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

The Dr. Scholl Nutrition Conference is sponsored by the Chicago Park District
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Proceedings of the Third Annual

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A Conference on the Nutrition of Captive Wild Animals

Edited by

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TABLE OF CONTENTS

FEEDING, FORAGING, AND MENTAL HEALTH

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INTRODUCTION

There is a clear and pressing need to carefully examine what the captive animal is fed and how it is fed within the broad context of how animals feed in nature. Although the major subjects of our research have been the nonhuman primates, the principles that we will develop herein are sufficiently broad to include many omnivorous and carniverous animals. For example, concerning ungulates, Dittrich (1976) has written as follows:

> The animal requires something more than a thoroughly nutritionally adequate diet. The habit so often observed in zoos of licking at fences and lattices, once generally regarded as a symptom of deficiency or mineral salts or other supplementary items, is rather to be explained as the instinctive lip and tongue movements employed during the long drawn out feeding in the wild state, which fail to find satisfaction in the rapidly consumed prepared feeds. (p. 49)

Consider a few representative facts about the feeding habits of free-ranging great apes:

(1) Jane Goodall (1968) discovered that the chimpanzees of the Gombe Stream Reserve spent six to eight hours per day feeding; they were known to travel from one to ten miles each day in search of food.

(2) Chimpanzees in Rio Muni have been observed to eat 43 different plants, while the Gombe chimpanzees apparently consume at least 80 different varieties.

(3) Chimpanzees characteristically feed most intensively from 0700 to 0900 and from 1530 to 1730 hours.

(4) The Spanish behavioral scientist Jordi Sabater-Pi (1979) estimated that 23% of the foods consumed by wild chimpanzees were orange in color, while 12% were red, and 18% yellow-green.

(5) Alan Goodall (1978) noted that Mt. Kahuzi gorillas consumed 104 species of plant life; Sabater-Pi counted 91 different types for gorillas in a different location.

(6) Orang-utans in East Kalimantan fed on fruit in at least 50 different species of trees and vines, according to Peter Rodman (1979). MacKinnon (1974) counted 105 distinct species as consumed by orang-utans in Sabah. Rodman furthermore discovered that orangs typically feed 45.9% of the day, resting for 39.2% and traveling 11.1% of a given day.

Without adequate data on natural feeding habits it is impossible to do an adequate job of feeding animals in captivity. For every taxon a "psychology of feeding" may be identified, so that the variables that control feeding behavior in nature can be modeled in the zoo environment.

The mental health of captive animals is enhanced when their living environment is complex and challenging. Likewise, the complexity of feeding is reflected in both the acquisition and consumption of food. When food is improperly presented, feeding pathology may develop.

FEEDING PATHOLOGY IN CAPTIVITY

In an excellent review, Fritz and Fritz (1979) discussed the behavioral problems of captive chimpanzees, including those that derived from feeding schedules and practices. They recommended feeding from three to four times daily on a varied schedule in order to prevent the development of a "food/time-clock syndrome." They attributed aggression at feeding to rigid scheduling such as that followed at the Holloman Air Force Base during the 1960's (cf. Reynolds and Luscomb, 1969; Wilson and Wilson, 1968).

At the Arizona Primate Foundation, Fritz and Fritz prevent diet boredom by feeding a large variety of produce in large enough quantities to permit a leisurely consumption. In addition to commercial monkey biscuits, high-fiber foods (e.g., celery and straw bedding) permit the animals to form a chewing "cud." Foraging behavior is encouraged by occasionally seeding the substrate with a dry breakfast cereal, chicken scratch, or popcorn. According to Fritz and Fritz, coprophagy has been nearly eliminated in this colony due to these efforts.

As fieldworkers have convincingly documented, feeding is a prominent behavior pattern in the repertoire of wild apes. Failure to permit lengthy feeding bouts leaves too much time for the development of conflict, coprophagia, regurgitation/reingestion, over-grooming, etc. There can be no doubt that the effective management of time is an essential feature of any successful colony of animals.

CHARACTERISTICS OF FOOD

Beyond the abundance, availability, and nutritive value of food, Alan Goodall (1978) identified a number of relevant characteristics of food. In his research he noted that great apes responded to food according to its taste, smell, size, shape, and texture. Each food type could also be characterized by the mode of preparation required to render it ingestible, the local group traditions associated with its acquisition and consumption, and individual preferences for the food itself.

There are additional variables that are associated with the feeding process such as:

(1) Duration of processing.
(2) Duration of consumption.
(3) Time (onset) of feeding.
(4) Frequency of feeding bouts.
(5) Locus of feeding activity.
(6) Color, size, weight of food.
(7) Amount and complexity of food.
(8) Manipulative characteristics.
(9) Dispersion and visibility of food sources.
(10) Contingencies and competitive factors.
(11) Stimulus change, novelty of food items.

About most of these variables there is little information to report. However, a recent study conducted by Dr. Robyn Barbiers (unpublished ms.) provides data concerning the response of captive apes to the color of food. In this pilot study, Barbiers demonstrated that colored monkey chow altered the feeding behavior of orang-utans, increasing their consumption and interest in the food. As the author explained, the provision of color is one means of increasing the complexity of food in captivity.

A few words about the processing of food are equally instructive. Two wild plants which require processing are Strychnos and Diplorhyncus, both found within the Gombe National Park in Tanzania. As McGrew (1974) observed:

> The hard-shelled, orange-sized fruit of the Strychnos requires strength and skillful technique to process it for eating. . . the leathery pod of the Diplorhyncus trees presented an even more interesting case. Each pod contains small amounts of the edible material in sticky sap, and adults may process hundreds of these in prolonged feeding sessions. (p. 307)

Fruits consumed by wild chimpanzees such as Musa sapientium and M. paradisica are particularly long-lasting. These foods are partially spat out in the form of a small ball after having been thoroughly masticated for a long time.

Of course, plant material functions as more than mere food. As Hediger (1950) long ago observed, plants may also be employed as support (e.g. as in sleeping nests), abrasives (for sharpening beaks, tusks, etc.), comfort (scratching posts), signalposts (for depositing secretions), stimulus substitutes (redierection of aggression), playthings, tools, building materials, cleansers, cover, and camouflage. Thus the psychological implications of plant materials are many indeed.

COPROPHAGIA AND REGURGITATION/REINGESTION

Great apes are particularly prone to the ingestion of their own feces and to the regurgitation and reingestion of vomitus (cf. Maple, 1979). It has been generally assumed that such behavior is an adaptation to the limited diet provided in captive settings. Interestingly, wild mountain gorillas consume their feces when they are confined to the sleeping nest due to inclement weather (Harcourt and Stewart, 1978). Thus, in the wild and in captivity, confinement, restriction, and stress seem to contribute to such habits (Maple and Hoff, 1982). In a recent survey conducted by Schildkraut and Akers (unpublished ms.) it was estimated that 68% of captive gorillas exhibited regurgitation/reingestion (R&R).

Bloomstrand, Riddle, Alford, and Maple (unpublished ms.) recently concluded an evaluation of a behavioral enrichment device designed to reduce or eliminate coprophagia. The device was designed by Dr. Ken Riddle for operation at the University of Texas/ System Cancer Center where laboratory chimpanzees are being systematically resocialized and rehabilitated under a contract from the National Institute of Health. It requires the animals to perform specific manipulations to obtain peanuts.

This study concluded that the device was very effective in eliciting activity by the chimpanzees and all animals attempted to use it. At the individual level, some chimpanzees decreased while others

increased their characteristic levels of aggressive display, coprophagia, R&R, and excessive grooming. Those animals that exhibited increases in undesirable behaviors did so when they were denied access to the device due to its monopoly by more dominant animals. Induction of competition-related stress could be altered by the placement of more than one device in a group enclosure.

DISPERSION OF FOOD

In a very useful study, Chamove, Anderson, Morgan-Hones and Jones (1982) provided eight taxonomically distinct primate groups (N=67) with a deep woodchip floor covering and furthermore seeded the litter with grain. These manipulations led to a reduction in inactivity and fighting, the animals spent more time on the ground, and the litter became increasingly inhibitory to bacteria with the passage of time.

The grain which was added to the woodchips was a mixture of millet seeds, peanuts, sunflower seeds, dried currants, wheat, and kibbled corn. Mealworms were also provided. The advantages of woodchips had been enumerated in a previous publication (cf. Chamove and Anderson, 1979) and may be summarized in the following passage:

> . . .the chips were shown to be inexpensive; after six weeks, odor was less than with bare floors, and the animals and walls appeared cleaner when woodchips were provided than when there was no floor covering but daily cleaning was performed. (p. 311)

Chamove and his associates also discovered that feeding solidly frozen fruits and vegetables led to a "better distribution and longer feeding times." An additional mode of distribution, burying the food in the litter, further prolonged feeding time. The conclusions of the University of Stirling team are well worth quoting:

> . . .we recommend deep litter as one technique of enhancing conditions for captive primates. It has real potential for promoting good health and induces positive kinds of behavior among species that invest a great deal of time and energy in foraging in their natural environment. (p. 316)

CONCLUDING REMARKS

We have reviewed here some of the major features of the feeding process, emphasizing its psychological characteristics. The way that we feed captive animals greatly affects their behavior.

Therefore it is imperative that we consider more than nutrition when selecting foods and feeding methods. In many instances, it will be important to experiment and to keep records on the results of such experiments. It is unfortunate the literature contains so little information on the psychological consequences of specific feeding regimes. The following conclusions provide a summary of our findings:

(1) The feeding process includes an acquisition and consumption phase, such that feeding must be recognized as a complex and time-consuming behavior pattern.

(2) To successfully provide challenging feeding experiences for captive animals, look to nature as the model; then be creative in attempting to replicate natural feeding situations.

(3) When you have developed a successful feeding program, evaluate it objectively and try to communicate your results to others through publication.

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COMPUTERIZED ANALYSIS OF DIETS FED TO ZOO ANIMALS

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INTRODUCTION

Advances in nutritional science in the past 40 years have been largely responsible for successful nutrition programs in zoological parks in the United States. This is primarily because of the expanded use of scientifically formulated complete rations, similar to those fed to domestic animals. The advent of specialty diets such as those formulated specifically for exotic game birds, ratite birds, birds of prey, primates, exotic felids, polar bears, exotic ruminants, etc., attests to this. These feeds have come into use because of the recognition that wild animal species, just like domestic ones, require specific nutrients and not specific feedstuffs (6). Nevertheless, the nutritional management of the diverse animal species housed in many zoos remains a challenge.

It is especially difficult to manage the nutritional intake of the animal species fed mixtures of many different food items rather than a simple formulated ration. This is often the case for the primate species, psittacine birds, herbivorous reptiles, and many other omnivorous, insectivorous, or frugivorous species. Diets fed to these type animals often consist of a commercially prepared product in addition to a variety of fresh fruits and vegetables, leafy greens, eggs, nuts, insects, meats, and dairy products. This cafeteria-style feeding may incorporate 15 or more food items in a diet. Discussions regarding the advantages and disadvantages of cafeteria feeding have been elaborated previously (1, 24), and it is acknowledged that foods and feeding methods are important for many reasons beyond providing essential nutrients (17,18). Yet there remains an overriding concern that each diet must be nutritionally sound.

This paper addresses this problem by presenting a method of computerized diet analysis which we believe will aid in evaluating the nutrient composition of diets composed of a complex array of food items.

METHODS

A computer computation program is utilized in this study to make calculations of the average nutrient composition of multiple food diets. Nutrient data for each food item are gathered from published reference material and entered into a data bank. Calculations are then made by the computer to give predicted average levels for each nutrient. Other interesting features of a diet are also revealed by the analysis. The method has been described in more detail in a previous publication (11).

Food Composition Data

There is an enormous volume of food composition data available from both published monographs (3,4,5,8,9,25) and from computerized data banks such as the International Feed Stuffs Institute (12). There have been discussions on the validity of such data, and for this concern the reader is referred to the paper entitled, "The Use and Misuse of Food Tables" (28). Since this type of data is widely utilized by nutritionists, both in North America (4,5) and throughout the world (9,10,15, 16, 25), we have assumed that the validity of this methodology has been established.

It must be emphasized that foods produced or grown in different geographic regions with variations in climate and soil composition can be expected to vary somewhat in nutrient levels. Accordingly, the nutrient values found in food composition tables must be regarded as indicating reasonable ranges and levels, and must not be considered to indicate absolute values. The computer calculations based on these data would hence reflect approximate nutrient levels. Additionally, there is a general lack of data for some nutrients such as vitamin K, vitamin B-12, biotin, choline, inositol, sulfur, cobalt, selenium, arachidonic acid, and linolenic acid. We nevertheless include these in our data base with the hope that more complete data will become available in the future.

The references used in collecting nutrient data for this study are as cited above. From these sources we have collected fairly complete data on over 100 food items commonly used in the feeding of zoo animals.

Data Analysis

The computer employed in this study is a Hewlett-Packard 85 Personal Computer with 32K bytes of memory. It is equipped with a cathode ray tube (CRT) display and printer. The program and relevant data are stored on magnetic tapes. One tape stores the

diet computation program, and another is used to store the nutrient composition data pertaining to food items and diet standards. The computer is programmed to calculate the average nutrient compositions for any diet that is chosen. The computation is based on data collected from food composition tables. We have collected data in these categories: gross composition, vitamins, minerals-elements, amino acids, and fatty acids (Table 1).

The computer printout first names the diet being analyzed. It then lists each food item and its quantity in grams, ounces, pounds or kilograms, as well as the per cent of each food item in the total diet. This is followed by a printout of the average chemical composition listing the parameters in Table 1. This can be selected for the diet as fed, or for any portion such as per 100 grams of diet. Finally, it provides the average composition per kilogram of dry matter. A printout of the nutrient contribution of each ingredient of the diet is given if desired.

Calculations can be made for diets of almost any complexity, providing fairly reliable nutrient data are available for each ingredient, and the quantities of each food are known. A comparison of diets fed versus diets actually eaten can be made if a food consumption study is undertaken (2). The method can also compare any diet to a standard if one is available.

This method of data analysis rapidly reveals a number of interesting features about a diet, in addition to the average composition of nutrients. These include: the predicted level of caloric intake for an animal; the energy density of a diet; the overall calcium:phosphorus ratio; the expected level of nutrient dilution of the commercial balanced componenet in the total diet; or the predicted need for vitamin/mineral supplementation based on comparison with a standard. Other diet parameters such as cost effectiveness of each ingredient can be addressed, depending on the interests of the nutritionist.

Diets Analyzed

The diets of five animal species housed at the Rio Grande Zoo were selected for analysis in this study. These include: 1) an adult male gorilla (15 food items); 2) an adult male orangutan (16 food items); 3) a troop of 7 colobus monkeys (14 food items); 4) an adult female lesser sulfur crested cockatoo (10 food items); and 5) a group of 3 Isla San Esteban chuckwalla (13 food items). These diets were selected because of their complexity, and our inability to accurately "eyeball" assess their nutrional composition.

The diets of the two apes give examples of how individual diets can be assessed. The colobus diet provides an example in which individual consumption is unknown, yet the average composition of the total diet is still informative. The cockatoo and chuckwalla diets demonstrate the necessity for accurate food consumption studies. In the latter cases the food consumed varies considerably from the diet offered. There is extensive food wastage in these cases, and consumption is impossible to estimate without a closely monitored food consumption study. Food consumption is determined by feeding in a controlled environment where food quantities are accurately weighed before and after feeding, with corrections made for evaporative moisture loss of fresh foods. This requires attention to detail and painstaking sorting of food wastage to determine the quantities of each food item eaten.

The intent of these analyses and the following discussion is not to criticize the diets, but rather to demonstrate how the computer-generated data can quickly reveal important nutrient parameters of a diet, and at the same time provide a basis from which to make diet modifications.

RESULTS

The total data generated in the analysis of each diet is excessive for the purpose of demonstration in this paper. We have thus selected portions of data from each set of computations to illustrate the potential usefulness of the computer analysis described herein. Tables 2-4 illustrate data for the gorilla, orang-utan, and colobus diets. Table 5 lists nutrient standards for non-human primates and the nutrient levels of monkey chow. Tables 6-8 give nutrient data on the cockatoo diet, and Tables 9-10 give similar data for the chuckwalla diet. Values calculated for the nutrients on which there is a lack of data, as stated above, are not listed in the Tables due to limited space and limited usefulness of the numbers at this time.

DISCUSSION

Examination of the computer-generated data for the diets chosen in this study reveals the potential usefulness of this method.

Data from the three primate diets show they are quite similar in overall nutrient composition (Tables 2-4). They also compare favorably to the NRC requirements for non-human primates, and there is no evidence of significant nutrient dilution of the monkey chow portion in the diet (Table 5). We can quickly see the predicted levels of all the vitamins, minerals, amino acids, and fatty

acids for which there are no data and make comparisons between the diets as well as to the standards in Table 5. The standards are not for these particular species, but for primates in general. Judging from these values, as well as from data on the nutrient requirements of many other species (22), and the generall principles derived from these data (23), there is no evidence that vitamin supplementation of these diets is necessary.

Comparison of the gross composition of the three diets shows the colobus diet to be higher in fat and protein than the ape diets. This reflects the increase in peanuts and eggs. Also apparent from the gross composition is a seemingly excessive caloric content and low fiber level for these species. The diets yield 38 Kcal ME(metabolizable energy)/ kg body weigh/day for the gorilla (@ 430 lbs.); 74 Kcal ME/kg b.w./day for the orangutan (@ 227 lbs.); and 65 Kcal ME/kg b.w./day for the adult colobus monkeys (@ 30 lbs.). Energy requirements are poorly defined. If animals are inactive and appear overweight, dietary items high in fat can be replaced with low-fat items. Overnutrition in captive great apes seems to be widespread in U.S. zoos (7,29), and computerized analysis of these diets may be a useful tool for rapidly identifying overfeeding and making diet modifications. For example, in our case, carefully reducing the amount of egg, using skimmed milk and removing the peanuts from the diet could be considered.

The fiber levels of the primate diets at 3.2%-3.7% appear quite low, considering the natural diet of these species (17,30). It may be advisable, as suggested previously (27), to formulate a high fiber complete ration for some primates as an aid to prevent overfeeding. The addition of browse and a reduction of the highly palatable fresh foods may also be a useful feeding strategy.

Concerning the cockatoo diet, we find that an evaluation would be quite erroneous without the food consumption study. Weighing each food item before and after feeding shows that no monkey chow or egg are eaten, which significantly affects the nutrient composition of the diet (Tables 6,7). We would not be aware of this by simply "eyeballing" the waste at the bottom of the cage. The diet we presented to the bird contains equal quantities of fat and protein (18.7%), which is probably excessive in fat on a long-term basis. More importantly, we find that the diet eaten is predicted to contain no vitamin D_3, very low calcium (.08%), a reversal of the calcium:phosphorus ratio (0.19), and low iodine (0.09 ppm). These are significant features of many psittacine diets from which birds can self-select. The complete list of values for the diet eaten (Table 8) can be compared to a standard such as the avian breeder

formula (21). This shows, in addition to the above comparisons, other minerals such as sodium, chlorine, copper, zinc, and manganese are predicted to be low; whereas, the remaining vitamins and amino acids are similar between the diets. Additionally, the diet is probably excessive in quantity since only 28% was consumed of the 102 grams fed. Some recommendations call for feeding 10-15% of body weight on an as fed basis, which would be 20-30 grams for this bird (26). The bird at 29 grams of food, which falls within this range.

The chuckwalla diet, similar to that of the cockatoo, required a food consumption study for evaluation. We found that less than 10% of the diet offered was consumed, and only 5 of 13 food items offered were eaten (Table 9). The gross composition shows the diet eaten is predicted to have less fat and more fiber and ash than the diet offered. This is a function of eating the dried alfalfa leaves. The quantity of vitamin/mineral mix in the diet eaten (estimated at 14 mg) is inadequate to significantly increase any nutrient or have any effect on the calcium:phosphorus ratio. This is found by running an analysis both with and without the vitamin/mineral supplement and comparing the numbers. The nutrient composition of the diet eaten (Table 10) compares favorably with the profile for simple stomached mammals such as the canine (20) or non-human primates (19). This correlation can be attributed to the chuckwallas eating a significant portion of canned marmoset diet. Clinical evaluation of the animals reveals all three are active, growing, and appear healthy.

In conclusion, we feel that there is significant potential for this method in evaluating the nutritional composition of diets composed of a complex array of food items.

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Table 1. Food Item Data Sheet Used to Collect Nutrient Data.

FOOD ITEM:_____ COMPUTER NO.:_____

REFERENCE:_____

MANUFACTURER:_____ HOW SUPPLIED:_____

INGREDIENTS:

GROSS COMPOSITION:		VITAMINS:		MINERALS-ELEMENTS:		AMINO ACIDS:	
Dry Matter.....	%	A.....	IU	Calcium.....	mg	Argenine.....	gm
		D.....	IU	Phosphorus....	mg	Histadine....	gm
Protein.....	%	E.....	IU	Magnesium.....	mg	Isoleucine...	gm
		K(Na-menadione)	mg	Potassium.....	mg	Leucine.....	gm
Fat.....	%	Thiamine (B1)	mg	Sodium.....	mg	Lysine.....	gm
		Riboflavin(B2)	mg	Chlorine.....	mg	Methionine...	gm
Fiber.....	%	Niacin.....	mg	Sulfur.....	mg	Phenylalanine	gm
		Pantothenic acid	mg	Cobalt.....	mg	Threonine....	gm
Ash.....	%	Pyridoxine(B6)	mg	Copper.....	mg	Tryptophan...	gm
		Cobalamine(B12)	mg	Iodine.....	mg	Valine.....	gm
Nitrogen Free Extract	%	Folic Acid...	mg	Iron.....	mg		
		Biotin.....	mg	Manganese.....	mg	FATTY ACIDS:	
Energy (kcal/100gm)		Choline.....	mg	Selenium.....	mg	Arachidonic Acid	gm
		Inositol.....	mg	Zinc.....	mg	Linoleic Acid	gm
		Ascorbic Acid	mg			Linolenic Acid	gm
		Carotene.....	IU				

All values per 100 gm edible portion.

Table 2. Gorilla Diet - Adult Male

A. Average Daily Diet as Fed*

Apple	544 gm
Banana	508 gm
Orange	879 gm
Raisins - dried	11 gm
Beans - string	178 gm
Cabbage - white	459 gm
Carrots	1010 gm
Lettuce	143 gm
Onions - white	530 gm
Spinach	31 gm
Sweet Potato	353 gm
Peanuts	73 gm
Egg	22 gm
Cows Milk	478 gm
Monkey Chow - Purina	1386 gm
Total Wt. of Diet	6605 gm

*edible portions

B. Gross Composition of Daily Diet as Fed

Dry Matter	2064 gm(31%)
Protein	307 gm
Fat	138 gm
Fiber	74 gm
Ash	103 gm
NFE	1441 gm
Energy (ME)	7480 Kcal

C. Gross Composition D.M. of Diet

Protein	14.8%
Fat	6.7%
Fiber	3.6%
Ash	5.0%
NFE	69.8%
Energy (ME)	3631 Kcal/Kg

D. Fatty Acids/Kg D.M. of Diet

Linoleic Acid**	5.2 gm

** data for peanuts only

E. Vitamins/kg D.M. of Diet

A - Pure A	18,130 IU
Carotene	75,653 IU
D_3	4,535 IU
E	53 IU
Thiamine	7 mg
Riboflavin	7 mg
Niacin	75 mg
Pantothenic Acid	50 mg
Pyridoxine	11 mg
Folic Acid	3 mg
Ascorbic Acid	982 mg

F. Minerals-Elements/Kg D.M. of Diet

Calcium	.68 %
Phosphorus	.42 %
(Ca:P Ratio = 1.62)	
Magnesium	.14 %
Potassium	1.02 %
Sodium	.19 %
Chlorine	.29 %
Copper	10 ppm
Iodine	1 ppm
Iron	112 ppm
Manganese	37 ppm
Zinc	22 ppm

G. Amino Acids, D.M. of Diet

Arginine	0.9 %
Histidine	0.4 %
Isoleucine	0.7 %
Leucine	1.2 %
Lysine	0.7 %
Methionine	0.3 %
Phenylalanine	0.7 %
Threonine	0.5 %
Tryptophan	0.2 %
Valine	0.8 %

Table 3. Orang Utan Diet, Adult Male

A. Average Daily Diet as Fed*

Apple	544 gm
Banana	508 gm
Orange	439 gm
Raisins - dried	38 gm
Beans - string	178 gm
Cabbage - white	459 gm
Carrots	1010 gm
Celery - stalk	1281 gm
Lettuce	143 gm
Onions - white	397 gm
Spinach	124 gm
Sweet Potato	529 gm
Peanuts	73 gm
Egg	22 gm
Cows Milk	478 gm
Monkey Chow-Purina	1356 gm
Total Wt. of Diet	7612 gm

* edible portions

B. Gross Composition of Daily Diet as Fed

Dry Matter	2147 gm(28%)
Protein	318 gm
Fat	139 gm
Fiber	80 gm
Ash	113 gm
NFE	1495 gm
Energy (ME)	7661 Kcal

C. Gross Composition D.M. of Diet

Protein	14.8 %
Fat	6.4 %
Fiber	3.7 %
Ash	5.2 %
NFE	69.6 %
Energy (ME)	3568 Kcal/kg

D. Fatty Acids/Kg D.M. of Diet

Linoleic Acid **	5 gm

** data for peanuts only

E. Vitamins/Kg D.M. of Diet

A - Pure A	19,674 IU
Carotene	82,028 IU
D_3	4,360 IU
E	57 IU
Thiamine	7 mg
Riboflavin	7 mg
Niacin	74 mg
Pantothenic Acid	51 mg
Pyridoxine	12 mg
Folic Acid	3 mg
Ascorbic Acid	928 mg

F. Minerals-Elements/Kg D.M. of Diet

Calcium	.67 %
Phosphorus	.43 %
(Ca:P Ratio-1.57)	
Magnesium	.16 %
Potassium	1.19 %
Sodium	.25 %
Chlorine	.37 %
Copper	9.7 ppm
Iodine	1 ppm
Iron	111 ppm
Managanese	37 ppm
Zinc	21 ppm

G. Amino Acids, D.M. of Diet

Argenine	0.9 %
Histidine	0.4 %
Isoleucine	0.7 %
Leucine	1.2 %
Lysine	0.7 %
Methionine	0.3 %
Phenylalanine	0.7 %
Threonine	0.5 %
Tryptophan	0.2 %
Valine	0.8 %

Table 4. Colobus Diet, Troop (7)

