APPLIED NUTRITIONAL STUDIES WITH ZOOLOGICAL REPTILES

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"Until one has loved an animal, a part of one's soul remains unawakened."
-Anatole France

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-Romans 15:13, NIV

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The first clinical case evaluated the body condition estimates of Brothers Island Tuatara (*Sphenodon guntheri*) housed at the San Diego Zoo to that of the wild. The tuataras that are cared for by the San Diego Zoo (SDZ) are larger and have a greater conditioning score than that of the tuataras sampled in the wild. Over the 9 year period of sampling the SDZ tuatara had a mean increase in mass of 125.2 %, SVL of 31.7 %, and BCE of 7.5%. The second clinical case evaluated ascorbic acid, vitamin E, vitamin A, and trace elements in serum of zoo crocodilians. For the 20 individuals from four species (*Alligator mississippiensis*; *Alligator sinensis*; *Crococdylus johnsoni*; and *Gavialis gangeticus*), serum nutrient concentrations averaged 11.06 mg Ca/dl, 91.66 μg Cu/dl, 36.88 μg Fe/dl, 2.86 mg Mg/dl, 4.03 mg P/dl, 3.97 mEq K/L, 153.88 mEq Ma/L, 41.43 mg Zn/dl, 0.50 μg vitamin A/dl, 46.70 μg vitamin E/dl, and 0.66 mg ascorbic acid/dl. The third study evaluated the nutrient composition of banana tree (*Musa sp*.) leaf, petiole, and pseudo-stem at the San Diego Zoo. In a zoo environment, different banana tree components are commonly fed to various animals. At the San Diego Zoo, banana tree (*Musa sp*.) petiole and leaves are part of the gorillas' (*Gorilla gorilla gorilla*) browse rotation, and the pseudo-stem is fed once weekly to the Galapagos tortoises (*Chelonoidis nigra*). Knowing nutritional composition of different banana tree components can improve diet formulation for captive zoo animals. For this study, the length, weight, and nutritional composition of banana tree, leaves, petiole, and pseudo-stems was analyzed. The last study compared the estimated digestibility of two commercially-available herbivorous tortoise pellets versus historic San Diego Zoo Global diets. Trial 1 consisted of a series of digestibility trials on produce-based and commercial pelleted diets, using animals in the SDZG collection. Trial 2 examined the same range of information in a more controlled fashion by simulating the herbivorous tortoise's digestive system within a test tube. The results may be used to improve husbandry at zoos and to better educate pet owners on appropriate care of their companions.

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CHAPTER I

INTRODUCTION

Captive Wildlife Nutrition: a Review

History and Overview

Nutrition is a crucial area of management for captive animals. In livestock, advances in nutrition have been an integral component with increasing production efficiency across species. In captive wildlife, the science of animal nutrition is not always held as a major antecedence (Kleiman et al., 2010). Prior to 1970, zoos primary purpose in North America was entertainment. During this time zoos were able to receive wild-caught animals as needed; survivability was not a priority. Prior to 1970, roughly 60-70% of all animals in zoos died from poor husbandry and management with ~25% dying from nutritional problems (Robbins, 1993). Lack of proper nutrient composition and supplementation resulted in poor reproduction, or abnormal fetuses (Bartlett, 1899; Dierenfeld, 1997). Zoos are still slow to catch on to the need of an on staff nutritionist.

Almost every zoo in North America has a veterinarian on staff; however, very few have a trained nutritionist. As of 2010 only 10% of all zoos in North America employ a trained nutritionist or work with a nutrition consultant (Kleiman et al., 2010). Modern

zoos purpose is conservation, species survival and public education. Zoos are an excellent asset to further captive wildlife nutrition (Robbins, 1993). Zoo institutions across the world are committed to providing diets that promote longevity, health and well-being (Kleiman et al., 2010). Wackernagel (1968) stated "We want to make it clear that, in planning diets, the physiological considerations should be a priority." Wackernagel's quote holds true today. Feeding captive wildlife is challenging, considering the obstacles that are faced. Many captive wildlife's nutrient requirements are unknown, complete natural diet consumption can be unknown, and feeding behavior based on observations of animals in their natural environment can be limited. The knowledge gained from observing diet consumption only tells of what they consume, and rarely quantities (Leopold, 1933). In the early 20th century many diets fed to animals housed in zoos where nutritionally inadequate causing poor health, little to no breeding and decreased behavior. Diets were based on natural history and observed feeding habitats of wild animals than modified to ingredients that were available (Dierenfeld, 1997). The nutrition responsibility for captive wild animals was commonly held by veterinarians and pathologists. The person who started zoo nutrition was Dr. Ellen Corsen-White in 1918, who was a pathologist by training and credited for developing the recipe for "Zoo Cake," designed to be fed with produce (Crissey, 2001). Another pathologist, Dr. Herbert Ratcliffe, developed further use of Zoo Cake in 1936 and developed new feeding strategies in 1937 (Crissey, 2001). These feeding strategies remained until 1966 when H.F. Matthysen and Wackernagel further improved the feeding strategy for Zoo Cake by incurring the use of browse and supplements (Ratcliffe, 1966; Wackernagel, 1966; Crissey, 2001). Traditionally diets fed to wild animals housed in captivity such as zoos

has been a source of controversy within the zoo community, with different approaches to diets being suggested. Prior to 1966 a few unknown scientists proposed the "retort" theory which suggests if animals are fed a nutritionally complete diet then increased health, longevity, and reproduction success will ensure (Lane-Petter, 1966; Lindburg, 1985). However, Hediger strongly voiced that a more natural diet should mimic wild free grazing nutritional fluctuations. This suggestion is based on the theory that the function of a zoo is to preserve the animals natural state of inherit behavior, reproduction, and survival (Lane-Petter, 1966). The natural approach raises the question, can a zoo successfully imitate every natural variation that an animal would be exposed to in their natural environment? Furthermore, as caretakers of captive wild animals would it be morally justified to knowingly put animals through a nutrient deficiency that would not otherwise be a health benefit to the animal? In the past and less frequently in current times, captive wild animals have had clinical or subclinical nutritional deficiency or toxicity not deliberately, but from ignorance (Lane-Petter, 1966).

Ratcliffe's work in 1936 and 1937 helped to decrease nutritional deficiency in some captive wild animals and improved overall fitness (Lane-Petter, 1966). By making the animals more nutritionally fit, Dr. Ratcliffe may have unintentionally developed a need for mental stimulation (enrichment). Enrichment was not needed before nutritionally complete diets were fed due to the animals' inability to participate caused from nutritionally inadequate diets (Lane-Petter, 1966). Ratcliffe (1966) was one of the first scientists in a zoo setting to suggest feeding diets that surpass adequate for growth and reproduction to support common parasite and other disease resistance. The idea of utilizing nutrition to aid in disease control in zoos prior to 1966 was met with uncertainty

despite published scientific evidence dating back to 1931 with tuberculosis and internal parasites (Ratcliffe, 1966).

Wackernagel (1961) published a highly popular paper at the time, suggesting diet formulas with detail to vitamins for herbivorous, omnivorous, carnivores and fish-eating animals. In 1966 the vitamin levels were increased (Wackernagel, 1966). Wackernagel (1966) described in detail diet formulations, mixing directions, manufacture, storage and distribution of the feed. Some of his suggestions are common place in modern zoos. Such as separate freezer and refrigerators for meat, fish and vegetables; use of a central commissary for daily feed pickup and dispersal; and limit human element to decrease mistakes (Wackernagel, 1966). However, the diets may have been oversimplified by feeding the same diet to multiple species. For example to feed the same diet to gorillas, capuchins, and sloths (Hediger, 1966).

Hediger (1966) believed that feeding zoological animals the same as livestock is unhealthy, meaning that he felt very strongly that the retort theory is false for a zoo. The goals are different, livestock production pushes animals to carry as much protein and fat needed for human consumption with low cost, and in the shortest time possible (Hediger, 1966). Zoos have the opposite goal for wild animals housed in captivity, preserve the animal from the domestication effects, provide them with as natural conditions as possible, and not be pushed for growth, reproduction, and performance (Hediger, 1966). Meaning the diet quantity and quality, time of feeding, method including type of intake, and social factors must be considered to make the diet as natural as possible (Hediger, 1950). Although, a completely natural diet is impossible, substitutions can be made such as whole carcass feeding to carnivores. At the current time in the United States, carcass

feeding is a controversial subject with very opinionated views for and against it. Feeding a quartered carcass can be frowned upon by the viewing public, yet the health benefits to the carnivore should outweigh the publics frown. Consuming a large chunk of carcass requires the use of different teeth causing less plaque buildup, encourages stronger muscle development around the jaw, and claws to hold and further break up the meal (Hediger, 1966). However, the public perceives watching a large cat overtake and consume its prey on television as natural, but finds observing any form of carcass feeding in person as gross negligence. Zoos can utilize carcass feeding as an educational tool for the public and post signs warning guests of the event while providing trained staff to educate the public and answer questions.

Currently nutritionists working for or with zoos are eager to exchange nutritional information. Information exchange became easier with the formation of the American Zoo and Aquarium Association (**AZA**) Nutrition Advisory Group (**NAG**) in 1994 (Crissey, 2001; NAG, 2015). The NAG holds biannual meetings that provide scientific information and research dedicated to applied applications. In 1996, The Comparative Nutrition Society (**CNS**) was founded to create a line of communication between different disciplines of laboratory and field scientists interested in comparative nutrition (Crissey, 2001; CNS, 2015). The CNS also holds biannual meetings focusing more on scientific research. The first Biannual Zoo Animal Nutrition Conference of the European Zoo Nutrition Research Group was held in 1999 (Crissey, 2001). The founding of these organizations have provided an outlet of communication not only during the meetings, but also through publications, seminars, online material, and member communication.

Approach to the Unknown: Basic Procedure for Formulating a Diet for an Animal With Unknown Nutrient Requirements

A zoo nutritionist can be presented with many difficulties. One major difficulty can be frequent. What would a nutritionist do when needing to formulate a diet for an animal with unknown nutrient requirements and has not been previously housed in a zoo? First, review the AZA, TAG and NAG for recommendations and possible Species Survival Plan (**SSP**). Also, use contacts with other zoos to verify they have never housed a similar animal. Then review natural ecology from publications of known feeding habits. Reviewing natural feeding behavior provides a basic understanding of possible feeding approaches and a guide to using a similar animal (Dierenfield, 1997). For example, using a goat as a model for some antelope species.

Depending on the species of exotic ruminants kept in captivity, the diets can be comparable to that of domestic ruminants. The science of formulating diets is the same between exotic and domestic ruminants. However, understanding the three morphological ruminant feeding types is essential in order to formulate healthy diets for captive exotic ruminants. All ruminant species can be split into three feeding types: concentrate selectors (the term "browser" is deceptive), roughage (grass) selectors, and intermediate selectors (Hoffman, 1989, Figure 1.1 and 1.2).

Concentrate selectors feed on plants and plant parts that are highly digestible and contain high nutritious plant cell contents. The highly nutritious plant cell contents include starch, protein, and fat, which is similar to that of concentrate feeds such as grain. Some example species include roe deer, white-tailed deer, mule deer, duikers, dik-diks, kudus, and giraffes (Hoffman, 1989, Figure 1 and 2). There are no domestic ruminants

that are concentrate selectors. Species are that are concentrate selectors are very limited with their ability to digest cellulose, unlike roughage selectors.

Roughage selectors are ruminants that depend on grasses and fibrous plant material. Roughage selectors are considered advanced ruminants because of the ability to rapidly ferment fiber, cellulose and cell contents. Almost all domestic ruminants, wild sheep, bison, and savannah antelope species of Africa are roughage selectors (Hoffman, 1989, Figure 1.1 and 1.2).

Lastly, the intermediate selectors or mix feeders, have adapted to one or the other extreme selector. Typically, these animals have the ability to change their feeding behavior according to season. This allows the animals to be highly variable and flexible in their ability to find nutritious food. A great example of an intermediate selector is the North American elk, red deer, and artic caribou (Hoffman, 1989, Figure 1 and 2). The only domestic ruminant that is an intermediate selector is the goat. Goats prefer to select concentrate plant material, but have a limited ability to digest cellulose (Church, 1988; Hoffmann, 1989). With a basic understanding of different ruminant feeding types, we can now discuss diets of captive exotic versus domestic ruminants.

The nutritionist first has to accept the fact that in captivity, it is impossible to provide the variety of ingredients or selection of items that an animal would have in its native environment. However, the nutritionist can provide a complete and balanced diet that can provide all the needed nutrients and mental enrichment for the animal. Being trained as a livestock nutritionist, one learns to us the NRC's: nutrient requirement of dairy, beef, small ruminant, poultry, swine, non-human primate, cats and dogs, and so on.

For most exotic animals their specific nutrient requirements are unknown. Quoting Evens and Miller (1968), “The number of species whose nutritional requirements are known with any precision is relativity few. Of the mammals only about a dozen species have been studied out of the total 5,000; the situation with birds is worse.” Sadly, this situation is still a problem today, but some advances in nutrient requirements for exotics have been made. With this in mind, a nutritionist will use the known nutrient requirements for livestock as a model and research the animal’s ecology and feeding type for balancing diets for exotics.

The easiest ruminants to balance a diet for would be the roughage selectors. These animals’ diets would be very similar to a loose fed diet of a non performing domestic ruminant. Cattle and sheep are used as the model for determining nutrient requirements. Typically, loose hay (Bermuda, or another type of grass hay, sometimes alfalfa), and a complete pellet are fed. The complete pellets of choice are based on the level of acid detergent fiber (ADF) of 16 or 26%. There are a few feed companies that supply these pellets or some zoo’s will have them custom made and are designed for exotic ruminants. These animals will typically receive free choice hay or limit fed hay with a high or low fiber pellet fed once or twice daily, depending on life or production stage (maintenance, growing, and reproduction) of the animal.

Animals that are intermediate selectors are slightly more difficult, but maintain a similar diet to that of roughage selectors. The goat is used as the model for nutrient requirements. These animals are provided a highly digestible grass hay, a 16% ADF pellet and some browse. ADF (16%) is offered over 26% ADF due to the animal’s limited ability to digest cellulose. Offered browse is typically a branch of a high growing

woody tree or shrub containing leaves, soft shoots, or fruits that are deemed safe by the nutritionist. The browse not only provides essential nutrients, but also enrichment by providing a more natural feeding behavior.

The most challenging animals to formulate diets for are concentrate selectors because no domestic animals are found in this feeding group. The closest animal is the goat and is commonly used as a model for nutrient requirements, but the nutritionist takes into account that these animals have even less ability to digest cellulose than the goat. These animals are still offered a highly digestible grass hay, but the proportional amounts are less than that of an intermediate feeder and replaced with a higher percentage of browse. Concentrate feeders may also be offered rotation of produce such as leafy greens. ADF (16%) pellet is still offered, but these animals may receive a special low starch formulation of ADF to compensate for the extra starch they are receiving in browse and produce. Concentrate feeders normally feed throughout the day and require feed to be present during active hours (Figure 1.1).

No matter the feeding type all animals' body conditions are carefully monitored to make needed diet adjustments. From trial and error some known nutrient sensitivities have been determined in some species of exotic ruminants, similar to copper sensitivity in sheep or, in the case of *Damaliscus pygargus*, copper deficiency. This requires the nutritionist to pay close attention when balancing diets for one species or mixed species exhibits.

As mentioned earlier, there are limitations to using domestic animals as nutrition models for captive wildlife. Nutritionally caused disease still occurs today in captive

wildlife. Unfortunately, some of these health disorders are discovered during an annual exam or necropsy. The result would be a rapid diet change, documentation, and possible publication to prevent further health disorders in other collections. These health disorders could arise from the lack of information on nutrient requirements for wildlife, composition of dietary ingredients of the free ranging wild animal's intake, and certainly the imperfect fit of a domestic animal model.

Dr. Schlegel summarized the complication of utilizing domestic animals as a model in his title "A rhinoceros is not always like a horse…" from a 2013 symposia (Schlegel, 2013). The horse NRC (2007) estimation of milk replacer works for hand raising black rhinos (*Diceros bicornis*); however, it overestimates milk intake. Overestimating milk intake leads to an over estimation of daily digestible energy requirement which leads to over feeding of ~30,300-37,000 kcal/ME/d (Schlegel, 2013). This information came from years of hand raising black rhinos with strict recording of consumption and growth (Schlegel, 2013). Black rhinos are also susceptible to iron stage diseases such as hemosiderosis, and hemochromatosis (Mylniczenko et al., 2012). Black rhino's gastrointestional physiology is similar to that of a horse, but they have drastically different feeding ecology, concentrate selector versus grazer. This has resulted in the suggestion that black rhinos receive a diet lower in iron (<6 g/d) than that of horses or even their grazing cousins the white rhino ([*Ceratotherium simum*] Clauss et al., 2012; Schlegel, 2013).

The Challenges of Formulating Diets for Captive Wild Animals

As a livestock nutritionist we are taught that there are three versions of the diet being formulated. The first version is the final diet formulated by the nutritionist. Second, the one made by the employee feeding the animals. Third and final, is the one the animals actually consume. This scenario is also true in the zoo setting except more complicated. There are many factors that affect the diet offered and the nutritional status of captive wild animals. In order to provide a healthy diet to captive wild animals, the nutritionist must study the foods to be offered and consumed, nutritional requirements, current health status, and management restrictions for the given species (Crissey, 2005). A summary of these areas is summarized in a matrix (Figure 1.3).

The main areas that affect consumption are diet, client, and animal preferences. Diet is affected by quantity and quality of the diet items. Sorting of favorite food items can occur if more food is offered than the animal can consume and results in a nutritionally inadequate diet (Crissey et al., 1998). Management can affect the quantity of food offered by cost, availability, quality, waste during storage or feed refusal, and client point vantage point (Crissey, 2005). Quality of the diet is affected by nutritional requirements, digestive morphology, and animal feeding strategies. For example, the quality of the insect being fed is not only its nutritional content, but also the physical condition of the insect (Bernard and Allen, 1997; Crissey, 2005). The amount of food and feeding times can change accordingly, dependent on the specific animal and feed type. An example offered by Dr. Edwards (1997) is a leaf-eating primate requiring a high fiber, low fermentable carbohydrate diet that is offered several times a day. An omnivorous primate should be fed a diet consisting of readily available nutrients. Feed

items come in many different forms for zoo diets. Feed forms can be live insects, rodents, or frozen/thawed fish or raw meat. The size, texture, and processing ability can change the nutritional quality of the item. Nutrient quality of browse, forage and produce changes by season, location, soil type, and processing (Crissey, 2005). The supplier, storage, diet, life stage, life cycle, and age can change the nutrients within invertebrates or other live, whole prey and frozen/thawed feed forms (Bernard and Allen, 1997; Crissey et al., 1999). The quality of meat products change depending on storage conditions, quality of products, and processor/manufacture (Crissey, 2005). Commercially manufactured feeds are affected by type, manufacturing method, supplies, and institution storage capability (Crissey, 2005). Supplements must be included if the diet does not meet the nutritional requirements of the animal. Lastly the nutritionist must look at non-food items such as bedding that are sometimes consumed, which can provide fill to the animal that can be often unaccounted (Crissey, 2005). The client can also have an effect on diet. Client refers to the animal caretaker (keeper), manager, or veterinarian. The client's point of vantage is affected by experience, knowledge and dedication. Keepers can sometimes choose not to include a given feed item, or feed more of a particular item that the animal likes and can result in unintentional nutritional deficiencies or toxicities. In this way the client is similar to the employee making a livestock diet on the farm. Lastly, the ingredients the animal consumes is prejudiced by animal preference. Animal preference is influenced by animal behavior, competition between other animals and past experiences (Crissey, 2005). For example, in the pet and companion animals it is common for parrots to develop a predisposition to consuming seeds. These parrots are commonly referred to as "seed junkies." Seed junkies will choose to consume mostly

seeds even when other feed items are placed in the enclosure. Seeds alone are nutritionally incomplete for parrots and over time will cause health problems.

