Proceedings of the Ninth Dr. Scholl Conference on the Nutrition of Captive Wild Animals

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BODY WEIGHT, DIGESTION, AND FOOD SELECTION IN CAPTIVE MARMOSETS AND TAMARINS: IMPLICATIONS FOR DIET FORMULATION.

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The small New World primates known as marmosets and tamarins (family *Callitrichidae*) are popular exhibit animals in zoological parks and gardens. Many are endangered species. Captive propagation can be an important facet of conservation efforts for these species. Although several species, such as the common marmoset and the golden lion tamarin, have thrived in captivity, some species have experienced difficulties in adjusting to captive life (Barnard et al. 1988). Several medical problems, such as alopecia and "wasting marmoset syndrome", are suspected of having nutritional components (Shimwell et al. 1979, Escajadillo et al. 1981, Barnard et al. 1988).

In this paper we discuss several aspects of the biology of marmosets and tamarins that should be considered when devising diets and feeding regimes for these species. The first part of the paper examines digestive function in five species: golden lion tamarins (*Leontopithecus rosalia*), cotton-top tamarins (*Saguinus oedipus*), saddle-back tamarins (*S. fuscicollis*), common marmosets (*Callithrix jacchus*), and pygmy marmosets (*Cebuella pygmaea*).

The second part of this paper presents data on the nutritional implications of dietary self selection in golden lion tamarins fed a "cafeteria" style diet.

I. Digestion in Captive Marmosets and Tamarins

There are over 20 species of marmosets and tamarins (Mittermeier et al., 1988), ranging in body weight from 100 gram pygmy marmosets to 600 - 700 gram lion tamarins. Many field studies have confirmed the omnivorous nature of these species, however there are dietary differences among them. Marmosets generally are thought of as gum feeders (Hershkovitz 1977). Their dentition enables them to gouge trees and vines, stimulating the flow of gum and sap (Coimbra-Filho and Mittermeier 1977).

The diets of tamarin species mainly are composed of fruit and insects. Opportunistic exudate feeding by tamarins has been reported (Garber 1980, 1984, 1988, Rylands 1983, Terborgh 1983, Ramirez 1985, Soini 1987), and for certain *Saguinus* species exudates are significant seasonal dietary components (Garber 1980, 1986, Ramirez 1985, Soini 1987). The tamarins lack the tree-gouging ability of the marmosets.

The following study was predicated on the hypothesis that digestive function in the gum-feeding marmosets would differ from that found in the largely frugivorous tamarins.

Definitions

transit time - the elapsed time from the ingestion of markers until the first appearance of the markers

in the feces (Warner 1981). Also referred to as the time to first appearance (TFA).

coefficient of apparent digestibility - the ratio of the net assimilation of a nutrient (defined as the amount of nutrient ingested minus the amount defecated over a given time period) to the amount of nutrient ingested (Van Soest 1982).

Table 1
Composition of the diets.

	Basic Diet		Gum Arabic Diet	
	as fed basis	dry matter basis	as fed basis	dry matter basis
Water	69.80%	------	67.90%	-----
Energy	1.34	4.45	1.4	4.37
	kcal/g	**kcal/g**	**kcal/g**	**kcal/g**
Crude Protein	6.20%	20.40%	6.00%	18.70%
Ash	1.80%	5.90%	1.70%	5.20%

Methods

Digestion trials with complete collections of feces and uneaten food were conducted on 39 animals (nine golden lion tamarins, ten cotton-top tamarins, seven saddle-back tamarins, eight common marmosets, and five pygmy marmosets). Plastic beads (1 mm in diameter) and chromic oxide were used as markers. To eliminate variation due to individual food preferences, the animals were fed two homogeneous diets. The baseline diet (basic diet) was a standardized mixture of canned marmoset diet (Hill's Pet Products, Topeka, KS), banana, and gelatin. The experimental diet (gum arabic diet) was made by adding spray dried gum arabic powder to the basic diet recipe until gum arabic constituted 9% of the dry matter. The diets were refrigerated overnight to gel. The average amounts of water, energy, crude protein, and ash (total minerals) for each diet are given in Table 1.

Analysis of covariance was used to test for differences among species in the allometries of transit time and apparent dry matter and energy digestibility. For the complete methods for these experiments see Power (1991).

Results

Table 2 lists the species averages from the basic diet digestion trials for body weight, transit time, coefficients of apparent dry matter digestibility (ADMD) and apparent energy digestibility (AED), and dry matter intake as a percentage of body weight. Ignoring the pygmy marmosets, transit time and both coefficients of apparent digestibility increased with body weight, and intake as a percentage of body weight decreased with body size. Pygmy marmosets do not fit this pattern.

Pygmy marmosets, the smallest species, had transit times absolutely longer than those of all of the other species ($p<.001$, ANCOVA). Transit times did not differ among the common marmosets and the three tamarins when corrected for body size ($p=.519$, ANCOVA). These results held true even when the correlations of dry matter intake with weight and transit time were considered. After correcting for both weight and dry matter intake, transit times in common marmosets and the three tamarin species were still not different ($p=.744$, ANCOVA), while transit times in pygmy marmosets were significantly longer than those in the other species ($p<.001$, ANCOVA).

Similarly, common marmosets and the three tamarins did not differ in their coefficients of apparent energy and dry matter digestibility when the effect of body weight was accounted for ($p=.992$, ANCOVA). Pygmy marmosets had significantly higher apparent digestibilities than would be predicted if they followed the pattern of the other species ($p<.001$, ANCOVA), equal to those of tamarins four times their size.

The digestibility of gum arabic can be estimated from the differences in the apparent digestibilities between the basic diet and the gum arabic diet (Llyod et al. 1978). Judging from these estimates, the marmosets appeared to have little difficulty digesting gum arabic (ADMD = 80.5% and 86.5%, AED = 89.5% and 88.2%, for common marmosets and pygmy marmosets respectively). The ability of the tamarin species to digest gum arabic increased with body size, but was always below that of the marmosets (ADMD = 33.2%, 52.0%, and 62.1%, AED = 27.7%, 53.7%, and 61.3%, for saddle-back tamarins, cotton-top tamarins, and golden lion tamarins respectively).

Discussion

Gums are resistant to endogenous mammalian digestive enzymes (Van Soest 1982), and presumably require a longer time to digest, on average, than fruit pulp. Species with a high proportion of exudate in the diet could benefit from longer retention times if the net gain from a given quantity of food eaten increases with retention time.

Pygmy marmosets are true exudate specialists, relying on exudates from tree and lianas as their principal food source (Hernandez-Comacho and Cooper 1976, Moynihan 1976, Coimbra-Filho and Mittermeier 1977, Ramirez et al. 1977, Soini 1982). Although high frequencies of gum-feeding have been observed in wild common marmosets, when fruit is plentiful feeding on exudates becomes a small proportion of their feeding time (Alonso and Langguth 1989). During those times the natural diet of common marmosets resembles the diets of tamarins in that fruits comprise the majority of the plant portion of the diet (Alonso and Langguth 1989, Stevenson and Rylands 1988). Therefore it is not surprising to find that common marmosets share a pattern of digestive function with the tamarins.

The rapid passage rates of common marmosets and the three tamarin species in this study enable them to ingest large amounts of food. The long transit times in pygmy marmosets reduce their ability to ingest large quantities of food, but result in high assimilation efficiencies, even on a gum-free diet.

The higher estimates of apparent digestibility of gum arabic for pygmy marmosets compared to the tamarins are consistent with the difference in digestive function between these groups. The apparent ability of common marmosets to digest gum arabic readily cannot be explained as easily. Clearly some aspect of digestive physiology must differ between common marmosets and the tamarin species in this study.

II. Dietary Self Selection in Golden Lion Tamarins

A homogeneous diet, as in the above experiment, ensures that the animals receive a known mix of nutrients. In zoos, marmosets and tamarins are usually fed a "cafeteria" style diet, consisting of "marmoset" food (either a commercial product or an in-house mix of commercial foods), fruits, vegetables and insects. In all cafeteria diets, the possibility exists that the consumed diet will differ

from the offered diet. The consumed diets can vary greatly among animals as well. The greater the variety of choices offered, the greater the probability that an individual's diet will differ from that intended. This study was designed to investigate food preferences in golden lion tamarins, and the nutritional implications of those preferences.

Methods

The subjects in this study were eight adult golden lion tamarins from the Departments of Mammals and Zoological Research at the National Zoological Park, Washington D.C. Four animals were singly housed; the other four were housed as pairs, one pair being a father and son and the other pair consisting of a vasectomized male and a female (Table 3).

Table 3
Subjects in food selection study.

Animal ID	Studbook Number	Sex	Age (yrs)	# of days Observation	# of days Intake
TB	1573	M	3.5	6	5
KF	1723	F	2.5	6	5
MA	1725	F	2	6	5
JA	1644	M	3	3	2
BA	1367	M	4	6	5
MM	1415	F	4.5	6	5
DO	763	M	10	4	3
HE	1328	M	5.5	4	3

Animals were fed twice per day. The morning feeding consisted of canned marmoset diet (Hill's Pet Products, Topeka, KS) solely. The afternoon feeding consisted of 12 food items: marmoset diet, five fruits, four vegetables, and two insects (Table 4). All foods were weighed out to the nearest tenth of a gram. Samples of all foods were taken at the time the food trays were prepared. These samples were weighed and then dried overnight in a 100° C oven to obtain a dry weight. All uneaten food was collected the following morning, separated by food type, dried and weighed. The amount consumed of a food was calculated by multiplying the amount offered by the fractional dry matter content and then subtracting the dry weight of the uneaten portion. The food trays were watched for thirty minutes after they were placed in the enclosures. All foods that were eaten (placed in the mouth) by animals were recorded as to food type and time of ingestion.

Results

Table 5 ranks the foods by the proportion of offered that was consumed. By this measure, fruits and insects generally were preferred to marmoset diet and vegetables. Banana and raisins were essentially completely consumed by every animal. The only portion of grapes that was not always consumed was the skin. The legs and heads of crickets were occasionally left uneaten. Orange (without peel) was chewed and then the pulp (sans juice) was discarded. Thus the uneaten portions of these foods indicate that the animals make choices within foods as well as between foods.

Table 4 Amounts of foods (per animal) offered during study.

	% of KCALS		
	AM	PM	Offered
MARMOSET DIET	62.00	62.00	72.50
BANANA	14.40	4.90	
APPLE	20.50	4.00	
ORANGE	12.20	1.40	
KALE	3.10	0.60	
CARROT	5.30	0.70	
SWEET POTATO	15.20	5.80	
BEANS	4.30	0.50	
GRAPES	4.30	1.00	
RAISINS	4.20	4.10	
MEAL WORMS	4.10	3.50	
CRICKETS	2.10	1.10	

Table 5
Rankings of food types by the proportion of offered that was consumed. Foods above the dashed line contributed calories to the consumed diet in excess of their proportion in the offered diet.

	Consumed as % offered
BANANA	100.00
RAISINS	99.60
CRICKETS	93.50
GRAPES	86.50
MEAL WORMS	84.90
APPLE	77.40
MARMOSET DIET	65.60
ORANGE	58.20
SWEET POTATO	44.60
BEANS	23.80
KALE	23.00
CARROT	20.40

The preference for fruit and insects was also demonstrated by the observational data (Table 6). In the first thirty minutes the five fruits and two insects accounted for 99% of the "bites" taken. Grapes, raisins and banana were usually completely consumed before the end of the thirty minute observation. Apple and orange tended to be taken later than the other preferred foods, probably being chosen after the most desired foods had been consumed.

One animal (TB) had very low intakes during this study (Table 7). His gross energy intake was roughly half of most of the other animals, and was similar to that of common marmosets half his body weight from the digestive function study. Another animal (KF) had moderate intakes, while the rest of the animals had what we consider to be high intakes.

This range of intakes demonstrates what can happen as the relative proportions of marmoset diet to supplemental foods change. Because banana, grapes and raisins were essentially completely consumed, as intake decreased their relative contribution to total calories increased. These three foods accounted for 28.8% of the gross energy intake (GE) for animal TB, 20.6% of GE for animal KF, but only 11.7% of GE for the other animals.

Discussion

Table 6 Relative proportion of "bites" for each food type during the thirty minute observation periods (in %).

	first 10 min.	second 10 min.	last 10 min.	full 30 min.
MARMOSET DIET	0.10	1.10	0.00	0.30
BANANA	16.80	21.00	15.00	17.40
APPLE	1.50	13.80	19.70	6.00
ORANGES	1.00	9.80	12.70	4.00
KALE	0.10	0.00	0.00	0.00
CARROT	0.20	0.70	0.00	0.30
SWEET POTATO	0.20	0.40	1.20	0.30
BEANS	0.10	0.00	0.00	0.00
GRAPES	8.80	2.50	0.00	6.60
RAISINS	19.30	15.60	13.30	17.90
MEALWORMS	47.40	26.40	27.70	41.10
CRICKETS	4.50	8.70	10.40	6.00

Table 7 Percent of intake in kcals by food type.

	TB	KF	Others
MARMOSET DIET	59.10	60.80	73.20
BANANA	14.50	10.40	5.80
APPLE	2.40	5.90	4.40
ORANGE	2.20	1.70	1.00
KALE	0.30	0.30	0.20
CARROT	0.30	0.20	0.20
SWEET POTATO	1.40	1.30	3.80
BEANS	0.30	0.20	0.10
GRAPES	2.50	1.60	1.00
RAISINS	11.80	8.60	4.90
MEAL WORMS	2.80	6.60	4.10
CRICKETS	2.40	2.40	1.30
TOTAL KCALS PER ANIMAL	100.00	100.00	100.00

Golden lion tamarins showed consistent preferences for fruit and insects over vegetables and marmoset diet. Within the first 30 minutes of being presented with the food tray animals ate virtually no other foods besides fruit and insects. This implies that, given sufficient amounts of these supplemental foods to satisfy energy requirements, they would be the only foods eaten. This implication is supported by the consumed diet of the animal TB. That animal only ate marmoset diet during the morning feeding, when no supplemental foods were offered.

The results of this study do not support the notion that, under a cafeteria style feeding regime, golden lion tamarins will self-select a balanced diet. The risk of compromising the nutritional quality of the diet can be minimized, however, by keeping the quantities of supplemental foods offered small compared to energy requirements.

Implications for Feeding Practices

Marmosets and tamarins are typically fed at least twice per day. The morning feeding, when the animals are the most hungry, is a good time to offer a single item, complete feed. Subsequent feedings may be more varied, as long as the total quantity of supplemental foods does not exceed about 30% of total energy intake. If the morning feeding consists solely of a canned product, subsequent feedings should include foods high in vitamin C, as the canning process destroys this required nutrient.

Supplemental foods are generally of little nutritional importance for marmosets and tamarins, except as a vitamin C source. Care must be exercised, however, to ensure that they

do not compromise the nutritional balance of the offered diet. Marshmallows and other candy-like foods have no place in marmoset and tamarin diets. If food is to be offered as a training reward, insects or fruits can be used.

The food choices of animals with low intakes should be monitored carefully. Efforts should be made to increase the intake of the complete feed. The danger in attempting to increase intake through offering more of the preferred supplemental foods is that total intake may be little changed, but the proportion of caloric intake due to the supplemental foods can increase, compromising the nutritional quality of the consumed diet.

Marmosets and tamarins are customarily housed in family groups, often containing six or more animals. Foods are not equally shared among family members. Studies on common marmosets and cotton-top tamarins have shown that the breeding female generally consumes a disproportionate amount of all preferred foods (Tardif and Richter, 1981, Petto and Devin, 1988). As the amounts of supplemental foods offered to a group will be greater than those offered to an individual, the possibility exists that the consumed diet of the breeding female will largely consist of supplemental foods. Ironically, the animal which presumably has the greatest nutritional demands also (Oftedal, 1991) has the highest probability of consuming an imbalanced diet. To guard against this, multiple food bowls should be used, and the amounts of supplemental foods strictly controlled.

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PROTEIN NUTRITION OF CAPTIVE HUMMINGBIRDS

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The nectarivorous feeding habits of hummingbirds have been well documented (Bent 1940, Bene 1947), and results from time-energy budget (Pearson 1954, Stiles 1971,) and doubly-labeled water studies (Powers and Nagy 1988) have provided a thorough assessment of the carbohydrate requirements of hummingbirds. Their needs for protein, however, had not been well studied. The little information available indicated that, in addition to nectar, hummingbirds feed to some extent on arthropods and pollen (Bent 1940), and that time spent foraging for insects by non-reproducing adults is a relatively small percent of total foraging time (Gass and Montgomerie 1981). I studied the protein nutrition of hummingbirds and evaluated the contributions of arthropods, pollen and nectar itself toward meeting the protein needs for adult maintenance.

Development of a Purified Diet for Long-term Maintenance

Captive hummingbirds have been maintained either by solutions containing sugar and vitamins plus a supply of live fruit flies or complex mixtures that include ingredients such as baby food, pollen and ground worms. Scheithauer (1967) recorded detailed behavioral observations of captive hummingbirds fed a variety of diets. Young birds have been raised, adults have been maintained, and some species have bred under these various regimes, but which of the nutrients are essential in such diets and at what levels have not been determined.

The purified diet presented here permitted long-term maintenance, with no supplemental feeding, of two species of captive, adult hummingbirds, Anna's (*Calypte anna*) and Costa's (*Calypte costae*) (Brice and Grau 1989). Because the amount of each nutrient was known, and thus could be varied under controlled conditions, the development of the diet was a first step toward defining actual nutritional requirements for these species.

The liquid diet was prepared by combining 25% dry ingredients (Table 1) with 75% distilled water. The dry mixture contained sucrose as the principal energy source at a level approximating sugars found in hummingbird flower nectars, which average a little over 20% sucrose-equivalents weight by total weight (Baker 1975). The other nutrients were supplied by ingredients commonly found in purified diets at levels approximating those required by poultry (National Research Council 1986), with two exceptions: the isolated soybean protein and the modified starch. The nitrogen source was a food-grade sodium proteinate that was flocculate rather than gritty. It was used because, even if finely ground, diets that were gritty were rejected by the birds. The soybean protein contained 1% sodium, hence the diet contained only a small amount of additional sodium. The modified starch with its high water-holding capacity was used to aid in maintaining the ingredients in suspension.

Protein Requirements of Costa's Hummingbirds

Hummingbirds are thought to have evolved from an insectivorous ancestor (Johnsgard 1983), but spend most of their foraging time probing flowers for nectar to fulfill the high energy demands of small homeotherms. It is generally accepted that hummingbirds are "energy limited", that is, their major behavioral and physiological responses are based on the availability of their high carbohydrate food source (Gass and Montgomerie 1981). Other nutrients--water, proteins, lipids, vitamins and minerals--are also essential for all animals, but, with the exception of water (Calder and Hiebert 1983) their significance to hummingbirds has not been documented.

TABLE 1. Composition of the dry hummingbird diet

Ingredients	%
Sucrose	79.45
Isolated soy protein [1]	3.50
DL-Methionine	0.05
Food starch, modified[2]	5.00
Corn oil	3.70
$CaCO_3$	1.00
$CaHPO_4 2H_2O$	3.00
NaCl	0.25
Choline Chloride (60%)	0.40
Mineral premix	1.30
Vitamin premix	0.10
Total	100.00

[1]Supro 620, 87.4% crude protein, 1.1% sodium, Ralston Purina Co., St. Louis, MO.
[2]Instant Clear-Jel, National Starch and Chemical Co., Bridgewater, NJ.
[3]Supplied the following in mg/kg diet: $MnSO_4 H_2O$, 297; $CuSO_4 5H_2O$, 97; Co Acetate $4H_2O$, 20; KIO_3, 9; $MgSO_4 7H_2O$, 3970; KCl, 2970; K_2HPO_4, 4950; $Na_2MoO_4 2H_2O$, 9; Na selenite, 0.66; ZnO, 120; and $FeSO_4 7H_2O$, 644.
[4]Supplied the following in mg/kg diet (except as noted): vitamin A palmitate, 18,720 IU; cholecalciferol, 4200 IU; d,l-alphatocopherol acetate, 119 IU; menadione sodium bisulfite complex, 7.8; vitamin B_{12}, 14 ug; thiamin HCl, 9.3; niacin, 231.3; riboflavin, 18.7; pyridoxine HCl, 18.7; calcium pantothenate, 75; folic acid, 4.7; and d-biotin, 0.9.

It appears that arthropods provide the principal source of nutrients other than carbohydrates, and, indeed, arthropod remains were found in 95% of the individuals from 140 species examined by Remsen et al. (1986). However, Gass and Montgomerie (1981) reported that non-breeding birds spend only 2-12% of their foraging time arthropod-catching when the nectar supply is adequate.

It can be hypothesized from the small percentages of time reported for arthropod foraging that the hummingbird's requirement for protein is low in relation to carbohydrate needs. The purpose of this study was to determine the protein requirement of a representative hummingbird, the Costa's (*Calypte costae*), in the laboratory and compare it with estimated amounts of protein supplied during foraging in the wild. The methods used were responses to dietary protein levels as determined by body weight maintenance, nitrogen balance and endogenous nitrogen loss. In the current paper I will only discuss the body weight maintenance method. A full discussion of all three techniques and results is found in Brice and Grau (1991).

Methods

Eight Costa's hummingbirds, mean body mass of 3.1g, which had been maintained for two years on the purified diet discussed earlier, were used in the study. During the nitrogen balance studies, four diets were fed with zero, 0.75%, 1.5% or 3% protein of the solids (see Table 2). The

diets were kept isocaloric by interchanging protein and sucrose as required. Each gram of dry diet was calculated to provide 3.6 kcal (15.1 kJ) of metabolizable energy. Liquid diet and deionized water were provided ad libitum.

TABLE 2. Composition of dry diets with four levels of protein

Ingredient	3% protein	1.5% protein	0.75% protein	0% protein
Sucrose	81.920	83.700	84.582	85.470
Isolated soy protein[1]	3.500	1.750	0.875	0.000
DL-Methionine	0.050	0.025	0.013	0.000
Food starch, modified[2]	5.000	5.000	5.000	5.000
Corn oil	3.700	3.700	3.700	3.700
$CaCO_3$	1.000	1.000	1.000	1.000
$CaHPO_4 2H_2O$	3.000	3.000	3.000	3.000
Choline chloride (60%)	0.420	0.420	0.420	0.420
Mineral premix[3]	1.310	1.310	1.310	1.310
Vitamin premix[4]	0.100	0.100	0.100	0.100
Total	100.000	100.005	100.000	100.000

[1]Supro 620, 87.4% crude protein, 1.1% sodium, Ralston Purina Co., St. Louis, MO.
[2]Instant Clear-Jel, National Starch and Chemical Co., Bridgewater, NJ.
[3]Supplied the following in mg/kg diet: $MnSO_4 H_2O$, 297; $CuSO_4 5H_2O$, 97; Co Acetate $4H_2O$, 20; KIO_3, 9; $MgSO_4 7H_2O$, 3970; KCl, 2970; K_2HPO_4, 4950; $Na_2MoO_4 2H_2O$, 9; Na selenite, 0.66; ZnO, 120; and $FeSO_4 7H_2O$, 644.
[4]Supplied the following in mg/kg diet (except as noted): vitamin A palmitate, 18,720 IU; cholecalciferol, 4200 IU; d,l-alphatocopherol acetate, 119 IU; menadione sodium bisulfite complex, 7.8; vitamin B_{12}, 14 ug; thiamin HCl, 9.3; niacin, 231.3; riboflavin, 18.7; pyridoxine HCl, 18.7; calcium pantothenate, 75; folic acid, 4.7; and d-biotin, 0.9.

There were four experimental periods of 24 days. Each comprised 10 days during which one of the protein diets was fed and 14 days for recovery after the birds were returned to the 3% protein diet. For each experiment there were four randomly assigned groups of two birds, and each group was fed one of the four diets. The length of the recovery period was set by a pilot study during which the birds were weighed every few days to determine the time needed to re-establish normal body weights after losing mass on a low protein diet. During subsequent experiments the birds ate diets with different levels of protein so that by the end of the study every bird had been fed each of the four levels. The birds were weighed on a Mettler Analytical Balance (accuracy $\pm$.01g) before first feeding (0700 h) on days 1, 5 and 10 of each experiment.

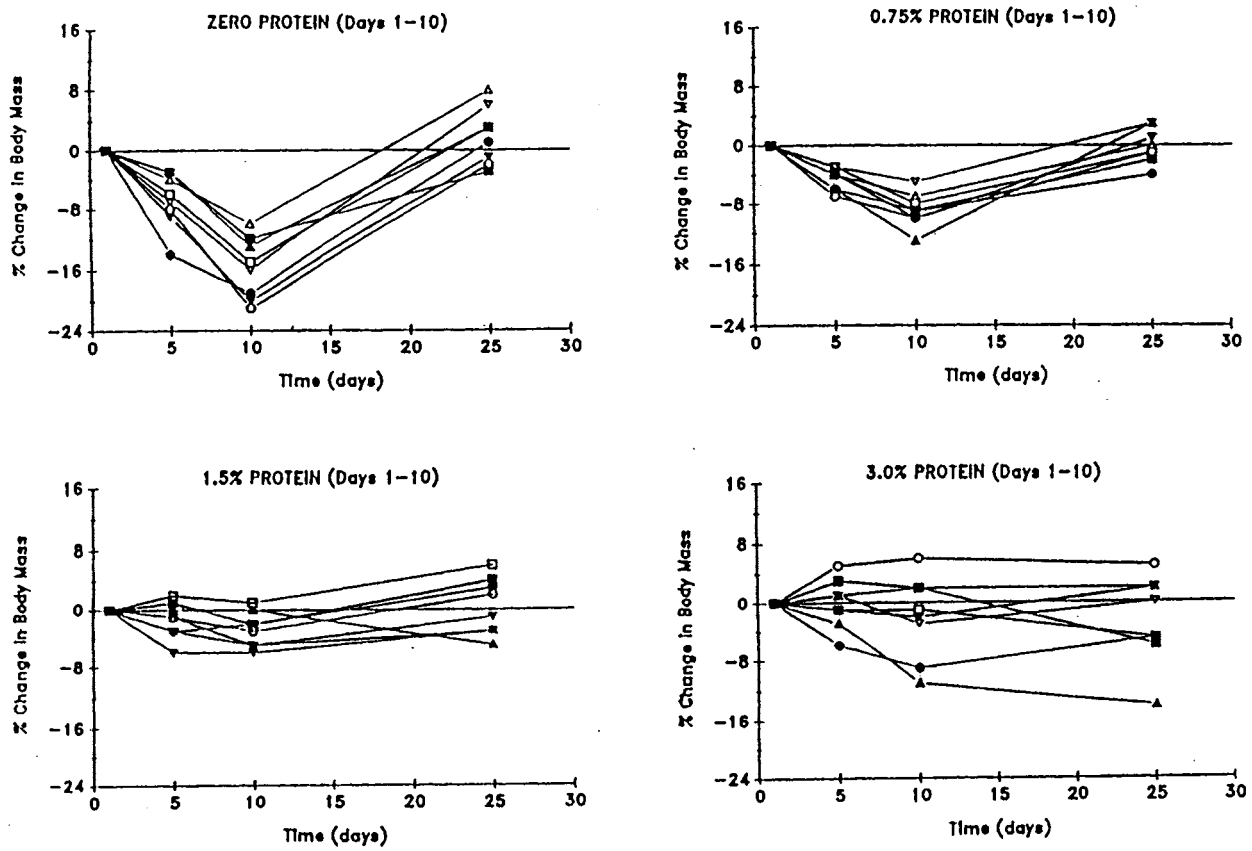
Results and Discussion

After eating the zero protein diet for 5 days, all birds lost an average of 7% of their initial body mass; and after 10 days there was a 16% loss. With the 0.75% protein diet the birds lost 4.6% of their body masses on day 5 and 8.8% by day 10. With the 1.5% protein diet they lost 1.4% and

2.6% on Days 5 and 10, respectively. When fed the control diet of 3% protein, the birds showed a weight losses of 0.1% and 1.6% on days 5 and 10, respectively. Based on body mass changes alone, the diets containing 0 or 0.75% protein were clearly inadequate nitrogen sources, whereas 1.5% and 3.0% were adequate (Fig. 1). On day 10 we returned all the birds to the control diet for 15 days. By day 25, on average, the birds regained their initial masses, except for the 3% protein group which had one bird that lost weight during the entire third experimental period when fed the control diet. When fed the lower protein diets, this particular bird did not deviate so dramatically from others in the group.

Although many animals do not maintain their normal energy intakes on nitrogen-free diets (Robbins 1983), we found no significant differences in energy intakes in a comparison of the four protein levels over the entire experimental period. The mean energy intake was 6.70 $\pm$ 0.18 kcal/day (28.1 $\pm$ 0.75 kJ/day). This intake level agrees well with the work on the similarly sized free-living Black-chinned Hummingbird (*Archilocus alexandri*), which was found by the doubly-labeled water method to have a daily energy expenditure of 6.90 kcal/day (28.5 kJ/day) at a mean temperature of 24°C (Powers pers. obs.).

Figure 1. Percent body mass change of hummingbirds from initial mass when fed each of four dietary protein levels for 10 days and the 3% protein control diet for another 14 days. Each symbol represents the same bird in all four graphs.



The results of the weight maintenance data on four levels of dietary protein indicate that the

minimal protein requirement for the Costa's hummingbird is only 1.5% of the total dry weight of the diet or about 4.50 mg N/day.

Hummingbirds acquire the majority of their non-energy nutritional needs from arthropods. The size of these prey items may vary according to availability. Montgomerie and Redsell (1980) reported that a female Broad-tailed hummingbird (*Selasphorus platycercus*) fed from a swarm of insects with an average dry weight of 2.1 mg. This would represent an insect with a wet weight of about 7 mg, which seems large for a 3.8 g bird to consume. It was found that 99% of the observations of a 20 g New Holland honeyeaters insect hawking involved catching insects that had a dry weight of 0.7 mg or less (Paton 1982). If one assumes that 60% of the dry weight of an insect is protein and 80% of the protein is absorbed (Paton 1982), a Costa's hummingbird would have to consume 28 arthropods weighing 7.5 mg or 90 weighing 2.5 mg per day. Hainsworth (1977) reported that *Colibri coruscans* could make 20 flycatching attempts per minute if the hummingbird spent all its foraging time flycatching rather than nectar feeding. Assuming a similar flycatching attempt rate for the Costa's with a 75% success rate and an adequate supply of insects, the nitrogen requirement could be met in 6 minutes/day. Stiles' (1971) time-budget data indicate that the male Anna's he observed for two days averaged only 8 minutes/day flycatching or a little over 12% of the total foraging time, which agrees well with the amount of time estimated for Costa's to fulfill its protein needs.

The protein requirement of Costa's Hummingbirds presented here is for maintenance. During reproduction, the female Costa's lays two 0.5 g eggs. If one assumes 12.2% protein for a hummingbird egg (Romanoff and Romanoff 1949), the female will need an extra 19.5 mg N for egg development plus a small amount for ovary and oviduct growth. This extra requirement would be spread over an approximately 6-7 day period (48 hour laying interval, 4 days for yolk deposition) and thus during that time would require around 3.0 mg N/day in addition to the normal levels. This amount can be obtained by catching an extra 19 to 60 arthropods a day using the size categories for insects described earlier. The female alone feeds the young totally for about 21 days in the nest and at least partially for some days after they fledge (Bent 1940). It has been reported that female hummingbirds spend more time flycatching when they have young (Hainsworth 1977), so the need for more arthropods may stay elevated above maintenance levels until the young are independent. However, the overall protein requirement for the Costa's hummingbird is so low in relation to its carbohydrate needs that the increase for the female during reproduction may be relatively insignificant in terms of foraging time and prey availability. Two years of data from the Colorado Desert indicate that peak aerial-insect biomass coincides with the spring annual plant bloom (Walsberg 1977), at the time when the Costa's breed and raise young.

The observation that hummingbirds spend most of their foraging time feeding on nectar supports the assumption that they are "energy limited". The results of this study also appear to justify this assumption and to indicate that the Costa's Hummingbird has a remarkably low protein requirement when expressed as a percentage of the diet.

Pollen Digestibility by Hummingbirds and Psittacines

In the course of feeding on flowers to obtain nectar, hummingbirds collect pollen on their bills, ingest it (Bent 1940), and feed it to their chicks (Carpenter and Castronova 1980). However, little

is known about the nutritional role of pollen for any avian species. Pollen grains contain significant levels of protein (7% to 40% of dry weight) and carbohydrates (13% to 39% sugars and starch of dry weight), and lesser amounts of fats and minerals (Todd and Bretherick 1942).

Although there have been numerous observations of pollen eating by birds and some reports of pollen grains found in the gastrointestinal tract, only two papers have considered whether the pollen is actually digested. Churchill and Christensen (1970) examined the alimentary canals of two purple-crowned lorikeets (*Glossopsitta porphyrocephala*) that had been feeding on pollen in the wild and reported that the pollen grains found in the duodenum were empty. No controlled feeding trials were conducted, however, and there were no data reported on the percentages of the grains that were already empty when the birds consumed them. The New Holland honeyeater (*Phylidonyris novaehollandiae*) had been known to ingest pollen, but upon analysis of pollen grains in the gastrointestinal tracts of wild birds and in pollen feeding trials with captive birds, Paton (1981) found that the majority of pollen grains passed through the birds unchanged.

This paper reports on the results of pollen digestion trials in adults of two and nestlings of one species of hummingbirds and in nestlings and adults of two species of psittacines. A more complete treatment may be found in Brice et al. 1989. Initially hummingbirds were the focus of the study to confirm in the laboratory the observations of pollen feeding in the wild. Lorikeets were then added for comparative purposes because of their unusual brush-tongues, which had long been thought to enhance nectar feeding (Gould 1865) and were associated with pollen harvesting by Churchill and Christensen (1970). The non-nectarivorous cockatiels (*Nymphicus hollandicus*), which have not been reported to feed on pollen in the wild, were also selected to compare with the nectarivores.

Methods

The birds used in the pollen digestion study were (1) five male Anna's hummingbirds and two male and one female Costa's hummingbirds (*C. costae*) that were wild caught but had been in captivity for at least a year; (2) two fifteen-day-old and two nineteen-day-old Anna's hummingbird nestlings which were being reared at a wildlife care facility; (3) two male and two female captive-bred adult rainbow lorikeets (*Trichoglossus haematodus haematodus*); (4) one male and one female captive-bred, hand-reared nestling Moluccan lorikeets (*T. h. moluccanus*), which were used in the feeding trials as nestlings (one month old) and later as adults (9 months old); (5) one male and one female captive-bred adult cockatiels; and (6) four parent reared nestling cockatiels.

The pollen samples were (1) bee-collected (*Eucalyptus calophylla*) from Australia, (2) bee-collected local almond (*Prunus dulcis*), (3) hand-collected bottlebrush (*Callistemon citrinus*) and (4) hand-collected California fuschia (*Zauschneria californica*).

The experimental diets were fed in liquid form containing 75%-80% water. The hummingbirds were fed the purified liquid diet described earlier, and the lorikeets and cockatiels were fed a hand-feeding diet (Handrearing Diet, Roudybush, Inc.). In each of the feeding trials, pollen constituted approximately 20% of the dry weight of the diet.

Reference samples of the diet were collected and frozen before each feeding trial. To minimize possible germination of pollen grains in a liquid medium (Stanley and Linskens 1974), water was not added to the dry diet until just before each feeding trial began.

Samples of excreta were collected from the adult birds by placing plastic sheeting under the

wire cage bottoms. Samples were collected for one hour three times during each trial except for the nestlings where timing was less precise. All samples were frozen and stored for later study.

After pollen reference and excreta samples were thawed, a portion of each was placed in a small vial and saturated with Alexander stain (Alexander 1969) for at least 30 minutes, thereby staining the outer coat of the pollen grain green and the contents red. Two or three drops of the stained sample were then pipeted onto a slide and a cover slip put over it. Microscopic examination of approximately 1000 pollen grains per sample were scored, except the *Zauschneria* samples which averaged 500 grains each. Pollen grains were scored as "full" (similar in shape and contents to the diet sample reference); "partially full" (some protrusion of pores, slightly misshapen, some contents gone); or "empty" (no red stained contents left in grains, collapsed outer coat).

Stanley and Linskens (1974) found that all samples of pollen contain some grains that are aborted, that is, completely or partially devoid of contents. We estimated, based on observations of thousands of grains, that partially full grains contained 85% of the contents of full grains. Thus, for the purpose of calculating digestion, 85% of the partially full pollen grains were assigned to the "Full" category and 15% to the "Empty" category. The percent of nonaborted pollen grains assumed to be digested was then determined by the following formula:

$$\%\ digested = \frac{\%\ fecal\ \ sample\ \ entry - \%\ diet\ \ sample\ \ empty}{\%\ diet\ \ sample\ \ full} \times 100$$

Results and Discussion

Table 3. Pollen digestion by hummingbirds: adult Anna's (A); nestling Anna's (NA); and adult Costa's (C). "Partially full" pollen grains were partitioned as indicated in the "Methods" section. Values with standard errors (±S.E.) are means of 3 collection periods.

		Empty Pollen Grains		Pollen Grains Digested	
Pollen Source	Bird	Diet (%)	Feces (%)	Per Bird (%)	Per Trial (%)
Zauschneria	A1	4.8	6.4	1.7	
	A2	11.0	16.2	5.8	3.8 ± 2.1
Zauschneria	C1	1.6	1.1	-0.5	
	C2	1.1	0.9	-0.2	
	C3	0.0	0.3	0.3	-0.1 ± 0.2
Callistemon	A3	1.0[1]	4.8 ± 1.2	3.9 ± 1.2	
	A4	1.0[1]	4.6 ± 1.3	3.7 ± 1.3	
	A5	1.0[1]	7.3 ± 1.0	6.4 ± 1.0	4.7 ± 0.7
Eucalyptus	A3	4.6[1]	10.1 ± 0.2	5.8 ± 0.2	
	A4	4.6[1]	12.0 ± 0.8	7.7 ± 0.8	
	A5	4.6[1]	11.5 ± 2.2	7.2 ± 2.4	6.9 ± 0.8
Eucalyptus	NA1 & 2	5.2	13.1 ± 3.4	8.6 ± 3.5	
	NA3 & 4	6.0	8.0 ± 2.2	2.3 ± 2.5	5.4 ± 2.4
Eucalyptus	C1	5.8[1]	3.3 ± 0.4	-2.6 ± 0.4	
	C2	5.8[1]	4.4 ± 0.2	-1.4 ± 0.2	
	C3	5.8[1]	4.7 ± 1.7	-1.2 ± 1.8	-1.7 ± 0.4

[1]One sample of diet was the control for all three birds.

The largest percentage of pollen grains that were digested by the hummingbirds was only 6.9% by adult Anna's eating *Eucalyptus* pollen, and no digestion was found in the Costa's eating *Eucalyptus* or *Zauschneria* (Table 3). The Anna's digested less *Callistemon* pollen, 4.7%, than *Eucalyptus* pollen. The adult and nestling Anna's did not differ significantly in their ability to digest *Eucalyptus* pollen .

Like the hummingbirds, the adult lorikeets of both subspecies digested low percentages of the *Eucalyptus* pollen, with the adult rainbow lorikeets digesting 4.5% and the adult Moluccans 6.6% (Table 4). However, the nestling Moluccan lorikeets digested 26% of the *Eucalyptus* pollen, significantly more than the adults, and substantial differences were also seen between the adult and nestling cockatiels, which digested 18.1% and 38%, respectively. The nestling cockatiels digested twice the percentage of *Eucalyptus* pollen as did the adults, and the Moluccan lorikeets digested more than three times the percentage of *Eucalyptus* as nestlings than they did as adults.