A. Average Daily Diet as Fed*

Apple	362 gm
Banana	253 gm
Grapes	190 gm
Raisins - dried	39 gm
Beans - string	178 gm
Potato	316 gm
Carrots	404 gm
Celery - stalk	11 gm
Onions - white	177 gm
Spinach	1086 gm
Sweet Potato	236 gm
Peanuts	130 gm
Egg	207 gm
Monkey Chow - Purina	1134 gm
Total Wt. of Diet	4727 gm

* edible portions

B. Gross Composition/100 gm of Diet as Fed

Dry Matter	36 %
Protein	6.3 %
Fat	3.2 %
Fiber	1.1 %
Ash	1.9 %
NFE	23.4 %
Energy (ME)	131 Kcal

C. Gross Composition D.M. of Diet

Protein	17.5 %
Fat	9.0 %
Fiber	3.2 %
Ash	5.2 %
NFE	65.0 %
Energy (ME)	3638 Kcal/kg

D. Fatty Acids/Kg D.M. of Diet

Linoleic Acid**	12 gm

** data for peanuts only

E. Vitamins/Kg D.M. of Diet

A - Pure A	21,423 IU
Carotene	91,420 IU
D_3	4,481 IU
E	66 IU
Thiamine	7.6 mg
Riboflavin	8 mg
Niacin	83 mg
Pantothenic Acid	51 mg
Pyridoxine	12 mg
Folic Acid	3 mg
Ascorbic Acid	967 mg

F. Minerals-Elements/Kg D.M. of Diet

Calcium	.68 %
Phosphorus (Ca:P Ratio=1.53)	.45 %
Magnesium	.17 %
Potassium	1.26 %
Sodium	.22 %
Chlorine	.30 %
Copper	10 ppm
Iodine	1 ppm
Iron	128 ppm
Managanese	40 ppm
Zinc	25 ppm

G. Amino Acids, D.M. of Diet

Arginine	1.1 %
Histidine	0.4 %
Isoleucine	0.8 %
Leucine	1.4 %
Lysine	0.8 %
Methionine	0.4 %
Phenylalanine	0.9 %
Threonine	0.6 %
Tryptophan	0.2 %
Valine	0.9 %

Table 5. Nutrient Requirements of Non-Human Primates and Nutrient Levels in Monkey Chow

	Published Standard(19)	Monkey Chow(13)*
A. Gross Composition/Kg D.M. of Diet		
Protein	15% [1]	15.2%
Fat	---	5.2%
Fiber	---	2.5%
Ash	---	4.8%
NFE	---	63.3%
Energy (ME)	---	4173 Kcal
B. Vitamins/Kg D.M. of Diet		
A - Pure A	10,000-15,000 IU	28,000 IU
Carotene	---	---
D_3	2,000 IU	6,600 IU
E	50 IU	55 IU
Thiamine	---	7.5 mg
Riboflavin	5 mg	9.3 mg
Niacin	50 mg	98.2 mg
Pantothenic Acid	15 mg	63.3 mg
Pyridoxine	---	12.7 mg
Folic Acid	0.2 mg	4.0 mg
Ascorbic Acid	100 mg	758.0 mg
C. Minerals-Elements/Kg D.M. of Diet		
Calcium	0.5 %	.86 %
Phosphorus	0.4 %	.47 %
(Ca:P Ratio)	1.25	1.8
Magnesium	0.15 %	.11 %
Potassium	0.8 %	.56 %
Sodium	0.2-0.4 %	.22 %
Chlorine	0.2-0.5 %	.27 %
Copper	---	11.3 ppm
Iodine	2 ppm	1.6 ppm
Iron	180 ppm	144.0 ppm
Manganese	40 ppm	46.2 ppm
Zinc	10 ppm	19.9 ppm
D. Amino Acids, D.M. of Diet		
Arginine	---	0.8 %
Histidine	---	0.4 %
Isoleucine	---	0.8 %
Leucine	---	1.3 %
Lysine	---	0.7 %
Methionine	---	0.4 %
Phenylalanine	---	0.7 %
Threonine	---	0.6 %
Tryptophan	---	0.2 %
Valine	---	0.8 %
E. Fatty Acids/Kg D.M. Diet		
Linoleic Acid	1% total calories	

* Purina 5038 [1]Estimate of requirement for old world primates

Table 6. Lesser Sulfur Crested Cockatoo Diet, Adult Female

A. Food Consumption Study

Food Item	Amount Offered	Amount Eaten
Apple	6.5 gm	4.0 gm
Banana	9.0 gm	1.5 gm
Orange	13.5 gm	4.0 gm
Carrot	4.6 gm	1.3 gm
Corn	17.0 gm	12.0 gm
Beans - string	5.0 gm	1.7 gm
Peanuts	7.0 gm	.5 gm
Sunflower Seed	16.0 gm	4.0 gm
Egg, h.b.	10.0 gm	0
Monkey Chow	14.0 gm	0
Total Weight	102.6 gm	29.0 gm
Dry Matter Weight	42.0 gm	8.0 gm

Percent of diet eaten = 28%

B. Diet Analysis - Gross Composition (dry matter basis)

	Diet Offered	Diet Eaten
Protein	18.7 %	14.3 %
Fat	18.7 %	18.4 %
Fiber	3.0 %	3.6 %
Ash	3.7 %	2.3 %
Energy	4368 Kcal/Kg	4366 Kcal/Kg

Table 7. Lesser Sulfur Crested Cockatoo Diet, Adult Female

Comparisons of selected nutrients between diet offered, diet eaten, and a published standard for an avian breeder diet; values expressed per kilogram dry matter of diet.

Vitamins	Diet Offered	Diet Eaten	Published Standard Avian Breeder (21)
A	12,785 IU	- 0 -	10,000 IU
Carotene	15,524 IU	29,106 IU	---
D_3	2,500 IU	- 0 -	1,500 IU
E	60 IU	74 IU	120 IU
Minerals			
Calcium	.7 %	.08 %	2.3 %
Phosphorus	.6 %	.4 %	0.6 %
Ca:P Ratio	1.2	.19	3.8
Copper	8.6 ppm	2.6 ppm	10 ppm
Iron	100 ppm	37 ppm	100 ppm
Iodine	.8 ppm	.09 ppm	.4 ppm

Table 8. Lesser Sulfur Crested Cockatoo Diet, Adult Female
Average Chemical Composition of Diet Eaten

A. Gross Composition D.M. of Diet

Protein	14.3 %
Fat	18.4 %
Fiber	3.6 %
Ash	2.3 %
NFE	61.2 %
Energy (ME)	4366 Kcal/Kg

B. Vitamins/Kg D.M. of Diet

A - Pure A	-0-
Carotene	23,215 IU
D_3	-0-
E	86.8 IU
Thiamine	7.2 mg
Riboflavin	2.5 mg
Niacin	45.6 mg
Pantothenic Acid	15.2 mg
Pyridoxine	4.2 mg
Folic Acid	0.5 mg
Ascorbic Acid	453 mg

C. Minerals-Elements/Kg D.M. of Diet

Calcium	.085 %
Phosphorus	.47 %
(Ca:P Ratio=0.18)	
Magnesium	.28 %
Potassium	1.06 %
Sodium	.01 %
Chlorine	.05 %
Copper	2.1 ppm
Iodine	.07ppm
Iron	41.7 ppm
Manganese	5.2 ppm
Zinc	14.3 ppm

D. Amino Acids, D.M. of Diet

Arginine	2.2 %
Histidine	0.7 %
Isoleucine	0.6 %
Leucine	1.0 %
Lysine	0.5 %
Methionine	0.2 %
Phenylalanine	0.6 %
Threonine	0.5 %
Tryptophan	0.1 %
Valine	0.6 %

Table 9. Isla San Esteban Chuckwalla Diet

A. Food Consumption Study

Food Item	Amount Offered in grams	Amount Eaten in grams
Apple	12.5	0
Banana	10	2
Orange	14	0
Grapes	7	2
Watermelon	32	0
Carrots	7.5	5.5
Spinach	17.5	0
Celery	11	0
Sweet Potato	6.5	0
Egg	6	0
Marmoset Diet, canned	21	3.0
Alfalfa Leaves, dried	5.5	2.5
Mixed Vegetables, frozen	15	0
Total Weight	165.5	15
Dry Matter Weight	30	5
Vitamin/Mineral Mix	0.15	0.014

Percent of diet eaten = 9.4 %

B. Diet Analysis - Gross Composition (dry matter basis)

	Diet Offered	Diet Eaten
Protein	16.8 %	18.0 %
Fat	6.0 %	3.4 %
Fiber	5.6 %	10.2 %
Ash	6.6 %	8.5 %
Energy (ME)	3412 Kcal/kg	3120 Kcal/kg

Table 10. Isla San Esteban Chuckwalla Diet: Average Chemical Composition of Diet Eaten

A. Gross Composition	D.M. of Diet
Protein	18 %
Fat	3.4 %
Fiber	10.2 %
Ash	8.5 %
Energy (ME)	3120 Kcal/Kg
	b

B. Vitamins/Kg D.M. of Diet	
A - Pure A	12,600 IU
Carotene	211,000 IU
D_3	5,942 IU
E	100 IU
Thiamine	9.5 mg
Riboflavin	13.7 mg
Niacin	68.5 mg
Pantothenic Acid	42 mg
Pyridoxine	6 mg
Folic Acid	3.8 mg
Ascorbic Acid	119 mg

C. Minerals-Elements/Kg	D.M. of Diet
Calcium	1.2 %
Phosphorus	.36%
(Ca:P Ratio=3.34)	
Magnesium	.25%
Potassium	1.9 %
Sodium	.25%
Chlorine	.31%
Copper	11 ppm
Iodine	0.5 ppm
Iron	248 ppm
Manganese	37 ppm
Zinc	20.6 ppm

D. Amino Acids, D.M. of Diet	
Arginine	0.7 %
Histidine	0.2 %
Isoleucine	0.5 %
Leucine	0.8 %
Lysine	0.6 %
Methionine	0.2 %
Phenylalanine	0.5 %
Threonine	0.4 %
Tryptophan	0.2 %
Valine	0.6 %

PROTEIN AND AMINO ACID UTILIZATION IN CARNIVORES

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INTRODUCTION

Protein has long been recognized as an important dietary component for all animals. However, animals do not require protein per se, but an array of amino acids. Not only is the total amount of each amino acid in the diet important, but also the pattern of the amino acids in the diet. A number of factors are important in determining the availability and utilization of proteins and amino acids in carnivores.

DIGESTION OF PROTEIN

The digestive tract of the carnivore can be divided into five major areas: the mouth, esophagus, stomach, small intestine, and large intestine. Once obtained, food is chewed in the mouth. The teeth of the carnivore are specially adapted for tearing and chewing the typical carnivore diet. Saliva is added to the food in the mouth. While saliva has no role in protein digestion, it does help lubricate the food, thus making it easier to chew and swallow.

The stomach has three major functions. First, it acts as a food reservoir. The stomach of the typical carnivore makes up 60-70% of the volume of his gastrointestinal tract, whereas the stomachs of the pig and horse make up only 29% of their respective gastrointestinal tracts. The large stomach volume of the carnivore allows him to consume food in large meals rather than eating continuously. Secondly, the stomach regulates the passage of food into the intestine. In addition, the stomach is involved in the initial stages of protein digestion. Ingestion of food initiates the release of gastric juice which contains hydrochloric acid, mucus and proteases-enzymes involved in protein digestion. Furthermore, the presence of food in the stomach causes gastric pH to increase from it's fasting level of 2 to a pH of approximately 3-5. This pH shift results in release of the hormone gastrin, which in turn stimulates further hydrochloric acid secretion. The acidic conditions of the stomach cause pepsinogen to be converted to it's active form, pepsin. Pepsin, the major proteolytic enzyme in the stomach results in the hydrolysis of protein to peptides.

The presence of acid causes peptides and fats in the duodenum of the small intestines to release the hormones secretin and cholecystokinin from the intestinal mucosa. Consequently, the pancreas is

stimulated to release pancreatic juice which contains a number of proteolytic enzymes: carboxypeptidases, elastase, chymotrypsin and trypsin. At this time, a number of proteolytic enzymes (enterokinase, aminopeptidases, and dipeptidases) are also released along with intestinal juice from the mucosa of the small intestine. The combination of proteolytic enzymes from the pancreas and intestine result in rapid hydrolysis of peptides to small peptides and amino acids.

The majority of amino acid absorption occurs in the distal duodenum and proximal jejunum. Specific transport processes exist in these areas of the intestine for the absorption of amino acids and small peptides, and their transport to the portal vein. From here, the amino acids are carried to the liver where they can be recombined to form protein, metabolized to other important metabolic products or catabolized. The liver releases a certain amount of free amino acids which can then be taken up by skeletal muscle and used for protein synthesis and growth. It is obvious, therefore, that the animal does not require protein *per se*, but instead requires an array of amino acids that make up proteins. While there are 22 specific amino acids, the animal can meet his needs for some of these amino acids by *de novo* synthesis. Ten amino acids are required in the diet of the carnivore.

FACTORS AFFECTING AMINO ACID UTILIZATION

A number of factors affect the utilization of amino acids and proteins. When dealing with carnivores it is important to consider the nutritional idiosyncrasies of this animal concerning amino acid metabolism. First, the cat has a high protein requirement. Second, he requires taurine, an amino acid derivative which is not known to be a dietary essential for any other species; and, thirdly, the cat responds severely to a deficiency of the amino acid arginine.

The protein requirement of the cat is higher than that of any other mammalian species (Table 1). While the protein requirement of most species decreases dramatically with age, the cat's requirement remains relatively high; the protein requirement does not seem to be related to a high requirement of any one amino acid. Instead, it is apparent that hepatic amino acid catabolic enzymes are fixed at a high level of activity (Rogers *et al.*, 1977). Therefore, cats cannot adapt metabolically to various levels of protein intake. Additionally, the enzymes of the cat have minimal ability to adapt to starvation.

A second idiosyncrasy of the cat is his dietary requirement for taurine (Anderson *et al.*, 1979; Hardison *et al.*, 1977; Knopf *et al.*, 1978). Taurine is known to be involved in many metabolic processes

although the mechanism behind many of these functions remains obscure at this time. A major function of taurine is bile acid conjugation. While most species can increase the incorporation of glycine into bile acids in the abscence of taurine, the cat cannot use glycine in lieu of taurine. Taurine is also thought to be involved in membrane excitability and neurotransmission. Brain taurine levels have been found to be low in neuropsychiatric patients and in epileptics. The threshold for convulsive seizures in experimental animals is increased by taurine. Therefore, taurine may have some use in controlled epilepsy. Since taurine concentrations are high in the heart muscle of hypertensive rats and patients dying from congestive heart failure, taurine appears to somehow be involved in these diseases. It may be involved in the regulation of potassium in heart cells. Additionally, taurine is involved in vision and olfaction. The most striking sign of taurine deficiency is central retinal degeneration, resulting in blindness.

Most species can synthesize taurine from the amino acid cysteine. The cat, however, has a deficiency of the vitamin B_6 dependant carboxylase involved in the synthesis of taurine. Additionally, the cat synthesized felinine and isovalthine from cysteine, products which are not found in the urine of any other mammalian species. Therefore, some of the cysteine flux is toward these compounds and away from taurine. This, along with the abligatory use of taurine in bile acid conjugation, creates a higher physiological need for taurine in the cat than in other species.

Taurine deficiency can be of practical importance. While taurine is found in high concentrations in most fish products (Table 2), it is essentially nonexistent in vegetables and cereal products. Muscle meat contains, at best, marginal levels of taurine. In addition, commercial dog foods which are based on cereals are extremely deficient in taurine when fed to the cat.

Another nutritional idiosyncrasy of the cat concerns urea cycle metabolism. The cat is extremely sensitive to arginine deficiency (Morris and Rogers, 1978 a,b; Costello *et al.*, 1980). Some of the symptoms observed are: hyperammonemia, frothing at the mouth, vomiting, ataxia, convulsions, and rapid death. These signs appear within two of ingesting a single meal of an arginine-free diet. Therefore, the cat appears to have little or no ability to synthesize arginine *de novo*. Because of this, the level or urea cycle activity is inadequate to handle the flux of excess nitrogen resulting from the consumption of a high protein diet. Uncer these conditions, urea cannot be formed and the cat dies of hyperammonemia. It is interesting to note that while the ferret also shows severe hyperammonemia in response to arginine deficiency, he does not die (Deshmukh and Shope, 1983).

Likewise, the chicken is unable to synthesize arginine due to a lack of carbamyl phosphate synthetase, ornithine transcarbamylase, and arginine synthetase in the liver (Tamir and Ratner, 1963). The chick, however, does not rely on the urea cycle and arginine synthesis to rid the body of excess nitrogen. Therefore, his response to an arginine-free diet would not be as severe as the cat's. The dog apparently has the ability to synthesize limited quantities of arginine de novo. While he cannot grow on an arginine-free diet and will show signs of vomiting and impaired urea cycle function, he can survive on the diet (Czarnecki and Baker, 1983).

As alluded to earlier, the essential amino acids must not only be present in the diet in the proper quantity, but also in the proper ratio to one another. Ideally, the amino acids should be present in the feed in the same ratio as the needs of the animal for protein synthesis. If these conditions are not met, the protein is said to be imbalanced and a depression in growth will occur if the protein is not properly supplemented.

In experiments conducted with dogs (Table 3), the addition of excess lysine to the feed resulted in a severe depression in gain and feed efficiency. Classic signs of arginine deficiency: orotic aciduria, depressed urea formation, hyperammonemia and emesis were observed in the dogs fed the high lysine diet (Czarnecki and Baker, unpublished data). These symptoms were alleviated somewhat by the addition of excess arginine to the high lysine diet. Therefore, lysine was depressing performance by inducing arginine deficiency. While it is unlikely that lysine would be present in a practical feedstuff in sufficient quantity to depress performance, the supplementation of commercial feeds with crystalline lysine and the possibility of a calculation or mixing error makes this interaction of practical importance. It also illustrates the importance of having the correct balance of amino acids in the feed.

Unfortunately, the perfectly balanced protein does not exist. Egg protein (white or yolk) is probably the closest to being perfectly balanced and is often used as the reference to which other proteins are compared (Table 4). However, even egg protein has it's problems, containing a great excess of the amino acid, isoleucine. Likewise, beef, and wheat gluten contain imbalances of amino acids. Wheat is deficient in many amino acids including lysine, the sulfur amino acids, threonine and tryptophan. The amino acid deficiencies of beef become readily apparent when it is compared to the amino acid requirements of the cat (Table 5). Beef is limiting in the sulfur amino acids methionine and cysteine, and in tryptophan. Therefore, the feeding of an all-beef diet, even if it is properly supplemented with vitamins and menerals, will result in depressed performance if it is not supplemented with the limiting amino acids.

FACTORS AFFECTING AMINO ACID AVAILABILITY

While the absolute amount of each amino acid in the feed and the ratio of the amino acids to each other are extremely important, the availability of the amino acids in the feed must also be considered. Although a feed may contain enough total lysine to meet the needs of an animal on paper, if only 50% of this lysine is available to the animal, it may be deficient. A number of factors affect amino acid availability: 1. Resistance of the protein to digestive enzymes; 2. Presence of inhibotors of digestive enzymes; 3. Processing conditions.

A protein may be resistant to digestive enzymes due to physical inaccessibility of the protein to the enzymes. Proteins are normally found intracellularly. If the cell wall is highly resistant to mammalian enzymes, the protein within the cell may never get the chance to be digested. Likewise, the presence of gossypol, an natural pigment found in cottonseed meal reduces amino acid availability. Gossypol combines with free amino groups in the protein to render them unavailable. Gossypol is extremely toxic to the pig, levels as low as 0.04% can be detrimental to performance and can even cause death.

The presence of inhibitors of digestive enzymes can also affect protein digestibility and, consequently, amino acid availability. Raw soybeans contain a compound which inhibits the actions of the protease, trypsin (Table 5). Feeding raw soybeans will, therefore, depress protein digestibility as evidenced by a reduction in weight gain and protein efficiency ratio (Rackis et al., 1975). Destroying the trypsin inhibitor by heating the soybeans results in increased efficiency of protein utilization and rate of weight gain.

Amino acid availability may also be affected by storage and processing conditions (Table 7). Heat treatment of feeds often results in the destruction of amino acids. Under conditions of heat and moisture, free amino groups of amino acids can react with sugars present in the feed by way of the Maillard or "Browning" reaction. The resultant amino acid-sugar complex is unavailable to mammalian enzymes. Common examples of Maillard proteins are toasted bread and the brown material that forms around eggs when you fry them. The amino acid lysine is especially susceptible to the Maillard reaction because it contains two free amino groups. Heating a protein source can result in a three or more-fold reduction in the bioavailability of lysine. Additionally, certain amino acids, such as the sulfur amino acids, can be directly destroyed by the heating process resulting in decreased bioactivity.

The protein digestibility of various feeds is presented in Table 8 (Kendall, 1982). Digestibility studies are typically conducted by feeding the feed in question for a period of 14 to 21 days then measuring

the amount of protein excreted in the feces and comparing it to the amount of protein consumed. While some proteins have a relatively high digestibility, eg., fish meal and meat meal, the digestibility of other protein sources is relatively low. It is important to note that digestibility of a protein merely represents the ability of the protein to be digested and absorbed by the animal. It says nothing about the amino acid pattern of the feed and, thus, the ability of the animal to truly use the protein for growth after it is digested. Meat meal, for example, has an apparent crude protein digestibility of 90% which would indicate that it should be a fairly good protein source. However, as mentioned earlier, meat is deficient in sulfur amino acids and tryptophan. Therefore, an all-meat diet would not be recommended despite it's apparently high digestibility.

CONCLUSIONS

In conclusion, we can see that many factors must be taken into consideration when examining the ability of an animal to utilize a specific protein source. Not only are the digestibility of the protein and the total content of amino acids of importance, but also the ratio of amino acids and the specific amino acid needs of the species in question are critical. All of these factors must be taken into consideration when formulating a diet for an animal.

Table 1. Protein requirement of selected species

Species	Requirement (%)[a]
Cat[b]	28
Dog[c]	22
Hamster[d]	15
Mouse[d]	12
Pig[e]	13-20
Rat[d]	12

[a]Dry basis

[b]NRC, 1973

[c]NRC, 1974

[d]NRC, 1978

[e]NRC, 1979

Table 2. Taurine content of selected feeds[a]

Item	Taurine content [b]
Beef muscle	362
Chicken muscle	337
Cod	314
Oysters	698
Clams	2400
Vegetables	--

[a] From Roe and Weston, 1965

[b] Expressed as mg/kg wet basis

Table 3. Effect of excess lysine on performance of the growing dog[a]

Treatment	Gain (g)	Gain/Feed
Basal	1138	.433
Basal + Arg	1150	.440
Basal + Lys	320	.171
Basal + Lys + Arg	870	.354

[a]Czarnecki and Baker, unpublished data

Table 4. Amino acid pattern of selected protein sources[a]

Amino Acid[b]	Egg Albumin	Beef	Wheat Gluten
Arg	374	402	230
His	151	203	124
Ile	403	323	284
Leu	548	488	450
Lys	450	537	126
Met	260	169	104
Phe	371	243	315
Thr	305	278	175
Trp	72	63	48
Val	466	321	266
Nutritive Value	100	80	44

[a] From Allison, 1964

[b] Expressed as mg/g N

Table 5. Comparison of beef to the amino acid requirements of the cat

Amino	% (Air-dry basis) Requirement	% (Air-dry basis) Beef
Arg	.83	1.50
His	.30	.75
Ile	.30	1.20
Leu	1.20	1.83
Lys	.80	2.01
Met & Cys	.90	def
Phe	.60	.90
Thr	.80	1.05
Trp	.15	def
Val	.60	1.20

Table 6. Effect of trypsin inhibitor on performance of rats[a]

Trypsin Inhibitor Content mg/100g diet	% Destruction	Body wt (g)	Protein Efficiency Ratio
887	0	79	1.59
532	40	111	2.37
282	68	121	2.79
157	82	134	2.97
71	--	142	3.03
Casein		145	3.35

[a]From Rackis *et al.*, 1975

Table 7. Amino acid composition of fish meal after heat treatment[a]

Amino Acid[b]	No Heat	Heat
His	2.10	1.30
Ile	4.20	3.65
Leu	7.15	6.60
Lys	6.50	2.15
Phe & Tyr	6.95	6.25
Met & Cys	4.65	3.05
Thr	4.20	2.70
Trp	.90	.80
Val	5.35	5.00

[a]From Madsen et al., 1965

[b]Expressed as g/16 g N

Table 8. Protein digestibility of selected feeds (dog)[a]

Feed	Crude Protein (%)	Apparent Digestibility of Crude Protein (%)	Digestible CP (% of DM)
Soybean meal	54	85	46
Soybeans	55	71	39
Fish meal	73	90	66
Meat meal	78	92	72
Meat & Bone meal	56	77	43
Rice	56	77	7
Wheatgerm meal	22	72	16
Potato by-product	11	65	7

[a]From Kendall, 1982

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Q: Dr. Watkins, I was just wondering for someone not quite so sophisticated in approaching these diets, what would be a good table or tables to start out with? Our approach is, we present a lot of food items and they eat whatever they want, and I would like to get away from this, but don't know where to begin.

Dr. Watkins: There is no single food table which is the best. I would say USDA Handbooks #8 or #456 are the most commonly used. You need to realize the limitations of the tables, particularly the energy levels, fiber levels and the USDA itself. You have to be able to interpret those values to some extent. You can't just take them at face value because there are analytical problems with that. I personally use all of the ones I talked about and sometimes if I have time I will even calculate the diet using different tables just to see what kind of difference I might get. I gives me an idea of the variability that could occur in the diet.

Q: Is it possible to compare data determined with one table to that which is prepared with another table?

Dr. Watkins: You have to be cautious because there are differences from one table to another. The food composition tables are just a tool. They have to be used along with practical experience, with observation of the animals, the animals' response, and so forth. The whole point of my talk is that you shouldn't use the food composition tables exclusively. I'm afraid there might be a tendency to do that when these data bases become computerized. It would be very easy to go and get a computer on a diet and then use that solely to evaluate the idet. I think over time our data base will improve and increase to the point where we can put more reliance on the food composition tables, but right now they can only be used as an adjunct to other methods.

Q: Ms. Czarnecki, is it possible to make up for imbalances in the amino acid composition by simply raising the protein level in the diet?

Ms. Czarnecki: Increasing the protein level actually will exacerbate existing problems because you are increasing the relative imbalances which you already have. You have to use a variety of

protein sources that compliment each other like corn and soy bean meal. These have very good complimentary patterns in amino acids, and what is deficient in one of them is usually made up for by the other one. An alternative would be to supplement with crystalline amino acids to overcome the deficiencies or imbalances that you might have.