There are roughly sixty-four or more nutrients that have to be considered when formulating animal diets. Many publications list the nutrient requirements for domestic and laboratory species; however, as mentioned earlier, little is known about the nutrient requirements of captive wildlife. Despite the differences in species, nutrient requirements appear to be similar at a cellular level (Crissey, 2005). Nutrient deficiencies and toxicity are inclined to demonstrate comparable patterns among some species like fish (Li and Robinson, 1999), and possibly large differences in others such as an African Elephant (*Loxodonta Africana*) and the horse (Crissey, 2005). Seasonality, life cycle and activity level are important considerations for adjusting nutrient requirements. Some animals experience seasonal changes in body mass, appetite, and body condition. Wild animals in captivity must be assessed to determine if they undergo similar changes, and apply appropriate diet changes. Life cycle can impact feed intake and nutrient requirements. A lactating animal typically requires much higher energy intake than that of maintenance. Young animals have specific requirements for growth and more active animals utilize more energy than inactive counterparts (Nagy, 2001; Crissey, 2005). Feeding ecology must also be researched for each species. Feeding ecology is extremely complex that can be affected by the animals metabolic needs, habitat, animal size, gut morphology, season, climate, food competition, and the physical form (Mautz and Nagy, 2000; Nagy, 2001; Crissey 2005). Literature can provide much about the feeding habits of animals, sometimes including types of food consumed. Knowing what types of food are consumed is of little use unless quantities and/or nutrients consumed were measured. Example,

knowing a free ranging animal consumed 16 plant species tells a nutritionist very little; however, providing more detail such as the animal consumed leaves, flowers and fruit that equates to 18% nitrogen and 32% ADF provides a good start to formulating a captive diet (Crissey, 2005). Animals in their native habitat change their feeding strategy for the varying seasons (Foster, 1978). For example, a fruit eating bird starts consuming insects during breeding and chick raising season (Crissey, 2005). Lastly, the animals' morphology can affect nutrient requirements. Gastrointestinal morphology and digestive strategies can be very different between species. Cats and dogs have relatively simple gastrointestinal morphology leaving them little ability of microbial fermentation and restricting them to more digestible food items. The Okapi (*Okapia johnstoni*) has a large foregut fermentation similar to goat that has the ability to digest fibrous material from plants, utilize microbes as protein and utilize microbial by-products for nutrition. In contrast, a Przewalski's horse (*Equus przewalskii*) utilizes hindgut fermentation just like a domestic horse. These animals consume large amounts of fibrous plant material throughout the day, and extract as much nutrients as possible with a rapid rate of passage (Crissey, 2005). Understanding the difference in morphology and feeding ecology is imperative to formulating diets for captive wildlife.

Health of the animal can drastically change required nutrients of an animal. The health of the animal can be affected by an infectious agent such as virus, bacteria, and/or parasite; noninfectious such as diet toxicity or deficiency, contamination, trauma, and/or toxins; preventative health measures such as vaccinations, sanitation, deworming, and health monitoring; lastly the diagnosis and treatment of the above (Crissey, 2005). A nutritionist has to be able to change the nutrients and diet to assist the animal. These

diets should be able to adapt to the animals changing needs and conditions. Working closely with veterinary staff to collect physiological data such as blood values and body condition score assures the tracking of nutritional status to determine diet success, allow for future diet planning, and determining if the diet needs to be modified (Crissey, et al., 1999). The best tool a nutritionist has is body condition scoring. When used properly body condition scoring can estimate energy intake and monitor the health of an animal. When used with diet consumption data, eventually the nutritionist can monitor seasonal variations in intake or body mass and potentially health disorders (Crissey, 2005).

Management of an animal in captivity can affect diet formulations or presentation. The environment is managed in every way possible, lighting, temperature, housing, breeding, enclosure size and type, and shelter (Crissey, 2005). The design of an enclosure can affect diet availability and intake. Easy access to food items may lead to quick consumption and boredom. Food items placed too difficult can result in little intake, and *ad libitum* can result in sorting (Crissey, 2005). Small enclosures may have less active animals requiring less dietary energy. Temperature can increase or decrease food intake in animals, especially ectotherms (Crissey, 2005). Management of animals can also be affected by other animals, food storage and preparation, and feeding timing and location. Food can be offered as rewards during training, but must be accounted for in relation to the maintenance diet. The presentation of food items can encourage or hinder intake. Smaller pieces of food can be spread throughout the enclosure to ensure more equal intake in group housing, while large pieces allow for more manipulation of the feed item. Food presentation is important for training and enrichment, however health should not be compromised for treats (Crissey, 2005).

Every institution housing captive wild animals and every animal is different. Every factor must be considered to properly formulate diets. A change in any factor can affect other factors and eventually an animal's diet. Diet formulation for captive wild animals is fluid. The process is very complex, always changing, and continuous. The nutritionist must work closely keeping good communication with other health staff such as veterinarians, animal managers and keepers to insure the appropriate diet is being offered and to quickly modify the diet if needed.

Figure 1.1. European and North American ruminant feeding types (Hofmann. 1989).

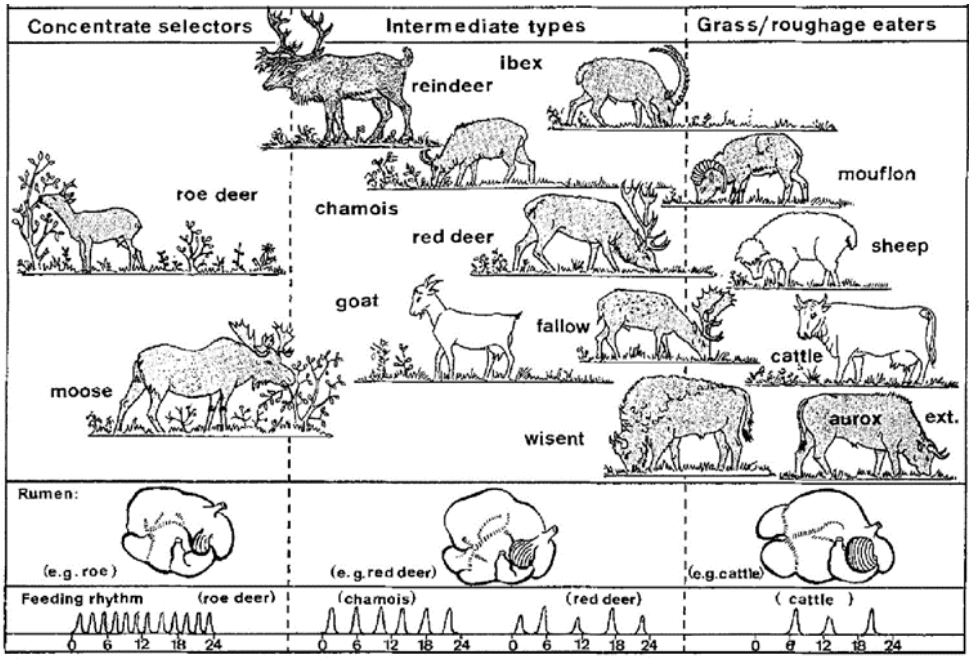


Figure 1.2. African ruminant feeding types (Hofmann. 1989).

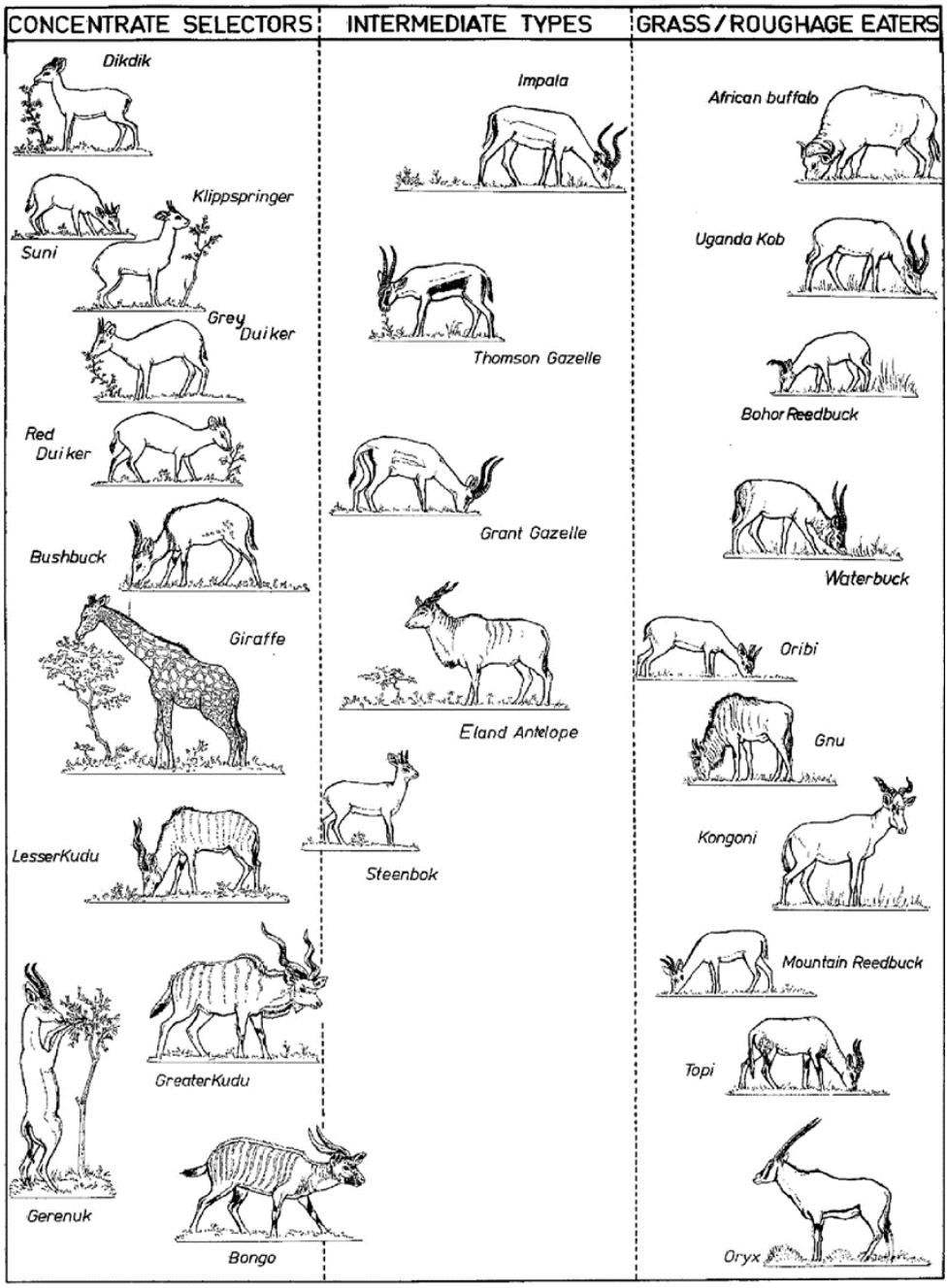
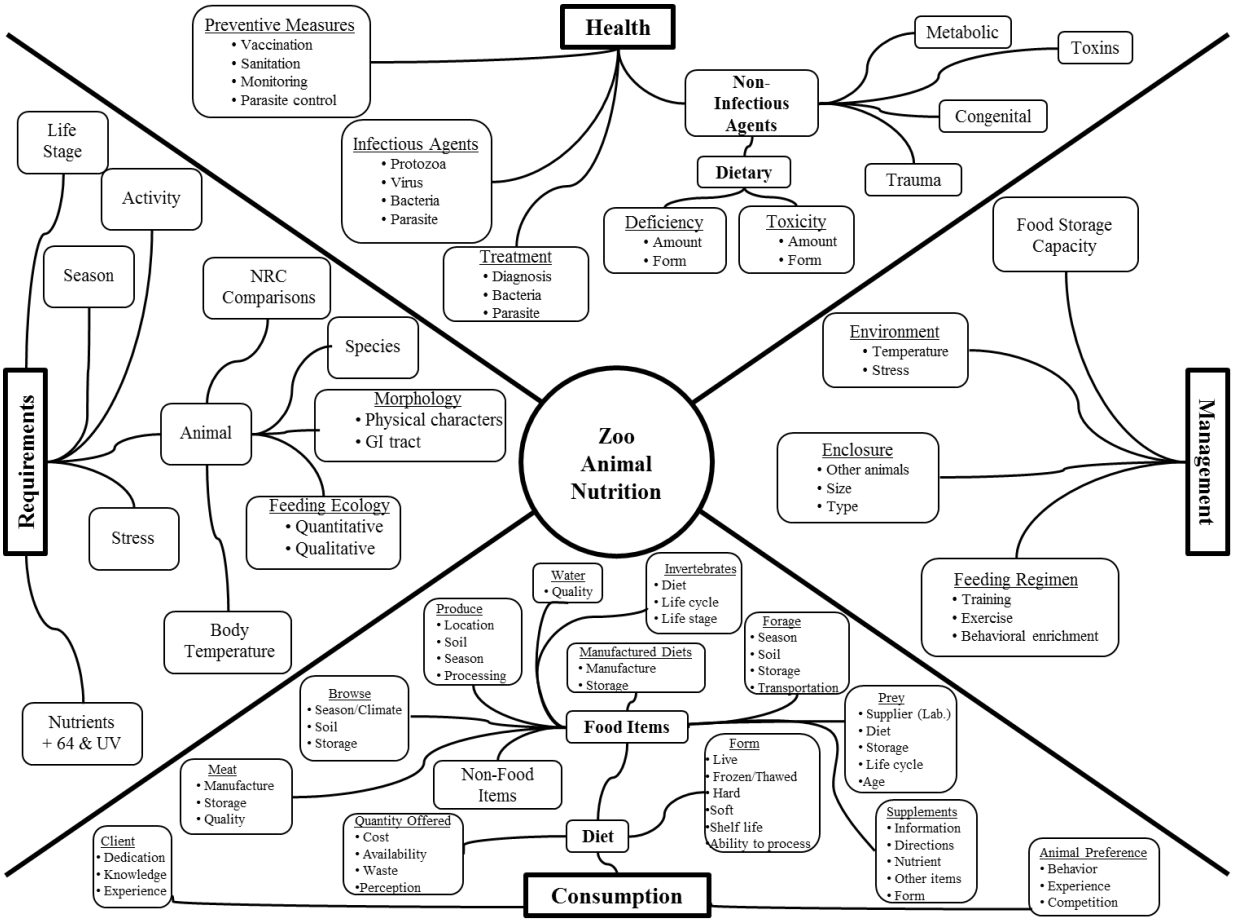


Figure 1.3. Captive wildlife nutrition matrix (adopted from Crissey, 2005)



CHAPTER II

REVIEW OF LITERATURE

Reptile Nutrition Review

One of the most common diseases found in insectivorous, omnivorous, and herbivorous reptiles caused by improper nutrition is metabolic bone disease (**MBD**). Metabolic bone disease is used to describe many bone associated diseases in reptiles: osteoporosis, osteomalacia, rickets, and secondary nutritional hypothyroidism. The cause of MBD is typically associated with calcium deficiency, but the disease can be caused from other nutritional imbalances. Most commonly MBD is caused from too little calcium, too much phosphorus (reversed calcium to phosphorus ratio), too little or too much vitamin D_3 in the diet or too little ultraviolet light (**UVB**), too much protein or a combination of these factors. Metabolic bone disease can also be caused by diseases of the kidney, intestines, liver parathyroid and thyroid gland (Kaplan, 2014), but only the nutritional sources are discussed.

Ultraviolet B from a provided UVB light bulb or natural sunlight, is absorbed in the skin, causing 7-dehydrocholestrol to form pre-vitamin D_3, than vitamin D_3. Vitamin

D_3 is transported to the liver and is synthesized to 1,25 $(OH)_2D_3$ to be utilized in calcium absorption. (Wallis et al., 2008). Vitamin D_3 can also be supplemented in the diet, normally over the counter premixed containing calcium and vitamin D_3. However, a UVB light should always be provided even with oral D_3 supplementation. Lack of UVB or supplementation of vitamin D_3 will result in an inadequate absorption of calcium from the gastrointestinal tract leading to a calcium deficiency. Overtime the lack of calcium can lead to metabolic bone disease.

Ultraviolet B specific light bulbs must be provided when housing most reptiles indoors. These special light bulbs come in different concentrations and in different forms, compact fluorescent, T8 or T5 fluorescents are the most common. A reptile keeper has to educate themselves on which concentration and bulb type works best in there management plan and optimal health for the animal. Whenever the weather and temperature is appropriate with supervision the animal should be placed outside to receive natural sunlight. The strategy for placing the animal outside depends on the species of reptile. Example: oustalet chameleon (*Furcifer oustaleti*) can be safely placed in an outdoor screen cage containing plants with a mixture of full sun and shade, with dripping or misting water when the temperature is above 13˚C and below 40˚C. Leopard tortoise (*Stigmochelys pardalis*) requires a slightly warmer at 16.5˚C with plenty of lawn to graze on and access to full sun, shade, and water.

Caring for insectivorous reptiles requires the keeper to also become an amateur entomologist. The reptile keeper must learn how to care for different species of insects in order to provide a well-balanced diet. The most common insect fed to reptiles is the common brown cricket. Easy to obtain from any pet store and ease of care makes them the first go to as a feed source. However, brown crickets have a natural reversed calcium to phosphorus

ratio, 1:2, which should be 2:1. A 1:2 Ca:P ratio causes insoluble calcium phosphate, which renders the calcium unusable (Kaplan, 2014), eventually leading to hypocalcemia metabolic bone disease. To combat the lack of calcium many in the hobby provide a phosphorus free calcium powder (usually from calcium carbonate or crushed oyster shells) to dust the insects (similar to shack and bake), or provide the insects with a high calcium diet with a technique called gut loading. Gut loading is the most effective way to provide calcium to the reptile. Crickets cannot be maintained over three days on a gut loading diet due to it causing impaction of the GI tract and death of the cricket. It is highly recommended to feed a variety of insects maintained on highly nutritious diets to insectivorous reptiles such as crickets, meal/super worms, wax worms, silk worms, horned worms, phoenix worms, grasshoppers, praying mantis, fruit flies and cockroaches. This approach done properly not only prevents MBD, but also provides enrichment to the reptile. Herbivorous reptiles can receive oral calcium and vitamin supplementation by sprinkling the supplement over there diet.

Omnivorous and Herbivorous reptiles can be sensitive to plants containing digestive or metabolism disruptors. Eliminating or light supplementation of any item within the cabbage family: cabbage, kale, collards, broccoli, brussels sprouts, and chard (Highfield, 2002). Due to thyroid interfering properties called goitrogen and anti-mineral factors that many reptiles are highly sensitive to the affects and tend to be predisposed to hypothyroidism in captivity (Frye, 1981; Starrett, 1992). Leafy greens high in oxalic acid should also be minimized within the diet. This includes: spinach, rhubarb leaves (contains toxic levels and should not be fed), parsley, and chives. Oxalic acid binds to calcium within the gastrointestinal tract forming indigestible oxalates that are expelled in the feces. All the leafy greens listed above should be eliminated or lightly used in insect gut loading diets.

In captivity herbivorous tortoises should have access to free choice grass hay such as Bermuda, native or timothy hay, a small amount of leafy greens, a high-fiber, low-starch commercial pellet and supplements as needed. Larger tortoises are ideally housed outside in pastures when the season is appropriate for the species of tortoise with mixed species grasses and a supplementation of a high-fiber, low-starch commercial pellet. Some root vegetables such as turnips and carrots may be included. Cacti, prickly pear paddles (Opuntia), may be fed as available. Items high in starch, protein and energy should be avoided due to potential negative health effects such as: accelerating growth, undesirable Ca:P ratio, pyramiding caused from reductions in shell calcium and can be linked to accelerated growth, and kidney stones (Paull. 1997; Paull. 1999). If the tortoise is housed outside, receiving a diet consisting of grasses, hays, and supplemented with the correct high-fiber, low-starch pellet, occasional produce and calcium, it should not require any additional supplementation. Assuming they are on pasture, or receiving fresh cut grasses, or appropriate hay (Paull. 1997; Paull. 1999). Young tortoises will have varying growth differences even if they are from the same clutch (Furrer et al. 2004).

