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

The Dr. Scholl Nutrition Conferences are sponsored by
The Lincoln Park Zoological Society and the Chicago Park District,
with funding from the Dr. Scholl Foundation.

# Proceedings of the Eighth DR. SCHOLL CONFERENCE ON THE NUTRITION OF CAPTIVE WILD ANIMALS

Edited by

Thomas P. Meehan, D.V.M.
Steven D. Thompson, Ph.D.
Mary E. Allen, Ph.D.

Copy Editor

Barbara E. Royal

December 8 and 9, 1989
Lincoln Park Zoological Gardens
Chicago, Illinois

**Library of Congress National Serials Data Program**

Lincoln Park Zoological Society
Proceedings of the Eighth Dr. Scholl Conference
on the Nutrition of Captive Wild Animals

**ISSN 1054-6650**

**Acknowledgements**

The editors would like to thank Dave Baer, Perry Barboza, Sue Crissey, Andreas Papas, Laura Jane Stewart, and Duane Ullrey for their assistance in reviewing papers.

## TABLE OF CONTENTS

## Eighth Dr. Scholl Nutrition Conference - 1989

TABLE OF CONTENTS (continued)

# DESIGNING DIETS FOR HERBIVOROUS MARSUPIALS

P.S. Barboza BSc (Hons), PhD[1]
&
I.D. Hume BSc (Agric), PhD, DSc[2]

1. Department of Zoological Research,
National Zoological Park, Smithsonian Institution,
Washington DC, USA 20008.
2. School of Biological Sciences,
Zoology Building A08, University of Sydney,
New South Wales, Australia 2006.

Herbivorous marsupials are popular exhibits in many zoos and often represent large investments of time and money in establishing the animals and their displays. The success of these exhibits depends upon the maintenance of the health and fecundity of the animals and thus upon the provision of appropriate diets.

Marsupials generally have lower requirements for energy (Dawson and Hulbert, 1970) and protein (Hume, 1982) than eutherian mammals. Thus marsupials may meet their nutrient requirements at lower feed intakes than a similar eutherian on the same diet. Alternatively, these low requirements may also enable marsupials to utilize poorer quality diets of higher fiber content than similar eutherians. In captivity, marsupial herbivores are often fed concentrated diets of low fiber content which may cause obesity or disrupt digestive function and normal metabolic processes.

Most herbivorous marsupials select the fibrous parts of plants such as the stems and leaves of grasses, sedges and herbs, and the leaves of shrubs and trees. These plant tissues are digested by disrupting the fibrous structure of the plant cell walls through mastication and by microbial fermentation in the digestive tract. The design of a captive diet must therefore consider the physical and chemical characteristics of the natural diet, and the digestive and metabolic adaptations of the species.

Herbivorous marsupials fall into three distinct groups: the macropodines (kangaroos and wallabies); the wombats (hairy-nosed wombats and the common wombat); and the arboreal folivores (brushtail possums, ringtail possums, koala and greater glider).

## Kangaroos and Wallabies

The macropodine marsupials comprise 37 species ranging in body weight from approximately 5kg in the forest wallabies to 70kg in the grazing kangaroos. Kangaroos mainly consume a variety of grasses whereas the smaller wallabies may also select the leaves of shrubs and forbs (Dawson, 1989). The molar dentition of grazing kangaroos is adapted to shearing fibrous material, whereas that of the browsing species is better suited to crushing and grinding herbage (Sanson, 1989). Some browsing wallabies such as the swamp wallaby (Wallabia bicolor) also possess premolars adapted for shearing coarse vegetation. In some grazers the molar dentition is replaced anteriorly (molar progression), thereby maintaining a sharp and efficient dental mill throughout life. Molar progression

is a feature of the genus Macropus, which includes commonly exhibited species such as the eastern grey kangaroo (M. giganteus), red kangaroo (M. rufus) and tammar wallaby (M. eugenii). The macropodine digestive tract features a haustrated forestomach which is the principal site of microbial fermentation. A secondary fermentation region also occurs in the hindgut along the cecum and proximal colon, but this is minor in comparison with the forestomach. Dietary fiber is digested by the retention and fermentation of coarse particles in the forestomach. The fermentation produces volatile fatty acids (VFA) and microbial cells. The VFA are absorbed directly across the stomach mucosa and are utilized as an energy substrate by the animal. The microbial cells are readily digested and absorbed in the hind stomach and small intestine and are a valuable source of protein and B-vitamins. The foregut fermentation of the macropodines is directly influenced by the dietary content of fiber and readily fermentable materials such as starches. Dietary composition will greatly influence the species composition of the microbial population and thus the products of the fermentation. Since fiber residues stimulate gut motility, digesta passage and mixing will also be influenced by dietary fiber content. Therefore the digestive function of the forestomach may be disrupted on diets comprised of vegetables and cereal grains which are low in fiber and high in starches.

Soft, concentrated diets have traditionally been provided to macropods in many collections as a precaution against 'lumpy jaw' (Fox, 1923; Wallach, 1971). This condition usually results from the infection of an injury to the mouth or gums and is associated with several bacteria especially Fusobacterium necrophorum (Samuel, 1983). The low incidence of 'lumpy jaw' in the wild, and its increased incidence with crowding in captivity suggest that stress and faecal contamination of enclosures may be contributing factors (Finnie, 1982; Munday, 1988). Although soft diets may minimize the chance of oral injury they may further stress the animals by disrupting their digestive function, and also weaken the teeth and gums by promoting the accumulation of plaque (Munday, 1988). Furthermore, these diets may be insufficiently abrasive to rapidly remove teeth shed through molar progression, thereby leaving a potential site for infection (Finnie, 1982). Thus these soft concentrate diets may make kangaroos and wallabies even more vulnerable to 'lumpy jaw'.

Grazing kangaroos such as wallaroos (M. robustus robustus) and euros (M. robustus erubescens) can be maintained on pelleted mixtures of straw and alfalfa hay (Freudenberger, 1990) (Table 1). Loosely pelleting this mixture maintains its homogeneity during feeding, handling and storage. This complete diet was provided ad libitum to a colony of female kangaroos for three years, during which time there were no cases of infectious or nutritional disorders (Freudenberger, 1990). The composition of the pelleted mixture is similar to that of many grasses, that is, 60.5% Neutral Detergent Fiber (NDF - dry matter basis) and 9.4% Crude Protein (CP - dry matter basis). Growing euros and wallaroos were provided with fresh grasses in addition to the pelleted mixture to increase the digestible energy content of the diet (Freudenberger,

1990). A similar feeding practice may also be appropriate for the smaller browsing wallabies.

The preparation of complete diets specifically for a small population of grazing and browsing macropodines may not be economically feasible for most collections. In these cases a pelleted supplement formulated for grazing ungulates (for example, containing 40% NDF and 12.5% CP) should be provided with a high quality grass hay. The diet should exclude sharp items such as oat or rice awns and over-dried hays as these may injure the animals' mouths. If the animals have been maintained on soft diets for prolonged periods their gums may be easily injured. Therefore it would be prudent to gradually introduce the hay with fresh grass to minimize the chance of injury. The greater time required to consume these forages may also reduce stress-related behaviors such as pacing and excessive grooming.

Wombats

The wombats comprise three species with adult weights ranging from 20 to 50kg. Like the macropodines, the wombats are grazers but also consume sedges which are high in fiber and low in protein contents. This abrasive diet is thoroughly milled by a dentition which grows throughout life and wears to leave extremely sharp facets for shearing the coarse vegetation. In contrast with the macropodines, the wombats have a small stomach and a voluminous proximal colon but only a vestigial cecum (Barboza, 1989). The prolonged retention of particulate digesta enables the fermentation of fiber along the proximal colon. Dietary starches and proteins are digested and absorbed in the small intestine, leaving mainly fibrous residues to pass to the fermentation region of the proximal colon (Barboza, 1989). Therefore, unlike the kangaroos, energy is not potentially lost by fermentation of these soluble components in the foregut. Conversely, the microbial cells produced in the hindgut fermentation are potentially lost with the feces of the wombat, since they are not coprophagous.

The maintenance requirements for dietary energy and protein in the wombats are the lowest among herbivorous marsupials (Barboza, Hume and Nolan, 1990). Consequently, wombats may become obese if maintained on concentrated, low fiber diets. Furthermore, dietary fiber is essential to maintain gut motility and function and to prevent over growth of the teeth. Common wombats (Vombatus ursinus) and hairy-nosed wombats (Lasiorhinus latifrons) were maintained for four years on pelleted mixtures of straw, alfalfa hay and cereal grains (Table 2) with high fiber grass hays provided ad libitum. These animals will accept extremely hard pellets of feed, and this may assist both tooth wear and behavioral occupation. Where a customized diet is impracticable, wombats can be maintained on a high fiber supplement for grazing ungulates (for example, containing 50%NDF and 12.5% CP) with a high fiber grass hay or straw provided ad libitum.

The micronutrient mix included in the pelleted mixtures for wombats and kangaroos is listed in Table 3. Levels of Vitamin E (acetate form) in these diets were apparently sufficient to prevent muscular dystrophy in both kangaroos and wombats for over 3 years. The mineral and vitamin levels in the complete diets are generally

similar to those recommended for domestic pigs, rabbits and poultry. However, copper levels are low and similar to levels recommended for sheep and cattle (7-8ppm) (Mertz, 1986); hairy nosed wombats (L. latifrons) may be poisoned by high levels of dietary copper (>30ppm) (Barboza, and Vanselow, in preparation). These low levels of copper were apparently adequate for maintenance of both the grazing kangaroos and the wombats.

Arboreal Folivores

Unlike the wombats, the arboreal folivores are small, with body weights ranging from less than 1kg in the ringtail possums (Pseudocheirus spp.) and greater glider (Petauroides volans), to 2.5kg in the brushtail possums (Trichosurus spp.), and 8kg in the koala (Phascolarctos cinereus). Eucalyptus foliage is the predominant or sole dietary item of the koala, ringtail possum and greater glider, but the brushtail possum may also include fruits and flowers in its diet. The small body size of these species may constrain their utilization of bulky dietary fiber because their digestive capacity to retain this slowly fermenting material may be low in relation to their energy requirements (Demment and Van Soest, 1985; Hume, 1990). The digestive tracts of arboreal folivores are characterized by a well developed cecum. Cecal complexity increases in progression from the brushtail possum, to the koala, with the greatest haustration evident in the ringtail possum and the greater glider. The proximal colon is well developed in the brushtail possum and the koala, whereas the ringtail possum and the greater glider have a simple proximal colon without haustrations. Therefore microbial fermentation is restricted to the cecum of these latter two species. Fine particles of digesta are selectively retained in the cecum and proximal colon of the koala, and in the cecum of the ringtail possum and greater glider. The selective retention of fine particles of digesta reduces the bulk of the less digestible coarse fibrous material in the hindgut and also minimizes the loss of microbial cells with the feces.

Foley and Hume (1987) suggested that the absence of selective retention in the brushtail possum explained the inclusion of more digestible items than foliage in its diet. In the ringtail possum, the loss of microbial cells is further reduced by cecotrophy (ingestion of soft feces) which may also enhance the digestion of the fibrous fine particles (Chilcott and Hume, 1985). The brushtail possum will accept a variety of items in captivity and can be maintained on a mixture of cereals and ground alfalfa hay (2mm mesh) (W.J. Foley, personal communication 1989) (Table 4.). This complete diet includes infant milk replacer (lactose free) as a source of protein and micronutrients, with honey to enhance palatability and cohesion of the mixture. Similarly, the ringtail possum may also be maintained on a mixture of vegetable produce without fresh tree foliage (Roberts, Phillips and Kohn, 1990). However, insufficient dietary fiber may predispose this species to torsions and pathogenic infections of the hindgut (W.J. Foley, personal communication 1989). Dietary fiber levels for possums in the National Zoo (Research Collection) were increased by sprinkling ground alfalfa hay over the other dietary components of vegetable

produce and canned primate diet (Hills, Zupreem).

