Low calcium (n=6)	High calcium (n=6)	Control (n=4)
Intake:			
1	0.772^{A} ±0.043	0.797^{A} ±0.053	0.874^{A} ±0.036
2	0.678^{A} ±0.063	0.688^{A} ±0.074	0.795^{A} ±0.032
3	0.894^{A} ±0.135	0.970^{A} ±0.137	0.977^{A} ±0.061
4	0.644^{A} ±0.077	0.977^{A} ±0.210	0.987^{A} ±0.028
5	0.697^{A} ±0.038	1.232^{B} ±0.220	1.234^{B} ±0.063
6	0.487^{A} ±0.035	1.313^{B} ±0.204	1.385^{B} ±0.046
7	0.606^{A} ±0.052	1.471^{B} ±0.205	1.489^{B} ±0.139
8	0.307^{A} ±0.027	1.007^{B} ±0.177	0.892^{B} ±0.102
Intake as % BW:			
1	13.622^{A} ±0.577	14.390^{AB} ±0.990	17.203^{B} ±1.657
2	8.537^{A} ±0.533	8.905^{AB} ±0.542	10.662^{B} ±0.846
3	8.882^{A} ±1.010	9.565^{A} ±0.664	10.075^{A} ±0.987
4	5.503^{A} ±0.433	7.472^{A} ±0.797	7.805^{A} ±0.275
5	5.365^{A} ±0.234	7.895^{B} ±0.531	7.970^{B} ±0.487
6	3.457^{A} ±0.322	7.170^{B} ±0.469	7.490^{B} ±0.355
7	4.042^{A} ±0.457	6.927^{B} ±0.242	6.818^{B} ±0.491
8	1.940^{A} ±0.203	4.045^{B} ±0.330	3.530^{B} ±0.337

[1]Intake expressed as grams of live crickets per feed.
[2]Intake expressed as a percent of body weight (intake of live crickets per feed/weight at the midpoint of that month).

Means in a row with the same superscript are not significantly different at the 0.05 probability level (LSD).

the end of the study (Figure 14) may be related to a temperature change which occurred during April when the central heating to the reptile building was shut off (see Materials and Methods).

Radiography

Radiographs were taken of all geckos at the end of the trial. The skeletons of all animals on the low-calcium diets (Treatments 1 and 2) appeared to be poorly mineralized as indicated by a lack of contrast to soft tissue in the radiographs (Figure 16). Cortices were thin and there were folding fractures evident in the long bones of several low-calcium geckos. By comparison, the radiographs of geckos fed high-calcium diets, including animals in the control group, revealed well-mineralized bone, with well-defined cortices and no pathologic fractures (Figures 17 and 18). No qualitative differences could be discerned in the radiographs associated with different levels of dietary vitamin D.

Bone Composition

Dry, fat-free cranial bone of the geckos was analyzed for ash and calcium content. There was a highly significant effect of dietary calcium on the percentage of ash and of calcium in dry, fat-free bone (DFFB) (Table 25). Vitamin D also produced a significant effect on calcium as a percent of DFFB but not on ash. The mean values for geckos in the four experimental groups and for control geckos were compared by Bonferroni t-tests (Table 26). Geckos in treatments 1 and 2 had significantly lower ash than did geckos fed diets 3, 4 and 5. Calcium in DFFB was significantly lower in bone from the low-calcium, low-vitamin D geckos than in bone from the

Figure 16. Radiograph of treatment 1 leopard gecko.

Thin cortices of the long bones and a reduction in density of the skeleton are evident.

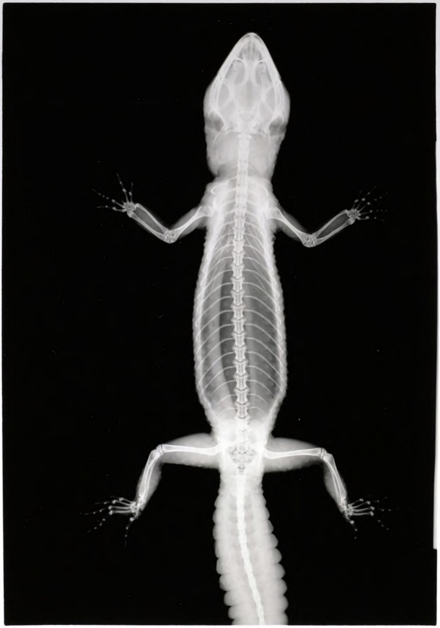


Figure 17. Radiograph of treatment 4 gecko.

Note the well-mineralized skeleton.

Figure 18. Radiograph of treatment 5 (control) gecko.

Note the well-mineralized skeleton, similar to that in Figure 17.

Table 25. Analysis of variance (2 x 2 factorial) of leopard gecko bone ash and calcium in bone.

		Ash as a percent of dry, fat-free bone		Calcium as a percent of dry, fat-free bone	
Source[1]	df	F value	Prob.	F value	Prob.
Calcium	1	119.85	0.0001	31.24	0.0005
Vitamin D	1	2.55	0.1487	7.73	0.0239
Interaction	1	0.08	0.7834	1.47	0.2597

[1]Source of variation

high-calcium and control geckos (Table 26).

Plasma Vitamin D Levels

As it was necessary to pool plasma samples from all animals in each treatment group to obtain sufficient amounts for analysis, only one analytical value could be obtained per treatment (Table 27). In the leopard geckos it appeared that higher levels of 25-OH D_3 occurred in animals consuming high-vitamin D diets (Treatments 2 and 3) than in those consuming low-vitamin D diets, but of course this could not be evaluated statistically. A similar but less pronounced pattern occurred in the levels of 1,25-OH D_3, although it is doubtful that the values for geckos on diets 3 and 4 are different. The plasma 25-OH D_3 levels of the control groups for both the leopard gecko and the day gecko appeared to be similar.

Table 26. Composition (mean $\pm$SE) of leopard gecko bone.

	Diet[1]				
	1 (n=3)	2 (n=3)	3 (n=3)	4 (n=3)	5 (n=3)
Ash in DFFB[2] (%)	25.68^{A} $\pm$5.56	29.67^{A} $\pm$2.26	58.12^{B} $\pm$.184	63.85^{B} $\pm$1.00	58.20^{B} $\pm$1.76
Ca in DFFB (%)	14.74^{A} $\pm$1.39	18.35^{AB} $\pm$0.67	20.89^{B} $\pm$0.82	22.30^{B} $\pm$0.48	20.61^{B} $\pm$0.47

[1]Diets are as follows: Diet 1, low calcium, no vitamin D; Diet 2, low calcium, high vitamin D; Diet 3, high calcium, no vitamin D; Diet 4, high calcium, high vitamin D; Diet 5, Control.
[2]DFFB = dry, fat-free bone.

Means in a row with the same superscript are not significantly different at the 0.05 probability level (Bonferroni T test).

Table 27. Concentration of vitamin D in gecko plasma[1].

Diet[2]	25-OH D_3 nanograms/ml	1,25-OH D_3 picograms/ml
Leopard Gecko:		
Diet 1	ND[3]	31
Diet 2	8.5	72
Diet 3	4.7	48
Diet 4	41.0	52
Diet 5	10.1	46
Day Gecko:		
Diet 5	16.0	80

[1]Plasma pooled within treatment group to obtain sufficient sample for analysis.
[2]Diets are as follows: Diet 1, low calcium, no vitamin D; Diet 2, low calcium, high vitamin D; Diet 3, high calcium, no vitamin D; Diet 4, high calcium, high vitamin D; Diet 5, Control.
[3]ND None detected.

DISCUSSION

Mortality in Phelsuma

All but one of the Phelsuma in the four experimental groups died, and the one that survived grew very poorly. Although day gecko bones were not evaluated chemically or histologically, by gross inspection they were found to be extremely soft in animals that died, suggesting a calcium or vitamin D deficiency.

It is not clear why the experimental day geckos experienced such high mortality when the leopard geckos did not, or why this mortality seemed to be associated with calcium or vitamin D deficiency. One possible explanation is that the level of vitamin

Table 27. Concentration of vitamin D in gecko plasma[1]

Diet[2]

D_3 was not sufficient for day geckos even in the high-D treatment groups (treatments 2 and 4). The control animals were exposed to an artificial source of ultraviolet light that might have allowed endogenous synthesis of vitamin D (see below). A second possibility, yet more remote, is that Phelsuma may be unable to utilize dietary vitamin D, and must be exposed to ultraviolet light of specific wavelengths for vitamin D synthesis to occur. A third possibility is that some nutrient is contained in the dusted supplement that is lacking in the experimental diets. All of these explanations require a significant physiological difference between Phelsuma, which did so poorly on experimental diets, and Eublepharis which did so well when sufficient calcium was supplied.

It is widely held among zoo herpetologists that dusting by itself (in the absence of artificial sources of UV light) will not result in adequate growth and development at least with delicate basking lizards such as the Phelsuma. The dusted supplement (Pervinal) provided a source of calcium and other nutrients, but not vitamin D_3 since this supplement contains vitamin D_2 rather than D_3. It is not known whether reptiles can utilize vitamin D_2, or whether they must be supplied with vitamin D_3 as must poultry (Scott et al., 1982).

The biological responses of reptiles exposed to artificial sources of ultraviolet light have not been adequately and objectively studied under controlled conditions. There are many different kinds of artificial lights commonly used in zoo reptile collections, and only recently has attention been paid to the

spectral characteristics and energy contained in such bulbs (Gehrmann, 1987) and their effect on biological systems (Allen, 1988; Bernard et al., in press). Townsend and Cole (1984) reported that growth and reproduction improved in a captive colony of whiptail lizards (Cnemidophorus) after new Vita-lites were installed, and they attributed earlier, poor performance to the use of aged bulbs. The authors acknowledged that dietary factors may have played a role in the incidence of metabolic bone disease seen in the Cnemidophorus colony, but the nutrient composition of the diet was not reported.

The interpretation of vitamin D status in poikilothermic animals, such as reptiles, may be complicated by the temperature-dependence of the reaction which transforms pre-vitamin D_3 to vitamin D_3 (Holick, et al., 1980). The sequence of events in the body which results in the formation of cholecalciferol (vitamin D_3) includes a thermal isomerization in the epidermis which, in homeothermic animals, occurs at 37° C (Miller and Norman, 1984). The fact that reptile body temperature is highly dependent on ambient temperature means that the skin temperature of reptiles may be a critical variable in studies of the effects of ultraviolet light on vitamin D status in captive reptiles. Basking behavior will clearly influence skin temperature as well as extent of exposure to ultraviolet light. It seems likely that the suspected physiological difference between day geckos and leopard geckos may relate to the fact that the former is a diurnal, basking species, while the latter is nocturnal, and is rarely exposed to direct

sunlight in the wild.

Phelsuma proved to be fairly difficult experimental animals, as they are delicate as well as quick. Nonetheless the striking difference in results obtained with the Phelsuma and the Eublepharis demonstrate that the latter cannot be considered a suitable model for the former, and that nutritionists must be extremely careful in extrapolating from one species or animal model to another. It is clear that further investigation into the calcium and vitamin D requirements of day geckos is warranted.

Calcium and Vitamin D in Leopard Geckos

Low-calcium diets produced abnormal development in leopard geckos. The leopard geckos fed low-calcium diets exhibited reductions in weight gain, feed intake, intake as a percent of body weight, skeletal radiographic density, bone ash content and bone calcium content. In general, these findings are consistent with reports of calcium and/or vitamin D deficiency signs in domestic animals (Maynard, et al., 1979; NRC, 1986; Blood et al., 1983).

Relatively few studies have been conducted on the effects of dietary calcium in reptiles. In a study of red-eared turtles (Pseudemys scripta elegans), Kass et al. (1982) did not find any significant differences in feed intake or weight change among diets containing 0.5%, 2.0% or 3.1% calcium, but both shell ash content and calcium as a percent of shell ash were significantly lower in the turtles fed 0.5% as compared to those fed 2.0% calcium diets.

Many of the findings in the present study with leopard geckos are consistent with those of Anderson and Capen (1976a,b) who

investigated the effects of dietary calcium and phosphorus in an herbivorous lizard, the green iguana (Iguana iguana). They reported that the weights of iguanas fed diets that were either low in calcium (0.1% calcium, DMB) and phosphorus (0.2% phosphorus) or just low in calcium (0.2% calcium, 1.1% phosphorus) were reduced relative to controls, but statistical comparisons were not reported. Radiographs of the iguanas fed low-calcium diets were similar to the ones of leopard geckos fed low-calcium crickets in the present study in that thin cortices and pathologic fractures were evident. Anderson and Capen (1976a) also reported features not seen in the leopard geckos, however: cartilaginous callus formation around pathologic fractures, an abnormal, rectangular shape of long bones in 5-month old animals fed calcium- and phosphorus-deficient diets and tetany.

Anderson and Capen (1976b) reported that bone ash was not significantly different when iguanas on low-calcium, low-phosphorus diets and low-calcium, adequate-phosphorus diets were compared to controls, although some sample sizes were small (n=1 - 3). Direct comparison to the present findings is complicated by the fact that dietary vitamin D concentrations were not evaluated in the iguana diets, but a clear effect of dietary calcium on bone ash was evident in the present study. The differences in radiographic and bone ash findings in the iguana study, as compared to those of the present study, suggest that there may be species differences in the pattern and degree of bone demineralization in calcium and/or vitamin D deficiency.

The plasma concentration of 25-OH D_3 is usually considered a good indicator of vitamin D status in humans (Horst, 1984). In the present study the plasma vitamin D results cannot be considered conclusive since samples were pooled within treatment groups. This was necessary due to the small body sizes of these geckos. However, plasma 25-OH D_3 of geckos fed diet 3 was similar to values reported for vitamin D-deficient humans (3.6 ng/ml) (Stanbury and Mawer, 1978). In addition, it is noteworthy that the geckos fed diet 1 had no detectable 25-OH D_3. There appeared to be higher levels of 25-OH D_3 in geckos fed diets 2, 4 and 5. Normal values for humans range from 16.2 to 29.9 ng/ml (Stanbury and Mawer, 1978).

While no specific conclusions can be drawn from the plasma vitamin D results, it is possible that dietary vitamin D did result in real differences in plasma D levels. Future work in the area of vitamin D and calcium in lizards should include the use of 25-OH D_3 as an indicator of calcium homeostasis.

In conclusion, diets low in calcium produced changes in growth, feed intake, radiographic results and bone composition in leopard geckos that are consistent with changes seen in domestic animals fed diets limiting in calcium (Blood, et al., 1983; NRC, 1986; Harris, et al., 1951; Bassett, et al., 1951). The results of this study also suggest that there may be an effect of dietary vitamin D, when added to cricket diets, on the concentration of calcium in dry, fat-free bone. It is also clear that <u>Phelsuma</u> <u>madagascariensis</u> responds in an entirely different manner to diets than does the nocturnal <u>Eublepharis</u> species. Future work on the calcium and

vitamin D requirements of lizards must include serious consideration of species differences with regard to apparent requirements of ultraviolet light as well as possible differences in the absolute requirements of calcium, phosphorus and vitamin D. While *Phelsuma* and *Eublepharis* are members of the Gekkonidae family, they do not apparently have the same requirements for calcium and/or vitamin D.

List of References

Allen, M.E. 1988. The effect of three light treatments on growth in the green iguana. Proceedings of the American Association of Zoo Veterinarians, Toronto, Canada.

Anderson, M.P. and Capen, C.C. 1976a. Nutritional osteodystrophy in captive green iguanas (*Iguana iguana*). Virchows Archives B Cell Pathology 21:229-247.

Anderson, M.P. and Capen, C.C. 1976b. Fine structural changes of bone cells in experimental nutritional osteodystrophy of green iguanas. Virchows Archives B Cell Pathology 20:169-184.

Bernard, J.B., Watkins, B.E. and Ullrey, D.E. In press. Manifestations of vitamin D deficiency in chicks reared under different artificial lighting regimes. Zoo Biology.