Herbivore Tortoise Nutrition Review

Herbivorous tortoises share many aspects of gastrointestinal anatomy and physiology with horses, elephants, and other species that house large populations of cellulolytic microbes in the large intestine (Barboza, 1995). Because these microbes require dietary fiber as a primary substrate, the health of the microbial population, and therefore optimal digestion in the tortoise, depends on a diet of sufficient fiber concentration. Diets for captive herbivorous tortoises are often too high in protein and too low in fiber. Both of these factors can contribute to juvenile growth that is too rapid (Jackson et al., 1976; Baer et al., 1997; Hatt et

al., 2005). Rapid growth may result in early sexual maturity and aggression (Jackson et al., 1976) and, particularly in the absence of adequate mineral supplementation, can lead to life-threatening mineral imbalances resulting in skeletal and shell deformities (Jackson et al., 1976; Highfield, 2010). Additionally, high-protein diets may contribute to visceral gout or kidney or bladder stones, whereas low-fiber diets may contribute to bacterial and cloacal infections (Jacobson, 1994). Too much dietary energy, either from excessive protein or fat, may similarly result in obesity in adult tortoises. Diets with high concentrations of non-fibrous carbohydrates (sugars or starches) may cause a significant imbalance in normal gut flora, leading to poor stool quality, inefficient digestion and absorption, potential colic, or other digestive disorders.

Nutritional caused disorders are a significant cause of mortality in captive hatchlings and juvenile herbivorous chelonians (Highfield, 1987). A summary of 144 pathological examinations of long term zoo housed herbivorous chelonians summarized cause of death. Out of the 144 exams mortality was reported as 27% from gastrointestinal tract disorders, 11% hepatic and 9.7% renal disease. An additional 22.2% died from other nutritionally causes disorders. A total of 77.8% of captive tortoises died from an improper nutrition caused disease (Keymer, 1978). Another survey conducted utilizing herbivorous tortoises kept as pets showed similar causes for mortalities was presented. Majority of mortalities was caused from hepatic disorders, respiratory disease (mostly pneumonia), gastrointestional tract disorders, renal disease and cardiac ailments at 72.6%, 53%, 50.7%, 40.6% and 34.3% respectively (Rosskopf, 1981). In both publications gastrointestinal disorders are substantial and pet owned chelonians have a higher occurrence of hepatic and renal diseases. Examinations of living and deceased captive herbivorous tortoises support the surveys by

presenting, primary acute secondary nutritional osteodystrophy from inadequate dietary calcium:phosphorus ratio (Wallach and Hoessele, 1968, Lickel, 2010) with hepatic and renal dysfunction containing uric acid deposits within renal tubules (Highfield, 1988). Hepatic and renal dysfunction is commonly associated with extremely high protein diets in which herbivorous tortoises would not encounter in their native habitat. Excess protein combined with low calcium:phosphorus ratios and/or inadequate UVB lighting, can cause pyramidal shaped scutes (Kirsche, 1984; Gerlach, 2004) progressing to primary acute secondary nutritional osteodystrophy also referred to as metabolic bone disease (Highfield, 2000). Metabolic bone disease is described by abnormal carapace morphology, with thick spongy bone leading to kidney dysfunction and death. Captive hatchlings and juveniles are more susceptible to nutritional excess and deficiency, exhibiting deformities within 12-24 months, while adults may take 5-10 years to show clinical signs of nutritional disorders, despite having long term internal damage (Highfield, 1988; Gerlach, 2004). Feeding a varied diet that imitates that of wild is crucial to build a solid foundation for the long term health of captive herbivorous tortoises.

Wild free ranging herbivorous tortoises tend to be concentrate (browsers), or intermediate selectors. These animals travel a wide area consuming small amounts of a large variety of plant material, roughly 200 different plants (Highfield, 2002). The consumed quantity and combination of maturity status, part of plant, and dryness varies significantly seasonally, resulting in variable nutritional composition intake (Highfield, 2002). For example with savannah and semi-arid habitat species, availability of plant material peaks early spring when plant moisture and protein content are highest (Highfield, 2002). Than greatly reduces around the high peak temperatures and dry summer season when plants dry

matter and fiber content is very high, and protein is low (Highfield, 2002). During the hot summer season the tortoises may enter estivation to conserve energy (Highfield, 2002). Tortoises will change their eating habits to morning and evening, while sleeping during the day to avoid the heat (King, 2012). Tortoises consume a well-balanced diet with essential minerals required for proper growth, development and reproduction by traveling a wide area, and consuming a large variety of plant material (Highfield, 2002). Diets of herbivorous tortoises housed in captivity tend to be very limited compared to the observed natural feeding patterns (Highfield, 2002).

For any captive species, the overriding principle in formulating a captive diet should be a close approximation to the free-ranging diet. Each species' digestive anatomy and physiology is adapted for a specific set of nutritional conditions, often resulting from thousands of years of co-evolution with its habitat; altering those conditions, even to provide a diet that is perceived to be "better" (produce-based), could have serious health consequences. To that end, when available, information on nutrient composition of the wild diet should be used as an approximation of nutrient requirements. Such information is often limited for herbivorous chelonians, but there are a handful of peer-reviewed studies which report wild tortoises consuming a diet with 25 to 30% crude fiber, 22.6 to 32% acid-detergent fiber (ADF), 50% neutral-detergent fiber (NDF), 2 to 10.5% crude protein, 2.1% fat, 1.15% calcium, and 0.18% phosphorus (Moskovits, 1985; Highfield, 1988; Highfield, 2002; Hatt et al., 2005). Typical produce-based captive diets compare poorly, containing 17% ADF, 31% NDF, 19% crude protein, 3.5% fat, 1.1% calcium, and 0.45% phosphorus (preliminary data).

Traditionally captive herbivorous tortoises where fed a diet that was excessively high in protein and fat while being very poor in dietary fiber. Beginner tortoise keepers who

attempted to educate themselves on proper husbandry practices relied heavily on printed material during the early and mid-1990. Popular books for novice tortoise keepers would print diet recommendations based on current trends or authors experiences. While providing good introduction material for environmental husbandry, they failed in proper diet recommendations. Some of these popular books recommended supplementing the diet with wet or dry dog food, insects, hard boiled eggs, lean ground beef once a week, cereal with or without milk once a week (Coborn, 1995; Zimmermann, 1995; Wilke, 1991). Recommending milk to any reptile can cause digestive upset due to the lack of lactase. These recommendations were based on the idea that they may consume carrion or accidental insect while eating plant material in the wild (Coborn, 1995; Wilke, 1991). In addition some recommended feeding high amounts of fruits (Coborn, 1995; Zimmermann, 1995; Wilkie, 1991). These recommendations where to be added to a green produced based diet or in addition to free range grazing in outdoor enclosures (Coborn, 1995). Feeding highly fermentable carbohydrates (sugars) to herbivorous tortoises that are not adapted to such diets can result in disturbances to the entire animal's metabolism. Highly digestible carbohydrates in the form of starch (primarily from fruit) is degraded into sugars in the small intestine. High concentration of starch in the diet leads to an increase concentration of starch reaching the hind gut. The consequences will cause increase gut motility, accelerated rate of passage, increased gas production, acidification of hindgut digesta, altering of hindgut microorganisms and disturbing normal digestive processes (Highfield, 2000). Very similar to horses and ruminant animals who are susceptible to ruminal acidosis from highly digestible starches (fruit), which are also associated with clostridial infections, laminitis, liver

abscesses, keratinization of gastrointestinal tract disrupting absorption of nutrients, colitis, colic, sudden death, diarrhea, and dehydration.

Digestible fiber (NDF) is fermented and broken down in the hind-gut by symbiotic microorganisms into volatile fatty acids (**VFA**). VFA's such as acetate, butyrate, and propionate are absorbed through the hind-guts epithelium and are an important source of energy. VFA's as a source of energy represents ~30-40% of total energy requirements for herbivorous tortoises (McBee and McBee, 1982, Highfield, 2010). The normal pH range in the hindgut in herbivorous reptiles consuming a high fiber diet is 6.8-7.0 (Highfield, 2010). Similar to ruminant animals, a state of hindgut acidosis occurs at a pH <5.5 from an increased production of lactic acid. The low pH environment causes a shift in the microorganism population causing a high level of endotoxins as normal hindgut bacteria die off. As the epithelial lining of the hindgut degrades over time from exposure to acidosis resulting in decrease absorption of nutrients. Eventually, leading to the hindgut epithelium to deteriorate causing a severe colitis. Further epithelial degradation allows bacteria to enter the bloodstream and seed themselves in the liver resulting in hepatic disease. Hepatic disease is one of the primary cause of mortality in captive tortoises. Providing a diet high in fiber, low in starch will prevent hindgut acidosis.

The amount of time for digesta to pass through herbivorous chelonians digestive tract is slow compared to mammalian hindgut fermenters. Rate of passage is heavily influenced animal physiology and mass-specific metabolic rate, but also dietary intake of water and fiber, environmental and body temperature, as well as feeding frequency (Stevens and Hume, 1995; Boyer and Boyer, 1996). Previous reports have described mean retention time ranging from 6.7-14.8 days in *Gopherus agassizii* (Barboza, 1995), 2.2-8.7 days in *Geochelone*

pardalis (McMaster and Downs, 2008; Lickel, 2010), up to 12-14 days in *Geochelone nigra* (Hatt et al., 2002; personal observation). Increased fiber intake typically decreases overall digestibility while also decreasing rate of passage. Farlow (1987) observed that herbivorous reptiles are able to achieve digestion coefficients comparable to mammals. Furthermore Karasov et al., (1986) suggests that this is achieved due to their comparatively slower digesta rate of passage. The difference between reptiles and mammals may not be in the relative digestive coefficient, but the amounts of nutrients digested per unit of time.

The Tortoise Trust (BM Tortoise, London, UK) has been recommending appropriate healthy diets since the 1980's for herbivorous tortoises. As an organization they were the first to research the effects of high protein diets on herbivorous tortoises. In 1988 A.C. Highfield, member founder and international director of Tortoise Trust, published recommendations on proper diet husbandry for herbivorous tortoises. Highfield's recommendations are based from scientific research, observed wild populations of herbivorous chelonians, and personal experiences (Highfield, 2004).

Calcium

Calcium requirement for herbivorous tortoises is variable and increases during growth and in reproductively active females during egg formation (Highfield, 1988). Calcium requirement is also dependent upon environmental factors such as quantity of phosphorus in the diet, amount of phytic acid or oxalic acid, and vitamin D_3 availability. Highfield, 1988 notes that desert habiting tortoises consume 5:1 to 8:1 calcium:phosphorous ratio while mediterranean species have reported a 3.5:1 ratio. Consumed wild plants are also high in fiber and low in protein. In addition these tortoises consume soil, sand and grit resulting in additional trace elements. Others have reported that a critical calcium:phosphorus ratio

deficiency of 1.2:1 will result in osteoporosis, osteomalacia, and pyramiding (Zwart, 1987; Gerlach, 2004). Some plant material such as beans, legumes, sprouting seeds, and peas contain more phosphorus than calcium, contain phytic acid and should be avoided (Highfield, 1988). Phytic acid has a strong binding affinity to calcium preventing absorption. Plants high is oxalic acid should also be avoided as it binds with calcium to form insoluble calcium oxalate. Plants high in oxalic acid include beet greens, kale, spinach, the Goosefoot family (Highfield, 1988). Herbivorous tortoise keepers must review plants to exclude items that have a negative calcium to phosphorus ratios. Plants which contain neutral or positive calcium to phosphorus ratio can be supplemented with high quality multi-vitamin and mineral powder if needed to reach a ratio of 5:1. Juveniles raised on such a regime showed no signs of pyramiding and secondary nutritional osteodystrophy (Highfield, 1988).

Calcium can be supplemented by commercially available calcium preparations containing no phosphorus. Avoid calcium supplements centered on bone meal due to high phosphorus content as herbivorous diets tend to be rich in phosphorus, resulting in no additional supplementation needed. Calcium carbonate is the most common form of calcium. It's recommended for supplementation due to its high calcium content, is safe to use and effective (Highfield, 2003).

Vitamin D_3

Vitamin D_3 is essential in the formation of bone development, calcium absorption, and calcium regulation in within the body. Without adequate quantity and quality of vitamin D_3 the tortoise are unable to absorb calcium despite high calcium intake. Tortoises in their natural habitat are highly improbable to suffer from hypovitaminosis-D_3 (Highfield, 2003). Unlike their wild counter parts, captive tortoises housed indoors with no access to sunlight or

efficient artificial UVB can cause deficiencies. Keepers can also over supplement oral vitamin D_3 causing a toxicity. Vitamin D_3 toxicity can cause metastatic mineralization of soft tissues (Barten, 1982). Plant foods contain very little to no vitamin D (Highfield, 1988). Tortoise skin is abundant in oils containing sterols that react with UVB and produce vitamin D (Highfield, 1988). With adequate UVB exposure from sunlight or artificial, no oral supplementation of vitamin D_3 is needed. With adequate calcium, phosphorus ratios combined with high quality and quantity of UVB exposure the occurrence of hypovitaminosis-D_3 is unlikely (Highfield, 1988). Humans require ~400 IU per day of vitamin D which can be achieved from 3 hours of sunlight exposure. Tortoise requirement are unknown, however Zwart (1987) suggests 10-20,000 IU of vitamin D_3 per Kg of vitamin-mineral supplementation given at 4% of offered food volume when UVB exposure is inadequate.

Vitamin B Complex

Vitamin B complex includes thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, biotin, folic acid, and cobalamin. Deficiencies have occurred in herbivorous tortoises raised primarily on lettuce only diets causing poor neuromuscular coordination and pernicious anemia (Highfield, 1988). Deficiencies have also presented during severe colitis and malabsorption syndrome from pathogenic flagellate infection of the hindgut (Highfield, 1988).

Magnesium

Magnesium is an essential mineral in the bone matrix and is important for calcium absorption and effects the parathyroid hormone to maintain and regulate calcium metabolism (Highfield, 2003).

Iodine

Captive herbivorous tortoises are prone to hypothyroidism when fed a diet of green produce (cabbage family and kale) containing high levels of anionic goitrogens (Highfield, 1988). Plants containing high levels of anionic goitrogens should be limited in the diet and supplementation of a commercial multi-mineral product containing trace amounts of iodine. This supplement should be offered at a rate of 6-10 mg per Kg of supplement provided during every meal (Highfield, 1988).

Fat

Herbivorous tortoises in the wild consume a diet very low in fat, similar to ruminants and wild equids. Plant based diets are naturally low fatty acid content. Herbivorous chelonians do not have the ability to metabolize saturated fats (Highfield, 1988). When offered a high fat diet tortoises develop hepatosis, and steatitis causing jaundice and cannot retain vitamin A. High fat diets also lead to obesity. Wild herbivorous tortoises have been observed consuming a diet of plants consisting of 0.35 g per 100g of polyunsaturated fatty acids (Highfield, 1988).

Protein

Protein is commonly over-fed to captive herbivorous tortoises. High protein diets in combination with inadequate calcium intake is a direct cause for hatchling mortality, scute deformity, and metabolic bone disease. Protein rich diets increase calcium requirements due to accelerated growth, causing early sexual maturity, and aggression (Highfield, 1988). Feeding high levels of protein has two effects 1) directly effects the ability to absorb calcium (Margen, 1974) and excessive protein leads to high levels of blood urea nitrogen (**BUN**), which increases nitrogenous waste processing in the renal system (Highfield, 1988). Known

BUN and creatinine levels of wild tortoises are very limited. Reference values are from captive tortoises kept as pets and are unreliable to be used as a healthy range. Animals exposed to dehydration in combination with consuming an excessive protein diet are at higher risk of renal disease by accumulating ureic acid in the renal tubules, pericardium, liver and other organs (Highfield, 1988). Wild herbivorous tortoises consume a wide range of plant material, leaves, stems, bark, seeds, flowers, fruit, and grasses. The high variety of intake not only provides a wide range of minerals, vitamins, and fiber, but also improves the range of essential amino acids (Highfield, 1988). An increased intake range of essential amino acids increases the potential Net Protein Utilization (**NPU**) in the diet. A wide intake range of essential amino acids allows herbivorous tortoises to sustain themselves on a diet very low in protein percentage (Highfield, 1988). However, in captivity the occurrence of excessive protein intake is very common, not deficiency.

Protein requirement has not been established in most reptiles or herbivorous tortoises. Species of herbivourous tortoises have been observed in the wild consuming a diet averaging 1-5% plant protein as-fed (Rosskopf, 1982), and utilized at ~55% (Highfield, 1988). A safe upper limit to offer captive tortoises is 7% protein with a median intake of 4% on an as-fed basis (Highfield, 1988). It is accepted that herbivorous tortoises require less protein kg for kg than mammals which is ~0.5g of usable protein per kg per day. Daily protein requirements of a growing tortoise is suggested to be 0.2g of usable protein per kg (Highfield, 1988).

Energy

The kcal requirement for maintenance, growth, and reproductions is not well described in reptiles. Some have reported field metabolic rates of small species of wild

herbivorous tortoises (Brown et al., 2005). However, the values calculated for the field metabolic rate could possibly be overestimated. Estimating energy needs is difficult as it's widely accepted that the energy needs of herbivorous reptiles is similar to that of protein which is amazingly low. For example a 1 kg *Uromastyx aegyptius* can survive on ~11.8 kcal per day, ~3.7g of naturally consumed vegetation. A smaller animal weighing 65g requires 550 kcal or 0.17g of food in order to meet energy expenditure (Robinson, 1995). Average daily intake range between 2.3-2.7 times the bare minimum (Foley et al., 1992). *Iguana iguana* daily expenditure is 5.48 kcal at 28˚C to 14.26 kcal at 35˚C (Baer and Oftedal, 1995). Golley (1961) reported the energy content of plant parts and communities commonly fed to herbivorous tortoises range in energy content from perennial grasses (3.905 kcal/g), green leaves (4.229 kcal/g), stems and branches (4.267 kcal/g), roots (4.720 kcal/g), to seeds (5.065 kcal/g).

Water

Though some species of herbivorous tortoises rarely consume water in a liquid form, it is advisable to provide captive tortoises with cool, clean and abundant water on a daily basis. Herbivorous tortoises will readily drink and or soak in water when offered. Optimal hydration can potentially prevent renal disease when a high protein diet is fed.

Staple Diet/Fiber

It is impractical for a tortoise keeper to be able to provide as varied diet that herbivorous tortoises would consume in the wild. However, tortoise keepers are obligated to provide as much of a variety of diet items as feasibly possible. Herbivorous chelonians such as *Geochelone pardalis spp*., *Geochelone sulcata*, *Gopherus agassizii*, *Aldabrachelys gigantea*, *Chelonoidis nigra*, and *Testudo spp*. need access to mixed species free range areas

(pastures) to allow grazing and browsing of grasses and clovers (Highfield, 2004; King, 2012). In addition these tortoises should be offered wild plants. A composite list of safe wild plants that would be offered as a staple addition to graze and browse for herbivorous tortoises is found on Table 2.1. Wild plants must be washed prior to being fed to tortoises (King, 2012). Wild plants should not be harvested from areas treated with herbicides or pesticides. Table 2.2 provides a list of potentially toxic plants that should be avoided. The toxic plant list (Table 2.2) is derived from known plants toxic to mammals and should serve as a guide as identification of all toxic species is not possible in this document. The degree of toxicity to herbivorous tortoises is unknown for many of the plants found in the list (Highfield, 2000). Grasses, clovers and wild plants must comprise the staple diet for herbivorous chelonians (Highfield, 2004). Grasses, clovers, and wild plants provide the basis of a high-fiber diet and the correct calcium:phosphorus ratio (~5:1) that is suggested by many herbivorous tortoises researchers (Highfield, 2002; Highfield 2004). The fiber content provided by grass based diets contribute to digestive health (Highfield, 2000). Highfield, (2010) recommends never increase dietary fiber levels by offering bran (high phytic acid levels) or oats (extremely high phytic acid and readably digestible carbohydrate levels). Bread and corn should also be excluded because it is high in carbohydrates and phytic acid (Highfield, 2010). During the spring, summer and early fall, captive herbivorous chelonians diet can consist of 85% or more of graze, browse and wild plants. The remaining 15% is to be supplemented with green produce and high-fiber, low starch commercial pellets designed for herbivorous tortoises. (Highfield, 2004). During the remaining months a staple diet consisting of grasses, wild plants and dried hay (~75%) make up the majority followed by supplementation of green produce and high-fiber, low starch pellets (Highfield, 2004).