The diet consumed by these five animals (Table 5) contains approximately 78% moisture, 14.4% CP and 9% NDF (Barboza, unpublished data). These adult animals maintained condition and body weight (736 - 931 g) on this diet for approximately one year. It is hoped that higher dietary fiber levels similar to Eucalyptus foliage may be achieved by mixing higher levels of ground hay with the fruit items. The greater glider and the koala are more specialized folivores which require fresh Eucalyptus foliage in captivity. In the koala, species preferences for foliage vary both geographically and between trees within an area. The chemical characteristics of foliage selected by koalas are being studied by Hume and co-workers. Captive koalas in northern New South Wales (Australia)consumed 6 of 18 species of Eucalyptus and selected young foliage containing greater than 11.3% CP and 65% moisture,with 20-33% NDF (Pahl and Hume, 1988). Eucalyptus foliage also contains volatile oils which are potentially toxic and may influence selection by koalas. Selected Eucalyptus species contained 1-3% essential oils which were mainly comprised of monoterpenoids such as cineole and a mixture of more volatile groups (Hume and Rezl, unpublished data). These characteristics were used to compose and test an artificial diet for koalas based on ground alfalfa hay and presented in a flat, pliable form similar to Eucalyptus leaves (Pahl and Hume, 1988). This artificial feed was used as a supplement to fresh foliage and contained 11.9% CP, 62% moisture and 24% NDF. Hand feeding the artificial supplement reduced foliage consumption of koalas by up to 50% (Pahl and Hume, 1988). Further studies of artificial feeds with trained animals may result in a complete captive diet for koalas independent of fresh Eucalyptus foliage.

## Conclusion

Herbivorous marsupials are highly adapted to utilizing the fibrous parts of plants. The form and composition of captive diets must therefore address the digestive physiology and metabolism of these species. The design of improved diets and feeding practices will enhance the viability of captive populations and thus promote research, conservation and exhibition of these fascinating animals.

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Table 1. Pelleted maintenance diet for grazing kangaroos (Freudenberger, 1990).

| Component (% Air Dry) | Inclusion |
|---|---|
| Milled Straw | 50.0 |
| Chopped Alfalfa Hay | 47.0 |
| NaCl | 0.75 |
| $CaH_2PO_4$ | 0.75 |
| $CaCO_3$ | 0.75 |
| $MgSO_4$ | 0.12 |
| KCl | 0.22 |
| $NaSO_3$ | 0.21 |
| Micronutrient mix | 0.20 |

Table 2. Pelleted maintenance supplement for wombats (Barboza, 1989).

| Component (% Air Dry) | Inclusion |
|---|---|
| Milled Straw | 40.0 |
| Chopped Alfalfa Hay | 30.0 |
| Ground Maize | 13.9 |
| Ground Oats | 15.0 |
| NaCl | 0.21 |
| $CaH_2PO_4$ | 0.44 |
| $CaCO_3$ | 0.12 |
| $MgSO_4$ | 0.13 |
| Micronutrient mix | 0.20 |

Table 3. Micronutrient mix for grazing marsupials (Level per kg of air dry feed) (Barboza, 1989).

| | |
|---|---|
| Fe | 10 mg |
| Co | 1 mg |
| Mn | 58 mg |
| Zn | 50 mg |
| I | 0.7 mg |
| Se (ug) | 2 ug |
| Vitamin A | 12000 I.U. |
| Vitamin D | 2400 I.U. |
| Vitamin E | 30 I.U. |
| Vitamin $B_1$ | 2 mg |
| Vitamin $B_2$ | 6 mg |
| Vitamin $B_6$ | 2 mg |
| Vitamin $B_{12}$ | 20 ug |
| Pantothenic Acid | 5.5 mg |
| Niacin | 26 mg |
| Choline | 200 mg |
| Vitamin K | 0.6 mg |
| Biotin | 320 ug |
| Folic Acid | 0.4 mg |

Table 4. Maintenance diet for brushtail possums (W.J.Foley, personal communication 1989).

| Component (% Air Dry) | Inclusion |
|---|---|
| Ground Alfalfa Hay | 24.0 |
| Crushed Weetbix (breakfast cereal) | 16.0 |
| Rolled Oats | 16.0 |
| Infant Milk Replacer (lactose free) | 4.0 |
| Honey | 24.0 |
| Water | 16.0 |

Table 5. Diet consumed by common ringtail possums at the National Zoo (Barboza, unpublished data).

| Component (g Air Dry) | Daily Intake |
|---|---|
| Ground Alfalfa Hay | 1.6 |
| Banana (without skin) | 16.5 |
| Carrot | 17.6 |
| Celery Stems | 9.7 |
| Grapes | 12.1 |
| Kale | 9.8 |
| Primate Diet (Hills, ZuPreem) | 12.7 |

# Eucalyptus Production

Robert J. Frueh
Koala Browse Inc.
Boynton Beach, FL

## GENUS EUCALYPTUS

The genus Eucalyptus is the largest genus of trees in the world. There are over 500 species of eucalyptus, all endemic to Australia. They vary from low shrubs to the tallest hardwood trees in the world. Koalas feed exclusively on eucalyptus and are known to eat over 30 species. Eucalyptus is a great economic resource in Australia and now is found in over 50 countries in the world. Numerous chemicals are extracted from eucalyptus. There are medicinal oils for the nose and throat, industrial solvents such as disinfectants and deodorants, and perfumery oils like menthol. In addition, the trees are used for firewood, charcoal production, shelter, ornamental ** , some are great honey trees, and most are good post or lumber and furniture woods.

## SEED

Eucalypts have varied flowering patterns. The flowers are generally inconspicuous, small, and whitish. Fertilized flowers result in a seed capsule somewhat like an acorn except the seed in most cases is like coffee grounds. There can be from 200 to 60,000 seeds per ounce. The seed can be stored for years in refrigeration. This seed is lightly sprinkled over moist, sterile potting soil in trays. Kept damp and warn, the seed will germinate in 5 to 10 days.

## SEEDLINGS

As soon as seedlings appear we start applying liquid fertilizer every 7-10 days. By the time the third set of leaves appear (4-6 weeks) the seedlings are placed in separate containers and then placed in the sun. The seedlings grow 12-18" in the next 2-3 months and are ready to be transferred to a larger container or planted in the ground.

## FIELD

Florida's soil is extremely sandy, very low in organic matter and needs supplementing. We dig a trench 1 foot x 1 foot x 100 feet and fill it with potting soil with long term, slow release incorporated fertilizer. All grass and seeds are removed as the seedlings do not compete well. The most aggressive species will grow 8-9 feet in the next 6-8 months. The top four feet is removed as a shippable cutting. The existing side branches will produce more cuttings in another 4-6 months.

SHIPPING

We are now harvesting approximately 1600 4 foot stems per week. These stems are collected into bundles of about 10 stems each. The base of the bundle is wrapped with paper and soaked in water and an identification tag is attached. The bundles are placed in plastic sleeves to conserve moisture and to protect the leaves in the cold months. Finally, four or five bundles are placed in cardboard boxes and shipped to the airport.

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# Feeding and Browse Selection in Koalas

Pearl Yusuf
Lincoln Park Zoo
2200 N. Cannon Drive
Chicago, IL 60614

Until 1988, koalas were scarcely seen in American zoos. It was a privilege reserved only for 3 Californian zoos and the occasional institution who could keep them on a temporary loan basis. The primary obstacle institutions had to overcome concerned Eucalyptus browse - the koalas' nearly exclusive diet. Holding these animals on a permanent basis involves the high cost and potential unpredictability of obtaining and maintaining a considerable volume and selection of species.

Lincoln Park Zoo overcame this obstacle by connecting with horticulturalist Bob Frueh of the now established Koala Browse, Inc. in Boynton Beach, Florida. On this 5 acre plantation, Frueh is able to maintain and supply Lincoln Park (and since, other institutions) with a variety of Eucalyptus species which are flown to Chicago twice weekly.

In September of 1988, Lincoln Park's cooperation with San Diego Zoo and Bob Frueh resulted in our acquiring 2 female koalas (and recently, a breeding male). These animals are on permanent loan from San Diego Zoo in an attempt to establish the first breeding group of koalas in an institution unable to cultivate its own supply of feed. And though, as in many undertakings, things have not gone as smoothly as ideally possible, the system has worked.

Working with San Diego Zoo staff, we have been able to establish a routine for the best care of these animals. But beyond that this routine allows us to collect information on their browse preference and to monitor any effects it may have on their weight and fecal output.

This paper will report mainly what we have found to be our female koalas' browse preferences on a month by month basis in the time we have been holding them and (in admittedly less conclusive data) show their correlating weights and combined fecal output.

Twice a week we receive a shipment of 80-100 Lbs. of several varieties of Eucalyptus from Florida. The branches are placed loosely in large barrels of water and stored in a zoo commissary cooler. It is the responsibility of the koala keeper to daily hand-pick 20-30 Lbs of at least 4 varieties of Eucalyptus for feeding. This selection is based on a branch by branch assessment of how well varieties were eaten on the previous day. Each species is judged to be accepted as "excellent", "good", "fair", "poor", or "none". Each keeper must therefore be capable of recognizing all species available and to communicate the need for an increase or decrease of specific varieties to Bob Frueh in Florida.

With this system it was possible to compile a monthly "percentage of acceptance." Tables were created counting the number of days a species was "offered" and how many of those days they were "preferred" (meaning a judgement of either "good" or "excellent"). This data was then charted for every species offered between September 1988 and September 1989, as seen in Table I. Information on the number of days a species was judged to be either "excellent" or "none" (none eaten) is also included in this table.

Species that were offered at least 12 of these 13 months for at least an average of 15 days were used to create a percentage graph (days preferred/days offered). This graph then included E. citriodora, E. rudis, E. robusta, E. tereticornis, E. cinerea, E. grandis, E. melliodora, and E. camaldulensis. This data is seen in figures I and II.

To examine the possibility of correlation with the animal's monthly average weight and combined fecal output, similar graphs were made. (See figures III and IV.)

It is obvious from figures I and II that selections were not as conclusive as we would hope. However, with a few explanations, things may be made a little clearer:

- In spring and early summer of 1989, E. citriodora for some reason rarely survived shipment well and therefore only a few surviving branches were offered. Thus the 100% acceptance in May 1989 is only for 3 days of availability and not very conclusive. The species was not offered at all in July 1989.

Though the fecal weight dropped consistently between November 1988 and March 1989, the weights of the animals and the general drop in preference of several browse species occurred January through March 1989. It must be noted that the decrease in fecal weight is due in part to the decreased humidity in the exhibit during those months. But also the actual volume of pellets collected decreased as well. Obviously, more could be discovered if we were able to collect dry weight data. Also, it should be said that none of these decreases reflected ill-health in either animal.

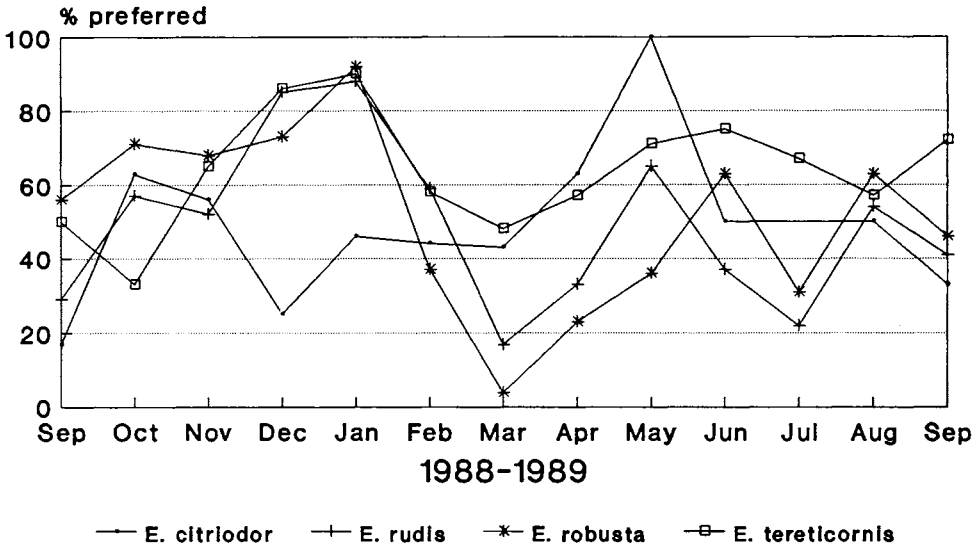
Neither E. tereticornis nor E. camaldulensis (2 of our more consistently offered species) dipped below 30% and represent our most consistently accepted varieties.

Some species which are accepted well over 50% of the time have not been available in abundance throughout the year (e.g., E. occidentalis, E. ovata). It would be interesting to see if they would be able to sustain their level of acceptance year-round.