Bassett, C.F., Harris, L.E. and Wilke, C.F. 1951. Effect of various levels of calcium, phosphours and vitamin D intake on bone growth II. Minks. Journal of Nutrition 44:433-442.

Blood, D.C., Radostits, O.M. and Henderson, J.A. 1983. Veterinary Medicine. Bailliere Tindall, London, U.K.

Coulson, R.A. and Hernandez, T. 1965. Amino acid metabolism in the alligator. Federation Proceedings 24(4):927-940.

Dacke, C.G. 1979. Calcium Regulation in Sub-Mammalian Vertebrates. Academic Press, London, UK.

Demeter, B.J. 1976. Observations on the care, breeding and behavior of the Giant day gekco. International Zoo Yearbook 16:130-133.

Digney, T. and Tytle, T. 1982. Captive maintenance and propagation of the lizard genus *Phelsuma*. Proceedings of the 6th Annual Reptile Symposium on Captive Propagation and Husbandry. Washington, D.C.

Elsey, T.M. and Lance, V. 1983. Effect of diet on blood selenium and glutathione peroxidase activity in the alligator. Comparative Biochemistry and Physiology 76B(4):831-837.

Fowler, M.E. 1986. Metabolic bone disease. In: M.E. Fowler, ed., Zoo and Wild Animal Medicine. W.B. Saunders, Co., Philadelphia, PA.

Frye, F.L. 1981. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Veterinary Medicine Publishing Company, Edwardsville, KS.

Gehrmann, W.H. 1987. Ultraviolet irradiances of varous lamps used in animal husbandry. Zoo Biology 6:117-127.

Gomorri,G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. Journal of Laboratory Clinical Medicine 27:955-960.

Harris, L.E., Bassett, C.F. and Wilke, C.F. 1951. Effect of various levels of calcium, phosphorus and vitamin D intake on bone growth. Journal of Nutrition 43:153-165.

Holick, M.F., MacLaughlin, J.A., Clark, M.B., Holick, S.A., Potts, J.T. Jr., Anderson, R.R., Bland, I.H., Parrish, J.A. and Elias, P. 1980. Science 210:203-205.

Horst, R.L. 1984. Recent advances in the quantitation of vitamin D and vitamin D metabolites. In: R. Kumar, ed. Vitamin D. Martinus Nijhoff Publishing, Boston, MA.

Horwitz, W., ed. 1980. Methods of Anaylsis. Association of Official Analytical Chemists. Washington, D.C.

Horwitz, W., ed. 1982. Changes in Methods. Journal of Association of Official Analytical Chemists 65(2):494-495. Supplement to Methods of Analysis XIII edition (1980).

Kass, R.E, Ullrey, D.E. and Trapp, A.L. 1982. A study of calcium requirements of the red-eared slider turtle (Pseudemys scripta elegans). Journal of Zoo Animal Medicine 13:62-65.

Lance, V., Joanen, T. and McNease, L. 1983. Selenium, vitamin E and trace elements in the plasma of wild and farm-reared alligators during the reproductive cycle. Canadian Journal of Zoology 61:1744-1751.

Laszlo, J. 1969. Observations on two new artificail lights for reptile displays. International Zoo Yearbook 9:12-13.

Maynard, L.A., Loosli, J.K., HIntz, H.F. and Warner, R.G. 1979. Animal Nutrition. McGraw Hill, New York, NY.

McCrystal, H.K. and Behler, J.L. 1982. Husbandry and reproduction of captive giant Ameiva lizards. International Zoo Yearbook 22:159-163.

Miller, B.E. and Norman, A.W. 1984. Vitamin D. In: L.J. Machlin, ed., Handbook of Vitamins. Marcel Dekker, Inc., New York, NY.

Nichols, D., Montali, R.J., Pickett, C. and Bush, M., 1983. Rickets in double-crested cormorants (Phalacrocorax auritus). Journal of Zoo Animal Medicine 14:115-124.

NRC (National Research Council). 1986. Nutrient Requirements of Cats. National Academy Press, Washington, D.C.

Packard, M.J. and Packard, G.C. 1984. Comparative aspects of calcium metabolism in embryonic reptiles and birds. In: R.S. Seymour, ed. Respiration and metabolism of embryonic vertebrates. Dr. W. Junk, Dordrecht, Netherlands.

Packard, M.J., Packard, G.C. and Gutzke, W.H.N. 1984. Caclium metabolism in embryos of the oviparous snake Coluber constrictor. Journal of Experimental Biology 110:99-112.

Scott, M.L., Nesheim, M.C. and Young, R.J. 1982. Nutrition of the Chicken. M.L. Scott and Assoc., Ithaca, NY.

Simkiss, K. 1967. Calcium in Reproductive Physiology: A Comparative Study of Vertebrates. Chapman and Hall, London, U.K.

Stanbury, S.W. and Mawer, E.B. 1978. Physiological aspects of vitamin D metabolism in man. In: D.E.M. Lawson, ed., Vitamin D. Academic Press, New York, NY.

Townsend, C.R. and Cole, C.J. 1985. Additional notes on requirements of captive whiptail lizards (Cnemidophorus), with emphasis on ultraviolet radiation. Zoo Biology 4:49-55.

Zwart, P. 1980. Nutrition and nutritional disturbances in reptiles. Proceedings of the European Herpetological Symposium, Oxford, U.K. Pp. 75-80.

5. CALCIUM BALANCE IN THE LEOPARD GECKO *EUBLEPHARIS MACULARIUS*

Introduction

In a previous study I demonstrated that leopard geckos fed crickets unsupplemented with calcium developed signs of rickets and had significantly lower ash in bone as compared to geckos fed crickets supplemented with calcium (see Chapter 4). Similar signs of abnormal bone development are observed in other species of captive reptiles and are often attributed to nutritional deficiency, specifically insufficient concentrations of calcium and/or vitamin D (National Zoological Park, Department of Pathology Records; Fowler, 1986; Jacobson, 1984). It is believed that mealworm larvae and crickets, if unsupplemented with calcium and fed to insectivorous reptiles, will produce poor growth and skeletal abnormalities, especially in young animals (D. Marcellini and B. Demeter, National Zoological Park, personal communication; Frye, 1981). Although there is general agreement that crickets, when used as food, should be supplemented with calcium, the optimal dietary calcium level for insectivorous reptiles is unknown. With the exception of the leopard gecko study mentioned above (see Chapter 4), the effects of low calcium diets on insectivorous reptiles have not been determined by controlled experiments. The present study was designed to attempt to define the dietary calcium requirement of young leopard geckos.

Nutrient requirements can be defined in a number of ways. Feeding trials may be used to compare the response of a productive function (e.g., growth, egg production, milk yield or other measures of reproduction) to dietary manipulation (Maynard et al., 1979; Church and Pond, 1978). In the comparative slaughter method the accretion of nutrients is estimated through analysis of body constituents before and after defined diets containing graded levels of a nutrient are fed (Maynard et al. 1979). A third method used to define requirements is the balance trial in which graded levels of a specific nutrient are fed and the amount of nutrient excreted in urine and feces is measured (Maynard et al., 1979; Church and Pond, 1978). As nutrient concentration in the diet increases, the amount of that nutrient retained in the body increases until it reaches a break point, at which time retention levels off or begins to decline. It is assumed that the change in retention indicates that requirements for that nutrient have been met, at least under the conditions of the study. Balance or retention studies have been used extensively to help determine nutrient requirements and nutrient bioavailability for domestic and lab animals (Maynard et al., 1979; Church and Pond, 1978; Nelson and Tillman, 1967; Miller et al., 1965; Greenaway, 1974; Combs and Miller, 1985).

There are inherent problems associated with balance trials. Technical errors such as inaccurate measurements of food intake, incomplete collection of excreta and contamination of excreta with food may lead to biased conclusions (Asplund, 1978). The results of balance trials may also be influenced by prior nutritional state of

the animals, the degree of correspondence between intake and excretion, interactions with other nutrients, the nature of the adaptation period prior to the onset of the study, and the duration of the study in relation to excretion kinetics and nutrient pool size (Asplund, 1978; Mertz, 1987; Jeejeebhoy, 1986). Although balance trials have been criticized for being imprecise, they can be an important tool, if conducted properly (Asplund, 1978; Mertz, 1987; Jeejeebhoy, 1986). They are still widely used by human and animal nutritionists, especially in the study of mineral bioavailability and requirements (Combs and Miller, 1985; Linkswiler et al., 1981; Recker and Heaney, 1985).

The present study was designed to measure calcium balance in leopard geckos that were in a state of moderate calcium deficiency at the onset of the study. Geckos were fed a dietary item (crickets) containing graded levels of calcium. The calcium content of crickets can be systematically altered by manipulation of the amount of calcium in the feed given to the crickets (see Chapter 2). The specific objectives of the experiment were:

1. To demonstrate that a calcium balance trial could be conducted with an obligately insectivorous animal by manipulation of the calcium content of live, invertebrate prey.
2. To determine the effect of dietary calcium level on calcium retention.
3. To determine if calcium excretion and retention are influenced by the duration of feeding of experimental diets.

4. To estimate calcium requirements of growing leopard geckos from calcium balance data.

Materials and Methods

Twenty-one geckos that had been hatched at the National Zoological Park were used in this study. From hatching until eight weeks before the start of the experiment, geckos were fed crickets that had been maintained on 8% calcium diets for two days prior to being offered to the geckos (see Chapter 2). Initially, when geckos were small (from 2-4 g at hatching to 10-12 g at five to six months) 'half-grown' crickets were used (average weight = 0.25 g). Thereafter, adult crickets (average weight 0.35 g) were used. Eight weeks before the balance trial began the geckos were switched to crickets maintained on 2% calcium diets so that the geckos would be in a state of calcium depletion at the start of the balance trial (see Chapter 2). Thus, geckos were fed diets low in calcium and with inverse calcium to phosphorus ratios for 56 days prior to the beginning of the balance trial.

Crickets to be fed to geckos were purchased from a commercial supplier (Jiminy Cricket, Richmond, VA) and maintained as described in Chapter 2. Four bins of crickets were established, with each bin receiving one of four diets that were formulated to contain 2, 4, 6 or 8% calcium, as described in Chapter 2. The diets were manufactured by Zeigler Brothers (Gardners, PA). Crickets were maintained on these diets for at least 48 hours prior to being used as food for the geckos. Thus four dietary treatments were provided

4. To estimate calcium

from

to the geckos by using crickets fed the four diets. For simplicity, these treatments will be referred to as 2% TRT, 4% TRT, 6% TRT and 8% TRT in the remainder of this chapter.

At 6-8 months of age the 21 geckos were randomly assigned to one of the four treatments. Six geckos were assigned to the 2% TRT and five assigned to each of the 4%, 6% and 8% treatments. Geckos were fed live crickets twice per week between 14:00 to 15:30. Crickets were weighed, placed in the gecko cages for 30-45 minutes, and then uneaten crickets were removed and weighed. Cricket consumption was calculated by weight for each gecko. Geckos were weighed and measured (snout to vent length) once a month.

From hatching, and during the balance trial, each gecko was individually housed in a plastic box with a tightly fitting lid. Boxes were approximately 35 cm L X 12.5 cm W X 19 cm H. Air holes had been provided by melting about 25 small holes (0.5 cm) in each plastic lid, and along the top edge of the sides of the box, using a soldering iron. Distilled, deionized water was provided at all times in a plastic jar lid 5 cm in diameter and approximately 10 mm high. A green plastic leaf (approximately 8 X 14 cm) was provided in each cage for hiding and climbing. Cool-white, fluorescent lights, mounted in ceiling fixtures, maintained photoperiod at 14 hours light and 10 hours dark, from hatching until the end of the balance trial. Geckos were misted once per day, five days per week, with a light spray of distilled, deionized water to increase humidity within each box.

The balance trial was conducted from mid-February to mid-May.

From hatching until mid-April the geckos were maintained at ambient temperatures of approximately 30° C. Heat tape was placed under each box to provide a thermal gradient which raised the temperature to 32-33° C at one end of the box. The heat tape was automatically regulated to be on from 12:00 to 17:00 hours daily. In April, two months after the start of the trial, the central heat was shut down in the building and room temperature dropped to 25-26 °C for the remainder of the trial. To compensate for this temperature change, the heat tape was left on 24 hours per day. Average cage temperature over the heat tape was approximately 30-32°C during this period.

The balance trial was conducted for 12 weeks with six, 2-week collection periods. Gecko cages were checked for excreta five times per week. Droppings were collected and placed in pre-weighed, acid-washed ashing crucibles, dried overnight at 100° C and the crucibles placed in a desiccator. Every two weeks the geckos were removed from the cages and held in a clean, acid-washed, 1 liter glass beaker while all residual excreta were collected from the cages. Adhering excreta were scraped free with an acid-washed, ceramic spatula. Cages were then rinsed with distilled, deionized water (approximately 10-15 ml). The scrapings and rinse water were transferred to the ashing crucibles. The rinse water was collected in the ashing crucibles which contained the gecko waste. A final, quantitative cage rinse was carried out with known amounts of 6 N hydrochloric acid (HCl). This rinse acid was collected into 30 ml polypropylene bottles that had been previously acid-washed, dried

and weighed. The bottles were capped and stored at room temperature. Gecko cages were thoroughly rinsed with distilled, deionized water before the geckos were returned to the cages.

The crucibles containing the excreta were dried to constant weight in a forced-air, convection oven at 100° C, cooled to room temperature in desiccators and weighed to the nearest 0.0001 g. The dried excreta were then ashed at 600° C overnight, cooled in desiccators and re-weighed. The ash was solubilized in a known weight of HCl by warming the crucibles over low heat for approximately 2 hours. Solubilized ash was transferred into pre-weighed, acid-washed, 30 ml polypropylene bottles and stored at room temperature. There was one acid-solubilized ash bottle and one acid cage-rinse bottle for each gecko for each two week period. Calcium determinations were carried out, in duplicate, on the acid-solubilized ash and acid cage-rinse material by flame atomic absorption spectrophotometry.

Crickets from each dietary treatment were sampled (40 g) once per week during the 12 week balance trial. They were collected into plastic bags and killed by freezing at -10° C. The dry matter content of crickets was determined on duplicate samples (5-10 g) by drying to constant weight in a vacuum oven at 100° C for approximately two days. For calcium and phosphorus analyses, duplicate 5-10 g samples of crickets were removed from each of the bags and weighed to the nearest 0.001 g into tared 250 ml Phillips beakers. The samples were digested by nitric (10 ml) and perchloric (3 ml) acids under a perchloric acid fume hood. Calcium

determinations were performed on the wet ash by flame atomic absorption spectrophotometry with duplicate readings per sample. Phosphorus was measured colorimetrically, according to Gomorri (1942), with duplicate readings per sample.

The data were analyzed using a repeated measures analysis of variance (ANOVA) model which tested both between subject (treatment) effects and within subject effects (period and treatment x period). The statistical analyses were conducted on a Zenith 183 microcomputer using the General Linear Models Program of PC SAS (SAS Institute, Cary, NC). The tests of sphericity showed the hypothesis of equality of covariance and variance matrices to be rejected. However, even when the Huynh-Feldt Epsilon correction was applied, the probabilities remained similar. In addition, the multivariate analyses produced rejection at similar probability levels (SAS, 1985; pp 230), and thus did not change the conclusion of the univariate F tests. Contrast variables were generated using reverse Helmert transformations to compare, among periods, the effects of dietary treatment on dry matter intake, dry matter intake as a percent of body weight, calcium intake, calcium output and calcium retention in milligrams and in percent. A one-way analysis of variance model was also used to separately evaluate the performance of animals at each time period and each treatment. Means were compared at each time period using Bonferroni T tests (SAS Institute, Cary, NC). A probability level of 0.05 was chosen to determine statistical significance of observed differences.