Traditional commercially available “complete” tortoise diets (canned, often dried) are unsafe and should be avoided (Highfield, 2002). These products tend to be very high in protein and starch, while being low in fiber (Highfield, 2002). Tortoises must also be provided with a high quality mineral and vitamin supplement (Highfield, 2002; Highfield, 2004; King, 2012). Keepers must be cautious in regard to mineral and vitamin supplementations as requirements vary from species to species. Herbivorous tortoises raised primarily on “complete” tortoise diets exhibit extreme deformities from high growth rates and inadequate calcium such as pyramiding and metabolic bone disease (Highfeild, 2002).

Natural and perfect growth of herbivorous tortoises can be achieved by limiting protein intake, provide adequate amounts of calcium and other trace elements, provide adequate quality and quantity of UVB and or vitamin D_3, offer a diet based on grasses, clovers, wild plants and hays, monitor feed intake to prevent over feeding, housing environment is optimal for specific species, provide cool, clean and abundant water.

Recent advances in feed manufacturing have made low-starch, high-fiber commercial pelleted diets available for the first time. These diets are attractive to captive tortoise managers (zoos and research facilities), as well as pet owners, due to their superior nutritional quality coupled with ease of feeding. However, little experimental data exist that either compare various new commercial products, or demonstrate the degree of improvement in digestive health and function. We seek to obtain data to satisfy both questions by performing an experimental transition from a produce-based diet to one of two commercial pelleted diets: Zoo Med Natural Grassland Tortoise Food (Grassland) and Mazuri Tortoise LS Diet (Mazuri 5E5L). Both of these products provide superior high-fiber substrate for

intestinal microflora, compared to traditional produce-based diets and should result in superior fecal quality and animal health over produce-based diets.

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Table 2.1. List of common wild plants that can be offered to captive tortoises. (Adapted from Cohen, 1992; Highfield, 2000; Highfield, 2002; Highfield 2004; King, 2012)[1]

Common Name	Scientific Name	Parts Offered
Dandelion	*Taraxacum officianale*	leaves, stems and flowers
Hibiscus	*Hibiscus spp.*	leaves, stems and flowers
Viola	*Viola spp.*	leaves, stems and flowers
Petunia	*Petunia spp.*	flowers
Prickly Pear	*Opuntia spp.*	De-spined paddles, flowers and fruit
Hawkbits	*Leontodon spp.*	leaves, stems and flowers
Sow thistles	*Sonchus spp.*	leaves, stems and flowers
Nipplewort	*Lapsana communis*	leaves, stems and flowers
Hawkweeds	*Pictis spp.*	
Hawk's-beards	*Crepis spp.*	leaves and flower
Plantains	*Plantago spp.*	leaves, stems and flowers
Clovers	*Trifolium spp.*	leaves, stems and flowers
Chicory	*Cichorium intybus*	leaves, stems and flowers
Honeysuckle	*Lonicera periclymenum*	flowers
Shepherd's purse	*Capsella bursa-pastoris*	leaves, stems and flowers
Cat's ears	*Hypochoeris spp.*	leaves, stems and flowers
Fescue Grasses	*Festuca spp*	leaves, stems and flowers
Buffalo Grass	*Bouteloua dactyloides*	leaves, stems and flowers
Bermuda Grass	*Cynodon dactylon*	leaves, stems and flowers
Couch Grass	*Elymus repens*	leaves, stems and flowers
Kikuyu Grass	*Pemmisetum clandestinum*	leaves, stems and flowers
Dallisgrass	*Paspalum dilatatum*	leaves, stems and flowers
Blue Grama Grass	*Bouteloua gracilis*	leaves, stems and flowers
Big Bluestem grass	*Andropogon gerardi*	leaves, stems and flowers
Rye Grass	*Lolium spp.*	leaves, stems and flowers
Bluegrass	*Poa spp.*	leaves, stems and flowers
Orchard grass	*Dactylis spp.*	leaves, stems and flowers
Western Wheat Grass	*Pascopyrum smithii*	leaves, stems and flowers
Vetch, Common, Bush, Tufted	*Vicia sativa, sepium* & *cracca*	leaves, stems and flowers
Sainfoin	*Onobrychis sativa*	leaves, stems and flowers
Creeping Bell-flower	*Campanula rapunculoides*	leaves, stems and flowers
Evening primrose	*Oenothera biennis*	leaves, stems and flowers
Bindweeds	*Convolvulus* & *Calystegia spp.*	leaves, stems and flowers
Stonecrops	*Sedum album* & *spectabile*	leaves, stems and flowers
Hedge mustard	*Sisymbrium officinale*	young plants, leaves and stems
Mulberry	*Morus spp.*	leaves
Bittercress	*Cardamine hirsute* & *feluosa*	leaves
Trefoils	*Lotus spp.*	
Ice Plant	*Mesembryanthemoideae spp.*	leaves and flowers
Crassula	*Crassula spp.*	
Mallows	*Malva spp.*	leaves and flowers
Bindweeds	*Calystegia spp.*	
Sedums	*Sedum spp.*	
Ivy-leaved Toadflax	*Cymbalaria muralis*	
Chickweed	*Stelaria media*	
Robinia	*pseudo-acacia*	leaves
Wild clematis	*Clematis spp.*	
Acanthus	*Acanthus spp.*	
Nettles	*Lamium album* & *purpureum*	
Bramble	*Rubus fruticosus*	shoots, tender leaves and fruit
Sow Thistle	*Sonchus ol-eraceus* & *as-per*	coarsely or finely chopped

[1] Wild plants should be offered in addition to large free range area with a variety of grasses and clovers to provide graze and browse. Plants picked should be thoroughly washed before offering to tortoises. Never collect plants from areas that have been treated with herbicides or pesticides.

Table 2.2. Herbivorous tortoise, common toxic plant list (Adapted from Cohen, 1992; Highfield, 2000)[1].

Item	Item	Item	Item	Item
Acokanthera	Coriara	Jerusalem cherry	Poison ivy[2]	Verbena
Aconite (monk's hood)[2]	Coral plant	Jessamine	Poison oak[2]	Virginia creeper
Amaryllis	Creeping charlie	Jimson weed (thorn apple)[2]	Poison sumac[2]	Water hemlock[2]
Amsinckia (tarweed)	Crocus[2]	Johnson grass (wilted)	Pokewood or pokeberry	Wild parsnip
Anemone	Croton	Lambkill (sheep laurel)	Poppy (except California)	Wisteria
Angel's trumpet	Crown of thorns	Lantana camara	Potato (leaves)[2]	Yellow star thistle
Apple (seeds only)	Cyclamen	Larkspur	Privet	Yew[2]
Apricot (seeds only)	Daffodil[2]	Lily of the valley[2]	Redwood	
Arrowgrass	Daphne	Lobelia	Rhubarb (leaves)[2]	
Asparagus fern	Death camus[2]	Locoweed[2]	Rhododenron[2]	
Autumn crocus[2]	Deadly nightshade[2]	Locust	Ripple ivy	
Avocado (leaves)	Delphinium	Lupin[2]	Rosemary	
Azalea	Destroying angel (death cap)[2]	Machineel	Russian thistle	
Baneberry[2]	Dieffenbachia	May apple	Sage	
Beach pea	Dogwood	Mescal[2]	Salmonberry	
Betal nut palm[2]	Easter lilly	Milk weed	Scarlet pimpernel	
Bittersweet	Elderberry	Mistletoe[2]	Scotch broom	
Bird of paradise	Elephant ear (taro)	Moccasin flower	Senecio	
Black locust	English ivy	Mock orange	Skunk cabbage	
Bleeding heart	Euphorbia	Monkshood[2]	Snapdragon	
Bloodroot	False hellebore	Moonseed	Soap berry	
Bluebonnet	Fiddle neck (Scenecio)	Moonweed	Spanish bayonet	
Bottlebrush	Fly agaric (amanita, death cap)[2]	Morning glory	Spider chysanthemum	
Boxwood[2]	Four o'clock	Mountain laurel	Sprangeri fern	
Buckeye horse chestnut	Foxglove[2]	Narcissus	Squirrel corn	
Buttercup	Gelsemium	Natal cherry	Sudan grass	
Caladium	Glocal ivey	Nectarine (seed)	Star of Bethlehem	
Calla lily	Golden chain	Needlepoint ivy	Sundew	
Cardinal flower	Hemlock[2]	Nicotine, tree, bush, flowering[2]	Sweetpea	
Caroline jessamine	Henbane[2]	Nightshades[2]	Tansy	
Casava	Holly	Oak	Taro (elephant ears)	
Castor bean	Horse Chestnut	Oleander[2]	Tarweed	
Chalice/trumpet vine	Horsetail reed (Equisetum)	Pear (seeds)	Tiger lily	
Cherry (seeds)	Hyacinth	Pennyroyal	Toad flax	
Cherry laurel	Hydrangea[2]	Peony[2]	Tomato (leaves & plant)	
China cherry tree	Impatiens	Periwinkle	Toyon berry	
Christmas berry	Iris	Philodendrons (some species)	Tree of heaven	
Christmas cactus (Euphorbia)	Ivy	Pinks	Trillium	
Christmas rose	Jack-in-the-pulpit	Plums (seeds)	Trumpet vine	
Columbine	Jasmine	Poinsettia	Umbrella tree	
Common privet	Jatropha	Poison hemlock[2]	Venus flytrap	

[1] Plants may be listed more than once under other names.
[2] Known plants that have caused mortality in tortoises after consumption.

CHAPTER III

COMPARING BODY CONDITION ESTIMATES OF ZOO BROTHER'S ISLAND TUATARA (SPHENDODON GUNTHERI) TO THAT OF THE WILD, A CLINICAL CASE

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Abstract

Brother's Island tuataras (*Sphenodon guntheri*) housed at the San Diego Zoo were measured and weighed routinely as part of the preventative medicine procedure. From June 2000 through April 2009, snout-vent length and mass were used from that data and compared to Brother's Island tuataras that were sampled by Hoare et al., 2006 in the wild from 1957 to 2001. Along with comparing snout-vent length (SVL) and mass, body condition was estimated using the ratio of the log-transformed mass to the log-transformed snout-vent length. The tuataras that are cared for by the San Diego Zoo (SDZ) are larger and have a greater conditioning score than that of the tuataras sampled in the wild (Hoare et al., 2006). Over the 9 year period of sampling the SDZ tuatara had a mean increased in mass of 125.2 %, SVL of 31.7 %, and BCE of 7.5%.

Introduction

Tuataras are a rare lizard looking reptile found only on the islands surrounding New Zealand. They represent the only living organism of the Order *Sphenodontida*.(Cree and Butler, 1993). There are three subspecies of tuatara, Northern (*S. p. punctatus*), Cook Strait (*S. punctatus*) and Brother's Island (*S. guntheri*, Bullar 1877; Cree and Butler, 1993).

The 4-ha island of Brother's is off the North coast of the Southern Island of New Zealand and is sustaining the population of about 470 adult Brother's Island tuatara (Hoare et al., 2006). The population is condensed to approximately 1.7 ha, due to the construction of a light house and supporting buildings in the late 18th century (Hoare et al., 2006; Thompson et al., 1992). In January 1998, the U.S. Department of the Interior expanded the endangered status to Brother's Island tuatara due to its separation from the Cook Strait tuatara (Clark 1998).

Tuataras are slow to reproduce and grow, which is possibly one of the many reasons for the declining numbers (Clark, 1998). Other reasons include habitat destruction, predation by introduced mammals such as rats, fires, and poaching from illegal visitation to the island (Clark 1998; Nelson et al., 2002). Females reach sexual maturity at approximately 13 years of age (Cree and Butler, 1993). On average, clutches of eggs are laid every 2 - 5 years (Cree and Butler, 1993; Nelson et al., 2002). It can take approximately 25 - 35 years for a tuatara to reach its potential length and mass (Nelson et al., 2002; Thompson et al., 1992). Females can reach a snout-vent length (SVL) of up to 225 mm and a mass of 480 g (Nelson et al., 2002; Thompson et al., 1992). While males can reach a SVL around 270 mm and a mass of approximately 790 g, but maximum size

is unknown prior to the construction (Castanet et al., 1988). The objective of this clinical study was to compare the body weight (mass), SVL, and body condition estimates (BCE) of a zoo population of Brother's Island tuataras housed at the San Diego Zoo (SDZ) to that of the wild.

Methods

Eight Brothers Island tuataras (3.5) have been housed at the SDZ since March 1995. All were born between March and September of 1992. The group was housed together in a 24.38 m long by 7.62 m wide by 3.05 m tall enclosure. Weekly diet (Table 3.1) consisted of a rotation of adult crickets, large Phoenix worms (black-solder fly larvae), wax worms (wax moth larvae), giant mealworms and earthworms. The diet was supplemented daily with calcium carbonate and Herptivite (Rep-Cal® Research Labs, P.O. Box 727 Los Gatos, CA 95031). Once a month they received pinkie mice. Periodic weights and measurements were recorded by animal care staff. Morphological measurements, SVL (mm) and mass (g) from June 2000 to April 2009, were used to calculate BCE, the ratio of log-transformed mass to log-transformed SVL.

Data was analyzed using a PROC GLIMMIX, repeated PROC mixed procedure of SAS and Two-tailed Paired T-tests of Microsoft Excel contrasts to separate significant differences. Pearson's correlation coefficients were used to determine the correlation between SVL and mass. Values were considered significant if at $P < 0.05$.

Results and Discussion

The mean increase ($P = 0.0009$) of mass for the SDZ females (Figure 3.1) from June 2000 to April 2009 was 232.64 g to 567.80 g (rate of gain (RG) = 6.96 g/yr or 144.07 % total increase). The increase ($P = 0.0008$) in SVL (Figure 3.2) from June 2000

to April 2009 was 173.00 mm to 235.60 mm (growth of length (GL) = 6.9 mm/yr or 36.19 % total increase). Males at SDZ had a mean increase in mass (P = 0.0009) and SVL (P = 0.01) change over the 9-year period (mass = 297.1 g to 797.8, RG = 55.62 g/yr or 106.29 % total increase, SVL = 187 mm to 260 mm, GL = 8.11 mm/yr or 27.17 % total increase). The SDZ Bother's Island tuataras have larger SVL and mass than that observed in the wild. As of April 2009, the largest male had a mass of 845 g with a SVL of 269 mm and the largest female's mass was 657 g with a SVL of 245 mm (Table 3.2). Compared with the results from Hoare et al., 2006, 4 of wild tuataras, the largest male sampled had a mass of 655 g and a SVL of 242 mm. The heaviest female sampled weighed 480 g with a SVL of 225 mm.

A high correlation ($r^2 = 0.95$) was detected between SVL and mass. The animals had a linear increase in body condition over time (Figure 3.3). A difference (P = 0.019) between males and female BCE (Figure 3.4 and Table 3.3) was detected after June 2006 to April 2009 with no differences (P > 0.05) detected before this time. Males had a greater increase over time in body condition than females [slopes = 3.31 × 10-5 (P < 0.0001, SEM = 0.004) for males and 2.65 × 10-5 (P < 0.0001, SEM = 0.003) for females] (Figure 3.4 and Table 3.3). The absolute mean increase of BCE for SDZ females was 0.106 (P = 0.002) and the absolute BCE mean increase of 0.118 (P = 0.02) for males. The mean BCE of the SDZ tuataras in June 2000 was 1.06 which is similar to that of the largest tuatara sampled by Hoare et al., 2006 (1957, BCE ~1.09). As of April 2009, the overall mean BCE was 1.14 ± 0.03 for the SDZ tuataras, a 7.5% increase in 9 years. Suggesting that not only are the captive tuatara continuing to gain length, but also gaining mass and conditioning.

By comparing the BCE of the SDZ tuatara of that found in the wild, the SDZ tuatara had more conditioning than that found in the wild. Many factors can contribute to the larger size and conditioning. The zoo tuataras receive a more consistent diet, with no major seasonal variation. According to Hoare, et al., 2006 the wild Brother's Island tuatara are decreasing in length (SVL), mass, and BCE (slope = $6.5 \times 10\text{-}5 \pm 2.5 \times 10\text{-}6$). The loss in total size and conditioning may be partially explained by the loss of habitat from man-made structures in the late 19th century consisting of a light house and supporting buildings thereby reducing the amount of feed resources available (Hoare et al., 2006). Though the SDZ tuatara are larger in size, mass, and BCE than that measured in the wild in 1957, a comparison of pre-construction wild tuatara could not be determined. No data could be found on wild Brother's Island tuatara prior to 1957 and the effects of the loss of habitat may have already taken place, therefore making it difficult to compare BCE of the SDZ tuatara to that of a healthy wild population prior to major human impact.

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Table 3.1. Diet rotation fed to tuatara (*Sphenodon guntheri*) at the San Diego Zoo.

Diet Item[1]	Base Diet per Animal		
	Frequency	No.	As-fed, g/d
Crickets, adult	3 d/wk	8	2.200
Phoenix worms, large	1 d/wk	8	0.900
Wax worms	1 d/wk	8	1.900
Mealworms	1 d/wk	8	3.000
Earthworms	1 d/wk	2	8.000
Mouse, pinkie	1 d/mo	2	3.500
Calcium carbonate	Avg. daily	-	0.046
Herptivite (Rep-Cal)	Avg. daily	-	0.046

[1] Nutrient composition of diet as a percent: CP = 57.0, Ca = 1.91, P = 0.91, Ca:P ratio = 2.10.

Table 3.2. Difference between individual growth in mass, snout-vent length (SVL) and body condition estimate (BCE) of Brother's Island tuatara (*Sphenodon guntheri*) from the beginning of data collection (June, 2000) and the most current (April, 2009).

		---------- Mass, g ----------			------- SVL, mm -------			---------- BCE[1] ----------		
ID	Sex	Jun 00	Apr 09	Diff.	Jun 00	Apr 09	Diff.	Jun 00	Apr 09	Diff.
1	♀	284.8	657.0	372.2	190.0	245.0	55.0	1.08	1.18	0.10
2	♀	188.0	554.0	366.0	160.0	245.0	85.0	1.03	1.15	0.12
3	♀	297.4	591.0	293.6	182.0	233.0	51.0	1.09	1.17	0.08
4	♀	222.2	431.0	208.8	171.0	220.0	49.0	1.05	1.12	0.07
5	♀	170.8	606.0	435.2	162.0	235.0	73.0	1.01	1.17	0.16
6	♂	224.0	689.0	465.0	167.0	246.0	79.0	1.06	1.19	0.13
7	♂	407.7	845.0	437.3	211.0	269.0	58.0	1.12	1.20	0.08
8	♂	259.6	859.0	599.4	183.0	265.0	82.0	1.07	1.21	0.14
$\bar{x}$[2]	♀	232.6[a]	567.8[b]	335.2	173.0[a]	235.6[b]	62.6	1.05[a]	1.16[b]	0.11
$\bar{x}$[2]	♂	297.1[a]	797.7[b]	500.6	187.0[a]	260.0[b]	73.0	1.08[a]	1.20[b]	0.12

[1] Ratio of log-transformed mass to log-transformed SVL.
[2] Mean of mass, SVL, BCE, and absolute change between June 2000 and April 2009.
[a,b] Means within category and sex with unlike subscripts differ ($P < 0.05$).

Table 3.3. A comparison overall mean, standard deviation, slope, r^2, and intercept of the overall mean body condition estimate (BCE), male and female of Brother's Island Tuatara (*Sphenodon guntheri*) over a 9-year period (June 2000 to April 2009).

	Mean BCE[1] Combined	Mean BCE Male	Mean BCE Female	Difference of Males & Females
Mean	1.1317	1.1476	1.1219	0.0256
St. Deviation	0.0307	0.0358	0.0285	0.0074
Slope	2.91 ± 10^{-5}	3.31 ± 10^{-5}	2.65 ± 10^{-5}	6.56 ± 10^{-6}
r^2	0.8828	0.8360	0.8508	-0.0148
Intercept	0.0544	-0.0771	0.1402	-0.2173

[1] Ratio of log-transformed mass to log-transformed SVL.

Figure 3.1. Mean mass, of Brother's Island tuatara (*Sphenodon guntheri*) from June 2000 to April 2009. Female = □, Male = •.