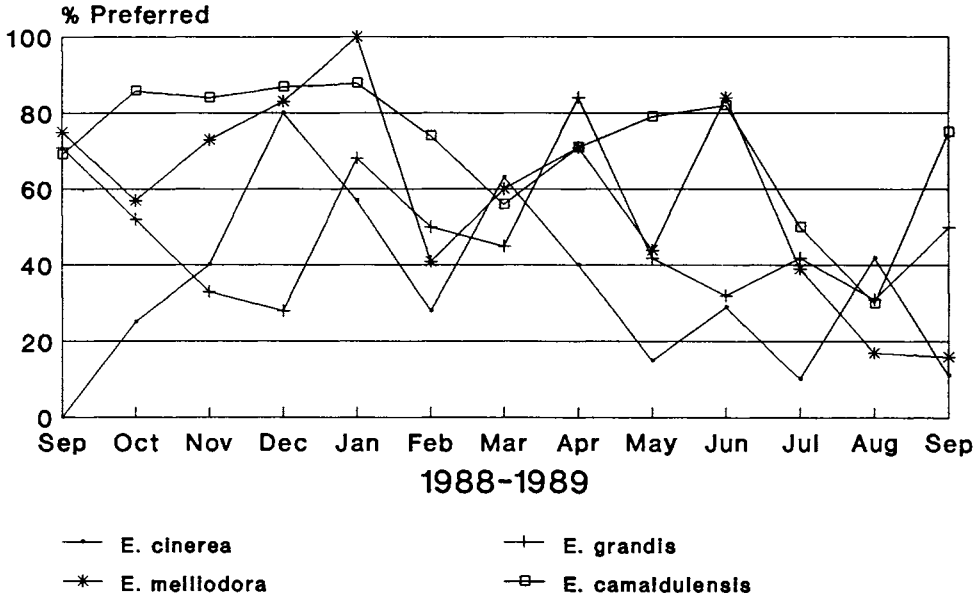
In years to come, hopefully, we will see that these preliminary numbers represent the beginnings of a more defined pattern in koala browse preference. But it should be apparent that with this routine and similar ones throughout the country that now we have the opportunity to discover a great deal and thus add to the relatively scarce literature on this subject.

# Eucalyptus % Preferred

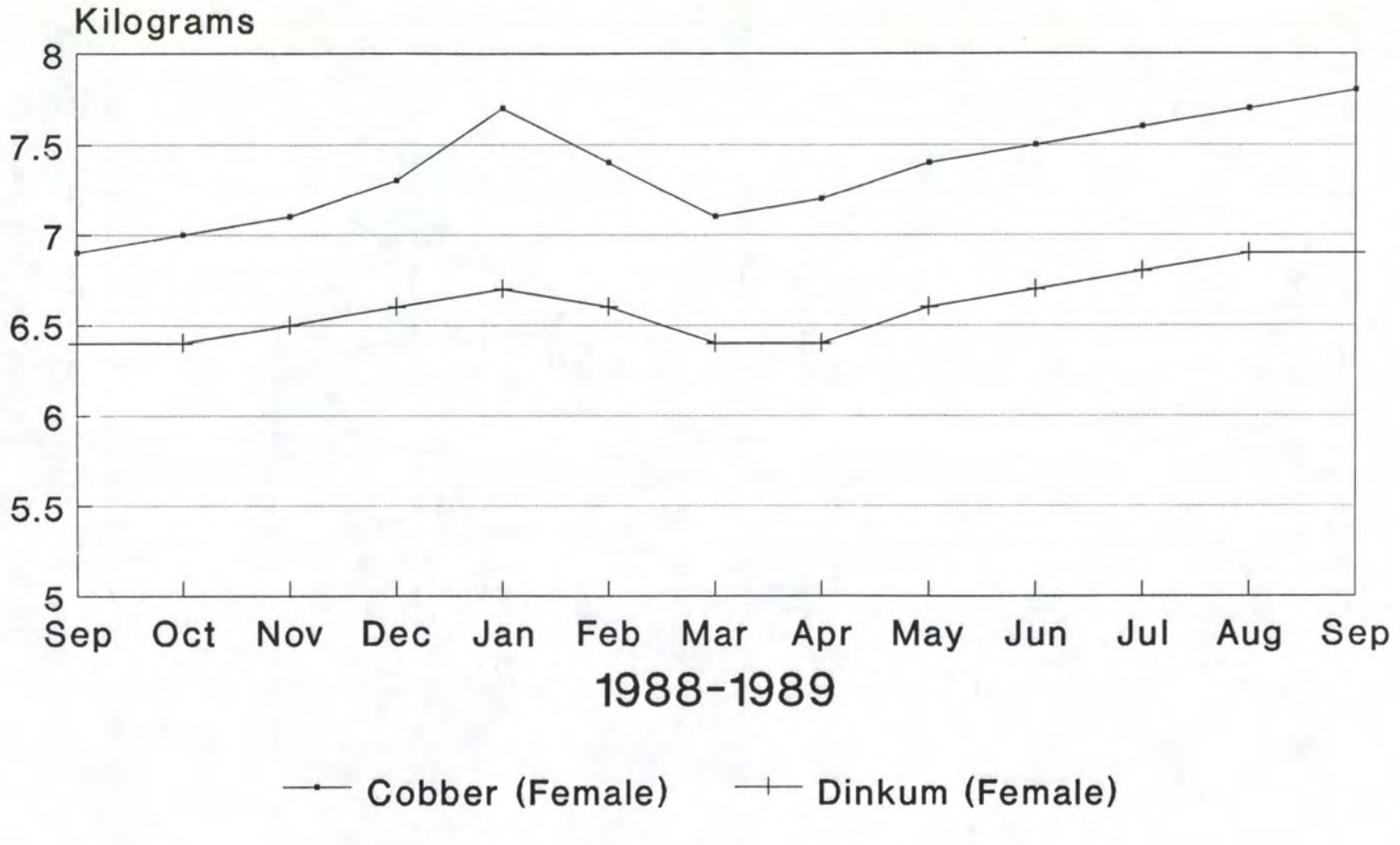
## Figure I




## Figure II



# Figure III
## Koala Body Weight



# Figure IV
## Combined Wet Fecal Weight

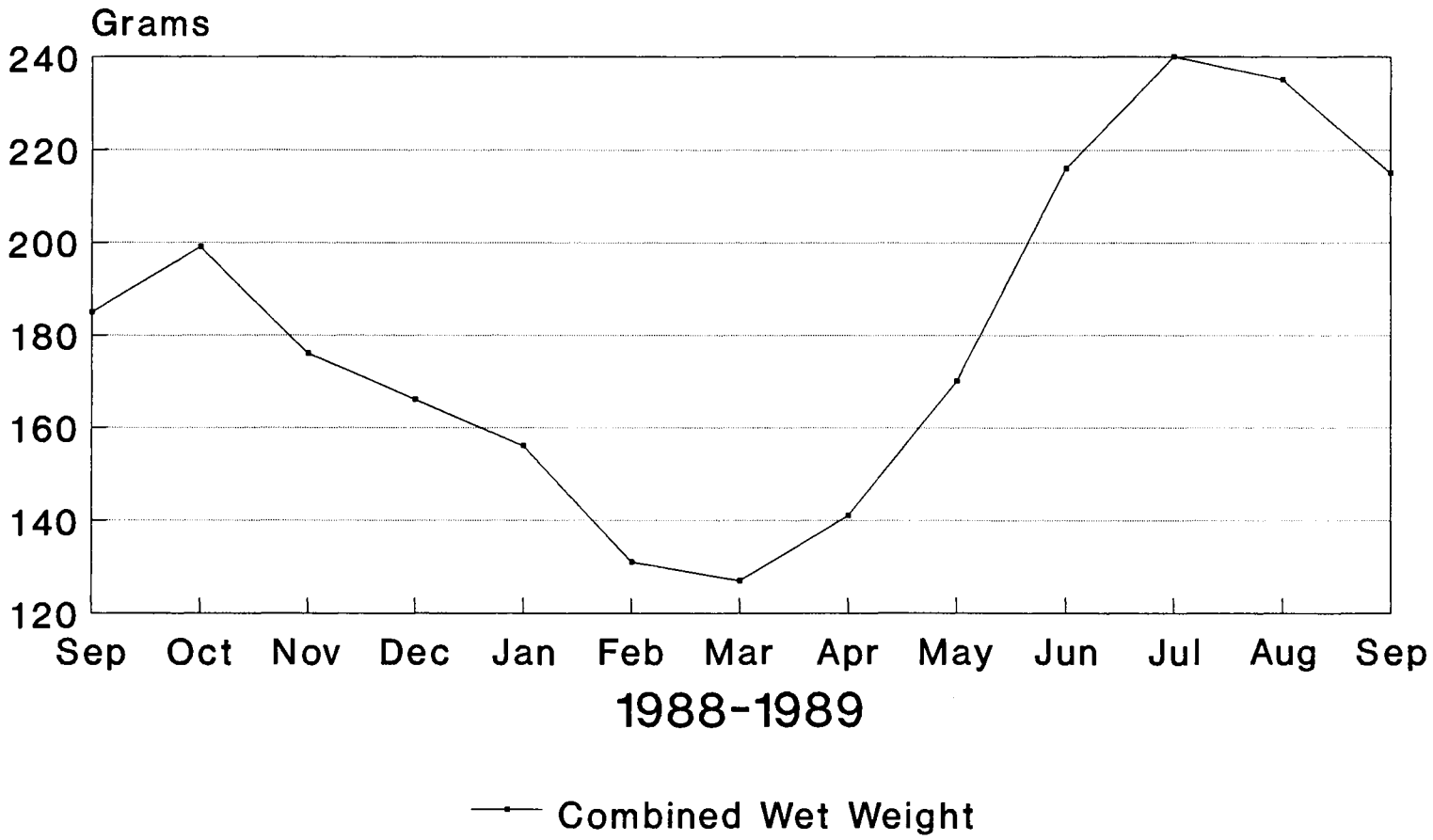


TABLE I

EUCALYPTUS SPECIES OFFERED TO KOALAS AT THE LINCOLN PARK ZOO: Sept. '88 - Sept. '89

O = Offered P = Preferred E = Excellent N = None

| SPECIES | | 88 SEP | OCT | NOV | DEC | 89 JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. grandis | O | 14 | 29 | 27 | 29 | 22 | 26 | 29 | 25 | 26 | 22 | 24 | 26 | 24 |
| | P | 10 | 15 | 9 | 8 | 15 | 13 | 13 | 21 | 11 | 7 | 10 | 8 | 12 |
| | E | 0 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| E. cinerea | O | 13 | 8 | 10 | 10 | 21 | 18 | 19 | 20 | 20 | 24 | 21 | 12 | 9 |
| | P | 0 | 2 | 4 | 8 | 12 | 5 | 12 | 8 | 3 | 7 | 2 | 5 | 1 |
| | E | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| | N | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 2 | 0 | 0 | 1 |
| E. mellio. | O | 4 | 14 | 15 | 6 | 7 | 17 | 20 | 21 | 27 | 25 | 23 | 12 | 19 |
| | P | 3 | 8 | 11 | 5 | 7 | 7 | 12 | 15 | 12 | 21 | 9 | 2 | 3 |
| | E | 1 | 0 | 1 | 0 | 1 | 1 | 3 | 3 | 0 | 6 | 0 | 0 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| E. camaldu. | O | 13 | 29 | 25 | 30 | 26 | 27 | 27 | 24 | 29 | 28 | 24 | 23 | 24 |
| | P | 9 | 25 | 21 | 26 | 23 | 20 | 15 | 17 | 23 | 23 | 12 | 7 | 18 |
| | E | 0 | 2 | 4 | 6 | 3 | 1 | 1 | 0 | 3 | 7 | 0 | 0 | 3 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E. robusta | O | 16 | 31 | 28 | 30 | 26 | 27 | 28 | 22 | 28 | 24 | 26 | 24 | 26 |
| | P | 9 | 22 | 19 | 22 | 24 | 10 | 1 | 5 | 10 | 15 | 8 | 15 | 12 |
| | E | 0 | 1 | 4 | 1 | 6 | 1 | 0 | 0 | 1 | 2 | 0 | 2 | 1 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| E. punctata | O | 2 | 9 | 15 | 4 | 1 | 0 | 2 | 1 | 4 | 9 | 6 | 9 | 9 |
| | P | 2 | 6 | 12 | 3 | 1 | 0 | 0 | 1 | 2 | 9 | 6 | 8 | 8 |
| | E | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 4 | 3 | 3 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E. siderox. | O | 3 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 9 | 0 |
| | P | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| | E | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE I (continued)