Weight and length data were analyzed using one-way ANOVA, and

weight gain was analyzed by analysis of covariance (ANCOVA) with initial weight as covariate. Means were compared using Bonferroni T tests with a probability level of 0.05. In order to compare intake to body weight in each period, weight at the midpoint of each period was estimated for each animal from regression of weight on time using the four weights taken at monthly intervals.

Results

Cricket Composition

The dry matter, calcium and phosphorus content of feed crickets are presented in Table 28. Cricket calcium content varied with the level of calcium in the diet, with mean values of 0.27% 0.44%, 0.61% and 0.85% for 2%, 4%, 6% and 8% calcium diets, respectively. The measured calcium levels were relatively invariant over the course of the study, as indicated by the small standard errors of the means (Table 28). Phosphorus and dry matter contents were not affected by the diet fed to the crickets.

Table 28. Dry Matter, calcium and phosphorus composition[1] (mean ±SE) of crickets.

	Diet			
	(n=12)	(n=12)	(n=12)	(n=12)
Nutrient	2% TRT	4% TRT	6% TRT	8% TRT
Dry Matter (%)	31.41 ±0.18	30.39 ±0.46	30.14 ±0.49	29.75 ±0.37
Calcium (%)	0.27 ±0.01	0.44 ±0.03	0.61 ±0.02	0.85 ±0.03
Phosphorus (%)	0.78 ±0.03	0.86 ±0.03	0.83 ±0.03	0.89 ±0.03

[1]Calcium and phosphorus expressed as a percentage of dry matter.

weight gain was analyzed by analysis of covariance (ANCOVA) with initial weight as covariate. Means were compared using Bonferroni T tests with a probability level of 0.05. In order to compare intake

Food Intake and Growth

All geckos ate well. Dry matter intake increased over the course of the 12-week study (P < 0.0001) but it was not affected by dietary treatment (P > 0.05, repeated measures ANOVA). The average dry matter intake per 2-week period was 1.92, 1.92, 1.77 and 1.91 g per animal for the 2%, 4%, 6% and 8% TRT groups, respectively. All geckos increased in weight and length (Table 29), but there were no significant differences among treatment groups in initial or final weights or lengths. ANCOVA confirmed that there was no significant effect of dietary treatment on weight gain although initial weight was significant as a covariate of weight gain. The increase in dry matter intake over the course of the study was only partially attributable to the increase in gecko weight, since dry matter intake also increased significantly as a percentage of body weight (P < 0.0001, repeated measures ANOVA). The calculated dry matter intake per 2-week period increased from 6.1 to 7.1% of body weight from the first to last periods, equivalent to an increase in calculated daily intake from 0.44 to 0.51% of body weight.

Calcium Balance Data

The means for calcium intake, calcium output, calcium retention, and percentage retention are presented by period in Table 30. One-way ANOVA tests demonstrated significant treatment effects in each period for calcium intake, output and retention. Repeated measures ANOVA for the entire study indicated that dietary treatment had a significant between-subjects effect for all calcium balance variables (Table 31). There were also highly significant within-

Table 29. Summary of means (±SE) and ANOVA results for weight (Wt.) length (SVL[1]) and weight gain of geckos by diet.

Item	Diet: 2% TRT	4% TRT	6% TRT	8% TRT	S of V[2]	F	Prob
Initial Wt. (g)	24.6 ±0.68	24.7 ±1.16	24.5 ±1.43	24.7 ±0.46	TRT	0.02	0.997
Final Wt. (g)	32.1 ±1.34	32.5 ±1.50	31.1 ±2.28	32.4 ±1.06	TRT	0.15	0.930
Initial SVL (mm)	102.5 ±0.99	100.2 ±1.66	98.8 ±2.48	102.6 ±0.81	TRT	1.38	0.284
Final SVL (mm)	108.2 ±0.98	106.4 ±1.33	106.6 ±1.33	109.2 ±0.66	TRT	1.21	0.336
Weight Gain (g)	7.47 ±0.91	7.75 ±0.57	6.66 ±1.32	7.67 ±0.74	TRT COVAR[3]	0.25 38.65	0.868 0.0001

[1]SVL = Snout to vent length
[2]S of V = Source of variation
[3]COVAR = Covariate (Initial weight)

Table 30. Summary of means[3] (±SE) of calcium intake, calcium output, calcium retained (mg) and calcium retained (%) and ANOVA results by treatment group over time.

Period[2]	Diet: 2% TRT (n=6)		Diet: 4% TRT (n=5)		Diet: 6% TRT (n=5)		Diet: 8% TRT (n=5)		ANOVA[1]: F Value	ANOVA[1]: Prob.
Calcium Intake (mg):										
1	4.32^{A}	±0.04	6.91^{B}	±0.06	9.24^{C}	±0.24	13.28^{D}	±0.17	450.8	0.0001
2	4.73^{A}	±0.03	7.56^{B}	±0.10	9.78^{C}	±0.53	14.25^{D}	±0.16	231.3	0.0001
3	5.14^{A}	±0.03	7.81^{B}	±0.22	10.43^{C}	±0.39	14.64^{D}	±0.39	214.1	0.0001
4	5.53^{A}	±0.25	8.64^{B}	±0.41	11.41^{C}	±0.58	16.33^{D}	±0.70	88.1	0.0001
5	5.46^{A}	±0.53	10.27^{B}	±0.12	12.03^{B}	±1.47	19.37^{C}	±0.33	56.2	0.0001
6	6.07^{A}	±0.44	9.87^{AB}	±0.67	11.61^{B}	±2.05	18.72^{C}	±0.64	23.7	0.0001
Calcium Output (mg)										
1	0.269^{A}	±0.025	0.357^{A}	±0.017	0.639^{B}	±0.069	0.649^{B}	±0.084	13.4	0.0001
2	0.247^{A}	±0.014	0.349^{AB}	±0.015	0.511^{B}	±0.076	0.534^{B}	±0.056	9.2	0.0008
3	0.211^{A}	±0.021	0.335^{A}	±0.062	0.643^{B}	±0.101	0.889^{B}	±0.077	20.7	0.0001
4	0.426^{A}	±0.034	0.599^{A}	±0.054	1.018^{B}	±0.110	1.083^{B}	±0.125	14.6	0.0001
5	0.441^{A}	±0.029	0.676^{A}	±0.076	1.629^{A}	±0.261	13.079^{B}	±1.940	42.1	0.0001
6	0.464^{A}	±0.047	0.758^{A}	±0.134	1.153^{A}	±0.071	15.777^{B}	±1.330	135.6	0.0001

Table 30 (cont'd.).

Period[2]	Diet: (n=6) 2% TRT		(n=5) 4% TRT		(n=5) 6% TRT		(n=5) 8% TRT		ANOVA[1]: F Value	Prob.
Calcium Retained (mg)										
1	4.05^{A}	±0.06	6.55^{B}	±0.07	8.60^{C}	±0.39	12.63^{D}	±0.22	282.6	0.0001
2	4.48^{A}	±0.03	7.21^{B}	±0.11	9.27^{C}	±0.53	13.72^{D}	±0.16	175.6	0.0001
3	4.93^{A}	±0.03	7.48^{B}	±0.25	9.78^{C}	±0.43	13.76^{D}	±0.42	149.2	0.0001
4	5.10^{A}	±0.26	8.04^{B}	±0.45	10.39^{C}	±0.62	15.25^{D}	±0.65	75.3	0.0001
5	5.02^{A}	±0.54	9.60^{AB}	±0.08	10.40^{B}	±1.63	6.30^{AB}	±1.85	4.7	0.0146
6	5.60^{AC}	±0.46	9.12^{AB}	±0.68	10.46^{B}	±2.07	2.94^{C}	±0.90	8.6	0.0011
Calcium Retained (%)										
1	93.76^{A}	±0.60	94.83^{A}	±0.27	92.94^{A}	±1.06	95.10^{A}	±0.66	2.0	0.1569
2	94.79^{A}	±0.29	95.37^{A}	±0.27	94.54^{A}	±1.20	96.26^{A}	±0.39	1.4	0.2728
3	95.89^{A}	±0.40	95.67^{A}	±0.84	93.74^{A}	±1.10	93.89^{A}	±0.59	2.4	0.1087
4	92.18^{A}	±0.79	92.89^{A}	±1.03	90.91^{A}	±1.19	93.39^{A}	±0.66	1.3	0.3136
5	91.43^{A}	±1.13	93.44^{A}	±0.68	84.67^{A}	±3.94	32.68^{B}	±9.79	31.7	0.0001
6	92.04^{A}	±1.10	92.14^{A}	±1.44	87.98^{A}	±3.05	16.08^{B}	±4.91	161.7	0.0001

[1] One way ANOVA, source of variation = Treatment (TRT); df=3.
[2] Period = 6 consecutive 2-week periods.
[3] Means in a row with the same superscript are not signigicantly different at the 0.05 probability level (Bonferroni (Dunn) T test).

Table 31. Repeated measures analysis of variance for calcium intake, calcium output, calcium retention (mg) and calcium retention (percent).

		CA IN		CA OUT		CA RET (mg)		CA RET (%)	
Source [1]	DF	F	Prob	F	Prob	F	Prob	F	Prob
Diet	3	134.4	0.0001	98.9	0.0001	33.8	0.0001	63.6	0.0001
Period	5	28.8	0.0001	77.9	0.0001	9.0	0.0001	87.9	0.0001
Interaction	15	2.9	0.0011	61.3	0.0001	18.8	0.0001	58.3	0.0001

1 Sources of variation:

Diet (between subjects effects),
Period = Repeated measure (6 consecutive 2-week periods) and reflects within subject effects.
Interaction = Diet x Period (within subject effects).

subjects effects for all calcium balance variables due to period and the interaction between treatment and period.

The changes with time in calcium intake, output, retention and percentage retention are illustrated in Figures 19-22. Contrast variables generated in the repeated measures ANOVA using reverse Helmert transformations allowed the calcium balance measures for each period to be compared to those of previous periods (Table 32). Although mean calcium intake increased in each successive period, treatment had no effect on this increase except in the comparison of period 5 to periods 1-4. By contrast, the pattern of calcium output was strongly influenced by dietary treatment. Mean calcium output increased with time, and exhibited a highly significant effect of dietary treatment ($P < 0.0001$) when period 5 was compared to periods 1-4, and when period 6 was compared to periods 1-5 (Table 32). The effect of dietary treatment is illustrated in Figure 20 which shows the steep increase in calcium output of the 8% TRT group in periods 5 and 6. In these two periods calcium output was significantly greater in the 8% TRT group than in the other TRT groups (Table 30, $P < 0.05$, Bonferroni t-tests). In periods 1,2,3 and 4 the calcium output of the 8% TRT did not differ from that of the 6% TRT. Calcium retention (mg) increased from period 1 through 4 in all treatment groups (Figure 21, Table 32), presumably due to the simultaneous increase in calcium intake. The amount of calcium retained was highest in the 8% TRT group in these four periods, but there were no significant differences among treatment groups in the percentage of ingested calcium that was retained. Percent retention

Figure 19. The relationship between calcium intake (mg) and time (6, 2-week periods) for leopard geckos fed 4 different diets differing in calcium content.

Figure 20. The relationship between calcium output (mg) and time (6, 2-week periods) for leopard geckos fed 4 different diets differing in calcium content.

Figure

FIG. 19

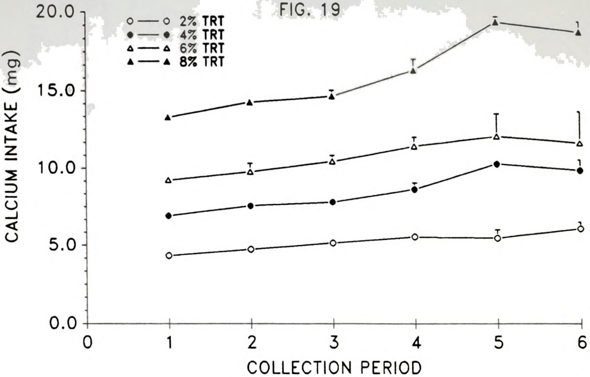

20.0
15.0
10.0
5.0
0.0
CALCIUM INTAKE (mg)
2% TRT
4% TRT
6% TRT
8% TRT
0
1
2
3
4
5
6
COLLECTION PERIOD

FIG. 20

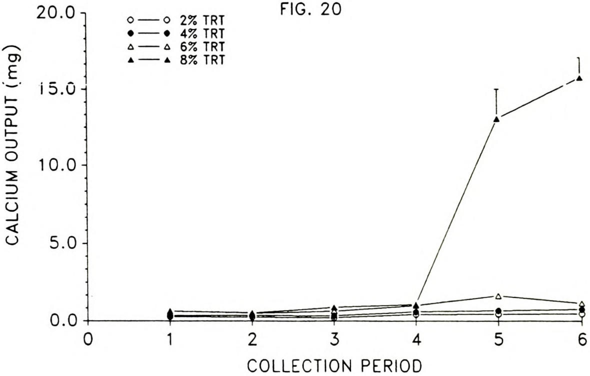

20.0
15.0
10.0
5.0
0.0
CALCIUM OUTPUT (mg)
2% TRT
4% TRT
6% TRT
8% TRT
0
1
2
3
4
5
6
COLLECTION PERIOD

Figure 21. The relationship between calcium retained (mg) and time (6, 2-week periods) for leopard geckos fed 4 different diets differing in calcium content.

Figure 22. The relationship between calcium retained (%) and time (6, 2-week periods) for leopard geckos fed 4 different diets differing in calcium content.

FIG. 21

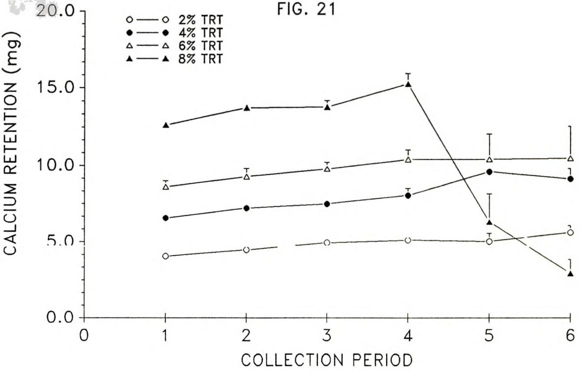

2% TRT
4% TRT
6% TRT
8% TRT
CALCIUM RETENTION (mg)
20.0
15.0
10.0
5.0
0.0
0
1
2
3
4
5
6
COLLECTION PERIOD

FIG. 22

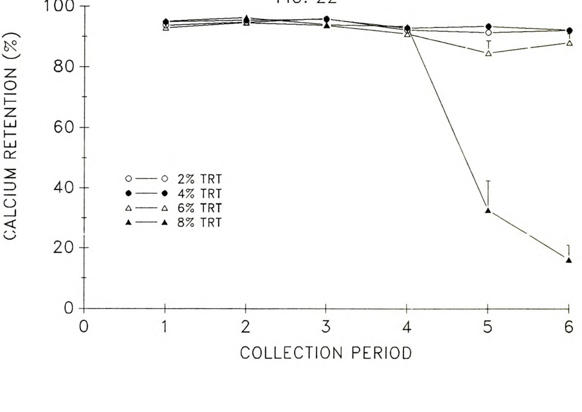

CALCIUM RETENTION (%)
100
80
60
40
20
0
2% TRT
4% TRT
6% TRT
8% TRT
0
1
2
3
4
5
6
COLLECTION PERIOD

Table 32. Analysis of variance of contrast[1] variables for calcium intake, output, retention (mg) and retention (%).