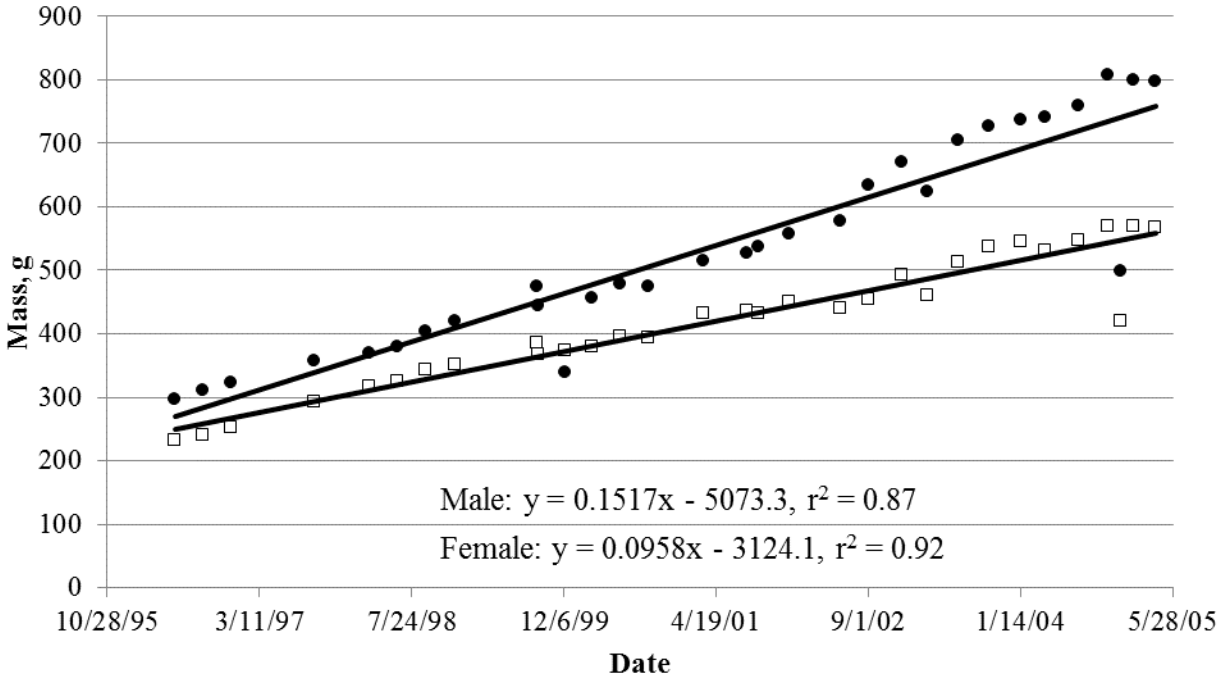


Figure 3.2. Change in snout-vent length (SVL) of Brother's Island tuatara (*Sphenodon guntheri*) from June 2000 to April 2009. Female = □, Male = •.

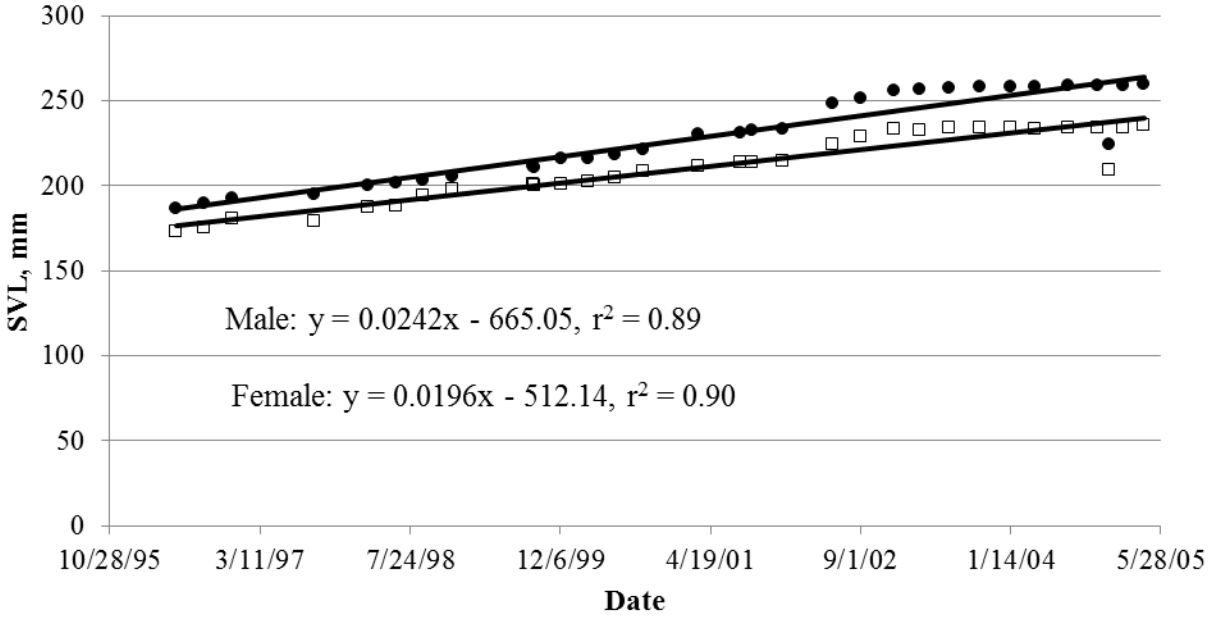


Figure 3.3. Change in mean body condition estimate (BCE) of the Brother's Island tuataras (*Sphenodon guntheri*) housed at the San Diego Zoo from June 2000 to April 2009. BCE = ratio of log-transformed mass to log-transformed SVL.

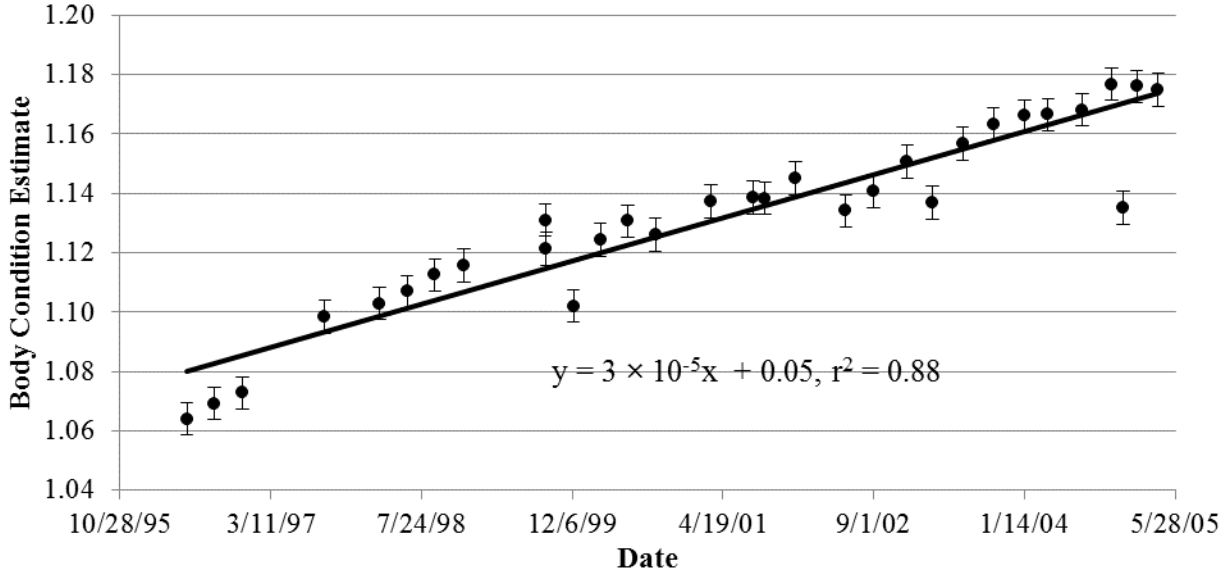
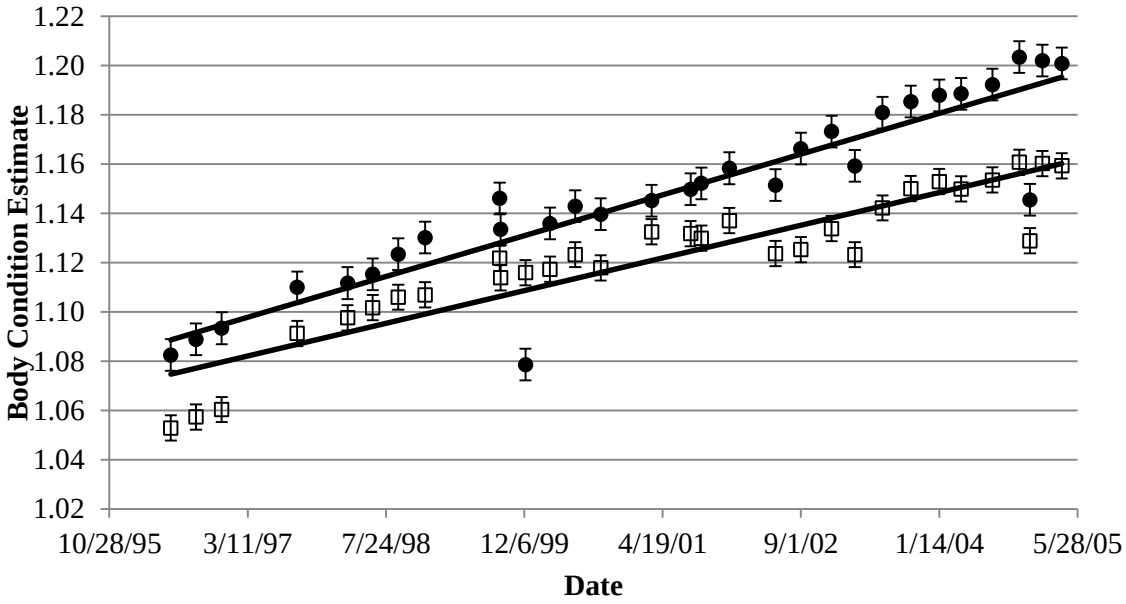


Figure 3.4. Change in mean body condition estimate (BCE) for males and females Brother's Island tuataras (*Sphenodon guntheri*) at the San Diego Zoo from June 2000 to April 2009. BCE = ratio of log-transformed mass to log-transformed SVL.
Female = ☐, Male = •.



CHAPTER IV

ASCORBIC ACID, VITAMIN E, VITAMIN A, AND TRACE ELEMENTS IN SERUM OF ZOO CROCODILIANS

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Abstract

A potential clinical case of ulcerative gingivitis in a male gharial (*Gavialis gangeticus*) initiated an investigation to determine if there was adequate ascorbic acid in the diet of crocodilians at the San Diego Zoo (SDZ), and San Diego Zoo Safari Park (SP). Reptiles can synthesize ascorbic acid and classic deficiency is rarely seen. The objective of this summary was to compare serum trace-mineral and vitamin concentrations of zoo-housed crocodilians to wild (Honeyfield et al., 2008; Huchzerymeyer, 2003; Huchzerymeyer and Huchzerymeyer, 2001) and farmed (Honeyfield et al., 2008; Huchzerymeyer, 2003; Lance et al., 1983) American alligators. For the 20 individuals from four species (American alligators, *Alligator mississippiensis*; Chinese alligators, *Alligator sinensis*; Johnston's crocodiles, *Crococdylus johnsoni*; and gharials), serum

nutrient concentrations averaged 11.06 mg Ca/dl, 91.66 μg Cu/dl, 36.88 μg Fe/dl, 2.86 mg Mg/dl, 4.03 mg P/dl, 3.97 mEq K/L, 153.88 mEq Ma/L, 41.43 mg Zn/dl, 0.50 μg vitamin A/dl, 46.70 μg vitamin E /dl, and 0.66 mg ascorbic acid /dl. No additional deficiencies or toxicities have been observed, although some values were above and below those of wild and farmed American alligators.

Introduction

A lack of reference information exists regarding the serum nutrient concentrations of crocodilians. The current report was initiated after a potential case of ulcerative gingivitis in a gharial (*Gavialis gangeticus*) at the San Diego Zoo (SDZ), and a subsequently-discovered lack of serum ascorbic acid reference ranges in crocodilians. In crocodilian species, cases of ulcerative gingivitis have been associated with ascorbic acid deficiency (Huchzerymeyer and Huchzerymeyer, 2001). Reptiles have the capacity to synthesize ascorbic acid and classic deficiency is rarely seen (Honeyfield et al., 2008). However, individuals can become deficient with continuous stress associated with captivity or sickness (Bendich et al., 1990; Huchzerymeyer, 2003). During times of combined stress and infection, the demand for ascorbic acid is increased and endogenous production cannot keep up with demand (Huchzerymeyer, 2003). To prevent deficiencies it is common to supplement the diet of farmed crocodilians with ascorbic acid (1000 mg/kg DM; Huchzerymeyer and Huchzerymeyer, 2001). The goal of the current report is to start developing serum nutrient reference ranges for zoo crocodilians.

Methods

Upon arrival into quarantine or during examinations at the SDZ or San Diego Zoo Safari Park, blood samples were collected from American alligators (*Alligator mississippiensis,*

0.0.2, 10-April-2013), Chinese alligators (*Alligator sinensis,* 0.2, 2-July-2013), Johnston's crocodiles (*Crococdylus johnsoni,* 0.5, 10-May-2012) and gharials (*Gavialis gangeticus,* 8.2.1, 23-February-2011 and 16-May-2012). Animals were fed frozen, thawed whole prey (mice or fish, with the fish supplemented with thiamin and vitamin E), and in the case of the American alligators, were also fed Mazuri Fish Analog 50/10 Mix (PMI Nutrition International LLC Saint Louis, MO 63108).

Blood was collected by venipuncture into an acid-washed blood collection tubes and centrifuged. Serum was harvested and shipped cool overnight, for analysis of trace mineral concentrations (Ca, Cu, Fe, Mg, P, K, Na, Zn) by ICP-MS, vitamin A, and vitamin E by HPLC at California Animal Health and Food Safety Laboratory System (University of California, Davis, Davis, CA 95617), and ascorbic acid by HPLC at Diagnostic Center for Population and Animal Health (Michigan State University, Lansing, MI 48910-8104). Individual animals with multiple samples were averaged. Serum nutrients were compared to reference values from wild (Honeyfield et al., 2008; Huchzerymeyer, 2003; Huchzerymeyer and Huchzerymeyer, 2001; Lance et al., 2001; Lance et al., 1983) and farmed (Honeyfield et al., 2008; Huchzerymeyer, 2003; Lance et al., 2001; Lance et al., 1983). American alligators. Because no crocodilian reference range for serum ascorbic acid was found in the literature, concentrations were compared among the three species in this report and poultry (Puls, 1994).

Results

American alligator (Table 4.1) serum copper and sodium concentrations were similar to wild and farmed American alligators. Serum calcium, iron, magnesium, phosphorus, potassium, vitamin A and vitamin E concentrations were below the range of

wild and farmed American alligators. Serum zinc concentration and the calcium to phosphorus ratio were above the corresponding ranges for wild or farmed American alligators.

Chinese alligator (Table 4.2) serum magnesium and potassium concentrations were similar to those in American alligators. Serum calcium, iron, phosphorus, sodium, zinc, and vitamin E concentrations were below the range of wild and farmed American alligators. Serum copper concentration was above the range of wild or farmed American alligators.

Johnston's crocodile (Table 4.3) serum calcium, magnesium and potassium concentrations were similar to those of American alligators. Serum iron, phosphorus, zinc, and vitamin E concentrations were below the range of wild and farmed American alligators. Serum copper and sodium concentrations were above the range of wild and farmed American alligators. The calcium to phosphorus ratio was substantially greater for Johnston's crocodiles than for wild or farmed American alligators (Lance, 2001).

Gharial (Table 4.4) serum iron, magnesium, potassium, and zinc concentrations were similar to those of American alligators. Serum calcium and phosphorus concentrations were slightly below the range. Serum copper and sodium concentrations were above the range. Calcium to phosphorus ratio was greater than that of wild or farmed American alligators.

Serum ascorbic acid concentrations were compared among the crocodilian species sampled, poultry (Puls, 1994), and cheetahs (Beckmann, 2013) due to a lack of crocodilian data from the literature. American alligators had the lowest ascorbic acid concentration, followed by gharials, than Johnston's crocodiles (Tables 4.1, 4.3, 4.4).

American alligators, gharials, and Johnston's crocodiles had lower serum ascorbic acid concentration than poultry (Puls, 1994), but higher than reported concentration in captive cheetahs (Beckmann, 2013). Ascorbic acid was not analyzed on serum collected from Chinese alligators.

Conclusion

No further deficiencies or toxicities have been observed despite some mineral and vitamin concentrations being above or below the referenced ranges. Continuing monitoring of health and feed will allow for any diet revisions as new data is obtained. The data provides preliminary data to use as a benchmark for future diet evaluations.

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Table 4.1. Serum mineral and vitamin concentrations of zoo American alligators (*Alligator mississippiensis*).

				Range		Reference values	
Nutrient	n[a]	Mean	SD	Low	High	Wild	Farmed
Ca, mg/dl	2	10.75	3.54	10.50	11.00	12.51[b]	11.50[b]
Cu, µg/dl	2	63.25	0.01	62.50	64.00	76.00[b]	60.00[b]
Fe, µg/dl	1	24.00	NA[d]	24.00	24.00	53.00[b]	54.00[b]
Mg, mg/dl	2	2.55	2.12	2.40	2.70	3.21[b]	2.58[b]
P, mg/dl	2	3.70	4.24	3.40	4.00	6.17[c]	5.73[c]
K, mEq/L	2	3.53	0.11	3.45	3.60	5.42[c]	3.71[c]
Na, mEq/L	2	152.50	10.61	145.00	160.00	154.62[e]	144.20[c]
Z, mg/dl	2	53.00	0.37	27.00	79.00	44.00[b]	42.00[b]
Ca:P ratio	2	2.92	0.24	2.75	3.09	2.03[b,e]	2.01[b,c]
Vit E, µg/dl	1	45.50	NA	45.50	45.50	53.60[b]	52.50[b]
Vit. A, µg/dl	2	0.50	NA	0.50	0.50	0.90[f]	0.85[f]
Ascorbic Acid, mg/dl	2	0.60	0.12	0.51	0.68	0.50[g]	1—2[h]

[a]Two animals sampled for serum minerals, vitamin E, vitamin A, and ascorbic acid, collected in 2013. Multiple samples from any given animal were averaged.
[b]Lance et al., 1983.
[c]Huchzermeyer, 2003.
[d]Not available.
[e]Honeyfield et al., 2008.
[f]Lance et al., 2001.
[g]Compared to cheetah serum concentrations (Beckmann et al., 2013).
[h]Compared to poultry serum concentrations (Puls, 1994).

Table 4.2. Serum mineral and vitamin concentrations of zoo Chinese alligators (*Alligator sinensis*).

			Reference values	
Nutrient	n[a]	Mean	Wild	Farmed
Ca, mg/dl	1	10.00	12.51[b]	11.50[b]
Cu, μg/dl	1	120.00	76.00[b]	60.00[b]
Fe, μg/dl	1	29.00	53.00[b]	54.00[b]
Mg, mg/dl	1	2.80	3.21[b]	2.58[b]
P, mg/dl	1	4.20	6.17[c]	5.73[d]
K, mEq/L	1	3.80	5.42[c]	3.71[d]
Na, mEq/L	1	140.00	154.62[c]	144.20[d]
Z, mg/dl	1	34.00	44.00[b]	42.00[b]
Ca:P ratio	1	2.38	2.03[b,c]	2.01[b,d]
Vit E, μg/dl	1	52.00	53.60[b]	52.50[b]

[a]Two animals total were sampled: samples from one animal were analyzed for serum minerals; samples from the other animal were analyzed for vitamin E; collected in 2012.
[b]Lance et al., 1983.
[c]Honeyfield et al., 2008
[d]Huchzermeyer, 2003.

Table 4.3. Serum mineral and vitamin concentrations of zoo Johnston's crocodiles (*Crocodylus johnsoni*).