The *Prunus* pollen, which was also fed to the Moluccan lorikeets as nestlings and then as adults, and to the nestling cockatiels, passed through all the birds with less than 15% digested (Table 4).

The results of this study indicate that none of the four pollens fed is likely to furnish significant amounts of protein or energy to the diets of the species studied.

When flowers are available, hummingbirds have an easily accessible source of energy from nectar; thus it would be expected that if pollen were important in their diets it would be as a source of nitrogen and other nutrients. They are also skilled at catching arthropods, the remains of which are normally found in their stomachs and crops at all times of the day (Remson et al. 1986). Arthropods contain a high percentage of protein on a dry weight basis (Leung 1968, Williams and Prints 1986), hence for hummingbirds pollen might best be considered as a supplement to, or as a partial replacement of, arthropod feeding.

It has been assumed that lorikeets feed on pollen as well as nectar and fruits (Forshaw 1981). As shown in Table 4, however, the adult rainbow and Moluccan lorikeets, like the hummingbirds, digested only small amounts of the pollen. Such low levels of digestion appear to be an inefficient way to fulfill energy requirements, especially with the availability of easily assimilable sugars in nectar. The protein requirements of lorikeets have not been studied, but even if they are low in relation to energy needs, as is the case with hummingbirds, the birds would have to consume very large amounts of pollen to meet their requirements. Lorikeets are known not to catch flies or feed on the ground (Forshaw 1981), but they may satisfy a portion of their protein needs from fruits and flower buds and from insects gleaned from leaves and flowers. Arthropod remains are often found in the stomachs of collected lorikeets (North 1911, Cleland 1969).

Adult cockatiels are primarily seed-eaters and pollen feeding is not considered part of their feeding regime (Forshaw 1977), yet the percent of *Eucalyptus* pollen digested by adults was almost three times the percentage digested by adult hummingbirds and lorikeets. The reason for this is not apparent.

The cockatiel chicks digested almost 40% of the *Eucalyptus* pollen (Table 4), more than any other group fed and twice the percentage of the adult cockatiels. Similarly, the Moluccan lorikeets digested three times the percentage of *Eucalyptus* pollen as chicks than they did as adults. Normally, more complete digestion of nutrients is seen in older birds.

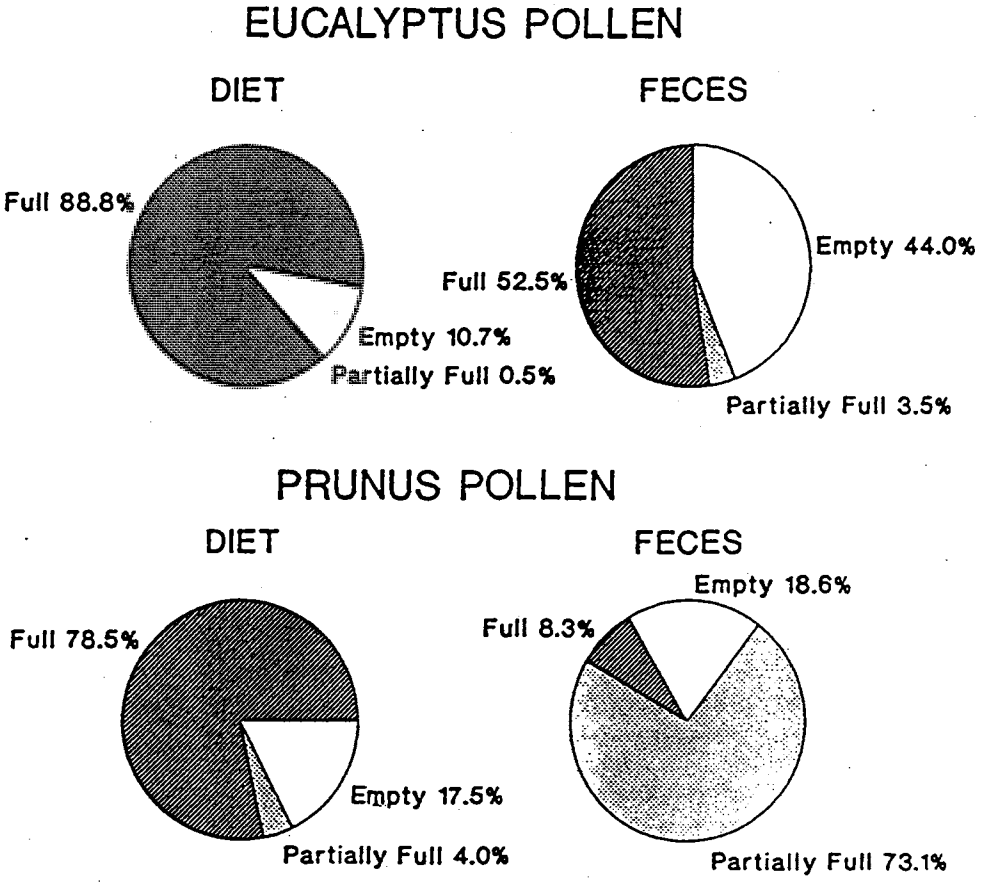
Table 4. Pollen digestion by psittacines: adult Cockatiels (AC); nestling Cockatiels (NC); adult Rainbow Lorikeets (ARL); adult Moluccan Lorikeets (AML); and nestling Moluccan Lorikeets (NML). "Partially full" pollen grains were partitioned as indicated in the "Methods" section. Values are means ±S.E. of 3 collection periods.

		Empty Pollen Grains		Pollen Grains Digested	
Pollen Source	Bird	Diet[1] (%)	Feces (%)	Per Bird (%)	Per Trial (%)
Eucalyptus	AC1	12.7	32.4 ± 3.7	22.7 ± 4.0	
	AC2	12.7	24.4 ± 2.3	13.6 ± 2.7	18.1 ± 2.8
Eucalyptus	NC1	10.8	44.9 ± 2.9	38.5 ± 3.3	
	NC2	10.8	40.2 ± 3.6	33.1 ± 4.0	
	NC3	10.8	41.1 ± 3.9	34.1 ± 4.4	
	NC4	10.8	51.6 ± 2.9	46.2 ± 3.2	38.0 ± 2.2
Eucalyptus	ARL1	1.8	5.6 ± 0.1	3.9 ± 0.1	
	ARL2	1.8	6.0 ± 1.3	4.3 ± 1.3	
	ARL3	1.8	6.0 ± 1.0	4.3 ± 1.0	
	ARL4	1.8	7.3 ± 1.2	5.6 ± 1.2	4.5 ± 0.5
Eucalyptus	AML1	12.5	17.9 ± 0.4	6.2 ± 0.5	
	AML2	12.5	18.4 ± 0.8	7.0 ± 1.0	6.6 ± 0.5
Eucalyptus	NML1	12.3	24.2 ± 3.9	19.5 ± 4.5	
	NML2	12.3	40.6 ± 6.5	32.4 ± 7.4	26.0 ± 4.9
Prunus	NC1	18.2	29.5 ± 0.6	14.5 ± 0.8	
	NC2	18.2	29.3 ± 1.7	14.2 ± 2.1	
	NC3	18.2	31.9 ± 0.2	17.5 ± 0.2	
	NC4	18.2	27.4 ± 1.4	11.8 ± 1.7	14.5 ± 0.9
Prunus	AML1	18.4	30.1 ± 1.2	15.2 ± 1.6	
	AML2	18.4	26.6 ± 1.9	10.7 ± 2.5	12.9 ± 10.6
Prunus	NML1 &2	20.6	30.0 ± 2.9	12.3 ± 3.7	12.3 ± 3.7

[1]One sample of diet was the control for each pollen feeding trial.

There is variation in the digestibility of different pollen species fed to the same birds (Fig. 2). Clearly the initial stages of digestion had begun in the *Prunus* pollen grains that were scored "partially full": swelling of the germination pores had occurred and in some cases had ruptured, but the majority of the pollen cytoplasm was still visible within the grains. An increase of partially full, but not empty, *Prunus* pollen grains was also seen in the diet samples allowed to sit at room temperature for several hours. *Prunus* pollen may be more sensitive than the others fed to the osmotic environment of both the diet and the birds' digestive tracts, but this did not result in larger numbers of grains that were completely digested. The nutritional importance of this initial digestion in the birds is unknown but based on direct observations (Y.-S. Peng, pers. comm.) is probably minimal. More research on the physiological and ecological aspects of avian pollen digestion would be useful, especially among those species of lorikeets that have been reported to forage actively for pollen in the wild.

Figure 2. ***Eucalyptus*** **and** ***Prunus*** **pollen grains scored before and after ingestion by Cockatiel nestlings.**



To what extent hummingbirds utilize arthropods as an energy source, as well as a source of amino acids, vitamins and minerals, has not been studied directly.. However, Wolf (1970) estimated that several species in Costa Rica spent 70% or more of foraging time flycatching during certain times of the year, and Montgomerie and Redsell (1980) observed a nesting female broad-tailed hummingbird (*Selasphorus platycercus*) that appeared to feed on nothing but insects for several days. They calculated, as has Hainsworth (1977), that, theoretically, hummingbirds should be able to meet their energy requirements from readily available arthropods within the limits of normal foraging time. Whether they are physiologically capable of metabolizing arthropods efficiently enough to fulfill their total nutritional needs has not been studied under controlled conditions.

To gain a better understanding of the role of arthropods in the diet of hummingbirds, I conducted studies which simulated two conditions hummingbirds might encounter in nature: a period of time when arthropods but no nectar were available and a period of time when nectar but no

arthropods were available. I report here on the ability of captive Anna's hummingbirds (*Calypte anna*) to subsist on these two dietary regimes. Please see Brice, 1992 for a more detailed report of this study.

Methods

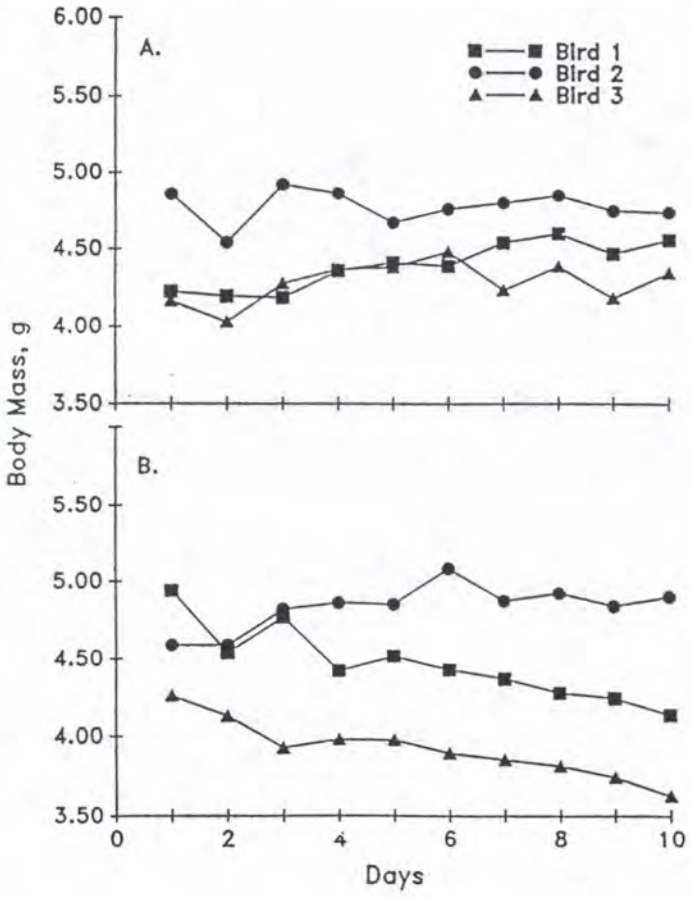
After more than one year in captivity, three male Anna's hummingbirds that had been maintained on the purified diet were gradually switched to an un-supplemented diet that contained 22% sucrose in deionized water plus an abundant supply of free-flying fruit flies (*Drosophila melanogaster*). The birds were housed in individual cages (38 cm x 38 cm x 92 cm) covered with mosquito netting, which completely contained the flies.

Each cage contained a perch attached to strain gauges. When activated by the weight of a birds, they generated a voltage output that was sent to the input terminals of a control unit, amplified and converted into grams, and displayed by a digital read-out on the front panel of the unit.

Throughout the feeding trials, the birds were weighed soon after the lights switched on, before feeding, and a few minutes before the lights went off at night. During the experiment when one of the birds was fed only fruit flies for four hours, all three birds were weighed every two hours throughout the day.

Each of the three birds was fed one of three dietary regimes for 10 days, thus after 30 days each bird had been fed each diet. The diets consisted of: 1) a 22% sucrose solution; 2) a 22% sucrose solution plus unlimited fruit flies; and 3) fruit flies alone during one of three four-hour time blocks (845-1245, 1245-1645, or 1645-2045) per day. For the remainder of those days the birds were fed fruit flies and sucrose solution, which they were also fed for at least 24 hours before being offered fruit flies alone for another 4 hour block. When fruit flies were included in the feeding trials, flies were shaken into the cages from jars every morning, and more were added later during the day as necessary. Before being fed the sucrose solution diet without fruit flies, the birds were removed from the cages, all flies were removed and the cages wiped clean before the birds were returned. The feeding syringes were weighed before

Figure 3. Morning body masses of Anna's during ten day experimental periods when fed A) 22% sucrose solution and fruit flies and B) 22% sucrose solution only.



placement on the cages and then 24 hours later to determine the daily intake.

Results and Discussion

All three birds fed the 22% sucrose solution plus unlimited fruit flies maintained their weights over the 10 day period (Fig. 3a). Bird 1, which spent more time flying than the others, ate a mean of 15.78g/day of sucrose solution, significantly more than bird 2 or 3, which ate 9.68g/day and 9.00 g/day, respectively.

When fed only the sucrose solution, birds 1 and 3 lost 16% and 15% of their initial weight, respectively, by day 10 (Fig. 3b). Bird 2, however, gained 7% more than his initial weight by day 10. All three birds maintained their normal activity patterns of flying and preening, and the daily food intake level over 10 days was comparable to the same diet when fruit flies were available. The amount of sucrose solution consumed per two hour block was essentially constant throughout the day regardless of the dietary regime (Fig. 4).

When the birds were offered fruit flies and water with no sucrose solution from 845 to 1245, none of them attempted to catch flies. During the first hour or two they flew around their cages with greater frequency than when fed the other two diets. Bird 1 drank an amount of deionized water equivalent to his morning sugar water intake (5.8g), but the other two drank negligible amounts of water. By 1245, however, birds 1 and 3 were fluffed up, sitting on the floors of their cages. They were revived by hand feeding 22% sucrose solution. Bird 1 ate 0.54g and bird 3 ate 0.66g of sucrose solution at this feeding. Within minutes they were back on their perches and preening. After 4 hours bird 2 was also fluffed up but was still on the perch and flew to feed himself when the sucrose solution syringe was offered, eating 0.34g.

When fed fruit flies and deionized water from 1245 to 1645, birds 1 and 2 were quiet by the end of the period, but were able to eat when the sucrose solution was available. Bird 1 drank 3.7g of water. Bird 3 was fluffed up and listless by 1545. When offered sucrose solution by hand, he ate 0.68 g and then resumed normal activity.

Due to signs of stress, bird 3 was not offered fruit flies and deionized water a third time, but the diet was provided to birds 1 and 2 from 1645 to 2045 during separate experimental periods. The birds were not observed feeding on fruit flies, and bird 1 drank 3.3g of water. They were quiet but not fluffed up at the end of the day, and they both fed normally the next morning when the sucrose solution was made available.

Perhaps the most striking results of this study were that the birds subsisted so well for ten days when the only food available to them was a 22% sucrose solution diet and that they appeared not to be able to replace that energy source with an ample supply of fruit flies.

Except for Montgomerie and Redsell's (1980) observation of a nestling hummingbird subsisting on flies for several days when no nectar was available, there is no documentation of hummingbirds routinely eating a diet composed solely of arthropods. The captive hummingbirds in this study made no apparent attempts to meet their energy needs from the flies, on which they were accustomed to feeding, whereas sugar appeared to be essential. Presumably, in a natural situation, nectar production of a given area would be slowly depleted. It is possible that the captive Anna's might have succeeded without sucrose solution if the amount available had been decreased gradually to permit the hummingbirds to adjust to an increased arthropod intake.

The birds' negative reaction to a diet of fruit flies alone appeared to be a behavioral response to the lack of sucrose solution, and thus it could not be determined here whether hummingbirds have the physiological capacity to metabolize the nutrients in arthropods efficiently enough to meet their energy demands. This study does provide evidence, however, that adult, non-reproducing hummingbirds are well adapted to living through periods of arthropod scarcity lasting at least as long as 10 days without any serious effects. Such unexpected temporal flexibility in fulfilling non-carbohydrate nutritional requirements allows hummingbirds to make ecological decisions that maximize immediate energy acquisition and provides some latitude for response to environmental variations as long as nectar is available.

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FOOD PREFERENCES IN SELECTED SPECIES OF LORIES

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Introduction

The striking colored plumage and playful nature of lories have made them favorites in the world of aviculture for decades. There are fifteen genera in the family of Loriidae. The two characteristics common to all are: a) diet, which consists of fruit, nectar and pollen; b) the unusual brush-like tongue, which was thought to be used to gather up the nectar.

In 1970 it was discovered that the tongue was an organ for harvesting pollen and pressing it into a form suitable for swallowing, and not primarily for extracting nectar (Churchill and Christensen, 1970). Nectar is consumed when available, but only as an addition to the pollen which is their primary source of nitrogen. When the birds ingest nectar, subcutaneous fat is accumulated (Churchill and Christensen, 1970).

Due to their specialized diet lories have evolved structural adaptations of the bill, tongue and alimentary canal. The bills are longer and slimmer than other parrots, the ventriculus or gizzard of the Loriidae is weak and not muscular. The compound glands are arranged linearly along the walls of the proventriculus (Steinbacher, 1934). The most fascinating modification is their tongue, which is long and tipped with papillae that expand like tentacles to feed on flowers (Serpell, 1985).

Everyone that breeds lories swears by a diet that is the definitive lory diet. The reality is that each is quite different and contain elements that lories would not find in the wild. There are no quantitative studies in the literature on lory diets in nature, although studies refer to lories feeding on the same fruiting trees in New Guinea as fruit pigeons and hornbills (Leighton, 1986). There are few studies of lories in captivity; all that is really known is that they appear to have a greater need for sugar than most other birds.

The San Antonio Zoo (SAZ) has a large collection of lories representing 7 species. They have all bred consistently over the years. In looking at their traditional diet, it is a combination of many 'tried and true' diets for lories that date back to 1925.

Lories and their food preferences have been a nutritional mystery. SAZ decided to take on the challenge; but with so little information in the literature it was difficult to choose a starting point. Research in zoos always presents problems along with its rewards. We could ask the questions that could be answered by research, but what about the variables such as the novelty factor in introducing a new diet, or, the opposite, suspicion or aversion to the new diet? We decided to test the palatability of a commercial lory diet, both dry and liquid in composition, against the traditional diet.

Methodology

Six lories were selected for the study, four Duyvenbodes (*Chalcopsitta duivenbodei duvenbodei*), and two Stella's (*Charmosyna papou goliathina*) lories. The birds, including 1.1 Stellas, 1.1 and 0.0.2 Duyvenbodes housed as pairs in separate cages in the Parrot House, were under three years of age and determined to be in good health. The three adjacent cages used in the study were all the same size: the outside enclosure measured 2.56 m (long) x 1.23 m (wide) x 2.61 m (high), and the indoor enclosure measured 1.12 m x .92 m x 1.82 m.

Table 1. Composition of SAZ Lory mix.

Ingredient	Weight (g)
Whole wheat bread	425
Sugar	198
Milk	198
Gerber Hi-Protein ceral	114
Pullet starter	83
Apple	435
Carrot	508
Flamingo pellets	22
Monkey biscuit (25%)	22
Powered egg	11
Limestone	4
Vitamin pre-mix	2
Brewer's yeast	2
Salt	1.5

Before the palatability of a new diet could be determined, a baseline was established by measuring the feed intake of the diet normally fed to lories at the SAZ for 13 days. The control diet consisted of a liquid mixture and a fruit and vegetable mixture. The liquid mixture (SAZ lory mix) contained fourteen ingredients (Table 1). The primary ingredients included sweetened condensed milk, dry baby cereal, whole wheat bread, sugar, apples, and carrots. The fruit mixture contained apples, oranges, peas, corn, blueberries, and occasionally bananas.

The liquid lory mix and fruit were prepared daily. The birds were offered 600g lory mix per day, divided evenly between two feedings, one in the morning and one in the afternoon. Seventy-five (75g) grams of fruit were offered in the afternoon. The remaining lory mix from the morning feeding was picked up and weighed during the afternoon, and the remains of the afternoon feedings of lory mix and fruit were picked up and weighed in the evening. On cold nights the afternoon feeding was left in the pens overnight and the remains weighed in the morning.

During the palatability trial a commercial nectar mixture, Roudybush's Hummingbird #1 was offered in dry powder form as well as in liquid form along with the SAZ lory mix and fruit mixture. The Roudybush nectar was prepared by mixing the powder with warm water in a 1:6 ratio in a blender. For 21 days the birds were offered 600g Roudybush nectar, 10g Roudybush dry, 600g SAZ lory mixture, and 75g fruit. As with the control study, the nectars were divided between a.m. and p.m. feedings, the fruit was offered in the afternoon, and the dry diet was offered in the morning and picked up in the evening. All remaining food items in the pans were weighed and recorded.

During the 34 day study the birds were weighed regularly in order to monitor their health status. It was determined at the beginning of the study that if any bird lost 20% of its original body weight it would be pulled from the study.

The data was analyzed using SPSSX software statistics program. Curves of the food consumption and weights of the three pairs of birds was prepared using Harvard Graphics. The

nutritional composition of the mean ingredients consumed was computed with the use of MIXIT-2+ nutrition software program.

Results

During the 13 days of the control study the Stellas consumed on average 166.5g SAZ lory mix and 41.4g fruit per day. During the 21 days of the feeding trial they consumed on average 105.2g SAZ lory mix, 22.9g fruit, 144.6g Roudybush nectar, and 0g Roudybush dry per day. The 1.1 Duyvenbodes consumed per day 429.0g SAZ lory mix, 17.5g fruit per day during the control, and 104.9g SAZ lory mix, 9.7g fruit, 272.9g Roudybush nectar, and 0g Roudybush dry. The 0.0.2 Duyvenbodes consumed 383g SAZ lory mix and 6.0g fruit per day during the control period. During the trial they consumed per day 89.0g SAZ lory mix, 6.0g fruit, 261.9g Roudybush nectar, and 0g Roudybush dry diet (Table 2 and Figures 1,2, and 3). Analysis of the data shows that the decreased consumption of the SAZ lory mix, and the increased consumption of the Roudybush nectar were both significant changes for all three pairs (Table 3). All three pairs of birds selected significantly different food items, and the food items selected were significantly different for the two trials. The difference in diet selection between the two pair of Duyvenbodes is not significant (Table 4). The consumption figures of the dry Roudybush diet was not included in the statistical analysis since all the figures were zero.

TABLE 2 Mean food consumption of Stellas and Duyvenbodes lories on control and trial diets

Bird ID	Diet	SAZ Lory Mix	STD. Error	Fruit Mix	STD. Error	Roudy Nectar	STD. Error	Roudy Dry	STD. Error
Stellas	Control	166.5	23.3	41.4	4.7	0	0	0	0
	Trial	110.0 *	20.2	22.9 *	3.3	144.6 *	22.8	0	0
Duyv 1.1	Control	429	23	17.5	2.2	0	0	0	0
	Trial	104.9 *	26.4	9.7	3.8	272.9 *	18.7	0	0
Duyv 0.0.2	Control	383	29.1	6	1.3	0	0	0	0
	Trial	89.0 *	28.7	6	3.8	261.9 *	21.2	0	0.0

* indicates significant difference ($p < .05$)

The dry matter intake and nutrient composition of the food consumed is presented in Table 3. The percentage of crude protein, fat, and fiber did not appear to vary much among the diets. The dry matter intake of the birds does appear to vary between the control and the trial diets which could possibly be explained by the fact that the average % dry matter for the control diets was between 42.3% and the average % dry matter of the trial diets was between 22.9 and 27.8%. The weights of the birds were plotted against food consumption in Figures 4, 5, and 6. Although there was some

fluctuations in the weights, overall they remained fairly constant.

Table 3. Dry matter intake and nutrient composition of food consumed.

	DUVY 1.1 Control	DUVY 1.1 Trial	DUVY 0.0.2 Control	DUVY 0.0.2 Trial	Stellas Control	Stellas Trial
Dry Matter Intake	212.9	91.7	188.9	81.7	88	75.3
% Dry Matter	47.7	23.7	48.4	22.9	42.4	27.8
Protein	13.7	14.3	13.7	14.2	13	13.6
Fat	5	4.4	5	4.3	4.9	4.5
Fiber	2.3	1.6	2.3	1.5	2.4	1.8

Discussion

Previous unpublished attempts to introduce a diet of extruded pellets to lories at the SAZ had been unsuccessful so it was no surprise when the lories in this food trial did not consume any of the dry diet. What was a surprise, was their immediate switch to the Roudybush nectar, even when their traditional diet was offered at the same time "cafeteria style". Although there have been instances of dry diets being fed successfully to lories, it is extremely difficult to wean them on to the dry food (Brice, 1987).

The Duyvenbodes seemed to prefer the Roudybush nectar, substituting the new nectar for the SAZ lory mix almost entirely. The Stellas accepted the new nectar readily, but continued to consume the SAZ lory mix as well. The Roudybush diet is a hummingbird diet and therefore most of its energy source is sugar, 73% as compared to 22% of the SAZ lory diet, which could account for its attractiveness to the lories.

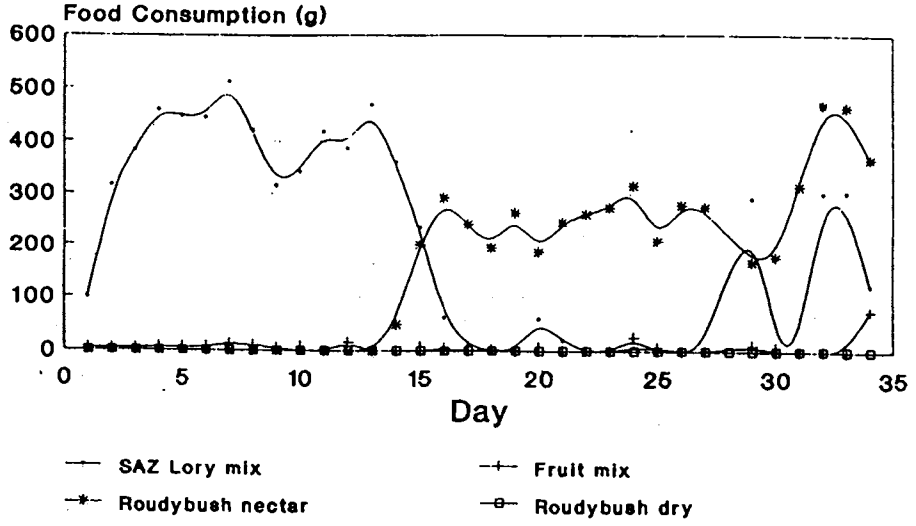
Why are lories so attracted to sugar? While they appear to adapt well to captive diets which vary considerably in sugar content, no one knows if there is a long term effect of feeding large amounts of sugar versus feeding more complex carbohydrates as an energy source. What do lories need as compared to what they like?

Although it is known that too high a level of protein can cause kidney and liver damage, as well as gout in lories, their actual requirement for protein and other nutrients is as yet unknown (Schroeder, 1991).

Conclusion

In this study we found a marked difference by species, but not by individuals, in food

LORY FOOD CONSUMPTION
0.0.2 Duyvenbodes

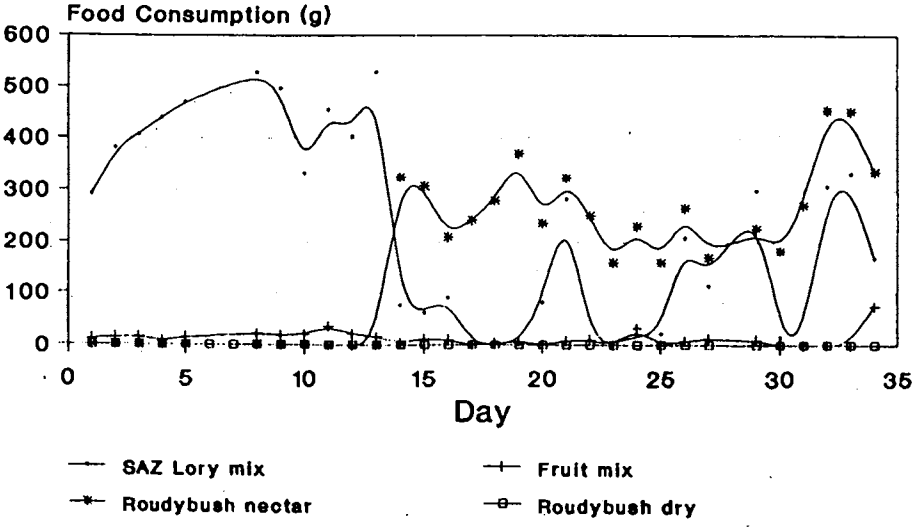


B 58

FIGURE 1

L. J. Stewart 1991

LORY FOOD CONSUMPTION
1.1 Duyvenbodes

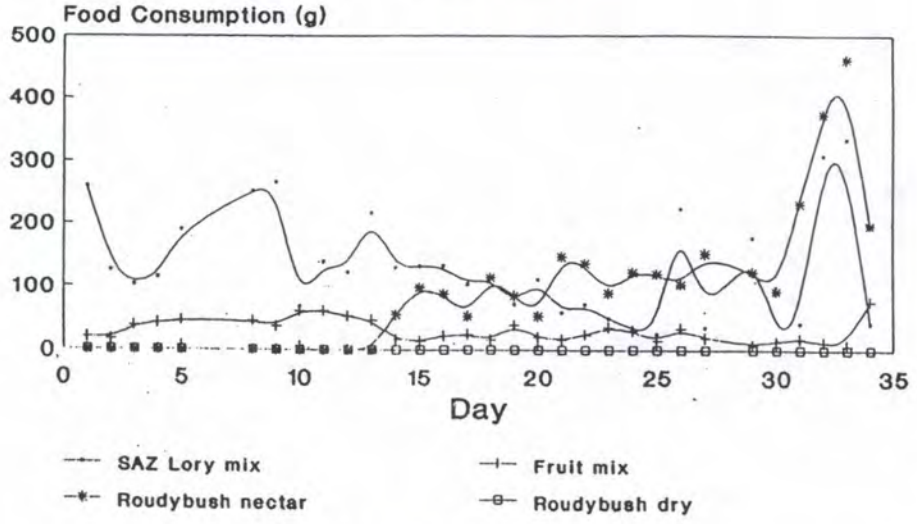


B 57

FIGURE 2

L. J. Stewart 1991

LORY FOOD CONSUMPTION
1.1 Stellas

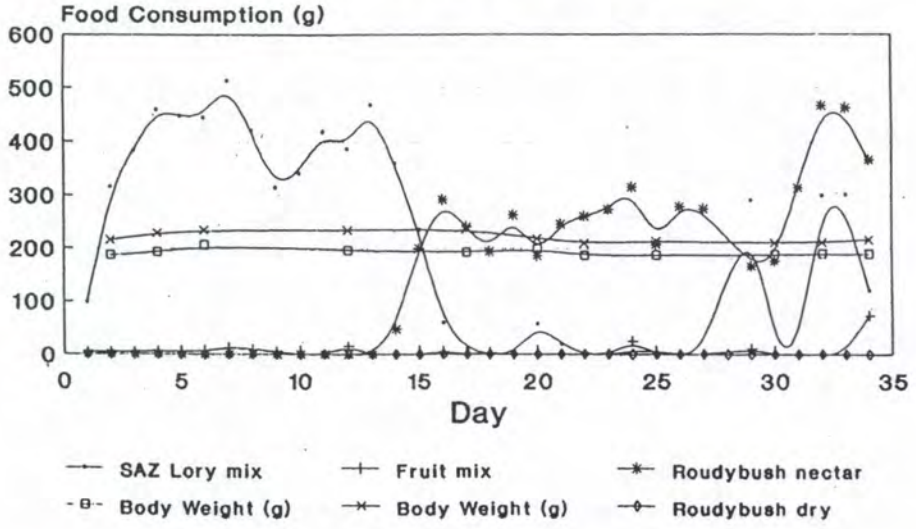


B 55

FIGURE 3

L. J. Stewart 1991

LORY FOOD CONSUMPTION & BODY WT
0.0.2 Duyvenbodes

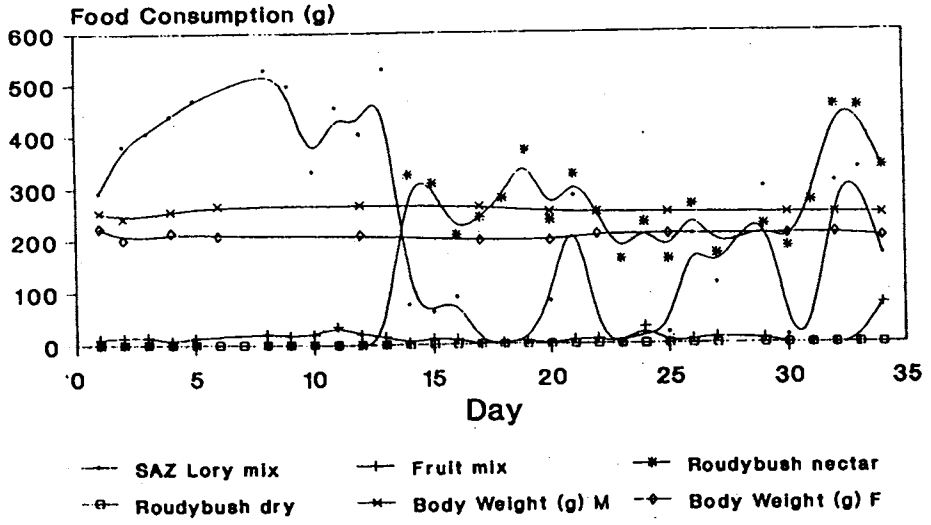


B 58 w/wt

FIGURE 4

L. J. Stewart 1991

LORY FOOD CONSUMPTION & BODY WT
1.1 Duyvenbodes

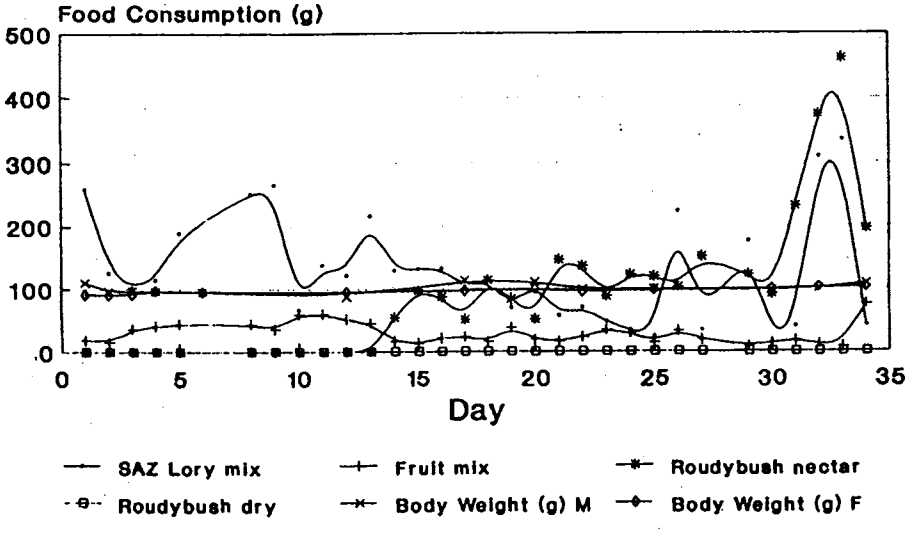


B 57

FIGURE 5

L. J. Stewart 1991

LORY FOOD CONSUMPTION & BODY WT
1.1 Stellas



B 55

FIGURE 6

L. J. Stewart 1991

preference, but there were too few birds to prove this supposition. Both species in this project are native to the island of New Guinea, and have separate, but parallel ranges (Cooper and Forshaw, 1973).

Analysis was completed for several nutrients. It appears that the birds were consuming one half the amount of the Roudybush liquid diet as compared to the control SAZ liquid diet and yet were receiving the same protein and fat intake and maintaining consistent weights (Figure 5) throughout the study. It is more likely, however, that due to the settling characteristic of the SAZ lory mix, the percent of dry matter content actually consumed may be closer to that of the Roudybush nectar intake.

In determining whether the lories in the SAZ collection would readily accept new food items and which was the preferred diet we were at the same time able to familiarize the bird department staff with procedures required for nutrition studies. The information gained from this study raises many questions and paves the way for further studies about the dietary needs of these vividly colored and charming psittacines.

Acknowledgments

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VITAMIN C REQUIREMENTS IN BIRDS

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Introduction

As birds and mammals have evolved some have lost the capability to synthesize vitamin C (ascorbic acid). In general, invertebrates and fish cannot synthesize ascorbic acid. Most amphibians, reptiles, birds, and monotremes synthesize ascorbic acid in the kidney. Most mammals such as the cow and pig synthesize ascorbic acid in the liver (Scott, 1986). Some birds including approximately one half of the passeriformes like the jungle myna and the Asian pied starling synthesize ascorbic acid in the liver. Other passeriformes such as the Indian house crow and common myna synthesize ascorbic acid in both the liver and the kidney. Humans, nonhuman primates (excluding prosimians), guinea pigs, the Indian fruit bat and some of the passeriformes including the vented bulbul cannot synthesize ascorbic acid in the liver or kidney (Robbins, 1983). Thus it may be necessary to provide some birds a dietary source of vitamin C (ascorbic acid). The purpose of this paper is to review the functions and metabolism of ascorbic acid, identify birds that are incapable of synthesizing ascorbic acid, examine species requirements and factors that may affect these requirements, as well as possible complications of excess intakes. Susceptibility of the vitamin to destruction with handling and processing will also be discussed.

Function

Ascorbic acid is a water soluble vitamin that can be classified as a carbohydrate. It was first recognized as the factor in fresh fruits and vegetables that prevented scurvy. Symptoms of scurvy include abnormal effects in collagen formation, fatty acid metabolism, brain function, drug detoxification, as well as infection, and fatigue. Ascorbic acid may be found in significant quantities in green leafy vegetables, fresh fruits, animal organs such as the liver and the kidney but only in small quantities in meats. Cereal grains have no ascorbic, and mature nuts have very little. Table 1 contains ascorbic acid quantities on a dry matter basis for a variety of foods.

One of the most significant characteristics of ascorbic acid is its metabolism to dehydroascorbic acid. This reaction provides the basis for its known physiological activities and stabilities. Specific reactions in which ascorbic acid participates include; collagen synthesis, steroid, lipid, and drug metabolism, carnitine biosynthesis, tyrosine metabolism and catecholamine synthesis, prevention of peroxidation, and histamine metabolism.