	Source[2]	DF	CALCIUM INTAKE (mg) F	Prob	CALCIUM OUTPUT (mg) F	Prob
Period 1	Mean	1	42.5	0.0001	7.6	0.0134
vs. 2	TRT	3	1.5	0.2530	1.6	0.2380
Periods	Mean	1	14.4	0.0015	4.2	0.0571
1-2 vs. 3	TRT	3	0.2	0.8970	4.5	0.0176
Periods	Mean	1	51.2	0.0001	40.6	0.0001
1-3 vs. 4	TRT	3	2.4	0.1054	1.4	0.2838
Periods	Mean	1	45.5	0.0001	53.1	0.0001
1-4 vs. 5	TRT	3	6.3	0.0047	39.2	0.0001
Periods	Mean	1	15.5	0.0011	154.0	0.0001
1-5 vs. 6	TRT	3	1.3	0.3096	129.6	0.0001

	Source[2]	DF	CALCIUM RETENTION (mg) F	Prob	CALCIUM RETENTION (%) F	Prob
Period 1	Mean	1	54.2	0.0001	14.4	0.0014
vs. 2	TRT	3	2.0	0.1472	0.6	0.6476
Periods	Mean	1	9.8	0.0060	0.1	0.8164
1-2 vs. 3	TRT	3	0.1	0.9700	2.9	0.0652
Periods	Mean	1	34.0	0.0001	25.0	0.0001
1-3 vs. 4	TRT	3	1.8	0.1784	0.3	0.8526
Periods	Mean	1	2.9	0.1060	54.8	0.0001
1-4 vs. 5	TRT	3	13.9	0.0001	32.8	0.0001
Periods	Mean	1	13.4	0.0019	276.5	0.0001
1-5 vs. 6	TRT	3	34.5	0.0001	203.4	0.0001

1 Contrast variables were generated from reverse Helmert transformations to allow comparison of balance data for each period to those of preceeding periods.

2 Source = Sources of variation.
Mean reflects a comparison of the average value of one period across diets to the mean of another period(s).
TRT reflects the effect of dietary treatment in comparing data from one period to others.

was very high, averaging 90.9% - 96.3% (Table 30). The transition to period 5 was marked by a sharp fall in calcium retention (both as a mg amount and as a percentage) in the 8% TRT group (Figure 21). The Helmert transformed variables indicated a very highly significant treatment effect in the comparison of retention and percent retention between the initial four periods and period 5 (Table 32). In period 5 the amount of calcium retained by geckos in the 8% TRT group did not differ from that of geckos in the 4% or 6% TRT groups (Table 30). Period 6 brought a further decline in both absolute and percentage retention in the 8% TRT group (Figures 21, 22). In period 6 the absolute retention of the 8% TRT group (2.94 mg) was significantly lower than that of the 4% and 6% TRT groups, and the percentage retention of the 8% TRT group (16.1%) was much lower than that of the other groups (88-92%; Table 30).

Discussion

The present study has attempted to overcome the errors of measurement or interpretation that commonly accompany balance trials (Mertz, 1987; Asplund, 1978). With respect to technical errors, the complete collection of gecko waste was accomplished through meticulous cleaning of cages followed by a quantitative acid rinse of the entire cage at each excreta collection period. The importance of the final acid rinse cannot be overemphasized. Although there appeared to be little mineral matter remaining in the cages after cleaning and a distilled water rinse, the amount of calcium recovered in the final acid rinse accounted for 25% of total excreted calcium, on average (n=126 collections). Since the geckos

were fed large, discrete food masses (crickets), the contamination of excreta with uneaten food was not a problem. However, it is possible that crickets may have defecated in the gecko cages prior to being eaten by the geckos, even though uneaten crickets were only allowed to remain in the cages for 30 to 45 minutes. This error would have produced overestimation of both calcium intake and calcium excretion, so the net effect on percent retention would have been small.

The geckos used in this study were relatively uniform in age and nutritional state at the onset of the balance trial as they had been maintained on identical diets from hatching until the beginning of the adaptation period. All too often balance trials are conducted on animals of unknown prior nutritional status even though prior status is known to affect calcium retention (Mitchell, 1962).

I designed the present study to take advantage of the fact that the calcium content of whole crickets can be varied systematically by manipulation of the composition of the feed on which the crickets are maintained. As observed previously (Chapter 2), there was a direct relationship between the calcium content of crickets and the diet consumed by them, and this proved to be a reliable method of altering the calcium intakes of leopard geckos.

In a previous study, leopard geckos consuming crickets containing 0.17% calcium developed clinical signs of rickets within 7 months (n=8; Chapter 4). The low-calcium crickets (2% TRT) in the present study were slightly higher in calcium (0.27%), but there is little doubt that this level was inadequate to sustain body calcium were fed large, discrete food masses (crickets), the contamination of excreta with uneaten food was not a problem. However, it is possible that crickets may have defecated in the gecko cages prior to being eaten by the geckos, even though uneaten crickets were only allowed to remain in the cages for 30 to 45 minutes. This error would have produced overestimation of both calcium intake and calcium excretion levels in growing leopard geckos. Although there was no clinical evidence of calcium deficiency in geckos maintained on these crickets, the preliminary 8-week period of feeding on low-calcium crickets led to extremely high calcium retentions for at least 8 weeks thereafter, suggesting that the animals started the balance trial in a state of calcium depletion.

In mammals the rate of calcium absorption by mucosal cells of the gut is reported to be enhanced when dietary calcium is limited, and it is possible that the same is true in reptiles. In contrast, when dietary calcium is provided in excess of requirements, the percentage of calcium absorbed is reduced (Goodhart and Shils, 1978; ARC, 1980). Vitamin D and phosphorus levels in the diet are also important factors influencing calcium absorption and in maintaining calcium homeostasis. In the present study remarkably high rates of retention were observed throughout the trial, with the exception of animals in the 8% TRT group during periods 5 and 6. These findings are consistent with the hypothesis that all the geckos were in a calcium-depleted state, but that the animals in the 8% TRT group became repleted after two months on a high-calcium diet. In the 8% TRT group the percentage of calcium retained ranged from 91 to 96% until periods 5 and 6 when the percentage of retained calcium declined precipitously to less than 30%. It was remarkable that all 5 animals in this group exhibited a decline in retention at about the same time.

Calcium retentions above 90% are rarely reported among birds and mammals, at least among animals that are no longer dependent on levels in growing leopard geckos. Although there was no clinical

evidence of

crickets

parental provisioning. However, milk-fed mammals are able to retain comparably high percentages of ingested calcium (ARC, 1980). In milk-fed calves this is apparently due both to very high absorption of milk calcium and to high rates of calcium deposition in developing bone. It has been reported that calcium retention in calves is 92% when the amount of milk offered produced weight gains of 1 kg/day; when fed at maintenance levels calcium retention was only 73% (Roy, 1980). Miller et al. (1962) observed retention rates of 77 to 87% in young pigs fed synthetic milk diets containing 0.4 to 1.2% calcium. As in the present study, differences in calcium retention were observed among dietary calcium treatments even though there were no differences in weight gain attributable to dietary calcium. In other studies with young domestic livestock, especially those using non-milk diets, retention rates are typically less than 80% (ARC, 1980; Hodge, 1973).

Mertz (1987) noted that nutrient pool and nutrient turnover rates have an important influence on balance trials and stressed the importance of conducting trials for an extended time, rather than the more typical trials of only a few weeks. This is especially important when the nutrient pool is large relative to nutrient intake, which is the case with calcium. The importance of this recommendation is evident in a comparison of the mean retention observed in successive periods (Table 30). During the first four periods, retention increased with increased calcium intake, suggesting that calcium requirements had not been met. It was not until 2 months had elapsed that the calcium pool had adjusted to the

higher intakes of the 8% TRT group, and retention declined. Thus, the duration of a balance trial may affect the conclusion reached.

The percentage of calcium retained by geckos in the 6% TRT group appeared to decline slightly in periods 5 and 6, although the mean percent retentions (84.7% and 88.0%, respectively) were not significantly lower than those of the 2% and 4% TRT groups because of the large standard errors. It is possible that continuation of the balance study would have revealed a significant drop in percent retention in the 6% TRT group as these animals might require longer to replete, but this is merely speculation. The present study indicates that 0.85% dietary calcium (8% TRT) meets the requirements for growth and calcium repletion in growing (7-9 month) leopard geckos, but 0.61% dietary calcium (6% TRT) does not.

Calcium regulation in reptiles is poorly understood (Simkiss, 1967; Dacke, 1979). Those reptiles studied possess parathyroid hormone and calcitonin, and it is assumed that these hormones serve a calcium-regulatory function as they do in birds and mammals. A feature unique to some amphibians and lizards is the presence of endolymphatic or paravertebral lime sacs. These are paired organs, continuous with the endolymph of the inner ear, which contain calcium carbonate. In amphibians these sacs appear to function as calcium reservoirs which are important in acid-base regulation (Dacke, 1979). Parathyroid hormone is known to be an important regulator of the calcium stores in the lime sac of the frog (Schlumberger and Burk, 1953). Endolymphatic sacs have not been reported in <u>Eublepharis macularius</u>, nor were extra-skeletal calcium

deposits seen radiographically in a previous study (Chapter 4). It can only be assumed that leopard geckos regulate calcium absorption and excretion in a fashion similar to that of domestic animals that have been studied.

Insects and many other invertebrate species do not possess calcified structural supports, unlike vertebrates in which the bony skeleton contains 98 to 99% of total body calcium (NRC, 1980). The calcium levels in invertebrates, both from the wild and from captive colonies, are typically very low (Chapter 1). It is not clear how insectivorous species obtain sufficient calcium to support growth, maintenance and reproductive functions. Some animals have been observed to select calcareous materials during reproductive activity (Jones, 1976; Ankey and Scott, 1980; B. Demeter, National Zoological Park, personal communication). It is possible that some insectivorous species have evolved metabolic adaptations that permit survival on diets that are low in calcium and that contain inverse calcium to phosphorus ratios. Perhaps the high rates of calcium retention seen in this study represent a specialized ability of insectivorous geckos to extract scarce calcium from an inherently low-calcium food supply. If insectivorous species in their natural habitats are indeed faced with chronic calcium depletion, high retention of ingested calcium may be the rule rather than the exception.

There are few studies which define calcium requirements of non-domestic animals (e.g., Ullrey et al., 1973; cf. Robbins, 1983) and even fewer that have utilized reptiles. Most studies have involved

the measurement of tissue or growth responses to dietary calcium manipulation. Kass et al., (1982) found that the red-eared slider performs better on diets containing 2.0% calcium, as compared to diets containing 0.5%, as judged by weight gain, food consumption, shell abnormalities and shell composition. Anderson and Capen (1976) observed that diets containing 0.2% calcium and 1.1% phosphorus produced severely demineralized bones, low serum calcium and poor growth in young iguanas. They found that diets containing 2.7% calcium and 1.1% phosphorus produced well-mineralized bone and higher bone ash percentages. The results of the present and a previous leopard gecko study (Chapter 4) indicate that the calcium requirement for growth of this insectivorous lizard lies between 0.61 and 0.85% calcium, when dietary phosphorus is 0.82%.

It is clear from the results of this study that the nutrient requirements of insectivorous animals can be estimated from balance trials using live invertebrate prey. Insectivorous reptile species remain one of the most difficult groups of zoo animals to maintain and propagate. Admittedly, it is difficult to conduct nutritional research with insectivorous animals that do poorly in captivity. However, this study has shown that species that are relatively easy to maintain and handle, such as the leopard gecko, can be used successfully as experimental animals for nutritional research.

the measurement of tissue or growth responses to dietary calcium manipulation. Kass et al., (1982) found that the red-eared slider performs better

diets

List of References

Anderson, M.L. and Capen, C.C. 1976. Nutritional osteodystrophy in the green iguana (*Iguana iguana*). Virchows Archives B. Cell Pathology 21:229-247.

Ankney, C.D. and Scott, D.M. 1980. Changes in nutrient reserves and diet of breeding brown-headed cowbirds. Auk 97:684-696.

ARC (Agricultural Research Council) 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, Slough, U.K.

Asplund, J.M. 1978. Interpretation and significance of nutrient balance experiments. Journal of Animal Science 49:826-831.

Combs, N.R. and Miller, E.R. 1985. Determination of potassium availability in K_2CO_3, $KHCO_3$, corn and soybean meal for the young pig. Journal of Animal Science 60:715-719.

Church, D.C. and Pond, W.G. 1978. Basic Animal Nutrition and Feeding. O and B Books, Corvallis, OR.

Dacke, C.G. 1979. Calcium Regulation in Sub-Mammalian Vertebrates. Academic Press, London, U.K.

Fowler, M.E. 1986. Metabolic bone disease. In: M.E. Fowler, ed., Zoo and Wild Animal Medicine. W.B. Saunders, Co. Philadelphia, PA.

Frye, F.L. 1981. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Veterinary Medicine Publishing Company, Edwardsville, KS.

Gomorri,G. 1942. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. Journal of Laboratory Clinical Medicine 27:955-960.

Goodhart, R.S. and Shils, M.E. 1978. Modern Nutrition in Health and Disease, 6th edition. Lea and Febiger, Philadelphia, PA.

Greenaway, P. 1974. Calcium balance at the postmoult stage of the freshwater crayfish *Austropotamobius pallipes* (Lereboullet). Journal of Experimental Biology 61:35-45.

Hodge, R.W. 1973. Calcium requirements of the young lamb II. Estimation of calcium requirements by the factorial method. Australian Journal of Agricultural Research 24:247-243.

Jacobson, E.R. 1984. Biology and diseases of reptiles. In: J.B Fox, B.J. Cohen and F.M. Loew., (eds). Laboratory Animal Medicine, Academic Press, N.Y.

Jeejeebhoy, K.N. 1986. Nutritional balance studies: Indicators of human requirements or adaptive mechanisms. Journal of Nutrition 116:2061-2063.

Jones, P.J. 1976. The utilization of calcareous grit by laying Quelea quelea. Ibis 118:575-576.

Kass, R.E., Ullrey, D.E. and Trapp, A.L. 1982. A study of calcium requirements of the red-eared slider turtle (Pseudemys scripta elegans). Journal of Zoo Animal Medicine 13:62-65.

Linkswiler, H.M., Zemel, M.B., Hegsted, M. and Schuette, S. 1981. Protein-induced hypercalciuria. Federation Proceedings 40:2429-2433.

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G. 1979. Animal Nutrition, 7th edition. McGraw-Hill, Inc., New York, NY.

Miller, E.R., Ullrey, D.E., Zutaut, C.L., Baltzer, B.V., Schmidt, D.A., Hoefer, J.A. and Luecke, R.W. 1962. Calcium requirement of the baby pig. Journal of Nutrition 77:7-17.

Mitchell, H.H. 1962. Comparative Nutrition of Man and Domestic Animals. Volume I. Academic Press, New York, NY.

Mertz, W. 1987. Use and misuse of balance studies. Journal of Nutrition 117:1811-1813.

NRC (National Research Council) 1980. Mineral Tolerance of Domestic Animals. National Academy of Sciences, Washington, D.C.

Nelson, T.E. and Tillman, A.D. 1967. Calcium status studies on adult sheep. Journal of Nutrition 93:475-479.

Recker, R.R. and Heaney, R.P. 1985. The effect of milk supplements on calcium metabolism, bone metabolism and calcium balance. American Journal of Clinical Nutrition 41:254-263.

Robbins, C.T. 1983. Wildlife Feeding and Nutrition. Academic Press, New York, NY.

Roy, J.H.B. 1980. The Calf. Butterworth and Company, Ltd. London, U.K.

SAS Institute Inc. 1985. SAS/STAT Guide for Personal Computers, Version 6 Edition. Cary, NC.

Schlumberger, H.G. and Burk, D.H. 1953. Comparative study of the reaction to injury II. Hypervitaminosis D in the frog with special reference to the lime sacs. Archives of Pathology 56:103-124.

Simkiss, K. 1967. Calcium in Reproductive Physiology. Chapman and Hall, London, U.K.

Ullrey, D.E., Youatt, W.G., Johnson, H.E., Fay, L.D., Schoepke, B.L., Magee, W.T. and Keahey, K.K. 1973. Calcium requirements of weaned white-tailed deer fawns. Journal of Wildlife Management 37:187-194.