				Range		Reference values	
Nutrient	n[a]	Mean	SD	Low	High	Wild	Farmed
Ca, mg/dl	5	12.20	13.04	11.00	14.00	12.51[b]	11.50[b]
Cu, µg/dl	5	86.80	0.10	73.00	98.00	76.00[b]	60.00[b]
Fe, µg/dl	5	36.00	0.06	26.00	40.00	53.00[b]	54.00[b]
Mg, mg/dl	5	2.68	2.49	2.50	3.10	3.21[b]	2.58[b]
P, mg/dl	5	3.84	10.50	2.80	5.10	6.17[c]	5.73[d]
K, mEq/L	5	4.22	0.35	3.60	4.40	5.42[c]	3.71[d]
Na, mEq/L	5	162.00	4.47	160.00	170.00	154.62[c]	144.20[d]
Z, mg/dl	5	34.00	0.15	21.00	57.00	44.00[c]	42.00[b]
Ca:P ratio	5	3.34	0.87	2.35	4.64	2.03[b,c]	2.01[b,d]
Vit E, µg/dl	5	42.60	1.28	32.00	64.00	53.60[b]	52.50[b]
Ascorbic Acid, mg/dl	5	0.78	0.04	0.73	0.84	0.50[e]	1—2[f]

[a]Blood samples were collected from five animals once in 2012 for analysis of minerals, vitamin E, and ascorbic acid.
[b]Lance et al., 1983.
[c]Honeyfield et al., 2008
[d]Huchzermeyer, 2003.
[e]Compared to cheetah serum concentrations (Beckmann et al., 2013).
[f]Compared to poultry serum concentrations (Puls, 1994).

Table 4.4. Serum mineral and vitamin concentrations of zoo gharials (*Gavialis gangeticus*).

				Range		Reference values	
Nutrient	n[a]	Mean	SD	Low	High	Wild	Farmed
Ca, mg/dl	11	11.28	9.81	9.80	13.00	12.51[b]	11.50[b]
Cu, µg/dl	11	96.60	0.15	73.00	120.00	76.00[b]	60.00[b]
Fe, µg/dl	11	58.50	0.24	15.00	81.00	53.00[b]	54.00[b]
Mg, mg/dl	11	3.40	4.64	2.70	4.00	3.21[b]	2.58[b]
P, mg/dl	11	4.36	14.19	2.40	6.50	6.17[c]	5.73[d]
K, mEq/L	11	4.32	0.30	3.80	4.80	5.42[c]	3.71[d]
Na, mEq/L	11	161.00	5.68	150.00	170.00	154.62[c]	144.20[d]
Z, mg/dl	11	44.70	0.17	32.00	84.00	44.00[b]	42.00[b]
Ca:P ratio	11	2.93	1.00	1.69	4.62	2.03[b,c]	2.01[b,d]
Ascorbic Acid, mg/dl	11	0.61	0.07	0.49	0.71	0.50[e]	1—2[f]

[a]Blood samples were collected from one animal in 2011 and 10 animals in 2012 for analysis of minerals, vitamin E, and ascorbic acid.
[b]Lance et al., 1983.
[c]Honeyfield et al., 2008.
[d]Huchzermeyer, 2003.
[e]Compared to cheetah serum concentrations (Beckmann et al., 2013).
[f]Compared to poultry serum concentrations (Puls, 1994).

CHAPTER V

NUTRIENT COMPOSITION OF BANANA TREE (*MUSA* SP.) LEAF, PETIOLE, AND PSEUDO-STEM AT THE SAN DIEGO ZOO

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Introduction

In a zoo environment, different banana tree components are commonly fed to various animals. At the San Diego Zoo, banana tree (*Musa sp*.) petiole and leaves are part of the gorillas' (*Gorilla gorilla gorilla*) browse rotation, and the pseudo-stem is fed once weekly to the Galapagos tortoises (*Chelonoidis nigra*). There have been a few studies that have examined the nutritional composition of banana tree components: crop dry-matter production (Turner, 1972), as cattle feed (Meyreles and Preston, 1977; Ffoulkes et al., 1977), as goat and sheep feed (Poyyamozhi and Kadirvel, 1986; Viswanathan *et al*., 1989), and ruminal dry matter digestibility (Kimambo and Muya, 1991). There is currently a lack of information on the nutritional composition of banana tree components fed as browse (NRC, 2003). Knowing nutritional composition of

different banana tree components can improve diet formulation for captive zoo animals. For this study, the length, weight, and nutritional composition of banana tree, leaves, petiole, and pseudo-stems was analyzed.

Methodology

Six banana trees were harvested in October and November, 2013 on grounds at the San Diego Zoo (San Diego, CA 92112). Once harvested, trees were measured from the tallest point to the end of the cut pseudo-stem. The diameter of the base of the pseudo-stem was measured using calipers. The top of the pseudo-stem was cut at the point where all the petioles separate from the pseudo-stem than measured the length. Each petiole and leaf was separated. Total leaf length was measured from the top of leaf to base of petiole. The leaf was removed from the petiole by cutting the petiole at the base of the leaf than each was measured. The diameter of the petiole was measured at the base using calipers. Each leaf was weighed, cut into 2.54 to 5.08 cm strips, and placed in individual freeze drier crucibles. Each petiole was weighed, cut into 2.54 cm pieces, and placed in individual freeze dyer crucibles. To determine dry matter, leaves and petioles were dried in a freeze drier (Labconco Free Zone 6, Freeze Dryer System, 8811 Prospect Ave, Kansas City, MO 64132) to a constant weight. Leaf sheaths were removed from the pseudo-stalk and weighed. The pseudo-stalk was weighed and cut into 2.54 to 5.08 cm cross sections. Dry matter was determined for the pseudo-stem and leaf sheaths in a forced-air oven at 55˚C to a constant weight. Once dry, all banana tree components were individually ground with a Wiley Mill through a 2 mm screen (Wiley Mill, Model 4, Thomas, 1654 High Hill Road, Swedesboro, NJ 08085). Once ground samples were stored in a -20˚C freezer. The samples of leaves, petioles, and pseudo-stems processed in

October were composited and the samples from November were individually sent to a commercial laboratory for analysis by wet chemistry, of protein, fiber, sugar, starch, fat, ash, and minerals (Dairy One, 730 Warren Road, Ithaca, New York 14850). Leaves and petioles were composited by weight and sent to a commercial laboratory to be analyzed by gas chromatography-MS for individual fatty acids, total saturated fatty acids (**SFA**), total monounsaturated fatty acids (**MUFA**), total polyunsaturated fatty acids (**PUFA**), total omega 3, 6, and 9 fatty acids, along with vitamin E and A by HPLC (Midwest Laboratories, Inc. 13611 B Street, Omaha, NE 68144). Banana leaves, petioles and pseudo-stems were averaged and standard deviation was calculated.

Results and Discussion

Banana tree component measurments are summerized in Table 5.1. The banana leaf and petiole had lower protein than required by primates (15-22%, DM basis; NRC, 2003; Table 5.2), but were high in ADF and NDF to balance low fiber produce items to meet the primate's needs (10-30% NDF, 5-15% ADF, DM basis; NRC, 2003). The banana leaf and petiol meet or exceed the primate's requirement for Ca, Mg, K, Fe, Mn and Cl, but lack P, Na, Zn, and Cu (NRC, 2003). Banana tree leaves are adequete in omega-3 polyunsaturated fatty acids, 3.09% of dry matter (NRC, 2003) to meet primate requirements, but lack the omega-6 fatty acids (Table 5.3). The banana petioles meet the vitamin E requirement of primates (100 IU/kg DM), but not the leaves (NRC, 2003; Table 5.4). Banana leaves contain more than 38 times the dietary primate requirment (8,000 IU/kg DM) of vitamin A in the form of beta carotene (NRC, 2003; Table 5.4). The pseudo-stem is an acceptable source of fiber (NDF: 30.57 ± 2.32, and ADF: 21.77 ± 1.74), but low in protein (>7.2 to 10.5%), and fat (2.1%) based on recommendations for

herbivour tortoises (Hatt *et al*., 2005; Highfield, 2002; Moskovits, 1985; Table 5.2). Psuedo-stem meets or excededs the dietary recommendations for Ca, P, and K, but low in Mg, Na, Fe, Zn, Cu, and Mn (Hatt *et al*., 2005; Allen, and Oftedal, 2003; Moskovits, 1985).

Conclusion

All banana tree components are a good source of fiber for gorillas and herbivour tortoises. Banana tree leaves are a good source of omega-3 fatty acids and beta-carotene. This information can be used to improve diet formulation for animals consuming banana tree components as part of their browse rotation.

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Table 5.1. Banana tree *(Musa sp.)* measurements.

Item	n	Mean	SD
Leaf			
Length, cm	32	100.71	12.01
Weight, g (as-is)	32	65.61	34.67
Petiole			
Length, cm	32	42.68	12.33
Weight, g (as-is_	32	48.80	27.55
Diameter, mm	32	26.08	9.57
Pseudo-Stem			
Length, cm	6	528.07	363.77
Diameter, mm	6	69.58	0.80
Weight, g (as-is)	6	2,788.14	1,888.94
Leaf Sheath, g	6	192.30	45.11
Total			
Leaf and petiole length, cm	32	143.18	19.12
Leaf and petiole weight, g (as-is)	32	114.41	54.31
Tree length, cm	6	230.12	60.61
Tree weight, g (as-is)[1]	6	3,551.52	1,984.46

[1]Leaves, petioles and pseudo-stem.

Table 5.2. Nutrient composition on a dry-matter basis of banana tree *(Musa sp.):* petiole, leaf, and pseudo-stem.

	Petiole		Leaf		Pseudo-Stem	
Item,	Mean	STD	Mean	STD	Mean	STD
Dry Matter (%, as-fed)	8.13	1.98	16.78	2.52	8.53	3.14
Crude Protein, %	4.07	0.31	11.77	0.93	3.03	0.58
Available Protein, %	3.13	0.49	10.33	1.01	2.20	0.70
Unavailable Protein, %	0.93	0.21	1.43	0.71	0.83	0.15
ADF, %	34.13	1.59	27.60	1.25	21.77	1.74
NDF, %	48.67	1.36	48.07	2.37	30.57	2.32
Lignin, %	2.60	0.44	3.13	0.15	2.07	0.12
Crude Fat, %	2.00	0.10	5.77	0.60	1.40	0.10
Ash, %	10.60	2.74	9.62	1.41	8.61	2.08
Calcium, %	0.92	0.16	1.14	0.32	0.55	0.15
Phosphorus, %	0.23	0.10	0.19	0.05	0.19	0.07
Magnesium, %	0.62	0.09	0.51	0.02	0.64	0.10
Potassium, %	2.77	1.30	1.97	0.91	2.44	0.98
Sodium, %	0.10	0.01	0.03	0.01	0.04	0.02
Iron, ppm	135.67	84.51	114.33	34.02	51.67	13.20
Zinc, ppm	12.33	2.52	18.00	3.46	8.67	3.06
Copper, ppm	5.67	1.15	8.33	2.52	4.33	0.58
Manganese, ppm	20.67	10.21	45.00	20.30	13.33	6.66
Molybdenum, ppm	0.23	0.06	0.43	0.21	0.20	0.10
Sulfur, %	0.14	0.06	0.23	0.09	0.11	0.03
Chloride, %	2.42	0.69	1.66	0.09	1.68	0.69
Starch, %	3.30	2.10	1.00	0.46	35.10	1.13
NFC, %[1]	36.00	3.64	28.07	4.31	57.83	4.16
ESC, %[2]	24.73	5.86	14.37	5.12	12.87	3.86

[1]NFC: Non-fibrous carbohydrates.
[2]ESC: Ethanol soluble carbohydrates

Table 5.3. Percent fatty acid concentration and total concentration of fatty acid classes on a dry-matter basis in banana leaves and petiole *(Musa sp.).*

Fatty Acid	Leaves	Petiole
12:0	ND[1]	0.01
14:0	0.02	0.01
15:0	0.04	0.02
16:0	1.09	0.33
17:0	0.04	0.01
18:0	0.14	0.03
18:1 Cis	0.14	0.07
18:2 Cis	0.68	0.43
18:3 alpha	3.09	0.31
20:0	0.02	ND
22:0	0.04	0.02
23:0	0.02	ND
24:0	0.06	0.02
SFA[2]	1.48	0.49
MUFA[3]	0.16	0.08
PUFA[4]	3.76	0.74
n-3 PUFA	3.09	0.31
n-6 PUFA	0.68	0.43
n-9 PUFA	0.14	0.07

[1]Not detected
[2]Total saturated fatty acids (SFA): all fatty acids without any double bond (12:0 to 24:0)
[3]Total monounsaturated fatty acids (MUFA): all fatty acids with a single double bond (12:1 to 18:1)
[4]Total polyunsaturated fatty acids (PUFA): all fatty acids with two or more double bonds (18:2 to 18:3).

Table 5.4. Total concentration of vitamin E and beta-carotene in banana leaves and petiole *(Musa sp.)* on a dry-matter basis.

Item	Leaves	Petiole
Alpha-tocopherol, IU/kg	32.02	120.27
Gamma-tocopherol, IU/kg	1.46	ND[1]
Total Vitamin. E, IU/kg	33.47	120.27
Vitamin A from beta-carotene, IU/kg	322.45	NA[2]

[1]Not detected.
[2]Not analyzed.

CHAPTER VI

ESTIMATED DIGESTIBILITY OF TWO COMMERCIALLY AVAILABLE HERBIVIROUS TORTOISE PELLETS VERSUS HISTORIC SAN DIEGO ZOO GLOBAL DIETS

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Abstract

Diets for captive herbivorous tortoises that contain high protein, high starch, low fiber and low calcium can lead to health disorders. Arid-zone herbivorous tortoises in a captive environment are traditionally fed a produce- based diet that is low in fiber, high in protein, and low in essential minerals (unless properly supplemented). Wild herbivorous tortoises typically consume high-fiber, low-protein diets. The disparity between wild and captive diets can lead to short- and long-term health risks. By comparing the palatability and digestibility of two commercially-available pelleted diets to the currently-fed diet, we can improve health and welfare of animals in the collection, provide data for public education on appropriate tortoise diets, and determine which commercial diet would be of

greater benefit. At San Diego Zoo Global, we have the opportunity to compare digestibility of diets fed to multiple arid-zone herbivorous tortoise species from around the world (African spur-thighed, pancake, California desert, Galapagos, leopard, and radiated tortoises). Two trials were conducted to determine diet digestibility and palatability, and to measure parameters of gastrointestinal health. Trial 1 consisted of a series of digestibility trials on produce-based and commercially pelleted diets, using animals in the SDZG collection. Trial 2 examined the same range of information in a more controlled fashion by simulating the herbivorous tortoise's digestive system within a test tube. The results may be used to improve husbandry at zoos and to better educate pet owners on appropriate care of their companions.

Introduction

Herbivorous tortoises share many aspects of gastrointestinal anatomy and physiology with horses, elephants, and other species that house large populations of cellulolytic microbes in the large intestine[1]. Because these microbes require dietary fiber as a primary substrate, the health of the microbial population, and therefore optimal digestion in the tortoise, depends on a diet of sufficient fiber concentration. Diets for captive herbivorous tortoises are often too high in protein and too low in fiber. Both of these factors can contribute to juvenile growth that is too rapid (Jackson et al., 1976; Baer et al., 1997; Hatt et al., 2005). Rapid growth may result in early sexual maturity and aggression (Jackson et al., 1976) and, particularly in the absence of adequate mineral supplementation, can lead to life-threatening mineral imbalances and skeletal and shell deformities (Jackson et al., 1976; Highfield, 2010). Additionally, high-protein diets may contribute to visceral gout or kidney or bladder stones, whereas low-fiber diets may

contribute to bacterial and cloacal infections (Jacobson, 1994). Too much dietary energy, either from excessive protein or fat, may similarly result in obesity in adult tortoises. Diets with high concentrations of non-fibrous carbohydrates (sugars or starches) may cause a significant imbalance in normal gut flora, leading to poor stool quality, inefficient digestion and absorption, and potential colic.

For any captive species, the overriding principle in formulating a captive diet should be close approximation to the free-ranging diet. Each species' digestive anatomy and physiology is adapted for a specific set of nutritional conditions, often resulting from thousands of years of co-evolution with its habitat; altering those conditions, even to provide a diet that is perceived to be "better" (produce-based), could have serious health consequences. To that end, when available, information on nutrient composition of the wild diet should be used as an approximation of nutrient requirements. Such information is often limited for herbivorous tortoises, but there are a handful of peer-reviewed studies which report wild tortoises consuming a diet with 25 to 30% crude fiber, 22.6 to 32% acid-detergent fiber (ADF), 50% neutral-detergent fiber (NDF), >7.2 to 10.5% crude protein, 2.1% fat, 1.15% calcium, and 0.18% phosphorus (Moskovits, 1985; Highfield, 1988; Highfield, 2002; Hatt et al., 2005). Typical produce-based captive diets compare poorly, containing 17% ADF, 31% NDF, 19% crude protein, 3.5% fat, 1.1% calcium, and 0.45% phosphorus (preliminary data, this study).

Recent advances in feed manufacturing have made low-starch, high-fiber commercially pelleted diets available for the first time. These diets are attractive to captive tortoise managers (zoos and research facilities), as well as pet owners, due to their superior nutritional quality coupled with ease of feeding. However, little experimental

data exists that either compare various new commercial products, or demonstrate the degree of improvement in digestive health and function. We seek to obtain data to satisfy both questions by performing an experimental transition from a produce-based diet to one of two commercial pelleted diets: Zoo Med Natural Grassland Tortoise Food (Grassland) and Mazuri Tortoise LS Diet (Mazuri 5E5L). Both of these products provide superior high-fiber substrate for intestinal microflora, compared to traditional produce-based diets and should result in superior fecal quality and animal health over produce-based diets.

Diet changes were already planned as part of clinical nutritional management of collection animals at San Diego Zoo Global (SDZG); digestibility trials were used to monitor the animals' gastrointestional health progress and collect data of interest. Comparisons were made utilizing two trials. Trial 1 compared the intake and digestibility of two commercially-available herbivorous tortoise pellets (Grassland and Mazuri 5E5L) to each other and to the SDZG traditionally-fed (produce-type) diet. Trial 2 compared *in vitro* fiber digestibility of Galapagos tortoises (*Chelonoidis nigra*) utilizing 3 commercial pellets (Mazuri Tortoise pellet 5M21, Grassland, and Mazuri 5E5L), Bermuda hay, produce, fruit and root mixes.

We hypothesize that Grassland and LS tortoise diets would improve overall apparent digestibility over produce based diets and that fecal quality would be superior for the two pellet diets. Our objective was to determine the effect of diet source (Grasslanf, Mazuri 5E5L, and Bermuda hay) on *in vitro* dry matter and fiber digestibility and fecal quality.

Materials and Methods

Trail 1

Trial 1 was conducted at the San Diego Zoo, utilizing 1.2 *C. sulcata*, 0.0.3 *Malacochersus tornieri* (pancake tortoise), 0.0.2 *Gopherus agassizii* (California desert tortoise), 1.1 *C. nigra*, 2.2.4 *S. pardalis* (4 adults and 12 juveniles), and 2.1 *Astrochechelys radiate* (radiated tortoise). Animals that were group-housed were treated as one individual and data were averaged for that group. Due to limitations of exhibitry, tortoises were group-housed by species in their typical enclosures at SDZG. *C. sulcata*, *C. nigra*, and adult *S. pardalis* were in exhibits open to the public. Zookeepers were responsible for enclosure maintance, with diet components and proportions provided by investigators. Animals were fed between 0800 and 0845. Feed ingredients were weighed and recorded prior to feeding. Orts were collected and weighed the same day between 1400 and 1430. Animals had *ad libitum* access to water in concrete ponds, automatic water bowls, or water bowls, depending on the enclosure. Water was provided to *M. tornieri* by soaking the *M. tornieri* in room temperature water for 30 to 45 min, biweekly, except during the collection period to prevent chances of defecating in the water. Hay normally used for bedding was removed and replaced with paper or nothing, depending on exhibit layout.

The study had 3 phases using 3 diets (A, B, C; Table 6.1 and Table 6.2). Diet A, the older diet for herbivourus tortoises at SDZG, consisted mostly of produce, roots, fruits, Bermudagrass hay and was supplemented with Mazuri 5M21 (PMI Nutrition International LLC Saint Louis, MO 63108). Diet B consisted of 5% produce, 5% Bermudagrass hay and 90% Grassland (Zoo Med Laboratories Inc., San Luis Obispo,

CA). Diet C consisted of 5% produce, 5% Bermudagrass hay and 90% Mazuri 5E5L (PMI Nutrition International LLC Saint Louis, MO 63108). To account for any error from intake estimation and to provide enough feed for all individuals housed in groups, amounts of Diets B and C offered corresponded to 120% of caloric intake of Diet A for each species. Diets B and C were formulated to be isocaloric (Table 6.2). Diets B or C were offered in phases 2 and 3 (see below), and were assigned in random order.