| SPECIES | | 88 SEP | OCT | NOV | DEC | 89 JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. tere. | O | 8 | 18 | 23 | 29 | 20 | 26 | 27 | 23 | 28 | 28 | 27 | 28 | 25 |
| | P | 4 | 6 | 15 | 25 | 18 | 15 | 13 | 13 | 20 | 21 | 18 | 16 | 18 |
| | E | 0 | 0 | 1 | 11 | 5 | 3 | 1 | 1 | 2 | 11 | 3 | 2 | 6 |
| | N | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| E. rudis | O | 7 | 30 | 21 | 20 | 26 | 27 | 23 | 21 | 23 | 27 | 22 | 26 | 27 |
| | P | 2 | 17 | 11 | 17 | 23 | 16 | 4 | 7 | 15 | 10 | 5 | 14 | 11 |
| | E | 0 | 1 | 0 | 2 | 8 | 3 | 0 | 1 | 1 | 3 | 0 | 2 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 3 | 3 | 1 | 0 |
| E. leuc. | O | 3 | 4 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | P | 1 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E. microc. | O | 4 | 3 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | P | 4 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E. citrio. | O | 6 | 19 | 16 | 20 | 24 | 25 | 23 | 8 | 3 | 4 | 0 | 8 | 18 |
| | P | 1 | 12 | 9 | 5 | 11 | 11 | 10 | 5 | 3 | 2 | 0 | 4 | 6 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | N | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| E. saligna | O | 0 | 1 | 0 | 6 | 13 | 20 | 27 | 15 | 16 | 11 | 7 | 13 | 9 |
| | P | 0 | 1 | 0 | 0 | 9 | 10 | 13 | 10 | 3 | 1 | 0 | 1 | 3 |
| | E | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| E. vimin. | O | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 8 | 20 | 18 | 15 | 8 | 5 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 7 | 11 | 12 | 0 | 6 | 3 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| E. resin. | O | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE I (continued)

| SPECIES | | 88 SEP | OCT | NOV | DEC | 89 JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. globu. | O | 0 | 3 | 11 | 10 | 16 | 17 | 15 | 6 | 11 | 7 | 8 | 1 | 0 |
| | P | 0 | 1 | 4 | 7 | 14 | 10 | 14 | 5 | 10 | 4 | 8 | 1 | 0 |
| | E | 0 | 0 | 2 | 2 | 1 | 0 | 4 | 3 | 2 | 0 | 3 | 1 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| E. occid. | O | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 4 | 20 | 16 | 24 | 17 | 5 |
| | P | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 3 | 19 | 16 | 24 | 17 | 5 |
| | E | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 8 | 15 | 18 | 14 | 4 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E. hybrid | O | 0 | 0 | 0 | 2 | 3 | 0 | 13 | 6 | 14 | 6 | 8 | 11 | 0 |
| | P | 0 | 0 | 0 | 2 | 1 | 0 | 9 | 6 | 5 | 2 | 2 | 2 | 0 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 0 |
| E. botryo. | O | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 6 | 7 | 8 | 0 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 4 | 7 | 0 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 3 | 0 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| E. polyan. | O | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 15 | 6 | 14 | 4 | 0 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 10 | 1 | 9 | 2 | 0 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 1 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 |
| E. ovata | O | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 12 | 16 | 6 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 12 | 15 | 5 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 7 | 8 | 4 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E. macart. | O | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 2 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 1 |
| | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| | N | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

# TOXINS IN PLANT AND ANIMAL PRODUCTS

David J. Baer
Allen and Baer Associates, Inc.
Silver Spring, MD 20904

## Introduction

Captive animals must rely on their caretakers to provide quality feedstuffs, yet many feedstuffs we provide zoo animals can contain toxins. The effect of these toxins can range from being very subtle to being very obvious. For instance, some toxins can interfere with normal physiological processes, such as reproduction. Other toxins can cause obvious signs of illness and in severe cases of intoxication, death can result. Plant and animal products can be a primary source of toxins. Some toxins are produced directly by plants or animals and other toxins can be produced by agents that are closely associated with these feedstuffs, such as bacteria, fungi or insects. Proper quality control and management are critical to ensure that only high quality feedstuffs are provided to captive animals.

## Plant toxins

Toxins associated with plants can be produced by the plants or can be produced by infectious agents that are very closely associated with the plants (e.g., fungi that produce mycotoxins). Both of these types of toxins can be found in natural forages (e.g., hays, pastures, or browse) and also in artificial diets (e.g., pellets). Toxins produced by plants are usually called secondary plant compounds and they can be classified by their chemical structure. These chemicals can have a wide variety of effects in animals that consume them but probably do not function in primary plant metabolism. It is assumed that many of these chemicals may act as feeding deterrents, especially for insects (Rosenthal and Janzen, 1979). The chemicals can be found in many plant tissues, including leaves, stems, seeds, and bark. The concentration of these chemicals, and hence their toxicity, is influenced by many factors including season, weather, soil conditions, stage of plant growth, and predation intensity.

There are many toxins that are produced by fungi and are closely associated with plants. The toxins that these fungi (principally molds) produce are called mycotoxins. Mycotoxins are very potent toxins and minute quantities can have devastating physiological effects. Specific mycotoxins can be found in grains or grain by-products and also in forages. Mycotoxins are referred to by either their common chemical name (e.g., aflatoxins, zearalenone) or by the toxicosis that they produce (e.g., staggers, slobbers) (Cheeke and Shull, 1985).

## Animal toxins

Meat, meat-based diets and fish can also contain toxins. These toxins can be naturally occurring in the animal product, they can be of environmental origin or they can be caused by infectious agents.

Toxins associated with fish have been known for hundreds and perhaps thousands of years (Dymsza et al., 1980). Several of the species of fish that can harbor toxins are used in zoos. Mackerels can contain an infectious agent produced by bacteria that causes illness but usually not death. This illness is referred to as scomboid poisoning. It is thought that bacteria cause a biochemical change in the fish muscle that generates high levels of histamine. This change may occur when fish are stored at too high a temperature. Thus, proper storage and handling that reduces the bacterial concentrations can help minimize the risk of scromboid poisoning. Another group of fish used in zoos that can contain toxins are the Clupea which includes herring, anchovies and sardines. Clupeoid poisoning may be related to another aquatic toxin, ciguatera. Ciguatera may be produced by a dinoflagellate and the toxin may increase in concentration along the food chain (Dymsza et al., 1980).

An example of a naturally occurring source of toxins in animal tissues is the potentially lethal concentrations of vitamin A found in the livers of polar bears and bearded seals (Rodhal and Moore, 1943). Although vitamin A (or its precursor) is required in the diet for vertebrate species, a high concentration of vitamin A can be deadly.

Some compounds found in animal tissues may originate in the environment. For instance, selenium concentration in fish appears to be relatively high (mean = 1.4 ppm, ranging from 0.5 to 4.5 ppm in 32 samples, dry matter basis, unpublished data). The maximum tolerable level for selenium in animals diets is 2 ppm (National Academy of Sciences, 1980). The selenium found in marine animals may be of environmental origin but it may serve other functions, such as in the detoxification of other heavy metals (Bradley and Hugunin, 1980). Thus, its potential toxicity may be reduced.

## Conclusion

The source of some toxins can be found in the plant and animal products that we feed to captive animals. There are other sources of toxins that must not be overlooked or ignored, such as improperly sanitized food preparation surfaces. Proper storage of feeds, frequent rotation of commissary stock and familiarity with potential toxins can help minimize possible poisonings.

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# ENERGY BALANCE IN ENDOTHERMIC VERTEBRATES

Steven D. Thompson, Ph.D.
Lincoln Park Zoological Gardens
2200 North Cannon Drive
Chicago, IL 60614

Energy balance, or the energy requirement, refers to the relationship between energy intake and allocation of that energy to metabolism, thermoregulation, activity, digestive processes, storage (fat), or growth (Oftedal 1985: Webster 1981, 1983). Gross or prolonged imbalances of energy intake and output can significantly impair health and/or reproductive performance. For example, when intake exceeds energy output, the storage of excess energy often leads to a variety of health impairments. These impairments are directly, or indirectly, attributable to the degree of obesity and thus may lead to suppression of female reproductive cycles (Bronson 1989; Lee 1987; Loy 1988; Frisch 1984). In contrast, when energy output exceeds intake, even by a relatively small amount, depletion of energy and corporal reserves may lead to a decline in physiological function, an inability to tolerate sudden energy demands associated with (i.e., sustained activity) reproduction, moderate cold stress, and/or a cessation of normal female reproductive cycles (Bronson 1989; Frisch 1984; Thompson in press). Although deleterious conditions are typically attributed to gross imbalances, the persistence of relatively small, chronic imbalances can have equally severe consequences (Thompson in press). Despite the many undesirable ramifications of energy imbalances, preventing or limiting the extent of such imbalances in captive wild animals remains difficult because balance points may vary [1] individually as a function of health, overall nutritional status, behavioral activity, age, morpho-physiological characteristics, reproductive state, social status, and the physical environment (Ekern and Sundstol 1982; Gittleman and Thompson 1988; Mount 1979; Milligan and Summers 1986; Waterlow 1986) and [2] interspecifically as a function of divergent strategies for maintenance of energy balance (McNab 1986a,b, 1988b).

While much is known about the maintenance and regulation of energy balance in domesticated mammals and birds (Brody 1964; Curtis 1983; Kleiber 1975; Mount 1979), relatively little direct information exists for wild species held in captivity (Thompson in press). Most of what is known about energy balance in wild animals is confined to a few small bodied species (e.g., mammals < 3 Kg, birds < 2 Kg), representing a narrow portion of the spectrum of strategies for maintaining energy balance; domesticated species (e.g., horses, cattle, pigs, sheep, dogs, chickens, etc.) also represent a narrow range of energy balance strategies. Moreover, many domesticated animals have been subjected to both "incidental" selection (e.g., genetic drift) and intensive artificial selection. The latter is usually designed to increase some aspect of net or gross productivity (e.g., nutritional efficiency, growth rate,

fecundity, etc.), any of which may be radically affected by the state of energy balance. These selective processes have been applied to animals held in well defined physical environments, fed diets of known compositions, and given access to food and water ad libitum. As a consequence, for a given diet and environment, it is often possible to predict, with considerable accuracy, the energy requirements of many domesticated breeds or species (Mount 1979; Yousef 1985a).

In contrast to domesticated species, the typical captive population of a wild species is often only a few generations removed from the wild (e.g., Glazier 1985). Captive wild species are often held under conditions that may differ greatly from their evolutionary mileau. While first order approximations of environmental and nutritional requirements are often easy to discern (e.g., warm-humid, cold-dry), the lack of precise information on the physical environment and diet in the wild often makes it difficult to predict energy requirements for wild animals held in captivity. Even for those "best known" species with a long history of intensive study in the field and captivity, it is often unclear how much, or often even in what respects, the captive situation differs from that of the wild (Yousef 1985a,b). It is even less clear how deviations from natural conditions affect wild mammals in captivity. For example, thermal stress and physical activity both can have significant contributions to daily energy budgets (Goldstein and Nagy 1985) yet they are seldom confronted directly in the requirements for wild species held in captivity. The effects of these factors may vary subtly, or dramatically, as a function of body size and ecology (e.g., natural diet, climate, social organization). As will be shown later in this paper, many tropical birds and mammals of small body size (< 3-5 Kg) may frequently be subjected to moderate or even substantial cold stress under the typical captive conditions of 20-25 °C and 40-50% relative humidity (e.g., Bartholomew 1977; Tracy 1977; Yousef 1985a,b).

This paper provides an introduction to the components of energy balance and emphasizes how interspecific variation in two of those components, basal metabolism and thermoregulation, can have profound impacts on perceived conditions of energy balance in exotic species held in captivity.