Q: Dr. Snyder, I wonder if you would comment on the effects of foraging and the opportunity to have food throughout the day rather than specific hours, on the obesity and overweight problems that you might have.

Dr. Snyder: I think that when these apes consume the natural type of diets that have been referred to they tend to eat a fairly large quantity of low nutritive value foods. Most animal will have a tendency to overeat when offered highly palatable foods ad lib. So, I think we have two problems; one is that we are offering them highly palatable foods, and the second is that we offer enough volume to allow them to select an imbalanced diet. I think we need to try and find the balance between how much highly palatable food would be required to meet the animal's nutritional needs and then offer low palatability, high bulk or fiber foods in order to manage their time with browsing behavior.

PROVIDING PROPER NUTRITION FOR CAPTIVE ALCIDS AND PENGUINS

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INTRODUCTION

Penguins and alcids represent the most popular avian exhibits in today's zoo. Their unusual appearance and amusing behavior stimulate the imagination and amuse visitors of all ages. The special requirements of these birds in captivity, however, make their exhibition a major investment for zoos and aquaria. Exhibits designed to provide proper environmental conditions including temperature, photoperiod and substrate can cost millions of dollars. After the initial capital investment, daily care is labor intensive, and the birds are themselves expensive (Beldegreen and Asper, 1980; Stoskopf and Beall, 1980). Contributing to the operating expense of the exhibit, are the specialized dietary needs of penguins and alcids which may often represent a disproportionate percentage of the total zoo feed budget (Oftedal, 1983).

The logistics of obtaining high quality feed fishes in appropriate quantities, and the difficulties involved in storing and preparing diets for fish eating birds are major challenges to any commissary operation. Considerable detailed information is available to aid in the selection of food species for feeding and in the design of preparation and storage programs, but unless this information is utilized, very expensive malnutrition can be the result. Attempts to ignore or to oversimplify this important and complex question invariably leads to a suboptimal exhibit performance and frequently to disaster (Conway, 1959; Conway et al., 1977; Samuelsen and Ektvedt, 1975). Diagnosing a nutritional deficiency at routine postmortem examination is difficult, and clinical diagnosis is often limited to response to therapy, two problems which contribute to the poor understanding of major mortality outbreaks in wild and captive seabirds. These problems also lead to the temptation to cut corners (Biester and Schwarte, 1967; Fiennes, 1967). This is unfortunate since investment in proper nutrition for fish eating birds represents a long term cost saving through improved longevity, decreased morbidity and increased reproductive success.

LESSONS FROM THE WILD

All aquatic birds do not eat fish, and even more important, all fish are not alike nutritionally. It is extremely important to have some idea of the normal diet in the wild when designing a diet for captivity. Wild Emperor and King penguins feed primarily on shoaling crustaceans, squid and small fish (Notothenia) (Simpson, 1976). Gentoo penguins have similar feeding habits while other pygoscelid penguins feed almost exclusively on krill (Euphausia spp.) during much of the year, and supplement their diet with small shoaling fish (Pleura gramma) the rest of the year (Volkman et al. 1980). Eudyptes penguins, alcids, and petrels also utilize small nonfish prey extensively (Dorst, 1974; Plumb, 1965; Simpson, 1976; Terres, 1980; Tschanz, 1959). Spheniscid penguins are also known for a wide variety of prey including invertebrates (Crawford and Shelton, 1978; Davies, 1956; Rand, 1960). A generalized summary of the composition of the diet in the wild for penguins and commonly displayed alcids is given in Table I.

A cursory examination of Table I shows that most of these birds are known to take a variety of prey. Diets utilizing several components with different nutritional compositions will more likely provide the required balance of fats, protein and calories, as well as a balance of trace nutrients. Mixed diets also avoid the severe problem of single fish dependence in a colony. Captive seabirds have a propensity for strong preferences for a single type of fish if it is fed exclusively for any period of time. This predisposes to nutritional problems, but is also catastrophic when the preferred fish becomes unavailable due to seasonal catch variations (Stoskopf and Beall, 1980; Swennen, 1977). The short storage times for frozen fish make this event probable. Balanced diets featuring a variety of fish, often in rotation minimize these problems and actually ease logistics in the commissary.

A FISH IS NOT A FISH

The nutrient value of fish varies considerably from species to species, and even between individual fish of the same species. Seasonal differences occur as well as age and sex related differences. These differences occur in every nutrient category and greatly complicate the compilation of a complete diet for captive fish eating birds year round.

PROTEIN

Fish are generally considered excellent sources of protein, and as a rule of thumb, lean fish tend to have more protein and better keeping qualities. Particularly high protein contents are found in plankton eating species (Geiger and Borgstrom 1962). The percentage of protein required in a diet depends heavily on the energy content of the feed since higher energy feeds increase the protein content required to provide adequate nutrition when energy limiting rather than bulk limiting is in effect. High calorie diets (fatty fish) will have markedly lower bulk intakes in free feeding situations. Given two diets with 20 percent protein content, but one with twice the calories of the other, only half the amount high calorie diet will be consumed, delivering only half as much protein. Considering this, fatty fish provide an even leaner protein supply than indicated by straight protein percentage. Discussions of protein requirements as protein/energy ratios rather than simple protein percentage of feed can be more appropriate when studying diets of captive animals.

The Kjeldahl method of protein determination is still the most commonly used in determining feed protein content, and this method interprets non-protein nitrogen as protein. Over estimation of protein availability in fish occurs, particularly with marine species, which have high non-protein nitrogen levels in their tissues to help with osmotic regulation. This results in a major error in sharks (40%) but can also be responsible for as much as 18% error in protein levels of members of the herring group (Geiger and Borgstrom 1962; Dyer and Dingle 1961). On the other hand, protein is not absorbed from the gut intact, but rather as free amino acids and dipeptides. This is a factor in the digestibility of feeds, particularly marine invertebrates which have more free amino acids than fish (Borgstrom 1962).

The amino acid composition of fish and invertebrates generally approximates that of any other animal protein (Geiger and Borgstrom 1962; Borgstrom 1962; Dyer and Dingle 1961). This data suggests higher lysine and histidine contents than terrestrial mammal meats and lower levels of phenylalanine, and isoleucine. Methionine and cystine are reported to be the limiting acids, particularly in marine fish (Geiger and Borgstrom 1962). This can be particularly important in feeding alcids and penguins since the sulfur containing amino acids along with tryptophan are needed for normal growth and feather production (Biester and Schwarte 1967)

Sulfur Containing Amino Acids

The apparent deficiency of sulfur containing amino acids in most fish makes provision of a good source important in designing diets for penguins and alcids. Squid is considered notably rich in methionine (3.2%) as is herring and several peruvian marine fish species (Borgstrom 1962; Dyer and Dingle 1961). Cystine is actually abundant in many freshwater fish, but crustaceans such as shrimp can be nearly devoid of both cystine and the singular form cysteine.

Aromatic Amino Acids

Tryptophan is usually considered low in fish and is particularly in sardines. Exceptions include some freshwater fish including pike-perch, and marine invertebrates such as squid and blue crab (Borgstrom 1962; Dyer and Dingle 1961). Tryptophan readily breaks down to indole in fish that are handled improperly, further reducing availability of the amino acid, but providing a good monitoring technique for fish and shellfish spoilage (Shewan 1961). Tyrosine is also broken down by oxidation in poorly stored fish through the action of tyrosinases which are enhanced by contact with havy metal ions such as copper. This breakdown and the conversion of other phenols by phenolases to melanins is responsible for the appearance of dark spots on improperly packed shrimp and squid (Shewan 1961). Both of these invertebrates are rich in tyrosine. Peruvian marine fish generally have high content of tyrosine along with freshwater species. Skates and sharks are particularly low in tyrosine. Although fish are thought to be lower than terrestrial meats in phenylalanine, adequate amounts are usually available (Geiger and Borgstrom 1962).

Basic Amino Acids

Lysine is abundant in all types of fish, and is more abundant in males. It also accumulates during the spawning season making fish caught at that time of year extra good sources. Shrimp are also excellent sources of this amino acid (Borgstrom 1962). Conversely, arginine levels tend to be reduced during spawning, with content levels dropping in older fish as the ovaries mature. Shark and skate are good sources and high levels are found in atlantic mackerel. Histidine behaves similarly to argenine, being more prevalent in females and dropping during spawning. Red meat fish

are potent sources as well as fresh water bream, perch and carp. A major concern in high histidine content fish is the bacterial conversion of the amino acid to histamine which can reach toxic proportions in fish held at too warm temperatures (Eilenmiller et al. 1982; Kimata 1961).

Acidic and Aliphatic Amino Acids

Glycine which is required for growth in avians (Bister and Schwarte 1967) is generally plentiful in fish. Valine and Alanine are also generally available, although some fish (carp) can be very low in valine. Leucine and Threonine are also relatively abundant in most feed fish, and glutamine is found in high concentration in proteins from shellfish (Borgstrom 1962; Geiger and Borgstrom 1962).

CARBOHYDRATES

Fish are for practical purposes devoid of carbohydrates, providing calories with the relatively plentiful fats. Shellfish and invertebrates, however, contain appreciable amounts of carbohydrate (Borgstrom 1962; Geiger and Borgstrom 1962) although much of this is in the form of complex chitin-like sugars. Their availability as an energy source in alcids is questionable but this is an important consideration when feeding species which normally prey on invertebrates in the wild.

LIPIDS

The fat content of fish is perhaps the most important aspect of its nutritional impact in an alcid or penguin diet. It is also the area which is least understood. Quantitative changes occur between feed species and within feed species. These variations are seasonal and may reflect dietary differences in the fish. Less frequently considered but extremely important are the qualitative differences in lipid composition which also vary between and within species.

Generally fish lipids are monobasic aliphatic compounds with ethylenic type unsaturations. Saturated fats are found, primarily palmitic acid (10-18%) and stearic acids in lesser quantities (1-2%), but fish lipids are highly unsaturated in comparison to vegetable and terrestrial mammal fats (Notevarp, 1961). Species differences in lipid quality are most marked between freshwater and marine species. Freshwater fish are essentially lacking in hexaenoic and pentaenoic acids. Their lipid is characterized by short chain fatty acids with low unsaturation. This can result in deficiencies of specific essential fatty acids when fed to marine

birds. For example, little or no linolenic acid is found in freshwater fish. Marine foods on the other hand, are high in long chain highly unsaturated fatty acids. This is particularly true of marine invertebrates. Krill is high in unsaturated fatty acids, and squid has two to four times the content of hexaenoic and pentaenoic fatty acids as marine fish (Fricke, H. et al., 1984; Notevarp, 1961).

Seasonal variations in lipid content and quality can be considerable, and bear careful consideration in the logistics of year round diet planning for penguins and alcids. Fat content is generally higher in young fat fish, typically caught in summer catch. Summer herring, mackerel and sprat have more trienoic and tetraenoic acids than fish caught at any other season. Older, post spawning fish are routinely lower in lipid content and quality (Notevarp, 1961). Supplementation with invertebrate feeds may be even more important in winter and early spring diets to achieve daily essential fatty acid requirements.

Recent experiences have indicated problems of weight loss and mortality in alcids fed a high percentage of smelt. The situation is apparently exacerbated by feeding lake smelt. Smelt are deficient in two essential fatty acids required for molt and reproduction in penguins and have been implicated in reproductive failures and increased feather matting in these birds (Gailey-Phipps and Sladen, 1982; Penny 1978). It is possible that similar problems are being encountered in alcids. Examination of such subtle effects of the diet on the physiology of these birds will undoubtedly uncover the answers to other confusing contradictions.

VITAMINS

Vitamin A

Fish are generally considered to be excellent sources of Vitamin A, but vitamin A is a fat soluble vitamin with an easily oxidized unsaturated side chain connected to a six carbon ring. It is highly susceptible to poor handling and storage, its fate being linked with the availability of antioxidants including vitamin E. Specific requirements for vitamin A in penguins and alcids have not been established, but levels determined in poultry (4400 - 5500 IU per Kg feed) fed fish oils, provide a reasonable starting point (Bister and Schwarte, 1967). Vitamin A is important to retinal function and is required for proper growth an embryonic development. It has a stabilizing effect on membranes and may affect the synthesis of mucopolysaccharides and cholesterol. It is

important in steroid interconversions which can affect reproduction, as well as the proper development and maintenance of epithelial structures.

The symptoms of vitamin A deficiency are slow to develop due to the general ability of the body to store the vitamin, and many months on a deficient diet may be required to elicit symptoms in adult birds. Symptoms may begin subtly, first becoming significant by affecting the bird's resistance to infectious dieases including viral infections (Bang, et. al., 1972). Signs of overt hypovitaminosis A include emaciation, poor feather condition, decrease in egg production and hatchability. Poor growth, ataxia, loss of pigmentation in beaks, and suppression of endochondral bone growth are other signs. Only one report of vitamin A deficiency is reported in seabirds, in a poorly substantiated case of mortality in penguin chicks (Dekker, 1967).

Vitamin A accumulation in fish is thought to be dietary in origin. It is more abundant in older fish and more plentiful in liver and skin than in meat. Seasonal changes associated with plankton availability occur as well as variations based on catch locality. No strict taxonomic relationships have been correlated with high levels of vitamin A, but predatory fish seem to have relatively high levels. Muscle levels range between about 50 and 150 IU per 100 g tissue with the low values being seen in skates, and carp and high values in swordfish and lamprey. Mackerel is a potential feed fish which contains relatively low amounts of vitamin A and also contains an oxidizing enzyme which destroys vitamin A in its tissues (Higashi, 1961).

In judging fish for vitamin A potential, certain rules can be applied. Most vitamin A is stored in the liver, making whole fish vastly preferable to cleaned fish, and dark colored livers generally contain more vitamin A than light livers. Essentially 90% of the vitamin A in fish is in the form of esters. Marine fish contain almost entirely retinol (vitamin A_1) while freshwater fish tend to contain 3-Dehydroretinol (vitamin A_2) which has only 30% of the vitamin potency of retinol (Goodman and Gilman 1975; Scott and Norris, 1967)

B Vitamins

The water solubility of the B vitamins makes them very susceptible to loss in fish juices during thawing, and the symptomatology of B vitamin deficiency is often confused since frequently more than one B vitamin is depleted in this manner.

Deficiencies of B vitamins have been identified as the cause of death in young alcid chicks being fed thawed smelt (Conway et al., 1977), and in adult rockhopper penguins receiving herring fillets (Samuelsen and Ektvedt, 1975). The disease in rockhoppers presented as ataxia, abnormal posture, and increased respiratory effort, followed by seizures and coma leading to death within 24 hours. Antibiotic therapy was unsuccessful, but affected birds recovered when injected with B vitamins.

The vagaries of thiamine (B_1) in fish diets are well known and have been discussed in detail by Geraci, 1974 and Higashi, 1961. In general, fish contain adequate amounts of thiamine, dark meat containing two to ten times as much of the water soluble vitamin as white flesh. The largest concentration of thiamine in feed fish is found in the eyes. The true problem with thiamine in total fish diets involves the presence of degradative thiaminase enzymes in fish flesh. Freshwater fish have a single thiaminase enzyme while marine fish have at least two with different pH maxima. Freshwater pond smelt and ayu have little or no thiaminase activity, nor do most invertebrates. Sardines have relatively little thiaminase as well. Thiamine deterioration in stored fish due to bacterial origin thiaminases is a factor to consider in these fish. The issue of thiamine supplementation must be decided by an institution's ability to utilize proper thawing techniques on well frozen fish. If this is not routinely the case, it is reasonable to consider supplementing thiamine at a level of 25-30 mg per kilogram of fish fed (Geraci, 1974).

Riboflavin (vitamin B_2) and its two coenzyme forms (FMN and FAD), are quite plentiful in fish with the bulk being in the form of FAD. The signs of riboflavin deficiency are difficult to differentiate from those of other vitamin deficiencies, and include glossitis, dermatitis anemia and cataract formation. Of particular note is the possibility that riboflavin deficiency might be a factor in lymphopenias frequently seen in captive alcids just prior to and during episodes or respiratory disease. Riboflavin deficiency is known to cause a marked decrease in lymphocytes along with heterophilia and increased hematocrit in poultry (Goff et al., 1953). Although most fish have considerable riboflavin, excessive loss of water and water solubles in the thawing process can grossly deplete riboflavin. Again, riboflavin requirements are not worked out in penguins or alcids and must be extrapolated from poultry recommendations, of 2 to 4 mg per kg feed (Biester and Schwarte, 1967).

Niacin (vitamin B_3) is a relatively abundant vitamin in fish with fatty fishes being particularly good sources. It is found in all tissues but is particularly abundant in the liver. Nothing is known of the requirements for this vitamin in alcids or penguins, and poultry recommendations vary between species significantly.

Pyridoxine (vitamin B_6) is important for the conversion of protein and carbohydrates to fat and particularly the synthesis of arachidonic acid from linoleic acid. Arachidonic acid is an important precursor of protaglandin synthesis. Prostaglandins are involved in reproductive hormone modulation, clot formation, polymorphonuclear leukocyte reactivity, renal blood pressure control and respiratory passage muscular control among undoubtedly many other important homeostatic mechanisms.

Fish muscle is a very good source of this vitamin, providing an average of about 7 micrograms per gram of tissue. Mackerel is particularly rich while herring provides an average of 5 ug/gm and cod only 1.5 ug/gm. Shellfish are generally a poor source of pyridoxine (Higashi, 1961). Poultry requirements for this vitamin range between 3 and 4.5 mg per kg feed (Biester and Schwarte, 1967).

Pantothenic acid is a part of coenzyme A and as such is very important to fat metabolism in alcids and penguins. Sardine, saury, mullet, ayu, and pelagic fish in general are good sources of the vitamin. Rockfish, sea robins and bottom dwelling fish are poorer sources. The highest concentration of pantothenic acid in fish is in the gonads but otherwise, dark meat provides more vitamin than white flesh. Poultry requirements range between 4.5 and 17.5 mg per kg feed (Biester and Schwarte, 1967).

Folic acid is found in most organs in fish. It is more abundant in white meat than dark meat, and mobile species such as eels, mullets and gobies tend to have higher contents of the important coenzyme than other fish. In general fish are a good source. Folic acid is susceptible to oxidation and therefore the content of antioxidants is important to help maintain high levels in stored fish.

Choline is an essential component of lecithin. Fish is an excellent source of the vitamin, providing 10 times as much as beef or mutton. It is possibly the source of the breakdown spoilage factor TMA.

Cobalimin (vitamin B_{12}) is generated in fish by intestinal microflora, and therefore the highest levels are found in internal organs, particularly the intestines. Fish with considerable dark meat are generally richer in this vitamin with herring (1.9 ug/100 gm) providing nearly three times as much as sardines (0.74 ug/100 gm) which provide yet half again as much as mackerel (o.3 ug/100 gm) (Higashi, 1961). Molluscs are particularly poor sources of this vitamin (Jacquot, 1962). Recommended levels in poultry are relatively poorly established at 0.012 mg per kg feed (Biester and Schwarte, 1967).

Vitamin C

Fish are particularly poor as a source of vitamin C, but this is not particularly critical since birds generally synthesize the vitamin in sufficient quantity for their needs. Vitamin C content is important, however, because of its antioxidant properties which play an important role in preventing the oxidation of vitamins such as vitamin A and folic acid. Generally speaking, the larger the fish, the less vitamin C per gram it will contain. The bulk of the available vitamin is found in brain and eggs, with higher levels being found in summer months. Marine fish have a hgiher vitamin C content than freshwater fish (Higashi, 1961; Jacquot, 1962).

Vitamin D

Vitamin D exists in many forms, each having different vitamin potency. Vitamin D_2, D_3, D_4, and D_5 are all present in fish, but most of the D vitamin is found in the form of Vitamin D_7, a ketone of D_3 with 5 to 6 times less potency than D_3. It is most abundant in the liver, and less fatty specimens are better sources of the vitamin. Good sources of the vitamin include menhaden, carp, bream, lamprey, pike-perch, roach, sardine, sheatfish, and atlantic redfish, particularly if caught in the early summer (Higashi, 1961).

Vitamin E

Vitamin E is an important antioxidant in fish flesh and as such affects the availability of other easily oxidized vitamins. It is found in most abundance in the gonads of fish, and is largely depleted in fish caught after spawning (Higashi, 1961). The importance of alpha tocopherol (vitamin E) in avian diets is still controversial. Many of the effects attributed to vitamin E deficiency including reproductive effects can be prevented by the provision of other antioxidants in the diet. It is possible that the main role of vitamin E stems from its ability to protect from

lipid peroxidation and free radical generation, an important attribute in any species feeding primarily on fish with its high content of unsaturated fatty acids.

Vitamin K

All fish are relatively rich sources of vitamin K. Deficiencies of these quinone ring compounds which play a role in blood clotting should be relatively rare in fish eating birds, and would most likely be attributable to malabsorption of the vitamin.

HANDLING AND STORAGE OF FISH AND SHELLFISH

Purchasing Fish

The initial step in obtaining feed fish, placing the order, may well be the most critical. The best storage and handling protocols will not improve the nutritional value of fish which arrives in poor condition. It is important to know your suppliers, and impress them with the fact that you will accept only first rate, table grade fish. Beware of bargain prices, they seldom if ever represent a true savings by the time waste is culled (Oftedal, 1983). If at all possible you want to deal with a supplier which catches and packs the fish. This will often allow you to specify packaging and handling techniques which will optimize the value of the fish to you. Individually quick frozen fish (IQF) are best and are worth the extra expense if the process is available. IQF fish are frozen soon after catch and individually glazed in a process which helps retard storage oxidation and dehydration while at the same time making the fish easier to separate for thawing later on.

The size of the fish should be specified to avoid as much cutting as possible. This not only takes man hours but causes greater vitamin and nutrient loss. Not all species will be available in small sizes, a factor dependant upon season and fisheries regulations. A good supplier can help you examine the legal options. If IQF is not an option, consider the size of the individual packages of fish. Ideally they should provide exactly one day's feed with no leftovers. This will rarely be the case, but request packaging sizes which will minimize waste when complete boxes are thawed. Insist that each box be dated with the catch day. Always request a history of the catch. Details such as the occurrence of a red tide shortly before or during the catch could have significant impact on your colonies' health (Armstrong et al., 1978). Knowledge of the catch can be extremely helpful epidemiologically as well, since local and periodic occurences of

pesticide and heavy metal pollution can have a health impact on alcids and penguins feeding on these fish (Bourne, 1976; Scott et al., 1975).

You should carefully consider the time between catch and freezing. While salmonella organisms are generally inhibited in seawater, human contamination of the catch becomes more important as time is spent in holds and processing bins. Other bacterial contamination (native flora, etc.) will also increase exponentially with time between catch and freeze. These bacteria are involved in vitamin degradation as discussed previously. They may also pose a health risk to your birds (Kapperud et al., 1983; Oelke and Steiniger, 1973). Sanitation during this entire process and speed will affect the bacterial load of the end product. Bacterial counts are generally not affected by freezing at proper fish storage temperatures (Buttiaux, 1961; Shewan, 1961). When you thaw, you will start with essentially what was present when the fish were frozen

Receiving Fish

The next step in obtaining feed fish is receiving the shipment at the loading dock. It is imperative that a knowledgeable person inspect the fish on arrival. Boxes should be checked for acceptable catch dates, and the condition of the cartons examined. They should be intact and protect against moisture loss. Boxes should be opened and fish examined in the frozen state. Fish that have been properly frozen, stored and transported at -30 C or colder will normally exhibit a cloudy lens. Fish frozen at higher temperatures will have a high percentage of clear eyes. Fish should be of the specified size and have uniform color in the center and on the edges of the box. Rarely will the truck wait for a more complete examination, so suppliers should be aware that you will reject a shipment after delivery on the basis of a more complete examination of the fish.

After the shipment has been moved to storage, a box should be picked randomly and thawed properly. The thawed fish should be firm, with intact skin. It should withstand handling without falling apart. The gills should be red and moist. The freshly thawed fish should be checked for odors and then cut. Several blood smears from hearts should be made and examined for hemolysis.

Properly frozen fish will have many intact red cells. Fish which have undergone thawing and refreezing will be hemolyzed. Finally the cut surfaces of the fish should be examined for dark spots and bruising. No shipment of fish is going to be perfect, but bruising and thaw damage should be held to economically acceptable levels or the shipment refused.

Storing Frozen Fish

Storing fish is the next major consideration which will greatly affect a nutrition program. Fish should be held below -30 C. Temperatures above this allow lipid peroxidation, and protein denaturation to occur. At -10 C definite deterioration occurs in even relatively stable fish species over just 3 months storage (Geiger and Borgstrom, 1962). If possible, a high humidity atmosphere in the freezer will help hold down inevitable dehydration. Different feed species should be stacked separately, and each carton marked with a shipment code which identifies the date the fish was received, or a predetermined disposal date. All boxes should be kept closed. Reasonable storage times vary from species to species. In general fatty fish are less stable than lean fish due to lipid peroxidation and free radical generation. Protein remains relatively stable at proper temperatures. Herring should not be held longer than 6 months. Mackerel is even more volatile and should not be used after 3 to 4 months storage. Very few fish provide reasonable levels of micronutrients after 6 months storage, and should be discarded.

Thawing is an important process in maintaining optimal fish quality. Improper thawing can increase vitamin loss, lipid peroxidation and bacterial growth, essentially reducing a good quality fish to a deficit. The common procedure of thawing frozen food in water contributes heavily to vitamin loss. Water soluble vitamins, and free amino acids are leeched out dramatically by this type of improper thawing which is often rationalized as a way to speed up the process, or to achieve a complete thaw. It should not be allowed. Thawing should be planned far enough in advance to allow air thawing under refrigeration, or if necessary, air thawing at room temperature. A complete thaw is desirable, but not necessary for polar penguins and alcids which are accustomed to feeding on prey which is the temperature of the frigid waters they inhabit. Fish for temperate and subtropical penguins may require more complete thawing.

DESIGNING DIETS FOR PENGUINS AND ALCIDS

There is no single food fish which can provide all of the nutrients required by captive seabirds. It is obviously necessary to utilize a variety of species to achieve a complete nutritional program. This means that some form of nutritional calculations are needed to balance a mixed feed diet. Which fish and invertebrate species are fed is going to depend heavily upon local availability. A number of different combinations will achieve the same result. This is a major advantage, since rarely is a given fish species available year round, and storage factors make it imperative that fish be bought in lots small enough to feed in 3 to 6 months.