To decrease the number of diet transitions, all tortoises in the study remained on the SDZG traditional diet (Diet A) during phase one. Because no diet transition was needed, the study started with a 14-d collection period. Fecal quality was evaluated prior to the start of the study. Every 24 h during the collection period, samples of diet ingredients, orts, and feces were collected, weighed, dried (to constant weight at 55°C), ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) equipped with a 2-mm screen, than stored in a -20°C freezer until analysis. Samples of feed, orts and feces were analyzed for apparent digestibility of dry matter, crude protein, crude fat, NDF, ADF, and ash. Values of NDF and ADF were determined via Van Soest method (Van Soest et al., 1991; Ankom Technology Corp., Fairport, NY) than corrected for ash. Ash was determined after 5 h of oxidation at 500°C in a muffle furnace. Crude protein was analyzed via combustion (Model FP-2000, LECO, St. Joseph, MI) at the ruminant nutrition laboratory at Oklahoma State University (Stillwater, OK). Crude fat was analyzed by ether extract at Dairy One Forage Lab Services (Ithaca, NY). Apparent digestibility was calculated based on equations provided by Galyean, 1997.

Feces were scored daily during the collection period and averaged by treatments using an adopted method utilizing a panda fecal scoring system (Edwards and Nickley,

2000). After Phase 1, animals were gradually introduced to the Phase 2 diet. Transition took 1 to 3 weeks, depending on keeper feedback on animals' consumption of the new diet. After the diet change there was a 28-day acclimation period, prior to the 14-day collection period, to allow the animals' digestive physiology to adjust to the new diet (Bjorndal, 1987; Meienberger, 1993; Hatt et al., 2005; Tracy et al., 2006; Lickel et al., 2010). A similar process occurred between Phases 2 and 3 with a 1-week diet transition and a 28-day acclimation period followed by a 14-d collection period.

Trail 2

Trial 2 was conducted at SDZG utilizing fresh feces from *C. nigra* as a microbial source for *in vitro* dry matter and fiber digestibility. Dry matter digestibility was analyzed using a common method (Tilley and Terry, 1963). Fiber digestion was analyzed by placing 0.5 g of feed into F57 filter bags (Ankom Technology Corp., Fairport, NY). NDF was analyzed as described by Van Soest et al. (1991; Method A for NDF; Ankom Technology Corp., Fairport, NY). Bacterial inocula comprised of 60 g of wet feces collected within 30 minutes of defecation per liter (Omed et al., 1989) of McDougal's buffer (McDougall, 1948). To determine the *in vitro* digestive efficiency of each tortoise species, 0.5 g of individual feed ingredients were tested and compared across species. Feed samples included Mazuri 5M21, Grassland, Mazuri 5E5L, SDZG green produce mix, SDZG fruit mix, SDZG root mix, and Bermudagrass hay. During fecal collection, animals were offered Diet B. A 48-h fecal inoculum digestion was utilized to replicate hindgut fermentation, allowing for interspecific comparison of dry matter and fiber digestion. The *in vitro* results were compared to the estimated apparent digestibilities from Trial 1.

Trail 3

Trial 3 was conducted at the ruminant nutrition laboratory at Oklahoma State University (Stillwater, OK) utilizing fresh fecal samples collected from *Aldabrachelys gigantean* housed at Tulsa Zoo and Living Museum (Tulsa, OK) as a microbial source for *in vitro* kinetic gas production, dry matter (**IVDMD**) and NDF digestibility (**IVNDFD**). Tortoises were offered there standard diet (Table 6.3). After collection the fecal samples were transported in a thermos pre-warmed to 39˚C. Fecal samples were utilized within 1.5 h of collection. Bacterial inocula was comprised of 60 g of feces per liter (Omed et al., 1989) of McDougal's buffer (McDougall, 1948).

For *in vitro* kinetic gas production; 18 gas production modules (Ankom Technology Corp., Fairport, NY) were used to incubate feed samples in duplicate with one additional module used as a blank. Feed samples included Mazuri 5M21, Grassland, Mazuri 5E5L, SDZG green produce mix, SDZG fruit mix, SDZG root mix, and Bermudagrass hay. Each 250 mL module contained 0.7 ± 0.01 g of feed sample plus 50 mL of the bacterial inoculum. Each flask was flushed with CO_2 for 30 seconds followed by immediately attaching the module. Modules were than placed in a 39˚C shaking water bath set at 45 rpm (Thermo Fisher Scientific Inc., Marietta OH) for 48 h. Each module sends data wirelessly to the computer every 10 min for the 48 h period. To prevent gas pressure buildup, the modules were set to release pressure at 20.7 kPa, allowing the program to determine cumulative gas pressure for each time point. Cumulative gas pressure was recorded in psi and converted to milliliters of gas produced per gram of dry matter digested at each time point using the equation provided by Ankom (Ankom Technology Corp., Fairport, NY):

$$G = (Vh/Pa) \times Pt$$

where G is gas volume, Vh is headspace volume, Pa is atmospheric pressure, and Pt is pressure measured by the transducer. The rate of gas production (slope) was than calculated using peak of milliliters of gas produced per gram of dry matter digested over the peak time.

IVDMD and IVNDFD: To determine the IVDMD efficiency, individual feed ingredients were tested in duplicate using 2 digestion jars. Feed samples included Mazuri 5M21, Grassland, Mazuri 5E5L, SDZG green produce mix, SDZG fruit mix, SDZG root mix, and Bermudagrass hay. IVDMD and IVNDFD was analyzed by placing 0.5 g of feed into F57 filter bags (Ankom Technology Corp., Fairport, NY) and placing into a Daisy Incubator jar (Ankom Technology Corp., Fairport, NY) with 1600 mL of bacterial inocula pre-warmed at 39˚C and flushed with CO_2 for 45 seconds. Daisy incubator was set at 39˚C and samples digested for 48 h. The F57 filter bags were pre-labeled and soaked for 5 min in acetone and dried at 55˚C for 12 h prior to adding samples. The pH was checked at the end of 48 h. Samples were gently rinsed with distilled water. IVDMD was analyzed by drying the samples post digestion at 55˚C for 12 h than weighing. NDF for IVNDFD was analyzed as described by Van Soest et al. (1991; Method A for NDF; Ankom Technology Corp., Fairport, NY). Results are compared to Trial 1 and Trial 2.

Data were analyzed in a PROC GLM procedure of SAS using LS means and orthogonal contrasts to separate significant treatment differences. Values were considered significant at ($P < 0.05$); if the value was between 0.05 and 0.10 it was considered a tendency towards significance. Data presented are Least squares means. Daily fecal

nutrient analysis was unable to be achieved resulting in all fecal material per period within group to be composited.

Results

Trial 1

Dry matter intake improved with all tortoises on diets B and C over Diet A. Diet C had a higher dry matter intake than Diet B: *C. sulcata*: 448.59, 557.61, 723.74 g/d, *M. tornieri*: 5.76, 8.52, 10.97 g/d, *G. agassizii*: 7.88, 16.05, 20.89 g/d, *C. nigra*: 704.84, 919.12, 1143.69 g/d, *S. pardalis* adults: 333.62, 475.71, 604.63 g/d, *S. pardalis* juveniles: 3.74, 10.41, 13.24 g/d, and *A. radiate*: 20.68, 48.49, 62.45 g/d respectfully (Table 6.4).

Total fecal output (DMB) and fecal percent DM results can be found on Table 6.5. The only tortoise to produce more g/d of feces while consuming Diet A was *C. sulcata* at 103.23 g/d. *C. nigra* had the greatest increase in fecal output with Diet A (167.79 g/d) compared to Diets B (318.48 g/d) and C (311.71 g/d, respectfully). This occurred while fecal DM also decreased from 42.15% when consuming Diet A to 24.07 and 24.75% with Diets B and C. *A. Radiate* demonstrated similar fecal output for Diets A and B (10.59 and 10.54 g/d), while Diet C produced less at 5.87 g/d. *S. pardalis* adults secreted more fecal material while consuming diet B (103.90 g/d) while the juveniles produced more on diet C (1.80 g/d). Most fecal DM where similar across diets; however, *M. tornieri* demonstrated a trend to increase DM percentage from Diet A (36.92%) to Diets B (54.49%) and C (59.47%). Overall fecal quality was averaged for all tortoises on Diets A, B, and C. The mean fecal score improved with Diets B (4.0 ± 1.0) and C (4.0 ± 1.5), over A (2.5 ± 1.0; Table 6.5 and Figure 6.1).

Apparent dry matter (DM) digestibility results can be found in Table 6.4. Apparent dry matter digestibility for *C. sulcata* was similar between the 3 diets (66.93, 65.06, and 67.34% respectively). *A. radiate* apparent dry matter digestibility for Diets A (78.59%) and B (77.93%) where similar while Diet C (64.23%) was less. Slight difference was observed between the diets fed to *M. tornieri*, with Diet A having the greatest apparent DM digestibility (93.75%) followed by Diet B (87.86%) and Diet C (83.00%). Diet A had a greater DM digestibility than Diets B and C when consumed by *G. agassizii* (85.00, 53.22, 68.18 %), *S. paradalis* (adults [73.37, 62.91, 64.05 %] and juveniles [95.54, 81.19, 76.16 %]). *C. niga* had the largest observed difference in DM digestibility between Diet A (72.24%), Diet C (26.82%), and Diet B (44.36%). Overall dry matter digestibility varied between all tortoises on diet A (SEM ± 10.57), Diets B (SEM ±15.69) and C (SEM ± 17.89).

Apparent CP digestibility (Table 6.4) differed slightly between all tortoises fed Diet A (SEM ± 4.29). The largest difference was observed between all the tortoises fed Diet C (SEM ± 20.40), followed by Diet B (SEM ± 13.39). Diet A (91.53%) had the highest CP digestibility compared with Diets B (60.12%) and C (59.25%) with all animals. *C. niga* had the lowest CP digestibility at 15.95% when consuming Diet C.

Apparent NDF digestibility (Table 6.4) was similar between all diets for *M. tornieri* (90.93, 85.70, and 86.85%, respectively). *S. pardalis* juveniles demonstrated the highest NDF digestibility at 93.91% when consuming Diet A, Diet B at 91.54% and Diet C at 69.30%. Adults tended to be similar at 68.79, 59.03, and 60.44% respectfully. *C. Sulcata* had a low NDF digestibility while consuming Diet A (45.23%), while little

difference was detected between Diets B (59.54%) and C (61.32%). *C. nigra* had the lowest observed NDF digestibility while consuming Diet C (15.39%).

Acid detergent fiber (ADF) apparent digestibility (Table 6.4) was improved between Diet A, B, and C: *C. sulcata*: 19.55, 47.70, 55.81% and *A. radiate*: 42.73, 69.41, 62.07%. ADF digestibility decreased between Diet A when compared to Diets B and C for *M. tornieri*: 90.93, 85.70, 86.85%, *G. agassizii*: 94.61, 90.13, 78.08%, and *C. nigra*: 37.54, 17.11, 14.43% respectfully.

Trial 2 and 3

In vitro dry matter digestibility (IVDMD) and *in vitro* NDF digestibility (IVNDFD) results are in Table 6.7. No difference was observed with IVDMD between Zoomed Natural Grassland Tortoise Food (54.64%), Low Starch Mazuri Tortoise Diet (57.47%), and Mazuri Wild Herbivore Plus Diet (59.20%). Bermuda hay was significantly greater IVDMD than the rest of the ingredients at 64.35% ($P < 0.05$). No difference was detected with IVDMD when compared to large, small, and composite produce chop at 35.95%, 36.25%, and 35.98%, respectfully ($P > 0.05$). A difference was detected in fruit and root mix IVDMD at 23.73% and 17.11% ($P < 0.05$). Zoomed Natural Grassland Tortoise Food (34.71%), Mazuri Wild Herbivore Plus Diet (39.76%), and Bermuda hay (50.78%) had similar IVNDFD. Low Starch Mazuri Tortoise Diet (35.44%) was not different than Zoomed Natural Grassland Tortoise Food ($P > 0.05$). No difference in IVNDFD was detected for large (8.81%), small (10.50%) and composite (16.75%) produce chop, and fruit mix (11.89%). IVNDFD for root mix (9.37%) was significantly different than all other feed ingredients except fruit mix and large produce

chop ($P < 0.05$). Large and composite produce chop had a tendency to be different in IVNDFD ($P < 0.10$).

Results of *in vitro* Gas production of digested individual feed ingredients fed to herbivorous tortoises are shown in Table 6.7. The two commercially complete extruded pellets, Zoomed Natural Grassland Tortoise Food (4.14 mL of gas/100 mg of DM) and Low Starch Mazuri Tortoise Diet (5.56 mL of gas/100 mg of DM) were not significantly different than that of Bermuda hay (6.98 mL of gas/100 mg of DM). Mazuri Wild Herbivore Plus Diet produced the least at 3.02 mL of gas/100 mg of DM. The SDZG fruit and root mix where similar at 11.22 and 14.25 mL of gas/100 mg of DM, respectfully. Zoomed Natural Grassland Tortoise Food, Low Starch Mazuri Tortoise Diet, and Bermuda hay where significantly different ($P < 0.05$) than fruit mix, root mix, large produce chop, and small produce chop.

Discussion

Trail 1

Diet dry matter intake was highest in diet C, followed by diet B, than A. The produce diet (diet A) had the lowest mean dry matter (~21.12 ± 9.87), than the pellet based diets (B: 89.36 ± 0.63; C: 91.64 ± 0.39), suggesting that the tortoises could have reached gut capacity faster on the produce diet. Nutrients including fiber were diluted in the produce diet due to high moisture content. The difference observed between the dry matter intakes of diet B and C could be partially explained by the slight increase in dry matter in the LS tortoise pellet over the Grassland pellet. Also, the higher consumption may suggest a greater palatability/preference for the LS tortoise pellet than the Grassland

pellet. Tortoise group's *G. agassizii*, *M. tornieri*, and *S. pardalis* juveniles consistently had the highest DM digestibility than the other species. The higher DM digestibility is possible because the diets provided the only source of water that these groups received during the collection periods. This forced the animals to be more efficient and absorbing water and perhaps reducing digesta rate of passage. The exception is *G. agassizii* while being fed diet B. Dry matter digestibility decreased while consuming diet B most likely due to the animals accidently consuming small rocks found in there exhibit which forced them to be relocated to another location. Overall, DM digestibility for all tortoises consuming all three diets where similar to that found in previous papers (Hatt et al., 2005; Lickel et al., 2010). Lackel et al. (2010) demonstrated that *S. pardalis* had an apparent DM digestibility of 83.6 to 85.4%. Others have demonstrated a DM digestibility for *C. nigra* ranging from 65% (Liesegang et al., 2001), 49 to 80% (Hatt et al., 2005) and *G. agassizii* 53 to 76% (Barboza, 1995; Meienberger et al., 1993). Fermentation of cell wall components within the hindgut is much slower than cell contents digested by endogenous enzymes within the small intestine (Van Soest, 1987). The two test diets contained high levels of cell contents could also explain the high apparent digestibility of DM. Diet A having a higher DM digestibility may also be explained by limit feeding. Diet A was not fed on a daily basis with two fast days given per week. Lackel et al. (2010) suggested that limit feeding tortoises a complete diet may decrease rate of passage while increasing digestion efficiency. In the wild herbivorous tortoises consume small quantities of browse and graze throughout the day. Tortoises that are housed without access to free range pasture may benefit from a limit feeding program.

Fecal quality was greatly improved by feeding the two commercially available extruded pellet based diets over the traditional produce based diet, supporting the hypothesis that feeding a similar amount of fiber found in wild herbivorous tortoise diets will improve fecal quality over a low fiber, high moisture produce based diets (Jacobson, 1994; Highfield, 2010). Increasing fecal quality will decrease the occurrence of bacterial and cloacal infections (Jacobson, 1994). The improvement of fecal quality is an effect of good gastrointestinal and hindgut microbial health from increasing dietary fiber (Baer et al., 1997; Hatt et al., 2005; Highfield, 2010). The increase in fiber and feed dry matter also decreases the digesta rate of passage (Bjorndal, 1987). Decreasing the rate of passage allows more time for nutrient breakdown and utilization by the hindgut microbes and improving digestibility (Bjondal, 1987; Meienberger, 1993; Lickel et al., 2010). The exception to this explanation can be found in the Galapagos tortoises where the higher DM and fiber diets resulted in lower CP, NDF, and ADF digestibility. These diets also increased fecal output (Diet A: 167.79 g/d, Diet B: 318.48 g/d, and Diet C: 311.71 g/d) while simultaneously decreasing fecal DM content (Diet A: 42.15 %, Diet B: 24.07 %, and Diet C: 24.74 %) when compared to the high moisture low fiber diet A. The higher DM diets most likely caused an increase in water consumption and or soaking, resulting in the decrease fecal DM content observed with diets B and C. Combined with a higher DMI in Diet C (1143.69 g/d) and Diet B (919.12 g/d) compared to Diet A (704.84 g/d), may suggest an increase in digesta rate of passage despite the increase in measurable fiber. Perhaps in giant tortoise species fiber length plays an important role in slowing down rate of passage. Further research needs to be conducted on fiber particle length for giant herbivorous tortoises.

Overall apparent crude protein and fiber (NDF, ADF) digestibility was highest for Diet A, followed by Diet B than C for most tortoise species (Table 6.4). The biggest change in CP, NDF and ADF digestibility was observed in *C. nigra* (Table 6.4). The group of *C. nigra* drop in digestibility's while consuming Diet C might be explained by the animals consuming an unknown amount of tree leaves on a daily basis that where falling into their exhibit. Zoo staff would remove leaves on an hourly basis during zoo hours; however, a large amount was still seen being consumed based from the leaves found in the feces. Hatt et al. (2005) reported juvenile *C. nigra* apparent CP digestibility of 48 to 78%. No other past research could be found on CP digestibilities in herbivorous tortoises. Previous studies have reported similar results on NDF digestibility's for *G. agassizii* with 29 to 67% (Meienberger et al., 1993; Barboza, 1995), *C. nigra* with 55 to 93% (Liesegang et al., 2001), and 75.4 to 78.5% in *S. pardalis* (Lickel et al., 2010). Apparent digestibility of ADF was similar to previously reported papers *C. nigra* 19 to 71% (Hatt et al., 2005), 62% (Liesegang et al., 2001) and S. pardalis at 78.2 to 81.4% (Lickel et al., 2010). Herbivorous tortoises had very comparable dry matter and fiber digestibility when fed the two pellet-based diets to that of horses (NRC, 2007). As mentioned above, with improved microbial health comes increase in digestibility and fecal quality (Bjorndal, 1987; Meienberger et al., 1993; Lickel et al., 2010).

The apparent digestibility of fiber for the juvenile *S. pardalis* was consistently higher than the adults of their own species and the other species of tortoises, while the dry matter digestibility was similar for all diets. The ability to efficiently break down fiber is related to gastrointestinal health, passage rate and overall gut length (Tracy et al., 2006). These young tortoises have a smaller, not fully developed gastrointestinal tract,

that may possibly have less microbial population and variety than that of their adult counter parts (Hatt et al., 2005; Tracy, 2006; Highfield 2010; Lickel, 2010). However, the juveniles showed greater efficiency at digesting fiber and dry matter possibly suggesting that the fiber length found within the pellets might be of appropriate size to maximize digestion by slowing down rate of passage.

Trail 2 and 3

No past research could be located describing *in vitro* dry matter digestibility, *in vitro* NDF digestibility and *in vitro* gas production in herbivorous tortoises. IVDMD of Zoomed Natural Grassland Tortoise Food, Low Starch Mazuri Tortoise Diet, Low Wild Herbivore Plus Diet, and Bermuda hay compared to apparent DM digestibility was within the range of that reported past research of *G. agassizii* on a high fiber pellet or produce 52 ± 2 to 69 ± 4% (Barboza, 1995) and C. nigra on a high fiber diet 49 to 80% (Hatt et al., 2005). This suggests that the higher amount of fiber provided by the pellets and hay deliver a superior substrate for microbial fermentation than that of produce. This is supported by the high dry matter, NDF, and ADF apparent digestibility. O'Niell et al. (2013) used barley which is a high starch grain and rumen fluid as the bacteria inocula source. Feedstuffs utilized in the present experiment are not comparable to barley as most contain higher fiber and low starch, except the fruit and root mixes.