Collagens are proteins that are primary components of skin and connective tissue, the organic substances of bones and teeth, as well as the ground substances between cells. Ascorbic acid is required to protect the enzymes necessary for the formation of a stable extracellular matrix and the formation of cross links in the fibers for the formation of collagen . It may not be needed for

"growth" collagen but for "repair" collagen which stimulates connective tissue formation required by the body for repair of tissues and promotion of healing.

Sterols and steroids are lipid containing compounds that act at many different sites within the body, performing a wide variety of functions. These compounds include cholesterol which, among other things, functions as a constituent of plasma membranes and lipoproteins to corticosteroids which function in carbohydrate metabolism and maintenance of the body's water and electrolyte balance. Ascorbic acid functions in the adrenal glands in the enzyme reactions that cause the formation of corticosteroids. In addition, ascorbic acid partakes in the reactions to convert cholesterol to bile salts which are necessary for digestion and absorption of lipid. Detoxification of aromatic drugs and other xenobiotics such as carcinogens, pollutants, and pesticides requires ascorbic acid.

Table 1. Vitamin C levels in selected foods on a dry matter basis.

	Mg/kg	Source
Green pepper	15,444	Souci, et. al., 1981
Broccoli	10,071	Souci, et.al., 1981
Cauliflower	8,310	Souci, et.al., 1981
Kale	7,742	USDA, 1982
Parsley	7,692	USDA, 1984
Strawberries	6,500	USDA, 1982
Papaya	5,529	USDA, 1982
Tomato	4,172	Souci, et.al., 1981
Orange	3,497	Souci, et.al., 1981
Spinach	3,345	USDA, 1984
Lettuce	2,600	Souci, et.al., 1981
Green beans	1,852	Leveille, 1983
Celery	1,189	USDA, 1984
Cucumber	1,183	USDA, 1984
Onion	913	USDA, 1984
Sweet potato	839	USDA, 1984
Apple	816	Souci, et.al., 1981
Carrot	761	USDA, 1984
Peach	533	USDA, 1982
Banana, no skin	352	USDA, 1982
Thompson green grapes	200	Leveille, 1983
Seedless raisins	39	USDA, 1982

Carnitine is an important transporter of fatty acids into mitochondria where they provide energy to the cell. Ascorbic acid executes a similar function in carnitine synthesis as it does in collagen synthesis; to protect enzymes involved in the process. A deficiency of ascorbic acid may reduce the formation of carnitine and consequently cause an increase in triglycerides in the blood and physical the fatigue that occur in scurvy.

Tyrosine is an amino acid whose metabolism is important in nerve and endocrine system functions. In the absence of ascorbic acid, tyrosine is not metabolized to homogentisic acid similar to metabolic disorders involving mental retardation observed in those with phenylketonuria. Ascorbic acid is necessary for the production of the catecholamines from tyrosine which are required for adaptation to acute and chronic stress, such as the "fight or flight" syndrome.

Ascorbic acid acts synergistically with vitamin E and selenium to combat peroxidation and free radical damage to cellular and subcellular membranes.

In addition, ascorbic acid binds some metals to increase or decrease their absorption

in the gastrointestinal tract. Ascorbic acid binds iron to increase its absorption and binds copper to decrease its absorption. Ascorbic acid not only reduces iron to the ferrous form, which is more rapidly absorbed from the intestinal tract, but binds it such that the resulting complex is more easily absorbed than iron alone.

Ascorbic acid is important in histamine metabolism which has been associated with allergies concurrent with the onset of scurvy. Studies have shown ascorbic acid, in the presence of copper prevents the accumulation of histamine and contributes to its degradation and elimination. It also may participate by modulating prostaglandin synthesis such that those prostaglandins produced are those that mediate histamine sensitivity and cause relaxation (Jaffe, 1984).

Metabolism

In birds that can synthesize ascorbic acid it is synthesized from D-glucose by the glucuronic pathway. The inability to synthesize ascorbic acid is due to lack of L-gulono-lactone oxidase, the essential enzyme for the conversion of L-gulono-lactone into 2-oxo-L-gulono-lactone which spontaneously transforms into L-ascorbic acid (Chaudhuri and Chatterjee, 1969).

Studies performed on rats and guinea pigs revealed that ascorbic acid is oxidized to respiratory carbon dioxide. Other metabolites of L-ascorbic acid include; unchanged ascorbic acid, dehydro-L-ascorbic acid, 2,3-dioxo-L-gulonic acid, and oxalic acid. The proportions of these metabolites vary from species to species and by amounts ingested. In humans, an intestinal block prevents the body from absorbing excessive amounts of ascorbic acid. Thus, when intakes are of considerable excess of nutritional requirements a large portion of the excess is found unchanged or as dehydroascorbic acid in the feces, and to a smaller extent in the urine. Some may be converted to oxalic acid (Jaffe, 1984).

Evolution of Synthesis in Birds

The change in the biosynthetic pattern for the synthesis of ascorbic acid with evolution of birds is similar to that observed in mammals. The crucial enzyme L-gulono-lactone oxidase is located in the kidneys of birds placed in older orders and then becomes localized in the liver of more advanced passeriformes. This is demonstrated in Chaudhuri and Chatterjee's studies (1969). Their work indicates a large number of birds synthesize ascorbic acid in the liver or are incapable of this biosynthetic capability. Kidneys and livers of species in the orders; ciconiformes, psittaciformes, cuculiformes, strigiformes, coraciformes, piciformes, and several species of passeriform were examined. The higher orders of the piciformes had activity in the liver and the passeriformes had activity in both liver and kidney, the liver, or were incapable of this biosynthetic capability. It appears that the Indian house crow and the common myna exist near the transition point of biosynthetic capacity from kidney to liver with synthesis in both organs.

Requirements and Factors Affecting these Requirements

Requirements for humans, domestic, and laboratory animals that have been shown to lack the capability to synthesize ascorbic acid are; 60 mg/day for adults and children greater that 4 years (RDA 1980), 111 mg/kg of diet for nonhuman primates (NRC 1978), and 222 mg/kg of diet for the guinea pig (NRC 1978). A lack of sufficient data exists to determine maximum tolerance levels of ascorbic acid for most domestic animals. Reported studies have shown no adverse effects on growth in long term studies of 60 days or more in chickens fed 3300 mg of ascorbic acid/kg of diet. In shorter term studies of less than 60 days no adverse effects were seen in growth of swine and fish fed 10g of ascorbic acid/kg of diet. Other short term studies including dogs determined ascorbic acid intakes of 0.5 to 3a/day to not adversely affect these animals. In laboratory animals it appears that growth is not adversely affected in rats and guinea pigs until a level of 1g of ascorbic acid/kg of diet is fed (NRC, 1987).

Factors that may increase an animal's requirement for ascorbic acid include acute and chronic stress that may cause the "fight or flight" syndrome in animals or possibly high environmental temperatures, and the stress of growth and reproduction. Many processes occur during stress; release of ACTH from the pituitary acting on the adrenal glands causing the release of hormones that in turn increase glycogenolysis, gluconeogenesis, amino acid mobilization, and lipolysis. Not surprisingly, simultaneously, large amounts of ascorbic acid are released from the adrenal glands. It was previously explained that ascorbic acid plays an important role in many of these processes. In such a situation ascorbic acid may have to be continually replenished to replace the large amount used to adapt to stress. Even those animals that synthesize ascorbic acid may not be able to supply the vitamin fast enough in some instances. The stress of high environmental temperatures effects the endocrine systems responsible for retention and proper metabolic functioning of the vitamin. It has been shown in many experiments that young chicks and hens subjected to high environmental temperatures showed improved growth, egg production, and eggshell strength with 100-200 mg/kg diet supplemental ascorbic acid (Scott, 1975).

Even those animals that are capable of synthesizing ascorbic acid may have an increased need requiring an exogenous source during such stressful situations. During reproduction and growth there is an increased demand for energy and nutrients such as protein, including collagen. For example, though the willow ptarmigan produces ascorbic acid in its kidneys, it still requires an exogenous source for survival. Chicks fed a diet containing 265 mg/kg diet developed scurvy. To avoid the symptoms of scurvy and cause those chicks already afflicted with the disease, the ascorbic acid content of the diet was raised to 750 mg/kg of dry diet (Hanssen et. al., 1979). No intermediate levels between 265 and 750 mg/kg were tested.

Excessive Intakes

Excessive intakes have been observed to have deleterious effects including possible binding of divalent cations such as copper by ascorbic acid itself or oxalate, rendering these minerals unavailable. Studies performed with chickens fed copper deficient diets showed decreased growth and an increase in the incidence of aortic rupture. A decreased length of time required to produce aortic ruptures was found with the addition of 5g of ascorbic acid per kg diet. Even on copper

adequate diets, decreased growth was observed when ascorbic acid was fed at this level (Carlton and Henderson, 1965). Another study feeding chicks copper deficient diets with 0.1% ascorbic acid caused decreased growth and decreased elastin content of the aorta. These effects did not occur with copper sufficient diets plus added ascorbic acid. Ascorbic acid did reduce the uptake of labeled copper by the liver, whether the copper was given orally or intraperitonealy suggesting ascorbic acid may influence movement of copper into or out of the liver (Hill and Starcher, 1965). A study performed with pigs on a nutritionally adequate diet with added ascorbic acid at 0.5% of the diet, showed a decrease in copper absorption and an increase in iron absorption (Gipp et. al, 1974).

An additional effect of ingestion of large amounts of ascorbic acid seen in humans is a conditioned increase in the requirement for the vitamin. Case histories of adults have shown the development of symptoms of scurvy, when those consuming very large doses over a long period of time return to recommended levels of ascorbic acid. Development of scurvy was shown in infants who's mothers had ingested megadoses during pregnancy. Those infants were receiving recommended levels of ascorbic acid (Cochrane 1965). Some researchers gave evidence indicating an induction of ascorbic acid catabolizing enzymes in animals receiving megadoses of ascorbic acid while others have not. This induction also was shown to be passed from mother to young. Guinea pig pups of dams that received that high levels of ascorbic acid during the last half of pregnancy showed a marked increase in labeled carbon dioxide excretion compared with control pups, following an intraperitoneal injection of carbon labeled ascorbic acid into both groups of pups (Norkus and Rosso 1975). Significance of these findings appear to indicate that the possibility of an increased requirement for ascorbic acid with excess intakes of the vitamin and that this may be passed from mother to young.

Susceptibility to Destruction with Handling and Processing

Due to the water soluble nature of ascorbic acid, and its oxidative properties it is susceptible to destruction during handling and processing. When cooking in boiling water for a short period of time, ascorbic acid content remains quite stable. More ascorbic acid is destroyed by heating at low temperatures for long periods of time than at high temperatures for a short time. However, large amounts of water will cause losses due to leaching. Shelf life of dry feeds decrease with increases in temperature and humidity. There may be some loss by oxidation during blanching but only a small loss by leaching. Greater losses are found with ascorbic acid than other vitamins in frozen foods if some processing occurs before freezing. Processing that ruptures tissue exposes ascorbic acid to air losses due to oxidation. Rupturing of tissues may occur during thawing also. Some loss may continue during storage in the frozen state. Little ascorbic acid is destroyed if oxygen is low. Tin protects ascorbic acid in solution. Ascorbic acid is stable during normal pelleting processes. It has been shown that pellets sprayed with an emulsion of ascorbic acid in animal fat on the outside of extruded pellets caused less leaching of the vitamin when immersed in water that those pellets where the ascorbic acid was added directly to the dry diet before pelleting (Scott, 1986). During drying water soluble vitamins are partially oxidized. Rapid drying retains greater amounts of ascorbic acid than slow drying (sun drying) and freeze drying is best. Vitamin potency decreased during storage

of dried foods (Desrosier, 1977).

Summary

It appears as birds have evolved, the capability to synthesize ascorbic acid has passed from the liver in the older orders of birds to the kidneys of those in the newer orders and even to the loss of this biosynthetic capability in either organ in some Passerines. Those birds incapable of synthesis of ascorbic acid due to the absence of the enzyme L-gulono-lactone oxidase, must receive the vitamin in their diet. Additional data are needed to support actual ascorbic acid levels needed in the diets. Requirements of those species incapable of synthesis and maximum tolerance levels of others, should be considered when selecting a range due to deleterious effects observed with excessive intakes of the vitamin. Caution should be exercised in the handling and processing of foods to prevent ascorbic acid losses with light, heat, physical damage of the tissue structure of the food, and prolonged storage.

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EFFECT OF DIET AND ENVIRONMENT ON NUTRIENT UTILIZATION IN REPTILES

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Background

Environmental factors can influence nutrient utilization in both homeotherms and poikilotherms. Perhaps the most often studied environmental factor is temperature, especially the effect of temperature on energy expenditure and feed intake. Many studies of homeotherms have been conducted and have established the relationship between heat production, feed intake and feed digestibility. Few studies have been conducted to establish the effect of temperature on heat production and nutrient utilization in poikilotherms. Since temperature is a factor that can be easily manipulated in captivity, it is important to investigate the relationship of temperature and energetics of poikilotherms to improve captive management. Diet composition can also be manipulated in captivity and the effects of substrates that can provide energy (e.g., protein, fat, carbohydrates) should also be considered in diet formulation.

Many homeotherms respond to changes in environmental temperature in a similar manner. In the thermoneutral zone, body temperature is maintained by behavioral and physical means. As the temperature decreases below the lower critical temperature, heat production increases (by chemical means) to help maintain thermal balance. Heat production peaks at the summit metabolism. At temperatures below the point of summit metabolism, animals are not able to maintain body temperature and death by hypothermia occurs. Above the upper critical temperature, heat production increases as a result of the work performed by the animal (e.g., panting) to dissipate the excess heat. Heat production increases with increasing temperature in a linear manner. Death can occur from hyperthermia. In general, below the lower critical temperature food intake increases and it decreases above the upper critical temperature. The effect of temperature on nutrient utilization (nutrient digestibility) is somewhat dependent on the feeding strategy of the particular species (e.g., ruminant verses nonruminant) and the type of diet (e.g., forage verses concentrate). Relative to the thermoneutral zone, energy digestibility tends to decrease with lower temperatures and increase with higher temperatures. The changes in digestibility are linked with changes in gut motility and rate of digesta passage.

The effect of temperature on energy expenditure of poikilotherms is different than in homeotherms. Many studies have been conducted cover a limited range of temperatures and studies that cover a wide range of temperatures are limited. In general, the curve of a plot of energy expenditure verses temperature is "J" shaped. This relationship has been found in reptiles and fish. With regard to reptiles, few studies have been conducted to determine the relationship between temperature and feed intake and nutrient digestibility. In two recent studies with the herbivorous

lizard *Dipsosaurus dorsalis*, animals were maintained in the laboratory and were forced-fed diets at different temperatures. In one study, an attempt was made to adjust the amount of feed being forced-fed to the calculated maintenance energy requirement (Zimmerman and Tracy, 1989). In the other study, animals were force-fed the same amount of feed at each temperature (Barlow et al., 1986). Unfortunately, force-feeding animals may affect nutrient digestibility.

Purpose

Two studies have been conducted using captive green iguanas (*Iguana iguana*). In the first study, animals were fed three levels of dietary fiber to determine the effect of dietary fiber on intake, digestibility and growth rate. In the second study, animals were housed at different temperatures to determine the effect of temperature on intake, digestibility, energy expenditure, and efficiency of growth. In both studies, animals were offered a meal-based diet and they consumed the diet ad libitum without the need for force-feeding.

Materials and Methods

In the first study, twenty-one individually housed animals were used in a Latin-square crossover design with animals block in homogeneous squares. Three levels of dietary fiber were fed (20%, 25% and 30% neutral detergent fiber). Each animal received each diet in a random order. Animals were fed each diet for approximately eight weeks prior to fecal collections for the determination of nutrient digestibility. Each animal was weighed, snout-vent length was measured and cloacal temperature was measured at the beginning and end of each period. Animals were housed in a room with a 12:12 light cycle with a infrared bulb suspended above each cage to provide a source of radiant heat. Feed consumption was measured by offering a weighed amount of feed and reweighing the uneaten portion 24 or 48 hours later. Total fecal and cloacal waste were collected together. A marker was used at the beginning and end of the fecal collection period to minimize end-point errors.

In the second study, twelve animals were individually housed in environmental chambers at either 28°C or 35°C and 60% relative humidity. Feed intake, energy expenditure, nutrient digestibility, and growth rate data were collected in a crossover designed study. The data from the first period are discussed. The animals were fed a diet similar to the medium fiber diet of the first study. Feed consumption and fecal collections were made similarly to the first study. Energy expenditure was determined using a closed system respirometer. Carbon dioxide and hydrogen production and oxygen consumption were measured on each animal for three twenty-four hr at the beginning and end of each 12 week period. Animals were allowed to acclimate to the respirometer chambers for 24 hour prior to the collection of the first samples.

Results and Discussion

Fiber level of the diet affected daily intake, growth rate and feed efficiency ($p<0.05$). Mean cloacal temperature during these studies was 31.8°C. As the fiber level increased, growth rate, dry matter and metabolizable energy decreased. The decrease of growth rate and increase in feed efficiency (feed/gain) are probably a result of the lower metabolizable energy content associated with increased dietary fiber of the diet.

Energy expenditure and daily dry matter intake was lower in animals housed at 28°C than 35°C ($p<0.05$). There was no apparent effect of temperature on dry matter digestibility, metabolizable energy digestibility, or on the efficiency of use of metabolizable energy for gain. Animals at lower temperatures apparently consume less food, expend less energy but they can apparently use energy above maintenance as efficiently as animals at higher temperatures.

The use of nutritionally complete feeds is important for maintenance of reptiles in captivity. Under more physiologically demanding times such as growth or reproduction, properly formulated diets are critical. Diet and environment are important factors to consider in captivity since under many circumstances they can be manipulated easily. Reptilian responses to temperature are not well established, especially nutritional responses. At higher temperatures, small increases can have a large metabolic impact. At lower temperature, the rate of change may be lower but other problems can occur (Coulson and Hernandez, 1983).

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DIETARY MANAGEMENT OF A DIABETIC SUMATRAN ORANGUTAN (*Pongo pygmaeus abelii*)

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Diabetes mellitus is a chronic disease caused by insufficient secretion of insulin or by target tissue resistance to insulin; it leads to impaired metabolism of carbohydrate, fat, and protein. In humans, the primary forms, Insulin-Dependent Diabetes Mellitus (IDDM) and Non-Insulin-Dependent Diabetes Mellitus (NIDDM), have differing etiologies, pathologies, genetics, ages of onset, and treatments. IDDM accounts for only 10 to 15% of humans with diabetes (Pemberton et al., 1988). It has an abrupt onset and clinical signs reflect the lack of insulin. IDDM usually occurs in individuals under the age of 40 years. Obesity is rarely a problem with human IDDM patients (Hollenbeck et al. 1990). NIDDM accounts for almost 90% of the diabetes in humans. NIDDM usually occurs in individuals over the age of 20 years and has an insidious onset. It may exist with few or no symptoms for many years before diagnosis (Hollenbeck et al., 1990). In contrast to IDDM, the production of insulin is adequate. The problem is the insensitivity of target cells (muscle, fat) to endogenous and injected insulin (Nemchik, 1983). Obesity and NIDDM are metabolically interrelated. Not all people who become obese will develop NIDDM (Howard, 1989); however, approximately 80% of humans with NIDDM are considered obese.

In humans, both forms of diabetes mellitus are well defined and diet plays a critical role in the management of both. The occurrence of NIDDM in domestic mammals is questionable, and diet has a secondary role in the management of IDDM in dogs and cats. Diabetes mellitus in non-human primates is not as well understood as in humans. We present a case demonstrating our approach to dietary management of diabetes mellitus in a non-human primate and the difficulties encountered.

Case History

In the spring of 1986, the Philadelphia Zoo opened a new exhibit, the "World of Primates". "Bung", PZG ISIS # 100008, a 29 year old intact male Sumatran orangutan (*Pongo pygmaeus abelii*) was one of the primates moved from the "Rare Animal House" to the new "World of Primates". All the animals to be moved were placed under anesthesia and complete medical examinations were done. At the time of the move, Bung was considered obese (114 kg), but in general good health. Blood samples were collected. The serum glucose level was 128 mg/dl. Normal human values are between 65 - 135 mg/dl, normal orangutan levels from ISIS are 85.3 $\pm$ 24.6 mg/dl (Mean $\pm$ SD) (Wells et al., 1990).

In the three months following the move, Bung began to slowly lose weight. He was lethargic, lack of appetite and seemed depressed. He showed no interest in his food or new surroundings. The

curator and veterinarians decided to place Bung under anesthesia in early August 1986, for a repeat examination. At this time it was discovered that Bung had lost 27% of his body weight (Table 1). Bung was found to be hyperglycemic (211 mg/dl), glucosuric (1 gm/dl), and ketonuric (+2), with trace proteinuria. Diabetes mellitus was diagnosed.

Medical Management

Bung was started on 5 units of NPH Iletin I (beef-pork) isophane insulin suspension (NPH), injected once/day before the first feeding. The level of insulin was based on the amount of glucose excreted in the urine; dosage was adjusted daily . At the time Bung was diagnosed with diabetes, he was underweight (83 kg) and had a urinary tract infection. His appetite improved after several days of insulin administration. Food intake was monitored closely, and the amount of food offered was increased initially but its composition was unchanged. In order to give Bung a daily insulin injection, it was necessary to house him in a squeeze cage. The confined space did not allow the animal much movement. He soon began to regain the weight he had lost.

After 6 months, Bung had regained 15 kg. The level of insulin he required steadily increased (Table 1). In early February 1987, the glucose concentration in his blood was 189 mg/dl (Table 1). By late February 1987, his insulin dosage had reached 100 units (Table 1), a level which suggests insulin resistance in humans (Moller, 1991). The veterinarians deemed it necessary to establish a diabetic diet for Bung in order to control his weight and manage his apparent resistance to insulin.

Dietary Management

The nutritional goals were to provide nutrients sufficient for a 91 kg diabetic mature male orangutan, moderate calories, reduce glucosuria and hence moderate blood glucose levels.

To begin, prior feeding records were examined. Records of specific food intakes during Bung's 24 years of good health at the Philadelphia Zoo are lacking. From general dietary records for primates at the Zoo, it is likely that he was fed Zoo Cake (Ratcliffe, 1977) twice daily with a moderate amount of produce, from 1962 -1986.

Zoo Cake is a barley- and corn-based mix, made daily at the Philadelphia Zoo, which contains grains, oil seed meals, chicken by-product, minerals , and vitamins. It averages 21% protein, 6% fat, 58% carbohydrates on a dry matter basis.

Bung's diet was reformulated in February 1987, keeping Zoo Cake as the primary source of calories and essential nutrients. Carrots, apples, oranges, escarole, celery and popcorn were given to supplement the diet. Bung's caloric intake was calculated as 1.7 times basal metabolic rate (BMR), based on Kleiber's equation: $BMR = 70W^{0.75}$, where W = body mass in kg (Kleiber, 1961). The

Table 1. Weight, serum glucose level, urine glucose level and NPH insulin dose of a male Sumatran orangutan with diabetes mellitus.

Date	Weight (a)	Serum Glucose	Urine Glucose	NPH Insulin (b)
	Kg	mg/Dl	g/Dl	units
2 Dec 1982	119	*	*	0
10 Oct 1985	120	*	*	0
14 May 1986	114	128	*	0
5 Aug 1986 (c)	83	210	1	5
4 Dec 1986	*	*	1	16
3 Feb 1987	98	189	1	40
21 Feb 1987	*	*	1-2	100
27 Apr 1987 (d)	*	255	*	X
17 Aug 1987 (e)	113	160	neg	46
10 Oct 1987	109	45	*	181
10 Jan 1988	*	*	neg	201
10 Apr 1988	*	*	1/ 10	276
10 Jul 1988	*	*	1/ 4	223
10 Oct 1988	*	*	neg	218
10 Jan 1989	*	*	1/ 4	223
10 Apr 1989	*	*	1-2	220
10 Jul 1989	*	*	1/ 4	193
10 Oct 1989	*	*	½	109
23 Dec 1989	115	180	neg	50
30 Jan 1990	113	306	1/ 4	113
10 Apr 1990	*	*	neg	110
10 Jul 1990	*	*	1/10	100
10 Oct 1990	*	*	neg	82
10 Jan 1991	*	*	1/ 4	70
10 Apr 1991	*	*	1/ 4	3
4 Jun 1991	76	112	neg	0
3 Jul 1991	76	120	neg	0
27 Jul 1991	71	154	neg	0
13 Aug 1991 (f)	59	*	*	0

*-no data, X-refused injection, a-actual weight, b-NPH Iletin I (Beef-Pork) isophane insulin suspension, c-diabetes mellitus diagnosed, d-initial release into exhibit, e-permanent release into exhibit, f-euthanized

distribution of calories was modified from the recommendations of the American Diabetes Association and others for humans (Garrow, 1981; West, 1983). The proportion of calories from fat and carbohydrates were adjusted to better reflect the high fiber, low fat contents of diets of wild Sumatran orangutans. (Rijksen, 1978). Total daily kcal intake averaged 3455 kcal, 19 percent (metabolizable energy (ME) basis) protein, 12% fat, 69% carbohydrates and 7.4% (dry matter (DM) basis) crude fiber (Table 2).

Table 2. Diet fed to a mature male Sumatran orangutan with diabetes mellitus. (N-Squared Computing, Silverton, OR.)

Ingredient	AMT (g)	KCAL (ME)	Protein	Fat	Carbohydrate	Fiber
			% kcal ME			% DM
MAIN DIET						
Zoo Cake	1362	2771	22	14	64	8.7
Oranges	570	260	6	2	92	3.0
Apples	450	240	2	9	89	2.0
Escarole	230	46	24	9	77	14.4
Carrots	170	71	11	5	85	8.4
FORAGE FOODS						
Carrots chopped	81	34	11	5	85	8.4
Celery chopped	40	3	15	6	79	13.2
Popcorn	6	28	11	26	63	0.0
TOTAL	2909	3455	19	12	69	7.4

Long Term Dietary Management

Weight control was a primary concern. Since Bung was kept in a squeeze cage to allow for administration of insulin injections, he had no opportunity for exercise. In addition, despite the veterinary staff's best efforts to keep the animal entertained (TV, magazines, toys etc.), he developed a lick granuloma on his wrist, presumably out of boredom and/or stress. In sympathy, the animal often received additional treats from his caretakers.

Six months after Bung was diagnosed with diabetes, the veterinary staff began training him to take his insulin injections voluntarily in preparation for moving him back onto exhibit. Bung was accepting his insulin injections voluntarily within two weeks, and he was released into his former exhibit at the Rare Animal House. Unfortunately, two days after his release he started to refuse to

accept the insulin injections. He was once again placed in the primate squeeze cage. His exhibit at the Rare Animal House was modified with a squeeze and twelve months after Bung was diagnosed he was permanently released into his former exhibit. During the time Bung was in the squeeze cage, one year and two weeks, he had gained 31 kg and was back up to his original weight. During this period he also suffered recurring urinary tract infections requiring aggressive treatment. Once Bung was released into the exhibit, an element of activity was added by cutting the fruit and vegetables in the diet into small chunks and spreading them throughout the exhibit. Browse was also offered whenever available. The diet was fed over the course of the day, divided into 5 scheduled feedings. Although Bung was above what was considered to be a desirable weight, the veterinarians believed his diabetes was under control. He was not showing any overt signs of complications from his diabetes, and was alert and taking his insulin injections voluntarily. No further adjustments were made in the diet for three years.

In January of 1991, the daily insulin dose was reduced gradually, and by mid-April, treatment with insulin was no longer required. In late May of 1991, almost 5 years after Bung was first diagnosed with diabetes, the keepers reported that he had a significant decrease in appetite over a five day period. Upon visual inspection, it was noted that Bung appeared very thin. The veterinarians decided to place the animal under anesthesia for a complete examination. Bung's weight had decreased from 113 kg in January 1990, to 76 kgs in June 1991. Diagnostic tests failed to reveal any abnormalities. In order to promote feeding, exchange lists were set up for fruit, vegetables and forage food (Table 3). The lists allowed the keepers to vary the types of fruit, vegetables and forage foods offered, without significantly changing the nutritional characteristics of the diet. Although Bung's appetite improved again for a time after the new diet was implemented, his condition continued to deteriorate. In mid August 1991, after inconclusive blood tests, radiography, ultrasonography and biopsy, the veterinarians decided exploratory surgery was necessary .

Surgical laparotomy revealed marked icterus and an extremely firm mass (approximately 12 cm diameter) attached to the right body wall immediately caudal to the right kidney. It was not possible to examine the entire small intestine, the cecum, or the proximal colon, as they were involved with the mass. An excisional biopsy of one ileocecal lymph node was collected and examined by the staff pathologist while the surgical exploration continued. Metastatic neoplastic adenocarcinoma was diagnosed in the ileocecal lymph node. Based on the grave prognosis this diagnosis carries, Bung's poor condition, and the fact that resection of the mass was impossible, the decision was made to euthanize the orangutan while he was still under anesthesia.

Necropsy Report:

A necropsy was performed (accession # 29910), immediately after Bung was euthanized. There were no grossly obvious secondary complications associated with diabetes. His arteries and veins were grossly normal, no gross lesions were apparent on his urinary bladder, and the pituitary and pancreas were grossly normal. Incidental findings at necropsy included pulmonary anthracosis, mild chronic interstitial nephritis, mild ventricular endocardiosis and marked hepatic congestion. The firm mass palpated by the surgeons was a 9 cm length of proximal colon, which had fibrous

Table 3. Examples of food exchanges utilized in the dietary management of Non-Insulin-Dependent Diabetes Mellitus (modified from Pemberton, et. al., 1988).

Vegetable - Each serving contains 2 g protein, 5 g carbohydrate, and 25 kcal. One exchange is ½ cup (100 g) juice or ½ to 1 cup raw vegetable.

Asparagus (5 - 7 sprouts)	Leeks (2 medium)
Bamboo shouts	Pea pods
Bean sprouts	Spinach
Beets	String beans
Beet greens	Tomato (1 large)
Broccoli	Turnips
Carrots	Water chestnuts

The following vegetables contain trace protein, fat, or carbohydrate. One cup is considered "free" and can be added to the daily diet.

Cabbage	Lettuce
Celery	Mushrooms
Cucumber	Radishes
Endive	Romaine
Escarole	Zucchini

Fruits - Each serving contains 15 g carbohydrate and 60 kcal.

Apple (2" diameter)	Grapes (15 small)
Apple juice (½ cup)	Kiwi (1 large)
Apricots - dried (7 halves)	Mango (½ small)
Blueberries (¾ cup)	Orange (2.5" diameter)
Cherries (12 large)	Peach (1 medium)
Dates (2 large)	Pear (1 small)
Grapefruit (½ medium)	Raisins (2 tbsp)

Fats - Each serving contains 5 g of fat and 45 kcal.

Primarily Polyunsaturated Fats	
Almonds (6 whole)	Pumpkin seeds (5)
Pecans (5 halves)	Sunflower seeds (1 tbsp hulled)
Walnuts (4 halves)	
Primarily Monosaturated Fats	
Green olives (9 medium)	Hazel nuts (5)
Ripe olives (5 large)	Peanuts (20 small)
Brazil nuts (2 medium)	Pistachio nuts (20)
Primarily Saturated Fats	
Cashews (4 large)	Macadamia nuts (3 large)

attachments to the body wall immediately caudal to the right kidney. In addition, there were several fibrous attachments between parts of the jejunum and ileum and the mass. All ileocecal lymph nodes were markedly enlarged and were very firm. On cut section, the lymph node architecture was completely replaced by glistening white fibrous material. The small intestine was grossly normal. The 9 cm of the neoplastic segment of colon began 3 cm distal to the cecum. The lumen of this segment of colon was severely constricted by polypoid and papilliferous mucosal projections. There were numerous mucosal ulcers, some of which were transmural. The wall of this segment of colon was greatly thickened (up to 7 mm thick) by glistening white fibrous material. The histologic diagnosis was colonic adenocarcinoma with metastasis to ileocecal lymph nodes. The cause of death was recorded as euthanasia and colonic adenocarcinoma with regional lymph node metastasis.

Discussion and Conclusion:

Weight control of our diabetic orangutan proved to be extremely difficult. Treats given to the animal for insulin injection training, a sedentary life, a confined living space, and the inability to weigh the animal except under anesthesia compounded the problem. Although the need for insulin was not eliminated until the last four months of life, when Bung was failing due to colon cancer, the use of diet and insulin appeared to manage his diabetes for 50 months. No secondary complications associated with diabetes were found at the time of death. However, in humans, complications to the disease do not usually occur until the disease has been present for 10 to 15 years (Cotran et al. 1989).

Published experience with dietary management of diabetes mellitus in orangutans is limited. The Brookfield Zoo currently has one diabetic orangutans (Chrissy 1991, personal communication). Initially the animal was placed on oral hypoglycemic drugs and the fibrous items (green leafy vegetables, browse and some fruits) in the diet were increased. A diet rotation offering a much wider variety of fruits and vegetables than the original Philadelphia diet was established. About a year after the animal was diagnosed, a chromium supplement of 500 ug/animal/day was added to the diet. In some humans and laboratory animals, NIDDM has been helped by the administration of chromium. Insulin injections were started at the Brookfield Zoo eighteen months after the animal was first diagnosed. At this time a more strict diet was implemented. The diet contained 10% fruits, 20% vegetables, 10% starchy vegetables, 47% leafy vegetables and 13% monkey biscuit. The diet was later adjusted to 7% fruit, 17% vegetables, 7% starchy vegetables, 61% leafy vegetables and 8% monkey biscuit, based on what the animal actually consumed. The schedule of feeding was modified to complement the activity of the insulin.

Obesity and NIDDM have been found to be metabolically interrelated in humans, and may be linked to diabetes in orangutans. Captive orangutans are often overweight. For example, the weights of captive adult male orangutans range from 75 - 189 kg (Martin, 1986), compared to their wild counterparts whose weights usually do not exceed 90 kg (Rijksen, 1978). Of 24 responses to a survey done by Dierenfeld (1990) on the diets of orangutans in North American zoos, 25% (n=6) listed obesity as a health problem attributable to diet. Wells et al. (1990) surveyed 41 zoos in North America. Interestingly, in a population of 249 animals, 6 animals over the age of 20 years were recorded to be suffering from diabetes (approximately 2% of the population). If obesity is a risk

factor for diabetes mellitus, then weight control should be a primary consideration in the nutritional management of the captive orangutan. Dierenfeld (1990), suggested the diet for captive orangutans should contain (by weight, as-fed basis) 50% green produce/browse, 20% fruit, 15% yellow/orange vegetables, and 15% dry high-fiber commercial primate biscuits. Although some humans and non-human primates may be genetically predisposed to obesity (Howard, 1989), we should be able to maintain the majority of animals at an optimal weight. Physical activity has also been shown to help people maintain an optimum weight, and can also reduce the occurrence of NIDDM (Helmrich et al., 1991). Feeding strategies which require the animal to search for his food and provoke some level of activity may be helpful as well.

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CLINICAL NUTRITION IN A CRANE CAPTIVE PROPAGATION PROGRAM

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Clinical veterinary nutrition provides a link between nutritional science and veterinary medicine. At the International Crane Foundation (ICF) in Baraboo, WI, veterinary nutrition has been integrated into the medical management program. Three examples demonstrate its role: i) evaluation of diets fed to crane chicks, ii) development of nutrition support programs for chicks, and iii) nutritional management of individual cranes with medical problems.

To begin integrating veterinary nutrition into crane management, attention focused on Siberian cranes (*Grus leucogeranus*). Elusive and endangered, Siberian cranes winter from late September in southeastern China and nest from May in Siberia, 5000 km away. Their nesting habitat is lichen and moss covered tundra, and sphagnum bogs, near open water. Siberian cranes are omnivorous, eating either predominantly plant or animal foods depending on location and seasonal availability. Diets appear to be varied -- twenty different foods have been reported (Perfilyev, 1965). During breeding in spring, lemmings and voles are the primary food items consumed. Fish is eaten as well. Later in the spring, when chicks hatch, abundant insect life is present and probably constitutes the majority of the chick diet. Plants (cloudberry, bearberry, cowberry, and horsetail) are primary in summer, then animals primary again in autumn (Perfilyev, 1965). Body condition of wild Siberian cranes is reported to be almost always lean, and 'plump' for only a short time during the breeding season (Perfilyev, 1965).

In captivity, Siberian cranes appear to be extremely susceptible to stress. In addition, at ICF, reproduction has not kept pace with other crane species, and body condition of individuals has become progressively heavier. Concerns about continued breeding success with captive Siberian cranes led to our focus on this species for clinical nutrition.

Crane Chick Diets:

Chick diets fed to Siberian cranes and other species at ICF were compared to diets fed successfully to chicks at the crane breeding center at the Oka State Nature Reserve, Soviet Union, and at Vogelpark Walsrode, Germany. Ingredients, calorie density, and fuel source contents were examined.

Marked differences were observed in diet ingredients. Crane chick diets from Walsrode and Oka contained primarily meat, insect, and dairy ingredients. Plant ingredients were limited to greens, berries, and sprouts. In contrast, the ICF chick diet contained almost entirely plant ingredients (Table 1).

Diets from Walsrode and Oka contained mostly fresh ingredients, including fish, eggs, grasses and berries, and freshly killed insects. The diets were in the form of moist crumbly meals. The chick

diet at ICF, however, is a fixed formula containing processed and milled ingredients in crumbled and pellet forms (Table 1). The ICF ingredients tend to be by-products. For example, corn distillers solubles are used instead of ground corn, and whey is used instead of dried milk powder. The ICF ingredients are inexpensive, readily available by-products of the milling industry in the US, and are used frequently in commercial poultry feeds. Such ingredients assure diets of consistent composition with minimal expense, but quality and wholesomeness is not guaranteed. While essential to profitable poultry farming, by-product ingredients may be less appropriate for captive propagation of rare species. With use of by-product ingredients, pelleting affords a greater margin of safety than fresh ground meals due to heat treatment.

Table 1. Ingredients utilized in crane chick diets fed at Vogelpark Walsrode, Germany, at the crane breeding center, the Oka State Nature Reserve, Soviet Union, and at International Crane Foundation (ICF), Baraboo, WI. Ingredients are listed in order by amount (as fed).

Walsrode	Oka	ICF
Insect mix[a]	Fresh whole fish	Soybean meal
Beef heart	Fresh meat[b]	Corn meal
Quark	Curds	Wheat midds
Fresh greens	Eggs	Ground oats
Pellets	Fresh greens	Alfalfa meal
Yeast	Fresh berries	Corn dist. sol.
Crickets	Wheat sprouts	Corn oil
Mealworms	Insects	Yeast
Calcium	Calcium	Whey
Vitamins/TM[c]	Vitamins/TM	Calcium
		Vitamins/TM

[a] Commercial mixture of dried insects and pellets
[b] Mice, quail, and beef
[c] TM = trace minerals

Commercial poultry diets are fed to meet goals of very rapid growth and maximal production of meat or eggs. Goals at ICF are to feed for optimal growth and health in chicks, and longevity and superior reproduction in adults, and to minimize the untoward effects of stress. At ICF, the expense of producing and growing chicks is secondary to optimal production of healthy chicks. Cranes are typical of many long-legged bird species in their tendency to develop long bone abnormalities and angular limb deformities as they grow. Research has suggested that controlling the daily rate of growth below 10-15% body weight minimizes the prevalence of these problems (Serafin 1982). This study suggests that in greater sandhill cranes (*Grus canadensis tabida*) optimal growth was attained with a relatively high protein diet (24%), containing controlled levels of sulphur containing amino acids. So for crane chicks, optimal, not maximal, growth is the goal.