6. INTAKE AND DIGESTIBILITY OF CRICKETS BY THREE SPECIES OF SMALL MAMMALS

Introduction

The earliest mammals that evolved from therapsid reptiles in the Triassic were probably small (ca 30 grams), nocturnal and insectivorous (Crompton, 1980). Many extant mammals consume invertebrates opportunistically or exclusively (Nowak and Paradiso, 1983; cf. Redford, 1987).

Some invertebrate prey commonly fed to captive insectivorous animals include crickets (*Acheta domestica*), mealworm larvae (*Tenebrio molitor*), fruit flies (*Drosophila* spp.) and earthworms (*Lumbricus* spp.). Reports on the nutrient composition of these and other invertebrates are scarce and incomplete (See Chapter 1). Available data indicate that insects are high in protein content. Protein has been measured as total nitrogen multiplied by 6.25, a conversion factor that was developed for an average mix of food proteins in human diets. Yet the insect cuticle contains chitin as well as protein. Chitin is a structural, nitrogen-containing polysaccharide of N-acetylglucosamine found in close association with cuticular proteins (see Chapter 1) (Chapman, 1982). Protein and chitin together account for up to 50% of the dry weight of the insect cuticle, the exact proportions varying with species (Hackman, 1976). Cuticular protein is often sclerotized (tanned) with phenolic compounds. The extent to which chitin or sclerotized

chitin-protein complexes are digested is not known. Measurement of total nitrogen may thus result in an overestimate of the truly available nitrogen for the insectivore.

Chitin is similar in composition and structure to cellulose (see Chapter 1). It has long been known that symbiotic bacteria and protozoa found in the foreguts and hindguts of herbivores can effectively degrade the cellulose in plant cell walls, the host animal being incapable of de novo cellulase synthesis. In the case of ruminants, the result of this bacterial degradation is a substantial contribution to the energy budget of the animal.

Benecke (1905) was one of the first to report chitin degradation by a bacterium, and subsequently invertebrates were found to possess enzymes that degraded chitin (Elyakova, 1972; Jeuniaux, 1963; Jeuniaux and Amanieu, 1955; Tracey, 1951). Initially, it was believed that such chitinases were of bacterial origin. However, chitinases synthesized by the host animal have been found in the gastrointestinal tracts and salivary glands of invertebrates and vertebrates (Febvay et al., 1984; Waterhouse and McKellar, 1961; Jeuniaux, 1961). The issue of whether gastrointestinal tract chitinases are of bacterial origin or endogenously synthesized by the predator may have significance when considering gut morphology. In herbivores, specialized gastrointestinal tract anatomy may permit ingested fibrous material to remain in the gut for prolonged periods and thereby encourage an effective bacterial degradation of cellulose. The guts of insect-eating vertebrates are typically simple and lack specialized

compartments, similar to the guts of carnivores. (Clemens, 1980; Schieck and Millar, 1985). Animals with simple guts typically retain ingesta for very brief periods of time, by comparison to herbivores. Even if bacterial chitinases are present in the guts of insectivorous vertebrates, there is little time for chitin digestion and usually no specialized gut structures to promote degradation of chitin.

From the perspective of the insectivorous predator, the issue of chitinase origin is perhaps not as important as is the degree to which, and the efficiency with which, nutrients can be extracted from chitin-containing prey species. While it has been demonstrated that chitinases are found in vertebrate guts, the extent to which nutrients in insects are digested by facultative or obligate insectivorous mammals has been little studied.

The objectives of this study were:

1. To determine if insectivorous mammals were selective in their consumption of crickets, avoiding the heavily sclerotized parts.
2. To determine if differences existed in the abilities of three species of insectivorous mammals to digest chitin and other nutrients in crickets.
3. To determine if the rate of ingesta passage, as measured by the time of first appearance of markers, was different in these mammals.

Three species of mammals were used in these experiments: the house or musk shrew (Suncus murinus, Insectivora: Soricidae), the southern grasshopper mouse (Onychomys torridus longicaudus, Rodentia: Muridae) and the pygmy hedgehog tenrec (Echinops telfairi, Insectivora: Tenrecidae). The two species of Insectivora were selected because they represent two distinctly different body sizes and phyletic origins and are fairly easily maintained in captivity. Shrews are reported to have relatively high metabolic rates, whereas the metabolic rates of tenrecs are reported to be especially low (Vogel, 1980; Thompson and Nicoll, 1986). Grasshopper mice are among the most insectivorous of rodents (McCarty, 1975), although they can be successfully maintained on more typical rodent diets.

Material and methods

Digestion Trials

Five house shrews and seven Southern grasshopper mice were individually housed in plexiglass cages, 27.5 cm H X 22.5 cm W X 34.62 cm L, equipped with water bottles. A plastic 250 ml bottle, with cap removed, was placed horizontally in each cage and used by the animals for shelter. Each shelter bottle was lined with one paper towel which was changed daily. Two sections of plastic pipe, 5 cm in diameter and 8.8 cm long, were also provided as shelter in each cage. Mice were provided with a ceramic bowl, 7.5 cm in diameter and 5 cm high, which was filled with a mixture of 90% coarse sand and 10% 'chinchilla dust' (Blue Cloud) for 30 minutes each day. This helped the mice maintain normal pelage characteristics although hair still appeared somewhat matted and

Three species of mammals were used in these experiments: the house or musk shrew (Suncus murinus, Insectivora: Soricidae), the southern grasshopper mouse

Rodentia: Muridae)

greasy during this study.

Eight hedgehog tenrecs were individually housed in stainless steel cages, 90 cm X 50 cm H X 60 cm L. Each cage was provided with a ceramic water bowl and a wooden 'nest' box containing pieces of paper towel which were changed daily.

Acclimation periods for the shrews, mice and tenrecs consisted of a two-week period during which crickets (Orthoptera: Acheta domestica) were gradually introduced into the diet while the previous diet ingredients were reduced. Shrews had been previously fed ad libitum on a mixture of pelleted mink feed (Ross Wells, Madison, WI) and extruded cat food (Ralston Purina, St. Louis, MO) (90:10). Mice had been previously fed ad libitum on a mixture of pelleted rodent feed, corn, millet and sunflower seeds and had been offered 2 crickets per day. Tenrecs had been previously maintained on a diet comprised of equal parts of a canned zoo carnivore preparation (Hill's Feline Diet, Hill's, Topeka, KS) and horsemeat as well as 2-3 crickets per day. They had been fed at the rate of 1 to 1.5% of their body weight per day on a dry matter basis (DMB). All animals were on a 12 hour:12 hour light:dark cycle prior to and during the digestion trials.

The crickets used in the digestion trials were obtained from a commercial supplier (Jiminy Cricket, Richmond, VA). Before use, the crickets were fed an 8% calcium diet for two days and given access to clean water (see chapter 2). Crickets to be used for the feeding of mice and shrews were immobilized by chilling at 5^{o} C for at least 2 hours prior to feeding. As live crickets could escape from the

tenrec cages, these crickets were first immobilized by chilling and then killed. The crickets were killed by crushing the head and partially dislocating the head from the thorax, using a putty knife, before being offered to the tenrecs. For approximately one week before digestion trials were started, the mice, shrews and tenrecs were allowed ad libitum consumption of crickets, and voluntary consumption of crickets was determined for each animal. No other food was offered during this period. During the digestion trials the animals were offered only crickets at a level of about 95% of previously defined voluntary intake. The daily amounts offered (DMB) were about 10% of body weight for shrews, 1.4% for tenrecs and 8% for mice. Given that adult crickets contain 29.5% dry matter (see Chapter 2), these amounts are equivalent to fresh weights of offered crickets of approximately 35%, 5% and 28% of body weight in shrews, tenrecs and mice, respectively. During the trials food intake was measured daily to the nearest 0.01 g using an electronic balance. All orts (uneaten food) and feces were collected 24 hours after feeding, prior to the next feeding. There were two consecutive digestion trials per animal. Trials for the shrews were from 5 to 7 days, those for tenrecs were 7 days and those for mice were from 4 to 7 days. All animals were weighed to the nearest 0.01 gram at the beginning, middle and end of each of the two trials.

Ambient temperature of the rooms in which the mammals were housed was measured and recorded daily. Means and standard errors (SE) of ambient temperatures for the tenrecs during the first and second 7-day collection periods were 27.2^{o} C ± 0.37 (n=7) and 27.6^{o} C tenrec cages, these crickets were first

then killed.

± 0.14 (n=7) respectively. Ambient temperatures for the mice and shrews were 28.5° C ± 0.13 (n=7) and 28.5° C ± 0.23 (n=7) for the first and second collection periods, respectively. Since there was reason to believe the tenrecs might become torpid during the period of study, cloacal temperatures of the tenrecs were taken with digital anal thermometers at the beginning and end of the trials.

Eight 50-gram samples of crickets were randomly sampled over a three-week period from the supply being used for the digestion trials. The crickets were killed by freezing (-10° C), dried to constant weight (ca. 3 days) in a forced-air, convection oven at 100° C, ground to a powder in an electric mincer (Varco Inc., Belleville, NJ) and frozen for subsequent analyses. Orts were pooled within each digestion trial, as were feces. After drying to constant weight at 100° C, orts and feces were sealed in plastic bags, frozen at -10° C and subsequently ground.

Crickets, orts and feces were analyzed for dry matter (DM), nitrogen (N), gross energy (GE) and acid detergent fiber (ADF). All subsamples were weighed with an electronic balance to the nearest 0.001 gram. To correct for moisture gained during grinding, dry matter of the ground material was determined by oven-drying at 100° C on 0.5 to 0.7 gram subsamples. Nitrogen was measured by the Semi-automated Method, # 7.025 (Williams, 1984) on 0.1 gram subsamples. Gross energy was determined on 0.15 to 0.25 g subsamples by complete combustion in a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). After combustion, the residue was titrated with 0.0725 N sodium carbonate to account for the heat of

combustion of nitric acid. The amount of chitin in a sample was estimated as the acid detergent residue (ADF) as suggested by Stelmock et al. (1985). The ADF method has been shown to recover chitin quantitatively, at least in shellfish meals (Stelmock et al., 1985; Watkins et al., 1982; White, 1981). The ADF fraction will be hereafter referred to as 'chitin'. ADF analyses were performed on 0.5 to 0.7 g subsamples according to Goering and Van Soest (1975) and Robertson (1978). The ADF fraction was corrected for ash content by igniting the ADF residue in a muffle furnace at 450^{o} C overnight. All analyses were performed in duplicate.

Two-way nested analysis of variance, general linear model, (species x animal within species) of the digestion and animal weight data was performed using the PC SAS statistical program (SAS Institute Inc., Cary, NC) and a 183 Zenith personal computer. Means were compared using Bonferroni's Multiple Comparison tests. The composition of intake and of orts were compared among mammals using Bonferroni T tests (SAS Institute, Cary, Inc.).

Time of First Appearance Measurements

Five shrews and four grasshopper mice were housed individually and fed crickets which had been 'dusted' with red and green ultraviolet reflective pigments (Radiant Color, Oakland, CA). Due to logistical constraints it was not possible to conduct similar trials with tenrecs. Each shrew and mouse was fed one red and one green cricket which had been killed by freezing. After the dyed crickets were consumed (within five to ten minutes), the mice and shrews were fed the remaining portion of their cricket rations, at

the rates described above. The time at which the 'dusted' crickets were consumed was noted to the nearest minute. Two observers watched the animals continuously until all animals had voided stool containing red and green pigment. The pigment was detected with a hand-held UV light.

The time of first appearance of the dye in the feces of the grasshopper mice and shrews was compared by a T test.

Results

Cricket Composition

The nutrient composition of whole crickets is presented in Table 33. Mean values (dry matter basis) for nitrogen, 'chitin' and gross energy were 11.51% ±0.112, 8.86% ±0.235, and 5.65 kcal/g ±0.052, respectively. Cricket legs, heads and bodies were also analyzed separately. Due to the small sample weights of legs and

Table 33. Composition[1] of whole crickets and cricket parts.

		N(%)[2]		'Chitin'(%)[3]		GE (kcal/g)[4]	
		X	±SE	X	±SE	X	±SE
Crickets	(n=8)	11.51	±.112	8.86	±.235	5.65	±.052
Legs	(n=2)	12.61	±.027	15.25	±.247	5.39	±.082
Heads	(n=2)	11.87	±.061	16.28	±.093	5.06	±.195
Bodies	(n=2)	10.73	±.009	7.99	±.082	5.41	±.100

[1]Dry matter basis; mean ±SE.
[2]N = Nitrogen
[3]'Chitin' = Acid detergent fiber
[4]GE kcal/g = Gross energy (kilocalories/gram)

heads, it was not feasible to conduct many analyses, so that statistical analyses of these data were not warranted. However, it appears that cricket heads and legs may be higher in 'chitin' concentration than are cricket bodies.

Selectivity

The animals did not always consume all parts of the crickets. Shrews and tenrecs usually consumed the crickets in entirety. The small amount of orts in shrew cages consisted of wings and legs. Some tenrecs left substantial amounts of uneaten crickets, but others consumed nearly all offered crickets and left orts consisting of legs, wings and rarely, heads. In both species the detachment of legs and wings during feeding on crickets seemed to occur by accident rather than by directed activity. By contrast, grasshopper mice rarely consumed entire crickets but rather appeared to deliberately discard heads and legs before consuming the softer body (thorax and abdomen). Shrews consistently consumed almost all offered food; orts comprised only 2.04% of offered food (dry matter basis). Tenrec and mice orts comprised 23.2% and 20.8% of offered food (dry matter basis), respectively.

The composition of orts is compared to that of ingested food in Table 34. Comparisons between tenrec and mouse orts indicate that mouse orts were significantly higher in 'chitin' than tenrec orts ($P < 0.05$). Statistical comparisons with shrews were not possible because the amount of orts per shrew was so small that all shrew orts were pooled in order to obtain a single sample that was large enough for analysis. Three out of the eight tenrecs produced no

Table 34. Composition[1] of orts and intake: differential selectivity by speices (mean ±SE)[4].

	Shrew		Tenrec		Mouse	
	X	±SE	X	±SE	X	±SE
Orts	(n=1)[2]		(n=10)		(n=14)	
Nitrogen (%)	13.05	--	11.32^{A}	±.179	12.63^{B}	±.209
'Chitin'(%)	15.29	--	11.14^{A}	±.567	19.01^{B}	±.586
GE (kcal/g)[3]	5.09	--	5.08^{A}	±.068	4.94^{A}	±.081
Intake	(n=10)		(n=16)		(n=14)	
Nitrogen (%)	11.48^{AB}	±.018	11.82^{A}	±.156	11.21^{B}	±.061
'Chitin'(%)	8.72^{A}	±.073	8.55^{A}	±.181	6.27^{B}	±.187
GE (kcal/g)	5.66^{A}	±.006	5.84^{B}	±.050	5.83^{B}	±.021

1 Dry matter basis
(n) = Number of samples analyzed
GE (kcal/g) = Gross energy (kilocalories/gram)
Means in a row with the same superscript are not significantly different at the 0.05 level (Bonferroni T test).

orts in either of the two collection periods. This is reflected in an n of 10 (5 animals, two digestion trials per animal) for tenrecs in Table 35. The 'chitin' in shrew orts, 15.29%, appeared similar to the 'chitin' content of legs and heads analyzed separately (Table 1).

The nutrient composition of intake is presented in Table 34. The 'chitin' content of ingested food was significantly lower for mice, 6.27% $\pm$0.187 ($P < 0.05$) as compared to that for shrews and tenrecs, 8.72% $\pm$0.073 and 8.55% $\pm$0.181, respectively. The gross energy and nitrogen content of ingested food was somewhat lower for shrews than for tenrecs or mice, but the nitrogen content did not differ between the shrews and the other two species.