## COMPONENTS OF ENERGY BALANCE

In its simplest form, energy balance can be expressed as:

$$\mathbf{ME = H + S} \quad \mathbf{(1)}$$

where **ME** is net (metabolizable) energy intake (i.e., total energy intake less energy loss in feces and urine), **H** is energy expenditure (total heat production), and **S** is energy "stored" as protein and fat (Curtis 1983; Mitchell 1977; Mount 1979; Webster 1984). Under **maintenance** conditions, when no energy is stored (e.g., body mass is constant in an adult),

$$\mathbf{ME = H_m} \quad \mathbf{(1a)}$$

Under these conditions, $\mathbf{H_m}$ is sometimes misidentified as the basal metabolic rate (**BMR**), however $\mathbf{H_m}$ is actually a maintenance energy expenditure which includes energy allocated to both physical activity and thermoregulation. The basal or standard metabolic rate (**BMR** or **SMR**) is determined by short-term (2-4h) direct (calorimetry) or indirect (oxygen consumption) measurements of **H** made under standardized conditions. The criteria demand adult, non-growing animals, at rest and unrestrained, postabsorbtive, and measured within the region of thermoneutrality (Fig. 1a; see also Kleiber 1975). Because many wild mammals show a substantial difference in **BMR** when measured during the active ($\propto$) or inactive ($\delta$) phase of the 24h cycle, most researchers working with wild species have expanded the definition of **BMR** to include the criterion of measurement during the $\delta$-phase of the day (Aschoff and Pohl 1970). Measurements of heat production or oxygen consumption made under conditions that deviate from the standard criteria are often denoted as resting metabolic rates (**RMR**; provided the animal was at rest). In contrast to **BMR**, **ME** is typically measured as net energy (food) consumption over periods of days, not hours. Unlike the specific criteria of **BMR**, measurement of **ME** is subject to considerable (uncontrolled) variation due to circadian and daily changes in thermoregulatory stress and levels of activity. This can be seen in a more precise representation of **ME**:

$$\mathbf{ME} = BMR + MR_{nr} + MR_a + \mathbf{HIF} + (BMR \cdot \underline{h}_T) + \mathbf{RE_p} + \mathbf{RE_f} \quad \mathbf{(2)}$$

where **HIF** represents specific dynamic action and the heat increment of feeding, $\mathbf{RE_p}$ is the energy stored as protein, $\mathbf{RE_f}$ is the energy stored as fat (including the small amount of other non-proteinaceous organic compounds), $\mathbf{MR_{nr}}$ (non-resting metabolic rate) is energetic cost of alert (over resting) behavior, $\mathbf{MR_a}$ is the energy expended for activity, $\underline{h}_T$ is the coefficient of heat transfer (also known as "thermal conductance," C, or sometimes "wet thermal conductance;" see McNab 1980a), and $(\underline{h}_T \cdot (T_{lc}-T_a))$ is the energy expended for thermoregulation (see Brody 1964; McNab 1980a; Mitchell 1977; Thompson 1985; Webster 1983; also Fig. 1a). **BMR**, **HIF**, $MR_{nr}$, and $MR_a$ are components of **H** and $\mathbf{H_m}$ in equations **(1)** and **(1a)** and $(\mathbf{RE_p}+\mathbf{RE_f}) = \mathbf{S}$.

While, factors in **(2)** may vary independently, there are well documented linkages and clear differences between domestic and wild species. Under some conditions, domesticated species may balance their energy budgets by manipulation of $\mathbf{MR_a}$, however, the small housing space and low activity levels characteristic of most domestic mammals often make $\mathbf{MR_a}$ a relatively minor component of **ME** (Milligan and Summers 1986; Webster 1983). In contrast, $MR_a$ is highly variable in both free-living and captive wild species (Carl and Robbins 1988; Gittleman and Thompson 1988). Among mammals, **ME** in captivity averages about 2 x **BMR** and trade-offs between $MR_{nr}$ and $MR_a$ are instrumental in modulating energy requirements in captivity (e.g. Wang 1925).

**BMR** and $\underline{h}_T$ covary directly among wild mammals and birds, however, large body size and the broad region of thermoneutrality of many domesticated species often minimize thermoregulatory expenditures (i.e., $\underline{h}_T \cdot (T_{lc}-T_a)$) many captive environments (e.g.,

McNab 1983; Yousef 1985a,b). Moreover, because [1] the complexity of both ($\underline{h}_T \cdot (T_{lc}-T_a)$) and $MR_a$ often make precise measurement very difficult and [2] thermoregulatory and activity costs are minimal in domestic mammals, energy balance studies are often reduced to:

$$ME = BMR + HIF + RE_p + RE_f \quad (3)$$

**HIF** is particularly important for larger herbivores with either hindgut or foregut fermentation. This perspective has focused much attention on [1] variation of **ME** per se rather than the components in equation **(2)** and [2] the efficiency of storage as major factors in energy balance. Because energy use is often viewed as the movement of energy from food to stores, the costs of that movement, and the maintenance energy needs of body tissues, much of the variation in energy use has been attributed to size related and/or "apparent" variation in **BMR** (Milligan and Summers 1986; Webster 1981). Implicit in this approach are the implicit assumptions that the contributions of activity and thermoregulatory costs to maintenance energy requirements vary little between species.

Among birds and mammals, **BMR** has traditionally been thought to scale allometrically with body mass raised to the .75 power (Kleiber 1932, 1975; Lasiewski and Dawson 1967). Low variation about these allometric relationships for domesticated mammals (Kleiber 1932) has promoted confidence in the practice of extrapolating from allometric relationships for domesticated species to wild species for which no energetic data are available (Calder 1985; Robinson et al. 1983). However, studies of wild species reveal a remarkable array of behavioral, physiological, and morphological strategies and tactics for adjusting energy intake, the use of stores, and energy expenditures in response to chronic and/or acute local conditions of food shortage (e.g., Hinds and MacMillen 1985; McNab 1986a,b). Prominent among these strategies is size independent variation in the **BMR**, which is often the major component of total energy requirements among wild animals (McNab 1986a, 1988b).

**ALLOMETRY AND THE BMR**

The predominant approach for estimating maintenance energy requirements of captive wild birds and mammals rely on extrapolation from allometric equations relating **BMR** to body mass. The best known of these equations is Kleiber's curve (Kleiber 1932, 1975) relating **BMR** and body mass for domesticated mammals. The initial premise for relationship was that basal energy expenditure in mammals was directly derivable from first principles (e.g., surface area, mass, diameter, etc.; see Bartholomew 1977; Huesner 1987; McNab 1988b). The slope of Kleiber's exponential relationship was believed to reflect a manifestation of these underlying principles and thus gave rise to the concept of metabolic body size: the practice of removing the effects of body mass on **BMR** by expressing the **BMR** as a function of body mass$^{3/4}$ (e.g., Robbinson et al. 1983). Implicit in this approach is that, as with domesticated mammals, the **BMR**s of wild mammals cluster tightly around the equation describing Kleiber's relationship

between mass and metabolic rate.

In studies of domesticated mammals, minor deviations of measured **BMR** from that predicted by allometric equations have typically been attributed to "apparent" (as opposed to real) differences related to variation in body composition (Milligan and Summers 1986; Webster 1981, 1983). These "apparent" differences seem to fall into either of two categories related to body composition: (1) an apparent lowering of **BMR** (below that expected from body size alone) due to a high proportion of metabolically inert tissues such as dermal derivatives or fat or (2) an apparent elevation of **BMR** due to relatively large internal organs or an overall high proportion of proteinaceous tissues. Between domestic species and among breeds, differences in mass independent **BMR** are often greatly reduced, or even non-existent, when expressed as a function of lean body mass (Thompson and Grand, in preparation). Similar results may be obtained from an interspecific comparison of skeletal muscle mass and **BMR** in wild mammals, although in this case there is less indication that apparency per se is a primary cause of differences in **BMR**. However, despite similarities in the scaling exponent for this and other allometric analyses of energy use, energy use by any given species of bird or mammal may differ substantially from the values predicted from virtually all "standard" curves (McNab 1983, 1988b).

Among wild endotherms, deviations from standard metabolic curves are correlated with differences in food habits, climate, physical substrate (e.g., arboreal vs. terrestrial), and (perhaps), taxonomy (Derrickson 1988; Elgar and Harvey 1987; Kurland and Pearson 1986; Hennemann et al. 1983; McNab 1986a,b, 1988b). The most prominent correlations have been those involving food habits and **BMR**. For example, McNab (1980b, 1986a,b) has shown that mammals and birds that feed on the leaves of woody plants (folivores) have low **BMR**s relative to Kleiber's domestic standard; mammalian arboreal folivores such as the red panda (Ailurus fulgens), Matschei's tree kangaroo (Dendrolagus matschiei), sloths, and koalas, have exceedingly low **BMR**s (c. 30-50% of Kleiber's standard; McNab 1986a,b, 1988b). Carnivorous mammals, browsers, and those feeding on grass/forbs have high **BMR**s (> 95% of Kleiber's predicted; McNab 1986a,b). However, while some patterns are remarkably consistent, there are notable exceptions. Small ant and termite eaters have low **BMR**s (e.g., numbats, tamandua, armadillos) and within this food habit group **BMR** decreases futher at larger body size (e.g., giant anteaters, giant armadillos; McNab 1986a). Climate can clearly moderate or exacerbate food habit effects on **BMR**. For example, at a given body size and diet, species from arid habitats tend to have lower than predicted **BMR**s while those from colder habitats have higher than predicted **BMR**s (Dawson and Hulbert 1974; Hinds and MacMillen 1985; McNab 1979). These correlations make it easier to generate ballpark estimates for the energy requirements of many exotics, yet the precision of such estimates still suffers from factors affecting individual variation in the **BMR** of captive animals (e.g., variation in **BMR** due to sex, age, etc.). These food habits-**BMR** relationships are not explicable solely on the basis of variation in body composition. Moreover, extrapolations from the more general **BMR**-food habit patterns to

species must be made with care because [1] the proximal mechanism(s) relating food habits and **BMR** are unclear and [2] there are notable exceptions (e.g., Colobus guereza; Mller, Kamau, and Maloiy 1983) to the food habits-**BMR** patterns. Thus, there are at present no substitutes for direct measurement of **BMR**.

**BMR** is correlated with the maximal capacity to use energy. The maximum rate at which energy can be expended is about 3-7 times **BMR** during cold stress and 5-15 times **BMR** during locomotion (Dawson et al. 1986; Hinds and MacMillen 1985; Lechner 1978; Taylor et al. 1981). Mean energy intakes during lactation, for a variety of domesticated and a few wild mammals, cluster around 5 times **BMR** (Brody 1964; Kirkwood 1983; Kleiber 1975) and [2] energy expenditures are 4-6 times **BMR** among free-living female birds feeding nestlings (Drent and Daan 1980; King 1974; Roby and Ricklefs 1986). This limit is important because it relates the **BMR** to the absolute amount of energy that can be allocated to [1] reproduction (Fig. 2; Thompson in press) and [2] any component of equations **(2)** or **(3)**. Under the constraint of a maximum limit on energy use, as demands by components of equations **(2)** or **(3)** vary, so does the energy available for reproduction. For example, as mild or moderate cold stress increases the need to allocate energy to thermoregulation detracts from abilities to allocate energy to reproduction (e.g., Leon et al. 1978, 1983; Leon and Woodside 1983) or growth (e.g., Clark 1981; Verhagen et al. 1986, 1987a,b).

**THERMOREGULATION**

The energy required to maintain a desired body temperature ($T_b$) varies greatly as a function of ambient temperature ($T_a$). This is because below thermoneutrality, metabolic heat production must increase (Fig. 1a, b) to compensate for increased heat loss attributable to an increased difference between $T_a$ and $T_b$. The resistance to this heat loss is reflected by $\underline{h}_T = (\mathbf{H}/(T_b-T_a))$, which is often approximated by the slope of the line relating RMR to $T_a$ below thermoneutrality. $\underline{h}_T$ is actually a complex function of direct energy exchange with the environment,

$$\underline{\mathbf{h}}_T = \underline{\mathbf{h}}_{convective} + \underline{\mathbf{h}}_{conductive} + \underline{\mathbf{h}}_{evaporative} + \underline{\mathbf{h}}_{radiative} \qquad \mathbf{(4)}$$

where $\underline{h}_i$ represents the rate of heat transfer through the respective avenue of exchange (Porter and Gates 1969). While radiative heat exchange can be substantial, especially for individuals exposed to direct sunlight or a "cold" night sky, where air movement is significant, convection is often the most important avenue of heat exchange with the environment. However, measurement of $\underline{h}_T$ is extremely difficult and for most purposes, the best estimate of $\underline{h}_T$ is the rate of change in heat production (e.g., oxygen consumption) as a function of $T_a$ (Fig. 1; see also McNab 1980a; Thompson 1985).

It is important to appreciate that $\underline{h}_T$ and the size of the thermoneutral zone vary with body size ($\underline{h}_T$ scales with body mass$^{-.50}$; McNab 1983, 1988a) and the level of **BMR**: larger body size and lower **BMR** are typically associated with lower $\underline{h}_T$ (Fig. 1b). While many species exhibit the general pattern in Fig. 1a, there is considerable variation in the size of the thermal neutral zone, and

the slope of the curve ($h_T$) below $T_{lc}$ (Fig. 1b). Moreover, for larger species, there may be substantial deviations from this pattern, and in many cases thermal stress in large mammals is, for all practical purposes (e.g., above -10 °C), restricted to heat stress (McNab 1988b; Thompson 1991). Other larger mammals, particularly arboreal species, show an even more unusual pattern in which RMR decreases below thermoneutrality (McNab 1988b).