Analysis

Achieving a balanced diet from several feeds, requires some knowledge of the feed components. It may be tempting to look up average values of nutrients from the literature and use those figures to calculate energy, protein, and water content for different feed fish in order to create a diet, but this shortcut can lead to a false sense of security. As mentioned above, seasonal and even geographic differences in a food species can be quite dramatic. Tabular data from the literature, while better than no data at all, does not reflect these variations. To get the most from expensive feeds, it is imperative that they be analyzed for at least proximate analysis and calories. Fine tuning of vitamin and amino acids requires even more extensive testing, but is usually not performed due to cost. Each new shipment of feed fish or invertebrates should be randomly sampled upon arrival and analyzed for calories, total protein, total fat, and ash content. The analysis can be performed in-house if equipment is available or the frozen fish shipped to commercial laboratories where the analysis cost will vary between 50 and 100 dollars per sample. This may seem like a large extra expense, but for larger shipments in the 1000 Kg range, it represents only about 5 to 10 percent of the purchase price. Savings realized through proper utilization can far exceed this expense by several fold. This data makes it possible to evaluate the shipping, handling and storage history of the lot as well as accurately calculate its contribution to the diet.

Abnormally high values for both protein and fat should be viewed with skepticism since this pattern often reflects low water content due to prolonged or improper storage resulting in dehydration. Fish arriving in this condition should not be accepted. While time for analysis makes refusal at the loading dock impractical, merchants and dealers supplying this type of product

can be served notice that you have documentation of delivery of unacceptable quality fish. Ethical suppliers will replace poor fish which has deteriorated in processing or transport. Others may think again before trying to pass off stale product on your institution, if they are informed that a repeat offense will result in being dropped from acceptable vendor lists. Remember, however, seasonal fluctuations in fat content can be dramatic, and are not under the control of the fish wholesaler.

It is possible to design penguin and alcid diets for energy limiting situations. This focuses attention on the most changeable component of the diet requiring it be managed closely. It also allows considerably more latitude in supplementation and places secondary concern on the most stable component, protein. Bulk limiting on the other hand, while easier to monitor, makes supplementation difficult and predisposes to negligence in lipid management. With energy limited diets, calories are abundant, and evidence of protein deficiencies can be corrected by decreasing the gross energy of the diet by substituting more lean foods. Note that food consumption measured by weight will increase dramatically if calories are decreased in an energy limiting diet. This may give an artificial sense of well being as the diet approaches bulk limiting energy levels. Likewise, a dramatic drop in intake may occur when high calorie fish are added to a diet, not necessarily indicating an impending disease problem. A new shipment of fatty herring, for example, can create such an intake drop if the proportions of the diet are not adjusted to compensate for the new energy source.

Monitoring Diets

Utilizing different species in the diet increases the complexity of calculations but helps assure availability of all essential lipids. A mixture of fresh and saltwater fishes or saltwater fishes and invertebrates will accomplish this as well as helping to balance sulfur containing amino acids and vitamins. It also increases the difficulty of monitoring intake, particularly in mixed exhibits.

Individual birds or species groups may not eat the different components in the same ratios as you provide. Unless birds are individually hand fed, and records of consumption kept, absolute dietary management will not be possible. This type of intensive management, although optimal, is not feasible in most institutions. Other means must often be used to monitor intake besides direct observation and recording. A feasible alternative is to weigh all feed delivered and all feed removed from the exhibit. With multiple

feed diets, an improvement involves weighing each diet item separately. Less exacting but acceptable is subjectively estimating the relative proportions of feeds offered and removed. This information combined with periodic spot observations on diet preference (noting first items selected), can help assess the true diet composition being delivered to the bird. This information should then be combined with routine weighings of the birds themselves before dietary corrections are made.

Birds that are gaining or holding weight appropriately while actively pursuing normal behavior are most likely receiving adequate nutrition. The lack of definitive knowledge of polar seabird nutrient requirements makes it necessary to overrule theoretical calculations when they conflict with actual observation of healthy disease resistant birds. In my experience this has rarely been the situation. More common by far is a situation where a theoretically reasonable diet does not support healthy birds. Most commonly, improper analysis, handling or delivery are discovered to be sabotaging the effort. Optimally weighing once a week under the same conditions of time and day and water exposure, will allow close fine tuning of diets as well as providing a sensitive indicator of other health problems. Birds are readily adapted to this type of procedure which also habituates them to handling in the event medical treatment is required. This type of manipulation has had no observable effect on reproduction in balckfooted penguins and puffins when sitting females are excluded from the activity. Monthly weighings are probably adequate in very stable situations, and should be considered the minimum monitoring acceptable in a well managed exhibit.

Supplementation

A major question concerning the nutrition of aquatic birds is, what vitamin and trace mineral supplements should be added to the diet? The documented occurrence of a variety of dietary deficiencies in captive seabirds argues for supplementation. (Dekker, 1967; Samuelsen and Ektvedt, 1975). The natural occurrence of antibiotic substances in the stomachs of Euphausia krill have led to the suggestion that krill eating species should be supplemented with antibiotics in their diet, (Burkholder, 1960) and extensive salt supplementations have been recommended in saltwater drinking species kept in captivity (Reuther, 1960). The ability of some institutions to maintain large breeding populations of penguins without any vitamin, mineral or salt supplementation argues against the need to provide diet supplements (Van Bocxstaele, 1978; Wheater, 1976). The quality of food fish provided, and its handling during

freezing, thawing, and presentation to the animal are the major factors which determine the need for supplementation. Supplementation should be based upon the quality of the diet available. Routine antibiotic supplementation is unwarranted. Examination of subtle effects of the diet on the physiology of alcids and penguins will undoubtedly uncover the answers to confusing contradictions. An example is the possibility that sodium chloride supplementation is more critical if birds are fed large amounts of invertebrates and therefore need to eliminate excessive magnesium and calcium loads (Gailey-Phipps and Sladen, 1982). This could help explain the different experiences of various institutions concerning salt supplementation. Considerable more research needs to be done before definitive recommendations in this area are possible.

SUMMARY

Wild penguins and alcids consume varied diets of a variety of prey species including fish and invertebrates. Different species of fish and invertebrates vary widely in both macro and micro nutrients, with no known single species providing all of the nutritional requirements of fish eating birds. Therefore, diets composed of several different species, optimally marine fish and invertebrates or marine fish and freshwater fish are necessary for the successful captive feeding of penguins and alcids. These diets should be monitored through feed analysis, monitoring of consumption and careful examination of body weights. The most critical aspect of dietary management of these birds is the knowledgeable purchase, storage and handling of high quality fish and invertebrates and the careful balancing of their nutrient properties.

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THE APPARENT DIET DIGESTIBILITIES OF CAPTIVE TIGERS (Panthera tigris spp.)

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Introduction

As zoological institutions expand their role as reservoirs for the world's dwindling genetic material, the nutritional aspects of their charges must be elucidated. The determination of the specific nutrients, their requirement and the attendant economics, is vital to the maintenance and propagation of the species within the captive environment.

Although felids are the most carnivorous family of the Order Carnivora (Theobald 1978; Scott 1968), nutritional investigations of wild felines have been limited, and diets have been developed by trial and error, or from extrapolation of the domestic cat's (Felis domesticus) requirements.

Tigers (Panthera tigris) as the largest of the felids, are consumate carnivores, requiring both a large quantity and high quality of protein diet. Conway (1980) notes that 1.2 million dollars are spent annually on feeding the captive Siberian tiger population. If the entire captive stock of all tigers were considered, the figure would jump to 6.0 million dollars (Hackenberger 1981).

Due to the nutritional and economic ramifications of a captive tiger population, furthur investigations are warranted. Studies which have dealt with the diet digestibilities of tigers to date (Barbiers, et. a., 1981; Morris et al., 1974; Wittmeyer Mills 1980) have only considered commercially prepared diets, with very limited sample sizes of tigers (n=4).

The purpose of this work was to investigate the digestibility of a chunk meat diet, supplemented with a vitamin and mineral mix, by a group of adult tigers. A second objective was to study the diet digestibilities among juveniles, of a commercially prepared feline diet and milk supplement mix.

Materials and Methods

The five day study was conducted at Hawthorn Circus Corp., Richmond, Illinois, from 19/01/81 until 23/01/81. No adjustment period was required as the study diet was identical to the diet which the cats had been receiving.

Initially the experimental group of adult tigers was comprised of 7 males and 7 females, however diarrhea and regurgitation of ingesta required the removal of two male Siberian tigers from the experiment; thus the final experimental group contained 5 male and 7 female tigers (Table 1).

The experimental tigers were identified by sex and subspecies (Table 1), according to their owner. As all tigers were captive born, their ages are well established (Table 1).

The tigers were housed indoors in individual cages (3.5 m x 3.5m) with concrete floors. All enclosures were maintained at 12 degrees C and provided a sleeping platform 1.8 meters above the floor.

The diet fed the adult tigers was chunk beef (Table 2). The amounts fed daily per tiger ranged from 3.86 to 5.12 kg on a wet weight basis (Table 3).

Feeding occurred every day at 1700 hrs. with the meat being dusted with a calcium lactate: Pervinal Vitamin mix (ratio 3:1) at the rate of 100 grams per kilogram meat.

The meat was individually weighed prior to being fed to the tigers (Table 3). Over the course of the trial, all feed introduced to the tigers was eaten and water was available ad libitum throughout the experiment.

Faeces were removed from the enclosures and weighed immediately following evacuation by the tiger. Upon weighing, the faeces were stored at -30 degrees D for subsequent analysis.

The juveniles were housed individually in enclosures identical to those of the adults. Five of the cubs (Table 1) (A-E) received similar diets of 1.58 kg (wet weight Nebraska Brand commercial feline diet (NB) (Table 2) and 320 gms. milk replacer (KMR) (Table 2) per feeding (Table 3).

One juvenile male slightly older than the rest was beginning to be fed chunk meat in addition to the NG and KMR, of which it received the same amount as the other cubs (Table 3). In addition this tiger was notable in that it was a white tiger (Table 1).

The feed and faeces of the juveniles were collected and weighed by the same procedure as the adults.

Total faecal collections of the 5 days of the experiment and samples of the diet were subjected to proximate analysis. The faecal samples were oven-dried at 43 degrees C while the feed samples were freeze dried. The dried samples were ground in a Wiley mill with a 1 mm screen and individually homogenized in a mixing machine.

The Kjeldahl method was used to determine protein N (protein = N x 6.25), and the total fat was extracted using andydrous ether in a Soxhlet apparatus. Samples were ashed at 600 degrees for 3 hours and the carbohydrate fraction of the diet was determined by the difference. Caloric determinations were made with a Parr adiabatic oxygen bomb calorimeter.

The calculated diet digestibilities of the captive tigers are presented in Table 4.

Results and Discussion

While tigers in the wild are intermittent feeders (Sankhala, 1977; Scott, 1968), within a captive regime they have traditionally been fed 6 days per week, with one day of fasting. Various methods of determining feed intake per day for captive felids have been proposed. Theobold (1978) suggests that tigers should receive 3.6 to 6.8 kg. per day, and that this amount be doubled for pregnancy and lactation. Scott (1968) observed that over a one week period, tigers ate one kilogram of meat for every 145 kilograms of body weight. Morris et al., (1974) fed approximately 3.6 kg/animal/day, while Wittmeyer Mills (1980) in her diet digestibility study with tigers fed at the rate of 0.92 kg. of DM/animal/day. Kailash Sankhala (1977), gives the average daily intake for a captive tiger as 10 to 12 kg. of meat. From these references, it is apparent there are wide discrepancies in the accepted amounts fed to tigers. The reason for the varied published figures is due to the practice of feeding large cats "to condition"; much as farmers in an agricultural milieu feed their livestock for production. In such a regime, the quality of the diet, for example protein and energy content, the condition of the animal, its level of production, thermal conditions, and many other factors are all considered in providing for the animals' nutrient economy.

Within a circus environment, this "feeding to condition" assumes very important status, for food is the reinforcer used in developing and maintaining the "tricks" or behaviors which tigers enact. These "tricks" result in an increased level of activity, thus engendering an increase in the animals caloric requirements (Farrel, 1968); Greaves, 1965). Thus there is the necessity of feeding performing animals "to condition" to avoid weight loss when performing and obesity when they are laid off.

The works of Morris (1974), Scott (1968) and Wittmeyer Mills (1980), suggest dietary intakes less than those received by our experimental tigers on a maintenance diet. These values were all derived from zoo maintained animals. Tigers housed in such environments would undergo significantly less physical activity than the circus animals, even though during the experimental period, the animals were not working. A general rule of thumb which is adhered to by the circus, is that once an animal returns to the performance, the amount of meat is doubled (Wade Burck, pers. com.).

The dry matter digestibilities (DM) and the proximate component digestibilities for the experimental adults are the highest to have been published for captive tigers (Table 5). The DM digestibility for the juveniles is approximately that recorded for adult tigers by other authors (Table 5).

In its role as one of the most carnivorous mammalian families, felids have evolved several unique features in conjunction with their protein requirements (Dickinson and Scott, 1956; Greaves, 1965; Scott, 1968).

The most significant facet of the cat's nutritional requirements is its high demand for protein (Scott, 1968). At the minimum, 30% by dry weight, or 29% of the calories must be available to a weaning kitten to permit growth (Dickinson and Scott, 1956). The requirement of the adult cat for protein falls to one quarter of the diet DM, or 22% of the calories (Greaves and Scott, 1960; Greaves, 1965). However, even the reduced adult requirement is in excess of the known requirements for other genera of the Order Carnivora (Scott, 1968).

The crude protein (CP) digestibility of the adult tigers is far higher ($p < .0001$) than any other reported value for this species (Table 5, although the values for tigers by Wittmeyer Mills (1980) and Morris, et al (1974) are close to the values of our juveniles (Table 5).

We would suggest that the adult tiger's higher CP digestibility is due to the diet. The meat which was fed the adult tiger was rigorously trimmed of any tendonous and connective tissue. While such tissue is protein, it is comprised largely of indigestible collagen, which, passing through the tiger and analysed in the faeces, would lower the apparent digestibility of the CP fraction. The lowered CP digestibilities of the juveniles of this study in Table 5 would support such an observation as all were on commercially prepared diets. However, while there are very signficant ($p < .0001$)differences between the adult and juvenile protein digestibility, two different factors could account for the different digestibilities. Both age of the tigers and the different diets could influence the digestibilities and within the experimental design there is no method for separating the two effects.

Silver, older than the other five juveniles, could have exhibited a diet or age effect if he had been fed as the other juveniles were. However, his feed consisted of meat-NB-KMR-mix, and as a result his digestibilities, which are higher than the other juveniles (Table 4), cannot be attributed to an age or diet function.

Within the adults there are no differences between the digestibilities of the species. The juveniles display a sexual differentiation of their digestive abilities ($p < .0001$) (Table 5). It must be remembered that the three females are siblings as are two of the males. Thus, rather than sex, the variation which does occur is perhaps the result of genetic considerations.

When compared to diets suggested for domestic cats (Greaves, 1965; NRC, 1978; Scott, 1968), the proximate analysis of both adult and juvenile diets reveals a high quality feed. However, economic concerns must be considered. Wittmeyer Mills (1980) in her investigation of lion and tiger diet digestibilities considered the cost of the various diets. Using a male lion's energy requirement of 10500 kcal/day (Schaller, 1972), Wittmeyer Mills calculated the feed costs.

Diet A: \$3.20/day[1]
Diet B: \$4.01/day[1]
Diet C: \$1.76/day[2]

While diet C is the least costly feed, is was unacceptable for consumption by lions and tigers as the tigers refused to eat it, and the lions which did, developed diarrhea.

The 10500 kcal/day which Schaller developed is based upon free living individuals. Using Schaller's value we would calculate:

1 gm. chunk meat = 6 kcal. (Table 2)
10500 / 6 = 1750 gms = 1.7 kilograms

As the cost of the chunk meat is \$.53/kg, it would seem that on average we could feed tigers for about \$.93/feeding.

The original rise in popularity of the commercially prepared diet came about under the auspices of Radcliff (Hediger, 1969), and was in response to the incorrect calcium:phosphorus ratio of animal meat. The ratio of calcium to phosphorus is important in both the absorption and utilization of these minerals. Scott and Scott (1967) have shown optimal utilization by the cat when dietary Ca:P ratios were 0.9:1 to 1.1:1. Supplementation of the meat as

[1] Horsemeat and meat by-product-based, frozen, commercial diet.

[2] Grain-based, dry, domestic cat commercial diet.

outlined within the methodology, should balance the Ca:P ratio and provide adequate amounts for optimal growth (see Table 2). Greaves (1965) notes that the highest kitten growth rate recorded was obtained on a beef heart diet, supplemented with Ca and Vit. A only. Cats require preformed Vitamin A in their diet since they lack the ability to effectively convert Beta carotene to Vitamin A.

It is apparent that if tigers are to survive in the future we must be their diligent keepers. By understanding their nutrient economy and the manner in which it interrelates with their anatomy, physiology and behavior, we can develop a holistic overview of the animal's biology. Through this understanding, hopefully, we can better facilitate their survival in the captive environment.

Table 1. Experimental Tigers

Subspecies	Sex	Age	Estimated Weight (kgs.)
Adults:			
Panthera tigris ssp.			
Jack	M	4 years	127
Babe	F	2 years	90
Overbite	F	4 years	95
Nasty	F	3 years	110
Indira	F	16 months	90
Panthera tigris tigris			
Bandola	M	22 months	123
Nazar	M	16 months	104
Saber	M	13 months	82
Honey	F	16 months	105
Mindy	F	22 months	110
Panthera tigris altaica			
Sheba	F	9 years	120
Panthera tigris sumatrae			
Roman	F	5 years	130
Juveniles:			
Panthera tigris ssp.			
Silver (white tiger)	M	9 months	50
A	F	180 days	23
B	F	180 days	23
C	F	180 days	23
D	M	165 days	23
E	M	165 days	23

* Weight estimation provided by owner J.F. Cuneo Jr. based upon known shipping weights of performing tigers.

Table 2: Diet Analysis (Dry Matter Basis)

Diet	DM %	CP %	EE %	TOTAL NFE %	ASH %	GE(kcal/g)
Meat[a]	29.7	60.8	32.7	2.8	3.7	6.0
Nebraska Brand[b]	39.5	50.0	28.5	10.5	11.0	5.8
Kitten Milk Replacer[c]	17.72	41.5	23.0	29.2	6.3	5.8

[a] Calcium lactate and vitamin/mineral supplement (Pervinal) added to give final concentration in diet of 0.93% calcium and 0.61% phosphorus (as fed basis).

[b] Guaranteed Analysis As Fed Basis

Crude Protein	Min. 19.0%
Crude Fat	Min. 12.0%
Crude Fiber	Max. 1.5%
Ash	Max. 4.5%
Calcium	Min. 0.6%
Phosphorus	Min. 0.5%
Moisture	Max. 62.0%
Vitamin A Min	16,500 USP units/kg
Vitamin D3 Min	1,870 USP units/kg

[c] Guaranteed Analysis As Fed Basis

Crude Protein	Min. 7.5%
Crude Fat	Min. 4.5%
Crude Fiber	none
Moisture	Max. 82.0%
Ash	Max. 1.5%

DM - Dry Matter
CP - Crude Protein
EE - Ether Extract (Fat)
NFE - Nitrogen Free Extract (carbohydrate)
GE - Gross Energy

Table 3. Total Feed Intake and Fecal Production - 5 Day Trial

Tiger	Feed Intake (kg) Fresh wt.	Dry wt.	Fecal Production (g) Fresh wt.	Dry wt.	Digestibility (DM) %
Adult					
Jack	22.7	6.7	455.90	155.33	97.7
Babe	21.7	6.5	516.03	137.68	97.9
Overbite	22.6	6.7	315.83	91.55	98.6
Nasty	21.7	6.4	504.61	140.59	97.8
Indira	19.3	5.7	845.51	165.10	97.1
Bandola	25.6	7.6	557.15	148.50	98.0
Nazar	19.5	5.8	484.47	131.22	97.7
Saber	19.4	5.7	438.91	114.09	98.0
Honey	22.4	6.6	394.40	108.78	98.4
Mindy	20.1	6.2	387.70	99.56	98.4
Sheba	22.0	6.6	1176.03	244.44	96.3
Roman	24.6	7.3	904.13	209.73	97.1
Juvenile					
Silver					
Meat =	6.9	2.00			
KMR =	1.6	0.28	1743.02	578.98	89.4
NB =	7.9	3.10			
	16.4	5.38			
A-E					
NB =	7.9	3.148			
KMR =	1.6	0.280			
A	9.5	3.4	2012.66	521.08	84.8
B	9.5	3.4	1834.18	514.22	84.9
C	9.5	3.4	1883.28	531.19	84.5
D	9.5	3.4	1612.25	480.08	86.0
E	9.5	3.4	1852.61	458.70	86.6

Table 4. Apparent Digestibility of Energy and Proximal Components.

Tiger	CP%	EE%	Total NFE%	DE%
Jack	98.2	99.3	75.4	97.9
Babe	98.2	99.6	77.0	98.1
Overbite	98.8	99.5	88.0	98.8
Nasty	98.2	99.3	79.6	98.1
Indira	97.6	98.9	73.1	97.5
Bandola	98.3	99.3	81.3	98.3
Nazar	98.0	98.9	83.6	97.1
Saber	98.4	99.1	80.4	98.2
Honey	98.6	99.4	85.7	98.5
Mindy	98.8	99.5	83.4	98.5
Sheba	97.2	98.5	67.0	97.4
Roman	97.9	98.9	73.9	97.6
Silver	93.2	98.2	67.5	93.3
A	90.9	97.9	66.9	91.1
B	92.4	97.8	63.4	90.1
C	91.1	97.7	60.3	90.5
D	91.5	97.6	66.7	91.5
E	92.2	97.7	66.6	91.9
$\bar{x}$ +/- sd	96.0+/-3.2	98.7+/-0.7	74.4+/-8.4	95.8+/-3.2

Table 5. Digestive Efficiencies of Captive Tigers

Sources	DM %	Energy[1] %	CP %	EE %	Total Carbohydrate[2] %
Hackenberger and Atkinson (present data)					
Adults (male)	97.7	97.8	98.2	99.1	78.9
Adults (female)	97.9	98.1	98.2	99.3	79.1
Cubs	86.0	91.4	91.7	97.8	65.2
Barbiers, et al.(1972)	88.7	91.8	88.2	98.9	----
Whitmeyer Mills (1980)	85.2	92.9	90.6	98.9	66.3
Morris, et al.(1974)	79.2	----	88.6	----	----

[1] DE (Digestible Energy) as a percent of GE (Gross Energy) (100 x kcal DE/kcal GE)

[2] Calculated by difference

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GENERAL SESSION DISCUSSION FRIDAY AFTERNOON

DECEMBER 2, 1983

Q: I have a question for Mr. Hackenburger. Do you notice any differences between the circus cats and the ones you see at the wildlife park as far as oral health? Do you see a difference in dental tartar buildup on the teeth between the animals that are taking chunk meat and the animals that are dressing down the food?

Mr. Hackenburger: I see a big difference in tartar buildup between those animals fed commercial diet and those which have been fed chunk meat. The tartar buildup is far less with a chunk meat diet than on the commercially prepared diet. The animals tend to be mentally healthier because of the occupational value of something to tear and work on. They tend to be far more active and not just slurping it up. The whole carcass diet seems better in that regard than the chunk meat diet. When we feed whole carcasses we don't even bother to supplement with calcium or phosphorous, and we haven't had any problems. Mind you, we don't start giving cats carcasses until approximately 14-15 months of age.

Q: Dr. Stoskopf, I am curious to know if you use salt supplementation for penguins or any sea birds?

Dr. Stoskopf: The answer is yes and no. In the penguins where we have been maintaining them in a fresh water situation we haven't supplemented them with salt. Traditionally at the zoo, the Baltimore Zoo, we started with salt supplementation a long time ago in dolphins, and that has just continued. We never put the alcids on a salt supplement.

Ms. Allen: An article by Geraci in 1972 pointed out the work he had done indicating that thawing fish in water, any kind of water will leach significant salt, what little there might have been.

Dr. Stoskopf: I guess my feeling is that if you handle the fish properly, storage wise and thawing wise, you probably don't have to supplement nearly as much. We have the advantage of being able to thaw under refrigeration. The food is held at -17° C and we store food for only about 2-3 months, depending on the food item and the food is thawed under refrigeration at 4° C. This system seems to be working.

Q: Do you store representative samples of each fish shipment to analyze in case of some future problem?

Dr. Stoskopf: That is an excellent point. It takes so long for some of these problems to show up, if you're dealing with a low level toxin, you may have completely used up the contaminated foodlong before you detect that you have a problem and it really is a good idea to save frozen fish back. The personnel that prepare the diet are also very important. We rarely have a fish get to an animal to be fed without everybody that is involved with that animal having dragged the boxes of fish out to show me one thing or another.

Q: Do you see any nutritional differences between fresh fish and frozen, and if so, what are the major differences?

Dr. Stoskopf: There is a remarkable difference in aplatability. There are some things that happen that I didn't really get into in my paper. There are oxidation processes occurring in freezing that can affect palatability and also effect the levels of certain nutrients. This is a surprise, but when you have the stuff frozen even at -30° C oxidation continues. You see a steady degradation of fats and some of the vitamins. There are color changes which we are interested in for display purposes. I'm not sure how life threatening it is if you reduce the color, but there is definite degradation. Dehydration is the other big thing that occurs. There are many other changes, but we can leave it at that. There is a big difference. Fresh fish is wonderful if you can get it.

Q: At what point does storing the fish become a nutritional problem, 6 months, 12 months?

Dr. Stoskopf: Some of the glazes that can be put on individually quick frozen fish can do a tremendous job of increasing storage time. The fat content of the fish is another consideration, some people suggest that capelin for example, is good for two years. On the other hand, most people will agree that mackerel has about a three month storage life. The general rule of thumb for herring is siz months turnover time. The deterioration of your fish is going to depend on what temperatures you are holding it at, how well it was handled before it was packed, and the bacterial contamination. It is very hard to judge the performance of a diet. To be really honest, most of us don't weigh our animals. We don't have many criteria for judging effectiveness of the diet other than if the animals are alive. That's the problem.

Comment: I have a comment along the lines of what you are talking about right now. One of the methods used by Mr. Oyarzun at the Metro Toronto Zoo involved requesting fish supplies to send single boxes of fish, with known batch numbers, to the zoo for inspection. This permits thawing and inspecting the fish before a large shipment is accepted. The other thing that can be done is called the seal test. We would actually feed these fish to the seals and wait 24 hours. You can observe whether they accept the fish and whether they have any diarrhea, vomiting, etc. If you go through that process, then you make your committement to the seller and have them ship your fish at that time. The problem with that is they can fiddle around with batch numbers and this type of thing, but if you get a reliable dealer, you may avoid getting stuck with a whole batch of fish that you have to have the dealer remove. This is difficult to do.

Q: You mentioned that you are economically farther ahead buying IQF rather than bulk frozen, are you basing this on thawing out, drying out, or what?