Use of meat, dairy, and insect ingredients, compared to plant ingredients, generally produces diets of greater calorie density, higher quality protein, lower fiber, and greater nutrient digestibility and bioavailability. These characteristics help to support growth, especially in species that are omnivorous and carnivorous.

Computer-assisted analysis of the Oka chick diet reflected the extensive use of meat ingredients. Calorie density was high and most of the calories derived from protein and fat (Table 2). Oka chicks are taken on 4 hr daily walks in surrounding forests, where live invertebrates are consumed as well. Lack of data precluded further analysis of the Walsrode diet, but the ingredient list indicates the calorie density was likely to be greater than 3 kcal metabolizable energy (ME)/g dry

matter (DM) and most of the calories likely derive from the animal products, i.e. from protein and fat.

Siberian crane chicks at ICF are fed more carbohydrate, 60-70% of calories, than crane chicks in the wild, at Walsrode, or at Oka (Table 2). While high carbohydrate utilization appears to suffice for many captive cranes, it may not be optimal for Siberian cranes when stressed by disease, confinement, or reproduction (see discussion of fuel sources below).

Table 2. Calories and fuel sources calculated for diets of wild cranes, captive crane chick diets fed at the Oka State Nature Reserve, Soviet Union, and captive crane chicks at ICF.

Diet	kcal (Me[a]/ g DM[b])	Protein	Fat	Carbohydrate
		-------% kcal ME-------		
WILD				
rodents	4.5	47	47	6
insects	3.7	59	39	2
cranberries	3.6	2	3	95
CAPTIVE				
Oka	4.6	47	42	11
ICF	2.9	31	14	55

Based on the contrasts in chick diets and the successes from Oka and Walsrode with animal-based diets and fresh foods, it was decided to introduce food of animal origin into the ICF chick diet. Animal ingredients would raise calorie density, protein, and fat, and lower carbohydrate and fiber. The addition would also add variety to the chicks' diet. The practice of feeding one diet as the sole source of nutrients is usually reserved for those species for which nutrient requirements have been studied rigorously by means of feeding trials and biochemical analyses. Nutritional data are incomplete for Siberian cranes, and increased variety may ensure completeness of diets.

To begin, killed neonatal mice were offered to all chicks from 5 days of age. Each mouse provided about 55% of ME kcal from protein and about 40% of kcal from fat. The mice were consumed readily by all chicks if introduced by 1 week of age; chicks exposed to this whole prey at later ages were more variable in their acceptance. Smelt were also offered, and were again readily consumed by chicks exposed early to the foods. Several years of chick production data are needed to evaluate the effects, if any, of this simple change in the chick diet. However, based on a very small sample size of all chicks produced in 1991 (n = 15), no significant developmental leg problems were seen and growth rates were more easily controlled than in previous years. Future plans include additional modifications of the chick diet, incorporating more fresh food and reducing the caloric contribution from the pelleted diet.

Nutrition Support:

Nutrition support of anorectic, severely sick, or injured cranes was considered inadequate at ICF. The enteral formula that was used (see below) kept adults alive, but was nutritionally suboptimal

for adults and had been clinically ineffective in the support of injured growing chicks. Because of several severely injured whooping crane (*Grus americana*) chicks needing nutrition support in the summer of 1990, a new chick enteral nutrition protocol was developed.

In general, provision of appropriate levels of calories and nutrients during illness improves recovery from disease and injury, enhances immune function, reduces hospitalization time, and improves mortality rates (Shils, 1988). Diseases, surgery, trauma, and stress affect calorie needs and the proportions of calorie utilization from protein, fat, and carbohydrate.

Fuel Sources: Cranes use carbohydrates, fats, and protein for energy. These three fuel sources are oxidized in amounts and proportions that can be altered by external or internal factors. The abundance of carbohydrate, 60-70% of calories, in a commercial poultry food is an example of external manipulation of fuel sources for a carnivore, such as the Siberian crane during breeding season. Stress from fasting or anorexia is an example of an internal factor that changes preferred fuel sources, increasing the catabolism of endogenous protein and fat.

Fasted birds exhaust glycogen stores by about 48 hours, then are entirely dependent on fatty acids from adipose and amino acids from lean tissue. Fat may be used for about 70-85% of calorie needs including up to 15% via ketones. Branched chain amino acids are oxidized in muscle. Certain amino acids are used to produce glucose, via hepatic and renal gluconeogenesis, for the cells with an obligate glucose need (cells in brain, blood, and renal medulla). Overall, protein may provide 25% of calories and carbohydrate less than 10%. Our goal was to design diets that matched our estimates of a crane's metabolism created by internal alterations of fuel sources.

With fasting, injury, and illness, tissue protein is used for energy, via gluconeogenesis, and for anabolism, such as wound healing and antibody production. Since all tissue protein is vital and none is stored, this use of tissue protein places the patient in negative nitrogen balance. The patient is catabolic and losing protein from skeletal muscle and other tissues, such as heart and intestine, to the detriment of the patient's health. A central goal of nutrition support is to minimize the loss of tissue protein by providing sufficient calories and protein. This is accomplished by providing a formula of fuels and nutrients in proportions that are utilized by the patient with maximal efficiency, and by delivering the formula in such a way as to minimize discomfort.

A bird that has not eaten for a few days already has adapted its fuel sources. Diagnostic tests or anorexia may prolong the fast even longer. The bird will be using endogenous fat more than protein for energy. Protein-sparing adaptations include decreases in metabolic rate and spontaneous activity.

Data from injured birds are lacking, but in mammals, injury and trauma are characterized by hypermetabolism, peripheral insulin resistance, prolonged and marked protein catabolism, and negative nitrogen balance. Endogenous amino acids vie with fatty acids as primary fuel sources.

Thus for nutrition support, predominant amounts of exogenous carbohydrate are contraindicated. Rather, fat and protein are provided to simulate the fuel mix already used by the patient. Exogenous fuels are provided in optimal ranges that maximize fuel utilization and minimize adverse effects, about 25-40% protein and 30-50% fat on a ME basis.

The ICF nutrition support diet was evaluated and found to contain too little protein (about 5% ME) and too much fat (about 70% ME). The high fat was derived from use of corn oil and Nutri-Cal^{R} as calorie supplements. Nutri-Cal^{R} (Evsco Pharmaceuticals, Buena, NJ) derives 62% ME, 1%

ME from protein, and 37% ME from carbohydrate in corn syrup. It is inappropriate as a balanced fuel source or supplement for nutrition support of many species.

During re-feeding of fasted patients, amino acid mobilization decreases rapidly and metabolism returns within one day to the pre-fasting state. In contrast to simple fasting, the metabolically stressed animal continues to exhibit negative nitrogen balance, accelerated gluconeogenesis, and insulin resistance when fed. These energetically expensive metabolic responses support the healing of wounds and resistance to infection for days and even weeks. The cumulative drain on tissues may continue for weeks, necessitating nutrition support for some time.

Calories: Sick patients require fewer, the same, or more calories than the average healthy individual of the same species, age, and weight. The corresponding metabolic states are termed hypo-, normo-, or hyper-metabolism. Crane chicks that are starved, moribund, or markedly inactive are likely to be hypometabolic. They require fewer calories, perhaps 40-90% of usual. Chicks that have infections, fractures, or large wounds are likely to be hypermetabolic. They require more calories, perhaps 5-50% more than usual.

For sick crane chicks, basal metabolic rate was calculated from: $Y = 91X^{0.73}$ where Y = basal metabolic rate, active phase, for nonpasserines in kcal per day and X = body weight in kg (Robbins, 1983). Increments are added for maintenance energy (activity, digestion, and excretion), for growth, and for infections, fractures, and wounds. Adjustments are made for inactivity, debilitation, and starvation.

Case Management The nutrition support plan for crane chicks outlined a 7-step system for case management (Donoghue, 1989):

1. Assess the patient's nutritional status;
2. Estimate the proportions of fuel sources;
3. Calculate approximate calorie needs;
4. Select diet and route of administration;
5. Initiate the support program;
6. Evaluate responses and modify as needed; and
7. Plan transition periods to usual diet.

Details of case management are provided elsewhere (Donoghue, 1989; Donoghue 1991).

Nutrition support may be provided via enteral or parenteral routes, and may be voluntary or involuntary. In domestic companion animals, most cases are managed with voluntary intakes, and over 90% can be managed with enteral diets (Donoghue, 1991).

Involuntary feeding is accomplished either enterally or parenterally. Involuntary enteral feeding is accomplished, depending on the species and patient, by syringe feeding directly into the patient's mouth, by repeated orogastric intubations, or by indwelling nasogastric, pharyngostomy, gastrostomy, and jejunostomy tubes. Indwelling tubes are recommended for many species, because repeated daily intubations inflict stress. For sick and stressed crane chicks, plans were developed for indwelling pharyngostomy tubes and for repeated orogastric intubation.

For nutrition support, diets should be of very high quality. Parenterals are not of highest quality because utilization of intravenously administered nutrients is suboptimal, and because enterocytes atrophy with intravenous nutrients. Enterals are of highest quality.

Table 3. Selected characteristics and indications of liquid enteral products.

Type	PRO FAT CHO %kcalME	Indications
Low CHO	>14 >39 <55	Hypermetabolism in most species. Normometabolism in carnivores.
High CHO	<20 <39 >40	Normometabolism, often require supplementation with protein. Protein restriction.
Low Fat	<20 <12 >70	Hypometabolism. Fat restriction. Malabsorption.

*CHO = carbohydrate

Enterals are liquid dietary products manufactured primarily for hospitalized humans. They are usually sterile and designed to be nutritionally complete or balanced for specific purposes. Certain enterals can be classified as "low carbohydrate" (Tables 3 and 4). These are relatively high in protein and fat, hence are often suitable for use in carnivores. Others are classified as "high carbohydrate" (Tables 3 and 4). These are low in protein and are used when dietary protein is restricted. A third category is "low fat" (Tables 3 and 4). These are elemental diets that contain as little as 2% fat (ME basis) and are useful when fat but not ME is restricted. A fourth group, fiber containing, was not considered appropriate for Siberian crane chicks.

Modules of protein, fat, carbohydrate, or fiber may be added to diets, including usual diets as well as enterals (Table 5). Carbohydrate modules are added rarely, because most patients utilize relatively more protein and fat, and carbohydrate should be minimized. Fiber may be added to moderate intestinal motility. Protein is the module added most often in nutrition support. It is used to raise the dietary protein content to more than 30% ME. This level is used for all carnivores, and for many omnivores that are catabolic, such as those with fractures, infections, or cancer.

Fats are added to increase calorie density. In addition, dietary fat reduces the work-load of the respiratory system because it produces less carbon dioxide than carbohydrate produces when used as a fuel. The most common are corn and vegetable oils. Next are medium-chain triglycerides, providing 8 kcal ME per gram, and requiring no lymphatics for absorption. They are useful when feeding patients with altered lymph function, as with chylothorax or lymphangectasia, and for patients with altered fat absorption. Vomiting and poor palatability are difficulties encountered when feeding medium-chain triglycerides. With fat supplementation, care is taken to ensure that protein levels, as a percent of calories, are maintained when fat is increased.

Nutrition support is always started gradually, no matter what the final calorie goal may be. Even the patient that will be fed 50% over usual intake is started at low levels, perhaps 40% of usual, for the first days of refeeding. Those who begin nutrition support by feeding at the full amount report

Table 4. Examples of nutrient contents of enterals manufactured for humans, dogs, and cats during illness.

Enternal	Kcal/ml	mOsm/kg	PRO FAT CHO %kcal	Distributer/Manufacturer
LOW CARBOHYDRATE				
Pulmocare	1.48	490	17 55 28	Ross Laboratories[1]
Magnacal	2	590	14 36 50	Sherwood Medical[2]
Traumacal	1.5	490	17 55 28	Mead Johnson[3]
Clinical Care Canine	1.0	340	20 55 25	Pet-Ag Inc.[4]
Clinical Care Feline	0.9	368	30 45 25	Pet-Ag Inc.[4]
HIGH CARBOHYDRATE				
Ensure	1.06	470	17 30 53	Ross Laboratories[1]
Attain	1.00	300	16 36 48	Sherwood Medical[2]
Isocal	1.06	300	13 37 50	Mead Johnson[3]
LOW FAT				
Vital	1.0	500	17 9 74	Ross Laboratories[1]
Pepti-2000	1.0	490	16 8 75	Sherwood Medical[2]
Criticare	1.06	650	14 3 83	Mead Johnson[3]

[1]Ross Laboratories, Columbus, OH 43216 (614)227-3333
[2]Sherwood Medical. St. Louis, MO 63103 (800)428-4400
[3]Mead Johnson, Evansville, IN 47721 (800)892-9201
[4]Pet-Ag Inc., Elgin, IL 60120 (800)323-0877

problems with intestinal pain, regurgitation, and diarrhea in patients. If diets are started slowly, and diluted for the first meals, there are fewer problems associated with refeeding.

In 1991, a whooping crane chick who developed a severe *Pseudomonas* keratitis was maintained intermittently on Clinical Care Feline[R] (Pet-Ag Inc, Elgin, IL 60120) by repeated orogastric intubation. During the periods of total enteral nutrition, the 600-800 g chick was fed approximately 100-120 kcal ME (1.4- to 1.7-times BMR) daily,

Table 5. Examples of modules used in nutrition support.

Module	Brand Name	Manufacturer
Whole Protein	Pro-Mod®	Ross Laboratories[1]
	Propac®	Sherwood Medical[2]
	Casec®	Mead Johnson[3]
	ProMagic®	American Nutritional[4]
Polyunsaturated Fatty Acids	corn oil	groceries
Medium-chain Triglycerides	MCT oil	Mead Johnson[3]

1,2, & 3 See Table 4 for addresses.
4 American Nutritional Laboratories, Burlington, NJ 08016 (609)387-3132

divided into four 25-30 ml feedings. The chick's daily growth rate during the 10-day period ranged from -2 to +5 percent of body weight. This was considered suboptimal but an improvement over the consistent loss seen previously with chicks requiring nutrition support.

Nutritional Management of Individuals

Weight loss was prescribed for a 21 yr old female Siberian crane. The crane had a 4 yr history of reproductive failure after having been a successful breeder. Remarkable findings on physical examination included a mildly distended abdomen and body weight at the high end of normal for female Siberian cranes and as compared to previous weights on this bird. Reproductive failure may be the result of behavioral (pairing) or physical problems. Complete blood counts, serum biochemistries, cloacal cultures, radiographs, and thyroid stimulation tests were unremarkable. Diagnostic laparoscopic examination to evaluate the reproductive tract failed because of massive abdominal fat. Weight reduction was prescribed, to facilitate laparoscopy and to test whether obesity might be a primary factor in the bird's infertility.

Weight reduction may be accomplished by restriction of the patient's usual diet or by use of special, low-calorie diets. Restriction of usual diets limits not only calories but also vitamins, minerals, and essential amino acids. Low calorie diets may be low carbohydrate, low fat, high protein, and high fiber.

Low carbohydrate diets function by reducing metabolic efficiency, with resultant ketonuria. Humans suffer from deficient total body contents of water, sodium, and calcium, elevated blood concentrations of ketones, lipids and uric acid, nausea and weakness, and, with long term use, bone demineralization. Low carbohydrate diets induce no adverse effects in carnivorous mammals, such as dogs and cats (Kronfeld et al, 1988).

Low fat diets contain low calorie density, low digestibility, and tend to be unpalatable for species used to diets containing moderate or high fat. Essential fatty acid deficiency may result from long term use of low fat diets containing animal fats as the sole fat source.

High protein diets maintain higher blood concentrations of amino acids and glucose, delaying or abating hunger and facilitating calorie reduction (Kronfeld et al, 1988). Liquid protein diets in humans have been associated with cardiac arrhythmia. Solid diets high in protein and fiber are used successfully for weight reduction in humans.

High fiber diets are most commonly used in domestic companion animals for weight reduction. Fiber decreases the bioavailability of minerals, and long term safety of the commercial high fiber products has not been demonstrated.

We selected a two-fold approach to the Siberian crane's diet. Her total intake of the usual diet was reduced, following extensive evaluation of her usual food intake and feeding behavior. Concurrently, fresh foods were added to increase protein content, and to provide variety and stimulate activity.

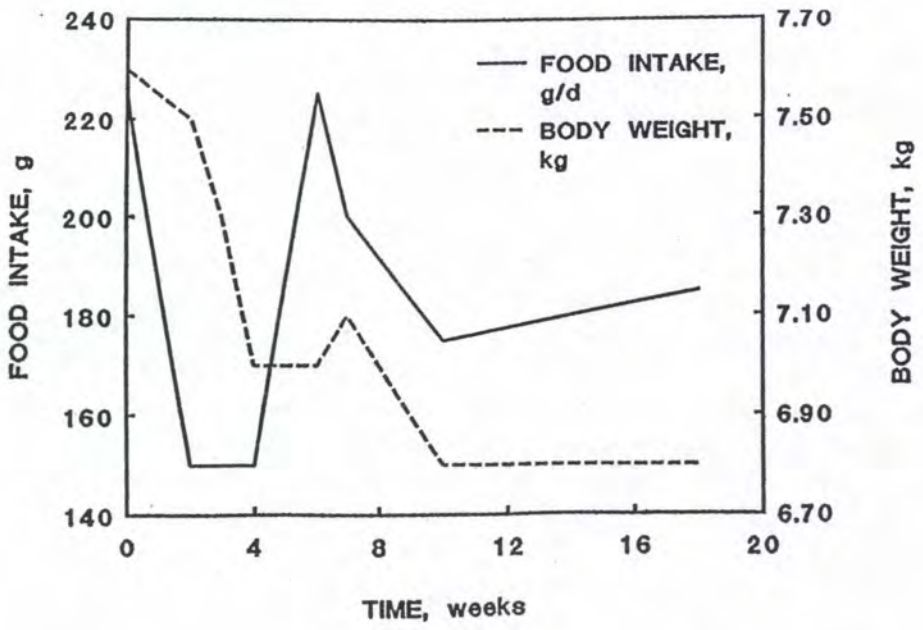
All weight lost, even in controlled weight reduction programs, comprises a mix of adipose and lean tissue. Too rapid weight loss leads to marked protein depletion. Safe weight loss is estimated to not exceed 1% body weight weekly (Stunkard, 1987). Our goal was to promote weight loss of no

more than 1% body weight per week with minimal behavioral perturbation and no observable loss of health.

Adverse effects of excessive weight loss are marked by excessive nitrogen excretion and hyperketonemia. Ketones are produced from metabolized fat. They are used readily as an energy source (by brain) in carnivores. In herbivores, ketones can accumulate in blood and signal disease. At each weighing, the crane was blood sampled to monitor for blood ketones.

Prior to the weight reduction program, the crane consumed about 225 g pelleted feed daily. For weight reduction, feed was reduced to 150 g (66% of initial), with daily additions of two killed neonatal mice and four to eight cranberries. Weight changes during the weight loss program are shown in Figure 1. By day 31, the crane had lost 7.9% of her body weight (1.8% per week). She was

Figure 1. Food consumption and body weight of a 21 year old Siberian crane with obesity and reproductive failure. Time 0 = start of dietary restrictions.



consistently negative for blood ketones, but developed depression and disinterest in her surroundings. We concluded that the rate of weight loss, 1.8% per week, had been too fast. Accordingly, food was increased to pre-weight loss intakes and behavior improved. Body weight responded slightly. After several weeks, food offered was reduced to 89%, then 78% of initial intake. From 7 to 10 weeks, weight loss was 0.3 kg or 4.2%, an average of 0.7% per week, with no observed adverse effects on health or behavior. From 10 to 18 weeks, the crane was fed 82% initial intake. Her body weight stabilized at 6.8 kg.

In summary, a 33% cut in food intake resulted in too rapid weight loss, but two steps of 11% each were tolerated. A total of 0.8 kg was lost in 10 weeks, a rate of 1.05% per week. This final weight has been stabilized until present (over 8 months). Due to the weight reduction program, laparoscopic and direct surgical examination of the bird's reproductive tract was successful. No anatomical cause of the infertility was detected. The female showed behaviors, such as courting, incubation, hatching and raising of a foster chick, compatible with normal hormonal cycling in 1991 (the first time since 1986), but no eggs were produced.

Addendum The female Siberian crane who underwent a controlled weight loss program in 1990, successfully laid and raised chicks in 1992 and 1993, for the first time since 1986 when her obesity problem started.

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THE USE OF GELATIN-BASED DIETS FOR SOFT-BILLED BIRDS

Mark S. Edwards, Joni B. Bernard, and Anita Cramm

Introduction

The advantages of nutritionally complete formulated diets have been well defined (Ullrey et al., 1991; Oftedal, 1980). An extruded or pelleted form of the complete diet is the most common vector used to deliver this to the animal. Unfortunately, these two manufactured forms are not always accepted by the species for which the diet has been formulated. Using a complete feed as a base, adaptations of the manufactured diet may be developed for feeding more selective feeders.

Background

Free-ranging birds have coevolved with the foods found in their native habitat. The foods selected in the wild are those that supply the nutrients which, after generations of experience, enable the species to survive. Studies of birds in captivity, specifically psittacines, suggest that this type nutritional wisdom has not been transferred from the wild (Ullrey et al., 1991). Therefore, there is the potential for detrimental effects to occur, when a species is offered numerous food items, each in itself nutritionally incomplete, from which to select.

Soft-billed birds are typically frugivorous and/or insectivorous. Historically, these animals have been fed mixtures incorporating these two food types, often heavily supplemented to improve the nutritional inadequacies of the individual foods. Typically, the individual birds will sort through the offered foods, selecting preferred items over nutritionally-complete feeds. Additionally, these diets are sometimes offered in quantities exceeding the daily dry matter intake capabilities of the species. The relatively recent availability of commercially-produced complete feeds for avian species has enabled the aviculturalist and nutritionist to offer a more balanced diet.

The National Zoological Park began using gelatin in avian diets as a binding agent for diets formulated for toucans (Oftedal, 1980). These birds had not shown an interest in the pelleted ration offered and are known to manipulate their foods prior to consumption, sometimes passing items between individuals. In attempts to provide a nutritionally-complete diet which could withstand this "billing" activity, gelatin was used, in combination with fruits and a complete pelleted diet.

Although most soft-billed birds do not exhibit this "billing" behavior, the adhesive properties of gelatin offer the possibility of creating artificial "fruits" or morsels which could be accepted by these species. A nutritionally-complete diet, either extruded or pelleted, may be ground to reduce the particle size. The complete diet may be mixed with gelatin and water, placed in a large pan, and allowed to set. The resulting gel may be cut or formed into the size and shape desired for the individual species.

Gelatin is produced, from highly-cross linked tissues such as mature bovine skin or bone, during various processes of the rendering industry. Gelatin is the water-soluble product which is

formed upon denaturation of the collagen triple-helix (Veis, 1964). Collagen is the principle component of white connective tissue fibers and is present in every tissue and organ. Gelation, the most desired property of gelatin solutions, is when the collagen structure is partially regained (Veis, 1978).

The addition of gelatin to a plant-based complete feed causes some nutrients to vary from estimated requirements. One example is the high proportion of the amino acid, arginine. The arginine concentration of unsweetened gelatin is 8.5 % on a dry matter basis. It was this amino acid concentration, plus low levels of tryptophan, which limits the addition of gelatin to the total diet. The minimum quantity of gelatin required to obtain the desired physical properties of the diet was used to avoid an amino acid imbalance.

Gelatin does not contribute a significant quantity of iron to the total dietary mixture. This is desirable since this diet is to be fed to several species of Sturnidae, a family which has shown a significant incidence of iron storage disease.

Two Midwestern zoos, the Potter Park Zoological Gardens (Lansing, MI) and the Lincoln Park Zoo (Chicago, IL) have recently had their avian diets evaluated, by Michigan State University's Comparative Nutrition Group and Allen and Baer Associates, respectively. Both institutions, separately, determined that the core of the avian feeding program should consist of a nutritionally-balanced complete feed which could be fed to a variety of species.

Diet Preparation

The components of the basic gel diet are ground extruded psittacine diet (Scenic Psittacine Diet), gelatin, and water. Selected nutrient concentrations of the extruded psittacine diet and of unsweetened gelatin are presented in Tables 1 and 2, respectively.

Gelatin is added at 5 % of the total diet. This quantity was found to provide the binding characteristics needed without altering the amino acid specifications. The gelatin is mixed with the ground psittacine diet and calcium carbonate. Hot water is then added to the dry ingredients, which are then completely mixed. Before the mixture begins to cool, it is poured into a pan or similar container. Once the gelatin-based diet has had time to set, it may be cut or shaped into the desired size. The components and selected nutrient concentrations of the basic gelatin-based avian diet are provided in Table 3.

The basic gel diet is only a foundation upon which one may create more specialized diets for selective feeders. The frugivore gel diet was the first variation to be tested. To the basic gel diet, fruits and/or vegetables may be added. The produce should never exceed 50 % of the as is or wet weight of the total diet. The produce is added to the dry ingredients, and thoroughly mixed. This coats the individual pieces of produce with the ground complete feed, gelatin, and calcium carbonate. The remaining procedure is the same as for the basic formulation. The imbedded fruits act to attract birds to feed upon the entire mixture. When an individual takes a bite of the fruit, it will inadvertently consume the ground complete feed as well. The components and selected nutrient concentrations of the frugivore gelatin-based diet are provided in Table 4.

As with other edible gelatin products, some fruits are not conducive to the gelation process.

Table 1. Nutrient concentrations of the extruded psittacine diet[1,2,3].

Nutrient	Concentration (DMB)
Crude Protein (%)	24
Arginine (%)	1.3
Isoleucine (%)	1.1
Lysine (%)	1.2
Methionine (%)	0.5
Methionine + Cystine (%)	0.9
Threonine (%)	0.95
Tryptophan (%)	0.24
Linoleic acid (%)	2
Calcium (%)	1.1
Phosphorus (%)	0.8
Potassium (%)	0.7
Sodium (%)	0.2
Magnesium (%)	0.15
Manganese (ppm)	65
Zinc (ppm)	120
Iron (ppm)	150
Copper (ppm)	20
Iodine (ppm)	1
Selenium (ppm)	0.3
Thiamin (ppm)	6
Riboflavin (ppm)	6
Pantothenic acid (ppm)	20
Niacin (ppm)	55
Vitamin B_{12} (ppm)	0.025
Biotin (ppm)	0.3
Vitamin A (IU/kg)	8000
Cholecalciferol (IU/kg)	1900
Vitamin E (IU/kg)	250

[1] From Ullrey et al., 1991
[2] All values are expressed on a dry matter basis (DMB).
[3] Formulated as a complete diet for psittacines.

Table 2. Selected nutrient composition of dry, unsweetened gelatin[1,2].

Nutrient	Concentration (DMB)
Dry matter (%)	77
Crude protein (%)	98.4
Arginine (%)	8.5
Isoleucine (%)	1.6
Lysine (%)	4.3
Methionine (%)	0.8
Cystine (%)	0.1
Threonine (%)	1.5
Trytophan (%)	0.1
Crude fat (%)	0.1
Ash (%)	1.5
Calcium (%)	0
Phosphorus (%)	0
Sodium (%)	0.1
Iron (ppm)	0
Copper (PPM)	4.5
Selenium (ppm)	0.2
Gross energy	3.9

[1]From Nutrients in Foods (1983)
[2]All values expressed on a dry matter basis (DMB)

Although pineapple is the most obvious of these fruits, it has been discovered that citrus fruits will also decrease the "strength" of the gelatin surrounding the piece of imbedded fruit. Because of this, several birds have been able to selectively remove the fruit from the gel diet, without consuming the complete feed with it, thus defeating the goal of the gelatin-based diet.

Some species may not be attracted to those gel diets containing fruit, such as the insectivorous birds. However, these birds can benefit from the consumption of the complete feed as much as the frugivorous species. A second variation, an insectivore- gelatin based diet, was developed for these animals. Again, utilizing the basic gel diet as a foundation, dried insects, both pupae and larvae have been added. These added insects should not exceed 10% of the total diet (as is basis). The components and selected nutrient concentrations of the insectivore gel diet are provided in Table 5.

Table 3. Components and selected nutrient concentrations of the avian gelatin-based diet/ basic formulation.

Component	Amount (g)[1]
Psittacine extruded diet, ground	94.5
Gelatin, unsweetened, dry powder	5
Calcium carbonate ($CaCO_3$)	0.5
Water	100

[1]All quantities expressed on an as is basis.

Nutrient	Concentration (DMB)[2]
Gross energy (kcal/g)	4.39
Crude protein (%)	28
Arginine (%)	1.61
Isoleucine (%)	1.12
Lysine (%)	1.35
Methionine (%)	0.53
Cystine (%)	0.39
Threonine (%)	0.98
Tryptophan (%)	0.23
Crude fat (%)	5.6
Ash (%)	6.8
Calcium (%)	1.28
Phosphorus (%)	0.76
Calcium : Phosphorus	1.68
Sodium (%)	0.27
Iron (ppm)	163
Copper (ppm)	23
Selenium (ppm)	0.45
Zinc (ppm)	114
Vitamin A (IU/kg)	8324
Cholecalciferol (IU/kg)	1862
Vitamin E (IU/kg)	270

[2]DMB = Dry Matter Basis

Species Acceptance

As was previously indicated, the initial use of the gelatin based diets was with the Piciformes (Toucans and Toucanets) which handle their food prior to consumption. The gel diets have been tested for their acceptance at both the Potter Park Zoological Gardens (Lansing, MI) and Lincoln Park Zoological Gardens (Chicago, IL). Those species which were tested and the results of their acceptance are provided in Table 6. Species specific intake levels of the gel diets have not yet been determined.

The gel diets are specifically useful in coaxing an animal to consume the complete feed when the original form of that complete feed is not typically accepted, for whatever reason. Although several species readily accepted the gelatin-based frugivore diet at the Potter Park Zoological Gardens, it was found that these species also accepted the manufactured form of the complete feed. Therefore, due to the

Table 4. Components and selected nutrient concentrations of the avian gelatin-based diet/frugivore formulation.

Component	Amount (g)[1]
Psittacine extruded diet, ground	50
Gelatin, unsweetened, dry powder	5
Calcium carbonate ($CaCO_3$)	0.5
Grapes	9.5
Banana	10
Strawberries	10
Apple	15
Water	100

[1]All Values expressed on an as is basis.

Nutrient	Concentration (DMB)[2]
Gross energy (kcal/g)	4.39
Crude protein (%)	27
Arginine (%)	1.64
Isoleucine (%)	0.99
Lysine (%)	1.28
Methionine (%)	0.47
Cystine (%)	0.33
Threonine (%)	0.87
Tryptophan (%)	0.2
Crude fat (%)	4.9
Ash (%)	6.4
Calcium (%)	1.22
Phosphorus (%)	0.63
Calcium : Phosphorus	1.91
Sodium (%)	0.22
Iron (ppm)	137
Copper (ppm)	20
Selenium (ppm)	0.37
Vitamin A (IU/kg)	6440

[2]DMB = Dry Matter Basis

Table 5. Components and selected nutrient concentrations of the avian gelatin-based diet/insectivore formulation.

Component	Amount (g)[1]
Psittacine extruded diet, ground	85
Gelatin, unsweetened, dry powder	5
Calcium carbonate ($CaCO_3$)	0.5
Fly pupae, dry	9.5
Water	100

[1]All values are expressed on an as is basis.

Nutrient	Concentration (DMB)[2]
Crude protein (%)31.4	
Arginine (%)1.07	
Isoleucine (%)1.01	
Lysine (%)1.26	
Methionine (%)0.49	
Cystine (%)0.35	
Crude fat (%)6.7	
Ash (%)6.7	
Calcium (%)1.2	
Phosphorus (%)0.8	
Calcium :	
Iron (ppm)187	
Copper (ppm)25	
Selenium (ppm)0.59	
Vitamin A (IU/kg)7452	
Cholecalciferol	
Vitamin E (IU/kg)242	

[2]DMB = Dry Matter Basis.

Table 6. Avian species which have shown acceptance of the gelatin-based diets under various conditions[1].

FAMILY/ Species	PPZG[2]	LPZG
GALLIFORMES		
Crested Wood Partridge (*Rollulus roulroul*)	+	+
COLUMBIFORMES		
Diamond dove (*Geopelia cuneata*)	+	
Jambu fruit dove (*Ptilinopus jambu*)		+
Green imperial pigeon (*Ducula aenea*)		+
Pinon imperial pigeon (*Ducula pinon*)		+
PSITTACIFORMES		
Cockatiel (*Nymphicus hollandicus*)	+	
Yellow-naped Amazon (*Amazona ochrocephalia*)	+	
CORACIFORMES		
Blue-crowned motmot (*Momotus momota*)	+	
PICIFORMES		
Green aracari (*Pteroglossus viridis*)	+	
Collard aracari (*Pteroglossus torquatus*)		+
Toco toucan (*Ramphastidae toco*)		+
Blyth's hornbill (*Aceros plicatus*)		+
PASSERIFORMES		
Common canary (*Serinus canaria*)	+	
Java sparrow (*Padda oryzivora*)	+	
Orange weaver (*Ploceus aurantius*)	+	
Bali starling (*Leucopsar rothschildi*)	+	+
Emerald starling (*Lamprotornis iris*)		+
Superb starling (*Spero superbus*)	+	+
Plush-crested jay (*Cyanocorax bracyrhynchos*)	+	
Laughing thrush (*Garrulax galbanus*)		+
Crimson back tanager (*Ramphocelus dimidiatus*)		+
Palm tanager (*Thraupis palmarum*)		+

[1]A (+) indicates species acceptance of gelatin-based diet.
[2]PPZG = Potter Park Zoological Gardens, Lansing, MI
[3]LPZG = Lincoln Park Zoological Gardens, Chicago, IL

increased cost of ingredients, storage requirements and labor, the use of the gelatin based diets was discontinued.

The acceptance of the dry, manufactured form has not yet been quantified for individual species at the Lincoln Park Zoological Gardens. Many of the soft-billed birds are kept in large, multi-species, free-flight aviaries; therefore, accurate data collection is inhibited.

Future Applications

The flexibility of the gelatin-based diets is one of their most desirable characteristics. Although only three diets have been presented here, numerous variations can be developed for further application. Enhancement of coloration with vegetable dyes or other edible pigments may increase consumption of the diets by those species which selectively feed upon specifically-colored food items in the wild. Various flavorings and scents may also provide a similar effect.

The extruded psittacine diet which was selected for these gelatin-based diets is produced in three flavors and two colors. Since the nutrient concentration of the complete feed is the same for all three flavors, these can be interchanged from time to time to provide variation in the diet.

New exhibits which display the animals in naturalistic surroundings often present conditions which may require further adaptations of the diet. Tropical species are most likely housed in exhibits with high temperatures and humidity levels. These environmental factors increase the rate of mold and/or fungal development on the moist gelatin-based diet. Such is the situation in the renovated bird house at the Lincoln Park Zoo.

Potassium sorbate ($CH_3CH=CHCH=CHCOOK$) has been identified as a potential mold inhibitor for the gelatin-based diet. Potassium sorbate, an effective antimycotic and antiyeast agent, is currently used in semi-moist pet foods up to concentrations of 0.3%. Laboratory results indicate no harmful effects of this substance at dietary levels up to 4%. Potassium sorbate is water soluble and could be easily mixed into the gelatin-based diet during preparation (Fischetti, 1980).

Acknowledgments

We would like to extend our thanks to the animal care staff at both the Potter Park Zoological Gardens and Lincoln Park Zoological Gardens for their assistance and expertise. Also, thanks to Mr. F. "Skip" Cockerum for providing the dried insect larvae.

Products Mentioned In Text: Scenic Psittacine Diet - Marion Zoological Inc., 113 North First, P.O. Box 212, Marion, KS 66861, 1(800) 327-7974 and Dried Insects - Skipio's, Cockerum Oregon Insects Corporation, P.O. Box 714, Tillamook, OR 97141-0714, 1(800) 633-5437

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SEEDS VERSUS FORMULATED DIETS FOR PSITTACINES

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Psittacines are often called seed eaters, even though foods eaten in the wild are diverse (over 80 different food plants for some species) and include fruit, flowers, cambium, insects and a variety of seeds (Cannon, 1981; Saunders, 1980; Snyder et al., 1982; Wyndham, 1980). Hardly any of the seeds found in the wild habitat are present in commercial mixes sold for psittacines. Ingredients in five commercial seed mixes are shown in Table 1. Other seeds that may be present include milo, rice, canary grass, flax, sesame, anise, fennel, lettuce, false flax, poppy, pea, caraway and teazle. Some psittacines are also fed Brazilnuts, English walnuts, cashew nuts, hazelnuts, almonds, macadamia nuts, pistachio nuts, beechnuts, pinyon nuts and pecans.

Table 1. Ingredients (%) in seed mixes for psittacines. Coded products sold in USA in 1990, (P,Q,R,S,T).

Ingredients	P	Q	R	S	T
Buckwheat	-	-	2.70	3.70	6.30
Canary grass seed	-	-	-	17.00	-
Corn grain	33.50	12.80	5.10	-	7.00
Hemp seed	-	2.20	0.20	-	-
Millet seed	-	-	21.90	32.20	6.20
Oat groats	-	10.60	11.20	3.70	6.20
Peanuts with shell	8.40	4.70	-	-	-
Peanuts without shell	-	-	1.50	-	25.40
Pellets	-	15.50	11.90	6.70	10.80
Pepper pods and seed	1.80	1.00	1.50	-	-
Pumpkin/ squash seed	10.00	-	0.40	-	-
Rape seed	-	-	-	13.00	-
Safflower seed	14.20	26.10	22.60	14.60	24.20
Sunflower seed	29.00	24.80	5.50	-	-
Wheat	3.10	2.30	13.40	4.90	14.00
Miscellaneous	-	-	1.90	4.30	-
Total	100.00	100.00	100.00	100.10	100.10

While psittacines generally exhibit a preference for seeds compared to pelleted or extruded diets, seed mixes are 30-60% waste (Table 2) and have serious nutrient deficiencies. Protein and lysine concentrations in seeds are below estimated requirements for growth, except in peanuts, pumpkin/squash and sunflower seeds. Peanuts are deficient in sulfur amino acids (methionine plus cystine) and marginal in threonine. In addition, peanuts, pumpkin/squash and sunflower seeds are so high in fat (45-60%) that their consumption leads to obesity. Other nutrient deficiencies in most commercial seed mixes include calcium, available phosphorus, sodium, manganese, zinc, iron, vitamins A, D and K, riboflavin, pantothenic acid, available niacin, vitamin B12 and choline. Supplies of iodine, selenium and vitamin E are variable and range from adequate to deficient.

Attempts have been made to correct the limitations of seeds by coating them with supplements

or by including a pelleted supplement in the mix. Supplement coatings are largely lost when the hull is removed as the seed is consumed. Supplement pellets are commonly not eaten or are consumed in such small proportions that they are ineffective.

Table 2. Proportions (%) of hulls/ shells and kernels in seeds.

Seed	Hull/shell	Kernel
Almonds	60	40
Beechnuts	39	61
Brazilnuts	52	48
Buckwheat	20	80
Canary grass seed	18	82
English walnuts	55	45
Hazelnuts	54	46
Macadamia nuts	69	31
Millet, common or proso	26	74
Peanuts	27	73
Pecans	47	53
Pinyon nuts	43	57
Pistachio nuts	50	50
Pumpkin/ squash seed	26	74
Safflower seed	49	51
Sunflower seed	46	54

Table 3. Fledgling percentage associated with feeding of seeds, fruit and vegetables or an extrusion, fruit and vegetables to 8 species of psittacines.