One example of this type of differential thermal stress involves three species of small mammals: a small, didelphid marsupial (Monodelphis domestica, 65-100g), an Australian opossum (Pseudocheirus peregrinus 800-1000g), and the rufous elephant shrew (Elephantulus rufescens, 65-80g). During the period 1983-1987, these three species were housed in the collection of the National Zoological Park's Department of Zoological Research. All three species were housed individually in a single large holding room at air temperatures that fluctuated between 19-25 °C. Thermoregulatory curves for each species were constructed from measurements of oxygen consumption under standard conditions (Nicoll and Thompson 1987; Thompson and Nicoll 1985; Thompson, unpub.). All three species have relatively low **BMR**s (respectively 64%, 64%, and 84% of Kleiber's predicted; Nicoll and Thompson 1987) but differ greatly in their respective $h_T$s (Fig. 3). The thermoregulatory curve of each species was standardized to **BMR**=1 and expressed as multiples of the **BMR** (Fig. 3); this permits direct comparison of thermal stress at any $T_a$. Among these three species, interspecific differences in the $T_{lc}$ and $h_T$ translate into substantially divergent thermal stress at air temperatures typical of many small mammal facilities. For example, at 25 °C (=77 °F), a typical $T_a$ for many small mammal facilities, resting energy use for E. rufescens, P. peregrinus, and M. domestica are, respectively, 2.09, 1.04, and 1.54 times the respective **BMR**s (Fig. 3).

If the limits on maximal energy use are 5-7 times the **BMR**, thermal stress at 22 °C could seriously reduce the ability of E. rufescens and M. domestica to allocate energy for reproduction (e.g., Fig. 2). At that $T_a$, Pseudocheirus is relatively unaffected due to its low **BMR** and low $h_T$ (which may be attributable in part to its larger body size). These differences can readily produce differential reproductive success.

Regardless of whether the limits to a female's abilities to allocate energy to reproduction are functional aspects of her biological design or the result of local resource availability, each female's ability to direct energy to reproduction is ultimately limited. Most females preferentially allocate energy to maintenance, thermoregulatory costs, and deposition of energy reserves necessary for future survival (e.g., during hibernation); once these needs are met any surplus energy is allocated to reproduction (Thompson in press). Flexibility in the minimal values of these requirements seems limited to compensatory shifts in behavior that reduce thermal stress and reduce energy spent on activity (e.g., locomotion). Once these minimal survival requirements are met, whatever additional energy can be obtained and processed, up to the prevailing ecological or morpho-physiological limit, can be allocated to reproduction. Thus, the

absolute amount of energy a typical mammalian female can possibly allocate to reproduction is dependent on both the limits of her abilities to obtain and/or process energy and her abilities to minimize her own requirements. Increased thermoregulatory costs are only one possible drain on energy that might otherwise be allocated to reproduction (Thompson in press). Thermoregulatory costs and/or high levels of physical activity (locomotion) have frequently been shown to detract directly from growth rates and net production in domesticated mammals (Mount, 1979; Pond and Maner, 1974; Yousef, 1985b). The manifestation of these detractions can be blatant, such as an absence of reproduction, or subtle, such as low birth weights and/or reduced litter sizes (e.g., Leon et al. 1978, 1983; Leon and Woodside 1983; Thompson in press).

**Conclusions**

It is impossible to overemphasize the potential for variation in energy requirements among wild species. Although the mechanisms, ecological bases for, and evolution of these variations are still poorly understood, it is clear that domesticated species often are poor indicators of energy use patterns and strategies of wild species. Conventional assumptions about scaling and prediction of the **BMR** and standard energy requirements are often grossly inaccurate when applied to wild species. Thus, considerations of energy balance, thermoregulation, or other aspects of energy use by captive wild animals, should, whenever possible, be based on direct measurement of relevant energetic parameters measured under the appropriate (e.g., most relevant) environmental conditions.

**Acknowledgments**

Frank Kohn, Miles Roberts, and Martin Nicoll assisted with various aspects of the small mammal study reported in Figure 3.

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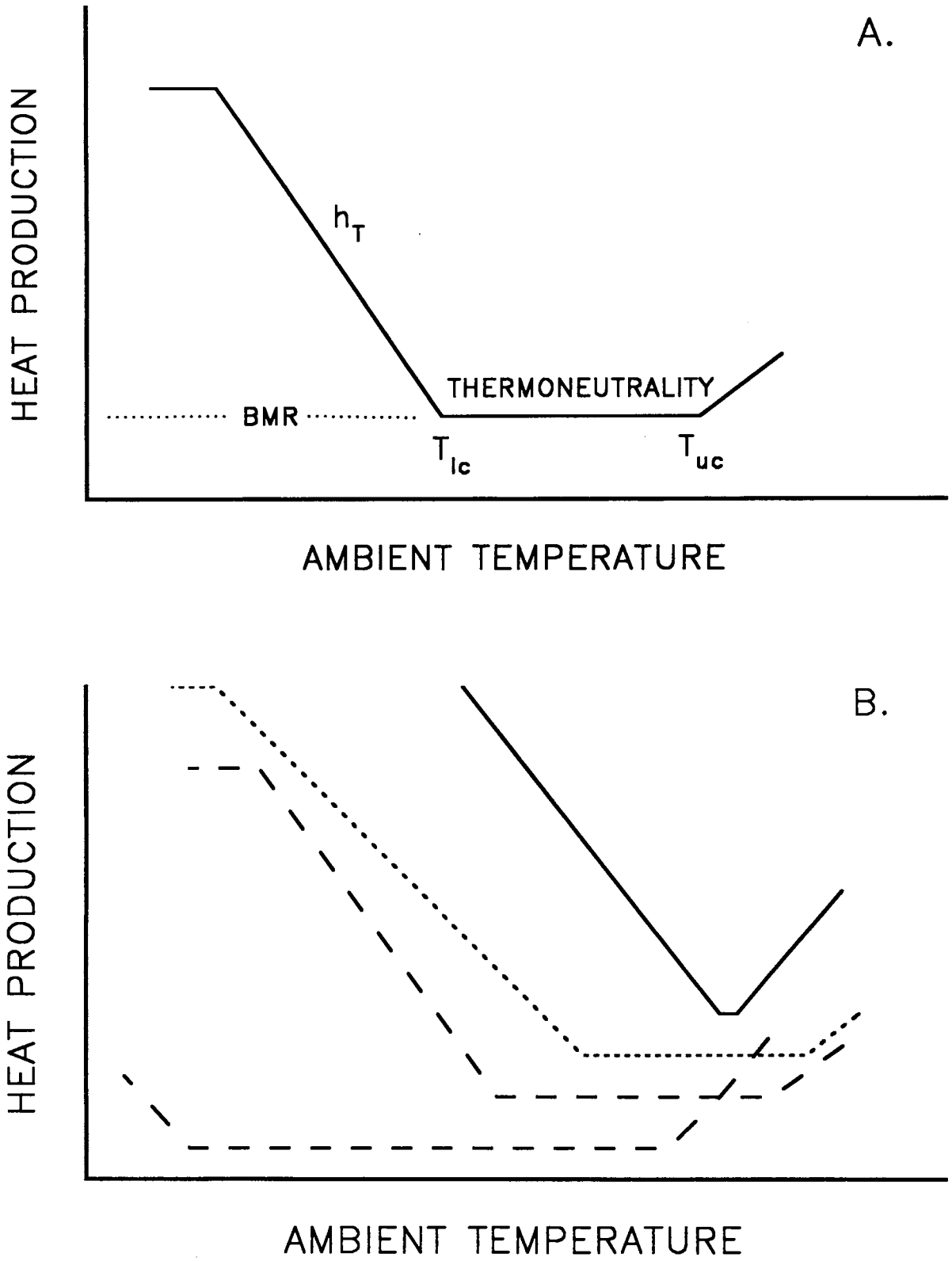
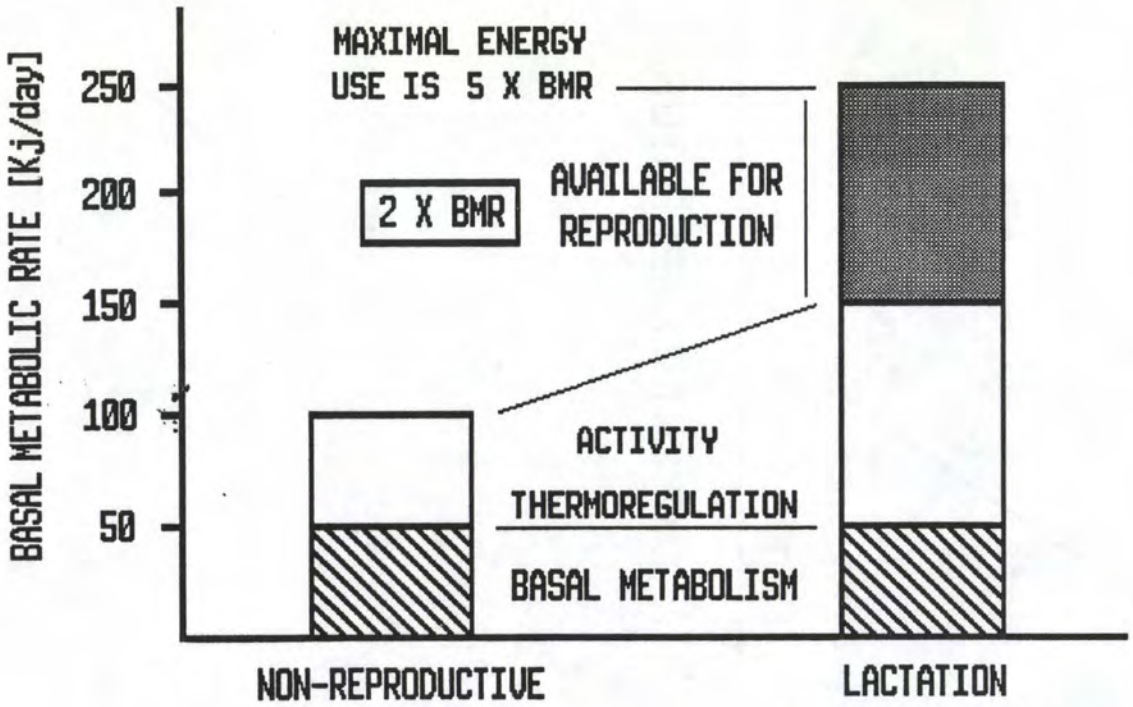
## FIGURE LEGENDS