Dr. Stoskopf: Basically, I'm basing it on impressions on percent wastage. We don't need a seal test because our mammologists are a whole lot pickier than our animals. If anything gets past our handlers, the animals are going to just love it. Wastage becomes a major economic factor.

Q: Do you adjust your supplementation programs with penguins and alcids depending on your analysis of the fish, with the variety of fish that you are feeding, or do you use straight across the board supplementation programs?

Dr. Stoskopf: We are supplementing as little as we can get away with. We feed about 8 species to the alcids, and they are split into groups, lean and fatty basically. The two key ingredients are the squid and herring, which we try to remain constant. The curators balance between these two groups according to our analysis to maintain the energy level of the diet. I balance some of the amino acids and other constituents by altering species within the fat and lean groups. That's about as fine tuned as we can get. Vitamin supplementation is minimal. Absolutely minimal. We don't even supplement thiamine at this point.

Q: Some individuals are now testing hair samples to see the various vitamin levels and mineral levels. Can you do that with birds or other animals?

Dr. Stoskopf: We haven't done any birds. Dr. Oftedal was good enough to get me some sea lion hair on one of his trips because I just was curious about it. We also shaved a few sea lions during their

physicals and sent it off for analysis. I got all these bizarre results back, like lead, nickel, and aluminum. I don't know what to make of these results, but I think the conclusion on a very small sample is that they are real tough to interpret.

Dr. Oftedal: I agree with that. Hair analysis is something you see a lot of advertisements around for, "Get your health analyzed." "Send your hair to someone, they will tell you what's wrong with you." I think that the consensus among professional nutritionists is that the value of hair analysis in most situations as an indicator of nutritional status is questionable.

Dr. Meehan: One of the things I had considered in setting up this panel initially was a whole morning for talking about computer analysis of zoo diets. There have been several talks today on computerizing zoo diets and there are others out there that are working on it that didn't talk and if you have anything to offer I would like your comments.

Dr. Culley: I would like to make one comment on this. We have done some work at Mississippi and the thing I think we're running into and I think you will too, is that in order to set up a computerized diet form a national standpoint, you need to decide what criteria you are going to use to evaluate a diet. The criteria you use affects the type of diet that you choose.

Dr. Meehan: Are you talking about the criteria for performance of the animal?

Dr. Culley: Yes. For instance, if we are evaluating a protein source for a tadpole diet and we measure metamorphosis, we select one source. If we measure growth, we select another protein, and if we are looking at percent abnormality, we select yet another source. We are trying to come up with an index of 5 performance factors that we can combine to get a numerical index of overall performance. I think you will have a hard time comparing diets with no performance measure to use.

Dr. Stoskopf: Thant's a very good point. I think we need to be educated by the nutritionists, as to performance and criteria that are finely tuned. I'm sure there are better measures of performance than survival or even reproduction.

Dr. Oftedal: Before you can compare diets you have to have the date on composition of diets. What we would like to do is get together some kind of data base where anyone can send in a diet. We would like to have a master data base and a program to generate the calculated composition of the diet, and then try to evaluate performance. Regardless of the performance standard

we use, I think the first step is to try to collect and evaluate some of this information. Most people when asked to submit a nutritional breakdown on a mixed diet for any animal would be pretty hard pressed.

Dr. Meehan: The computer data base would make the analysis of the diet easier but, in a zoo situation, it's a little bit hard to change all the variables and measure performance. One of the projects that Mary Allen and I talked about doing a long time ago is analyzing the diets of gorillas at Lincoln Park. These animals have reproduced, and they seem to be in good health. You can kind of work backwards from that. You've got performance, then you work backwards and find out what the diet is, and then we can almost fine tune the diet. What I would like to do with the analysis would be to pinpoint areas where we may be oversupplementing, We could probably eliminate some items entirely, and if, after a year,we find out the performance is unchanged, then we can hone the diet down to what we think it ought to be.

Dr. Oftedal: There are some points that Dr. Stoskopf made that are very valid. There are certain types of foods at the zoo that diet tables aren't very helpful with; fish is one of those, at least certain types of fish. Fish have large seasonal fluctutions that diet tables aren't going to show. You have a two fold difference in energy content with fish, from one shipment to the next. There are other types of things like browse. Browse can vary tremendously depending on when you cut it. You can't just have "oak leaves" in a computer. These are items that you really need to be getting data about on your own.

Q: I would like Dr. Stoskopf to comment on the accuracy of the analysis of repeated samples of fish. If you break a box into three pieces and have three analyses run, how repeatable will they be?

Dr. Stoskopf: Our replications have been really good. We sent in split samples early on because we don't have the facilities to run basic proximate analyses in-house. We just ship out triplicates which was within our budget and replication was exceedingly good. We are not analyzing routinely for some of more difficult things. We are doing the real easy ones. We definitely had 95% confidence in the three runs of three triplicates that we sent for analysis.

Dr. Culley: We haven't been that lucky with ours.

Dr. Stoskopf: There is a factor I should mention that I'm glossing over. If you take from the periphery of the box versus the center of the

of the box, you'll have variation. The condition of the box is also important. Our samples are taken right as the stuff comes in, and it's taken on a random series of three boxes, and then pulled out of the center of each box, because most of our wastage is discarded from the edges. Only then do we have good reproducability. You could have less luck, that's for sure.

Dr. Culley: Our luck has not been that good. We have our samples run at an independant lab and replicate samples of anything that has repeated poorly.

Dr. Stoskopf: I guess what it is going to come down to, is how much trouble you are going to, in order to get a good sample. Obviously, you have got to expect some variation.

CONSIDERATIONS IN FEEDING EXOTIC CATS[a]

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Many animals that have been classified by zoologists as carnivores are actually quite omnivorous in their dietary habits. This is certainly true of most canids, and the giant panda is even more miscast since it is primarily an herbivore. However, felids appear to be true carnivores, not just on the basis of their natural dietary habits but also because their tissue metabolism seems uniquely designed to accomodate a meat diet. Another way of saying it is that cats, exotic or domestic, are not well adapted to the consumption of plants. The felid digestive tract is simple in structure - without compartments for microbial fermentation that would allow for use of plant cell walls - and food passes through quickly.

Because the felid metabolic system has evolved to deal with animal flesh as food, inclusion of vegetable material may pose special problems in addition to those concerned with digestion. For example, benzoic acid is a compound found in plants to which cats are very intolerant (Bedford and Clark, 1972). Plants also tend to be lower in their protein concentration than do animal tissues. Felids have maintained a rather strict carnivorous diet throughout their evolutionary history, and there is little evidence of selection pressure to conserve nitrogen. Thus, enzymes involved in the catabolism of amino acids seem unregulated (Rogers et al, 1977, Rogers and Morris 1980). This is an advantage to the cat consuming large amounts of protein since any excess can be immediately disposed of without a time lag for induction of transaminases and urea cycle enzymes. However, this failure to regulate amino acid catabolism imposes a particularly high and continuing dietary protein requirement.

Young felids have qualitative amino acid requirements very much like other mammals, although quantitative needs may be higher, particularly for S-amino acids and arginine. Adult cats, as opposed to adults of other species, also have a high arginine requirement. This amino acid can be synthesized slowly in the tissues of the adult dog, and since needs are low, arginine is considered dispensible for maintenance. However, if the diet of the adult cat does not contain arginine, a hyperammonemia develops, with emesis, muscular spasms, ataxia, hyperaesthesia, and death (Morris and Rogers, 1977, 1978). This appears due to the failure of the cat liver to synthesize ornithine which is required for the detoxification of ammonia by the formation of urea.

Another unique feature of the cat is its requirement for the B-sulfonic amino acid, taurine. The retina contains high concentrations

[a] For more details, see Morriss and Rogers (1983)

of this compound, and in its absence, the cat will develop a central retinal degeneration, resulting ultimately in blindness (Hayes et al, 1975). Limited amounts of taurine can be synthesized by the cat from the S-amino acids, methionine and cystine (Knopf et al, 1978), but if dietary concentrations of these amino acids are low, then ingredients must be included in the diet which will provide taurine. Since taurine is not present in plants, animal products must be used.

Other features of felids which reflect their flesh-eating habits include their inability to convert B-carotene to vitamin A, their inability to convert tryptophan to niacin and their limited ability to convert linoleic acid to arachidonic acid. Plants do not contain vitamin A or arachidonic acid, and may contain only limited concentrations of niacin. Animal tissues, on the other hand, furnish all three.

In summary, it appears that exotic cats should be treated as obligatory carnivores, and their diets should be relatively low in fiber and high in protein. Their diets should also contain taurine (or generous supplies of S-amino acids) and preformed vitamin A, niacin and arachidonic acid. This is not to say that felid diets should not contain plant matter. Rather, if cereal seeds, by-products or plant protein supplements are used, the nutrients unique to animal tissue and that are not present or that are present only in limited quantities in plant tissue should be given particular consideration.

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FEEDING STRATEGIES AND METABOLIC ADJUSTMENTS OF THE POLAR BEARS

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SEASONAL ACTIVITIES OF POLAR BEARS IN SOUTHERN HUDSON BAY

Between mid-November to the end of June, males and unattached females of all ages, and females with yearlings are on the ice of Hudson Bay, hunting seals. Mating of males and unattached females both 3-4 years of age or older, occurs in April and May. The ice break-up occurs in July and Hudson Bay remains open to mid-November. During this period, all bears are ashore on coast or islands. In mid-October, the pregnant females who have come ashore in July make their winter maternity dens on the coast. The young are born in the maternity den in early December. The mother nurses them, and mother and cubs are ready to leave the den and move out into the Bay ice in early March. These cubs are weaned during the month of June (Ramsay, 1982).

SEASONAL FEEDING OF POLAR BEARS

Polar bear feeding is related to its seasonal activities. In winter, while on the ice, polar bears migrate long distances, killing and eating primarily seals. It has been estimated that bears kill a seal about once every six days and that about 15 kilograms of blubber are consummed, representing approximately 50,000 kilocalories (Sterling, 1974). This is the calculated quantity of calorie needs required for the subsequent 6-day period of physical activity. The feeding strategy of the adult non-pregnant polar bear is to eat only the blubber and hide of the seal. Several investigators have noted this behavior. For instance, Sterling (1982) estimated that bears kill a seal about once every 5 days. Sterling and McKuen (1982) state that "after a polar bear kills a seal, it feeds predominantly on the blubber and often abandons the meat." Blubber is the preferred part of the seal. Exceptions occur, such as when a small seal is killed by a particularly hungry bear or by a female bear with one or two cubs. Then, a large part or all of the carcass is consumed. Sterling further noted that "skin and fat" were eaten first, sometimes in a very exacting manner. For example, one bear about 0.5 km distance (Sterling, 1975) was observed "carefully using its incisors like delicate clippers to remove only the fat from the carcass, leaving the meat."

From knowledge of black bear and grizzly bear physiology, this behavior of limiting food intake primarily to that of fat protects

the polar bear from the need to take in great quantities of water. If polar bears ate protein, it would generate urea formation, which would increase urine volume (Nelson, 1980) and, thus, the need for water. This would impose thermoregulating stress, because the temperature of the ice and snow consumed would require a great quantity of polar bear metabolism to warm it up to its 37° body temperature. The metabolic generation of such heat would drain fat stores of the polar bear's body, thus impairing its ability to survive.

Although meat consumption would disrupt the metabolic adaptation of the polar bear, consumption of fat would not. The only end products of fat consumption are carbon dioxide and water. These are excreted via reapiratory exchange. Therefore, there is no increased need for water consumption.

The animals who require protein, that is, lactating female bears, cubs, yearlings, and sub-adults, then can feed on the protein part of the carcass to supply their growth needs. However, once the polar bear reaches adult size, it is apparent that very little protein intake is needed over the period of time it migrates on Hudson Bay ice between November and June.

Although wild polar bears require adequate vitamin, mineral, and trace metal intake for survival, it is thought that the blubber of the seals is the cheif storage depot for these substances. Blubber, along with the hide of the seal,could supply essential nutrients demanded by winter activity.

When ice break-up occurs in July and the bears come ashore on coast or islands, a profound difference in feeding and migratory activity occurs. In the Cape Churchill area, bears are observed walking along the water, swimming, or sitting by Hudson Bay. There is very little evidence that vegetation is eaten. There are many caribou and snow geese in this area, but the summer bears ignore these sources of food. Only occasional scats (bear feces) (Nelson, 1980) are observed.

Further south in the Cape Churchill area is a large polar bear denning area. Here again, food seeking behavior is at a minimum; some polar bears observed did not leave their dens at all to seek water or food. Summer observations have shown that polar bears spend as low as 3% of their time feeding and the energy content of the food is low. Others have noted that bears spend little time feeding on the island during summer, and they support this observation by the fact that fat condition in the animals is evident upon arrival. Currently, it is felt that these animals can survive a summer by utilizing (Nelson, 1980) their fat reserves.

Studies on a group of polar bears in the denning area of Cape Churchill in the late summer of 1977 indicated that these animals were in a biochemical state of hibernation similiar to that found in black bears. Black bears in hibernation do not eat, drink, urinate, or defecate, yet they exist at a normal body temperature burning 4,000 calories a day for periods of time up to 9 months. The metabolic adaptations (Nelson, 1980) to achieve this feat have been reviewed elsewhere. Thus, if the polar bear were in this type of biochemical adaptation (and current data support this concept), it would be protected from the need to eat and drink during the period of July through November (Nelson, 1980).

SUMMARY

Polar bears appear to have the ability to hibernate in the summertime and therefore reduce or abolish the need for food or water at a time when seals are not available. In winter, polar bears have incorporated a feeding behavior into their strategy, so that by eating primarily fat, the least physiological stress is produced on their physiological adaptation to the desert wastes of the artic.

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RESULTS OF A DIGESTION TRIAL EVALUATING SIX SPECIES OF CARNIVORE

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Introduction

Digestibility of a feed or of nutrients within a feed can only be accurately determined by conducting a digestion trial in which the specific feed is fed to the species of interest. Digestion trials have been conducted with domestic livestock for over a century. The accuracy of the standard digestion trial depends on the accurate measurement of total feed consumed and total feces excreted. To attain these measurements digestion stalls or crates are utilized. The use of such crates is, however, time consuming, laborious, expensive and stressful on the animal. Combine these drawbacks with the rarity, size, physical capabilities and temperament of zoo animals and the impossibility of conducting such a trial with zoo animals becomes obvious.

The Indicator Method

Digestion trials can also be conducted utilizing the indicator method. Though not as old as the standard digestion trial, it has also been in use for over a century. It is based on a simple mathematical principal, that of ratios. An inert substance, an indicator, is mixed into the feed at a constant percent lever or concentration. The ratio of that substance to the same nutrients in the feces is also determined. The change in ratio is used to calculate digestibility.

In the digestive tract the food is digested and then absorbed into the blood stream. The indicator, however, remains in the digestive tract. As the food is absorbed the indicator is, in effect, concentrated. The amount by which the indicator is concentrated indicates the amount of food absorbed.

Total feed consumption and total fecal collection are not required when the indicator method is used. All that is needed is a sample of the feed, the known concentration of the indicator in the feed, a sample of the feces and a means of determining the concentration of indicator in the feces.

For the indicator method to be reliable the substance used

as the indicator must not influence normal digestion processes and must be palatable (Chandler et al., 1964). It must be insoluble in the digestive tract and must pass uniformly through the digestive tract (Schneider and Flatt, 1975).

Favorable comparisons of the indicator method to complete collection methods have been made. Chandler et al. (1964), working with dairy calves, reported the means of the estimates from the two methods to be approximately the same. They noted more variation in the digestibility coefficients obtained with the indicator. Schurch et al.(1952) obtained similar results with both indicator and complete collection methods using pigs. Lloyd and McCay (1954) working with dogs, reported the indicator methods to give satisfactory results as long as the collection period was of at least 4 days duration to compensate for day to day variation in indicator and nutrient excretion.

An indicator has been used to obtain digestibility coefficients for a variety of different species. Studies using the indicator method with ruminants, horses, hogs, chickens, rabbits, fox and mink, (Schneider and Flatt, 1975) have been reported. It has also been used in studies with humans (Irwin and Crampton, 1951). Morris et al. (1974) used it in a study conducted with badger and thirteen species of felidae.

Experimental Procedure

This study was conducted at the Memphis Zoological Park and Aquarium during the summer of 1982. Feeding and collections were made at the zoo with the animals remaining in their exhibit enclosures. Procedures at the zoo duplicated, as much as possible, the daily routine the animals were accustomed to.

Four commercially prepared diets were fed to six species of carnivore. The quantities needed for the study were donated for use in the study. The species used are listed in table 1.

For this study, polyethylene was obtained from Phillips 66 Oil Corporation. It was spherical in form, was 3 to 4 mm in diameter and weighed 591 g per liter. The polyethylene was mixed into the feed at 3% of the total weight prior to feeding each day. Each diet was fed for a period of from 11 to 13 days. The animals were fed once a day, seven days a week (they were not fasted). The time during which each diet was fed was divided into two periods. The first four days were the preliminary period. The preliminary period allowed for uniform passage of either the polyethylene or the polyethylene and the diet fed. The fifth through either the eleventh or thirteenth day was the collection

period for each diet. Feces were collected from the exhibit enclosures once a day. Immediately following collection the areas were washed down. This insured each sample of representing a 24 hour period.

Fecal samples were frozen at the zoo immediately after being collected. Samples of the diet were also taken and refrozen. All samples were transported to Mississippi State University for laboratory analysis.

Chemical Analysis

In preparation for chemical analysis fecal samples were dried in a force draft oven for 4 days at 80° C. They were then allowed to air dry for 3 days. After drying, samples were ground in a Waring blender and sieved through a #8 standard sieve to recover polyethyleve and remove hair.

Diet and fecal samples were subjected to the same chemical analysis: dry matter (DM), crude protein (CP), gross energy (GE) and ether extract (EE). The procedures used are those stated by the AOAC (1981). CP was calculated as nitrogen content times 6.25. A Goldfisch apparatus was used for ether extraction and an Oxygen Bomb Calorimeter was used in GE determinations.

Calculations

All values reported and the percent indicator in each of the four diets were calculated on a DM basis. Daily digestion coefficients for DM, CP, GE and EE were calculated for species. Single daily values for digestible CP, digestible energy and digestible EE were also calculated from the results of the chemical analysis of the diets done at the laboratory. The chemical analysis of the diets are given in table 2. The digestion coefficient of DM was calculated using equation 1 and the digestion coefficients of CP, GE, and EE were calculated using equation 2.

Results

Species x diet interaction existed for all variables tested ($P<.001$). Means of the apparent digestion coefficient the DM, CP, DE and EE are given in tables 3, 4, 5 and 6 respectively.

The digestion coefficients for DM (table 3) for diets two, three and four are statistically the same for all species except the aardwolf. For the African hunting dog, hyena, lynx and caracal the coefficients determined for diets two through four are higher ($P<.01$) than for diet one. The coefficient for diet two for the

serval is the same as diet one. The digestion coefficients for DM determined with the aardwolf progressively increase from diet one to diet four. The only statistical increase, however, was between the coefficients of diets three and four.

The digestion coefficients for CP (table 4) for all 4 diets were statistically alike for all species except for the aardwolf and lynx. For the aardwolf the coefficient for diet four was higher ($P<.01$) than for diets one and two. And for the lynx the digestion coefficient for CP for diet two was higher ($P .01$) than for diet four.

The results of mean separation for the digestion coefficients for GE (table 5) are similar to those for DM. The coefficients for diets two, three and four are the same for all species except the aardwolf and except for diet two for the serval and for diet four for the African dog are higher ($P<.01$) than for diet one. The coefficients for GE for the aardwolf progressively increased from diet one to diet four with diet three being higher ($P<.01$) than diet one and diet four being higher ($P<.01$) than diet one or two.

The final set of coefficients given in this report are those for EE (table 6). The digestion coefficients for EE for diets two, three and four for the African hunting dog, hyena, lynx and caracal are statistically the same. And in this case except for the hyena are higher ($P<.01$) than diet one. Progressive increases of the coefficients from diet one to four were observed for the aardwolf and serval. The coefficient for diets two, three and four were higher ($P.<01$) than for diet one for the aardwolf and the coefficient for diet four was higher than for diet two. For the serval the digestion coefficient for EE for diets three and four were higher ($P<.01$) than for diet two.

Summary

The indicator method is a potentially valuable research method for obtaining data on zoo animals. Polyethylene meets all requirements for an indicator substance and was well suited to the animals and diets in this study.

There was species x diet interaction for all variables tested. No one diet gave significantly higher digestion coefficients for all six species. The aardwolf was the only species which consistently had the highest digestion coefficient occur for one diet, diet four. Diet three was the only diet for which the digestion coefficients for all six species were statistically alike for DM, CP, GE and EE. Species differences between digestion coefficients

for the other 3 diets were not consistent. They appeared to be primarily due to the digestion coefficients for the aardwolf and the hyena being significantly higher or lower than those for all other species.

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Equation 1

$$\% \text{ digestible DM} = \frac{\% \text{ indicator in feed}}{\% \text{ indicator in feces}} \times 100$$

Equation 2

$$\% \text{ digestibility of each variable} = 100 - 100 \times \frac{\left(\frac{\% \text{ indicator}}{\text{in feed}}\right) \times \left(\frac{\% \text{ variable}}{\text{in feces}}\right)}{\left(\frac{\% \text{ indicator}}{\text{in feces}}\right) \times \left(\frac{\% \text{ variable}}{\text{in feed}}\right)}$$

Table 1. IDENTIFICATION OF ANIMALS USED IN THE STUDY

Species		No. of individuals of species	Sex	Origin	Age (mo)	Housed[a] (mo)
Common name	Scientific Name					
African Wild Dog	Lycaeon pictus	4				
			M	San Diego Zoo	8	4
			F	San Diego Zoo	8	4
			F	San Diego Zoo	8	4
			F	San Diego Zoo	20	13
Spotted Hyena[b]	Crocuta crocuta	2				
			M	imported[b]	108[c]	55
			F	imported[b]	108[c]	55
Aardwolf[d]	Proteles cristatus	1	F	imported	82[c]	70
Serval	Leptailurus serval	2				
			M	Salt Lake City Zoo	16	12
			F	Salt Lake City Zoo	18	13
Lynx	Lynx lynx	2				
			M	Baton Rouge Zoo	15	9
			F	imported	42[c]	30
Caracal	Caracal caracel	2				
			M	imported	42[c]	30
			F	Los Angeles Zoo	34	22

[a] Length of time each animal was housed at the Memphis Zoo prior to the start of the study.
[b] Hyena were imported and owned by the Baton Rouge Zoo, LA. They were on loan to Memphis Zoo at the time of the study.
[c] Estimated age only, the exact birth date is unknown.
[d] Captured from the wild.

Table 2. RESULTS OF CHEMICAL ANALYSIS OF DIETS

Constituents [a]	Diets			
	1	2	3	4
Dry matter (%)	36.5	40.6	43.9	47.0
Polyethylene (%)	7.81	7.08	6.58	6.17
Crude Protein (%)	55.4	45.2	44.9	39.8
Ether Extract (%)	16.5	37.6	38.6	43.7
Gross Energy (Kcal/g)	5.4970	6.2832	6.6413	6.4568

[a] All constituents are given on a dry matter basis.

Table 3 . MEANS OF THE DIGESTIBILITY COEFFICIENTS FOR DRY MATTER

Species	Diet 1	2	3	4
African Dog	59.1[a]	74.9[b]	77.2[b]	72.4[b]
Hyena	63.1[a]	76.6[b]	78.1[b]	78.4[b]
Aardwolf	63.5[a]	70.3[ab]	76.2[ab]	89.7[c]
Serval	57.8[a]	66.3[ab]	75.7[b]	74.6[b]
Lynx	57.5[a]	75.5[b]	75.7[b]	73.7[b]
Caracal	61.4[a]	74.6[b]	75.1[b]	74.6[b]

[a,b,c] means in the same row or column followed by the same letter are the same ($P<.01$).

Table 4. MEANS OF THE DIGESTIBILITY COEFFICIENTS FOR CRUDE PROTEIN

Species	Diet 1	2	3	4
African Dog	84.0^{a}	83.5^{ac}	85.8^{ac}	79.8^{ac}
Hyena	89.5^{ab}	92.0^{b}	89.0^{bc}	86.4^{bcd}
Aardwolf	82.2^{a}	79.9^{a}	85.3^{ab}	92.7^{b}
Serval	86.3^{a}	85.0^{ab}	86.2^{ab}	83.6^{ac}
Lynx	85.9^{ab}	88.4^{bc}	82.5^{ab}	80.6^{ad}
Caracal	85.3^{ab}	88.3^{bc}	85.5^{ac}	84.0^{ac}

^{a,b,c,d} means in the same row or column followed by the same letter are the same ($P<.01$).

Table 5. MEANS OF THE DIGESTIBILITY COEFFICIENTS FOR GROSS ENERGY

Species	Diet 1	2	3	4
African Dog	79.7^{a}	87.0^{bcd}	85.5^{bd}	84.0^{ab}
Hyena	82.3^{a}	92.4^{c}	91.3^{cd}	90.0^{bc}
Aardwolf	79.2^{a}	82.9^{ad}	88.7^{de}	94.6^{ce}
Serval	78.7^{a}	84.4^{abd}	89.1^{bd}	88.3^{bc}
Lynx	78.1^{a}	89.8^{bc}	87.9^{bd}	87.5^{b}
Caracal	79.0^{a}	89.0^{bc}	88.6^{bd}	88.4^{b}

^{a,b,c,d} means in the same row or column followed by the same letter are the same ($P<.01$).

Table 6. MEANS OF THE DIGESTIBILITY COEFFICIENTS FOR ETHER EXTRACT

Species	Diet 1	2	3	4
African Dog	92.5[a]	95.5[bce]	95.7[b]	94.4[b]
Hyena	96.8[b]	98.0[b]	98.5[b]	97.4[bd]
Aardwolf	90.6[a]	94.6[ce]	97.3[bcd]	98.8[d]
Serval	91.1[a]	92.8[ae]	96.8[b]	97.2[bd]
Lynx	91.0[a]	95.5[bce]	96.0[b]	97.3[bd]
Caracal	92.7[a]	96.5[bc]	96.2[b]	96.9[bd]

[a,b,c,d,e] means in the same row or column followed by the same letter are the same ($P < .01$).