Intake and Digestibility

The statistical model used for this experiment allowed for the separate testing of effects due to species and due to animals within species. By accounting for animal within species as a source of variation, the effect of species was not confounded by variation due to animal. As indicated in Table 35, there were highly significant animal and species effects, explained by the model, for all variables except 'chitin' % digestibility (animal).

Mean values for the amounts of dry matter, nitrogen, 'chitin' and energy consumed and digested are presented by species in Table 36. There were significant species differences with respect to these variables.

Body weights of shrews and mice did not change appreciably during the digestibility trials, however there were net gains in the

Table 35. Analysis of variance for species and animal within species.

Variable[1]	Species (df = 2)		Animal within species (df = 17)	
	F Value	Prob.	F Value	Prob.
Body weight (g)	25,520.68	0.0001	208.52	0.0001
DM intake (g/day)	118.37	0.0001	4.87	0.0005
DM intake (% BW)	508.98	0.0001	4.11	0.0016
DM digestibility (%)	48.55	0.0001	2.26	0.0414
N digestibility (%)	28.46	0.0001	2.48	0.0274
'Chitin'digestibility (%)	32.96	0.0001	1.83	0.0980
Energy digestibility (%)	96.55	0.0001	2.26	0.0414
DE/MBS (kcal/kg$^{.75}$)	412.71	0.0001	2.86	0.0134

[1] g = gram
DM = dry matter
BW = body weight
N = nitrogen
DE = Digestible energy
MBS = metabolic body size (kilograms$^{.75}$)

tenrecs. The mean changes ($\pm$SE) in weight (g) from the beginning of the trials to the end, were -0.61 g ($\pm$0.92), -0.46 g ($\pm$0.51), and +5.46 g ($\pm$1.08) for shrews, mice and tenrecs, respectively. The mean cloacal temperature for all tenrecs except one, was 31.31^{o} C $\pm$0.175 SE. One animal which began to show signs of torpor and lower feed intake at the beginning of the second collection period had a final cloacal temperature of 28.4^{o} C.

Dry matter intake and apparent digestibilities of dry matter, nitrogen, 'chitin' and energy are presented in Table 36. As a percentage of body weight, dry matter intake differed significantly among the three species. Mice digested 71.04% $\pm$0.47 of dietary dry

Table 36. Comparisons of mean values[1] for intake and digestibility of crickets by three mammal speices.

Variable[2]	Shrew (n = 5)		Tenrec (n = 8)		Mouse (n = 7)	
	Mean	±SE	Mean	±SE	Mean	±SE
Body Weight (g)	35.00^{A}	2.15	162.61^{B}	6.76	28.02^{C}	1.21
DM intake (g/day)	3.68^{A}	0.21	1.75^{B}	0.15	2.19^{C}	0.07
DM intake as % BW (%)	10.58^{A}	0.29	1.08^{B}	0.08	8.06^{C}	0.52
DM digestibility (%)	62.47^{A}	0.96	63.82^{A}	0.94	71.04^{B}	0.47
N digestibility (%)	61.55^{A}	1.96	58.23^{A}	2.31	72.77^{B}	1.03
'Chitin' digestibility (%)	1.79^{A}	1.92	19.77^{B}	1.93	12.00^{C}	1.34
Energy digestibility (%)	68.47^{A}	0.95	72.17^{B}	0.52	78.62^{C}	0.51
DE intake rel. to MBS ($kcal/kg^{.75}$)	176.99^{A}	6.48	28.42^{B}	1.84	149.19^{C}	7.32

1 Means in a row with the same superscript are not significantly different at the 0.05 level (Bonferroni T test).

2 DM = dry matter
N = nitrogen
DE = digestible energy
BW = body weight
MBS = metabolic body size ($kg^{.75}$)

matter which was significantly higher than that of shrews and tenrecs. The percentage of dry matter digested by the shrews (62.47% ±0.96) and tenrecs (63.82% ±0.94) did not differ. The percentage of nitrogen digested by grasshopper mice was significantly higher (72.77% ±1.03, $P < 0.05$) than the percentage of nitrogen digested by the shrews (61.55% ±1.96) or the tenrecs (58.23 ±2.31) which did not differ from one another (Table 36). Although the grasshopper mice digested a larger percentage of 'chitin' (12.0 ±1.34) than did the shrews (1.79 ±1.92), 'chitin' digestibility was highest in the tenrecs (19.77% ±1.93, $P < 0.05$; Table 36).

Mice digested significantly more energy (78.62% ±0.51) than did either the shrews (68.47% ±0.95) or the tenrecs 72.16% ±0.52, $P < 0.05$). Tenrecs digested a higher percentage of dietary energy as compared to the shrews ($P < 0.05$). Digestible energy intake relative to metabolic body size is compared among species in Table 36. Shrews had a higher DE intake relative to metabolic body size (176.99 ±6.48 kcal), than mice (149.19 ±7.32) and tenrecs (28.42 ±1.84, $P < 0.05$). Mice and tenrec values were also significantly different from each other ($P < 0.05$).

Time of First Appearance

Markers administered to shrews appeared in the feces more rapidly than did those administered to grasshopper mice (1.21 hours ±0.264 vs. 3.89 hours ±0.198, $P < 0.05$).

Discussion

Selectivity in Feeding

I have suggested that some parts of insects may be more digestible than other parts (see Chapter 1) so that selective ingestion of the higher quality insect parts may lead to an increase in diet digestibility. Both chemical analysis and visual inspection of orts indicated that southern grasshopper mice ate crickets in a more selective manner than did shrews and tenrecs. The grasshopper mice were observed to reject hard, sclerotized body parts (legs, heads) which appear to be more than twice as high in 'chitin' content than were the thorax and abdomen of the crickets. As a consequence, the food ingested by the mice was significantly lower in 'chitin' than that consumed by the shrews and tenrecs.

It might be argued that the animals in this study exhibited atypical feeding behavior as they were fed chilled or killed crickets in a constrained, artificial environment. However, grasshopper mice are known to be adept at discrimination among the body parts of prey. Horner et al., (1964) observed that both wild and captive southern grasshopper mice tended to leave uneaten the appendages and other heavily sclerotized body parts of insects and other arthropods. The method of predation varies in response to the defenses of the insect prey (Langley, 1981; Whitman et al., 1986). Southern grasshopper mice direct the first bites on or near the head when offered either stink beetles (which have anal stink glands) or crickets (which have no chemical defenses). By contrast, scorpions are initially bitten on the tail, severing the stinger (Langley,

1981). In the present study grasshopper mice were observed to initially sever the head from the thorax and then eat the thorax and abdomen, usually discarding the hind legs as mastication proceeded. This pattern was consistent with prior reports.

The shrews and tenrecs did not appear to discriminate; that is, they did not deliberately reject specific body parts of crickets. Both species appeared to consume the crickets head-first, but did not use their front legs or paws to orient the cricket as the mice typically did. Legs and wings would sometimes become detached as the crickets were ingested. Balakrishnan and Alexander (1979) fed cockroaches to captive Suncus murinus and reported that the shrews discarded the wings, tibia and tarsals. They did not report the species of cockroach fed to the shrews and did not discuss the mechanism by which the shrews rejected these body parts. Pernetta (1977) observed that the shrews Crocidura suaveolens, Sorex araneus and Sorex minutus masticate and ingest the entire body of most arthropod prey with minimal loss of appendages. However, he also observed that, when presented with beetles and large insects, the shrews are able to orally manipulate the prey which resulted in the rejection of heads, wings and other hard body parts. He observed that the shrews rarely use their legs or feet to orient their prey.

Although feeding behavior was not evaluated quantitatively in the present study, these observations and descriptions of feeding behaviors reported elsewhere indicate that insects may be eaten in different ways by different animals.

Digestibility of Insects

Relatively few studies have been conducted comparing the digestibility of insects among different mammalian species. Available data on the dry matter and energy digestibility of insects are summarized in Table 37, and include results for 4 prey types and 10 predator species. Comparison of the results of different studies are complicated by differences in methodology and terminology, as well as the small sample sizes studied.

The only prior study to compare energy digestibility of an insect fed to various predator species was that of Buckner (1964) who fed sawfly nymphs to four shrew species. While there appeared to be some differences among the shrew species, sample sizes were small (n=1 for _Microsorex_ and n=2 for _Blarina_) and no statistical comparisons were made by the author. Both Buckner (1964) and Balakrishnan and Alexander (1979) analyzed feces that had been preserved in 70% ethanol, which would lead to extraction of soluble carbohydrates and some lipids. Thus, fecal energy content may have been underestimated and energy digestibility overestimated in these studies. In both studies the energy content of insects and feces were calculated from chemical analyses of protein, fat and glycogen, rather than by direct measurement using a bomb calorimeter (see Table 39, footnote 3). The omission of chitin and use of energy conversion factors determined for other types of food further emphasize the imprecise nature of these calculations.

The present study demonstrated highly significant interspecific differences in the digestibility of all nutrients measured when

Table 37. Dry matter and energy digestibility of insects by insectivorous mammals

Prey species[1]	Mammal species[2]	Dry matter digestibility (%)	Energy digestibility (%)	Reference
Sawfly nymph				
	Microsorex hoyi (n=1)	--	83	Buckner (1964)[3]
	Sorex cinereus (n=5)	--	93	"
	Sorex articus (n=3)	--	88	"
	Blarina brevicaudus (n=2)	--	78	"
Mealworm larvae				
	Sorex araneus (n=2)	91.3	--	Hawkins and Jewell (1962)[4]
	Croccidura suaveolens cassiteridum (n=10)	85.8	89.4	Pernetta (1976)
	Antechinus stuartii (n=10)	82.7	87.3	Nagy etal., (1978)
Cockroach				
	Suncus murinus (n=16)	76.7	79.9	Balakrishnan and Alexander (1979)[3,5]

Table 37 (cont'd.).

Prey species[1]	Mammal species[2]	Dry matter digestibility (%)	Energy digestibility (%)	Reference
Cricket				
	Onychomys torridus longicaudus (n=7)	71.0	78.6	This study
	Suncus murinus (n=5)	62.5	68.5	"
	Echinops telfairi (n=8)	63.8	72.2	"

[1]Scientific names, Feed:
Cockroach = not given (Insecta: Dictyoptera)
Cricket = *Acheta domestica* (Insecta: Orthoptera)
Maggot = *Calliphora erythrocephala* larvae (Insecta: Diptera)
Mealworm = *Tenebrio molitor* larvae (Insecta: Coleoptera)
Sawfly nymph = *Pristiphora erichsonii* (Insecta: Hymenoptera)

[2]Common names, Mammals:
Antechinus stuartii = Brown antechinus ("marsupial mouse")
Blarina brevicaudus = Short-tailed shrew
Crocidura suaveolens cassiteridum = Scilly shrew
Echinops telfairi = Pygmy hedgehog tenrec
Microsorex hoyi = American pygmy shrew
Onychomys torridus longicaudus = Southern grasshopper mouse
Sorex araneus = Common shrew
Sorex arcticus = Arctic shrew
Sorex cinereus = Masked shrew
Suncus murinus = Musk shrew

[3]Energy values for feed and feces were calculated. Protein, fat and glycogen were determined by chemical assay and the values 4.3, 9.3 and 4.1 kcal/g respectively, were used to estimate the energy in feed and feces.

[4]Maggots offered with mealworm larvae.

[5]Cockroach legs and wings removed before feeding.

crickets were fed to three small mammal species (Table 35). The percent of dry matter, nitrogen and energy digested by the mice was greater than that digested by shrews and tenrecs (Table 36). However, the mice selected a diet lower in 'chitin' (Table 34) and presumably lower in sclerotized chitin-protein complexes. Thus, the higher digestibility may reflect the characteristics of the diets selected as much as differences in ability to digest dietary nutrients. To accurately compare differences in diet digestibility among different species, the ingested diet should be identical. If the mice had been forced to consume entire crickets (i.e., if their diets had contained more sclerotized protein-chitin complex) the observed differences in digestibilities may have been less pronounced.

Comparison of the shrews to the tenrecs is more meaningful as there was little evidence of selectivity and the ingested diets did not differ as markedly in composition (Table 34). Whereas the 'chitin' fraction proved to be indigestible for the musk shrew, the hedgehog tenrec digested about 20% of it. Nonetheless, the contribution of 'chitin' nitrogen to the total nitrogen budget of tenrecs is small. Tenrecs consumed 0.15 grams 'chitin', of which they digested 0.03 grams per day. The nitrogen provided by 'chitin' digestion was about 2 mg per day (assuming that chitin contains approximately 7% nitrogen) as compared to 120 mg total nitrogen digested per day by tenrecs.

The energy contribution of digested 'chitin' is also small in tenrecs, about 0.12 kcal per day (assuming that digested 'chitin'

provides 4 kcal per gram) or 1.7% of daily digestible energy (7.3 kcal per day). Thus 'chitin' digestion can account for only about one third of the observed difference in energy digestibility between the musk shrew and hedgehog tenrec. The remaining difference must be due to differences in digestibility of other constituents such as lipids, which comprise about 15% of cricket dry matter (see Chapter 1).

The mean values for dry matter (62.5 to 71.0%) and energy (68.5 to 78.6%) digestibility observed in the present study are lower than most previously reported values for mammals consuming insects (Table 37). Although some of the discrepancies may stem from methodological problems with some of the earlier studies (see above), there are undoubtedly real differences in nutrient digestibilities of various insects. For example, a careful study by Nagy et al. (1978) of a small insectivorous marsupial (Antechinus stuartii) consuming mealworm larvae indicated that dry matter and energy digestibilities were about 83% and 87%, respectively. Other studies have also suggested relatively high digestion coefficients for mealworm larvae (Table 37).

Mealworm larvae and crickets are considerably different in composition. Tenebrio larvae may contain as much as 30-40 % ether extract (fat) which is about two and a half times the fat contained in Acheta (see Chapter 1). Licht and Jones (1967) reported higher gross assimilation efficiency (dry matter digestibility) when insectivorous lizards (Anolis carolinensis) were fed mealworm larvae (88.9%) than when fed crickets (69.5%). Hanski (1984) also found provides 4 kcal per gram) or 1.7% of daily digestible energy (7.3 kcal per day). Thus 'chitin' digestion can account for only about one third of the observed difference in energy digestibility between the musk shrew and hedgehog feces. The remaining difference must be due to differences in digestibility of other constituents such as that when six species of shrews were fed either sawfly cocoons, ant pupae or beetles, food utilization efficiency (estimated from carbon balance) differed depending on the prey. Carbon utilization ranged from 47% to 62% when beetles were fed, compared to ranges of 71% to 83% for ant pupae and 76% to 85% for sawfly cocoons (Hanski, 1984). While nutrient composition values for these prey were not provided, it is likely that differences in composition, including 'chitin' content and extent of sclerotization, may have influenced digestibility measures.

There is some evidence that adult cockroaches may also be more digestible than crickets. Balakrishnan and Alexander (1979) fed musk shrews an ad libitum diet of cockroaches with the legs and wings removed. Dry matter digestibility was calculated to be 76.7%, considerably higher than the mean value of 62.5% found in the present study when musk shrews were fed crickets. Unfortunately, the authors did not report whether nutrients in orts were accounted for, or whether the legs and wings had been removed from the cockroaches that were chemically analyzed. As noted above, the methods of fecal preservation and energy determination are also suspect. Thus, this research needs to be repeated with more appropriate methodology before conclusions can be drawn.

Gastrointestinal Tract Structure and Function

Differences in digestibility of nutrients by insectivorous mammals may also be related to variation in gastrointestinal tract (GIT) structure and function. The GIT's of insectivorous mammals are typically short and simple (Clemens, 1980). Measurements of the

GIT's from a shrew, a tenrec and a Northern grasshopper mouse (Onychomys leucogaster) are presented in Table 38. Both the shrew and tenrec GIT lacked any clear demarcation between small and large intestines. No ceca were present in Suncus or Echinops, which is typical of many species of Insectivora (Schieck and Millar, 1985). The GIT of Onychomys leucogaster is somewhat larger than that of Onychomys torridus longicaudus, reflecting a larger body size (43.3 g vs. 28.0 g, respectively), but is otherwise similar in structure as described by Horner et al., (1964).