Species	Seed fruit, veg	Extrusion fruit, veg
Yellow-headed Amazon	75	100
Forster's lorikeet	62	100
Goldie's lorikeet	45	83
Blue and gold macaw	62	80
Scarlet macaw	62	100
Ring-necked parakeet	80	100
Rock peplar parakeet	88	80
Blue-crowned hanging parrot	50	75
Mean	66	90

Seed mixes for domestic poultry were abandoned long ago and were replaced by nutritionally-complete mashes, pellets or crumbles. The result was a remarkable improvement in growth, reproduction and health. While properly-formulated pellets provide the same benefits to psittacines as they do to poultry, many psittacines do not accept pellets readily. The sensory system used in the selection of food includes the tactile bill-tip organ, which is important for identification of expected food size, shape and texture. In several respects, a properly manufactured extrusion more nearly mimics the physical characteristics of preferred food items. Even so, when an extruded diet is offered with seeds, many psittacines select the latter.

In a study with Timneh African Gray Parrots (Ullrey et al., 1991), a nutritionally-complete extrusion was offered with corn, sunflower seeds, peanuts, safflower seeds, oranges, sweet potatoes, celery, green beans, carrots and spinach. Dry matter intake from seeds and nuts comprised 82% of the total, the extrusion comprised 11% and fruits and vegetables the remainder. As a consequence, the diet was marginal or deficient in methionine, calcium, available phosphorus, sodium, manganese, zinc, riboflavin, vitamin B12, available niacin, pantothenic acid, vitamin A and vitamin D. On the other hand, when seeds and nuts were removed from this mixture and the extrusion was offered with the same fruits and vegetables, dry matter intake from the extrusion was 81% of the total. Because the extrusion was formulated to accommodate this dilution, nutrient requirements were met. To verify the suitability of this dietary strategy, a comparison was made of the fledgling percentage of parent-

raised chicks of eight psittacine species whose parents had been fed seeds, fruits and vegetables for 2 years and were then fed an extrusion, fruits and vegetables, but no seeds, for 1 year (Table 3). The numbers of chicks hatched per year were not significantly different ($P>0.05$), but fledgling percentage was improved ($P<0.01$) from a value of 66 to a value of 90% by the substitution of an extrusion for seeds.

Table 4. Nutrient specifications (dry basis) for a nutritionally-complete extruded diet (Marion Zoological, Inc., Marion, KS).

Nutrient	Concentration	Nutrient	Concentration
Protein	24%	Zinc	120 ppm
Arginine	1.3%	Manganese	
Isoleucine	1.1%	Iodine	1 ppm
Lysine	1.2%	Selenium	0.3 ppm
Methionine	0.5%	Vitamin A	8000 IU/kg
Meth + cystine	0.9%	Vitamin D_3	1900 IU/kg
Threonine	0.95%	Vitamin E	250 IU/kg
Trytophan	0.24%	Vitamin K	4 ppm
Linoleic acid	2%	Thiamin	6 ppm
Calcium	1.1%	Riboflavin	6 ppm
Phosphorus	0.8%	Pantothenic acid	20 ppm
Potassium	0.7%	Niacin	55 ppm
Sodium	0.2%	Pyridoxine	6 ppm
Chlorine	0.2%	Choline	1700 ppm
Magnesium	0.15%	Folacin	900 ppb
Iron	150 ppm	Biotin	300 ppb
Copper	20 ppm	Vitamin B12	25 ppb

May be fed with fruits and vegetables but should constitute at least 40% of the diet by weight as fed (wet basis) or at least 80% of the diet dry weight.

The nutrient specifications for this extrusion are presented in Table 4. Nutrient levels were based on limited published research with psittacines (Roudybush and Grau, 1986; Grau and Roudybush, 1986) and the large body of data on precocial birds (NRC, 1984). Consideration was given to the special needs of reproducing altricial species, since parent birds feed their young. It was also recognized that psittacines are often fed a variety of foods, requiring that an effective formulated diet compensate for potential nutrient dilution and imbalance.

The extrusion may be fed as the sole diet or mixed with fruits and vegetables, as long as it constitutes $\geq$40% of the weight of the diet as fed (wet basis) or $\geq$80% of the diet on a dry basis. The process of extrusion involves heat and pressure sufficient to destroy pathogenic microorganisms and to partially dextrinize starch, thus improving digestibility. The finely ground extrusion can be mixed with water and used as a handrearing diet, as described by Howard et al. (1991a). When fed in this way to 85 chicks of six psittacine species, 84 (99%) of the chicks were successfully raised to weaning (Howard et al., 1991b).

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THE EFFECT OF DIETARY VITAMINS A AND E ON SERUM PLASMA STATUS OF HUMBOLDT PENGUINS

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The Humboldt penguin (*Spheniscus humboldti*) inhabits the coastal areas of Chile and Peru and is highly threatened because of loss of food and breeding grounds (Hays, 1984). It is currently listed on Appendix I of the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora. Although actual numbers are unknown, their total wild population prior to the 1982-83 El Nino was estimated to be approximately 20,000 (Modinger, 1983). Population studies after El Nino suggest that the Peruvian population may have been reduced by as much as 65% as food supplies carried by the current shifted (Hays, 1986).

The decreasing wild populations have sparked interest in captive propagation of Humboldt penguins in most zoological institutions in North America and Europe. The Humboldt penguin is the first species of penguin designated for captive management under a Species Survival Plan (SSP). It is, however, one of the more delicate species to maintain in captivity, because of low reproductive success and chick survivorship. The Spheniscus Newsletter (1991) stated that the captive population of Humboldt penguins is declining with successful reproduction rates below mortality rates despite a sizeable portion of the population in its peak reproductive years. This poor propagation record has spurred many zoos to search for reasons for the low success rate. Facilities (building structures, air handling systems), behavioral, and nutritional factors are being investigated. Nutrition has received considerable attention, in part because of the limited diet of frozen fish penguins often receive in captivity, and because of the role vitamins A and E play in reproduction.

Reproductive success rates at the Milwaukee County Zoo (MCZ) have not been encouraging. Over 6 breeding seasons, the Humboldt penguins had a hatching rate of 52% with approximately 50% of the hatchlings surviving to greater than 3 months of age (at fledgling).

Reproduction at Brookfield Zoo (BZ) consists of two breeding pairs over the last four years. An average of 15 eggs have been laid with a hatch rate of 23% with 70% surviving to over 30 days. The purpose of this paper is to describe the circulating levels of vitamins A and E in Humboldt penguins and to correlate those levels with diet, if possible.

Materials and methods

MCZ:

At the beginning of 1991, the MCZ began a comprehensive nutritional study of the Humboldt penguins in the collection. The MCZ has 2 outdoor Humboldt penguin exhibits. The Krause exhibit, holds 3.3 adults, 2.5 subadults and 0.0.2 chicks. The Taylor exhibit, houses 5.5 adults. Both flocks are hand fed a variety of fish twice daily: anchovies, capelin and smelt (freshwater or Columbia River). Daily vitamin supplementation per bird includes 100 mg thiamin and 1 Sea-Tab, formulated for bird which contains 1000 IU vitamin A acelate and 10 IU vitamin E (alpha-tocopherol) acelate)

per tablet.

Six male and six female adult penguins were selected as study birds. To determine the daily quantity of vitamins A and E ingested, a cassette was used to record the number and type of fish eaten by each study bird. Each time a fish was eaten, the keeper called out the color of the identification band located on the bird's flipper. The beginning of each recording identified the date, the exhibit, morning or afternoon feedings, which birds were counted, and the type of fish offered. To facilitate communication between keepers, a monthly chart logged which birds were counted on a particular day, the cassette number and the length of feeding per the recorder's counter. This log also provided a cross reference for transcription of the tape. Volunteers transcribed the cassettes 3 times weekly, tallying the number and type of fish eaten/bird/day.

Up to 6 ml heparinized blood were collected every 2 months from the medial metatarsal vein of the study birds. Birds were fasted a minimum of 12 hours before being bled. The study did not alter routing penguin husbandry and management procedures. Nesting birds on eggs or young chicks were not disturbed for blood sampling. Dietary changes occurred based on availability of fish types. Plasma was harvested at the MCZ hospital and frozen at -70 degrees. Within 40 days, samples were sent to the University of Illinois, Chicago, Nutrition Laboratory to be assayed by high performance liquid chromatography (HPLC) for alpha-tocopherol, retinol and retinyl palmitate levels.

Frozen fish were sent every 2-3 months to 2 laboratories to determine their content of vitamins A and E. Laboratory 1 prepared the fish for assay while they were still frozen, and performed the assays at cool temperatures to minimize vitamin loss. Vitamin content was determined by HPLC. Laboratory 2 thawed the fish at room temperature until it was soft enough to grind, then performed the assays at room temperature using a colorimetric method to determine vitamin content. (MCZ decided not to disclose the name of its laboratory. BZ identified its laboratory as Hazelton Labs, for methodology refer to AOAC 1990).

BZ:

In 1990, several banked Humboldt penguin serum samples were sent for analysis of vitamins A and E as part of a BZ routine survey. These preliminary results proved very interesting and prompted further study of both diet and serum samples. Brookfield Zoo holds one indoor exhibit for 5.6.3 Humboldt penguins. Birds were fed a combination of anchovies, capelin, herring, and Columbia River smelt (CR smelt). Supplementation consisted of vitamin E and thiamin plus a multi-vitamin/mineral supplement. The diet and supplementation regime were manipulated twice. All food was fed by hand. Intake calculations were averaged among individuals within the group. BZ's sampling procedure and handling techniques were similar to MCZ except that serum was used. Frozen fish also were sampled similarly. The laboratory using the colorimetric procedure was the same for both institutions. Those performing analysis by HPLC differed.

Results and Discussion

Fish:

Data presented in Tables 1 and 2 show vitamin A and E concentrations of the fish fed at each institution. There was a large difference among labs. This may be due to differences in sample

preparation as well as differences in assay procedures. Those data with the greatest values were performed by colorimetric assay which usually provides higher figures than HPLC. The colorimetric determination includes retinol, retinyl palmitate, and carotenoids while the HPLC generally includes just free retinol, (Bowen, Pers.Comm.).

Table 1. Vitamin A (IU/100g dry weight) of fish fed to Humboldt penguins (anchovies, capelin, CR smelt, RW smelt and herring), (3.33 IU/ mg retinol).

Milwaukee					
HPLC					
10/90	306	1089	-	473	-
1/91	260	619	-	296	-
5/91	263	609	-	273	448
7/91	1185	-	2354	-	-
8/91	220	167	2667	-	-
Colorimetric					
9/91	250	400	7290	-	-
Brookfield					
HPLC					
9/91	-	-	3408	-	-
Colorimetric					
4/91	2360	6920	17440	-	6600
5/91	680	1760	38120	-	4520
9/91*	-	-	22760	-	-

*Duplicate samples: i.e one homogeneous sample was halved and the halves sent to the different labs.

Table 2. Vitamin E (IU/100g et weight) of fish fed to Humboldt penguins (anchovies, capelin, CR smelt, RW smelt and herring), (1.49 IU mg D - ∝ - tocopherol).

Milwaukee					
HPLC					
10/90	2.98	7.54	-	5.15	-
1/91	4.01	5.68	-	4.77	-
5/91	3.36	5.31	-	4.50	2.57
7/91	3.26	-	3.32	-	-
8/91	2.24	3.4	2.55	-	
Colorimetric					
9/91	<0.5	<0.5	4.10	-	-
Brookfield					
HPLC					
9/91	-	-	3.3	-	-
Colorimetric					
4/91	<0.5	0.7	3.3	-	0.9
5/91	<0.5	0.5	4.6	-	0.8
9/91*	-	-	3.1	-	-

*Duplicate samples: i.e one homogeneous sample was halved and the halves sent to the different labs.

Regardless of assay used, for both institutions, CR smelt contained up to 10 times more vitamin A than the other fish. Vitamin E levels were also highest in CR smelt fed at BZ while they were mid-range to other fish at MCZ.

DIET:

At both institutions, fish were fed in combination with supplements. The vitamin concentration in the total diets are shown in Tables 3 and 4. Regardless of supplementation regime, those diets which included the most CR smelt also had the highest vitamin A levels. Vitamin E content did not differ markedly among diets within the institution because supplements of vitamin E remained constant. However, given the different feeding and supplementation regimes between the two zoos, vitamin E content of the BZ diets was considerably higher than the MCZ diets.

When expressed on a dry matter basis the vitamin A concentration of the MCZ diets ranged from 14 to 72 IU/g prior to adding CR smelt to the diet. After adding CR smelt, the concentration ranged from 75-85 (Lab 1) or 240 - 290 IU/g (Lab 2). The BZ diets ranged from 98 to 202 IU/g.

Table 3. Milwaukee County Zoo Humboldt penguin diets, estimated % dry matter, vitamins A and E (DMB).

Diet	estimated dry matter	estimated A (IU/g)	estimated E(IU/g)
1[a] (HPLC)	25	16 - 25	109 - 211
2 (HPLC)	25	86 - 97	130 - 150
2[b] (Color)	25	240-290	104 - 159

[a]Pre-CR smelt, [b]Post-CR smelt.
Probable requirements: 1.5 IU/g vitamin A and 400 mg/kg vitamin E.
* values are estimations: to date the actual moisture content of the fish has not been reported.

Table 4. Brookfield Zoo Humboldt penguin diets, % dry matter, vitamins A and E (DMB).

Diet	dry matter	A (IU/g)	E (IU/g)
1[a]	25	202	604
2[b]	25	183	590
3[c]	27	98	480

[a]65% CR + suppl., [b]65% CR + no vitamin A, [c]25% each + ½ vitamin A.
Probable requirements: 1.5 IU/g vitamin A and 400 IU/kg vitamin E.

Published vitamin A requirements range from 1.5 - 10 IU/g dry diet for most species studied (NRC, 1978-85). Presumed safe levels were levels at or less than 10 times the requirement (NRC, 1987). For domestic birds presumed safe levels range from 15-40 IU/g. For the carnivorous cat, toxic levels are 100 IU/g.

The alpha - tocopherol content of the diets (dry matter basis) ranged from 73 to 145 mg/kg at MCZ and from 0.024 - 16 IU/g at BZ. Minimal changes were noted due to diet, since supplementation remained relatively constant. For most domestic animals, the published requirements are 0.222 - 0.5 IU/g dry diet. However, requirements have been shown to be inversely related to the content of polyunsaturated fats (PUFA) (Machlin, 1984). Marine fish have considerable levels of PUFA. Given this and the fact that most fish fed to penguins are stored frozen for months which can cause depletion of vitamin E, (Geraci 1986), recommended levels of 400 IU/g vitamin dry diet appears appropriate. Vitamin E is considered relatively non-toxic. However, Nichols et.al. (1989)

Table 5. Plasma/ serum* Humboldt penguin retinol and retinyl palmitate (R.P.) levels (mcg/ dl), (mean and standard deviation).

	MCZ		BZ		
	retinol	R.P.	retinol	R.P.	
MCZ date	mean / SD	mean/ SD	mean/ SD	mean/ SD	BZ date
1/91 (n=10)	90 / 19		249 / 9	13 / 0.8	10/90 (n=2)
3/91 (n=9)	76 / 21		232 / 25	12 / 1.6	5/91 (n=10)
5/91 (n=7)	88 / 22		199 / 22	9 / 0.5	8/91 (n=6)**
7/91 (n=11)***	184 / 46		202	12	12/91 (n=8)*
9/91 (n=10)	209 / 27				

* MCZ = plasma; BZ = serum.
** Multi-vitamin supplement discontinued.
*** CR smelt added to diet (6/91).
* CR smelt decreased in the diet (11/91 to 25%).

reported problems with pink-backed pelicans receiving from 0.5 - 10.51 IU/g and decreased growth was seen with domestic chicks fed 2.2 IU/g (NRC, 1987).

Blood values

Vitamin A:

Plasma/serum values are presented in Table 5. As CR smelt, and thus more vitamin A was added to the diet of the penguins at MCZ, the plasma levels increased.

When the supplementation of vitamin A was decreased at BZ there was a corresponding serum decrease of both retinol and retinyl palmitate. However, when the proportion of CR smelt was decreased for approximately one month, no change was seen in retinol and there was an increase in retinyl palmitate.

Retinol values for free-ranging rockhopper, Magellanic and gentoo penguins ranged between 68 and 137 mcg/dl (Ghebremeskel and Williams, 1989). Captive jackass penguins receiving supplements possessed 86 mcg/dl circulating retinol (Gulland et al., 1988). Although plasma/serum levels may not be a true reflection of liver stores, the National Research Council states (for many species) that persistence of a plasma retinol concentration above 100 mcg/dl is indicative of toxicity.

Table 6. Plasma/ serum* Humboldt penguin alpha-tocopherol levels (mcg/ dl), (mean and standard deviation).

	MCZ	BZ	
MCZ date	mean/ SD	mean/ SD	BZ date
1/91 (n=10)	1794/ 461	2516/ 122	10/90 (n=2)
3/91 (n=9)	1263/ 571	2516/ 299	5/91 (n=10)
5/91 (n=7)	1832/ 344	2538/ 434	8/91 (n=6)**
7/91 (n=11)***	1705/ 380	2938	+12/91 (n=8)^
9/91 (n=10)	2227/ 535		

* MCZ = plasma; BZ = serum.
** Multi-vitamin supplement discontinued, vitamin E supplement continued.
*** CR smelt added to diet (6/91).
^ CR smelt decreased in the diet (11/91 to 25%).

Generally, vitamin A metabolism provides protection from the toxicity of free retinol by: 1) a large storage capacity in the liver in the more stable, less toxic ester form, retinyl palmitate, 2) circulation of retinol bound to retinol binding protein (RBP) and 3) when RBP is saturated, conversion of retinol to retinyl palmitate, (Machlin, 1984, Bowen, pers. comm.). If any of these processes are overwhelmed by excessive vitamin A intakes or the metabolic processes are hindered, vitamin A toxicity may occur. Thus, one indicator of potential vitamin A toxicity in the fasting animal is the presence of circulating retinyl palmitate. It may be possible that due to the vitamin A content of the diet, penguins normally have retinol levels over 100 mcg/dl. Retinyl palmitate may normally circulate at low levels. Values can appear excessive if blood samples are taken post-prandially. However, it appears prudent to suggest that sustained serum/plasma levels over 200 mcg/dl with an accompanying retinyl palmitate value of over 10 may indicate potentially toxic dietary levels.

At BZ, serum retinol dropped from averages of 240 to 200 mcg/dl and retinyl palmitate was constant when dietary vitamin A values were reduced from 50 to 90% of original levels. MCZ mean

plasma retinol increased from 76-90 to 184-209 mcg/d and retinyl palmitate from 2.7-2.9 to 5.8-6.4 mcg/d when dietary vitamin A content increased by about 4 fold.

Vitamin E:

Plasma/serum values are presented in Table 6. There appear to be relatively limited differences in these levels over time. This is not surprising given that dietary intake remained relatively constant. There was some difference between zoos with average a-tocopherol levels of 1764 (MCZ) and 2522 mcg/d (BZ). These values correspond somewhat to the vitamin E content of the diet. BZ had a higher concentration of vitamin E in the diet (0.024 - 16 IU/g DMB) than MCZ (100- 210 mg/kg DMB).

The normal serum/plasma range of domestic animals is 100-500 mcg/dl (NRC, 1987-85). Levels found in selected fish eating birds was 953-1891 mcg/dl (Dierenfeld, 1989). Jackass penguins receiving vitamin E at 61.7 IU/g body weight had an average of 3710 mcg/dl (Gulland, et al., 1988). Thus it is probable that healthy penguins possess a-tocopherol levels reflective of a high tocopherol diet where "normal" may be 1500-2500 mcg/dl.

Since vitamin E is considered relatively non-toxic, it doesn't appear that these high values should be cause for alarm. Somewhat lower levels however, probably do not signal a deficiency.

Conclusions

Vitamin A:

*Circulating serum/plasma retinol levels between 68 and 130 mcg/dl were typical values found in captive Humboldt penguins.

*Levels over 200 mcg/dl accompanied by retinyl palmitate values over about 10 mcg/dl may indicate excessive levels.

*Vitamin A content of whole fish is usually high compared to other types of foods.

*Of fish used at these institutions, CR smelt contained the highest vitamin A (by 4 to 10 times).

*Care must be taken to assess the vitamin A content of the diet before supplementation or recommending diet changes. When assessing, type of analysis used should be considered.

*In these studies, plasma/serum retinol and retinyl palmitate concentrations were correlated with dietary intake of the vitamin.

*In these studies, depending on the laboratory conducting the analysis, over 75 IU/g (DMB by HPLC) or over 200 IU/g (DMB by Colorimetric) dietary vitamin A levels were correlated with excessive retinol and increased retinyl palmitate values in Humboldt penguins.

Vitamin E:

*Circulating a-tocopherol levels between 1500-2500 mcg/dl were typical values in supplemented captive Humboldt penguins.

*Excessive levels are unknown at this time.

*Vitamin E content of stored whole fish used in these institutions ranged from 0.012 - 16 IU/g DMB.

*Given the inverse relationship of PUFA: Vitamin E with respect to requirement and frozen fish storage time, it may be wise to continue the recommendation of Geraci (400 IU/g, DMB).

Suggestions for Further Study

More investigation of captive penguin vitamin status is needed. This should include plasma/serum values and be extended to liver values from animals which die or are euthanized. It is important to correlate status with dietary intake. Among laboratory variation should be investigated further. MCZ hopes to collect blood samples for retinol, retinyl palmitate, and a-tocopherol assays from free-ranging Humboldt penguins inhabiting the Chilean coast. The data obtained from free-ranging Humboldt penguins may help establish a baseline from which to compare data obtained from captive penguins.

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ASSESSMENT OF SKELETAL DEVELOPMENT IN LEOPARD GECKOS

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Abstract

Leopard gecko lizards (*Eublepharis macularius*) were fed crickets differing in their calcium and vitamin D_3 contents from 3 weeks to 8 months of age. Feed crickets contained either 0.16% calcium and <400 (Diet 1) or 700 IU D_3/kg (Diet 2), or 0.95% calcium and <400 (Diet 3) or 700 IU D_3/kg (Diet 4). A fifth group of crickets were "dusted" with mineral powder shortly before being fed to lizards. After 7 months on these diets, the breaking strengths of left femurs of animals fed diet 3, calculated from the results of transmission ultrasound measurements, were an average of 107% greater than those of the animals fed diets 1 or 2. Increasing the vitamin D_3 content of feed crickets from <400 to 700 IU/kg (diet 4) increased femur breaking strength by another 50%, to a level not different from that attained by lizards fed "dusted" crickets. These differences were confirmed when the left femurs were dissected free of soft tissue and their properties tested by 4-point mechanical loading. The effects of dietary differences on bone strength were reflected in changes in bone densities estimated optically from radiographs, mid-shaft cortical widths and cortical indices, and total mid-shaft diameters. These data indicate that the limb bones of gecko lizards fed diets inadequately supplemented with calcium become significantly compromised in only a few months, suggest that increasing the calcium content of food crickets can substantially improve the skeletal integrity of growing leopard geckos, and demonstrate that transmission ultrasonics and optical analysis of radiographs can be used to monitor skeletal status in small lizards.

Introduction

Gecko lizards are obligatory insectivores, requiring supplemental dietary calcium when maintained in captivity (Allen, 1989). However, dietary thresholds for calcium, interactions between dietary calcium and vitamin D, and the sensitivity of the growing skeleton to dietary calcium supply are poorly understood in reptiles. Attempts to better understand the reptilian skeleton have been hindered by the lack of an accurate, sufficiently sensitive, non-invasive, and inexpensive method of monitoring bone development in vivo.

Two solutions to this problem have been developed in recent years. Transmission ultrasonics now provides a practical method of estimating the density of selected limb bones in live, unanesthetized, minimally restrained animals (including humans), allowing the calculation of the mechanical breaking strengths of these sites (Jeffcott and McCartney, 1985; Glade et al, 1986; Wright et al, 1987; Antich et al, 1991). This technology utilizes the relationships described by Ficke's Law (the velocity of a sound wave is directly proportional to the density of its transmitting medium), and can be applied in experimental situations in which it is desirable to measure longitudinal changes

within the same animals, and for routine monitoring of the effectiveness of husbandry practices.

Computerized digital image analysis of standardized radiographs also allows access to the growing skeleton without requiring animal sacrifice. This technique can also be used to determine a number of anatomic characteristics that directly reflect bone growth and structural integrity, including diameter, cortical index, length, and shape. In addition, the relative optical densities of different bones or sites within a bone can be estimated with this technique,; from these data, relative bone densities can be calculated. Bone densities determined optically can then be related mathematically to mechanical breaking strengths, using empirical formulas obtained experimentally.

These techniques were applied to groups of growing juvenile leopard geckos (*Eublepharis macularius*) in order to characterize the anatomic and mechanical properties of growing reptilian limb bones. This investigation was part of a larger project (Allen, 1989) that examined the effects of the calcium and vitamin D_3 content of live feed crickets on the growth, development, and mineral contents of the skeletons of growing juvenile leopard geckos (*Eublepharis macularius*).

Materials and Methods

Fifteen individually housed geckos were randomly assigned to 5 diet groups as they reached 3 weeks of age. For 7 months the geckos were fed ad lib twice weekly. The geckos received only crickets whose nutrient composition had been manipulated through their diets (for details, see Allen and Oftedal, 1989), such that the crickets comprising gecko diet 1 provided 0.16% calcium and <400 IU D_3/kg; those in gecko diet 2 provided 0.16% calcium and 700 IU D_3/kg; gecko diet 3 provided 0.95% calcium and <400 IU D_3/kg; and gecko diet 4 provided 0.95% calcium and 700 IU D_3/kg. A fifth group of crickets were fed a standard avian maintenance diet and were dusted with a vitamin/mineral mix (Pervinal) prior to being fed to the fifth group of geckos. This group of lizards were considered controls and reflected the National Zoo's Reptile House protocol for supplementing crickets with calcium.

The velocities of standardized pulses of sound through selected anatomic sites were measured in vivo after 4, 5, 6, and 7 months of the experiment using transmission ultrasonics. The time required for the transmission of a standardized pulse of sound to travel through a specimen was measured on an Apple II-plus micro-processor (Apple Computer, Inc., Cupertino, CA 95014) that had been modified so that it could function as a digital oscilloscope with image-storing and comparing capabilities (Equine Biomechanics, Inc., Unionville PA 19375). The signals were generated (Pulser Receiver 5055 PR, Panametrics, Waltham, MA 02154) at a rate of 5/sec for 4 sec (440 nsec/signal) and were transmitted and received by transducers (Amdek Corp., Arlington Heights, IL 60005) mounted on a custom-built steel caliper rig. The received signals were superimposed on the transmitted waveform by the computer, signal-to-noise ratios were maximized, the 20 resultant complex waveforms were averaged, and the final result was displayed on a video monitor. The average elapsed time between the transmission and reception of a signal was measured as the transmission time. The transmission distance was measured simultaneously by an electronic caliper mounted into the rig. The velocity of sound pulses through the specimens (V) was calculated and expressed in m/sec.

After 7 months, the animals were sacrificed and their left humeri and femurs were dissected free of skin and soft tissue. The velocities (V) of standardized sound pulses were measured mediolaterally at the mid-diaphysis of these bones, and their ultimate mechanical breaking strengths (BS) were calculated (Pratt, 1980):

$$BS\ (kg/mm^2) = (0.10197\ kg/mm^2)\ \frac{22834\ km^2/sec^2}{34.6\ km^2/sec^2 - V^2} - 700$$

where V = average velocity of a transmitted sound pulse in km/sec. BS were also independently estimated by subjecting the bones to 4-point loading to failure (Floor Model TT, Instron Co., Canton MA 02021) (Schryver, 1978).

Standardized whole-body radiographs were taken at 7 months. These images were electronically entered into a computerized digitizing system (Drexel Image Processing System, Drexel University, Philadelphia PA) and the digitized images were used to measure the following attributes of the left femurs and tibias: medial and lateral cortical widths at the mid-diaphysis, total diameter at mid-diaphysis, and the distances between the proximal and distal growth plates along the longitudinal axes of the bones. Total cortical widths and cortical indices at mid-diaphysis were calculated from these measurements. The digitized images were also used to estimate the peak optical densities of the medial and lateral cortices of these bones at mid-diaphysis, relative to the background optical densities of each respective radiographic plate. The averages of the medial and lateral peak relative optical densities were calculated and expressed in arbitrary linear units for comparison.

Data were analyzed by computerized analyses of variance and linear regression analyses (Hintze, 1986).

Results

After 7 months, sound transmission velocities (V) through intact left hindlimbs in vivo (Figure 1) were least in the animals fed diets 1 and 2, and were greatest in animals fed diets 4 and 5 ($p<.05$).

V through cleaned left femurs maintained these relative differences post-mortem (Figure 1). In all cases, V through cleaned bones were 14 to 32% faster than through the corresponding limbs in vivo ($p<.01$).

The breaking strengths of the left femurs, estimated from V measured through cleaned bones post-mortem, were significantly greater ($p<.01$) in groups 3, 4, and 5 (averaging 3.95 to 6.11 kg/mm^2) than in groups 1 and 2 (averaging 2.15 and 1.66 kg/mm^2, respectively) (Figure 2).

The ultimate breaking strengths measured by 4-point loading to failure (Figure 2) were nearly identical to those calculated from V through cleaned bones ® = 0.998, $p<.001$). The breaking strengths determined by loading to failure were linear functions of V, whether measured through

Figure 1. Velocities of sound pulses (m/sec) through intact left hindlimbs and cleaned left femurs of juvenile leopard geckos after 7 months of experimental feeding. For intact limbs, pooled SEM = 25 m/sec; for cleaned bones pooled SEM = 31 m/sec. Mean velocities through intact limbs for diets 1 and 2 differ from means for diets 3,4, and 5 ($p<.05$). Mean velocities through cleaned femurs for diets 1 and 2 differ from means for diets 3,4, and 5 ($p<.01$). Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg; 4) 0.95% CA, 700 IU D_3/ kg; 5) "dusted" crickets.

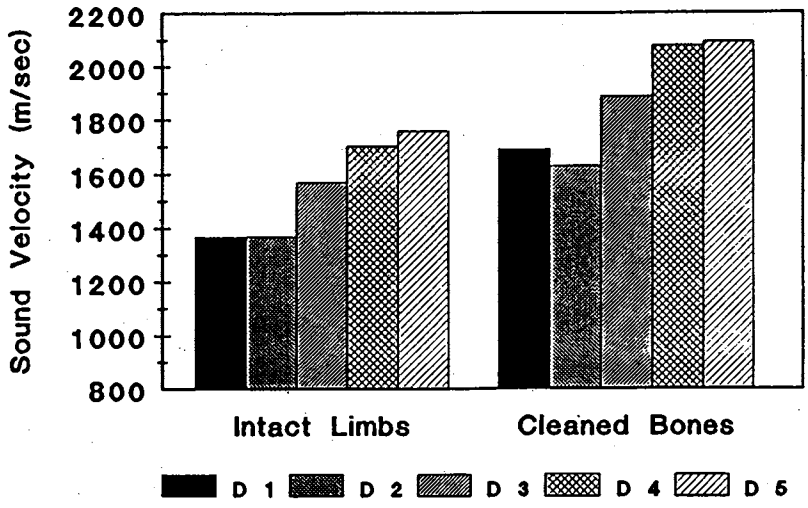
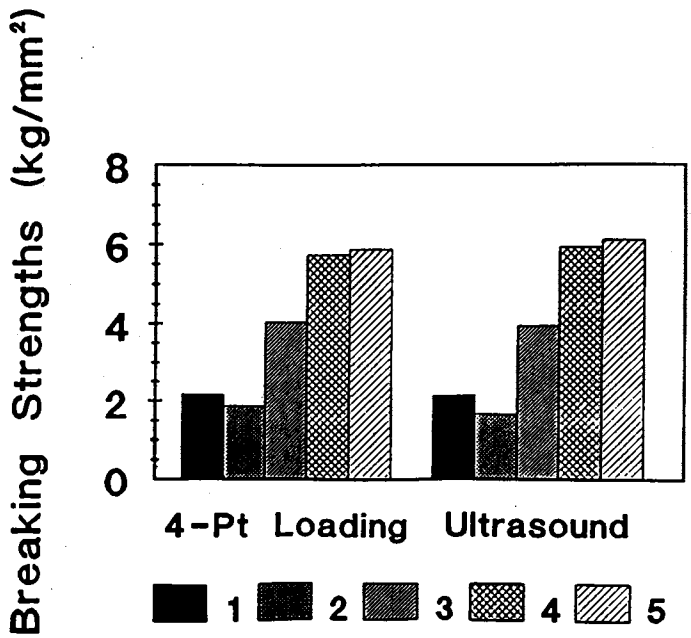


Figure 2. Ultimate breaking strengths (kg/mm²) of cleaned left femurs of juvenile leopard geckos after 7 months of experimental feeding. Left: measured by 4-point loading to failure; pooled SEM = 0.26 kg/mm². Right: calculated from V through cleaned bones; pooled SEM = 0.29 kg/mm². For breaking strengths determined by either method, means for diet 1 and 2 differ from means for diets 3,4, and 5 ($p<.01$). Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg;



cleaned bones (BS (kg/mm^2) = 0.087716 (m/sec) - 12.61792, r = 0.990), or <u>in vivo</u> (BS (kg/mm^2) = 0.009472 (m/sec) - 10.8105, r = 0.962).

The average peak relative cortical optical densities estimated from radiograph images by digital image analysis were greater in the limb bones of the animals fed diets 3, 4, and 5 than they were in the animals fed diets 1 and 2 ($p<.01$; Figure 3).

Average peak relative optical densities (r.o.d.) were highly correlated with breaking strengths estimated by 4-point loading to failure: BS (kg/mm^2) = 0.1026 (r.o.d.), r = 0.861.

The total cortical widths at mid-diaphysis were significantly greater in the left femurs of the animals fed diets 3, 4, and 5 ($p<.05$; Figure 4), and in the left tibias of the animals fed diets 4 and 5 ($p<.05$).

Total mid-diaphyseal diameters were not significantly different among diet groups, although they tended to be larger in the animals fed diets 3, 4, and 5 (Figure 5).

Figure 3. Average peaks relative optical densities (arbitrary units) of left femurs and tibias of juvenile leopard geckos after 7 months of experimental feeding, estimated by image analysis of standardized radiographs. Pooled SEM = 21 m/sec. Means for femurs for diets 1 and 2 differ from means for diets 3,4, and 5 ($p<.05$). Means for tibias for diets 1 differ from means for diets 2,3,4, and 5 ($p<.01$). Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg; 4) 0.95% CA, 700 IU D_3/ kg; 5) "dusted" crickets.

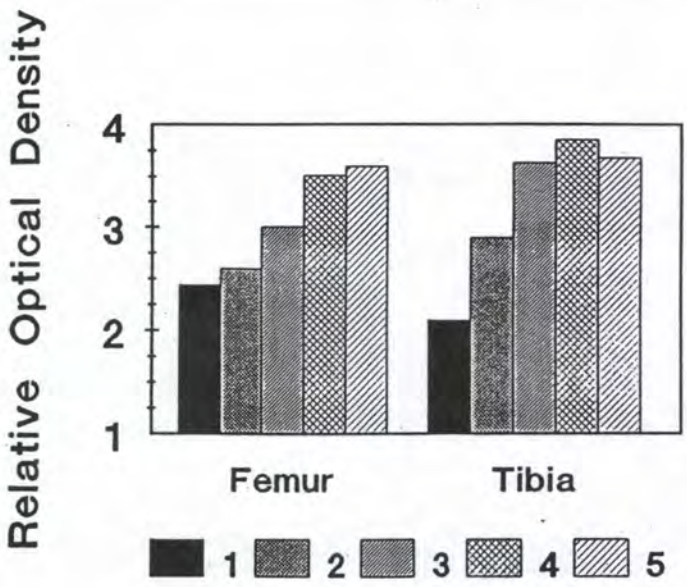


Figure 4. Total cortical widths (mm) at mid-diaphysis of left femurs and tibias of juvenile leopard geckos after 7 months of experimental feeding, measured optically from standardized radiographs. Pooled SEM = 0.06 mm. Means for tibias for diets 1 and 2 differ from means for diets 3,4, and 5 ($p<.05$). Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg; 4) 0.95% CA, 700 IU D_3/ kg; 5) "dusted" crickets.

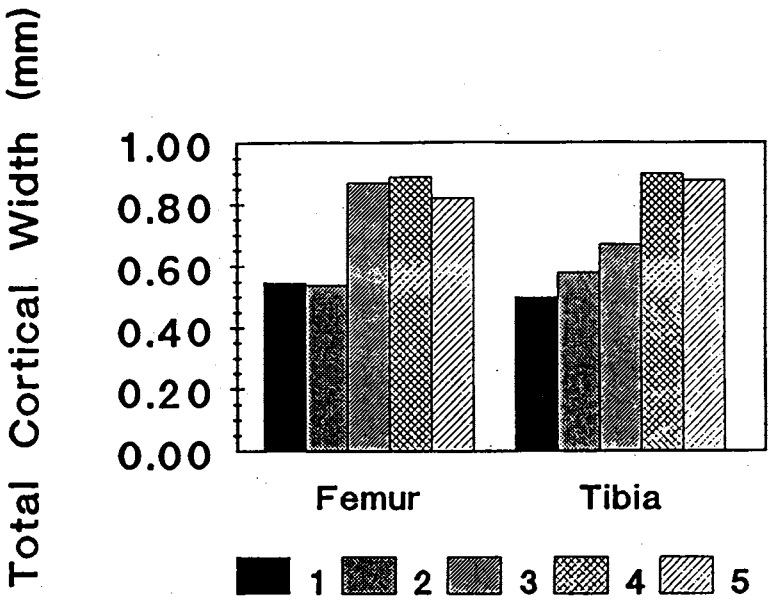
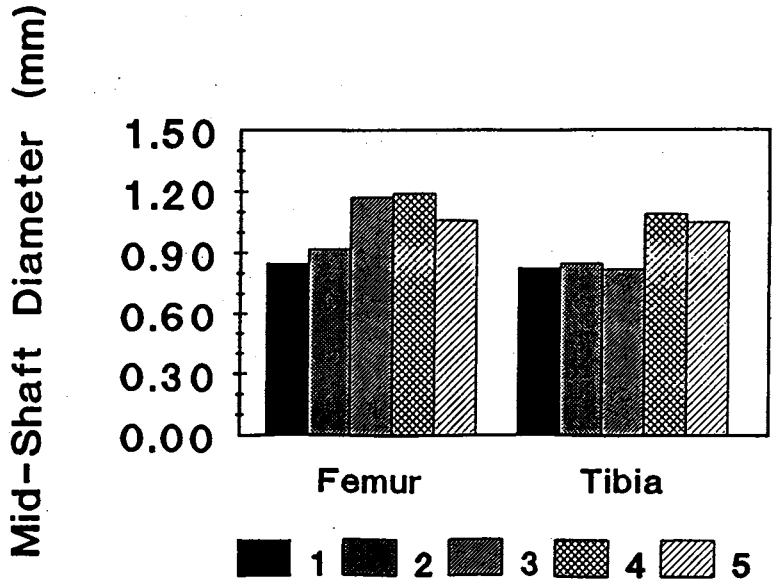


Figure 5. Total mid-diaphyseal diameters (mm) of left femurs and tibias of juvenile leopard geckos after 7 months of experimental feeding. Measured optically from standardized radiographs. There were no significant differences among means. Pooled SEM = 0.06 mm. Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg; 4) 0.95% CA, 700 IU D_3/ kg; 5) "dusted" crickets.