DIET AND ORAL HEALTH IN CAPTIVE AMUR TIGERS (Panthera tigris altaica)

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Eight Amur tigers, aged 11 to 17 years, were assigned at random from age and sex outcome groups to one of two treatments after an initial oral health examination and teeth cleaning. One group received a commercial meat-based diet alone while the second group received that diet plus beef leg bones one day per week. After 25 weeks, the tigers were immobilized and oral health was assessed 72 hours after the last contact with bones. Erythrosin disclosing agent was used to stain plaque and calculus, and photographic slides were taken of the buccal and lingual surfaces of the maxillary premolars and of the lateral and frontal surfaces of the maxillary canines. Using planimetric measurements of projected images, combined plaque and calculus area was calculated as a percentage of tooth area and notes were made of the thickness and consistency of calculus. Plaque and calculus were present in both treatments, with a tendency toward less plaque and calculus area and thinner and softer calculus on teeth of tigers receiving bones. However, these

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differences were not statistically significant. Measurements of gingival sulcus depth, made in four quadrants around all macillary and mandibular premolars and canines, established that gingival sulcus depths were significantly ($P<0.05$) greater in control animals than in those receiving bones. Thus, to further evaluate the usefulness of bones, the study was continued. The teeth of all tigers were cleaned and polished except for those of three animals which were euthanitized for other health reasons. The rest of the tigers were reassigned to their respective groups. However, bones were offered twice a week, on Mondays and Fridays, in the second phase of this study. After 42 weeks, oral health was reassessed as before except plaque and calculus area were calculated both together and separately. Marked differences were evident in favor of the three tigers receiving bones as compared to the two tigers receiving the meat-based diet alone. While both groups exhibited plaque (since the examination was made 24 to 48 hours after bones were removed), there was little calculus, and it was of softer consistency on teeth of tigers receiving bones. Plaque and calculus accumulation was significantly ($P<0.01$) greater in the control group. Gingival sulcus depth was relatively less in tigers receiving bones, and the difference between groups was significant ($P<0.05$). It is concluded that when commercial meat-based products constitute the primary diet for tigers, the feeding of bones will improve oral health. Although direct comparisons were not made between once- and twice-weekly feeding of bones, the data suggest that the latter practice favors more frequent plaque dislodgement and markedly reduces calculus formation and gingivitis.

Q: I was curious in hearing Dr. Nelson speaking about the polar bears in the wild. It seemed he was saying that during this time of year they were utilizing fat, and they were actually shutting down the protein metabolism. It seemed they were selecting a diet that was very, very low in protein, normally. I was just wondering if as a diet therapy, would it be any good to mimic this?

Dr. Nelson: I guess if you tried something like this it would be important to get seal hair, hide and fat because I don't know enough of what's in that, but someone else in the room may. I think that's intriguing, but these are bears that have been raised in captivity for some time and we know that the polar bear does quite well on the diets that are fed to them in zoos. It would be interesting to consider trying something like that, lowering the protein certainly wouldn't hurt an animal over a period of time, if it were done gradually, because the mammal adjusts its metabolism to the amount of protein it eats. If a person were to try something like you suggested, it would be just a very slow decrease of protein and a gradual increase in the fat content as an initial trial to observe whether something happens.

Dr. Meehan: Just a comment. I've got before me several analyses of diets. These are product data for the commercial diets and I welcome anyone that's got better information or information on newer diets they are coming out with. Generally, the omnivore diet has considerably lower protein than what we feed polar bears. What we have done is adjust up on the amount of protein. The bears may do very well on that, but it seems to be getting away from the more natural diet. Since whole meat makes up the protein part of the diet, you could make a diet that was certainly less expensive if you cut the protein in half and as a former zoo keeper, the idea of bears that don't urinate or defecate is very appealing.

Dr. Nelson: We had the cheapest experiment going when we had bears in captivity. They were charging us for care each day and I pointed out that we don't have to do anything for them for months, so our budget went down to zero at that time. Good point.

Dr. Ullrey: I wonder if I could ask another question of Dr. Nelson. In the regions where polar bears live you have got probably the most extreme photoperiodic changes that exist anywhere in the world and the regulation of some of these physiological processes is certainly under the influence of photo period. If you move a polar bear out of the artic to the northern temperate regions here or subtropical regions of Florida or Southern California, it is possible that the triggers that govern metabolic processes might be quite different. In this case, if you tried to simulate the arctic pattern of food supply and behavior you might be wrong too.

Dr. Nelson: That's a good point, but from our studies of the bears in Colorado, about 50% of the bears appeared to be in the biochemical state, as demonstrated by urea: creatinine ratio in August, at a time when there was still food available. They stopped eating, would roam about and not eat anymore and then go in the den when it appeared they felt like it. I don't know how photo period really affects denning behavior in bears in the U.S. We know the ambient temperature appears to be not a factor, because they hibernate in Arizona and Tennessee at temperatures 50-60° Farenheit. The only time we do know that the polar bear's food intake is zero, and we know that it's zero where they keep the bears, is in the summertime when there is quite a bit of light, in the July-November time. Your question is a good one and I think that we have to know what is in seal blubber and hide and hair, so forth, and then carry our a controlled feeding experiment to get an idea of what are the essential nutrients.

Dr. Meehan: I have a question regarding that for Dr. Nelson. How reliable is a one shot serum sample for getting urea: creatinine level, and I would suggest if that is rarely reliable, there are probably very few veterinarians in zoos that don't get routine CBC's and serum chemistries when they have bears immobilized. It might be a little bit tedious, but it would be possible to do a fairly good retrospective study to look at what times of year and in what areas the bears are metabolically hibernating.

THE EFFECT OF CRICKET CALCIUM LEVELS ON CALCIUM LEVELS IN TREE FROGS AND GECKOS*

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Introduction

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

Certainly, there are documented examples of unusual characteristics of the calcium homeostatic mechanisms of some reptiles and amphibians. For example, some possess well-developed endolymphatic sacs. These calcium carbonate-filled sturctures are continuous with the endolymphatic system of the inner ear. In amphibians (e.g., ranid and hylid frogs) they are sometimes referred to as paravertebral lime sacs. They may be found as bilateral sacs adjacent to, but not continuous with, the vertebral column. Dacke (1979) and others (Robertson, 1972; Schlumberger and Burk, 1953) have demonstrated that a number of substances, namely calcium, vitamin D_3, parathyroid hormone and calcitonin seem to influence the developmental state of these calcium carbonate deposits. Ruth (1918) was apparently the first to document the presence of the endolymphatic sac in Philippine house lizards and speculated as to their significance. They are sometimes visible as paired white structures in the ventral neck region in some lizards, particularly gekkonid lizards. It has been

* This paper was also presented at the annual meeting of the American Association of Zoo Veterinarians, Tampa, Florida, October, 1983.

suggested (Simkiss, 1967) that these deposits might respond to an ovarian hormone and function much as medullary bone seems to for birds.

It is well established that some captive insectivorous animals develop rickets, osteomalacia and depressed reproductive performance as a result of calcium-deficient or calcium-imbalanced diets. Some species of geckos appear to be particularly prone to the stress imposed by a low calcium diet (R. Montali, D. Marcellini, National Zoological Park, pers. comm.).

The present study was designed to investigate the effects of two dietary calcium levels on captive tree frogs (Hyla septentrionalis) and parthenogenic house geckos (Hemidactylus garnotii). The species were selected because of the likelihood of their having endolymphatic or paravertebral lime sacs, their insectivorous habits and their relative availability in southwest Florida. Radiography was used to demonstrate the existence of these structures relative to dietary treatment. Chemical analyses were conducted to measure differences in total body calcium. This study provided an opportunity to use crickets supplemented by feeding them either a high- or low-calcium diet, rather than using crickets supplemented by dusting with a calcium source. An 8% calcium level when fed to crickets has been shown to increase the cricket calcium level to at least 1% on a dry matter basis (Allen and Oftedal, 1982).

Materials and Methods

Crickets - Crickets (Acheta domesticus) approximately 3/8 to 1/2 inches were obtained from a commercial supplier*. They were maintained in aquaria on either an 8% or 1.2% calcium diet (Tables 1 and 2) with free access to water. Cricket calcium was determined at monthly intervals to confirm that the diets were having the desired effects. As determined earlier, keeping the crickets on the diets for at least two days (before offering them to the frogs and geckos) was required to consistently achieve the desired calcium levels (Allen and Oftedal, 1982).

Tree frogs - Thirty adult tree frogs, eighteen males (ave. weight 7.5 g) and twelve females (ave. weight 26.9 g) were purchased in late July of 1982 from a commercial supplier**. The tree frogs were caught

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* Flukers Cricket Farm, Baton Rouge, LA

** Herpetefauna, Ft. Meyers, FL

approximately ten days prior to air shipment. Upon arrival in East Lansing they were individually housed in 4 liter glass jars with vented tops and with approximately 150 cc of distilled, deionized water. Paper towels were provided for shelter. The jars were thoroughly cleaned twice weekly. After a two-week acclimation period with an ambient temperature of 25° C the tree frogs were weighed and randomly assigned to one of two treatment groups. At this time four frogs were randomly selected from the two treatment groups, two males and two females, and sacrificed by rapid freezing to provide baseline data for later comparison to the experimental frogs. The frogs were fed ad libitum and maintained for seven months on a 14:10 lighting schedule under fluorescent lights. The room temperature ranged from 20-26° C.

Geckos - Thirty parthenogenic house geckos (ave. weight 2.7 g) were purchased in late July from a commercial supplier. The geckos were caught approximately two weeks prior to air shipment. Upon arrival in East Lansing they were individually housed in 4 liter glass jars with vented tops. They were provided with paper towels for shelter and sponges moistened daily to maintain humidity. They were misted daily with distilled, deionized water. The jars were thoroughly cleaned at two-week intervals. After a three-week acclimation period, the geckos were weighed and randomly assigned to one of two treatment groups. Since losses during the acclimation period were significant (60%) five of these nineteen animals were randomly selected and frozen for later analyses (to provide baseline calcium levels). The experimental geckos were fed ad libitum and maintained for seven months on a 14:10 light schedule under fluorescent lights. The ambient temperature ranged from 20-26° C. An electrically heated pad (pig warmer) placed directly under the glass jars provided a jar temperature gradient of 22-30° C.

Radiography - The tree frogs and geckos were radiographed at approximately six-week intervals. Industrex (Kodak) M-2 high contrast film was exposed in a Faxitron (Hewlett-Packard) x-ray unit. Exposures were made at 40 kv (10 ma) for geckos, 45 kv (10 ma) for small frogs and 50 kv (10 ma) for large frogs. Exposures were 0.3 minute. The animals were restrained in vented platic bags to render them sufficiently immobile for radiography. Femur densities were evaluated from the radiographs with a densitometer*.

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* X-Rite (model 301), Grand Rapids, MI

Calcium analysis - Tree frogs and geckos were killed by rapid freezing at -20° C, freeze dried, quartered and oven dried. They were pre-digested overnight in nitric acid, subsampled and further digested with hot nitric and perchloric acids. Calcium was determined by atomic absorption spectrophotometry.

Results and Discussion

Tree Frogs - Dietary treatment did not appear to affect whole body calcium levels in the frogs. Mean calcium on a dry matter basis (DMB) was 4.20% ± 0.19 (SE) in the high calcium group and 3.97% ± 0.20 (SE) in the low calcium group. The control mean was 4.62% ± 0.34 (SE) (Table 3). These values were not significantly different from one another when examined by factorial analysis of variance allowing for unequal replication. It would appear that the sexually mature tree frog is able to maintain adequate calcium levels, when compared to whole body calcium levels of other animals, even when placed on 0.14% calcium diets. For comparison, a 30 g mouse contains 3.44% calcium on a dry matter basis (data of Widdowson, 1964, assuming 10% body fat on a fresh weight basis).

There was a significant difference ($P < 0.001$) between the means of male and female tree frogs. The mean calcium level of male tree frogs in control, high and low calcium groups was 5.04% ± 0.20 (SE). The combined mean value for females was 3.47% ± 0.20 (SE).(Table 4). Gender differences in calcium content in the rat have been reported (Spray and Widdowson, 1950). Females at 120 days evidenced a 25% higher calcium level when compared to males of the same age. The difference disappeared during pregnancy and lactation. The gender difference observed in the present study may be a function of comparisons made on a dry matter basis rather than on a fat-free body basis. For example, if one assumes that male tree frogs contained 20% fat (DMB) and females 40% fat (DMB), the respective calcium levels on a far-free, fresh weight basis would be 1.17% and 1.16% respectively. Unfortunately, preparation methods used in the present study precluded determinations of ether extract (fat), but there were distinct differences in the gross appearance of the tree frogs with females appearing much fatter. All female frogs had egg-filled abdominal cavities.

Differences in bone density in the frogs were not evident as measured by densitometry. It is recognized that the organic matrix of bone must be fairly well depleted, perhaps as much as thirty percent, before conventional radiographic methods can detect the reduced density of osteoporotic bone (Jubb and Kennedy, 1970). Photon densitometry, because of its increased sensitivity, may prove a more useful technique but is, as yet, not widely in use (J. Soares, U. of Maryland, pers. comm.).

Although hylid frogs share with ranids the likelihood of having well developed paravertebral lime sacs these could not be demonstrated radiographically in any of the frogs in this study.

Geckos - The mean calcium concentration (DMB) of geckos maintained on high-calcium crickets was 4.03% ± 0.10 (SE). This was significantly higher ($P < 0.025$, t-test) than 3.43% ± 0.27 (SE), the mean for the low-calcium geckos (Table 5). One of the five geckos in this group died after 2 1/2 months and a second died at 4 1/2 months after laying 2 eggs six weeks earlier. Otherwise one gecko in each group laid a single egg. These eggs appeared approximately three months after treatments were initiated.

The five geckos selected to provide baseline calcium levels had a mean calcium level (DMB) of 5.20% ±0.11 (SE) (Table 5). This was significantly higher than the means of either the low ($P < 0.025$, t-test) or the high (P 0.05, t-test) calcium groups. Since these animals were noticeably leaner when they died than were the geckos that were killed after seven months, this difference might be reduced or removed if comparisons were made on a fat-free basis.

There were no bone density differences, based on radiographic results, between high- or low-calcium treated geckos. The development and regression of endolymphatic sacs of geckos from both groups followed no clear pattern. Endolymphatic sacs developed and regressed from one to three times in each gecko over the course of the trial. However in those geckos that produced eggs, the endolymphatic sacs were well-developed and clearly visible six to eight days prior to egg laying. They were observed to be much reduced in size when viewed within 24 hours after egg deposition.

Conclusion

Unless additional calcium demands are placed on an animal by lactation, pregnancy, egg-laying or growth, acceptable dietary calcium levels might be as low as 0.2% (NRC, 1975). Therefore it is not surprising that adult tree frogs did not apparently suffer from a dietary intake of 0.14% calcium. All females from both treatment groups produced eggs. It would be of interest to determine whether reproductive efficiency as measured by viable offspring would have been different between the two groups. Further studies to determine if male and female tree frogs differ in whole body calcium on a fat-free basis would likewise be of interest.

It would appear that a dietary intake of 0.14% calcium is insufficient to maintain body calcium levels in the gecko equal to levels in animals receiving 1.10% dietary calcium. The calcium status in this species, and other species laying hard-shelled eggs, may be more subject to dietary calcium levels. While this study did not demonstrate a definite role of the endolymphatic sac in the gecko it is still possible that this structure serves as a calcium store to be mobilized prior to egg-laying. It would be useful to determine in more long-range studies if a definite and indispensable role is played by the endolymphatic sac and if dietary treatment can influence this structure in a growing gecko.

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Table 1.

CRICKET DIET

	Calculated Analysis	
	Low Calcium	High Calcium
Crude Protein, %	20.1	20.1
Calcium, %	1.2	8.2
Phosphorus, %	0.7	0.7
Vitamin A, IU/kg	61,500	61,500
Vitamin D3, IU/kg	7,200	7,200
Vitamin E, IU/kg	330	330

Table 2.

FORMULATION OF CRICKET DIETS

Item (%)	Low Calcium	High Calcium
Corn	28.6	6.7
Alfalfa meal	9.7	9.7
Soybean meal	23.7	27.8
Wheat	29.1	29.1
$CaCO_3$	1.65	19.4
Mono-dical phos	1.8	1.9
Salt	0.4	0.4
Corn oil	2.9	2.9
MSU VTM premix (a)	1.6	1.6
Se 90 premix (b)	0.11	0.11
Vit E premix (c)	0.12	0.12
Vit A premix (d)	0.17	0.17
Vit D_3 premix (e)	0.17	0.17

(a) 300,000 USP Vitamin A/lb; 60,000 USP Vitamin D_3/lb
(b) 90.8 mg Se/lb
(c) 125,000 IU Vitamin E/lb
(d) 30,000 IU Vit A/g
(e) 3,000 IU Vit D_3/g

Table 3.

CALCIUM CONCENTRATION OF WHOLE FROGS
COMBINED SEXES

	Treatment		
Males & Females	Control (n=4)	High Calcium (n=13)	Low Calcium (n=11)
% Calcium, DMB	4.62	4.20	3.97

Table 4.

CALCIUM CONCENTRATION OF WHOLE FROGS
COMBINED TREATMENT GROUPS

	Males (n=16)	Females (n=12)
% Calcium, DMB	5.04	3.47

Table 5.

CALCIUM CONCENTRATION OF WHOLE GECKOS

	Treatment		
	Control (n=5)	High Calcium (n=6)	Low Calcium (n=5)
% Calcium, DMB	5.30	4.03	3.43

PROGRESS AND PROBLEMS ASSOCIATED WITH BULLFROG TADPOLE DIETS AND NUTRITION

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Introduction

The systematic involvement of the Louisiana State University, School of Forestry and Wildlife Management in the development of culture techniques for the bullfrog (Rana catesbeinana) started in 1968. The National Institutes of Health joined the efforts in 1971 and in 1976 the Office of Sea Grant Development initiated support on the nutritional requirements of amphibians. Since the inception of the project techniques for both laboratory- and commercial-scale production have been developed. Even though many problems remain to be solved, particularly with regard to nutrition and disease control, the techniques and facilities developed on this project are now being incorporated in universities, medical schools, zoological parks, and biological supply industry. Hopefully, this increased culture effort will rapidly yield more efficient culture techniques.

Controlled culturing of bullfrogs, as with other "confined" animals involves providing an optimal experimental environment, reducing the inherent variability of the animals, and providing food of acceptable form and suitable nutrient composition (Zein-Eldin and Meyers, 1972). Captive animals are totally dependent on the culturist to provide all environmental requirements. For example, it is absolutely essential that the diet meet the animals' nutritional requirements as the animals are prevented from foraging (Lovell, 1975). In addition, many other environmental requirements must be satisfied (temperature, photoperiod, meeting behavioral needs, sanitation, etc.). The requirements become more difficult if succeeding generations of a species are to be produced and maintained, as techniques for meeting the reproductive needs of the species are required. The culture of aquatic and semi-aquatic animals, such as amphibians, is most complex because you must provide adequate conditions within two entirely different environments, aquatic and terrestrial.

This paper reviews some of the research designed to meet the nutritional requirements of bullfrog larvae (tadpoles). The design of a nutritionally adequate diet is complicated by the very nature of the animal. They are affected by density, water

quality, photoperiod, timed disturbances, water currents, genetic variability, and physical characteristics of the feed. Poor control of any of these factors can affect survival, development, growth, and metamorphosis, all measurable responses in nutritional studies.

THE CULTURE SYSTEM

For management procedures we know some things about meeting environmental requirements for bullfrog tadpoles. The information provided, in most cases is not necessarily optimum, but has at least been useful for experimental purposes, and allowed us to maintain our colony through succeeding generations for over ten years.

Egg Procurement

Egg production is accomplished through artificial ovulation, spermiation and fertilization of laboratory raised or wild conditioned animals (Easley et al. 1979; Culley et al., 1982). The fertilized eggs are retained in 10% Ringer's solution, pH 7.0, for 1½ to 2 hours and then transferred to shallow, screened culture tanks suspended in flowing water (Culley et al., 1977). The water is dechlorinated and its pH adjusted 6.5-7.0 with dilute HCl. Infected eggs are siphoned out daily. Feeding of the newly hatched tadpoles starts as soon as feeding behavior is evident (5 to 9 days depending on temperature).

Density

Although there is still an incomplete understanding of why density reduces growth and causes tadpoles to metamorphose at small sizes, the effect on growth and development of varying densities is real, and measurable (Richards, 1958, 1962; Rose, 1960; Wilbur and Collins, 1973; Gromko et al., 1973). Table 1 shows results of our earlier studies of density effects in a system with continuously flowing water. At the present time we do not exceed a density level of about 13 tadpoles/liter volume with water exchange approximately every four hours (Culley et al., 1977). With this system we have cultured 250 tadpoles within a 13 l.(19 tadpoles/liter volume) wire basket suspended in a 47 l. tank from hatching through metamorphosis. Total weight of the tadpoles approached 1900 g, or 158 g tadpoles/liter volume within the basket.

Tadpole density is one of the most important factors to consider when rearing anurans in the laboratory. At low densities filamentous fungi and bacteria often overgrow on the walls and bottoms of the baskets and entangle small tadpoles. High initial densities will alleviate this problem as bullfrog tadpoles effectively graze the flora (filter feeding tadpoles will not solve the problem).

Water Exchange

The exchange of water is essential for intensive culture. The main purposes are to remove waste materials and maintain desired water quality. The minimum limits of exchange are not known, but we have maintained an exchange rate of about 4 hr at the density levels covered above. Under commercial systems out-of-doors the exchange rate is as low as every 24 hrs and good growth is maintained. However, other factors come into play. Algae, fungi, and bacteria continuously consume and tie up toxic waste components (particularly nitrogen compounds), generate oxygen (algae), and help buffer the water pH by absorbing and releasing carbon dioxide and minerals.

Photoperiod

As a standard practice we operate our culture system on a 12L:12D regime photoperiod. Fluorescent 40W lamps are placed 50 cm above the tanks in order to provide uniform lighting conditions. Tests involving a series of tanks in replicated studies often show what we call "tank effects". Presumably one or two tanks may have, or develop slightly different environmental conditions which affect the tadpoles. As a result we get variability in responses, making interpretation more difficult. Light differences can play a role in this variability as Horseman et al. (1976) demonstrated different growth and metamorphic rates for tadpoles held on 8 and 12 hr light.

Temperature

For normal culture procedures we maintain water temperatures between 22 and 26° C. Temperatures above 26° C frequently are associated with increased bacterial infection and increased growth of bacteria and fungi in the culture tank. Temperatures below 20° C result in reduced growth rates.

Tadpoles are sensitive to sudden temperature changes. If they are to be transferred from one tank to another we always blend the water if the temperature difference is over 3-4° C.

Tadpoles do have temperature preferences, which may vary over time. Given a choice between 20 to 35° they cluster in water with temperatures of 20-24° C (Culley, unpublished data). Growth responses are roughly comparable within a temperature range of about 20 to 28° C. Above 28° C disease problems are more likely to be encountered and when temperatures are above 34-35° C growth and survival are reduced.

Water Quality

We probably know as little about water quality requirements as nutrition. Clearly, all aspects of water quality must be maintained within acceptable limits if nutritional studies are to be meaningful.

Water pH: We have had better growth and survival responses in our laboratory when the pH is between 6.5 to 7.0 (Marshall et al., 1980; Leibovitz et al., 1982). Equal success has been achieved in Brasil where incoming well water varied from 3.8 to 4.2 and culture water ranged from 4.5 to 5.5 (Culley, unpublished). Tadpoles have survived pH levels of 10 for short periods (several hours), but we have not attempted long term culture at this level.

Oxygen: The skin is the major site of O_2 uptake and CO_2 release in water (Burggren and West, 1982), and undoubtedly other materials. In early developmental stages oxygenated water is critical to survival. Bullfrog tadpoles develop lungs fairly early, and become less dependent on dissolved oxygen as they grow. Thus low oxygen is seldom a problem per se, but low oxygenated water usually is caused by accumulation of excess feed and waste or reduced water flow, and the resulting toxic materials (ammonia, nitrites, hydrogen sulfides, etc.) adversely affect the tadpoles.

In commercial systems where the tadpoles are cultured in concrete tanks with exposure to the sun another problem may develop; supersaturation of the water with oxygen. Such a condition can be caused by high density algae releasing oxygen which goes into solution. Under such conditions the oxygen is forced into the tadpoles tissues, and if the condition persists for several hours the tadpole dies. Oxygen concentrations between 17 to 23 mg/l. produce this type of condition, and similar effects have been produced in the laboratory by Colt et al. (1983).

Minerals: Calcium is apparently absorbed through the gut, gills, and skin (Baldwin and Bentley, 1980). If calcium is present in the water the route of entry is primarily through the gills and skin. Frequently water may be low in calcium. In such cases compensatory calcium must be present in the diet or skeletal development will be altered (Marshall et al., 1980). Brown (1964) also indicated that when R. temporaria is placed in high calcium water, calcium carbonate crystals accumulate in the lime sacs.

Of particular interest is the use of spinach as a tadpole diet. Spinach contains oxalic acid which reduces calcium availability (Briggs and Davidson, 1943; di Berardino, 1967).

Tadpoles require the same minerals as other vertebrates, but we have not established the quantities or ratios required. Snider et al., 1983 (manuscript in preparation) showed the mineral content of wild tadpoles collected in south Louisiana from a pond with excellent aquacultural qualities. These values can serve only as a guide for nutritional studies, and give us little insight as to the quantities needed in culture water. In mg/g of dried whole body analysis the following mean values were determined: Ca 26, Mg 6.8, K 1.8; in ug/g MN 59, Cu 3.3, Zn 19.3. Table 2 shows mineral balance in water that has been successfully used to culture tadpoles (Snider obtained tadpoles from the Baton Rouge pond).

We routinely incorporate a complete mineral packet in our tadpole diets in an effort to correct any mineral deficiencies in our culture water. However, we have no evidence the practice is necessary. Calcium and magnesium are the major minerals we are concerned with and recommend at least 20 mg/l. calcium and 4-6 mg/l. magnesium be in the culture water until further information is known.

Chlorine: Chlorine is a potent disinfector for bacterial control, but also toxic to tadpoles. In a commercial system in Brasil we routinely treated eggs and young tadpoles with 5 mg/l. chlorine for 10 minutes and achieved some success in reducing bacteria. Longer times of treatment were frequently lethal. In the laboratory we use activated charcoal to remove chlorine from tapwater.

Disturbance

Horseman et al., (1976) clearly showed the effect of timed-disturbance (disturbing the animals at the same time each day during routine maintenance). In these studies the animals

were simply fed and the tanks cleaned each day at the same time, some tanks in the morning and some in the afternoon. The controls consisted of random disturbance times. The tadpoles responded to the timed disturbance by delayed metamorphosis, growth, and increased fat storage. In some cases the storage of fat in the body cavity was so great that when the tadpoles went through metamorphosis the body size could not decrease (normally there is about a 50% loss of body weight) and the legs were too small to support the new frog. Furthermore, the effect was altered by 8 or 12 hours of light. We recommend a random time of disturbance as the controls resulted in very normal appearing frogs.