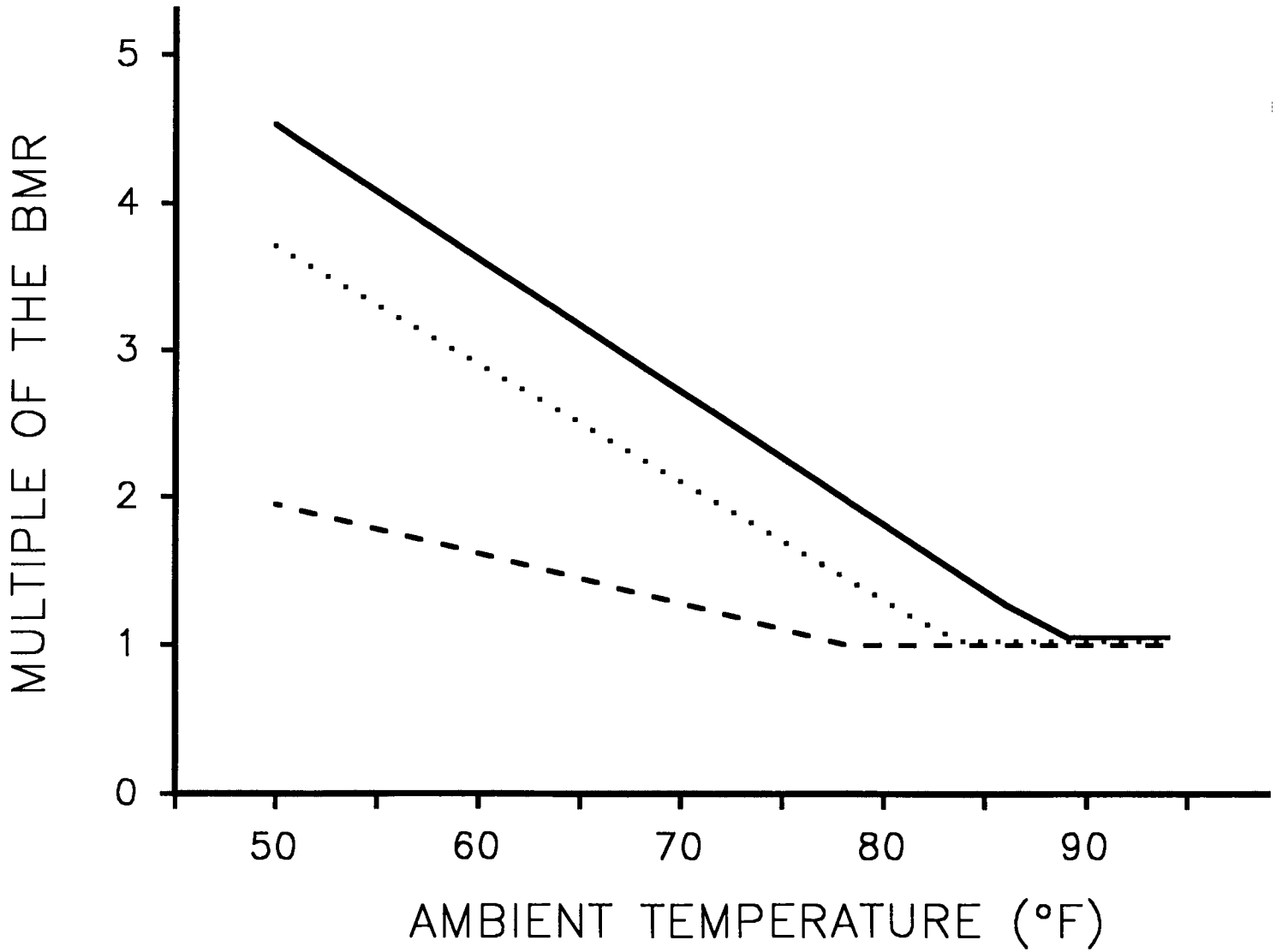
**Figure 1.** (a) Typical mammalian pattern of heat production as a function of ambient (air temperature). **BMR** is the level of heat production within thermoneutrality (=the thermoneutral zone). The upper and lower limits of thermoneutrality are designated as $T_{uc}$ and $T_{lc}$, respectively. $h_t$ can be approximated by the slope of the relationship between heat production and $T_a$ below $T_{lc}$. Maximum levels of heat production are indicated by the leveling of curve at lowest $T_a$, (b) representative variation in mammalian and avian thermoregulatory curves for very small (3-10g) to very large (50+ Kg) species; body size increases from solid, to dotted, to dashed lines. The solid line represents a small passarine bird and the long dashed line a sloth bear.

**Figure 2.** Representation of limits on energy processing in a female mammal. The increade energy demands of reproduction are met after the female meets her own requirements (Kenagy 1987). Thus, relative to the minimum possible, reductions or increases in thermoregulatory or other energy expenditures detract from the energy available for allocation to reproduction. Ecological limits reflect abilities to locate and consume sufficient food; food availability should not be a limiting factor in captivity. Morpho-physiological limits reflect relative gut size, digestive efficiencies, metabolic pathways, etc. (Demment and Van Soest 1985).

**Figure 3.** Comparison of thermoregulatory responses in three small mammals: Monodelphis domestica (Didelphidae: Marsupialia; dotted line), Pseudocheirus peregrinus (Pseudocheiridae: Marsupialia; dashed line), and Elephantulus rufescens (Macroscelididae: Macroscelidea; solid line). Oxygen consumption was measured under standard conditions (see Nicoll and Thompson 1987; Thompson and Nicoll 1986) and standardized on BMR=1. Lines below $T_{lc}$ are least square regressions from piecewise regression determinations of $T_{lc}$. BMR is mean of all points between $T_{lc}$ and $T_{uc}$.


A.
HEAT PRODUCTION
$h_T$
THERMONEUTRALITY
BMR
$T_{lc}$
$T_{uc}$
AMBIENT TEMPERATURE
B.
HEAT PRODUCTION
AMBIENT TEMPERATURE


BASAL METABOLIC RATE [Kj/day]
250
200
150
100
50
MAXIMAL ENERGY
USE IS 5 X BMR
2 X BMR
AVAILABLE FOR
REPRODUCTION
ACTIVITY
THERMOREGULATION
BASAL METABOLISM
NON-REPRODUCTIVE
LACTATION


MULTIPLE OF THE BMR
5
4
3
2
1
0
50
60
70
80
90
AMBIENT TEMPERATURE (°F)

# METHODS OF ESTIMATING BODY COMPOSITION, WITH PRELIMINARY OBSERVATIONS ON THE RELATIONSHIP BETWEEN OBESITY AND FERTILITY

Meehan, T.P.*, Wolff, P.L.#
Lincoln Park Zoo, Chicago, 60614

#current address: Minnesota Zoo
Apple Valley, MN 55124

Baer, D.J.
Allen and Baer Associates, Inc.
14159 Castle Blvd., #102
Silver Spring, MD 20904

Rumpler, W.V.
USDA Energy and Protein Nutrition Lab
Beltsville, MD

Raphael, B.R.
Dallas Zoo, Dallas, TX

Oftedahl, O.T.
National Zoo, Washington, D.C. 20008

## ABSTRACT

Obesity is common in captive gorillas and may have a significant impact on health and reproduction. A simple method of determining body composition would allow measurement of body fat levels and correlation with health and reproductive problems.

Deuterium oxide ($D_2O$) analysis, bioelectric impedance analysis (B/A) and skin fold thickness were compared in 9 female and 5 male gorillas. $D_2O$ analysis involves the infusion of a stable isotope into the animal and determination of total body water (TBW) by measuring the dilution of the isotope in the body. This method is very accurate but relatively time consuming and expensive. B/A involves passing a small alternating current (approximately 80 microamps) through the body and measuring impedance and phase shift to determine skinfold thickness at various sites to correlate to body composition.

The percentage body fat as measured by the three methods is shown in Table 1. The B/A method is not statistically different from $D_2O$ body composition analysis. Skinfold measurements were too variable to correlate with body composition.

Bioelectric impedance analysis appears to be a reliable method for the quick determination of body composition in gorillas.

Table 1. BODY FAT (%) DETERMINED BY THREE METHODS

| Method | Mean +/- SE | Min | Max | Range |
|---|---|---|---|---|
| FEMALES (n=9) | | | | |
| $D_2O$ | 29.5 +/- 4.1[a] | 6.7 | 44.5 | 37.8 |
| BIA | 35.4 +/- 2.3[a] | 27.0 | 50.4 | 23.4 |
| Skinfold | 20.3 +/- 1.1[b] | 16.8 | 25.9 | 9.1 |
| MALES (n=5) | | | | |
| $D_2O$ | 15.9 +/- 2.2[a] | 8.1 | 19.8 | 11.7 |
| BIA | 22.1 +/- 3.1[a] | 12.4 | 30.2 | 17.8 |
| Skinfold | 19.9 +/- 0.7[a] | 18.1 | 22.1 | 4.0 |

a,b Means with the same letter (within sex) are not different ($p<0.05$, Tukey)

# IS VITAMIN E REALLY THE KEY TO SEXUAL SATISFACTION?

Duane E. Ullrey
Department of Animal Science
Michigan State University
East Lansing, MI 48824

## INTRODUCTION

The vitamin era began shortly after the start of the twentieth century. By the 1920's, vitamins A, B (thiamin) and C had been discovered, and studies were progressing on a relatively heat-stable compound that would prove to be vitamin D. When rats were fed semipurified diets supplemented with these vitamins, they appeared healthy and grew well but failed to reproduce (Osborne and Mendel, 1919; Mattill and Conklin, 1920). By 1922, Evans and Bishop had found a fat-soluble factor in wheat germ, lettuce and alfalfa meal that prevented fetal death and resorption in the rat. Likewise, testicular degeneration in the rat was prevented (but not cured) by this factor (Mattill and Stone, 1923). It was ultimately named vitamin E (Sure, 1924; Evans, 1925).

## NATURAL FORMS OF VITAMIN E

By 1936, Evans et al. had discovered that two different compounds in wheat germ oil had vitamin E activity. These were named alpha- and beta-tocopherol after the Greek words "tokos" (offspring), "pherein" (to bear) and "-ol" (alcohol). Within the next 10 years, gamma- and delta-tocopherol were isolated from cottonseed oil and soybean oil, respectively (Emerson et al., 1937; Stern et al., 1947). In the 1960's, four structurally-related compounds (alpha-, beta-, gamma-, and delta-tocotrienol) were found in several vegetable oils (Green et all., 1960; Pennock et al., 1964).

The primary structures depicted in Figure 1 are the bases for the eight naturally-occurring vitamin E compounds. Specific structures vary with respect to the number and location of methyl groups. These structural differences and specific trivial names are presented in Table 1.

The biopotency of these natural forms varies considerably, although alpha-tocopherol seems to be the most active for the majority of measured functions. While published values differ, the relative biopotencies of six natural forms in rat fetal-resorption bioassays are shown in Table 2 (Brubacker and Wiss, 1972; Bieri and McKenna, 1981).

The highest concentrations of tocopherols and tocotrienols are found in seed oils, although the proportion that is alpha-tocopherol varies with plant species. In corn oil, alpha-tocopherol is about 14%, while in wheat-germ oil, alpha tocopherol is about 48% of total tocopherols and tocotrienols (Bauernfiend and Cort, 1974). Green vegetation is an important source of vitamin E for grazing or browsing animals. Field curing and storage results in declines in alpha-tocopherol concentration, and alfalfa hay contains about 50 mg/kg (Bunnell et al., 1968). Corn that has been artificially dried usually contains less than 10 mg/kg (Young et

al., 1975), while solvent-process soybean meal has about 3 mg/kg (Bunnell et al., 1968).

## COMMERCIAL FORMS OF VITAMIN E

Tocopherols of natural origin are collected by molecular distillation during the refining of vegetable oils. The tocopherols other than alpha-tocopherol can be converted to RRR-alpha-tocopherol, by methylation of the aromatic ring. This stereoisomer (formerly known as d-alpha tocopherol) has greater biological activity than the product derived from condensation of trimethylhydroquinone with synthetic isophytol, all-rac-alpha tocopherol (formerly known as dl-alpha-tocopherol). This product is a racemic mixture of eight possible diastereoisomers.

To improve stability, commercial preparations of alpha-tocopherol, either of natural or synthetic origin, are usually esterified to form acetates or hydrogen succinates. The free alcohols and acetate esters are light yellow, clear viscous oils. The succinate esters are white, granular powders.

The above forms of vitamin E are fat soluble, and their absorption is facilitated by bile and pancreatic lipase (Wiss et al., 1962). Thus, in humans with a variety of lipid malabsorption syndromes, vitamin E deficiency has been described. When a water soluble form of vitamin E, RRR-alpha-tocopherol succinate polyethylene glycol-1000, was administered orally to children with cholestatic hepatobiliary disorders, plasma and tissue tocopherol concentrations were restored to normal (Traber et al., 1986; Sokol et al., 1987).

## BIOEQUIVALENCIES OF COMMERCIAL FORMS

The relative bioequivalencies of these compounds, as found in the U.S. Pharmacopeia and National Formulary (1980), are shown in Table 3. These relationships were established on the basis of the relative potency of all-rac-alpha-tocopheryl acetate and RRR-alpha-tocopheryl acetate in the rat fetal-resorption bioassay. Ames (1979) stated that these bioequivalencies were invalid and proposed the revised values that also are shown in Table 3. However, the observations of Weiser and Vecchi (1981) supported the U.S. Pharmacopeia relationships.

In humans, using the elevation of plasma alpha-tocopherol as a measure of bioavailability, Horwitt (1980) and Horwitt et al. (1984) reported that a single oral dose of RRR-alpha-tocopheryl-acetate was 2.16 times more effective than all-rac-alpha-tocopheryl acetate. However, when Baker et al. (1986) observed the effect of continuous oral dosing for 28 days on plasma alpha-tocopherol concentrations in humans, RRR-alpha-tocopheryl acetate had a relative biopotency of 1.36 as compared to 1.0 for all-rac-alpha-tocopheryl acetate.

When beef cattle were given a daily oral dose of 1,000 IU of various vitamin E forms for 28 days, mean plasma alpha-tocopherol concentrations indicated that RRR-alpha-tocopheryl acetate had a relative biopotency of 1.80 as compared to 1.0 for all-rac-alpha-tocopheryl acetate. Using mean tissue concentrations of alpha tocopherol as criteria, these two vitamin E forms had a relative biopotency of 1.60:1 (Hidiroglou et al., 1988a).