Consequently, the cortical indices of the left femurs and tibias were significantly greater in the animals fed diets 3, 4, and 5 ($p<.05$; Figure 6).

The distances between the growth plates along the longitudinal axes of these bones were not significantly different among diet groups (Figure 7), although the femurs from the animals fed diets 3, 4, and 5 tended to be longer.

Figure 6. Cortical indices (total cortical width/ mid-diaphyseal diameter) of left femurs and tibias of juvenile leopard geckos after 7 months of experimental feeding, measured optically from standardized radiographs. Pooled SEM = 0.04. For both femurs and tibias, means for diets 1 and 2 differ from means for diets 3,4, and 5 ($p<.05$). Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg; 4) 0.95% CA, 700 IU D_3/ kg; 5) "dusted" crickets.

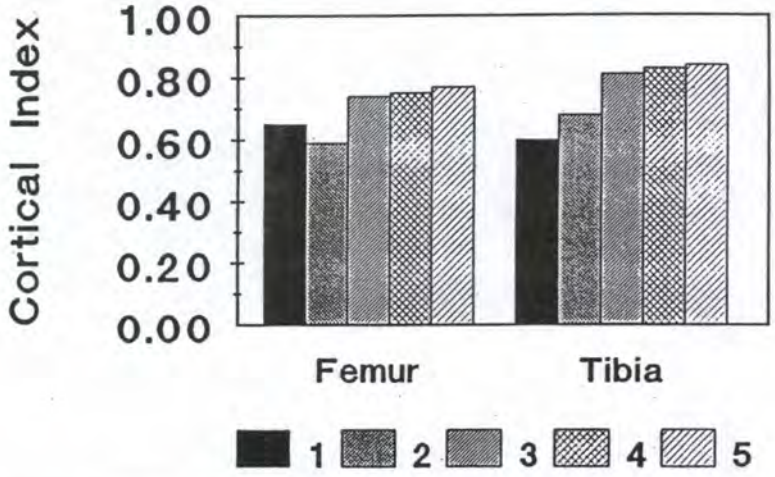
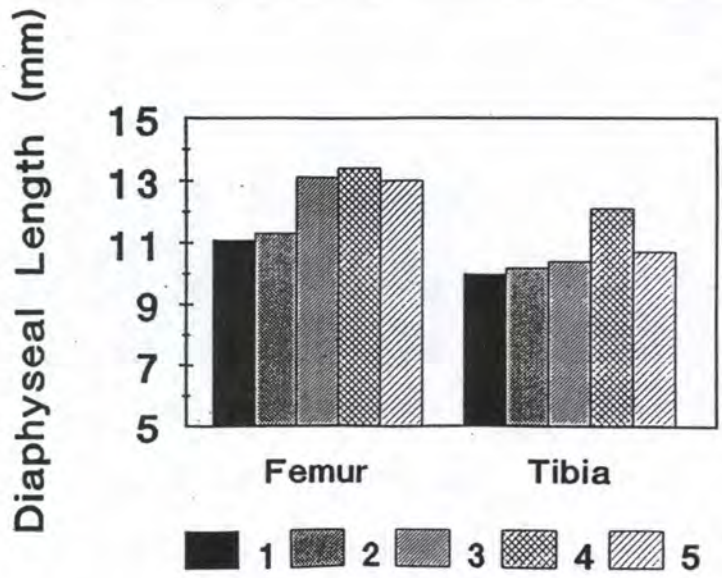


Figure 7. Diaphyseal lengths (mm) of left femurs and tibias of juvenile leopard geckos after 7 months of experimental feeding, measured optically between the proximal and distal growth plates along the longitudinal axes of the bones. Means for femurs for diets 1 and 2 differ from means for diets 3,4, and 5 ($p<.05$). Means for tibias were not significantly different. Pooled SEM = 0.63 mm. Key to diet groups: 1) 0.16% CA, <400 IU D_3/ kg; 2) 0.16% CA, 700 IU D_3/ kg; 3) 0.95% CA, <400 IU D_3/ kg; 4) 0.95% CA, 700 IU D_3/ kg; 5) "dusted" crickets.



Discussion

This experiment has demonstrated the practical usefulness of monitoring reptilian skeletal development with standardized transmission ultrasonics. The data obtained during this study are fully consistent with those published earlier (Allen, 1989) and confirm that the size and structural integrity of reptilian bones are sensitive to dietary calcium and vitamin D_3 content. All measured variables were proportional to the calcium status of these growing leopard geckos. The bones of those animals fed diets inadequately supplemented with calcium became significantly compromised within a few months. These bones not only failed to grow as rapidly, but their cortices also became thinner and less dense, resulting in significantly diminished ability to withstand loading.

In addition, portions of the data (sound velocities through intact limbs, sound velocities through cleaned left femurs) further suggest that when increased calcium availability results in denser bone, vitamin D_3 supply can become limiting. Approximately doubling the D_3 content of the diet exerted an additive positive effect on bone strength, in addition to the effect observed when D_3 intake was kept constant and calcium intake alone was increased.

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PROBLEMS IN MOOSE NUTRITION

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The North American moose has been maintained in captivity in U.S. zoological institutions since the early 1900's. Published reports on captive moose care from the 1960's allude to the difficulties of maintaining this large cervid in captivity (Speidel, 1966). The average life span of captive moose in North America is 4 - 6 years (Schwartz, 1989b), yet wild moose can live up to twenty years (Nygren et. al., 1990; Peterson, 1955). Captive moose appear to be extremely prone to intestinal disease with resulting diarrhea. Reports of intractable diarrhea and high mortality are common in hand reared calves (Addison et al., 1983; Schwartz, 1989b; Speidel, 1966; Welch et al., 1985). Chronic diarrhea, wasting and premature death have been reported in adults (Bjork, 1986; Ellis, 1987 Schwartz, 1989b). In a survey of moose husbandry in North America, (Schwartz, 1989b) reported that 31% of moose died of "digestive upset". Diarrhea has been so common at the Minnesota Zoo, that a stool consistency chart was developed to accurately track our moose stools (Table 1). The common feeling among moose managers is that there is a dietary component to this diarrhea / wasting syndrome. Historically, diets fed to captive moose have consisted of concentrates, hays, fruits, vegetables and browse (Baines, 1965; Speidel, 1966; Voss, 1959). The majority of North American institutions that are currently holding moose feed either a pelleted diet developed by the Moose Research Center or Purina Mills, Mazuri Moose Diet. These diets are fed with or without browse (Schwartz, 1989b). To better understand the nutritional theory behind these two moose diets and the role nutrition may play in diarrhea in captive moose, it is valuable to look at the natural diet.

Free ranging moose have been found to consume a wide variety of plant species (Peterson, 1955). Deciduous and coniferous trees, shrubs, forbs, grasses, aquatics, lichens and mushrooms have all been reported to be eaten by moose in varying quantities (Peterson, 1955; Jordan, 1987; LeResche et. al., 1973). As a resident of circumpolar regions the moose has evolved to cope with a changing food supply throughout the year. The summer diet primarily consists of the leaves of deciduous trees, including willow (*Salix sp.*), aspen (*Populous sp.*) and birch (*Betula sp.*). Shrubs, forbs, sedges, grasses and aquatics are also consumed in varying amounts depending on the habitat region (Leresche et. al., 1973; Jordan, 1987; Peterson, 1955). Winter diet is composed of the twigs and buds of many of the same deciduous trees that are consumed in the growing season. Conifers, primarily balsam fir (*Abies balsamea*), also constitute a major portion of the winter browse in some moose habitat (LeResche et. al., 1973; Peterson, 1955; Risenhoover, 1989; Thompson et al., 1989). The diet of the moose is very high in lignin. Nutrient - rich cell contents are limited to leafy vegetation and to the outer bark and buds of twigs (Hoffmann, 1988; Schwartz, 1989).

The moose is classified as a seasonal concentrate selector (Hofmann, 1989). Anatomical and physiological adaptations allow this large browser to survive seasonal extremes in diet. The forestomach conformation of the moose is consistent with that of other concentrate selectors. The rumeno - reticulum is simple with few and shallow pillars. The reticulo - omasal opening is relatively large and the omasum is small with few laminae. The hindgut (cecum and spiral colon) act as a

secondary fermentation chamber (Hofmann, 1989; Nygren et al., 1990). Moose as well as other browsers have a decreased rumen retention time. This allows fermentation of soluble cell contents with rapid passage of less digestible cell wall components out of the forestomach (Hofmann, 1989; Nygren et. al., 1990; Schwartz, 1989). Moose were found to pass lignin - rich browse diets more rapidly than cellulose - rich grass and alfalfa hays (Renecker et. al., 1990; Schwartz et. al., 1980). This is believed to be because lignin shatters upon mastication more readily than cellulose, thus resulting in particles of a more optimum shape and size to pass through the reticulo - omasal orifice (Renecker et. al., 1990; Schwartz et. al., 1980, Schwartz, 1989). Moose can pass large particles out of the forestomach and increase the volume of hindgut fermentation when presented with large amounts of undigestable cell wall components. These physiological adaptations allow this large browser to survive on a highly lignified diet (Hofmann, 1989; Nygren et. al., 1990; Schwartz et. al., 1980; Schwartz, 1989).

Table 1. Minnesota Zoological Garden moose stool consistency chart.

Grade	Definition
#1	Firm pelleted stools.
#2	Stool pelleted but soft, clumped together.
#3	Stool somewhat loose, no visible pellets. The stool stacks 2-3 inches high.
#4	Stool loose, no pellets, forms a flat "pie" or "pancake", but does not splash.
#5	Controlled diarrhea. Stool very loose, appears to have a high water content. Will splash on a 2 - 3 foot area as it is eliminated.
#6	Uncontrolled diarrhea. Stool very high water content. Defecation frequent or continual. Legs and hind quarters soiled with feces. Stool dribbled throughout holding pen.

It is from the above discussed adaptations that two pelleted moose diets have evolved. Schwartz et al.,1980) based their pelleted Moose Research Center (MRC "Special") diet (Table 2A and 2B), on the concept that moose have adapted to high lignin browse diets and will become bulk limited by grass and forb diets that are high in cellulose. Thus, captive moose maintained on traditional zoo herbivore diets would be unable to extract enough nutrients from grass and alfalfa hays. The MRC diet uses lignin - rich aspen sawdust as a fiber source. Energy is provided by corn, soybeans and barley. For five years, eleven captive moose were maintained and reproduced on the MRC pelleted diet (Schwartz et. al., 1985). Moose were found to have soft-formed stools during the summer when diet consumption increased. During the winter feces were consistently pelleted. No moose developed the diarrhea and chronic wasting syndrome (Schwartz, pers. comm.).

Purina Mills, Mazuri Moose Diet, also incorporates aspen as the fiber source. It differs from the MRC diet in that it contains no starch. (Table 3) Energy is provided by beet pulp, sucrose and cane molasses. The moose, whose diet consists of highly soluble cell contents and lignin (Hofmann,1989), may not be adapted to handle diets high in complex carbohydrates and starch. Rapid passage of the pelleted diet out of the forestomach would allow a significant amount of fermentable starch to reach the hindgut, possibly resulting in diarrhea (Saddler, pers. comm.).

Many institutions have not been able to repeat the success of Schwartz et. al., while feeding either the MRC diet or a similar approximation. Continued reports of diarrhea and wasting are not uncommon (Frank, pers. comm., Haigh, pers. comm.). The Minnesota Zoo has fed MRC since 1982

and has continued to loose moose from diarrhea and wasting.

Table 2A. Moose Research Diet - "MRC Special.

Diet Composition Percent As Fed:	
Ground yellow corn	28.7%
Aspen sawdust*	25.9%
Rolled oats	17.2%
Soybean meal	7.8%
Dried cane molasses	5.7%
Barley	5.7%
Ground beet pulp	5.7%
Dicalcium phosphate	1.3%
Trace mineral salt	0.7%
Vitamins A, D_3, and E	0.3 %
Ground limestone	0.28%

*Aspen sawdust from sawmill.

Table 2B. Moose Research Diet - "MRC Special.

Standard Analysis Results Dry Matter Basis:	
Dry matter low corn	80.0%
Crude protein	11.75%
Cell wall constituents	47.2%
ADF	26.5%
TDN	67.0%

From: Schwartz, et. al. 1980.

Table 3. Mazuri Moose Maintenance 5658.

Guaranteed Analysis:	
Crude protein	11.0%
Crude fat	4.0%
Crude fiber	32.0%
Ash	6.0%
Added minerals	2.0%

From: Mazuri - The complete zoo feeding resource manual. Purina Mills, Specialty Business Group, P.O. Box 66812, St. Louis, MO 63166-6812.

There have been anecdotal reports that moose fed browse with the pelleted diet tend to have a lower incidence of diarrhea. Schwartz (pers. comm.) feels that institutions that have kept moose in the same enclosures for many years, have a higher incidence of loose stools and that there may be an infectious component to the diarrhea.

A review of histopathology records on ten moose that died of diarrhea at the Minnesota Zoo revealed similar cecal and colonic lesions in all animals. Histopathological lesions include, vasculitis, ulcerations, plasma cell and eosinophil infiltrates within the cecum. The etiology of these lesions is unclear at this time. The high number of plasma cells and eosinophils suggests an immunological stimulation.

In summary, it is clear that further research needs to be conducted on diets fed to captive moose. Questions that remain to be answered include: 1) What is the significance of hindgut fermentation in moose that are fed pelleted diets? 2) Do moose have an intolerance, or a potential intolerance to high starch diets? 3) Is there a differences in the digestion of the MRC diet and Mazuri diet? 4) What role is browse playing in the health of the digestive tract? Are secondary plant compounds involved? 5) What is the significance of the histopathology lesions reported in the moose at the Minnesota Zoo, and have other institutions seen similar lesions in their moose that have died

from diarrhea? 6) Are their other factors besides nutrition that may be contributing to the diarrhea, (Bacterial or viral diseases, parasites, immunological factors or stress)? The puzzle of moose nutrition has yet to be solved, but undoubtedly this problem provides much for moose managers and nutritionists to ruminate upon.

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DIETARY INFLUENCES ON CALCIUM STONE DISEASE IN CAPTIVE ASIAN SMALL CLAWED OTTERS (*Aonyx cinerea*)

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The Asian small-clawed otter (*Aonyx cinerea* - ASCO) is the smallest member of the otter family. These otters live in mangrove swamps, rice fields and small streams from the Philippines through the Himalayan foothills. They are gregarious, tractable animals, and until the mid 1970's were exported from Thailand to the US and Britain as pets. Exportation stopped when declining numbers of animals in the wild caused Thailand to classify them as protected. In 1981, a Species Survival Plan program was instituted to ensure conservation of the species.

According to the 1990 ASCO North American Regional Studbook, "...problems continue to plague the captive otter population and captive conservation efforts: (one of the two largest is) the high incidence of urinary stones which continues to be the most frequently reported cause of death..." (Engfer, 1991). In 1988, Calle reported that renal calculi had been diagnosed in 66, and cystic calculi in 23, percent of the captive ASCO population that had been radiographed or necropsied. Stones that had been analyzed were found to be calcium oxalate, or urates. Interestingly, examination of 30 otters in the wild revealed the presence of only one renal calculus, associated with a parasite (Daengsvang, 1973; reported by Calle, 1988) suggesting that stone disease may be associated with captivity.

The cause of the stones is unknown, and many causes of lithiasis are recognized in human and veterinary medicine. Because the disease appears to be more prevalent in captive animals, it is thought to be related to the diet, although a diet "moderately restricted" in protein, calcium and oxalate (Hill's Prescription Diet k/d) did not prevent the disease (Calle, 1988). Diet-related factors most likely to influence calcium stone formation include the amounts of calcium, protein, sodium, oxalate, and vitamins A and D present in food (although many other diet-related factors have been suggested to play a role in lithogenesis) (Goldfarb, 1988, Robertson, 1987). Increasing the amount of calcium in the diet increases net calcium absorption, and then excretion in the urine. Acid metabolites of protein catabolism may enhance calcium excretion in the urine, as can sodium (at least in humans). Vitamin D promotes calcium uptake by the intestine, which would then be excreted in the urine. Excess oxalate, either directly from the diet or as a result of protein (glycolate, glycine, hydroxyproline, alpha-hydroxy-beta-ketoadipate) and ascorbic acid metabolism, also could increase urinary oxalate excretion (Menon, 1982). Vitamin A deficiency has been reported to promote urolithiasis (Osborne, 1917), but the amounts of this vitamin supplemented to captive otters makes the probability of a deficiency unlikely.

To better evaluate the appropriateness the diets fed to captive ASCOs, one may look at their natural foods. In the wild, ASCOs apparently eat crustaceans, molluscs and small fish (Calle, 1988). These foods are high in moisture (80%) and protein (80%) dry matter, low in calcium (0.2% of the

dry matter), and the flesh of these animals has an inverse calcium:phosphorus ratio. A comparison of the estimated dry-matter nutrient content of ASCO diets in the wild with that fed to captive otters is presented in Table 1.

Table 1. Nutrient analysis of some otter diets.

Diet	Protein	Calcium	Phosphate	Ca:P	Vitamin D IU/kg diet
Wild ASCO	79%	0.2%	1.0%	0.2:1	100-1000(?)
Columbus Zoo	36%	1.3%	0.6%	2.2:1	6000
Bronx Zoo	43%	1.7%	1.2%	1.4:1	3600
Nebraska Feline 45	1.4%	1.2%		1.2:1	4450
Hill's Canine k/d	16%	0.8%	0.3%	2.7	700
Pet foods	20 - 50%	00.8 - 2%	07. - 1.8%	>1:1	900 - 1500
Sea otter	73%	0.12%	1.0%	0.12:1	100-1000(?)
Proposed diet	80%	0.17%	0.7%	0.2:1	200

If the analyses of the various diets presented are correct, one can see that captive ASCOs are fed less protein, more calcium and vitamin D, and amounts of phosphorus similar to what is consumed in the wild. The high protein content of the diet consumed in the wild argues against oxalate synthesis from protein being a significant pathogenetic factor. The low calcium content assumes that calcium is primarily derived from the flesh of prey. Calcium is present in the carapace of crustaceans, the shells of molluscs and the bones of fish, but may not be highly available to the otter. Intestinal transit time is quite fast (3-4 hours) in otters (Kenyon, 1969), and exoskeletons and bone appear undigested in the feces. Other sources of calcium in the environment of these animals have not been identified, suggesting that they may have adapted to relatively low calcium intakes (Karesh, 1983).

The inverse calcium:phosphorus ratio also deserves comment. The importance of Ca:P ratios of > 1:1 is well accepted in animal nutrition. Diets containing inadequate calcium alone, or low or normal concentrations in combination with a relative or absolute dietary excess of phosphorus (Ca:P ratio less than 1:1), can result in bone resorption. Krook, et al. (1971) reported that adult beagles fed approximately 35 mg calcium and 300 mg phosphorus per kilogram body weight per day for 42 weeks lost significant amounts of interradicular bone in the mandibular molar region. The incisor teeth also become mobile. Jowsey et al. (1974) similarly reported finding increased bone porosity in biopsy specimens collected from the ulna of adult dogs fed approximately 85 mg calcium and up to 260 mg phosphorus per kilogram body weight per day for 28 weeks. Serum immunoreactive PTH also was significantly increased. In both growing (Scott et al., 1961) and adult (Roberts et al., 1961) cats, feeding unsupplemented beef heart has been reported to produce negative calcium balance and, in growing animals, histologically proven osteoporosis. The adults consumed approximately 7 mg calcium and 135 mg phosphorus per kilogram body weight per day for 12 weeks. Interestingly, calcium balance became positive when the adult cats were supplemented with 100 ug iodine per day. Growing kittens fed unsupplemented beef heart had thyroid enlargement and histological

abnormalities compatible with iodine deficiency. The parathyroid glands of these animals also were enlarged, but were histologically normal.

Osteoporosis due to inadequate dietary calcium, alone or relative to high dietary phosphorus content, is known as nutritional secondary hyperparathyroidism (NSHP). NSHP is a relatively common nutritional disease of domestic animals (Gershoff et al., 1958); alveolar bone loss appears first, and becomes most severe (Krook et al., 1971). The disease is most common in growing animals because of the calcium requirements for bone accretion (Capen, et al., 1989). Osteoporosis develops because hypocalcemia induces secretion of parathyroid hormone, which mediates increases in osteoclast activity to release bone calcium to attempt to maintain normo-calcemia.

Although the above studies might lead one to predict that the wild ASCO diet would result in NSHP, there is some evidence to the contrary. Gershoff et al.(1958) fed two dogs from 3 to 36 months of age a diet containing 0.11% calcium (10 and 20 mg/kg BW/day)and approximately 0.55% phosphorus (50 and 100 mg/kg BW/day) with no apparent ill effects. Anderson et al.(1977) also successfully fed a diet with a Ca:P ratio of 1:5 to young Cebus albifrons monkeys, although the amounts of both were quite large (450 mg calcium and 1800 mg phosphorus per day to animals weighing 0.95 to 2.58 kg). Adult chickens (Norris et al., 1972) and humans (Rivlin, 1991) also may have quite low calcium needs, and apparently tolerate "inverse" Ca:P ratios for extended periods of time.

Table 2. Proposed diet for captive Asina small-clawed otters: 1000 gram Beef heart; 1 gram Centrum Jr.® (vitamin supplement); 1 gram calcium carbonate; 10 gram cellulose powder.

Weight: 1002 gms (35.3 oz.) Water weight: 641 gms
Calories 1741 Protein 288 gms
Carbohydrate 4.2 gms Fiber 0 gms
Fat 56 gms

Vitamin A	1000 RE	Calcium	567 mg
Vitamin D	200 IU	Phosphorus	2527 mg
Vitamin E	27.2 mg	Potassium	2329 mg
Thiamin	2.4 mg	Sodium	631 mg
Riboflavin	22 mg	Magnesium	227 mg
Niacin	54 mg	Iron	87 mg
Pyridoxine	3.4 mg	Zinc	41 mg
Pantothenic acid	15 mg	Copper	8.7 mg
Folic acid	287 µg	Selenium	212 µg
Vitamin B_{12}	148 µg		

% of calories from protein = protein - 69, carbohydrate - 1, fat - 30. Ca:Phos = 0.2:1

Analysis of the diet of captive Sea Otters (Williams et al., 1991) is included because they also consume an invertebrate diet in the wild. In contrast to ASCOs, however, they continue to be fed invertebrates in captivity because they refuse to eat other foods. I was unable to locate any reports of calcium stone disease in captive sea otters.

This information supports the idea that ASCOs have lower requirements for calcium and vitamin D than they are forced to consume in captivity. To test this hypothesis, we plan to we feed our ASCOs a diet more similar in analysis to that of captive sea otters. The diet presented in Table 2 (ESHA 1991) was designed to approximate the macronutrient analysis of diets fed to Sea Otters, and that consumed by ASCOs in the wild. Diet therapy, if successful,

should stop growth of the stones currently in the kidneys, but is not likely to result in dissolution of oxalate stones. We plan to eliminate the current stones with lithotripsy, and attempt to prevent their recurrence in known stone-forming otters. We will evaluate their calcium regulatory hormone status by measuring serum concentrations of parathyroid hormone, calcitriol, and 25- and 1, 25- vitamin D before and after therapy. This may provide some evidence for or against the diet as a cause, and will permit evaluation of the animals during diet therapy to avoid nutritional secondary hyperparathyroidism. Radiographic evaluation also will be used to monitor the otters for NSHP.

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THE EFFECT OF NEONATAL INJECTIONS OF Bo-Se (blood selenium) ON VIETNAMESE POT-BELLIED PIGS

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Abstract

During 1989 and 1990, four litters of Vietnamese pot-bellied pig, (*Sus scrofa*) were studied to determine the effect of neonatal injection of Bo-Se. Protocol was standardized for all litters including the neonatal exam performed on all piglets. Treatment consisted of 0.25 cc Bo-Se IM, administered during the exam to randomly selected individuals. Blood was taken weekly from all piglets and α-tocopherol concentration was analyzed by high performance liquid chromatography. Statistical differences were assessed using a t-test. Overall mean differences ($P < .008$) in serum vitamin E concentrations were found due to treatment for the six week study period. One sow was supplemented with 14 I.U. vitamin E daily for a period of 129 days prior to farrowing. Results of ANOVA ($P < .05$) indicated interaction between supplementation and treatment.

Introduction

Pot-bellied pigs are popular exhibit animals in many zoos. At the time of this study both Zoo Atlanta and St. Louis Zoological Park were exhibiting them. The boar Zoo Atlanta acquired in 1987 was related to the animals at the St. Louis Zoological Park. Commercially available pig feeds, formulated to meet NRC requirements for domestic swine, were routinely used at both facilities. Prior to this study, Zoo Atlanta's boar died suddenly during a routine handling procedure. Cause of death was suspected to be a vitamin E - related deficiency and/or Porcine Stress Syndrome (PSS). PSS is a genetically - related trait that has been linked with vitamin E deficiency (Duthie et al. 1987). It had not previously been reported in this particular breed.

St. Louis Zoological Park had several instances where piglets suddenly collapsed. Three of the piglets subsequently died, others responded to treatment. Necropsy indicated skeletal myopathy (Junge and Miller, 1989). It was postulated these animals died of a vitamin E related deficiency and that vitamin E requirements for pot-bellied pigs may be greater than for other breeds.

Upon comparison of husbandry procedures and incidence of mortality, the following observations were made: Related pigs appeared to have a higher incidence of mortality than non-related, and Zoo Atlanta routinely administered Bo-Se to all piglets during neonatal exams; St. Louis Zoological Park did not. It was decided both zoos would cooperate to investigate the effect of neonatal supplementation of Bo-Se on the vitamin E concentration of the blood levels of pot-bellied pigs and to investigate any influence on these blood levels by genetic relatedness. It was also decided not to test Se levels. Though Se can have a sparing effect on vitamin E, it is not interchangeable with

the vitamin (Lloyd et.al. 1978, Dierenfeld 1989). Many studies of vitamin E deficiency indicate normal blood levels of Se (Liu et.al. 1982, Liu et.al. 1983, Liu et.al., 1984, Liu et.al. 1985). The indications which prompted this study were of vitamin E deficiency and did not implicate Se.

Methods and Procedures

Four litters farrowed during 1989 and 1990 were included in the study, one at St. Louis Zoological Park, and three at Atlanta-Fulton County Zoo. For purposes of clarification the litters will be referred to numerically as identified in Table 1. Litters one and two are indirectly related through the sire of litter two. This boar belongs to the bloodlines in which the incidence of sudden death occurred. Litters three and four are directly related to each other (same sire and dam) and are related to litter two through the dam. They are not, however, related to the boar in question.

All adult pigs were maintained on a commercial diet. A commercial creep feed was introduced at approximately 14 d of age. Both diets (Table 2) are formulated to meet NRC requirements (NRC, 1988) for domestic swine breeds.

One sow (litter number four) had a dry skin condition necessitating daily supplementation with 7 cc EFA-Z Plus (Table 3). This treatment was initiated 129 d prior to farrowing, and resulted in a supplementation of 14 IU vitamin E daily. Despite this confounding variable, data from this litter were also collected and analyzed.

Table 1: Identification of litters.

Litter*	DOB	Location of Birth
1[a]	23 July 1989	St. Louis Zoo
2[ac]	5 June 1990	Zoo Atlanta
3[bc]	12 June 1989	Zoo Atlanta
4[bc]**	21 August 1990	Zoo Atlanta

* Litters with similar subscripts are related.
** Sow was supplemented.

Neonatal exams were conducted on all piglets within 48 hrs of birth. All piglets were weighed, tagged, had the naval dipped with iodine solution, were given 0.5 cc iron dextran, IM, and had milk teeth clipped. Also, blood (1 cc) was drawn from all piglets. During the exam, each litter was divided into a control and a treatment group; treatment group individuals received 0.25 cc Bo-Se, IM, (Table 4) suppling 14 IU vitamin E activity and .25 mg Se as sodium selenite.

Table 2: Analysis of Commercial Feeds (IU/kg DM)*

	Complete Sow** Chow III	Purina** Startena
Vitamin E (Acetate)	11.22	15.31
Vitamin E (α-tocopherol)	3.37	5.31
Total Vitamin E	14.59	20.6

* Animal Health Diagnostic Lab, Nutrition Section, College of Veterinary Medicine, Michigan State University, P.O. Box 30076, Lansing, MI 48909-7576
** Purina Mills, St. Louis, MO 63116

All piglets had blood drawn (1 cc) once weekly until four weeks of age. Piglets were then bled once every two weeks until weaning. The procedure was immediately discontinued if a piglet was distressed or exhibited significant discomfort. Serum samples were stored at -70 C for no longer than one year prior to shipment. Samples were shipped on dry ice to the Nutrition Laboratory at the Animal Health Center, New York Zoological Society.

Table 3: EFA-Z Plus*

7cc provides:	
Linoleic acid (as glycerides)	3.4 gm
Gamma-Lin	10.0 mg
Eicosapentanoic acid	50.0 mg
Docosahexaenoic acid	33.0 mg
Vitamin A	850 IU
Vitamin E	14 IU
Zinc	15.0 mg
Biotin	740.0 mcg
Inositol	2.5 mg

* Distributed by: Veterinary Specialties for Dermatology. Manfactured for: Allerderm, Inc., a subsidiary of Vibac, Inc., Ft. Worth, TX 76118

Table 4: Bo-Se^{R}* (selenium, Vitamin E)

Concentration/ ml.	
Sodium selenite	2.19 mg
Vitamin E**	50 mg (68 IU)
polysorbate 80	250 mg
benzyl alcohol	2%
water for injection q.s.	
sodium hydroxide and/or hydrochloric acid may aed to adjust pH.	

* Distributed in 1985 by: Schering Corp., USA, Kenilworth, N.J. 07033.
** as d-αtocopheryl acetate.

Table 5: Statistical observations.

Litter	1	2	3	4
Litter size	5	9	6	8
Trt group size	3	5	3	3
Non-trt group size	2	4	3	3
Weeks sampled	7	6	5	4
Observations/ litter:				
Trt group	17	29	13	14
Non-trt group size	16	18	10	12
Total observations	33	47	23	26

Samples were extracted following modified methods of Storer (1974). Two hundred µl of plasma were mixed with 200 µl methanol containing 0.2% BHT to precipitate proteins; 400 µl hexane containing 0.2% BHT were added, and a 200 µl aliquot of the hexane layer was evaporated under N_2 and reconstituted with methanol containing 0.025% BHT.

Alpha-tocopherol levels were measured by high performance liquid chromatography using a Series 400 Liquid Chromatograph (Perkin-Elmer, Inc., Norwalk, CT) equipped with a 30 cm C18 reversed-phase column. HPLC-grade methanol and water (98:2 v/v) were used as the mobil phase. The flow rate was 2.0 ml/min, and recovery exceeded 95%. Peaks were monitored by fluorescence detection (Model LS-1 detector; Perkin-Elmer, Inc., Norwalk, CT) with the excitation wavelength set at 280 nm and the emission >310 nm.

Data were analyzed either by performing t-test, or by using a two-way ANOVA. A two-way ANOVA was used to assess the interaction between piglet blood levels, vitamin E and oral supplementation of the sow. To minimize Type I error risk in these analyses, the standard α level was adjusted (new α level = .008).

Results and Discussion

When comparing treated and non-treated piglets, without the influence of the supplement x treatment interaction (litter four), the overall mean for blood levels of vitamin E was greater ($P<.05$; means = 1.98 and 1.59 µg/ml respectively) in treated piglets indicating a positive effect due to treatment alone (Figure 4).

When the influence of the supplement x treatment interaction is included (Figure 1), the overall mean differences of blood level vitamin E were also significant ($P<.005$; means = 2.17 µg/ml treated and 1.68 µg/ml un-treated).

In neither of the above comparisons was this difference significant when each week was viewed individually.

Upon comparing litter four to litters one, two,

and three combined there was significant interaction ($P<.05$) between supplementation of the sow and treatment of the piglets (Figure 3).

The mean of the blood levels of non-treated piglets of litter four was less than the mean of non-treated piglets of the other litters (means = 2.0 [SE=0.22] and 1.59 [SE=0.10] μg/ml). The mean of the blood levels of the treated piglets of litter four was greater than the mean of treated piglets of the other litters (means = 3.13 [SE=0.77] and 1.98 [SE=0.29] μg/ml).

Analysis indicated no effect on blood levels of vitamin E due to litters being related. Mean differences (Figure 2) between litters three and four were significant ($P<.01$); mean differences between litters one and two were not. Blood levels for litter three were not significantly different from litters one and two combined, but blood levels for litter four were ($P<.01$). This difference appears to be due to supplementation of the sow and not to relatedness of litters.

Percent weight gain on a weekly basis was non-significant between treatment and non-treatment groups.

Initial neonatal blood samples were drawn before treatment was administered. To determine if supple-mentation of the sow of litter four affected vitamin E blood levels of her piglets at birth, levels of vitamin E in the first samples were compared between all litters. No significant difference was found (.881 μg/ml and .669 μg/ml).

The current study indicates injection of vitamin E to neonatal piglets does significantly increase overall blood levels through six weeks of age. The blood levels decreased weekly, except at week four, throughout the six week period (figures 1 and 4). If this decrease continues, the effect of the neonatal injection would be undetectable soon after six weeks of age. However, weaning, shipping and other procedures which require handling the piglet are likely events during the first six weeks of life. All of these procedures cause stress, which increases vitamin E requirement (Maynard et. al. 1979).

Blood levels of vitamin E in piglets of domestic breeds of swine normally are 0.3 μg/ml (Chavez and Patton, 1986). Treated piglets in this study had a mean blood level of 2.05 μg/ml and un-treated piglets had a mean blood level of 1.59 μg/ml. The mean vitamin blood level of the untreated pigs in this study was above that of domestic swine. However, the vitamin E blood levels of the litter mates to the pigs which died at the St. Louis Zoo (Junge and Miller 1989) were also above levels accepted for other domestic swine. During this study period, no mortality due to vitamin E related causes was detected.

The most curious results of this study come from the unintentional supplementation of the sow of litter four with vitamin E. The oral supplementation interacted with treatment of the piglets ($P<.05$). There is little transfer of α-tocopherol across the placenta in swine, however, dietary levels fed to the sow do affect the levels found in colostrum and milk (Pharazyn et. al. 1990). In this study, there was no significant increase in the vitamin E blood levels of litter four at birth indicating the orally supplemented vitamin E did not cross the placenta. This study does, however, indicate that blood levels of treated piglets were enhanced when the sow was orally supplemented. The processes of the supplement x treatment interaction observed here, are not within the parameters of this study. Vitamin E is stored in various body tissues and circulating blood levels do not adequately reflect this (Maynard et. al. 1979). Neither institution has bred pot-bellied pigs since litter four was farrowed,

EFFECT OF TREATMENT
All Litters Combined

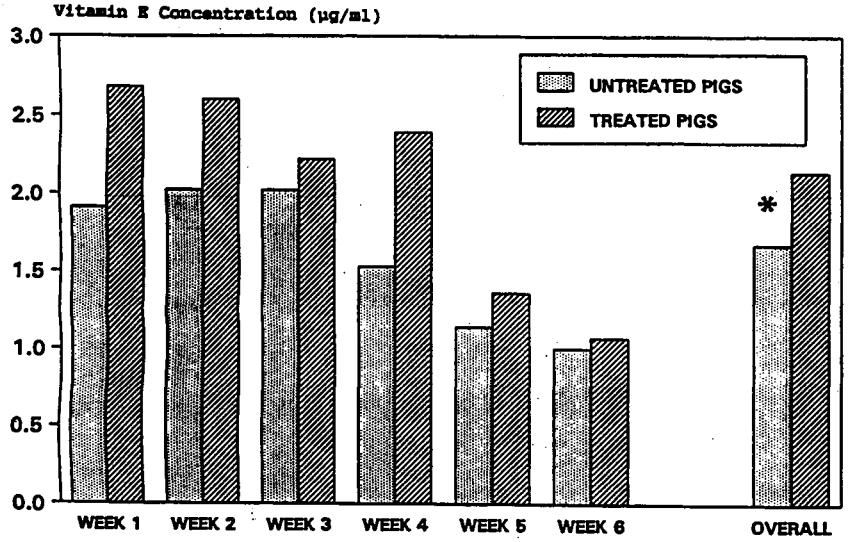

Figure 1

* $p < .005$

Interaction between Supplementation and Treatment

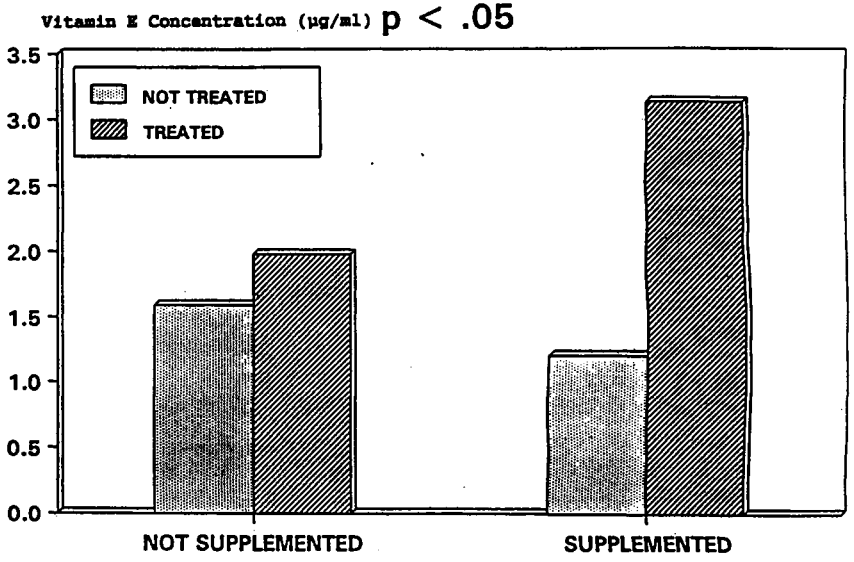

Figure 3

EFFECT OF RELATEDNESS OF LITTERS

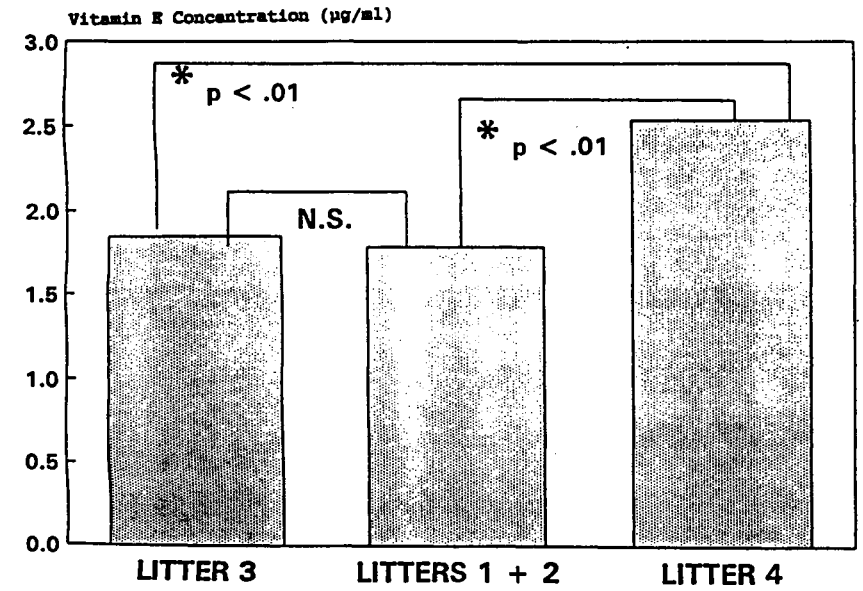

Figure 2

EFFECT OF TREATMENT
Litter 4 Excluded

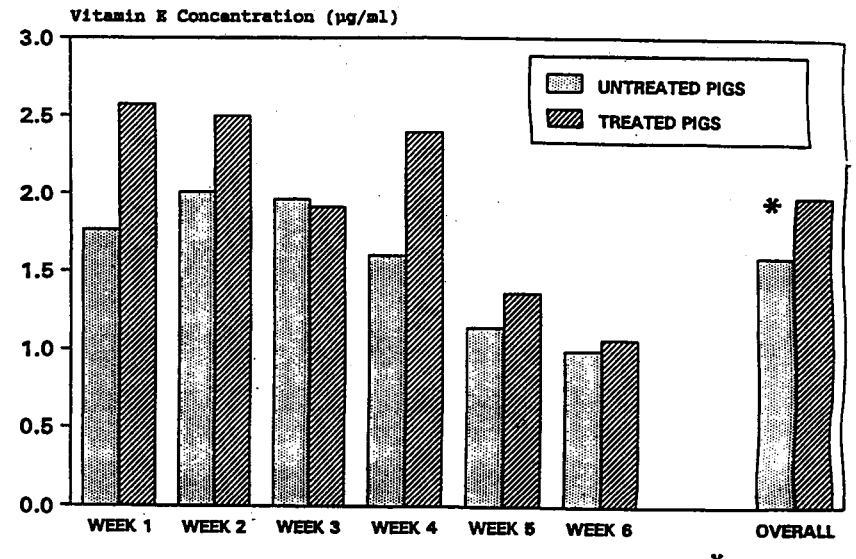

Figure 4

* $p < .05$

therefore these findings could not be verified by replicating oral supplementation of a s ow of other litters.