Mbangkollo and de Roos (1983) demonstrated the gentle handling of bullfrogs altered blood characteristics (plasma lactate, glucose, and hematocrit levels). More extreme handling produced responses that required 72 hours to return to normal.

The tadpoles used in Table 1 were part of a test in which we were also determining the efficiency of food utilization. In the initial tests we collected and weighed the tadpoles once each week. After three weeks we had practically no growth compared to the controls that were not collected and weighed. We began to weigh the animals daily and for the first week we noticed a weight loss for three days. By day six the tadpoles had recovered the weight loss and continued to show gains in weight as long as they were handled daily. The tadpoles adapted to a daily disturbance pattern, but were unable to do so with weekly disturbances.

The collection and transporting of tadpoles may cause a decline in some body minerals, even if the tadpoles are placed in the original water and given feed (Snider et al., 1983, manuscript in preparation). After 17 days, Mn, Cu, and Zn had not returned to original concentrations at the time of collection.

The point to all this should be clear, that many little things the animals are subjected to can have a pronounced effect, and effectively negate not only nutrition studies, but many other experiments.

Uniformity of Test Animals

In working with wild populations uniformity of animals for testing is impossible. Even with cultured animals uniformity is difficult to achieve, and analysis of test results is most difficult.

When we obtain eggs and sperm in our laboratory we get seemingly good fertilization and normal development of the embryos. However, as growth proceeds differences become apparent, and interpretation of nutritional studies becomes difficult.

Table 3 shows some of the problems confronting the culturist involved in tadpole nutrition. In this study tadpoles from two different spawns were used, produced four months apart. The culture system used was identical regarding photoperiod, temperature, container, waterflow, water quality, density, and feed. Yet the results obtained were different. The cause(s) of the differences are not understood, but the response is typical of what we have seen over the past ten years.

Working in a commercial-scale facility in Brasil we have been able to identify spawns and/or tadpoles within 15 days after hatching that will give a poor growth response. Spawns with a high percent of unfertilized eggs, abnormal morphology of the tadpoles at hatching (curved-shape body), and hydrocoel (accumulation of water in the body cavity) will normally result in poor growth as the population develops.

Each spawn carries its own genetic potential, and is affected by many factors of which we are not aware. Many replicated experiments are required in order to convincingly draw conclusions from a study.

Water Depth and Flow

Newly hatched tadpoles are opportunistic grazers, randomly feeding on any surface. They will also graze floating powdered feed placed on the water surface. Within a few days they "learn" to move directly to a prepared diet, thus distribution of food throughout the container is no longer required. However, it is most important in the first few days to maintain a small volume of shallow water (about 2-3 cm) to allow the tadpoles to quickly locate food with a minimum expenditure of energy. Once the yolk is absorbed they must receive food within about 24 hours or you can expect mortality.

A small volume of water also confines bacterial and fungal growth to a reduced surface area of the tank, allowing more intensive grazing due to the higher tadpoles density, thus effectively controlling the microflora expansion. As the tadpoles grow and store more energy the water depth can be increased to reduce

crowding. Normally within about ten days we increase the water depth to about 15 cm and divide the tadpoles so as to establish a density described in the previous section. At this time they are capable of locating the prepared feed and effectively control growth of microflora. Because they feed almost constantly it is most important to keep food present at all times.

Rapid exchange of water is required as pointed out previously, but currents must be minimized or growth will be reduced. In young tadpoles (first two weeks after feeding begins) little growth may result if currents are present. As they grow the problem becomes less severe until the tadpoles obtain a size of 8 to 10 g. At this time the larger tadpoles can and do create substantial currents by their feeding activity. Smaller tadpoles in the group have greater difficulty in feeding as they will be literally "swept away" from the feed due to these currents. Placement of these smaller tadpoles in a separate container results in normal growth rates (Culley et al., 1977).

Quantity and Replacement of Food

Bullfrog tadpoles are constant grazers, thus food should be present at all times. In nutritional studies it is important that the feed surface available to the tadpoles be approximately the same in all tanks. We do not know the ratio of tadpole density to available feeding surface, thus we attempt to maintain as large a surface area as possible in each tank. However, the quantity of feed should be adjusted so that all the feed is consumed in 24 hours or the excess feed removed daily unless there is evidence that it is being rapidly consumed. Spoiled feed can produce problems in nutritional studies.

Length of Experimental Study

Nutritional studies must be of sufficient length, beginning with the onset of feeding, to insure that a high percent of tadpoles complete metamorphosis. Further, it is necessary to continue the study for at least two months after metamorphosis to determine if any delayed effects are produced that can be related back to tadpole nutrition. Nutritional stress associated with improper tadpole nutrition (or culture conditions) may not show up until after metamorphosis, particularly developmental abnormalities.

Tank Effects

Even though serious efforts are made to set up a uniform test conditions, we must recognize that "tank effects" occur. Subtle factors occur within a tank that result in variation in tadpole growth, development, and mortality. In most cases we cannot identify these small differences between tanks.

Thus for all our tests it is imperative that each treatment occur within a single tank, and the test be run in at least three tanks. For example, if we set up three diets differing in only protein then we must have four compartments in a single tank; one for each of the protein diets and a control. At least two additional tanks are also set up. In this manner we can have some control over tank effects.

Feed Utilization

Bullfrog tadpoles consume feed almost continuously. Food consumption/24 hour period (wet wt/wet wt) exceeds 20% body weight/day during the first three weeks of development and gradually declines to about 7% during late metamorphosis (appearance of front legs). Feed conversion (wet wt/wet wt) ranges from about 2.0 to 2.5 until the tadpoles are half grown and then becomes rather erratic, ranging from 2.5 to 4.0 (Culley et al., 1977).

Marschall (1978) demonstrated that tadpoles provided a diet with 50% water consumed much less than feeding on a diet of 75% water, but on a dry weight basis the food consumed was equal. He also demonstrated that as protein content was increased in diets the tadpoles on a low protein diet consumed the same quantity of protein as tadpoles on a high protein diet by apparently regulating the quantity of feed consumed.

CURRENT KNOWLEDGE OF NUTRITION

Natural Feeds and Prepared Diets

One fact is obvious, when bullfrog tadpoles have access to natural microflora in ponds, hatcheries or laboratory tanks, with or without prepared supplemental diets, growth and development approaches normal proportions. Bacteria, fungi and attached phytoplankton are excellent grazing materials and add some unknown nutritional dimension to prepared diets.

Table 4 shows the percent deformed tadpoles from one spawn cultured in our standard laboratory tanks under identical conditions of water quality, light, prepared diet (Culley et al., 1977), and temperature. The only difference being that one group of tadpoles was confined within a screen basket and another group was placed outside each basket and allowed to graze on the container walls. The prepared diet was placed in the basket as well as outside. The data clearly indicate that tadpoles outside the basket developed no skeletal deformity (scoliosis). As tadpole density decreased within the baskets deformities decreased, indicating that microflora growing on the basket screen was sufficient to provide an additional source of food.

In a commercial operation in Brasil under hatchery conditions, scoliosis occurred in tanks where attached algal growth was not permitted to develop prior to the stocking of tadpoles (Culley, unpublished data). Scoliosis is occasionally observed in natural environments, but the occurrence is rare.

A variety of feed has been used in feeding amphibian larvae (Cairns et al., 1967; Mendell, 1963; Hirschfeld et al., 1970). Most of these diets incorporate poor nutrient sources. Spinach, despite its high oxalic acid content which reduces calcium availability, has been used in a number of instances (Pilkington and Simkiss, 1966; di Berardino, 1967; Briggs and Davidson, 1942). Other feeds commonly used were lettuce, cereals, brine shrimp, dried shrimp, rabbit feed, fish feed, liver, cabbage, etc. None of these materials should be serious candidates for meeting the nutritional requirements of tadpoles.

Formulating Diets

It was first necessary to produce a feed that was stable in water to prevent the deterioration of water quality. The feed had also to be soft enough to permit grazing. If bound too tightly tadpoles were ineffective in obtaining food. Leaching of minerals, vitamins, and water soluble amino acids and carbohydrates from the diet was an important consideration because unlike many aquatic animals, tadpoles feed incessantly. Thus, attention was given to obtaining an effective binder as diets were tested. The design was to produce a moist, soft feed that retained its integrity in water for at least 24 hours.

Culley and Meyers (1972) reported on a diet bound with an alginate binder, Kelgin, and a phosphate sequestrant (Calgon). Doucette (1973) tested various concentrations of this binder with diet formulations based on trout chow, catfish chow and rabbit chow. Culley et al., (1977) reported on a modified diet and a dual means of delivering the food. When the larvae hatch, powdered feed was suspended in the water, in later stages the feed was bound with the alginate and other binders. Marschall (1978) first experimented with the use of agar as a binder. The test was short-term but nevertheless agar proved to be a good binder and easy to work with. However, with the use of agar, and various gums (Culley et al., 1977) it was necessary to add a preservative (sodium benzoate) to increase shelf-life of the food when refrigerated and placed in tadpole tanks. Bioassays of sodium benzoate were performed by Culley (unpublished data) and safe levels for tadpole consumption confirmed by Leibovitz et al., (1982).

Meyers et al., (1980) tested ten different binders for larval diets with best results obtained with Kelgin and agar were incorporated in the diet at 2% of dry weight. Agar is currently used in our laboratories. It should be emphasized here that the percent of agar incorporated depends (a) on the size of tadpoles; (b) on the type of basic ingredients used; and (c) on the amount of added water. Diets with low protein and high fiber and carbohydrate content absorb more water, thus slightly less agar is required (1.5%). High protein diets with less fiber and carbohydrates absorb lesser amounts of water necessitating the incorporation of higher quantities of agar (3%). Since our diets contain 75% water, use of agar at 2% is acceptable for general culture practices. A new thickener is currently used in formulating the diets which enhances their water stability, decreases the water activity thus prolongs their shelf life, and can be used as filler instead of caloric compounds. The thickener, hydroxyl propyl methyl cellulose (Methocel^{R}, Dow Chemical Co.) is basically nutritionally inert and may be recommended when low fiber diets are formulated.

BUILDING A NUTRITIONALLY SOUND DIET

Developing a nutritionally balanced diet for animals is a difficult task. More often than not we turn to developing a diet that appears to give satisfactory growth and development, and as

problems arise try to improve upon the existing diet. Until recently, our laboratory has been primarily conerned with improving a diet we formulated around 1970. The major diet components changed somewhat until we established what we referred to as a "standard" diet (Table 5).

Marschall (1978) modified the above diet in a series of tests where protein was increased at the expense of fiber and carbohydrates, and undoubtedly many other feed ingredients. Protein values varied from 24 to 43%. Growth was acceptable on all the diets, but growth increased as the protein increased and fiber and carbohydrates decreased. Unfortunately the data were not very instructive. Marshall et al., (1980) and Leibovitz et al., (1982) working with diets similar to Marschall (about 40% protein) but with greater tadpole densities had severe scoliosis problems in nearly all of their test animals. Attention was thus turned to increasing calcium in the diet, and in other tests the addition of ascorbic acid (vitamin C). Some reduction in scoliosis resulted from adding calcium to the diet. Leibovitz demonstrated a significant reduction in scoliosis with the addition of ascorbic acid to the diet of tadpoles culture in high pH water (pH 7.5-8.3) but not in low pH water (pH 6.5-6.9). HCl was used to control pH and it apparently destroyed some ascorbic acid. Of equal interest is that we still use low pH water, but the data of Leibovitz et al. suggest that the high pH per se was not detrimental as both growth and survival were equal at both pH levels. The higher pH does favor pathogens however (Marshall et al., 1980). A modification of the diet of Leibovitz also has resulted in normal growth and development.

In a preliminary study, Sotiriadis (unpublished data) looked at growth through metamorphosis (emergence of front legs) as affected by protein levels in the diet. Excellent growth and metamorphosis occurred with diets containing 25, 30 and 35% protein (Table 6). Metamorphosis data indicate the 20 and 40% protein levels are not optimum, while the average weights in spawn 2 suggests that the 35 to 40% protein diets are optimum. Thus by evaluating different responses, i.e., average weight, deformities, stage of development, metamorphosis, and survival, different conclusions can be drawn about the performance of the diet. The use of an index to obtain an overall evaluation of various responses will perhaps provide the best conclusions on a preferred diet. Using the above responses in a preliminary indexing scheme the 25% diet appears to give the best performance (Sotiriadis, upublished data).

Although our diet is incomplete, Table 7 shows the test diet we are currently using. Modification of this diet is surely permissible, and perhaps some comercially prepared feeds could be powdered and reformed with agar. All evidence currently supports that ascorbic acid be added to the diet if some microflora is not available for grazing. Ascorbic acid is, however, water soluble and leaches rapidly into the water. We are currently testing coated ascorbic acid and new binders in an effort to stabilize the amount of vitamin C in the diet. Schafer (1982), using a modification of our 1977 standard diet (Table 8) and a coated vitamin C reported normal growth and development at the San Diego Zoological Garden. This diet appears suitable for general culture procedures.

Preparation of the complete diet in Table 7 is as follows:

A) Place 937 g dry mix components (1-10) in 1442 ml water and mix well.

B) Place 50 g fish oil, 4 g lecithin, 5 g linolenic acid, 2.1 g vitamin mix and 3 g vitamin C (50%) in container and mix well.

C) Add minerals(36.8 g), sodium benzoate (4.5 g) and agar (26 g) to 2000 ml water. Heat and stir continuously until agar dissolves (when boiling).

D) Combine A & C, mixing thoroughly. When temperature decliens to below 40° C blend in B (before food begins to get firm).

E) Pour into container and refrigerate (do not freeze) at 4° C when cool. The diet (about 4500 g) will remain good for about 8 days.

Even though many problems remain we are reasonably successful in culturing bullfrog tadpoles under laboratory conditions, and in commercial hatchery systems where algae, bacteria, and fungi are utilized.

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Table 1. Summary of growth characteristics of Rana catesbeiana larvae reared from hatching through emergence of forelimbs (13 weeks) at three density levels under defined laboratory conditions (Culley et al., 1977)

Number/tank[a]	100	175	250
Starting wt. (g)	.012	.012	.012
Avg. ending wt. (g)	11.4	10.8	9.1
Wt. range (g)	3-18	2.8-17	2.5-17
Mortality	5	7	6
Larvae completing metamorphosis (%)[b]	80	80	82

[a] Each density run in triplicate with continuous water exchange.

[b] Small animals did not enter metamorphic conditions.

Table 2. Mineral balance of water that has been used to culture bullfrog tadpoles successfully.

	Minerals (mg/1)											
	Ca	Mg	Mn	P	K	Na	Zn	Fe	S	B	Cl	Co
Baton Rouge, La												
Pond water	31	17	0.02	5	5	18	-	0.07	2	0.15	14	-
City water	2.1	0.8	-	5	1	82	-	-	2.7	-	228	0.06
Wisconsin												
City water	41	23	0.02	6	2	4	0.3	-	2.3	-	10	-
Belem, Brasil												
Well water	3.5	0.43	-	0.85	4	7.1	0.02	2.5	-	-	8	-

Table 3. Growth, survival and metamorphic response of bullfrog tadpoles from two spawns produced in the laboratory and cultured under equal environmental conditions on the same diet for 79 days.

	Spawn #1 Fertilization Date 8-24-82	Spawn #2 Fertilization Date 12-1-82
Initial Number	230	234
Final Number	214	210
% Survival	93	89.3
% Metamorphs[a]	51.9	60.3
Average Weight (g)	5.01	7.77
% with Hind Leg[a] Development	31.5	16.3
Average Weight (g)	4.47	7.2
% with no Leg[a] Development	16.8	23.4
Average Weight (g)	1.89	1.91
Average Weight of all Animals at End of Experiment (79 days) (g)	4.37	6.31

[a] Number of tadpoles/number of surviving tadpoles.

Table 4. Mortality and percent deformity (scoliosis) of laboratory cultured tadpoles confined and free swinning, provided a prepared diet, and with the free swimming tadpoles grazing on microflora of the culture tank walls.

	Larvae confined in baskets			Larvae free-swimming		
Tank No.	No. Stocked	At Metamorphosis Mortality	% Deformed	No. Stocked	At Metamorphosis Mortality	% Deformed
1	156	13	56	38	1	0
2	144	17	66	42	0	2
3	65	7	62	65	4	0
4	49	0	29	49	0	2
5	20	0	0	20	0	0
6	10	0	0	10	0	0

Table 5. Diet formulation for larval anurans[1].

Ingredient[2]	Percentage
Shrimp meal	16.5
Fish meal	22.0
Soy protein	5.5
Yeast protein (YEACO-20)	16.5
Rice bran (PROTEX-20)	21.0
Whey (N.M.C.)	5.0
Fish oil	2.0
Fish solubles	5.0
Vitamin premix[3]	2.0
Linolenic acid	0.5
Kelgin[4]	2.5 (to 4.0)
Sodium hexametaphosphate	1.0 (to 2.5)

From Culley et al., 1977.

[1] Antiobiotic mixture added: Oxytetracycline 1500 mg/kg, sulfamarazine 1000 mg/kg.

[2] Sources of ingredients: Shrimp meal (Blum and Bergeon, Houma, Louisiana; Fish meal, oil and solubles (Wallace Menhaden Co., Empire, Louisiana); Soy protein (Nutritional Biochemical Corp., Cleveland, Ohio); Yeast protein (Milbrew, Inc., Milwaukee, Wisconsin); Rice bran (Riviana Foods, Inc., Houston, Texas); Whey (Foremost Foods Co., San Francisco, California).

[3] Vitamin Diet Fortification (U.S. Biochemical Corp., Cleveland). Ingredients per lb.: Vitamin A (900 KIU), Vitamin D3 (100KIC), Vitamin E (5000 IU), ascorbic acid (45 gm), inositol (5 gm), choline chloride (86.4 gm), Vitamin K (6.75 gm), PABA (5 gm), niacin (4.5 gm), riboflavin (1 gm), pyridoxine HCl (1 gm), thiamine HCl (1 gm), D - Ca panthothenate (3 gm), biotin (20 mg), folic acid (90 mg), Vitamin B-12 (1.35 mg).

[4] Alginate high viscosity (HV) (Kelco Co., Sand Diego, California) combined with sodium hexametaphosphate. Other binders which can replace Kelgin and sequestrant are 2 to 3% Xanthin (Kelco Co.) added to 2 to 3% Locus bean gum (Meer Corp., North Bergen New Jersey).

Table 6. Percent metamorphosis and average weights of bullfrog tadpoles cultured for 79 days on diets varying in protein.[1]

Diet	Spawn #1 % Meta	Spawn #1 Avg. Wt. (g)	Spawn #2 % Meta	Spawn #2 Avg. Wt. (g)
20% protein	75	7.7	33	7.4
25%	87	8.5	69	8.7
30%	88	8.5	68	9.0
35%	85	8.3	76	9.8
40%	66	7.7	71	9.8

[1] Sotiriadis (manuscript in preparation)

Table 7. Tadpole diet currently used in the laboratory culture of bullfrog tadpoles.[1]

Ingredients[2]	% of Mix	FEED MIX Ingredients	% of Mix
YEACO 20	8.9	Soybean oil (43.3% protein)	13.6
Nutrex 540	5.0	Fish oil	5.0
(Saccharomyces fragilis)		Linolenic Acid	0.5
Corn meal (8.4% protein)	20.0	Lecithin	0.4
Whey	6.9	Mineral mix	3.68
Fish meal (menhadren)	4.0	Vitamin C (50%)*	0.3
Shrimp meal	4.0	Vitamin mix	0.21
Alfalfa (17.5% protein)	20.6	Sodium Benzoate	0.45
Bone meal	1.0	Agar	2.6
Rice bran	1.0		

* Coated Durkee Ind. Foods Group Ohio

1 All quantities are based on preparation of 1 kg of feed mix (before addition of water). Protein 25% (50% plant protein, 25% yeast protein, 25% animal and fish protein); fat and linolenic acid 7.5%; fiber 7.5%.

2 Source of materials: unless shown here ingredients are standard products and available from a variety of sources. YEACO 20 (Brewers yeast) Milbrew Inc., Wisconsin; Nutrex 540, Amber Laboratory, Wisconsin; Coated Vitamin C (50%) Durkee Co.

(Table 7 continued next page)

Table 7. Tadpole diet currently used in the laboratory culture of bullfrog tadpoles, continued.

MINERAL MIX

Mineral	Source	Quantity/kg Feed mix
Calcium	Calcium gluconate	2.505 g
	Calcium lactate	1.270
	$CaCO_3$	1.165
	$CaCl_2 \cdot 2 H_2O$	0.856
Phosphorus	$K_2HPO_4 \cdot 3 H_2O$	8.34 g
	$Na_2HPO_4 \cdot 7 H_2O$	13.085
	KH_2PO_4	4.988
Manganese	$MnSO_4 \cdot H_2O$	0.1142 g
	$MnCl_2 \cdot 4 H_2O$	0.05782
Iodine	$NaIO_3$	1.64 mg
	KIO_3	1.77
Iron	Ferric Ammonium Citrate	0.07714 g
	$FeSO_4 \cdot 7 H_2O$	0.0448
Zinc	$ZnCO_3$	0.04924 g
	$ZnCl_2$	0.05337
	$ZnSO_4 \cdot 7 H_2O$	0.11258
	Zn Acetate $\cdot 2 H_2O$	0.14366
Copper	Cupric Acetate $\cdot H_2O$	0.04713 g

Table 7. continued next page

Table 7. Tadpole diet currently used in the laboratory culture of bullfrog tadpoles, continued.

VITAMIN MIX	
Vitamin	Quantity/kg Feed Mix
Vitamin C (50%)*	3000 mg
Biotin	0.12
Choline	440
Folic acid	31
Inositol	305
Niacin	327
Pantothenic acid	172
Pyridoxine	130
Riboflavin	129
Thiamine	130
Vitamin E	319
Vitamin K	23
Vitamin B_{12}	49 ug

* Coated Durkee Ind. Foods Group, Ohio

Table 8. Special diet for larval anurans.[1]

Ingredient*	Amount (g)
Fish Meal	23
Shrimp Meal	14
Soybean Meal	17
Yeast Protein	14
Defatted Rice Bran	19
Vitamin Mix	3
Calcium Phosphate Monobasic	4
Coated Ascorbic Acid	3

Mix well first five ingredients. Bring to a boil 200 ml water with 4-5 g agar (Kelco Co., San Diego, or health food stores) and mix immediately with dry ingredients. Cool to below 40°C and mix well with the last three ingredients. Pour into several petri dishes and refrigerate for up to a week. If the diet is too solid, next time decrease the amount of agar used or, if too loose, increase the amount of agar used.

* Sources of ingredients: Fish Meal (Wallace Menhaden Co., Empire, LA), Shrimp Meal (Blum and Bergeron, Houma, LA), Soybean Meal (U.S. Biochemical Corp., Cleveland, Ohio 44128), Yeast Protein (Yeaco 20; Amber Laboratories, Juneau, WI 53039), Defatted Rice Bran (Protex 20; Riviana Foods, Inc., P.O. Box 2636, Houston, TX 77001), Vitamin Mix (Total Vitamin Supplement; U.S. Biochemical Corp., see above), Coated Ascorbic Acid (Roche Chemical Division, Hoffman-La Roche Inc., Nutley, NJ 07110).

[1] From Schafer (1982)

CONSIDERATIONS IN THE USE OF FISH AS FOOD[1]

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Fish quality presents a persistent problem as many vendors attempt to market inferior fish to zoos. Poor quality implies economic waste and compromised animal performance. At the National Zoological Park (NZP) fish purchases amount to $25,000 to $41,000 per year, or 8-14% of the annual food budget. About 92% of the fish purchased is for seals, sea lions, and bears. NZP pinniped keepers apply rigid quality standards, resulting in the disposal prior to feeding of 20-60% of the butterfish and 10-25% of the herring. Fish discards are usually greater in the summer months. When discard rates are high, fish consumption by seals and sea lions is significantly reduced (negative correlation $p<0.05$). Fish quality may be affected by seasonality in composition of many fishes as well as improper freezing procedures. Analyses of fish samples at NZP indicate marked compositional variation from month to month. Mackerel ranged from 3-20% fat (1.0-2,4 kcal ME/g), butterfish from 5-18% fat (1.1-2.1 kcal/g) and herring from 7-12% fat (1.2-1.6 kcal/g). To date both sea trout (10-12% fat, 1.6-1.7 kcal/g) and smelt (1-2% fat, 0.5-0.7 kcal/g) have been rather consistent. Variation in fish composition and in fish consumption result in varying energy intakes in both California sea lions (185-308 kcal/kg$\cdot^{75}$/day) and grey seals (78-214 kcal/kg$\cdot^{75}$/day). The relation of energy intake to animal performance needs further study. Poor fish quality may be accompanied by losses of nutrients, especially vitamin E. In 1981, vitamin E supplementation of pinnipeds was increased from 30 to 200 IU per animal per day, yet when vitamin E supplements were omitted for one month in the summer of 1983 a yearling California sea lion exhibited muscle weakness, respiratory difficulties and hyponatremia, followed by death. White muscle disease was diagnosed postmortem. High levels of vitamin E supplementation on an ongoing basis are essential when frozen fish is fed; thiamin (B_1) supplementation is also necessary.

Introduction

Fish is one of the least stable foods used in a zoo. Improper freezing procedures and inadequate storage conditions can lead to substantial deterioration in fish quality. Fish vendors may view zoos as

[1]This paper was also presented at the Annual Meeting of the American Association of zoo Veterinarians, Tampa, Florida, October, 1983.

an outlet for inferior quality fish that cannot be marketed for human consumption. Poor quality fish may lead to high discard rates, reduced food intakes and compromised animal performance. This paper discusses some economic, nutritional, and health aspects of fish as food for zoo animals.

Fish in Food Budgets

Fish purchases comprise a significant proportion of the annual expenditures on food of any zoo with a collection of marine mammals. In the past four years fish purchases at the National Zoological Park (NZP) have amounted annually to about $25,000-$41,000 or 8-14% of the total food budget (Table 1). The species of fish purchased have varied somewhat from year to year in accord with quality, availability, animal acceptance and experience, but the bulk has consisted of Atlantic herring (Clupea harengus)and butterfish (Peprilus sp.)(Table 2). In 1983 fish allocations for various animal groups were as follows: seals and sea lions 58,400 lbs (61.2%); polar bears, 15,400 lbs (16.1%); other bears, 14,600 lbs (15.3%); birds, 6700 lbs (7.0%) and others, 400 lbs (0.5%). Thus seals, sea lions, and bears account for nearly 93% of all fish used. Since seals and sea lions tend to be among the most selective fish eaters as well as the greatest volume consumers, most concerns about the types and quality of fish purchased by NZP emanate from staff involved with pinniped care.