Grossly, the two principal differences between the guts of the shrew and tenrec as compared to that of the mouse is the presence, in the mouse, of a cecum and of a reduced glandular area (fundus) of the stomach. Horner et al., (1964) describe this gastric feature as a glandular pocket with a small (1-2 mm) aperture protected by a mucosal fold, the majority of the stomach (antrum and corpus) being non-glandular and lined with stratified squamous epithelium. Onychomys torridus possesses cardiac and fundic glands but lacks pyloric glands. The unusual glandular pouch is known to occur in a few other Cricetid rodents but its functional significance is debated (Carlton, 1973; Horner et al., 1964). It is believed that gastric digestive processes take place in the corpus and antrum, with some mechanical degradation occurring in the antrum, aided by peristalsis (Carlton, 1973; Horner et al., 1964).

The short simple guts of insectivores promote rapid passage of digesta. The shrews in this study had more rapid rates of digesta passage than did the grasshopper mice. This factor, coupled with

Table 38. Measurements[1,2] of the gastrointestinal tracts of Suncus murinus, Echinops telfairi and Onychomys leucogaster

Species (Body weight)	Stomach (mm)	Small Intestine (mm)	Large Intestine (mm)	Cecum (mm)
Shrew (34.8 g)	20	178[3]	---	none
Tenrec (210.6 g)	29	410[3]	---	none
Northern Grasshopper Mouse (43.3 g)	38 (14 Cardiac) (9 Fundic) (15 Pyloric)	270	92	22

[1]Stomach (empty) measurements of shrew and tenrec made from anterior apex to pylorus. Mouse stomach measurements taken as straight lines: Cardiac region - from anterior apex along greater curvature to fundus; Fundic region - along greater curvature; Pyloric region - from fundus along greater curvature to pyloris.
[2]M.E. Allen, unpublished data.
[3]Shrew and tenrec had no discernible junction between ileum and colon.

the presence of a cecum and an unusual gastric pouch (both of which might promote chitin degradation) may help explain the higher digestibility of 'chitin' by the mouse as compared to the shrew.

Chitinases have been detected in the gastric and intestinal mucosa of a variety of insectivores and other vertebrates (Jeuniaux, 1961; Cornelius et al., 1975; Cornelius et al., 1976; Danulat and Kausch, 1984). Invertebrates also produce chitinases to aid in the breakdown of ingested chitin (Febvay et al., 1984; Martin et al., 1981). Given that significant amounts of 'chitin' were digested by both grasshopper mice and tenrecs, it is possible that the GIT of

these species contain chitinases, whether of microbial or endogenous origin. Unfortunately, the presence of chitinases has not been demonstrated in these species. Among rodents, Norway rats, golden hamsters and house mice have been shown to secrete chitinases, while guinea pigs do not (Frankignoul and Jeuniaux, 1965). The relative importance of endogenous versus microbial chitinases in the quantitative digestion of chitin is not known.

The importance of mechanical diminution of insect prey to the digestibility of insects also needs to be investigated. Based on visual scat analysis it appears that a substantial portion of the ingested invertebrate escapes mechanical or chemical degradation (Dickman and Huang, 1988; see Chapter 1). Given the similarity of chitin to cellulose, one might expect that chitin digestion would be improved by thorough mastication. Although southern grasshopper mice appear to masticate insects quite thoroughly, stomach contents of recently killed mice included numerous sharp cuticular fragments (Horner et al., 1964).

The teeth of Insectivora are specialized with cusps arranged to allow the rapid piercing of invertebrate cuticles (Pernetta, 1977). Rapid chewing helps to subdue prey more than to grind it, however. Dotsch (1986) reported that the masticatory cycle time of musk shrews was 5.5 orbits per second, which is relatively high compared to other mammals but is low for a shrew (Dotsch, 1982). While similar studies have been conducted with the tenrec, _Tenrec ecaudatus_, (Oron and Crompton 1985) comparisons are difficult because mastication times appear to depend on the substrate being

chewed.

Some species of shrews regurgitate and remasticate food (Geraets, 1978). While this practice has apparently not been observed in Suncus, Echinops or Onychomys, some insectivorous species may rely on remastication to further break down a substance that, like plant fiber, is resistant to degradation.

Dry Matter and Energy Intake

Dry matter intake is governed by many physical and physiological factors such as the physical form, energy density and palatability of food, the size and structure of the gastrointestinal tract (GIT), the physiological state of the animal and the species. In this study dry matter intake as a percent of body weight was shown to differ greatly among the three species. With adult domestic animals on typical maintenance diets, predictions of intake can be made in relation to body weight. Large herbivores like the horse (350-450 kg), typically consume the equivalent of 1.5 to 2% (DMB) of their body weight per day; small carnivores like the cat (3 kg) consume about 4-5% (DMB) of body weight per day. In general, dry matter intake relative to body size decreases as body size increases. Thus the relatively high measured dry matter intakes of musk shrews (10.6%) and grasshopper mice (8.1%) are consistent with trends in larger mammals; conversely, the dry matter intakes of hedgehog tenrecs are surprisingly low (1.1% of body weight).

However, it is known that shrews with elevated metabolic rates typically consume relatively large amounts of food per day, sometimes in excess of body weight when intake is expressed on a wet

weight basis (Buckner, 1964; Dryden et al, 1974; Hawkins and Jewell, 1962). A review of the published literature indicates that the reported dry matter intakes of shrews and other small insectivorous mammals are extremely variable and include both very high values (> 30% BW) and much lower ones (Figure 23a; Table 39). The findings of the present study tend to be in the low end of the range, but this study did not include small species of shrews, the smallest of which are the smallest living mammals. It appears that the smallest shrews have disproportionately high dry matter intakes (Figure 23a).

Most small insectivores consume insects and invertebrates that are relatively high in energy content (Chapter 1). Thus, high dry matter intakes imply high energy intakes. Many tiny shrews are known to have basal metabolic rates higher than the rates predicted from the Kleiber equation, BMR = 70 kcal/kg $^{0.75}$ (Dryden et al., 1974; Barrett, 1969; Hawkins and Jewell, 1962; Vogel, 1980). The reported gross energy intakes of some the smaller shrews are clearly exceptional, as many of the reported values are greater than 400 kcal/kg $^{0.75}$ (Figure 23b). Given the relatively high energy digestibilities of many of the insects used in these studies (Table 37), the calculated digestible energy intakes are also very high for shrews less than 20 g (291-563 kcal DE/kg $^{0.75}$; Table 39). As noted in the discussion of energy digestibility (page 176) some of these data may be influenced by methodological problems. For example, it is not clear whether the two-fold disparity in digestible energy intake between the present musk shrew study (177 kcal DE/kg$^{0.75}$) and that of Balakrishnan and Alexander (354 kcal

Figure 23a. The relationship between dry matter intake and body weight in small insectivorous mammals.

Each point represents a mean value for a species in an individual experiment. Dry matter intake is expressed as a percentage of body weight. Sources of the data may be found in Table 39.

Figure 23b. The relationship between gross energy intake and body weight in small insectivorous mammals.

Gross energy intake is expressed relative to metabolic body size ($kg^{0.75}$). See Table 39 for sources of data.

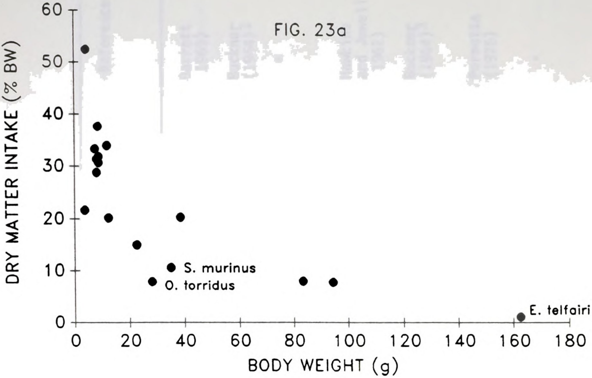


FIG. 23a
DRY MATTER INTAKE (% BW)
60
50
40
30
20
10
0
S. murinus
O. torridus
E. telfairi
0
20
40
60
80
100
120
140
160
180
BODY WEIGHT (g)

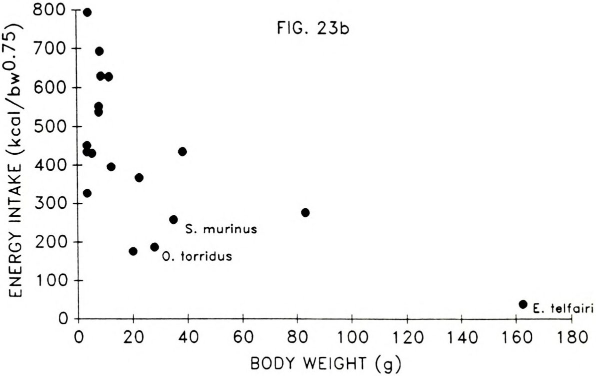


FIG. 23b
ENERGY INTAKE (kcal/bw0.75)
800
700
600
500
400
300
200
100
0
S. murinus
O. torridus
E. telfairi
0
20
40
60
80
100
120
140
160
180
BODY WEIGHT (g)

Table 39. Body weight, dry matter and energy intake by insectivorous mammals.

Species[1]	Feed[2]	Trial Duration (days)	Temp °C	BW (g)	DMI (g/day)	DMI AS % BW	GE INTK (kcal/day)	GE/MBS (kcal/kg)	DE/MBS (kcal/kg)	Reference
Cryptotis parva (n=1)	mouse	21	22	3.6	0.78	21.7	4.8	327	291	Barrett (1969)
Microsorex hoyi (n=1)	sawfly nymph	--	--	3.5	--	--	6.5	452	452	Buckner (1964)[3]
Sorex cinereus (n=5)	sawfly nymph	--	--	3.6	--	--	6.4	436	436	"
Sorex minutus (n=2)	maggot mealworm earthworm	15	16	4.2	2.2	52.4	13.1	794	--	Hawkins and Jewell (1962)
Sorex articus (n=3)	sawfly nymph	--	--	5.4	--	--	8.6	432	432	Buckner (1964)[3]
Sorex araneus (n=10)	mealworm	2	--	7.5	2.5	33.3	--	--	--	Pernetta (1976)
"	fly larvae	2	--	7.5	2.5	33.3	--	--	--	"

Table 39 (cont'd.).

Species[1]	Feed[2]	Trial Duration (days)	Temp °C	BW (g)	DMI (g/day)	DMI AS % BW	GE INTK (kcal/day)	GE/MBS (kcal/kg)	DE/MBS (kcal/kg)	Reference
Sorex araneus (n=1)	maggot mealworm earthworm mouse	7	14	8.0	2.5	31.3	14.4	538	--	Hawkins and Jewell (1962)
Sorex araneus (n=2)	maggot mealworm	11 14	15	8.5	3.2	37.1	19.4	693	--	"
Sorex araneus (n=1)	maggot mealworm earthworm	9	16	11.8	4.0	33.9	22.5	629	--	"
Crocidura suaveolens cassiteridum (n=1)	maggot mealworm earthworm	11	17	8.0	2.3	28.8	14.8	553	--	"
Croccidura suaveolens cassiteridum (n=10)	mealworm	--	--	8.8	2.8	31.9	18.1	630	563	Pernetta (1976)
"	fly larvae	2	--	8.8	2.7	30.7	--	--	--	"

Table 39 (cont'd.).

Species[1]	Feed[2]	Trial Duration (days)	Temp °C	BW (g)	DMI (g/day)	DMI AS % BW	GE INTK (kcal/day)	GE/MBS (kcal/kg)	DE/MBS (kcal/kg)	Reference
Neomys fodiens (n=2)	maggot mealworm earthworm mouse	8 28	11-14 12-19	12.4	2.5	20.4	14.7	396	364	Hawkins and Jewell (1962)
Blarina brevicaudus (n=2)	sawfly nymph	--	--	20.1	--	--	9.4	176	176	Buckner (1964)[3]
Antechinus stuartii (n=10)	mealworm	8	8	22.6	3.4	15.1	21.4	367	320	Nagy _et al_., (1978)
Onychomys torridus longicaudus (n=7)	cricket	4-5	26-28	28.0	2.2	8.1	12.8	187	150	This study
Suncus murinus (n=5)	cricket	5-7	26-28	35.0	3.7	10.6	20.9	258	177	This study
Suncus murinus (n=16)	cockroach[4]	10	--	38.5	7.8	21.1	37.9	436	354	Balakrishnan and Alexander (1979)[3]

Table 39 (cont'd.).

Species[1]	Feed[2]	Trial Duration (days)	Temp °C	BW (g)	DMI (g/day)	DMI AS % BW	GE INTK (kcal/day)	GE/MBS (kcal/kg)	DE/MBS (kcal/kg)	Reference
Talpa europaea (n=2)	maggot mealworm earthworm mouse	7 10 21	14-23	83.4	6.7	8.0	42.8	276	--	Hawkins and Jewell (1962)
Talpa europaea (n=1)	earthworm	5	--	94.5	7.4	7.8	--	--	--	Mellanby (1967)
Echinops telfairi (n=8)	cricket	7	27	162.6	1.8	1.1	10.1	39	28	This study

[1]Common names of animal species studied:
- *Antechinus stuartii* = Brown antechinus ("marsupial mouse")
- *Blarina brevicaudus* = Short-tailed shrew
- *Cryptotis parva* = Least shrew
- *Crocidura suaveolens cassiteridum* = Scilly shrew
- *Echinops telfairi* = Pygmy hedgehog tenrec
- *Microsorex hoyi* = American pygmy shrew
- *Neomys fodens* = Water shrew
- *Onychomys torridus longicaudus* = Southern grasshopper mouse
- *Sorex araneus* = Common shrew
- *Sorex arcticus* = Artic shrew
- *Sorex cinereus* = Masked shrew

Table 39 (cont'd.).

Sorex minutus = Pygmy shrew
Suncus murinus = Musk shrew
Talpa europaea = Mole

[2]Scientific names, Feed:
Cockroach = not given (Insecta: Dictyoptera)
Cricket = *Acheta domesticus* (Insecta: Orthoptera)
Earthworm = *Lumbricus* sp. (Oligochaeta: Haplotaxida)
Fly larvae = *Musca* sp. (Insecta: Diptera)
Maggot = *Calliphora erythrocephala* larvae (Insecta: Diptera)
Mealworm = *Tenebrio molitor* larvae (Insecta: Coleoptera)
Mouse = *Mus* sp., neonatal (Mammalia: Rodentia)
Sawfly nymph = *Pristiphora erichsonii* (Insecta: Hymenoptera)

[3]Energy values for feed and feces were calculated. Protein, fat and glycogen were determined by chemical assay and the values 4.3, 9.3 and 4.1 kcal/g respectively, were used to estimate the energy in feed and feces.

[4]Cockroach legs and wings removed before feeding.

Abbreviations used:

BW = Body weight
DMI = Dry matter intake
GE INTK = Gross energy intake
GE/MBS = Gross energy per metabolic body size (BW (kg) $^{.75}$)
G = Gram
KCAL/KG = Kilocalorie per kilogram body weight

DE/kg$^{0.75}$) is a result of differences in insect prey, method of energy analysis, environmental conditions, experimental technique or other factors. The DE intakes reported in the present study for musk shrews and grasshopper mice (155 kcal DE/kg $^{0.75}$) are similar to expected values for domestic carnivores (about 120-170 kcal DE/kg $^{0.75}$; NRC, 1982, 1985, 1986).