Conclusions

This study does show a positive effect on vitamin E blood levels through six weeks of age from neonatal administration of Bo-Se. However, since no mortality due to vitamin E related causes was observed in these particular litters, and mean blood values (even in untreated piglets) were higher than domestic piglet levels, this study did not demonstrate an actual need for supplementation. Since stressful procedures are likely events during the first six weeks of life, neonatal administration of vitamin E could be advantageous and is unlikely to be harmful. The dose used in this study was 14 IU vitamin E, and also included .25 mg Se as sodium selenite.

Since blood samples were only taken through six weeks, the study is not designed to show effect from neonatal administration past this age. The decreasing levels of vitamin E through the six week period, however, indicate declining effects of a single injection. Neonatal administration may be inadequate for stressful events which occur after six weeks of age.

The study did show an effect on vitamin E blood levels due to oral supplementation of the sow prior to farrowing - supplementation enhanced treatment effects. The processes for the supplementation x treatment interaction were not in the parameters of this study and warrant further investigation.

Treatment had no effect on mortality rate of piglets or percent weight gain during the course of this study.

The study indicated no correlation between bloodlines of pigs at or from St. Louis Zoological Park and blood concentration of vitamin E.

Acknowledgments

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DIETARY HUSBANDRY OF PSITTACINES HOUSED IN A COMMERCIAL AVIARY

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A dietary program initiated in July 1989 was tested for its ability to support maintenance and reproduction in adult psittacines at a commercial aviary in southern Oregon. The species studied were African gray parrots (*Psittacus erithacus*), yellow-naped Amazon parrots (*Amazona ochracephala*), double yellow-headed Amazon parrots (*Amazona ochracephala oratix)*, blue-fronted Amazon parrots (*Amazona aestiva aestiva*), blue and gold macaws (*Ara ararauna*), green-winged macaws (*Ara choloptera*), military macaws (*Ara militaris*), scarlet macaws (*Ara macao*), medium sulfur-crested cockatoos (*Cacatua galerita*), salmon-crested or Moluccan cockatoos (*Cacatua moluccenis*), white or umbrella cockatoos (*Cacatua alba*), sun conures (*Aratinga solstitialis*), and golden or Queen of Bavaria conures (*Aratinga guarouba*). Data were also gathered on hybrids (Catalina Macaws) of blue and gold and scarlet macaws.

All birds were housed in outdoor cages year round except African gray parrots which were housed in cages inside an unheated barn. The outdoor cages were located on the south slope of a small mountain (elevation 230 m [750 ft]) with the screened fronts facing east. Cages housing pairs were equipped with a nest box. Average annual rainfall was 89 cm (35 in), and average annual temperature was 18°C (65°F). Winter daytime temperatures tended to be near 0°C (32°F), while summer daytime temperatures generally ranged from 24°C (75°f) to 29°C (85°F).

Feed intakes were quantitatively determined at two separate time periods during two different seasons. The first collections occurred for two 24-hr periods between December 19 and 21, 1990 (Winter), while the second collection period occurred for three 24-hr periods between September 9 and 12, 1991 (Fall). During both periods, adult birds were offered a nutritionally-complete extruded diet (Table 1) in the morning between 8:00 and 10:00 a.m. and again in the afternoon between 3:00 and 4:00 p.m. During the first study period, apples, oranges, carrots, winter squash, broccoli, and sweet potatoes were offered in the morning while apples, oranges, broccoli, carrots, green peppers and honey dew melon were offered in the second study period. Uneaten fruits and vegetables were removed in the afternoon. Water was offered in ceramic crocks, and during freezing weather, which occurred in the first study period, the ice was broken three times per day.

Uneaten food items were weighed and removed. Recovery of uneaten items was accomplished by using plastic sheeting under and in front of the cages. Desiccation of the fruit and vegetables was not a problem in the first study since relative humidities were high and ambient

temperatures ranged from -7°C (20°F) to 0°C (32°F). In the second study period, representative food items were placed in similar environments to estimate moisture loss or gain which may have occurred. Mean daily dry matter intakes per bird are shown in Table 2 for both time periods. Using average body weights published by Flammer (1986), daily dry matter intakes ranged from 7% to 14%, and 7% to 18%, of body weight for Winter and Fall, respectively. Changes in dry matter intakes within species were not consistent between Winter and Fall; however, dry matter intakes across genus remained relatively constant. The percentages of dietary dry matter derived from each item are shown in Table 3 for Winter and Table 4 for Fall. The extruded diet accounted for 76% to 94% and 69% to 87% of the total for Winter and Fall, respectively. Intakes of individual fruit and vegetable items varied with species and birds, but the overall percentage of fruits and vegetables increased from Winter to Fall. In both periods, apples were most consistently consumed, providing 3.2 to 6.5% and 7.0 to 16.2% of dietary dry matter, respectively, for Winter and Fall. Table 5 presents the amount of each food item consumed as a percentage of the amount of the item that was offered. This table illustrates the birds preferences among food items, with all species consuming a mean of 93% of the apples offered. Nutrient intake ranges, expressed as concentrations in dietary dry matter, are shown in Table 6 for Winter and Fall, respectively. All nutrients were consumed in amounts sufficient to meet the needs of precocial birds (N.R.C. 1984) and presumably were adequate for psittacines as well. Although, nutrient concentrations were lower for Fall as

Table 1. Nutrient specifications (dry basis) for a complete extruded psittacine diet.*

Nutrient	Concentration	Nutrient	Concentration
Protein (%)	24	Zinc (mg/kg)	120
Arginine (%)	1.30	Manganese (mg/kg)	65
Isoleucine (%)	1.10	Iodine (mg/kg)	1
Lysine (%)	1.20	Selenium (mg/kg)	0.3
Methionine (%)	0.50	Vitamin A (IU/kg)	8000
Methionine + cystine (%)	0.90	Vitamin D_3 (IU/kg)	1900
Threonine (%)	0.95	Vitamin E (IU/kg)	250
Tryptophan (%)	0.24	Vitamin K (IU/kg)	4
Linoleic acid (%)	2	Thiamin (mg/kg)	6
Calcium (%)	1.10	Riboflavin (mg/kg)	6
Phosphorus (%)	0.80	Pantothenic acid (mg/kg)	20
Potassium (%)	0.70	Niacin (mg/kg)	55
Sodium (%)	0.20	Pyridoxine (mg/kg)	6
Chlorine (%)	0.20	Choline (mg/kg)	1700
Magnesium (%)	0.15	Folacin (µg/kg)	900
Iron (mg.kg)	150	Biotin (µg/kg)	300
Copper (mg/kg)	20	Vitamin B_{12} (µg/kg)	25

*May be fed with fruits and vegetables but should constitute at least 40% of the diet by weight as fed (wet basis) or at least 80% of the diet dry weight. Marion Zoological, Inc., P.O. Box 212, Marion, KS 66861, USA.

Table 2. Daily dietary dry matter intakes in grams or as a percentage of body weight (Winter:December 19-21, 1990; Fall:September 9-12, 1991).

		Daily dry matter consumption			
		Winter		Fall	
Species (No. of pairs, singles)[a]	Avg. BW(g)[b]	g	% of BW	g	% of BW
African gray parrots (14,0)[c]	554	49	8.8	40	7.2
Yellow-naped Amazon parrots (3,2)[d]	596	42	7.0	40	6.7
Double yellow-headed Amazon parrots (3,0)	568	40	7.0	49	8.6
Blue-fronted Amazon parrots[e]	432	-	-	40	9.3
Blue and gold macaws (10,0)[f]	1039	84	8.1	69	6.6
Green-winged macaws (3,0)	1194	83	7.0	117	9.8
Military macaws (2,0)	925	84	9.1	78	8.4
Scarlet macaws (10,0)	1001	78	7.8	86	8.6
Hybrid macaws (2,0)[g]	1078	116	10.7	112	10.4
Medium sulfur-crested cockatoos (2,0)	465	53	11.4	55	11.8
Salmon-crested cockatoos (1,0)	853	63	7.4	77	9.0
White cockatoos (1,0)	577	54	9.4	39	6.8
Sun conures (1,1)[h]	-	41	-	-	-
Golden conures (0,1)	262	37	14.1	47	17.9

[a] Animal numbers in parentheses refer to number of animals during winter collection period, December 19-21, 1990.
[b] Average body weights from Flammer (1986)
[c] Two pairs and a single African gray parrot were removed from the breeding colony prior to fall collection period.
[d] One female died after the winter collection period.
[e] One pair was added to the breeding colony after the fall collection.
[f] Four pairs were removed from the breeding colony after the winter collection period.
[g] One female was removed from the breeding colony after the winter collection period.
[h] One female died and the male was removed from the breeding colony after the winter collection period.

Table 3. Percentages of dietary dry matter from items consumed during winter.

	Percent of dry matter from -						
Species	Extruded diet	Apples	Oranges	Carrots	Winter squash	Broccoli	Sweet potatoes
African gray parrots	93.6	3.0	1.4	0.2	0.7	0.4	0.6
Yellow-naped Amazon parrots	90.3	6.1	1.2	0.8	0.0	0.8	0.7
Double yellow-headed Amazon parrots	83.4	7.2	3.0	0.6	0.0	0.5	5.3
Blue and gold macaws	93.0	3.2	1.7	0.4	0.3	0.3	1.1
Green-winged macaws	91.1	4.1	1.8	1.3	0.4	0.7	0.7
Military macaws	93.9	4.6	1.6	0.0	0.0	0.0	0.0
Scarlet macaws	93.5	4.1	1.4	0.4	0.4	0.1	0.0
Hybrid macaws	94.3	3.2	1.1	0.3	0.4	0.2	0.5
Medium sulfur-crested cockatoos	89.0	4.5	3.2	0.2	1.7	0.2	1.2
Salmon-crested cockatoos	89.2	5.1	2.5	0.2	1.2	0.3	1.4
White cockatoos	80.4	5.9	2.4	0.6	2.9	1.0	6.8
Sun conures	90.9	5.9	2.9	0.3	0.0	0.0	0.0
Golden conures	76.1	6.5	5.0	1.3	1.2	0.0	9.9

Table 4. Percentages of dietary dry matter from items consumed during fall.

	Percent of dry matter from -						
Species	**Extruded diet**	**Apples**	**Oranges**	**Carrots**	**Green pepper**	**Broccoli**	**Honey dew melon**
African gray parrots	83.6	9.3	2.3	2.4	0.5	1.6	1.6
Yellow-naped Amazon parrots	68.8	16.2	6.7	4.5	0.8	1.4	1.6
Double yellow-headed Amazon parrots	80.4	8.2	3.0	3.6	0.8	2.5	1.5
Blue and gold macaws	77.6	7.3	3.3	4.7	0.8	5.5	0.8
Green-winged macaws	84.2	9.5	2.5	1.5	0.3	1.3	0.7
Military macaws	86.4	7.0	3.1	1.1	0.2	1.4	0.8
Scarlet macaws	85.9	8.2	2.2	1.5	0.2	1.2	0.8
Hybrid macaws	87.1	7.9	2.0	1.2	0.2	0.6	1.0
Medium sulfur-crested cockatoos	81.1	8.6	2.7	2.9	0.5	3.4	0.8
Salmon-crested cockatoos	80.5	10.5	2.6	3.6	0.2	2.0	0.6
White cockatoos	75.4	14.3	3.1	2.9	0.4	2.4	1.5
Sun conures	76.2	11.8	2.4	3.6	0.5	4.4	1.1
Golden conures	81.6	7.2	5.9	1.5	0.4	1.4	2.0

Table 5. Consumption of feed items expressed as a percent of feed items offered during fall.

Species	**Extruded diet**	**Apples**	**Oranges**	**Carrots**	**Green pepper**	**Broccoli**	**Honey dew melon**
African gray parrots	43	94	51	48	32	28	75
Yellow-naped Amazon parrots	40	93	68	47	48	32	54
Double yellow-headed Amazon parrots	73	96	75	84	100	73	100
Blue and gold macaws	60	92	60	80	66	95	55
Green-winged macaws	49	95	57	38	32	30	61
Military macaws	84	96	79	47	47	53	74
Scarlet macaws	56	93	55	52	32	33	67
Hybrid macaws	64	93	54	43	42	22	97
Medium sulfur-crested cockatoos	51	92	53	72	69	65	70
Salmon-crested cockatoos	74	98	65	91	53	65	51
White cockatoos	52	87	56	67	56	48	67
Sun conures	46	93	72	60	77	52	55
Golden conures	60	96	100	51	79	54	90

Table 6. Range of nutrient concentrations in extruded diet, fruits and vegetables consumed.

	Concentrations in dietary DM	
Nutrient	**Winter**	**Fall**
Dry matter (%)	46.3 - 70.6	32.8 - 52.9
Crude protein (%)	20.1 - 23.7	18.9 - 22.4
Ether extract (%)	4.9 - 5.7	4.7 - 5.4
Crude fiber (%)	5.0 - 5.1	5.2 - 5.8
Ash (%)	5.8 - 6.3	5.6 - 6.3
Linoleic acid (%)	2.2 - 2.7	2.1 - 2.5
Lysine (%)	1.0 - 1.1	0.9 - 1.1
Methionine (%)	0.4 - 0.5	0.4 - 0.5
Methionine + cystine (%)	0.7 - 0.9	0.7 - 0.8
Tryptophan (%)	0.19 - 0.23	0.18 - 0.22
Calcium (%)	0.89 - 1.07	0.83 - 1.00
Phosphorous (%)	0.64 - 0.77	0.60 - 0.72
Sodium (%)	0.22 - 0.26	0.21 - 0.24
Potassium (%)	0.75 - 0.83	0.81 - 1.04
Magnesium (%)	0.15 - 0.16	0.14 - 0.15
Iron (ppm)	136 - 162	125 - 151
Copper (ppm)	19 - 23	19 - 26
Zinc (ppm)	93 - 115	83 - 106
Manganese (ppm)	53 - 64	48 - 60
Iodine (ppm)	0.9 - 1.1	0.8 - 1.0
Selenium (ppm)	0.35 - 0.44	0.32 - 0.40
Retinol (IU/kg)	6700 - 8300	6054 - 7654
Carotene (ppm)	13 - 22	20 - 45
Vitamin D_3 (IU/kg)	1500 - 1900	1355 - 1713
Vitamin E (IU/kg)	220 - 270	207 - 253

compared to Winter, the values remained relatively close between periods for the majority of the species. The lowest nutrient concentrations, with the exception of crude fiber, were consumed by Yellow Naped Amazons. The lower nutrient concentrations are a reflection of the increased fruit and vegetable consumption with a concomitant decrease in consumption of extruded diet (90% to 69%, for Winter and Fall, respectively). Selected nutrient concentrations in dietary dry matter for Winter and Fall, respectively, were: crude protein, 23% and 19%; lysine, 1.1% and 0.9%; calcium, 1.0% and 0.8%. Although the lower levels may have no affect on birds in a maintenance state, nutrient dilutions such as these may be adverse for parents rearing young.

Table 7. Reproduction records for proven breeding pairs in 1991 (to September 1)[a].

		Number of eggs				Chicks	
Species	**# of pairs**	**Laid**	**Fertile**	**Broken**	**Hatched**	**Died**	**Weaned**
African gray parrots	5	31	25	0	25	0	25
Yellow-naped Amazon parrots	2	7	5	2	4	0	4
Blue and gold macaws	7	40	19	0	39	0	39
Green-winged macaws	2	9	7	0	7	1	6
Military macaws	2	5	3	2	3	0	3
Scarlet macaws	3	7	0	0	0	0	0
Hybrid macaws	2	8	7	0	7	0	7
Medium sulfur-crested cockatoos	2	0	0	0	0	0	0
Salmon-crested cockatoos	1	0	0	0	0	0	0
White cockatoos	1	0	0	0	0	0	0

[a] Between September and December 1991, 2 pairs of blue and gold macaws laid 5 fertile eggs; however, at the time this manuscript was prepared the chicks were too young to hatch. Also, three pairs of African gray parrots laid an additional 11 eggs, of which 7 have hatched while the others were too young for hatching. Finally, a pair of military macaws laid 2 fertile eggs from which weaned chicks were reproduced.

At the time of preparing this manuscript (December 10,1991), the 1991 breeding season was not complete, but reproductive records for 1991 to this date are shown in Table 7. One hundred and seven eggs had been produced by proven pairs of six psittacine species and one hybrid pair. Of these 86 were fertile, 18 were infertile and 3 were broken prior to knowledge of their fertility. The eggs were artificially incubated from day 1 and were candled at day 4-5.

After hatching, all birds were hand reared on a finely ground extruded diet with nutrient specifications similar to those given in Table 1. Warm water was added to produce a slurry with a water to dry matter ratio of about 3:1. The newly-hatched birds were fed by syringe, starting no later than 6 hr post-hatching, after the first elimination of waste, which usually occurred at 4-6 hr. They were fed every 2 hr until midnight. Feeding resumed on a 2-hr schedule at 6 a.m. Gradually, the water-dry matter ratio was reduced and the time between feedings was increased. By 4-6 wk the young birds showed interest in solid food. The first solid food offered was a warmed mixture of diced vegetables and moistened morsels of the complete extruded diet. Finally, unwarmed diced fruits and vegetables and dry extruded diet were offered. When consumption was judged satisfactory,

the birds were weaned from the hand-fed slurry. Of the 86 fertile eggs produced thus far in 1991, one egg was broken (1%), one chick died (1%) and 84 birds were hatched (98%) and successfully reared to weaning.

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GROWTH, FEED CONSUMPTION AND FEED CONVERSION OF THE COMMON SNAPPING TURTLE ON TWO DIETS

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Abstract

Common snapping turtle (*Chelydra serpentina*) hatchlings were cultured over a 12 month period using 32% protein catfish and 45% protein alligator feeds. Feed conversion for turtles maintained on the catfish diet ranged from 1.5-2.1 over 12 months. The percent body weight of feed consumed daily was 2% in the second month and 0.76%, 0.49% and 0.53% for months 5,9 and 12, respectively. After 12 months, the average weight of the turtles was 261.0 g and the weights ranged from 68.0 to 441.0 g. Turtles on the alligator diet had a feed conversion ranging from 1.2 to 1.9 over 12 months. Percent body weight of feed consumed daily was 1.9%, 1.0%, 0.72% and 0.63% for months 2,5,9 and 12, respectively. Average weight of the turtles after 12 months was 431.0 g and weights ranged from 98.0 to 825.0 g.

Introduction

The common snapping turtle (*Chelydra serpentina*) is widely distributed throughout North America. In Louisiana, the common snapping turtle is highly desired for its meat which is used in the preparation of turtle soup. The species is not commercially cultured and there is concern that the population may be diminishing.

Although the species is considered to be carnivorous, consumption of vegetation has been observed (Lagler, 1943). Commercial trappers capture the turtles utilizing funnel traps baited with meat or fish. Some culture techniques have been defined utilizing diets consisting largely of fish and chicken parts, but these techniques have not been documented.

The common snapping turtle will breed in captivity if provided the proper environment (Falcon & Culley, personal observation). In general, all that is required is to confine the turtles to a pond and maintain a suitable shoreline for the female to excavate a hole in the soil to deposit eggs.

Some information has been reported on the biology, ecology, growth and breeding of wild populations (White & Murphy, 1973; Punzo, 1975; Christiansen & Burken, 1979; and Williamson, et al., 1989). This information is useful for establishing breeding populations. However, culture techniques have been poorly defined,(i.e., dietary requirements, growth rates and feed conversions, optimum temperature for culture, and natural and controlled reproduction).

This study was designed to obtain some basic information on growth, feed consumption and feed conversion by the common snapping turtle from hatchling through twelve months of age when provided two commercially available diets. The turtles were maintained in a recirculating system utilized for crawfish (*Procambarus spp.*) and channel catfish (*Ictalurus punctatus*) (Malone & Burden, 1988). Performance of the filter system was of interest throughout the study as the composition of the feed utilized in this study varied from feeds used to grow other animals in the system.

Methods

Seventy-three hatchling turtles were obtained from a commercial fisherman in Napoleonville, Louisiana on September 3, 1990. These turtles had hatched from eggs extracted from adult females in May and placed in a wooden box containing 6" (15 cm) of damp soil. The eggs were covered with 2" (5 cm) of soil and maintained at ambient temperatures ranging from approximately 22 to 27°C. Seven embryos died during the incubation period. The eggs hatched during the first week of August at which time the turtles emerged from the soil, but immediately burrowed back into the soil. After one month, the turtles were removed and transported to the School of Forestry, Wildlife, and Fisheries at Louisiana State University, Baton Rouge. The average weight of the turtles was 14.3 g and had a weight range from 9.6-19.4 g. The turtles were individually weighed and randomly placed in six fiberglass culture trays (11/tray) with a floor surface area of 0.62 m^2 (approximately 6.0 ft^2). The six trays were randomly selected to receive the two pelleted feeds containing either 32% or 45% crude protein (Table 1). The 32% protein feed was manufactured by MFC Corporation, Madison, Mississippi and was a standard catfish ration. The 45% protein feed was a standard alligator ration manufactured by Burris Mills, Franklinton, Louisiana. Initially, feed was provided daily (about 3% of body wt./day) to insure an excess of food. The turtles remained in the tanks approximately one month before consistent consumption of food was evident. The first feeding study was initiated October 15, 1990 and ended November 13, 1990. Thereafter, the study was repeated for 30 consecutive days each quarter until the turtles were 12 months of age.

Table 1. Proximate analysis of catfish and alligator feeds provided common snapping turtles.

Ingredients	Catfish feed[1]	Alligator feed[2]
Crude protein	32%	45%
Fat	3%	10%
Fiber	7%	4%

[1] Manufactured by MFC Corp., Madison, MS.
[2] Manufactured by Burris Mills, Franklinton, LA.

Growth and feed utilization

When the turtles were feeding consistently on a daily basis, they were individually weighed to the nearest gram. At the start of the second month, the turtles were initially fed 3% of their body weight per day. The turtles were allowed to feed for thirty minutes and then excess feed removed. The excess feed was dried, weighed and the dry weight subtracted from the quantity of feed initially supplied to determine daily feed consumption.

If all feed was consumed, then the quantity of feed was increased the following day by 10-20%. If the turtles did not consume all of the feed provided, then that amount in grams was reduced by 10-20% the following day. At the end of thirty days, total feed conversion was determined by dividing the total feed consumed by the weight gain of the turtles over the 30-day period. Weight gain was calculated by subtracting the initial weight of the turtles from the weight of the turtles after 30 days. The percent body weight of feed consumed per day was calculated by dividing the average feed consumed per day by the average daily weight. The percent efficiency of food utilization was determined from the weight gain divided by the food consumed times 100.

Recirculating system

Each recirculating system utilized in this study was designed using the criteria developed by Malone and Burden (1988). The system consisted of a fluidized bed of sand and an upflow sand filter. The flow rate through the two filters was approximately 20 gal/minute (75-80 l/min) with 90% of water going through the fluidized bed and 10% of the water going through the upflow sand filter. Both filters contained sands of 8-16 size mesh which were coated with bacteria. The bacteria in the fluidized bed functioned to convert Total Ammonia Nitrogen (TAN) to nitrate which is non-toxic to aquatic animals. The upflow filter functioned to remove solids from the system. The upflow filter was back-flushed as needed to discharge solids. Approximately ten gallons (35-40 l) of water were utilized in the recirculating system for each pound (0.45 kg) of living animal. The water moved through the filters to the culture tanks and was discharged from the culture tanks into a reservoir. A 1/4 h.p. pump transferred the water from the reservoir to the filter completing the circulation. Each filter consisted of a 6" (15 cm) diameter clear acrylic tube approximately 5' (150 cm) in height and containing approximately 1/6 ft^3 of sand. The culture trays received a continuous flow of recirculated and treated water from two identical but separate recirculating systems maintained at 28 ± 1°C. Lighting consisted of a 12L:12D photoperiod using Growlux florescent bulbs. The water quality parameters measured were dissolved oxygen, pH, NH_3, NO_2, and hardness. Throughout the study, the following values were maintained:

D.O. - not less than 6 mg/l
NH_3 - less than 0.5 mg/l
NO_2 - less than 0.2 mg/l
pH - 7.7-8.3
Hardness - approximately 40 mg/l as $CaCO_3$

Results

Table 2 shows average growth and feeding responses over 30 consecutive days of each

Table 2. Average weights, feed conversion, consumption, conversion efficiency and weight range of common snapping turtles from hatching to 12 months of age when fed a catfish feed containing 32% protein.[1]

Month	Initial Weight	Final Weight	Gain	FC[2]	%CE[3]	% Body Weight consumed/ day	Final Weight Range
2	22.5	33.8	11	1.7	59	2	17.9 - 50.7
5	118.8	131.9	21	1.6	68	0.76	43.4 - 265.8
9	151	169.4	17	1.5	69	0.49	48.7 - 308.69
12	238.2	260.9	22	2.1	52	0.53	67.88 - 440.9

[1] Figures are in grams/ turtle with 11 turtles/ tray. Averages are for 3 trays combined.
[2] FC = Feed Conversion
[3] %CE = Conversion Efficiency

quarter over a twelve month period for snapping turtles which were fed a 32% protein catfish diet. The average weight of the turtles at the beginning of month 2 was 22.5 g and weights ranged from 18 to 51 g. Over the 30 day period, there was a 50% weight gain. Average feed consumption was 2.0% of body weight/day. The feed conversion averaged 1.7 for a 59% consumption efficiency (CE).

For months 5, 9 and 12, the percent weight increase declined over time: 18% weight increase in month 5, 11% for month 9 and 9% for month 12. Over the 12 month period, the turtles increased their average weight by 18 times and the largest turtle by 31 times over the initial average weight.

Feed conversion (FC) remained relatively stable for nine months, but in the 12th month, the FC value increased showing a decrease in consumption efficiency (52%). The percent body weight of feed consumed/day declined over the 12 months which explains the decrease in percent weight gain over the year. Because the FC remained stable (through 9 months) and percent feed consumption declined, the percent weight increase declined. With feed consumption declining, the FC value would have to decrease for greater percent gain to occur. In the 12th month, there was a small increase in feed consumption (0.53% of body weight/day) but the FC increased to 2.1 to offset any gain efficiency.

The percent body weight of feed consumed daily is low when compared to other aquatic animals; catfish 3-5% and crawfish 1.5-2.0%. Altering the method of feeding, use of a more water-stable feed, different pellet form, and/or improvements in nutrient composition of the feed could stimulate a greater feed consumption.

There was a large variation in turtle weights. The variability occurred in all three trays. The reasons are unclear at this time. It is evident that growth can be quite rapid as some turtles approached 1 lb (0.45kg) in 12 months.

Table 3 shows growth and feed use patterns for common snapping turtles which were fed a 45% protein alligator feed for 30 consecutive days each quarter over 12 months. During month 2, the turtles increased their weight by 71%. The feed conversion (FC) averaged 1.9 for a 72% consumption efficiency (CE).

Table 3. Average weights, feed conversion, consumption, conversion efficiency and weight range of common snapping turtles from hatching to 12 months of age when fed an alligator feed containing 45% protein.[1]

Month	Initial Weight	Final Weight	Gain	FC[2]	%CE[3]	% Body Weight consumed/ day	Final Weight Range
2	21.1	36.0	14.9	1.9	72	1.9	20.0 - 56.8
5	157.7	197.8	40.1	1.2	83	1.0	30.0 - 437.3
9	151	169.4	16.8	1.5	69	0.49	48.7 - 308.69
12	238.2	260.9	21.6	2.1	52	0.53	67.88 - 440.9

[1] Figures are in grams/ turtle with 11 turtles/ tray. Averages are for 3 trays combined.
[2] FC = Feed Conversion
[3] %CE = Conversion Efficiency

The percent weight increase declined over time: 71% in month 2, 25% in month 5, 17% for

month 9 and 10% for month 12. The average total weight after the 12 months showed a 30 fold increase over initial average weight, with the largest turtle increasing its weight 59 times.

The FC was variable throughout the year with the average ranging from 1.2-1.9. The reasons are unclear but could be the result of several factors; improper nutrition, unstable pellets, inappropriate pellet form or the need to vary feeding regime. The percent body weight of feed consumed/day also declined over time (as with the turtles on the catfish feed) and could be related to any of the above factors.

As with turtles on the catfish feed, there was a wide range of weight differences. The largest turtle weighed 0.83 kg (1.8 lbs). Comparing turtles fed the two diets, several results were evident. Turtles receiving the alligator feed were 65% larger after 12 months than those on the catfish feed. Turtles on the alligator feed consumed 76% more than the turtles on the catfish feed, had a FC of 1.5 compared to 1.7 and had a higher daily feed consumption rate. These differences account for the larger size.

Size distribution of all turtles is given in Table 4. Turtles greater than 400 g after 12 months were considered to show sufficient growth rates to reach marketable size within 18 months, assuming the goal is 1 lb (0.45 kg) of meat/turtle. Of the turtles provided the catfish feed, 9% were considered potentially marketable.

Although the diets are referenced as 32% and 45% crude protein, other components of the two diets also differed. Protein alone was probably not responsible for the growth differences. An optimum diet must be developed.

Table 1. Proximate analysis of catfish and alligator feeds provided common snapping turtles.

Diet[1]	N	≤225 g(%)	226 - 400 g(%)	≥400 g(%)
Catfish feed	33	16 (48)	14 (43)	3 (9)
Alligator feed	30 [2]	5 (16)	11 (37)	14 (47)

[1] Catfish feed 32% protein; alligator feed 45% protein
[2] Three turtles escaped during the twelve month period

Discussion

It was evident that some snapping turtles grow rapidly. It was not determined whether growth differences were due to genetics, density, nutrition, or environmental culture conditions. Although chemical analyses of the turtles were not made at the end of the study, there was a visible difference in the two groups. Fat storage was very prominent on the posterior side of the back legs with turtles provided the alligator feed. This was not evident on turtles maintained on the catfish feed. This fat deposit was also conspicuous on wild females collected during the period of egg deposition.

Performance of the filter was as expected. Based upon work by Malone and Burden (1988), the filter system utilized was designed to handle approximately 150 lbs of crawfish when provided a 30% protein feed and fed at 1.5% of body wt./day. The turtles were fed an average of 0.75% body wt./day with the combined diets containing an average of 40% protein (32% and 45% protein with 75% of the feed provided being 45% protein). The 1/3 ft^3 (fluidized bed and upflow filters combined) of bacteria-coated sand converted the TAN load to nitrate until the turtle biomass approached 25 lbs (11.3 kg). When the bacterial coating on the sand grains thickened, this indicated that the system was overloaded. When all the sand became heavily coated with bacteria, the sand in the column would

either form a gel, or the particles would be flushed out of the filter due to a decrease in specific gravity and the high flow rate. Thereafter, TAN began to increase.

To properly design the filter, data on TAN excretion rates must be determined for the turtles. Based on the initiation of bacterial expansion in the upflow and fluidized filters, the 1/3 ft^3 of sand would handle the waste of about 20 lbs of turtle, or 60 lbs of snapping turtle biomass/ft^3 of sand. The back flush cycle had to be increased from one time/day when the study was initiated to 4 times/day at the end of the study.

The yield of turtle meat, bone in, ranged from 40-45% and 35-40% with bone extracted. These figures were based on 4 marketable-size wild turtles collected in May 1990. A 2.5 lb turtle should yield about one pound of meat. Based on growth rates for the turtles maintained on the alligator feed, most of the turtles would yield 1 lb (0.45 kg) of meat in 12-18 months. Improvement of culture techniques could reduce the culture time and favor economics of production.

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OF HOATZINS AND HORNBILLS: DUPLICATING NATURAL DIETS

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Introduction

Specific nutrient requirements of many avian species are unknown; nonetheless, we attempt to meet dietary needs by combining natural history information with captive management records and domestic animal comparisons. Chemical composition, as well as feeding behavior and environmental data, are essential for providing optimal diets. This paper focuses on two avian feeding extremes, using the specialized folivorous hoatzin and more generalist hornbills as examples of applying field and laboratory data to managed feeding programs.

Hoatzins (*Opisthocomus hoazin*): History in Captivity

Although specialized gut morphology (Böker, 1929; Gadow, 1891) and feeding habits (Beebe, 1909; Young, 1888) of the hoatzin were described over a century ago, early attempts to maintain this species in captivity were largely unsuccessful, likely due to improper dietary management. Young birds captured in British Guiana were fed a blenderized vegetable mixture with a syringe, but all gradually sickened and died; authors speculated on the lack of a vital dietary component provided only by adults (Grimmer, 1962). Of three adults shipped to the National Zoo, only a single bird survived in captivity for almost 6 months on a diet consisting of fresh lettuce, kale and spinach (Grimmer, 1962). Hoatzins collected during a later expedition (Bell and Dolensek, 1978) were initially fed a mixture of laying mash and powdered supplements (vitamins A & D, calcium) mixed with banana and beans, along with available green produce and fresh forages. Greens consumption gradually decreased as birds became lethargic, and oral antibiotics were administered. Twelve birds were received in New York for quarantine, but none survived more than 30 days. These early attempts, although failed, provided significant experiences for subsequent efforts which have led to the successful adaptation of hoatzins to captive diets.

Recent studies on its nutritional ecology and digestive physiology (Grajal et al., 1989; Grajal, 1991), and behavior and socio-ecology (Strahl, 1985) have contributed greatly to our knowledge of husbandry requirements for this species. Their unique digestive system and specialized diet make the hoatzin a special challenge.

Digestive Physiology & Feeding Ecology

It is quite possible that nutrient requirements of the hoatzin should be considered more similar to those of ruminants than poultry species. Hoatzins are obligate folivores (leaf eaters) and the only known birds with a well developed foregut fermentation system (Grajal et al. 1989). The relative capacity of the crop and esophagus is very high, with wet contents weighing up to 10% of the total adult body mass. Volatile fatty acid (VFA) concentrations (114.5 and 94.7 mM in crop and cecum, respectively) and pH measurements (6.3 - 7.5) are similar to those of other species with foregut

fermentation (Grajal et al, 1989). Bacteria (Grajal et al., 1989) and protozoan species (Dominguez and Michelangeli, pers. comm.) have been identified from crop contents, and a lysozyme that degrades bacterial cell walls has been identified from the stomach of hoatzins (Keanegay, pers. comm.). Each of these specializations is shared with numerous other foregut-fermenting species (Van Soest, 1982).

Anatomically, the crop of the hoatzin displays ridges with keratinized tissue, providing a mechanism for reducing feed particle size which is independent of the gizzard utilized by most other birds. Mean particle size decreased from 467 to 280μ in digesta sampled from the crop and posterior esophagus, respectively (Grajal, 1991). Turnover of gut contents is relatively slow in the hoatzin compared with other birds; liquid markers were excreted faster (mean retention 18 hr) than particles (24 to 44 hrs). Selective retention of larger particles in the crop allows longer time for microbial and physical breakdown of plant cell wall constituents. At the same time, the relatively faster liquid passage decreases microbial fermentation of the readily digestible cell contents, and makes these substrates available to the lower gut for digestion and absorption. Thus the hoatzin appears to be maximizing utilization of both components of its folivorous diet (Grajal, 1991).

In three separate trials, feed intake averaged about 40 g dry matter (DM) per bird per day, equivalent to a DM intake of 6.3% of body mass (Grajal, 1991). Relative dry matter intake levels in the hoatzin are thus similar to those reported for other herbivorous (but non-foregut fermenting) birds such as emus (4.6%; Herd and Dawson, 1984) and grouse (6%; Moss and Trenholm, 1987), yet above those of most ruminants (1.5 to 3.0% body mass; Van Soest, 1982). Due to its specializations, the hoatzin demonstrates fiber digestion capabilities similar to those of ruminants, and substantially greater than measured in most avian species. Dry matter digestibilities range from 70 to 80%, with apparent cell wall digestibility approximately 38% (alfalfa-based fiber source) or 70% (grass-based fiber source) (Grajal, 1991). The wide variation in cell wall digestion is due primarily to the large hemicellulose component present in grasses compared with alfalfa. Hemicellulose can be degraded both microbially and enzymatically (Van Soest, 1982), and may represent an important energy source for the hoatzin.

While current allometric equations propose that no flying endotherm weighing less than approximately 1 kg can rely solely on fiber fermentation to meet its energy needs (Demment and Van Soest, 1985), the hoatzin very effectively utilizes dietary fiber as a primary energy source. Herbivory in this species appears to be associated with a reduced basal rate of metabolism. Measurements have demonstrated a low basal rate of metabolism in the hoatzin -- about 70% of the expected rate for a bird its size (Grajal, 1991). Furthermore, hoatzins reduce time spent in flight, which may further minimize energy expenditure. Thus the ability to exploit a readily available food resource (i.e. leaves) may, in fact, produce physiological constraints.

Groups of 2 to 8 individuals forage regularly throughout the day, with about 3-hr gaps between feeding periods. On moonlit nights, nocturnal feeding is common (Strahl, 1987). Young birds are fed regurgitated leaves, and up to 5 non-breeding helpers may assist in all reproductive activities except egg production.

Wild Diet Composition

Hoatzins are selective feeders. While over 45 species of plants are consumed, new shoots and

leaves are preferentially eaten, comprising 69% of the observed diet. Mature leaves (13%), flowers (10%) and fruits (8%) make up the remainder of wild diets (Grajal, 1991). Samples of eaten portions of leaves are significantly higher in water content, crude protein, and hemicellulose, and lower in total cell wall, cellulose, lignin, and vitamin E than uneaten portions of the same plants (Grajal et al., 1989). Chemical composition of immature compared with mature portions of three preferred species of browse are found in Table 1, along with values for ornamental Ficus spp. leaves.

Table 1. Chemical composition of leaves from selected browse species consumed by hoatzins; birds displayed a preference for immature plant portions.