Conclusion

This study showed that captive herbivorous tortoises can improve their gastrointestinal health and fecal quality by increasing the diet fiber and dry matter to similar levels found in free ranging tortoises. Increased DMI, decreases overall

digestibility. Feed ingredients high in fiber can be compared using different digestibility methods resulting in similar IVDMD and IVNDFD. Feeding only a complete pellet may not be optimal for captive herbivorous tortoise. Further research is needed to evaluate appropriate diets for captive herbivorous tortoises housed with no access to graze or browse. The results can be utilized to help educate herbivorous tortoise caretakers in a zoo setting or a companion animal in the pet trade to prevent dietary based illnesses. Proper diet, hydration, and environment can prevent many diseases found in pet tortoises (Jacobson, 1994; Highfield 2002). Further study is needed to determine the long term health benefits of providing a high fiber complete pellet diet to captive herbivorous tortoises.

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Table 6.1. Foods offered to foods of herbivorous tortoises at San Diego Zoo Global, on as-fed basis.

Item	*Centrochelys sulcata*[1]	*Malacochersus tornieri*[1]	*Gopherus agassizii*[1]	*Chelonoidis nigra*[1]	*Stigmochelys pardalis* (Adults)[1]	*Stigmochelys pardalis* (Juveniles)[1]	*Astrochechelys radiate*[1]
Diet A[2]							
Produce, g/d[3]	1672	26	33	3055	1274	17	1289
Fruit Mix, g/d[4]	318	1	4	NA	NA	1	12
Root Mix, g/d[5]	NA[6]	NA	NA	NA	165	NA	NA
Bermuda, g/d	91	NA	NA	194	80	NA	46
Banana Tree, Trunk, g/d[7]	NA	NA	NA	703	NA	NA	NA
Pellet, g/d[8]	55	NA	NA	108	69	NA	30
Total, g/d	2136	27	37	4060	1588	18	1377
Diet B							
Produce, g/d[2]	30	1	1	372	31	1	5
Bermuda, g/d	30	1	1	130	31	1	20
Banana Tree, Trunk, g/d[7]	NA	NA	NA	703	NA	NA	NA
Pellet, g/d[9]	590	8	16	940	478	12	50
Total, g/d	650	10	18	2145	540	14	75
Diet C							
Produce, g/d[2]	37	1	1	387	31	1	5
Bermuda, g/d	37	1	1	90	31	1	20
Banana Tree, Trunk, g/d[7]	NA	NA	NA	703	NA	NA	NA
Pellet, g/d[10]	740	10	20	770	610	16	70
Total, g/d	814	12	22	1950	672	18	95

[1]1.2 *Centrochelys sulcata*, 0.0.3 *Malacochersus tornieri*, 0.0.2 *Gopherus agassizii*, 1.1 *Chelonoidis nigra*, 2.2 *Stigmochelys pardalis* (Adults), 0.0.12 *Stigmochelys pardalis* (juveniles), 2.1 *Astrochechelys radiate*.
[2]Diets offered on Sunday, Monday, Wednesday, Friday, and Saturday.
[3]Produce included: roman lettuce, dandelions, collard greens, spinach, and bok choy.
[4]Fruit Mix included: apples, grapes, turnips, and carrots.
[5]Root Mix included: turnips and carrots.
[6]Not available
[7]Offered on Thursdays
[8]Mazuri Tortoise Diet 5M21 (Land O'Lakes, Inc., PMI Nutrition International LLC's). Weight is equal to 50% water.
[9]Zoomed Natural Grassland Tortoise Food Zoo Med Laboratories Inc., San Luis Obispo, CA).Weight is equal to 50% water.
[10]Low Starch Mazuri Tortoise pellet (PMI Nutrition International LLC Saint Louis, MO 63108).

Table 6.2. Composition of diets fed to herbivorous tortoises (DMB).

Item	*Centrochelys sulcata*[1]	*Malacochersus tornieri*[1]	*Gopherus agassizii*[1]	*Chelonoidis nigra*[1]	*Stigmochelys pardalis* (Adults)[1]	*Stigmochelys pardalis* (Juveniles)[1]	*Astrochechelys radiate*[1]
Diet A							
DM, %	17.03	9.67	9.92	14.99	13.34	9.74	13.45
CP, %	18.13	21.80	20.36	17.31	16.59	21.34	19.74
NDF, %	34.69	16.96	16.55	39.62	30.44	16.83	28.43
ADF, %	19.17	12.06	11.61	21.96	16.21	11.92	16.67
ME, kcal/g	2.23	3.13	3.18	2.35	2.05	3.41	2.45
Fat, %	3.45	4.02	3.83	3.34	2.33	3.96	3.77
Ash, %	12.22	11.25	10.59	12.68	10.21	11.05	11.75
Ca, %	1.20	0.97	0.89	1.07	1.26	0.94	1.16
P, %	0.53	0.39	0.37	0.42	0.63	0.38	0.46
Ca:P Ratio	2.28	2.47	2.39	2.54	1.99	2.45	2.55
Diet B							
DM, %	45.97	46.39	46.08	39.74	46.10	46.26	54.91
CP, %	11.75	11.79	11.76	12.21	11.76	11.77	11.56
NDF, %	50.41	51.36	50.65	50.53	50.7	51.06	55.62
ADF, %	27.19	27.57	27.29	27.20	27.31	27.45	29.34
ME, kcal/g	2.12	2.09	2.11	2.10	2.11	2.10	1.96
Fat, %	3.26	3.15	3.23	3.11	3.22	3.18	2.74
Ash, %	10.44	10.68	10.5	10.87	10.51	10.61	11.48
Ca, %	1.36	1.29	1.34	1.23	1.34	1.31	1.07
P, %	0.6	0.56	0.59	0.53	0.59	0.57	0.45
Ca:P Ratio	2.28	2.29	2.28	2.31	2.28	2.29	2.35
Diet C							
DM, %	46.19	46.56	46.28	35.72	46.29	46.39	53.77
CP, %	13.73	13.60	13.69	13.88	13.69	13.66	12.93
NDF, %	47.49	48.59	47.76	47.12	47.78	48.08	52.97
ADF, %	30.94	30.98	30.95	29.76	30.95	30.96	31.63
ME, kcal/g	1.69	1.70	1.69	1.75	1.69	1.69	1.68
Fat, %	3.17	3.08	3.15	3.05	3.15	3.12	2.75
Ash, %	10.10	10.35	10.16	10.56	10.17	10.24	11.12
Ca, %	1.44	1.37	1.42	1.29	1.42	1.40	1.16
P, %	0.71	0.67	0.70	0.62	0.70	0.69	0.55
Ca:P Ratio	2.03	2.05	2.04	2.08	2.04	2.04	2.11

[1]1.2 *Centrochelys sulcata*, 0.0.3 *Malacochersus tornieri*, 0.0.2 *Gopherus agassizii*, 1.1 *Chelonoidis nigra*, 2.2 *Stigmochelys pardalis* (Adults), 0.0.4 *Stigmochelys pardalis* (juveniles), 2.1 *Astrochechelys radiate*.

Table 6.3. Diet of *Aldabrachelys gigantean* at Tulsa Zoo and Living Museum used as fecal donors for bacterial inocula, on as-fed basis.[1]

Item	Grams per day
Produce[2]	323.91
Fruit Mix[3]	22.76
Root Mix[4]	158.86
Hay[5]	*Ad libitum*
Pellet[6]	23.52

[1]Diet provided was given in produce bundles. Diet weights are estimated from previously weighed produce. Items offered 3 times per week and averaged for daily intake. Calcium and vitamins were supplemented via calcium carbonate 3 time a week and Vionate (ARC Laboratories, Atlanta, GA) 1 time a week.
[2]Produce included: turnip greens, collard greens, mustard greens, and kale.
[3]Fruit mix included: apples and 1 orange.
[4]Root mix included: sweet potatoes and carrots.
[5]Hay included: *ad libitum* prairie grass and alfalfa provided by a hay rack on a wall.
[6]Mazuri Tortoise Diet 5M21 (Land O'Lakes, Inc., PMI Nutrition International LLC's).

Table 6.4. Dry matter intake and apparent dry matter, crude protein, and fiber digestibility of herbivorous tortoises.

Item	*Centrochelys sulcata* [1]	*Malacochersus tornieri* [1]	*Gopherus agassizii* [1]	*Chelonoidis nigra* [1]	*Stigmochelys pardalis* (Adults)[1]	*Stigmochelys pardalis* (Juveniles)[1]	*Astrochechelys radiate* [1]	Mean	SEM
Intake									
Diet A, DM, intake g/d	448.59	5.76	7.88	704.84	333.62	3.74	20.68	NA[2]	NA
Diet B, DM intake, g/d	557.61	8.52	16.05	919.12	475.71	10.41	48.49	NA	NA
Diet C, DM intake, g/d	723.74	10.97	20.89	1143.69	605.63	13.24	62.45	NA	NA
Dry Matter Digestibility [3]									
Diet A, %	66.93	93.75	85.00	72.24	79.37	95.54	78.59	81.63	10.57
Diet B, %	65.06	87.86	62.19	44.36	62.91	81.19	77.93	68.79	14.66
Diet C, %	67.34	83.00	68.18	26.82	64.05	76.16	64.23	64.25	17.89
CP Digestibility [3]									
Diet A, %	85.02	93.91	97.09	91.35	88.40	95.61	89.32	91.53	4.29
Diet B, %	54.21	77.61	62.81	41.24	51.37	72.39	70.51	61.45	13.09
Diet C, %	65.81	80.82	62.48	15.95	59.57	69.30	60.79	59.25	20.40
Neutral Detergent Fiber Digetibilty [3]									
Diet A, %	45.23	90.93	44.90	60.38	68.79	93.91	60.46	66.37	19.78
Diet B, %	59.54	85.70	51.24	31.85	59.03	91.54	77.79	65.24	21.01
Diet C, %	61.32	86.85	64.52	15.39	60.44	74.29	65.10	61.13	22.19
Acid Detergent Fiber Digetibilty [3]									
Diet A, %	19.55	92.07	30.08	37.54	53.82	94.61	42.73	52.91	29.57
Diet B, %	47.70	83.38	38.49	17.11	47.41	90.13	69.41	56.23	26.00
Diet C, %	55.81	87.41	63.97	14.43	59.83	78.08	62.07	60.23	23.06

[1] 1.2 *Centrochelys sulcata* , 0.0.3 *Malacochersus tornieri* , 0.0.2 *Gopherus agassizii* , 1.1 *Chelonoidis nigra* , 2.2 *Stigmochelys pardalis* (Adults), 0.0.12 *Stigmochelys pardalis* (juveniles), 2.1 *Astrochechelys radiate* .

[2] Not applicable

[3] Calculations based on Galyean, 1997.

Table 6.5. Total fecal output (DMB), mean fecal DM, and mean fecal scores for each diet fed to herbivorous tortoises.

Item	*Centrochelys sulcata* [1]	*Malacochersus tornieri* [1]	*Gopherus agassizii* [1]	*Chelonoidis nigra* [1]	*Stigmochelys pardalis* (Adults)[1]	*Stigmochelys pardalis* (Juveniles)[1]	*Astrochechelys radiate* [1]
Fecal Output							
Diet A, g/d	103.23	0.37	2.36	167.79	47.96	0.31	10.59
Diet B, g/d	69.77	0.64	4.21	318.48	103.90	1.07	10.54
Diet C, g/d	51.16	0.55	5.11	311.71	75.82	1.80	5.87
MEAN	74.72	0.52	3.89	265.99	75.90	1.06	9.00
SEM	26.39	0.14	1.41	85.11	27.97	0.74	2.71
Fecal DM %							
Diet A	36.05	36.92	62.59	42.15	32.37	26.19	30.99
Diet B	33.16	54.49	37.38	24.07	31.95	31.95	41.11
Diet C	35.19	59.47	43.75	24.74	28.50	38.39	30.24
Mean Fecal Score [2]							
Diet A	2.0 ± 0.5	1.0 ± 1.0	2.0 ± 1.0	3.0 ± 1.5	3.0 ± 1.5	2.0 ± 1.0	2.5 ± 1.0
Diet B	4.0 ± 0.5	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	3.5 ± 1.5	4.0 ± 0.5	4.0 ± 1.0
Diet C	3.0 ± 1.5	4.0 ± 1.0	3.5 ± 1.0	4.0 ± 1.0	3.0 ± 2.0	4.5 ± 0.5	3.5 ± 1.5

[1] 1.2 *Centrochelys sulcata* , 0.0.3 *Malacochersus tornieri* , 0.0.2 *Gopherus agassizii* , 1.1 *Chelonoidis nigra* , 2.2 *Stigmochelys pardalis* (Adults), 0.0.12 *Stigmochelys pardalis* (juveniles), 2.1 *Astrochechelys radiate* .

[2] Fecal scoring guide adapted from Edwards and Nickley, 2000.

Table 6.6. Herbivorous tortoise IVDMD and IVNDFD using commonly used feed ingredients.

Item	% IVDMD	% IVNDFD
Zoomed Grassland[a]	54.64^{gx}	34.71^{lm}
Mazuri Tortoise[b]	57.47^{g}	35.44^{l}
Wild Herbivore[c]	59.20^{gy}	39.76^{m}
Bermuda Hay	64.35^{h}	50.78^{m}
Large Produce Chop[d]	35.95^{i}	8.81^{nox}
Small Produce Chop[d]	36.25^{i}	10.50^{n}
Composite Produce Chop[d]	35.98^{i}	16.75^{ny}
Fruit Mix[e]	23.73^{j}	11.89^{no}
Root Mix[f]	17.11^{k}	9.37^{o}

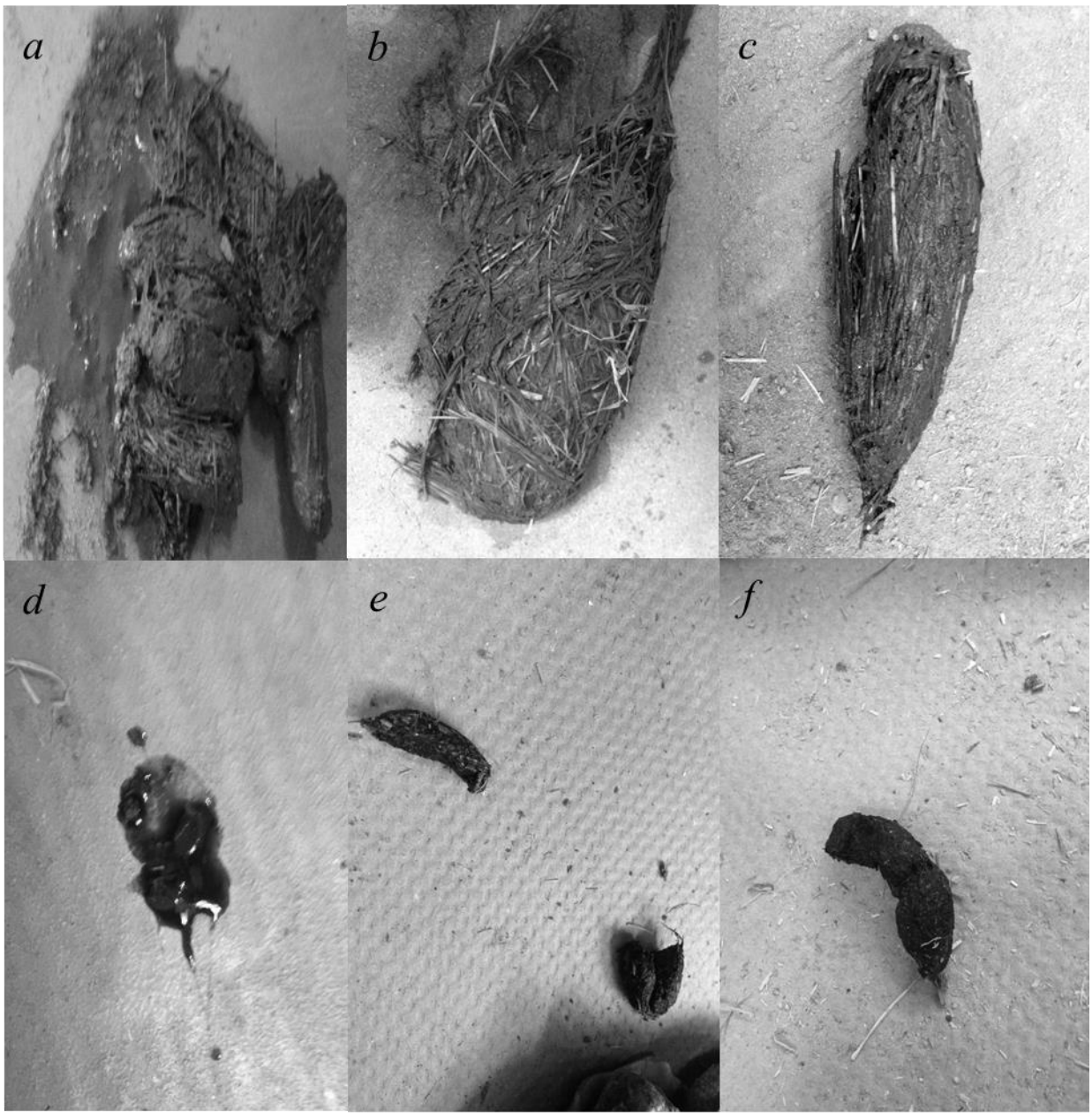
[a] Zoomed Natural Grassland Tortoise Food Zoo Med Laboratories Inc., San Luis Obispo, CA).
[b] Low Starch Mazuri Tortoise Diet (PMI Nutrition International LLC Saint Louis, MO 63108).
[c] Low Wild Herbivore Plus Diet (PMI Nutrition International LLC Saint Louis, MO 63108).
[d] Produce included: roman lettuce, dandelions, collard greens, spinach, and bok choy. Large is chopped with a knife. Small is the large chop blended in a food processor. Composite is a mix of each produce sampled after dried.
[e] Fruit Mix included: apples, grapes, turnips, and carrots.
[f] Root Mix included: turnips and carrots.
[g,h,i,j,k,l,m,n,o] Differing superscripts in a column are significantly different at $P<0.05$.
[xy] Superscript in column have a tendency to be different at $P<0.10$

Table 6.7. Herbivorous tortoise mL of Gas Production per 100 mg of feed sample (DMB) digested in fecal bacterial inoculum.

Item	mL of gas/100 mg substrate DM
Zoomed Grassland[a]	4.14[gi]
Mazuri Tortoise[b]	5.56[gi]
Wild Herbivore[c]	3.02[g]
Bermuda Hay	6.98[gi]
Large Produce Chop[d]	11.17[h]
Small Produce Chop[d]	8.74[hi]
Composite Produce Chop[d]	8.85[ghi]
Fruit Mix[e]	11.22[h]
Root Mix[f]	14.25[h]

[a] Zoomed Natural Grassland Tortoise Food Zoo Med Laboratories Inc., San Luis Obispo, CA).
[b] Low Starch Mazuri Tortoise Diet (PMI Nutrition International LLC Saint Louis, MO 63108).
[c] Low Wild Herbivore Plus Diet (PMI Nutrition International LLC Saint Louis, MO 63108).
[d] Produce included: roman lettuce, dandelions, collard greens, spinach, and bok choy. Large: chopped with a knife. Small: the large chop blended in a food processor. Composite: mix of each produce sampled after dried.
[e] Fruit Mix included: apples, grapes, turnips, and carrots.
[f] Root Mix included: turnips and carrots.
[g,h,i] Differing superscript are significantly different at $P<0.05$.

Figure 6.1. Herbivorous tortoise fecal quality comparison of diets A, B, and C.



[a]Fecal quality of *Chelonoidis nigra* on diet A (score 3).
[b]Fecal quality of *Chelonoidis nigra* on diet B (score 5).
[c]Fecal quality of *Chelonoidis nigra* on diet C (score 4).
[d]Fecal quality of *Stigmochelys pardalis* (Juveniles) on diet A (score 1).
[e]Fecal quality of *Stigmochelys pardalis* (Juveniles) on diet B (score 6).
[f]Fecal quality of *Stigmochelys pardalis* (Juveniles) on diet C (score 6).

VITA

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