In contrast to the shrews, the pygmy hedgehog tenrec appeared to ingest very low amounts of dry matter (1.1% BW) and digestible energy (28 kcal/kg$^{0.75}$). This dry mater intake is quite different from that of other small insectivorous mammals (Figure 23a) and is, in fact, equivalent to the intake of a reptile of similar size, such as Iguana iguana (D. Baer, unpublished data). The tenrec family (Tenrecidae) is considered to be one of the most primitive of extant mammalian families (Eisenberg and Gould, 1970) and includes species with low metabolic rates, such as Echinops (Thompson and Nicoll, 1986; Nicoll and Thompson, 1987). The digestible energy intake measured in the present study is only 40% of the expected metabolic rate of laboratory and domestic animals (70 kcal/kg$^{0.75}$; Kleiber, 1975). Nicoll and Thompson (1987) report that the resting metabolic rate of the pygmy hedgehog tenrec is about 19 kcal/kg$^{0.75}$ (n=2), or 68% of the digestible energy intake measured in the present study. It is possible that the energy intake of the tenrecs in the present study were somewhat depressed since these animals undergo a seasonal torpor, but only one animal showed a reduction in activity and a drop in body temperature below normal (31° C) during the study, and seven of the eight tenrecs gained weight. Thus, the maintenance

requirement of this species for digestible energy must be less than or equal to 28 kcal/kg$^{0.75}$, which is remarkably low for a homeothermic mammal.

Conclusion

In conclusion, insectivorous animals have evolved different feeding strategies which presumably allow them to maximize the benefit derived from invertebrate prey. The strategy of the musk shrew appears to be to eat a lot of food which is passed through the gut quickly. This may be typical of animals with simple GIT structures (Sibbald, 1962; Grant, 1988). For smaller shrews food intake may be even greater and transit times more rapid (Kolstelecka-Myrcha and Myrcha, 1965). The grasshopper mouse is more selective in what it eats and may be able to process nutrients more completely. This may be due, in part, to longer ingesta retention times and to a more specialized GIT. Although highly insectivorous, the grasshopper mouse has a feeding strategy that is perhaps typical of rodents, characterized by food manipulation and selection of digestible components. Finally, the pygmy hedgehog tenrec seems more reptile-like in having a low metabolic rate and extremely low rate of voluntary feed consumption. Our image of early insectivorous mammals is that they were shrew-like, when in fact the early insectivores of the Triassic may have more closely resembled the modern-day tenrec.

The large, specialized insectivores, such as the giant anteater, tamandua, echidna and numbat have not been included in the present study. These animals focus on social insects, such as ants

and termites. Their long, sticky tongues are adept at removing insects from nests, but in the process considerable amounts of detritus, soil and non-insect materials (e.g., nest parts) may be ingested (Griffiths, 1978; Hume, 1982; Redford, 1987). Digestive function has not been studied in these species, so it is not known whether the incidental matter affects digestive performance.

Insectivory is a unique adaptation within the Mammalia. Adult invertebrates may be quite different in composition than larval forms. The array of invertebrates from which predators select cannot possibly be duplicated for captive animals. We therefore can only guess as to the specific nutrient requirements of insectivorous species. However, by systematic study of the ways in which these unique animals deal with invertebrates as food, we may gain a more complete understanding of the evolutionary significance of entomophagy and better learn to care for them in captivity.

List of References

Balakrishnan, M. and Alexander, K.M. 1979. A study on aspects of feeding and food utilization of the Indian musk shrew (Suncus murinus viridescens Blyth). Physiology and Behavior 22:423-428.

Barrett, G.W. 1969. Bioenergetics of a captive least shrew, Cryptotis parva. Journal of Mammalogy 50(3):629-630.

Benecke, W. 1905. Uber Bacillus chitinovorus einen chitin zersetzenden Spaltpilz. Botanischer Zeitung 63:227-243.

Buckner, C.H. 1964. Metabolism, food capacity, and feeding behavior in four species of shrews. Canadian Journal of Zoology 42:259-279.

Carleton, M.D. 1973. A survey of gross stomach morphology in New World Cricetinae (Rodentia, Muroidea), with comments on functional interpretations. Miscellaneous publications No. 146. Museum of Zoology, University of Michigan, Ann Arbor, MI.

Chapman, R.F. 1982. The Insects, Structure and Function. Third Edition., Harvard University Press, Cambridge, MA.

Clemens, E.T. 1980. The digestive tract: insectivore, prosimian and advanced primate. In: K. Schmidt-Nielson, L. Bolis, C.R. Taylor, eds. Comparative Physiology: Primitive Mammals, Cambridge University Press, Cambridge, U.K.

Cornelius, C., Dandrifosse, G. and Jeuniaux, C. 1975. Biosynthesis of chitinases by mammals of the order Carnivora. Biochemistry of Systematics and Ecology 3(2):121-122.

Cornelius, C., Dandrifosse, G. and Jeuniaux, C. 1976. Chitinolytic enzymes of gastric mucosa of Perodicticus potto - Purification and enzyme specificity. International Journal of Biochemistry 7(9-10):445-448.

Crompton, A.W. 1980. Biology of the earliest mammals. In: Schmidt-Nielson, Bolis, Taylor, eds. Comparative Physiology: Primitive Mammals, Cambridge University Press, Cambridge, U.K.

Danulat, E. and Kausch, H. 1984. Chitinase activity in the digestive tract of the cod, Gadus morhua (L.). Journal of Fish Biology 24:125-133.

Dickman, C.R. and C. Huang. 1988. The reliability of fecal analysis as a method for determining the diet of insectivorous mammals. Journal of Mammalogy 69(1):108-113.

Doetsch, C. 1982. Der Kauapparat der Soricidae (Mammalia, Insectivora). Funktionsmorphologische Untersuchungen zur Kaufunktion bei Spitzmausen der Gattungen Sorex L., Neomys K., and Crocidura W. Zoologische Jahrbuecher (Anatomie) 108:421-484.

Dotsch, C. 1986. Mastication in the musk shrew, Suncus murinus (Mammalia, Soricidae). Journal of Morphology 189:25-43.

Dryden, G., Gebczynski, M. and Douglas, E.L. 1974. Oxygen consumption by nursling and adult musk shrews. Acta Theriologica 19(30):453-461.

Eisenberg, J.F. and Gould, E. 1970. The Tenrecs: A study in mammalian behavior and evolution. Smithsonian Contributions to Zoology No. 27, Smithsonian Institution Press, Washington, D.C.

Eisenberg, J.F. 1981. The Mammalian Radiations. University of Chicago Press, Chicago.

Elyakova, L.A. 1972. Distribution of cellulases and chitinases in marine invertebrates. Comparative Biochemistry and Physiology 43:67-70.

Febvay, G., M. Decharme and Kermarrec, A. 1984. Digestion of chitin by the labial glands of Acromyrmex octospinosus Reich (Hymenoptera:Formicidae). Canadian Journal of Zoology 62(2):229-234.

Frankignoul, M. and Jeuniaux, C. 1965. Effect of chitin on chitinase secretion by rodents. Life Sciences 4:1669-1672.

Geraets, A. 1978. Wiederkauen und enddarmlecken bei spitzmausen (Insectivora). Saugetierkundliche Mitteilungen 26(2):127-131.

Goering, J.K. and Van Soest, P.J. 1975. Forage fiber analyses: apparatus, reagents, procedures and some applications. Agriculture Handbook 8, U.S.D.A., Washington, D.C.

Grant, J.D. 1988. Food passage time in Nyctophilus gouldi (Microchiroptera: Vespertilionidae). Journal of Mammalogy 69(3):653-655.

Griffiths, M. 1978. The Biology of the Monotremes. Academic Press, New York, NY.

Hackman, R.H. 1976. The interactions of cuticular proteins and some comments on their adaptation to function. In: H.R. Hepburn, ed. The Insect Integument, Elsevier Scientific, Amsterdam.

Hanski, I. 1984. Food consumption, assimilation and metabolic rate in six species of shrew (Sorex and Neomys). Annales Zoologici Fennici 21:157-165.

Hawkins, A.E. and Jewell, P.A. 1962. Food consumption and energy requirements of captive British shrews and the mole. Proceedings of the Zoological Society of London 138:137-155.

Horner, B.E., Taylor, J.M. and Padukula, H.A. 1964. Food habits and gastric morphology of the grasshopper mouse. Journal of Mammalogy 45(4):513-535.

Hume, I.D. 1982. Digestive Physiology and Nutrition of Marsupials. Cambridge University Press, Cambridge, U.K.

Jeuniaux, C. and Amanieu, M. 1955. Proprietes chitinolytiques du liquide exuvial du ver a soie (Bombyx mori L.). Experientia 11(5):195-196.

Jeuniaux, C. 1961. Chitinases: An addition to the list of hydrolases in the digestive tract of vertebrates. Nature 192: 135-136.

Jeuniaux, C. 1963. Chitine et Chitinolyse. Masson, Paris.

Kleiber, M. 1975. The Fire of Life. Krieger Publishing Co., Huntington, NY.

Kostelecka-Myrcha, A. and Myrcha, A. 1965. Effect of the kind of indicator on the results of investigations of the rate of passage of foodstuffs through the alimentary tract. Acta Theriologica 10(17):229-242.

Langley, W. 1981. The effect of prey defenses on the attack behavior of the southern grasshopper mouse (Onychomys torridus). Zeitschrift fur Tierpsychologie 56:115-127.

Licht, P. and R.E. Jones. 1967. Effects of exogenous prolactin on reproduction and growth in adult males of the lizard *Anolis carolinensis*. General and Comparative Endocrinology 8:228-244.

McCarty, R. 1975. *Onychomys torridus*. Mammalian Species, No. 59:1-5. American Society of Mammalogists.

Martin, M.M., Kukor, J.J., Martin, J.S., O'Toole, T.E. and Johnson, M.W. 1981. Digestive enzymes of fungus-feeding beetles. Physiological Zoology 54:137-145.

Mellanby, K. Food and activity in the mole *Talpia europaea*. Nature 215(5105):1128-1130.

National Research Council, 1982. Nutrient requirements of mink and foxes. National Research Council, National Academy Press, Washington, DC.

National Research Council, 1985. Nutrient requirements of dogs. National Research Council, National Academy Press, Washington, DC.

National Research Council, 1986. Nutrient requirements of cats. National Research Council, National Academy Press, Washington, DC.

Nagy, K.A., Seymour, R.S., Lee, A.K. and Braithwaite, R. 1978. Energy and water budgets in free-living *Antechinus stuartii (Marsupiala:Dasyuridae)*. Journal of Mammalogy 59(1):60-68.

Nicoll, M.E. and Thompson, S.D. 1987. Basal metabolic rate and energetics of reproduction in therian mammals: marsupials and placentals compared. Symposia of the Zoological Society of London 57:7-27.

Nowak, R.M. and Paradiso, J.L. (eds.). 1983. Walker's Mammals of the World, Vol. 1. The Johns Hopkins University Press, Baltimore, MD.

Oron, U. and Crompton, A.W. 1985. A cineradiographic and electromyographic study of mastication in *Tenrec ecaudatus*. Journal of Morphology 185:155-182.

Pernetta, J.C. 1976. Bioenergetics of British shrews in grassland. Acta Therilogica 21(33):481-497.

Pernetta, J.C. 1977. Anatomical and behavioral specialisations of shrews in relation to their diet. Canadian Journal of Zoology 55:1442-1453.

Redford, K.H. 1987. Ants and termites as food. In: H.H. Genoways, ed. Current Mammalogy, Vol. 1., Plenum Press, New York.

Robertson, J.B. 1978. The detergent system of fiber analyses. In: G.A. Spiller, ed. Topics in dietary fiber research. Plenum Press, New York.

Schieck, J.O. and Millar, J.S. 1985. Alimentary tract measurements as indicators of diets of small mammals. Mammalia 49:93-104.

Sibbald, I.R., Sinclair, D.G., Evans, E.V. and Smith, D.L. 1962. The rate of passage of feed through the digestive tract of the mink. Canadian Journal of Biochemistry and Physiology 40:1391-1394.

Stelmock, R.L., Husby, F.M. and Brundage, A.L. 1985. Application of Van Soest acid detergent fiber method for analysis of shellfish chitin. Journal of Dairy Science 68:1502-1506.

Thompson, S.D. and Nicoll, M.E. 1986. Basal metabolic rate and energetics of reproduction in therian mammals. Nature 321(6071):690-693.

Tracey, M.V. 1951. Cellulase and chitinase in earthworms. Nature 167:776-777.

Vogel, P. 1980. Metabolic levels and biological strategies in shrews. In: K. Schmidt-Nielsen, L. Bolis and C.R. Taylor, eds. Comparative Physiology: Primitive Mammals. Cambridge University Press, Cambridge, U.K.

Waterhouse, D.F. and McKellar, J.W. 1961. The distribution of chitinase activity in the body of the American cockroach. Journal of Insect Physiology 6:185-193.

Watkins, B.E., Adair, J. and Oldfield, J.E. 1982. Evaluation of shrimp and king crab processing byproducts as feed supplements for mink. Journal of Animal Science 55:578-584.

White, R.L. 1981. Ruminant utilization of chitin and other components of Tanner crab meal. M.S. Thesis. University of Alaska, Fairbanks, Alaska.

Whitman, D.W., Blum, M.S. and Jones, C.G. 1986. Olfactorily mediated attack suppression in the southern grasshopper mouse toward an unpalatable prey. Behavioural Processes 13:77-83.

Williams, S. 1984. Official methods of analysis. Association of Official Analytical Chemists. Arlington, VA.

CONCLUSION

The fact that many diverse species of insects appear to be poor sources of calcium is not surprising since they do not possess internal, mineralized skeletons. Insectivorous vertebrates have obviously evolved with the ability to obtain the nutrients required to support growth, maintenance and reproduction, despite the apparent nutrient imbalances of many insects. It is not known how the calcium demands of lactation, bone growth and egg production are met by insectivorous vertebrates. There may be a diversity of behaviors and specialized physiological mechanisms by which adequate amounts of calcium are obtained and utilized.

For some insectivorous zoo predators one practical solution to the calcium problem is to feed crickets that have been maintained on diets fortified with calcium. It was demonstrated that when such diets are provided to crickets for a sufficient period of time, the calcium content of the crickets improved markedly. Some growing or egg-laying geckos are apparently able to derive sufficient calcium by this method to meet physiological demands. High calcium crickets were successfully used to prevent occurence of osteodystrophic signs in growing leopard geckos. However this method of providing calcium may not be sufficient, in and of itself, for heliotropic, insectivorous lizards, such as *Phelsuma madagascariensis*. It appears that vitamin D_3 may also be a critical nutrient when incident ultraviolet light is limited. The apparent difference

between nocturnal and basking lizards in their ability to use dietary vitamin D and/or artificial ultraviolet light is an important topic for future investigation.

It appears that the young leopard gecko, after initial calcium depletion, may be especially efficient at absorbing and retaining dietary calcium, at least when supplied as calcium carbonate. If this is a general ability of insectivorous animals it would help explain how some wild, insectivorous animals can meet their calcium requirements in the face of limited dietary supplies. The calcium balance data for growing leopard geckos do not indicate that calcium requirements are particularly low, however.

It has been shown that small mammals with diverse feeding and metabolic strategies digest insect nutrients in different ways. Consideration of these differences may be critical to the appropriate interpretation of studies with wild, insectivorous animals. Digestion trials, if conducted properly, can provide important information for zoologists who attempt to estimate the energy budgets of free-ranging insectivorous animals. Gross energy determinations are only approximations of the energy available to an insectivore, and may lead to misleading conclusions about the relative value of different types of prey if differences in prey digestibility are not taken into account.

The experiments described in this dissertation are a first attempt to examine some of the nutritional consequences of insectivory. Much more research is required on the factors that influence both the levels and availability of invertebrate

nutrients, and on the adapations of insectivores to cope with the nutrient imbalances that occur among wild prey items. Nonetheless it is clear that greater attention must be paid by zoologists and zoo staff to the need to provide supplemental nutrients (especially calcium) to captive insectivorous animals when they are being fed insect diets.