Species	Water	CP	NDF	ADF	Lig	Ash	Vitamin E
	%			% of dry matter			IU/kg
Enterilobium cyclocarpum							
immature	68.1	23.8	69.4	47.1	23.8	6.6	211.1
mature	53.0	17.2	62.0	46.9	23.3	9.7	605.4
Ficus spp.							
immature	72.5	14.0	53.2	45.7	29.4	9.8	NA
mature	69.1	12.5	55.2	49.0	25.2	14.0	NA
Phtyrhusa orioncensis							
immature	67.3	17.6	54.7	48.3	25.7	9.1	326.9
mature	58.8	15.1	57.3	45.3	21.6	10.4	558.4
Pithecellobium saman							
immature	64.6	24.9	53.2	38.0	15.0	5.1	126.8
mature	62.0	22.0	53.1	39.3	16.3	6.7	780.6

Abbreviations: CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; Lig = Lignin; Vit E = Vitamin E. Analyses were performed on samples as outlined in Robertson (1978); vitamin assays were conducted following extraction and analytical methods of Burton et al. (1985).

Diet Adaptation in the Field

From June 1986 through December of 1988, captive husbandry techniques for maintaining the hoatzin were explored (Strahl and Grajal, unpublished report). Two diets composed of romaine lettuce coated with a powdered mixture of ground alfalfa and ground roasted soybeans (fresh weight ratio 10:1 lettuce:powder), with a multiple vitamin/mineral supplement, were developed as acclimation diets. These high protein, low fiber content diets (Experimental Diets A and B, Table 2) maintained animals in stable condition and helped to overcome the initial stress of confinement. Intake, digestion, and passage trials were conducted on 2 birds in 1986 (Diet A), and 2 birds in 1988 (Diet B).

During 1989, a larger attempt was made to collect and adapt hoatzins to captive diets on-site at private facilities in Venezuela, prior to shipment to the New York Zoological Park for display and

propagation. Attempts were made to trap only adults, as previous preliminary attempts to

Table 2. Chemical composition of diets consumed by captive hoatzins (experimaental diet data from Grajal, 1991).

Species	Water	CP	NDF	ADF	Lig	Ash	Energy
	%	% of dry matter					Kj/g DM
Experimental diets							
A	58.6	25.6	29.2	22.0	6.6	15.1	17.8
B	57.8	28.8	35.7	21.3	7.4	14.3	18.3
C	43.0	18.1	39.0	18.7	4.6	12.3	17.9
Zoo diet offered							Vit. E (IU/kg)
D*	87.3	21.0	18.9	16.3	2.7	11.4	100 - 200

*Diet offered at the New York Zoological Park consists of a mixed green salad comprising ½ (by wet weight) kale and ½ other available produce including romaine, spinach, dandelion, mustard, and collard greens. Ornamental *Ficus spp.* trees are heavily browsed in the exhibit.

Abbreviations as in Table 1.

maintain fledglings by groups (or singly) were unsuccessful. Birds were collected using a wire and mesh trigger net placed over an active nest. Triggering, once adults were on the nest, was accomplished by remote radio. Eggs were removed and replaced with dummies prior to laying the trap; if adults had not returned within one hour, eggs were replaced on the nest and the attempt was aborted. Three chicks collected during this time period were adopted and successfully fed by unrelated adults. It is likely that innoculation of crop microbes occurs with ingesta transfer from adults to chicks; properly preserved frozen crop contents may be a useful supplement for future attempts if young birds are collected.

Newly captured hoatzins were held in an aviary close to their natural habitat. The aviary measured approximately 10m X 4m X 5m (L X W X H), with high and low perches provided at each end. Walls of the flight cage were concrete for the first meter, with one solid wall; remaining walls and top were wire mesh. Protection from rain was provided by sheets of plastic on top of the flight. Twice daily, birds were offered 3 species of preferred browses (see Table 1) along with the intended zoo diet of romaine (or other green leafy produce -- often iceberg lettuce was the only available fresh produce) supplemented with a ground commercial avian pellet, and ground timothy grass hay (to increase fiber content; Experimental Diet C). Chemical composition of all three experimental diets are found in Table 2. A third feeding of supplemented romaine was provided in the middle of the day. Consumption was monitored daily, and birds appeared to prefer lighter (iceberg) rather than darker lettuce. This may mimic natural browse preferences -- immature versus mature -- thus color may be an important palatability factor (along with other undocumented variables such as taste, texture, or moisture content). No water was offered, as hoatzins do not drink regularly in the field.

One major health problem occuring during this adaptation period was clogging of nostrils with the powdered avian pellets, necessitating regular handling and cleaning of the beaks. Unknown vitamin and mineral levels in diets fed to the hoatzins, compared with requirements established for

domestic poultry, prompted the inclusion of the powdered supplement. It has subsequently been discontinued in the diet, with no apparent negative health effects (see Table 3 and discussion below). Contribution of saliva or crop microbes to mineral, protein, and vitamin balance in these birds has not been documented, but is likely substantial as in other foregut-fermenting herbivores (Van Soest, 1982).

Table 3. Fat soluble vitamin concentrations and selected clinical chemistry parameters in captive hoatzin (*Opisthocomus hoazin*) plasma samples.

Component	n	X ± sem
Fat soluble vitamins:		
Retinol (μg/ml) (Vitamin A)	8	1.58 ± 0.10
25, hydroxy-D_3 (ng/ml) (Vitamin D)	1*	1.0 - 1.5
α - tocopherol (μg/ml) (Vitamin E)	8	10.34 ± 1.33
Vitamin K (nmol/l	1*	8.6
Clinical chemistry		
Cholesterol (mg/dl)	3	181.3 ± 47.4
Glucose (mg/dl)	4	299.5 ± 4.3
Calcium (mg/dl)	4	12.6 ± 0.6
Uric acid (mg/dl)	4	17.8 ± 5.2

* Pooled samples from 5 individuals; otherwise samples represent different individuals from a total population of 6 birds (i.e. only retinol + α - tocopherol samples as outlined in Robertson (1978); vitamin assays were conducted following extraction and analytical methods of Burton et al. (1985).

Zoo Husbandry

The birds were acclimated under the above diet for 60-90 days prior to shipment, and six birds were shipped to the New York Zoological Park in October 1989 for on-site quarantine. The 6-mo quarantine was uneventful, and birds have been exhibited in the Aquatic Bird House since February 1990. Hoatzins have been housed in a n enclosure with controlled temperature and humidity. Additional heat radiators have been installed to provide microclimates within the enclosure. Birds are separated from the public by a glass window that was originally covered with a see-through bamboo fence. After months of acclimation to the public, the fence has been partially removed.

The exhibit is planted with *Ficus spp.* trees which are heavily browsed, and birds are fed twice daily a mixed green salad consisting of (per bird) approximately 125 g chopped kale, and 125 g mixed seasonally available greens (mustard, dandelion, romaine, spinach, collards, turnips). A mixture of greens was preferred to minimize potential palatability, quality, or availability problems. Seeds and twigs of preferred browses were obtained for on-site propagation, and fresh browse will be offered when plants are mature enough for sustained harvest.

The diet offered to the hoatzins (Diet D, Table 2) is substantially lower in fiber content than either experimental or natural diets consumed by these birds. Domestic salad greens typically contain < 20% total cell wall, and very little (< 5%) hemicellulose (Van Soest, 1978). While not quantified, the zoo diet is estimated to be 85-90% digestible. However, consumption of Ficus in the exhibit cannot be measured, and likely contributes significantly to fiber intake.

On two occasions, blood samples were obtained for clinical nutrition and chemistry evaluation. Results of analyses are displayed in Table 3. No comparative blood levels have been obtained from free-ranging hoatzins; nonetheless, plasma vitamins A, E, and K concentrations are considered normal to high. The birds are not given a vitamin A supplement, therefore circulating retinol values are likely indicative of normal levels from physiological conversion of ß-carotene in the

diet. The vitamin E level is similar to that of other herbivorous (primarily granivorous) species of zoo avifauna (Dierenfeld and Traber, 1992), and vitamin K (> 8 nmol/l) is considered high compared with a human normal of 1.0 nmol/l. Both the natural dietary source (green plants), and microbial synthesis of vitamin K probably contribute to high circulating levels of this nutrient. The minimum normal level of 25, OH D_3 in mammals (humans, cattle, rats) and chicks is approximately 2 ng/ml (Rambeck, pers. comm.). Thus the 1.0 - 1.5 ng/ml level measured in our hoatzin colony may indicate a marginal to low vitamin D status. No clinical deficiency is indicated, but serum calcium (12.6 mg/dl) is higher than normal avian ranges (8-10 mg/dl; Fowler, 1986). Natural sunlight is available to the birds through glass skylights, and fertile eggs with normal thickness have been produced but were lost from the nest prior to hatching.

Clinical chemistry data (Table 3) includes glucose concentrations within domestic fowl norms (260-360 mg/dl, Fowler, 1986). Uric acid concentrations, however, appear elevated in the hoatzins (3-10 mg/dl norm, Fowler, 1986). Values > 15 mg/dl in other birds may be indicative of starvation, gout, dehydration, or tissue trauma (Fowler, 1986); none of these conditions is evident in the hoatzin colony. Plasma mineral assay of a single pooled sample from 5 birds indicated no obvious abnormalities. Concentrations (µg/ml) were; Ca - 137; Cu - 0.34; Fe - 1.95; Mg - 39.5; Na - 3870; total P - 188; Zn - 1.10.

The hoatzins have molted, built nests, and laid fertile eggs during the past 2 years. Continued monitoring of diet and health parameters will allow improved management of this species. The current diet contains considerably less fiber than natural forages; nonetheless, hoatzins can be adapted to, maintained, and reproduce on captive diets comprising a mixture of readily available green plants. As with other herbivores, dietary transition should be accomplished gradually to allow proper adaptation of crop microbes to new substrates. Identification of, and adjustment for, unique feeding behavior and digestive physiology of this species has proven successful.

Hornbills (*Bucerotidae*)

Hornbills represent a diverse group, comprising 45 extant species (Kemp, 1979). While most hornbills are found in forest habitats, African *Tockus spp.* and ground hornbills (*Bucorvus spp.*) are savanna-dwellers. Most species are omnivorous, although the savanna species tend to be more carnivorous, consuming a wide variety of insect and vertebrate prey items. In zoos, these species are fed meat- or insect-based diets, and display limited breeding success (Seibels, 1988; Turner, 1988; Tramontana and Rider, 1988). Habitats of most carnivorous hornbills are not considered endangered, and immediate conservation priorities are less critical than those of forest-dwelling species (Sheppard, 1988). Consequently, the more carnivorous hornbills will not be discussed in this review.

In recent years, hornbills have been identified as a group in need of good husbandry guidelines and a priority for captive breeding. Diet could be a key factor in developing successful programs. While numerous field observations from natural history accounts (Livingstone, 1857; Moreau and Moreau, 1940, 1941) or ecological studies (Kemp, 1976; Poonswad et. al., 1983, 1986, 1987) exist in the literature, few data exist on nutrient composition of wild diets. Captive diets reflect the perception that hornbills are fruit-preferring omnivores (Turner, 1988). Due to the lack of information, it has not been possible to reflect the actual composition of natural diets, and captive diets may differ substantially among zoos.

Several species of omnivorous medium to large-sized hornbills are the focus of captive propagation programs in North American zoos (Sheppard, 1988). These birds are spectacular exhibit animals, their habitats are seriously threatened or endangered in the wild, and species have only sporadic captive breeding success (Bohmke, 1988). Although wild breeding behaviors and physical nest characteristics have been carefully detailed for several of the southeast Asian species (Poonswad, 1983; Sheppard, 1988), the relationship between diet composition and reproduction warrants further examination. Fruit and non-fruit dietary components vary with species, and the proportion of animal-based foods increases during nesting in all hornbills (Shannon, 1988; Sheppard, 1988). Nutritional contributions of these dietary alterations, however, have not been specifically quantified and may be essential for designing optimal captive diets.

Wild Fruit Composition

Frugivores consume a plant part consisting of various numbers of outer, fleshy layers surrounding one or more seeds. Chemically and texturally, wild fruits tend to be quite dissimilar from more familiar cultivated fruits (Watt and Merrill, 1977) or domesticated tropical species (Morton, 1973). They may be either drier, less sweet, more oily, or chemically or physically protected. While various frugivorous small birds and bats have evolved digestive strategies to exploit watery fruits low in nutrient concentration, including rapid passage or wedging of pulp (Worthington, 1989; Morrison, 1980), some hornbills appear to have co-evolved as seed dispersers of calorically dense fruits. Two examples of predominantly frugivorous hornbills for which field data, including chemical analysis of foods, are detailed include the black and white casqued hornbill (*Bycanistes subcylindricus*; Kalina, 1988) and 2 *Rhyticeros spp.* (Leighton, 1982).

Table 4. Moisture, fat, and protein content of selected wild fruits (n=29) consumed by frugivores in Australia, Africa, and Latin America. [1]

Water	Fat	Crude Protein
%	% of dry matter	
13 spp. > 80% mean = 84%	13 spp. > 20% mean = 41%	6 spp. > 10% mean = 13%
16 spp. < 80% mean 57%	16 spp. < 20% mean = 8%	23 spp. < 10% mean = 6%

[1] Summarized from Leighton, 1982.

The few data that exist relevant to hornbill frugivory are summarized in Tables 4, 5, and 6. Information compiled by Leighton (1982; see Table 4) consists of nutrient values (moisture, fat, and crude protein) from Australian, Latin American, and African fruits in the same family as those eaten by the hornbills he studied in southeast Asia. Slightly more than half of these wild fruits contained < 80% water, substantially drier than typical domestic fruits fed in zoos (90+ % water, Watt and Merrill, 1977). Most of the fruits contained < 10% crude protein, and almost half displayed > 20% lipid (mean = 41%!). Thus while wild fruits were, in general, low in protein compared to avian requirements of 15-20% (NRC, 1984), they were nonetheless rich energy sources due to a high fat content.

Crude protein, condensed tannins, and fat in fruits eaten by black and white casqued hornbills in Africa over a single year are summarized in Table 5. *Tricilia splendida* fruit, which is moderate in protein (9%) and high in fat (32%), was consumed almost to the exclusion of other foods during

the two months prior to breeding (Kalina, 1988), and fruit choices switched significantly once nesting was initiated. Of the volume of foods delivered to the nest, 91% consisted of fruit (figs and *Diospyros abyssinica*) at the onset of breeding; only 9% was of animal (invertebrate) origin. A preferred fig species, *Ficus exasperata*, contained 21% crude protein (Kalina, 1988).

Table 5. Selected chemical constituents of fruits eaten by the black and white casqued hornbill (*Bycanistes subcylindricus*) in Kibale forest, Uganda.[1]

Species	CT	CP	Fat
	% of dry matter		
Blighia unijugata	0	17.5	55.1
Celtis durandii	0.5	28.4	NA
Ficus exasperata	0	21.1	NA
Trichilia spendida	11.9	8.8	31.6
Diospyros abyssinica	NA	NA	6.8
Pseudospondius microcarpa	NA	NA	2.9

[1] Summarized from Kalina, 1988.
Abbreviations: CT = condensed tannins; CP = crude protein; NA = not analyzed.

Table 6. Chemical composition of (Ficus spp. Collected in the Kibale forest, Uganda.[1]

Species	DM	CP	Fat	NDF	WSC
	%	% of dry matter			
F. brachylepis	12.6	10.2	2.8	28.2	15.5
F. natalensis	18.6	6.14	1.6	65.1	5.8
F. exasperata	NA	25.4	6.6	25.4	11.4
F. cyanthistipula	NA	8.2	2.4	33.0	14.3
F. mucuso	NA	4.4	4.5	34.9	23.2
F. urceolaris	NA	13.9	7.6	21.1	22.0

[1] Conklin and Wrangham, in prep.
Abbreviations: DM = dry matter; CP = crude protein; NA = not analyzed; NDF = neutral detergent fiber; WSC = water soluble carbohydrates

Kalina (1988) states that figs are preferred by hornbills during breeding periods due to their high protein value, but her statement must be interpreted with caution. Table 5 lists only one species of fig, yet more than a dozen *Ficus spp.* are available to hornbills in Kibale forest; chemical composition of the 6 most common species is found in Table 6. Variability in protein (4-25%), fat (1.6-7.6%), and water-soluble carbohydrate (6-23%) content among these species collected at the same site is worth noting. While the single species analyzed by Kalina was high in protein, other authors (e.g. Milton et. al., 1980; Leighton, 1982) consider figs as low protein food items. Without detailed field observations (recording the plant species consumed), in combination with specific laboratory analysis, interpretation of nutrient consumption is impossible. Despite these limitations, figs are in fact, relatively high in protein, particularly when compared with domestic fruit substitutes.

Feeding Ecology Relative to Dietary Composition

Both Leighton (1982) and Kalina (1988) report that percentages of animal (either vertebrate or invertebrate prey) items increased in the diets of their study birds during the breeding/nesting season, supporting earlier observations conducted with other hornbill species (Poonswad, 1983; 1987). Thus even the highly frugivorous (80-90% fruit) hornbill species must be considered omnivores. Protein supplied from meat sources during nesting periods may be particularly important for meeting nutrient requirements

of chick growth and female molt. Inclusion of snail shell fragments -- presumably to supply calcium for growth of chicks -- has also been documented during nesting periods (Poonswad, 1983; Kalina, 1988). Most zoos routinely add high protein items to hornbill diets during the nesting season, especially when chicks are present, but requirements have not been quantified.

While field data are limited, they nonetheless suggest that seasonal changes may be an important component of hornbill biology, and provide some nutrient guidelines. The fat content of natural hornbill foods, in particular, may be critical and are not typical of fruits or diets commonly fed to large hornbills in zoos. Prior to 1986, hornbill diets (non-breeding season) fed at the New York Zoological Park contained approximately 5% fat and 16% crude protein. Our current hornbill diets, as offered, contain 10 to 16% fat on a dry basis, and 25 to 36% crude protein (non-breeding compared to breeding periods, respectively). While hornbills have successfully reproduced on diets containing these nutrient levels, they may not be optimal.

Three distinct diets, differing in protein and fat content, are proposed for captive feeding of large, predominantly frugivorous hornbills (Table 7). A maintenance diet containing approximately 15% crude protein, and 15% crude fat is suggested for non-breeding periods. Based on analytical values from field samples, diets during courtship and breeding periods should contain at least 30% fat and approximately 20% crude protein (dry matter basis). Nesting diets, supporting chick growth and female molt, should contain at least 25% crude protein, and an increased calcium concentration. In addition to providing higher levels of nutrients required for molt and reproduction, these diet changes may themselves provide physiological cues needed to stimulate breeding. This is a testable hypothesis, provided accurate records of diet and reproductive success are kept both before and after any nutrient modifications.

Table 7. Suggested seasonal alterations of selected dietary nutrients for captive frugivorous hornbills.

Season	Protein	Fat	Ca	P
	% of dry matter			
Non-breeding	15	15	1.0	0.8
Courtship/ breeding	20	30	1.0	0.8
Nesting (molt/ chick growth)	25	25	2.0	1.0

Summary

The hoatzin work discussed above represents a successful application of wild feeding ecology to captive dietary management. As we learn more about the composition of wild diets, particularly of fruits and other plant materials, it is apparent that for many avian species, successful husbandry and propagation cannot rely on diets derived solely using domestic poultry models. Birds have specialized to utilize a wide variety of food items, and it is our responsibility to ensure that captive diets are neither inadequate nor dangerously rich. Our work with both hoatzins and hornbills illustrates how field data can be used to develop more suitable captive diets, and why chemical composition data are essential for achieving that goal.

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VISCERAL GOUT IN BIRDS AND REPTILES

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Introduction

Gout is a metabolic disorder that results in a white chalky substance called urate being deposited in many places throughout the body. There are two types of gout, visceral and articular. In man, articular gout is the primary form. It involves the deposition of urates in the tissues around the joints. In visceral gout, urates are deposited throughout the internal organs. Visceral gout is most common in reptiles and birds. Articular gout occurs occasionally in avian species, but usually not in the same animal at the same time as visceral gout.

Urates, an end product of protein metabolism, build up on the internal organs impairing function and eventually causing death. One problem with visceral gout is that few clinical symptoms are visible before death. Some birds appear healthy and die suddenly; others have prolonged general illness.(Siller, 1959) Reptiles occasionally show lethargy and anorexia.(Maixner et. al., 1987) Most cases of visceral gout are discovered upon necropsy, where there is diffuse deposition of urates on serous surfaces of the heart, lungs, liver, gut, and air sacs. These deposits range from tiny flecks to massive encrustations.(Siller, 1959) Early deposits are seen on the heart, where they appear as delicate white streaks on the epicardium. In advanced cases, a dense white mass completely covers the heart.

Visceral gout is thus of special concern in both birds and reptiles. The purpose of this paper is to describe gout, explain protein metabolism as it applies to gout and examine possible causes of gout and the dietary implications of those causes.

Protein Metabolism

Proteins, whether dietary or endogenous(gut microbes, enzymes, sloughed cells), are broken down into amino acids to be absorbed from the gut. The amino acids then travel to the liver via the portal circulation where they can: 1)be utilized for formation of liver and plasma proteins, 2)pass through unchanged, or 3)of primary importance here, have nitrogen removed from the molecule for use from the body amino acid pool. Ammonia is the end product of amino acid deamination and is toxic to tissues. In order to keep from poisoning itself, the body converts ammonia to a non-toxic form. In man this is primarily urea, but in many reptiles and birds the end product is uric acid. Uric acid is excreted as a thick slurry. It is less toxic than urea and is a concentrated form which is an adaptation of animals that don't retain moisture in their bodies.(Mc Donald 1990) The majority of uric acid synthesis occurs in the liver in both birds and reptiles. Some synthesis may take place in the kidney, but it is primarily the site of excretion.

Uric acid is the end product of purine degradation. Purines are synthesized in the body from simple precursors such as carbon dioxide, folic acid and other B vitamins, and the amino acids aspartate, glycine and glutamine. Purines(and pyrimidines) form the nitrogenous base portion of nucleotides, used in DNA and RNA. The purines adenosine and guanosine are broken down to hypoxanthine and guanine. These are then oxidized to xanthine, which is the ultimate precursor of

uric acid. This reaction is catalyzed by the enzyme xanthine dehydrogenase. Drugs used to treat gout in humans act here to inhibit xanthine dehydrogenase's (oxidase in man) action and decrease uric acid production, (Montgomery, et.al. 1990).

Physiology

The avian kidneys have three distinct lobes and are relatively larger than those of mammals, comprising 1 - 2.6% of body weight.(Sturkie, 1954) Lacking a urinary bladder, the ureters flow directly into the cloaca. Each lobe is divided into thousands of lobules. The cortical region of each lobule contains nephrons, while the medullary region is the site of the collecting tubules.(McDonald, 1990)

Uric acid comprises 60-80% of the total nitrogen excreted by the kidney. The renal tubules excrete 87-93% of this uric acid. As plasma levels of uric acid increase, the amount filtered continues to increase. However, at very high plasma levels, the ability of the tubules to secrete uric acid decreases.(Sturkie, 1954) Phalen et al (1990)states that blood uric acid concentration reflects the capacity of the proximal renal tubule and is minimally affected by hydration status.

In birds with gout, each kidney may be affected differently both in type and severity of gouty lesions. Shrinkage of the kidneys is common. Secondary lesions appear similar to the changes in human gouty kidneys. Visceral gout in birds is almost always accompanied by some degree of pyelonephritis. Thus, the consequence of severe or widespread kidney damage is the development of hyperuricemia.(Siller, 1959) Uric acid levels are elevated in avian monocytosis, with renal insufficiency, and acute nephritis. Because of these factors, Siller concluded that visceral gout must in most cases be regarded as following damage to the kidneys.

Reptiles normally excrete urates, either as ammonium acid urate, sodium urate or uric acid, depending on whether they are terrestrial, aquatic, or semi-aquatic, (Gans, et.al, 1977). The reptilian kidneys are triangular in shape, flattened and, like the avian kidney, lobulated. The reptile urogenital system is similar to birds, except that turtles and some lizards have a bladder. Compared to avian species, reptiles excrete a similar portion of nitrogenous waste as uric acid, ranging from 95% in lizards to 89% in the python.(Wallach and Hossle, 1967) Neither Wallach and Hossle (1967) nor Appleby and Siller (1960) found evidence of primary renal disease in reptiles with visceral gout. However, according to Appleby and Siller (1960), there appears to be a correlation between the amount of urate deposited and pathological changes in the kidney.

Studies - Birds

Excess dietary protein has been studied by numerous authors. Siller, (1959) used six week old chicks to determine the response of plasma uric acid to dietary protein. For 14 weeks test birds were fed a 30% protein diet containing gelatin and casein. This was followed by 11 weeks on a 60% protein diet. An 18% protein diet was used as a control. Blood samples were taken at four intervals and increased from 1.3 mg/dl(control) to 9 mg/dl. From this study Siller concluded that increased protein levels did not cause an increase in plasma urate above physiologic(<9.6 mg/dl) levels. However, this study did not monitor food consumption, which may have decreased with higher protein levels. Featherston and Scholz, (1967) conducted studies to determine the influence of

protein alterations on liver xanthine dehydrogenase, liver weight and liver nitrogen in the chick. Day old chicks were placed either on a 75% or 25% protein diet. Before sacrificing, half of each group was either starved for 24 hours or fed a diet devoid of protein for 24 hours. Xanthine dehydrogenase activity increased on a high protein diet or 24 hour fast, but not on a protein free diet. Because xanthine dehydrogenase catalyzes the conversion of xanthine to uric acid, increased xanthine dehydrogenase could cause an increase in uric acid production and thus increase the possibility of gout. A continuation of this study indicated that uric acid production does indeed increase due to greater xanthine dehydrogenase activity on a high protein diet. Austic and Cole, (1972) conducted studies to determine the uric acid excretion of chickens genetically selected for hyperuricemia and gout. For two weeks chickens were fed diets ranging from 20 to 60% protein. Plasma uric acid levels increased to 8, 19 and 18 mg/dl on the 20, 40, and 60% diets, respectively. When uric acid excretion was measured, it was found that although excretion increased as protein level increased, the amount excreted daily was similar for both lines. This indicated that chickens can excrete up to three times the requirement of protein. Okumura and Tasaki, (1968) investigated the effect of graded protein levels on uric acid content in blood, kidney, and liver. Five month old cockerels were fed varying protein levels. Blood was drawn periodically after feeding. Just before feeding, plasma uric acid concentration was constant across protein levels. After feeding, plasma uric acid concentration levels rose sharply and reached a peak at 2 hrs and then gradually decreased. At 5,10, 15 and 20% protein diets, the plasma uric acid concentration approached 0 hr levels at 3 hrs, whereas birds fed 30 and 40% diets maintained higher uric acid concentrations. We can conclude that increasing protein in the diet can increase plasma uric acid levels, but effects may be short lived. A fasting and refeeding study showed starvation causes dramatic increases in plasma uric acid, but levels return to normal quickly after refeeding.

Dietary protein imbalance was investigated by Miles and Featherston, (1974). This study compared uric acid excretion with weight gain as an indicator of amino acid requirement. Chicks fed an inadequate lysine diet excreted more uric acid and showed increased plasma uric acid(25 mg/dl). This may be an indication of increased catabolism of amino acids because of insufficient lysine. Uric acid excretion decreased, plasma uric acid dropped to 12 mg/dl and weight gain increased as the diets approached the requirement(approx .87%), then plateaued. These results suggest imbalanced protein levels in elevated plasma uric acid. This is especially important because seed diets are frequently deficient in lysine.

Impaired tubular uric acid transport was investigated by Zmuda and Quebbman, (1975). Chickens genetically selected for high incidence of articular gout and hyperuricemia were used to determine the site of defective uric acid transport in gout. The results suggest that uric acid transport is located at the peritubular membrane and that this mechanism is defective in gouty chickens.

Peterson, Hamilton and Lilyblade, (1971) studied heredity in a selected line of chickens susceptible to articular gout transmitted as a recessive trait. These chicks were fed diets of 20 or 80% protein for four weeks. Gout only occurred in chicks fed the high protein diet. It appears that heredity may have a role in susceptibility to gout.

Elvehjm and Neu, (1932) investigated avitaminosis A by examining the effect of Vitamin A deficient diets on uric acid levels in blood. Chicks were fed diets containing combinations of various levels of vitamin A and protein. Blood uric acid levels varied from normal(5 mg/dl) to 44 mg/dl. These high levels indicate normal elimination of uric acid is altered and extensive renal damage

occurred during vitamin A deficiency. The amount of uric acid in the blood was dependent upon the degree of kidney hypertrophy.

Other contributing factors to gout have been published. Dehydration may cause urates in the urine to become more concentrated and therefore more likely to precipitate. Nephrotoxic agents such as poisonous chemicals cause renal damage and thus impair excretion of uric acid. Seasonal changes, i.e. molt have been shown to increase uric acid levels.(Siller, 1959). Drugs that alter renal clearance (such as probenecid and phenylbutazone) decrease urate excretion and could cause hyperuricemia.(Shideman et. al., 1981 and Berger, et.al., 1960).

In summary, elevated protein levels may cause increased serum uric acid, but usually birds can excrete the excess. Diets deficient in lysine amino acids also cause increased serum uric acid. Fasting dramatically elevates serum uric acid, but levels return to normal shortly after refeeding. Heredity has been shown to be a factor in hyperuricemia and articular gout. It is possible that it could also be a factor in visceral gout. A defect in the tubular secretion mechanism of the kidney has been shown to contribute to hyperuricemia. Diets deficient in vitamin A have been shown to cause kidney damage which could contribute to gout.

Studies - Reptiles

Few studies have been conducted regarding gout in reptiles. The intake of uric acid and its corresponding levels in snakes with gout have not been established. Smeller et. al., (1978) is one of two studies that investigated the effects of feeding on plasma uric acid in various reptiles follow. Two gopher snakes and two black rat snakes were fed mice. Plasma uric acid increased following feeding, then decreased to a constant level 7-11 days from feeding and considered baseline. Baseline levels in fasting snakes increased from 2-5 mg/dl to levels 5-10x greater than baseline. It was concluded that plasma uric acid levels could be helpful in diagnosis of reptilian gout, but careful attention needs to be given to the feeding schedule. Maixner, Ramsey and Arp, (1987) used 5 monitor lizards, 5 black rat snakes and 4 gila monsters to study the effects of feeding on serum uric acid. Prefeeding serum uric acid concentrations of monitors and gila monsters were only half that of the snake. The most notable difference among species was the time required for maximum serum uric acid concentration to occur postfeeding. This was at day one for snakes and monitors and day two for gila monsters. Reestablishment of prefeeding serum uric acid levels was faster in snakes and monitors (3 days) than in gila monsters(4 days+). Over the course of this 4 week study, one monitor developed gout and died. It's serum uric acid was consistently higher, increasing to over 100 mg/dl. Maixner suggested that serum uric acid levels of 1.5-2 times greater than normal may indicate potential for gout before development of extreme uric acid levels or death.

Serum uric acid levels may be useful in indicating potential for gout in reptiles, however no studies were available which described the connection. Caution must be exercised because feeding schedules and species differences can lead to misinterpretation of results.

Nutritional implications

Extreme excesses of protein should be avoided, but there is no evidence that moderate levels produce gout in an otherwise healthy animal. Diets deficient in vitamin A or specific amino acids

have the potential to contribute to high uric acid levels. Blood uric acid levels may be helpful in assessing possibilities of gout in birds and possibly reptiles, but may change when fasted or fed. Thus assessment must take these factors into consideration.

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CROP MILK COMPOSITION AND SQUAB GROWTH IN THE PIGEON

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Introduction

Crop milk is an avian crop secretion unique to pigeons, doves, and a select group of penguins and flamingos. Both the male and female produce crop milk to feed their young. Squabs are fed by regurgitation of the milk by the parent. The process of formation of crop milk is under the control of prolactin and begins to thicken and becomes highly vascularized. There is a successive detachment of distal cells and lipid droplets within the crop epithelium which become the primary components of crop milk. By the fourteenth day of brooding, true crop milk is produced in preparation for feeding the hatchlings.

Crop milk has been characterized as a high protein and high fat secretion. Only trace amounts of carbohydrates are present and may be attributed to the adult avian diet becoming incorporated into the crop milk secretion. Crop milk differs from mammalian milk in that it does not contain lactose or caesin. The nutritive properties of crop milk have been investigated, but the results are difficult to assess. Differences in experimental methodology, milk collection procedures and analyses produce varying results. It has been established that important changes in the composition of crop milk occur during the first week of crop milk feeding. Th compositional changes of the milk reflect and support the rapid growth of the squab during the first week of life.

Typically, young *Columbidae* are difficult to raise in captivity on milk replacers. Appropriate formula composition for normal growth of the hand-raised squab has not fully been established. Trial and error mixed with luck has been the basis for milk replacer formulation. Knowledge of true milk composition and actual milk consumption will facilitate hand-raising pigeons, doves, flamingos and penguins in zoo settings.

Squab growth can be an indication if the adequacy if the formula used with hand-raised birds. Growth and body composition can be monitored in several ways. The use of isotopes and direct methods to measure body composition have been employed, but are not practical when dealing with exotic species since both of these are invasive or terminal procedures. It is crucial to develop methods that can be used in endangered species to assess body composition and condition. A new method that is used more frequently in laboratory animals and some wild avian species is electromagnetic scanning. This method involves the electrical conductance across body tissues of animals placed in a chamber. Electromagnetic resistance is measured in the empty chamber and measured again with the animal in the chamber. The difference in the two fields is the conductance of the animal. Lean body mass conducts more efficiently than fat, and therefore lean body mass can be estimated in an equation developed with the use of other measured parameters, such as body

weight or wing length. The objectives of this preliminary study were to develop a technique which allowed for the collection of consecutive samples of crop milk from the same birds and to measure growth rate and body composition of squabs using non-invasive methods.

Methods

Consecutive daily samples of crop milk were obtained on days one through four from squabs using a method that consists of gently mixing the crop contents of the birds by messaging the outside of the squab crop, and directing the contents from the crop back out through the bird's beak. Crop milk is semi-solid and therefore can be extracted from the young bird with little risk of aspiration. Samples were analyzed for fat, crude protein, carbohydrate and water. Fatty acid analysis of the crop milk fat was also performed using capillary column gas chromatography.

Growth was measured by weighing the squabs on a daily basis after the crop milk had been removed. Crop contents can contribute significantly to the weight of the squab and must be removed to obtain a valid live weight.

Body composition was estimated using electromagnetic technology. The instrument used was an EM-Scan designed for determination of body composition in small lab animals. The instrument allows measurements on live animals without restraint or the use of anesthesia.

Results

Table 1. Crop milk composition (dry matter {DM} basis).

Day	DM (%)	Fat (%)	Crude protein (%)	Carbohydrate (%)
1	31.6	46.8	49.3	trace
2	26.1	45.4	54.2	trace
3	25.9	37.2	51.1	trace
4	16.8	33.3	63.7	trace

Proximate analysis of crop milk sampled from white carneaux (*Columba livia*) squabs on days one through four is presented in Table 1. Fat and protein content of crop milk was characteristically high with ranges of 33-47% and 49-64% respectively. Fat and protein are high in "early lactation" and tend to decrease with time. This decreasing trend is evident in the analysis of the fat of these samples. Only trace amounts of carbohydrate was found in the crop milk.

Fatty acid analysis is presented in Tables 2 and 3. Table 2 compares the fatty acid composition of drop milk with that if ferret and dog milk. Crop milk contains oleic acid as the most

prominent fatty acid, which is common in most milks. Differences in linoleic acid between milks is most likely due to the composition of the diet, since mammals are not able to synthesize that particular fatty acid. A comparison of fatty acid composition of crop milk obtained from two different squabs of the same clutch (being fed by the same parent birds), is presented in Table 3. The fatty acid composition of the milks collected from the different squabs is almost identical. These data suggests that samples obtained from different birds is representative and indicates that the sampling technique is adequate for milk analysis. Growth data on five squabs over the first ten days after hatching are presented in Figure 1. Growth rate is exponential. Squabs tend to double their weight on a daily basis. This is certainly a significant growth rate, not common in most other avian species.

Table 2. Fatty acid composition of dog, ferret and pigeon crop milk.

FA (wt%)	Dog	Ferret	Pigeon
Total saturated	35.5	35.6	36.0
Oleic	40.7	29.5	35.7
Linoleic	11.9	12.8	18.8
Linolenic	0.5	0.1	1.1
> C 18	1.1	1.2	3.0

Table 3. Fatty acid composition of crop milk collected from two squabs in the same clutch (day 2).

FA (wt%)	Squab 1	Squab 2	Pellet
16:0	17.5	17.6	14.8
16:1	4.1	4.3	0.6
18:0	14.0	13.7	2.0
18:1	31.6	31.7	23.9
18:2	24.5	24.3	51.1
18:3	4.2	3.4	1.8

Body composition measurements were taken in squabs from hatching to ten days of age using EM-Scan instrumentation. Previous studies suggest that there may be differences in the accuracy of this method for estimating body composition in different species. No inferences can be made from the data obtained in this study until validation studies are complete. After validation, it will be possible to determine the accuracy of using total body electrical conductance to predict body composition in squabs.

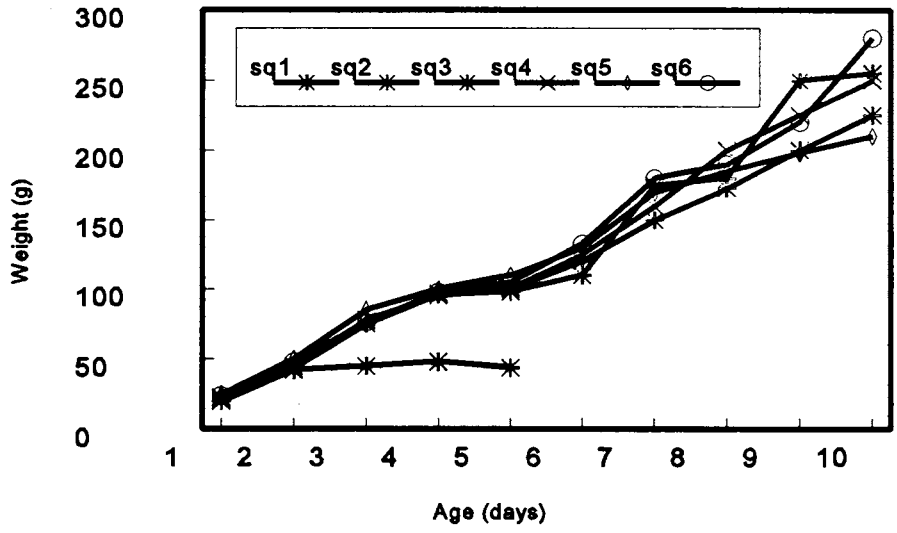
Conclusions

Crop milk is essential for the rapid growth of squabs during the first few weeks of life. The composition reflects the nutrient rich, energy dense diet required by these birds during the early stages of life.

Advances have been made in the techniques used to collect crop milk and measure body composition in squabs. In the past, either the adult bird or the squab was sacrificed or surgery was performed to obtain crop milk samples. Due to the nature of the procedures used in past research,

it has been impossible to obtain consecutive milk samples from the same bird. The new techniques described in this paper are non-invasive procedures that produce acceptable samples for analysis with respect to quantity and quality. The application of these techniques in exotic avian species may ultimately provide zoos with information essential to successful reproduction and rearing in captive breeding environments.

Figure 1. Squab growth rates



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