Quality Standards

NZP pinniped keepers apply rigid quality standards in evaluating fish received from the zoo commissary, and any fish deemed unacceptable is discarded. The criteria used are essentially derived from standards adopted by the National Marine Fisheries Service (Title 50, Code of Federal Regulations, Parts 260 and 261) and in 1984 are being specifically incorporated into NZP contract specification for fish purchases. To be acceptable, fish must conform to the following standards:

1. Mild odor - no strong, rotten or "iodine" smell;
2. Flesh intact and firm - not soft, spongy or with a tendency to separate;
3. Natural skin color and appearance - no discoloration, dehydration, or wrinkling of skin;
4. Fish intact - no breaks in skin, bloating, or protrusion of viscera.

Fish which do not comply with all standards are discarded (for breaks in skin, if other criteria are met, cuts are made an inch on either side of the break and the remainder of the fish fed). For the past four years discards have been weighed and recorded allowing calculations of discard rates for different types of fish and for different seasons of the year.

Discard data are available for 40,38,20, and 19 months for Atlantic herring, butterfish, smelt (Osmerus mordax) and mackerel (Scomber scombrus), respectively (Fig. 1). Poor quality was greatest for butterfish; discard rates were typically about 20-60% of the fish received ($\bar{X}$=32.9%±13.9 SD), By contrast only about 10-25% of the herring ($\bar{X}$= 15.7% ± 10.2 SD), 5-20% of the mackerel ($\bar{X}$ = 11.5% ± 8.8 SD), and 0-20% of the smelt ($\bar{X}$ = 9.8% ± 13.4 SD) was discarded. Discard rates are often, but not always, highest in the hot summer months.

Poor fish quality may result from many factors surrounding the handling, freezing, and frozen storage of fish (Symes 1966). Delays in freezing, slow rates of freezing, inadequate freezer temperatures, excessively prolonged frozen storage and improper packaging may all contribute to loss of fish quality. Reduced quality results not only in reduced human appeal and animal acceptance, but may imply oxidative destruction of nutrients, bacterial proliferation, and generation of potentially toxic compounds. Even after keeper discard of the apparently inferior fish in a lot, pinnipeds may be able to discriminate with respect to those remaining. A weak ($r= -0.33$) but significant ($P < 0.05$) negative correlation was found between fish discard rates (% of received) and fish consumption rates (fish intake as % of body weight) by grey seals and California sea lions at NZP over the period of 1980-1983.

Fish Composition and Energy Intake in Seals

The nutritional value of fish depends not only on freezing and storage conditions but also on variation in composition between and within fish species. In December 1982, a program of periodic analysis of fish obtained by the NZP Commissary for animal feeding was initiated. Fish are assayed at 3-month intervals in the NZP Nutrition Laboratory for dry matter, fat and protein [(total nitrogen - nonprotein nitrogen)x 6.25)] and metabolizable energy values are calculated. Data available to date (Table 3) indicate that fat content is highly variable in Atlantic herring (7-12%), mackerel (3-21%) and butterfish (5-18%), but much less variable in sea trout (10-12%) and smelt (0.9-1.8%). Both the absolute amount of fat and fatty acid composition may affect storage characteristics. The calculated energy contents varied markedly (up to twofold) in some species but much less in others (Table 3). The policy of feeding pinnipeds a quantity of fish equal to a certain percentage of their body weight ignores seasonal and interspecific variation in the energy content of fish.

Fish consumption rates of both captive grey seals and California sea lions tend to vary from month to month (Fig. 2). To some extent this variation may be of seasonal origin and relate to reproductive cycles. In many pinnipeds males and/or females deposit substantial body energy stores prior to parturition and mating on land. Reserves supply energy during periods of fasting on land. Appetite enhancement and suppression may coincide with these times of the year. Appetite reduction in summer months in Washington, D.C. may also relate to environmental temperatures

that far exceed the normal range for free living grey seals and sea lions as well as to changes in fish quality.

Data on fish consumption, fish intake and body weight allow calculation of energy intakes at various months (Table 4). The mean metabolizable energy intakes of the California sea lion range from 5550 kcal/animal/day to 9180 kcal/animal/day. Both species exhibit a pattern of rising energy intake from winter to spring and a decline in summer. These data are of a preliminary nature and need to be confirmed in other years and at additional institutions. On a metabolic body size basis, California sea lions consumed 185-308 kca./$kg^{\cdot 75}$/day, while grey seals had lower energy intakes (78-214 kcal/$kg^{\cdot 75}$/day). Further data are needed to determine if these differences represent true interspecific variation.

Nutrient Losses and Deficiency

A net result of purchasing inferior quality or low energy content fish is economic wastage, but of even greater consequence is the consumption by animals of fish that are low in nutrients or high in bacterial products. Two nutrients, in particular, are susceptible to degradation prior to feeding: thiamin (vitamin B_1) and vitamin E. Many, if not most, species contain enzymes (thiaminases) that result in the postmortem breakdown of thiamin (Geraci, 1978, 1981). It has been suggested that this breakdown may continue in the pinniped stomach after fish ingestion although several variables (time course, pH and temperature dependance, and relationship to gastric emptying) need clarification. In the absence of thiamin supplementation, thiamin deficiency characterized by anorexia, unresponsiveness, tremors, spasms, and death may result (Geraci, 1972). At NZP pinnipeds are supplemented with 250mg thiamin per day, or about 200 mg/kg fish dry matter. This supplementation level is greatly above the estimated requirements of terrestrial carnivores, 1-5mg/kg feed dry matter (NRC 1974, 1978, 1982). Recently Geraci (1978, 1982) has recommended incorporation of 25-35 mg thiamin per kg fish (freshweight) per day.

Animals fed thawed frozen fish are particularly susceptible to vitamin E deficiency. Marine and coldwater fish store body energy as polyunsaturated oils that remain fluid at cold temperatures. Polyunsaturation renders the oil highly unstable in the presence of molecular oxygen, with peroxidation and rancidity rapidly developing. The peroxidative process consumes vitamin E in the fish, and ingested polyunsaturated oils increase the vitamin E requirements of fish-eating animals. Thus fish which has been stored is apt to be low in vitamin E yet impose an elevated requirement. The poorer the quality of fish, the more likely the vitamin E status of the fish eater will be compromised.

The importance of regular and continued vitamin E supplementation is illustrated by the development of a deficiency syndrome in a yearling California sea lion at NZP. In 1981, it was deemed that the level of E

supplementation provided by a commercial marine mammal supplement (31 IU/tablet; Seatabs) was inadequate, yet the very high level of vitamin A (12,500 IU/tablet) in the same supplement made it inadvisable to increase the number of tablets given. Gelatin capsules providing 400 IU of vitamin E per capsule were substituted at a rate of 3-4 times per week. In June 1982, a female California sea lion produced and successfully reared a pup. At about 10 months the pup was finally weaned, and about two weeks thereafter vitamin E supplementation was initiated. Due to supply problems, vitamin E supplementation was discontinued after about one month (i.e. at about one year of age). The yearling developed poor appetite, ataxia (especially of front flippers), respiratory difficulties, hyponatremia and subsequently died 32 days following discontinuation of supplementation. Upon postmortem examination white muscle disease was diagnosed, with whitish streaks through most major skeletal muscle groups, especially in the subscapular muscles and muscle groups associated with the front flippers. Myopathy was also evident in the tongue and muscular portion of the diaphragm (NZP pathology report; #83-438).

The rapid onset of deficiency signs suggests that the vitamin E status of the yearling may have been marginal when the supplement was omitted. Little is known about maternal transfer of vitamin E to suckling pups in pinnipeds. Several samples of milk collected from wild California sea lions were found to contain 50-320 IU vitamin E per kg milk dry matter (O. Oftedal & D. Boness, unpublished data) but these may not be representative. Perhaps earlier initiation of supplementation or increased supplementation levels for lactating females may be indicated. At present supplementation levels for all pinnipeds have been doubled to 400 IU/day (or 800 IU every other day). On the other hand, rapid onset may simply be a product of the deleterious effect of partially oxidized fish oils: weaned kittens placed on a vitamin E deficient diet containing fish oils may die within 30 days (National Research Council, 1978).

It is unlikely that selenium deficiency was involved since marine fish are typically high in selenium. Milk from wild California sea lions contains about 0.7-1.5 ppm selenium on a dry matter basis (M. Allen and O. Oftedal, unpublished data), values which exceed expected requirements by 5-10 times.

Conclusion

If zoos are to breed and successfully rear fish-eating species, both economic and animal health considerations require that close attention be paid to fish quality. Fish is susceptible to rapid deterioration and requires immediate freezing and optimal freezer storage conditions prior to use. Vitamin supplementation should accompany use of thawed, frozen fish. Attention should be paid to both interspecific and seasonal variation in fish composition. Further studies are needed to document the effects of changing composition and quality on the nutrition, reproduction, and long-term health of fish-eating animals in zoos.

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Table 1. Annual Expenditures on Fish, National Zoological Park

Fiscal Year	Food Budget ($)	Fish Purchase ($)	Percent Food Budget (%)
1980	298,000	41,000	13.7
1981	297,000	24,950	8.4
1982	393,000	30,200	7.7
1983	357,000	33,700	9.4

Table 2. Fish Species Purchased by National Zoological Park (Percentage by Weight)

	Fiscal Year			
	1980	1981	1982	1983
Smelt	6.4	6.6	1.2	6.7
Sea trout	34.7	7.6	4.4	7.9
Mackerel	0.9	8.0	12.1	-
Herring	14.4	41.5	50.6	42.8
Butterfish	39.1	36.3	31.7	43.0
Whiting	4.5	-	-	-

Table 3. Analyses of Fish Purchased by National Zoological Park[1]

	Month[2]	Dry Matter (%)	Crude Fat (%)	True Protein[3] (%)	Metabolizable Energy[4] (kcal/g)
Atlantic Mackerel	4/82	40.5	20.7	12.7	2.4
	3/83	27.2	3.0	17.5	1.0
Butterfish	12/82	33.5	15.7	12.6	1.9
	3/83	36.4	17.7	11.7	2.1
	8/83	24.1	5.3	14.4	1.1
Sea Trout	12/82	30.7	11.9	15.2	1.7
	6/83	31.9	11.8	15.5	1.7
	9/83	31.2	10.3	17.4	1.6
Atlantic Herring	12/82	26.8	7.0	14.4	1.2
	3/83	33.5	12.2	13.6	1.6
	6/83	30.5	10.6	14.6	1.5
	9/83	32.2	11.4	14.9	1.6
Smelt	11/82	21.1	1.0	12.5	0.7
	3/83	18.7	0.9	10.7	0.5
	6/83	23.5	1.8	13.4	0.7
	8/83	23.5	1.3	14.5	0.7

[1] All assays performed in duplicate; means reported.

[2] Month received by NZP, not date of capture.

[3] True protein = 6.25 (total nitrogen - non-protein nitrogen).

[4] Calculated using 9 kcal/g fat and 4 kcal/g true protein.

Table 4. Calculated Metabolizable Energy Intakes of California Sea Lions and Grey Seals

Month	Group Composition (M.F.)	Mean Weight (kg)	Mean Fish Intake (kg/animal-day)		Energy Content (kcal/g)		Energy[1] Intake	
			Butterfish	Herring	Butterfish	Herring	a	b
California sea lions								
Oct. 82	1.5	94.1	3.3	1.1	1.92	1.21	7670	258
Mar. 83	1.5	93.4	---	5.6	2.06	1.64	9180	308
July 83	1.5	95.3	0.3	3.4	1.05	1.54	5550	185
Grey seals								
Oct. 82	1.5	107	1.3	2.7	1.92	1.21	5760	176
Mar. 83	1.5	124	---	4.8	2.06	1.64	7870	214
July 83	1.4	126	0.7	1.4	1.05	1.54	2890	78

[1] a = kcal/animal-day; b = kcal/$kg^{.75}$-day

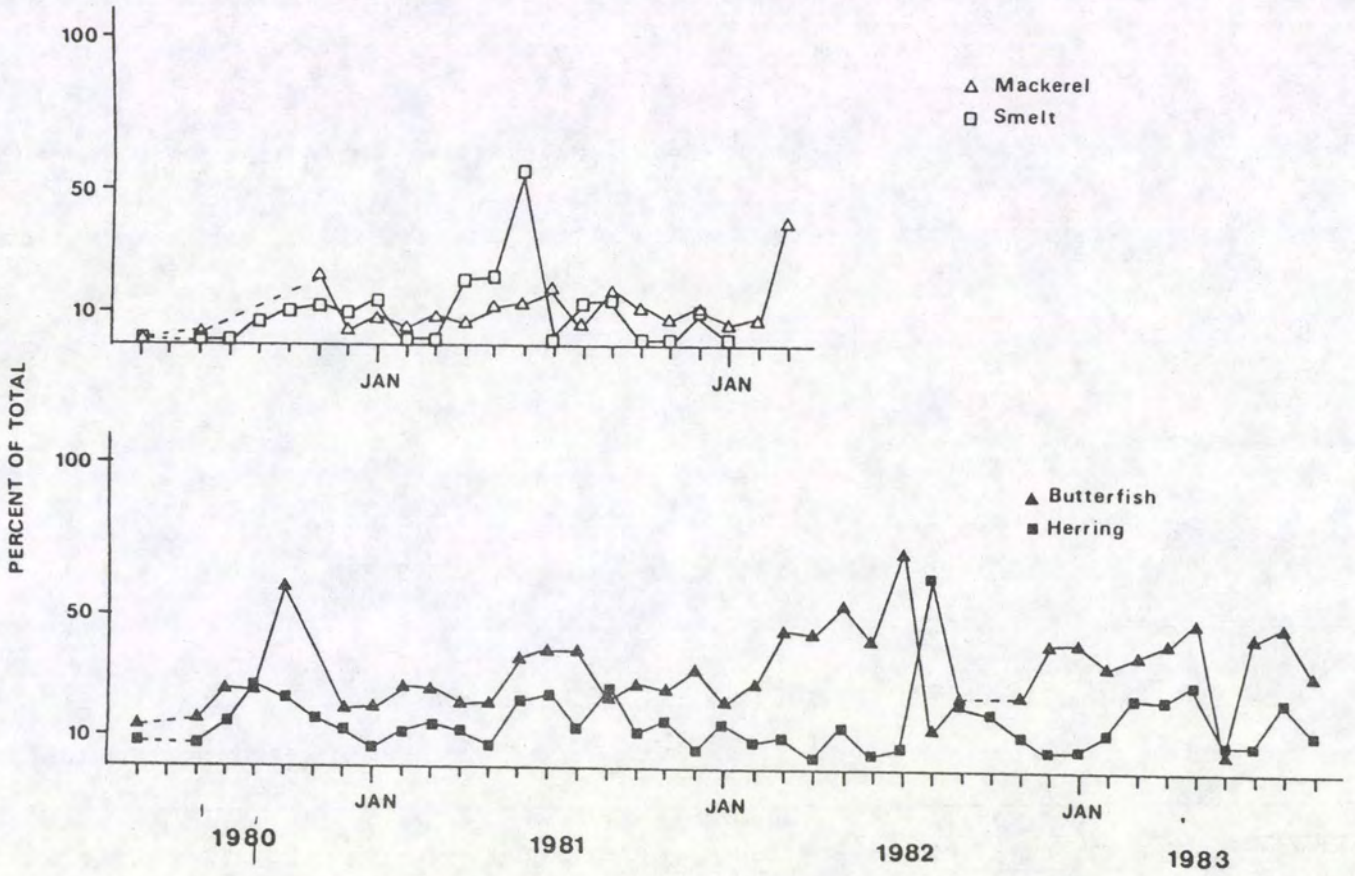

Fig. 1. Percentage of poor quality fish discarded at the National Zoological Park from May, 1980 to August, 1983

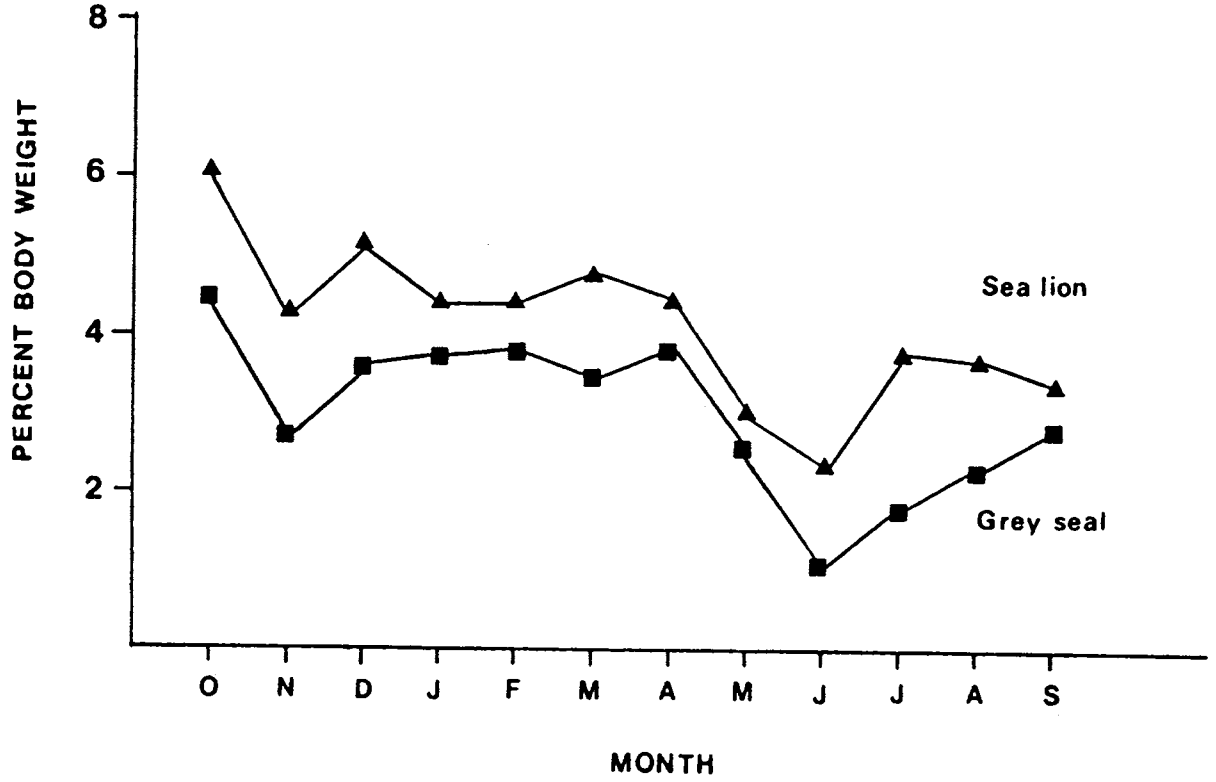

Fig. 2. Food consumption of California sea lions and grey seals at the National Zoological Park from October, 1982 to September, 1983

Q: Dr. Oftedal, I want to know if you could comment or maybe someone in the audience would comment on the correlations between serum vitamin E levels and clinical disease such as steatitis or white muscle disease in pinnepeds. Is there a good correlation with serum Vitamin E levels?

Dr. Ullrey: We are no great shakes at Vitamin E analysis, certainly. We have done quite a few plasma analyses, no attempt to differentiate between the various isomers that are present. We're currently working on high pressure liquid chromatography separation, with a good deal of success at this point. We do think there is a relationship between the signs and plasma concentrations. We have no experience with pinnepeds, it is practically all with domestic species and deer.

Q: Dr. Oftedal, regarding the case of the white muscle disease, was this animal chased extensively by another animal in the exhibit because of behavioral conflict, was there any chance of predisposition to capture?

Dr. Oftedal: I was out of town at the time this happened, so I have not made personal observations, but in discussing this with the keeper their only concern was that the animal might have been stressed by the vets collecting blood.

Dr. Meehan: Sounds familiar.

Dr. Oftedal: No, it was not chased around extensively. I just thought that might be an alternate explanation for why the striation was found in the muscles.

Q: I have a question for Mary Allen. On the frogs you showed the difference between sexes, male and female, you mention there is a difference in body fat. What if you were to express the calcium in terms of percent of body weight, are males larger than females?

Mary Allen: Males are noticeably smaller than females. The average weight of the males was 8 and the females was 27 grams.

Q: Question for Mary Allen. One of the biggest puzzles I think has been how the insectivorous reptile gets enough calcium in its environment to have bone structure. In your work, have you come up with any ideas in this area?

Mary Allen: Well, I think in starting I mentioned a couple. One idea is borrowed from Bilby & Widowson's paper, back in the 70's. They were looking at nestling thrushes and blackbirds in England and they noticed that in these particular nestlings there were phenomenal growth rates compared to other birds that they knew about. They thought the parent birds were bringing back insects and feeding them to the young and the nestlings were calcifying their skeletons very quickly on a diet of insects. How can that be? I haven't seen anything subsequent to that paper, but they suggested that at the time the parents were feeding on invertebrates that were terrestrial or sub-terrestrial earthworms for example, which might be ingesting soil. If that soil was particularly rich in calcium or other minerals then that in turn would be ingested by the earthworm, which is then ingested by the thrushes or the blackbirds. So, I guess, my ideas would be that the calcium is either coming directly into the insect eating animal, as in the anteater, which ingests a lot of soil when eating ants and termites, or the insect is allowed to or is obliged to eat calcium rich materials. Leafy materials are particularly high in calcium and since insects apparently don't have a calcium requirement per say as we vertebrates know it, that calcium would be found in the gut and passed on through. In my study, however, it took an 8% calcium diet in feeding a cricket to get a whole body cricket calcium level up to 1.5%. I don't know of any natural leafy material out there in the wild that an insect, a cricket or a locust would feed on that would have that high a calcium level. So we really don't know yet, but I suspect that there might be secondary ingestion of minerals especially by animals like tenrec or shrew who are terrestrial and feeding on the ground and ingest soil and minerals secondarily.

Dr. Culley: I would like to comment on this, just a little additional information. In working with frogs we did some studies where we were feeding crickets to frogs. It's been several years so I can't give you the exact figures, but if I recall there appeared to be enough calcium in the whole body analysis of the cricket but we were dealing with the calcium phosphorus ratio that was incorrect. We also have studied frogs fed a diet of fly larvae. Well, if you look at the fly larvae, there's not much of an exoskeleton. You really wouldn't call it exoskeleton at all, and yet there apparently is a tremendous amount of calcium in the larvae, so that we never had any kind of bone problems whatsoever with the frogs. I wonder if these insects do have a proper amount of calcium but it might

be the mineral ratio that is something to be looked at.

Dr. Oftedal: I'd like to make an addition and say it is remarkable when you think about it because some of the passerine birds have phenomenal growth rates. Aviculture people often talk about how birds switch to insects when they're rearing young babies because that's a good protein source. Well I think insects are a fairly good protein source, although there are some complications in interpreting protein values for insects, but insects certainly wouldn't appear to be a good source of calcium.

Mary Allen: I would also add that last summer some friends of mine were keeping some frogs as pets you might say and they were collecting wild crickets out by the mink farm in Michigan state and I had them collect about 200 grams of wild crickets for me and I did a few analyses on the crickets and found them to be just about what the calcium levels were from crickets I brought from Flukers Cricket Range in Baton Rouge. Maybe that changes throughout the year, or from season to season in wild crickets. I didn't do it on a seasonal basis, it was a one-time affair, but it would bear looking into. There are dipterin flies that are known to have calcium pockets and I think this might be in the larval stages as opposed to the adult. In general, from looking at the limited literature that is available on insect body composition, there doesn't appear to be a lot of calcium around in an insect. So, there are questions that still need to be answered, but we also have to start looking at different numbers and types of insects too.

Q: Dr. Oftedal, why did you get rid of mackerel and settle on butterfish?

Dr. Oftedal: I didn't do it, it was not my decision. I think the keepers felt about the same way Mike did about mackerel. I think there were problems with the quality of the fish, probably due to storage and handling, which led to problems of animal acceptance. I have to admit on all this, maybe I sound knowledgeable up here, I really feel like I know very little about what is really going on here with the fish. I think it might be that fish quality is the most important thing. It's a lot harder for me in my position to be sure we get good quality fish. Certainly we can supplement with vitamins, but I Think the bottom line is to really try and understand good quality fish and how to get it.

Q: Is it possible to use bacterial cultures to help determine the quality of frozen fish and how they have been handled?

Dr. Stoskopf: Strictly from a bacteriological standpoint we can culture. The starting counts that you deal with on most frozen fish are so astronomical that it's almost mind-boggling. Mostly what we do is just a search for salmonella.

Q: Regarding the histamine level that was mentioned with mackerel, Geraci or someone did a correlation that if the mackerel was fed at about 70° F, water temperature, that there was a high incidence of ulcers in dolphins. Do you know about that, is there much to that?

Dr. Stoskopf: There is, I'm not up on all those arguments. I do agree with Geraci that he has shown that you can analyze for histamine. Histamine content is certainly related to fish handling. The conversion to histamine, a lot of it being bacterial increases with temperature. The next part of the question is how histamine levels in the fish relate to ulcers. Geraci is very convinced that high levels of histamine can predispose to ulceration in cetaceans.

Q: Natural populations that feed on fish get fluctuations in dietary protein and fat levels. Should we be concerned with that in zoos? Should we actually be feeding fish from different seasons if there is some benefit to the varied diet?

Dr. Oftedal: Very good point. I think it is possible that there might be advantages to seasonal fluctuations in composition. We're actually doing that because the fish is fluctuating on us, whether we mean it to or not.

Q: Do you actually try to control when your fish are caught?

Dr. Oftedal: We right now are simply in a stage of gathering data. I'm not trying to do anything except get information. We are also trying to get better quality fish. Since I've been away from town , we have cancelled our contract with one of our vendors in Washington trying to force them to take us seriously when we say we won't accept bad quality fish. It is not an easy situation to deal with, particularly when you're in a contract system. We as part of the federal government award a contract to the lowest bidder and we say it has got to be grade A fish. We have listed all the things we will not accept, but I have a feeling some vendors think, "they're just a zoo, they just say that," and they bid it on the basis that they will give us

inferior quality fish and that's the way they get the award. Then we are stuck with dealing with vendors who are trying to give us poor fish, because the good vendor who wouldn't give us the poor quality fish wouldn't make such a poor bid so he never gets the contract. There are other institutions, however, that have a much more flexible system that encourages developing good vendor relations. You could actually get a box ahead of a shipment from several different vendors when you're trying to order and examine them all and let vendors know what you're looking for. You don't have to go on with the cheapest one, you go with the one you think is the better quality. When you have discard rates like we have, you're throwing away 20% - 30% - 50% of the fish you get in. You may be getting the fish for half price and still losing money, apart from the effect on the animals.