When sheep were given a daily oral dose of 400 IU of various vitamin E forms, the mean areas under the plasma alpha-tocopherol concentration curves indicated that the relative biopotencies of RRR-alpha-tocopheryl acetate and all-rac-alpha-tocopheryl acetate were 1.63:1. Mean tissue alpha-tocopherol concentrations suggested that relative biopotencies were 1.54:1 (Hidiroglou et al., 1988b).

CONCLUSIONS

It appears that the bioequivalency of RRR-alpha-tocopheryl acetate and all-rac-alpha-tocopheryl acetate, as determined by the rat fetal resorption assay, may not apply to all animal species or to all criteria of vitamin E adequacy. Whether plasma concentrations of alpha-tocopherol in captive elephants or rhinoceroses, that seem largely unaffected by dietary supplements of all-rac-alpha tocopheryl acetate, will respond to other forms of vitamin E is currently under investigation.

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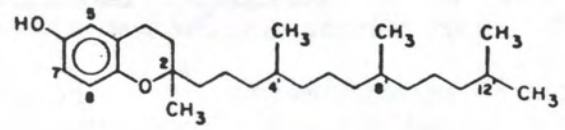
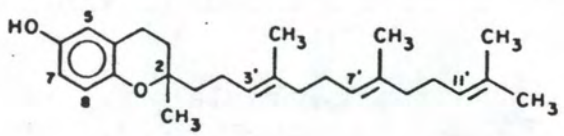



Tocol Structure




Tocotrienol Structure

Figure 1. Primary tocol and tocotrienol structures upon which eight naturally occurring vitamin E compounds are based.

TABLE 1. STRUCTURES AND NAMES OF NATURALLY OCCURRING TOCOLS AND TOCOTRIENOLS

| Position of methyl groups | Trivial name (abbreviations) | |
|---|---|---|
| | Tocol structure | Tocotrienol structures |
| 5,7,8 | α-tocopherol (α-T) | α-tocotrienol (α-T-3) |
| 5,8 | β-tocopherol (β-T) | β-tocotrienol (β-T-3) |
| 7,8 | γ-tocopherol (γ-T) | γ-tocotrienol (γ-T-3) |
| 8 | δ-tocopherol (δ-T) | δ-tocotrienol (δ-T-3) |

TABLE 2. RELATIVE BIOPOTENCY OF NATURAL FORMS

| Natural form | Relative biopotency[a] | Relative biopotency[b] |
|---|---|---|
| α-tocopherol | 100 | 100 |
| β-tocopherol | 15 to 40 | 40 |
| γ-tocopherol | 3 to 19 | 10 |
| δ-tocopherol | <1 | -- |
| α-tocotrienol | 17 to 21 | 25 |
| β-tocotrienol | 1 to 4 | -- |

[a]Brubacher and Wiss (1972).
[b]Bieri and McKenna (1981).

TABLE 3. RELATIVE VITAMIN E ACTIVITY OF 1 MG OF COMMERCIAL COMPOUNDS AS COMPARED TO 2-AMBO-α-TOCOPHERYL ACETATE

| Compound | Relative potency as given in the U.S. Pharmacopeia and National Formulary (1980) | Relative potency as reported by Ames (1979) |
|---|---|---|
| All-rac-α-tocopheryl acetate | 1[a] | .81 to .83 |
| RRR-α-tocopheryl acetate | 1.36[a] | 1.66 |
| RRR-α-tocopheryl hydrogen succinate | 1.21[b] | 1.12 |
| RRR-α-tocopherol | 1.49 | -- |
| RRR-α-tocopherol | 1.36[c] | 1.31 |

[a]Considered valid by Weiser and Vecchi (1981).
[b]Calculated stoichiometrically from corresponding acetate.
[c]Relative potency as compared to that of 2-ambo-α-tocopherol.

# VITAMIN E:
# CONSIDERATIONS IN PRACTICAL ANIMAL FEEDING AND CASE STUDIES WITH ELEPHANTS AND RHINOCEROS

Andreas M. Papas, Ph.D.
Eastman Chemicals Division
P.O. Box 431
Kingsport, TN 37662

Richard C. Cambre, D.V.M.
Denver Zoological Gardens
City Park
Denver, CO 80205

Scott B. Citino, D.V.M.
Miami Metro Zoo
12400 Southwest 152 Street
Miami, FL 33177

## INTRODUCTION

Renewed interest has been focused recently on the vitamin E nutrition of farm and captive wild animals. For example, the National Research Council (NRC, 13, 15) increased this year the recommended allowances for horses several fold over the 1978 levels. The recommendations for other species are the subject of considerable debate in light of better understanding of the functions of vitamin E such as its role in enhancing the immune system and preventing disease (14).

Scientists involved in the nutrition and health of captive wild animals have long been aware of the low circulating levels in several species and the potential role of vitamin E deficiency in the incidence of myopathy, hemolytic anemia and other diseases (2-5, 11). In some species, such as black rhinoceros and elephants, supplementation with the commonly used form dl-a-tocopheryl acetate either failed (unpublished data) or required a long period before increasing the circulating levels (3). New research with elephants and with black rhinoceros reported in this paper shows strikingly poor absorption by both species of the fat-soluble and water-dispersible forms of vitamin E. In contrast, both species exhibited good absorption of TPGS (d-a-tocopheryl polyethylene glycol 1000 succinate) a water soluble form (6). This is a vivid example of the factors that need to be considered in the vitamin E nutrition of animals and particularly captive wild animals for which research data are limited.

We will review in this paper some of the factors to be considered in practical nutrition programs and report preliminary results of studies with elephants conducted at the Denver Zoological Gardens and with black rhinos conducted at the Miami Metro Zoo.

## CHEMISTRY

In most cases, vitamin E has become synonymous with the most active form, a-tocopherol and its esters (7,12). Natural foods and feeds, however, contain, in addition to alpha-, the beta-, gamma-

and delta- tocopherols (Fig. 1) with some vitamin E activity (the NRC concluded that the non alpha tocopherols in a mixed diet contribute about 20% of the vitamin E activity indicated by the a-tocopherol content). Later, four additional compounds, analogous to tocopherols (tocotrienols) were characterized.

The tocopherols and tocotrienols are widely distributed in foods and feeds. The prevalent tocopherol forms also vary widely. For example, the alpha form is dominant in safflower oil while gamma and delta are more abundant forms in black berries and soybean oil. Latex from the Hevea rubber tree contains several of the tocotrienols. In animal tissues, the alpha form is by far the predominant form, but the role of the other forms in sparing alpha or in other biological functions is not well understood.

In their habitat, carnivores receive primarily d-a-tocopherol (natural source, also designated as RRR, see below) while all other species receive a mixture of all natural tocopherols and tocotrienols. Most commercial feeds today are supplemented with dl-a-tocopheryl acetate only.

NATURAL VERSUS SYNTHETIC

The tocopherol molecule has three asymmetric carbons marked by asterisks in Fig. 1. Only the RRR diastereoisomer is found in nature (RRR denotes that the methyl groups are positioned to the right). Synthetic a-tocopherol, a condensation product of trimethylhydroquinone and racemic isophytol, is a mixture of the eight diastereoisomers (Fig. 2; two possible positions R=right, S=left for three asymmetric carbons produce 2 x 2 x 2 = 8 diastereoisomers). Natural tocopherols are isolated from vegetable oil distillates (primary soybean oil). The non-alpha tocopherols can be converted to alpha by methylation. Alternatively, the tocopherols are isolated and are commercially available as the mixture of all forms (7,12).

The natural a-tocopherol is designated as RRR-a-tocopherol or, more commonly d-a-tocopherol. The synthetic is designated as all-rac-a-tocopherol or, more commonly, dl-a-tocopherol. Their esters are named in a similar manner. Non-alpha tocopherols are named also in a similar manner; only the natural mixed tocopherols are available in commercial quantities.

The differences in biological activity between natural source and synthetic forms are discussed below.

BIOLOGICAL ACTIVITY

The currently accepted biological activities, expressed as International Units (IU), are based primarily on the rat resorption-gestation bioassay. In this test, female rats raised on vitamin E-free diets are mated to normal males; in mid-gestation the test form of vitamin E is supplied and the biological activity of the dose is determined from the number of rats having at least one live offspring (all or none assay). Other bioassays are based on prevention of muscular dystrophy, erythrocyte hemolysis or encephalomalacia (12).

On the basis of the rat resorption-gestation bioassay, 1.0 mg of the synthetic all-rac-a-tocopheryl acetate was designated as 1.0 IU. One mg of the natural RRR-a-tocopheryl acetate was assigned

the value of 1.36 IU. The activities for the natural and synthetic a-tocopherol are 1.49 and 1.1 IU respectively. These activities have been disputed by Ames (1) who proposed relative potencies of .81-.83 IU for the all-rac-a-tocopheryl acetate and 1.66 IU for the RRR-a-tocopheryl acetate. His proposals, however, have not been accepted and the ratio of 1.36:1.0 is used to define the relative potencies of the natural and synthetic forms.

Recent research underscores major factors to be considered in using the IU values.

1. How well does the rat resorption-gestation test relate to other important functions of vitamin E such as a biological antioxidant and enhancer of the immune system of other species? Work by Ingold and his group at the NRC of Canada (10) demonstrated that rat tissues (with the exception of liver) show preference in their uptake of the natural RRR diastereoisomer over the synthetic SRR (over 2:1 by the red blood cells and over 5:1 by the brain).

2. Hidiroglou and his coworkers at the University of Florida (8) compared the natural and synthetic forms of a-tocopherol (as tocopherols or their acetate esters) in cattle. While all experimental cattle received 1,000 IU/day and similar circulating levels would be expected, the circulating levels were higher with the natural form. In addition, the tocopherol form produced an increase over the acetate form. Hidiroglou et al. observed similar trends favoring the natural forms in sheep (9).

3. The IU value does not predict the absorption of various forms by different species. It is important to note that, in general, vitamin E absorption is poor, ranging from 20-40%; a variety of factors such as biliary and pancreatic secretions (16) and polyunsaturated fatty acids affect the absorption. In general aqueous solutions are better absorbed than oil solutions. Differences in absorption between the species and vitamin E form are very well illustrated in the results with elephants and black rhinoceros reported below. Both species absorb very poorly the fat-soluble or water-dispersible forms tested, while they absorb well the water-soluble form, TPGS. The magnitude of the difference, based on the increase of circulating levels, is over 50 fold. These results also underscore the difficulties in extrapolating rat data to other species; for the same reason, we advise against extrapolating the rhinoceros and elephant results to other species.

4. In practical feeding, the non-alpha tocopherols are assigned zero IU value and are not included in feed supplementation because of their low activity in the rat fetal resorption and other bioassays in comparison to a-tocopherol. Yet as an antioxidant in vitro, gamma is more active than alpha; there is evidence that gamma is absorbed (12, 17) and that it may have a sparing effect on alpha. We need to learn more about the function of the non-alpha tocopherols and tocotrienols.

In developing a nutrition program, it is very important to consider the vitamin E form and its absorption by the supplemented animal in addition to the IU value. Failure to do so may lead to deficiency even with very high levels of supplementation as measured by the IU content.

FUNCTION

The function of vitamin E and disorders associated with its deficiency are well known, although its mechanism of action is not clearly understood. Its major function is that of a biological antioxidant with a special role of protecting the cell membrane from free radicals. Deficiency is associated with the following disorders:

Lesions of the reproductive system causing defective embryos or atrophy of the testicles.

Myopathy of the skeletal, cardiac and smooth muscles.

Lesions of the central nervous system manifested as encephalomalacia or axonal dystrophy.

Lesions of the cardiovascular system.

Erythrocyte hemolysis and anemia.

Liver necrosis, kidney nephrosis and discolored adipose tissues.

The incidence of similar disorders in captive wild animals and the possible contributory role of vitamin E deficiency has been well documented, and therefore, the selenium status should also be considered (18).

Of particular interest is the rapidly developing evidence of the role of vitamin E and other biological antioxidants (beta-carotene, vitamin C) in enhancing the immune system and preventing disease (12, 14). The role of vitamin E and other biological antioxidants in the prevention of cancer and in delaying the onset or alleviating the symptoms of diseases of the central nervous system is being actively investigated.

COMMERCIAL FORMS

As indicated earlier, the synthetic dl-a-tocopherol acetate (all-rac) is by far the most commonly used commercial supplement in animal feeds. In light of the evidence showing major species and vitamin E form differences in absorption and utilization, it is important to be aware of other commercial forms of interest to animal nutrition. These vitamin E forms can be classified as follows:

I. Fat Soluble

1. Mixed tocopherols. Commercially available in the natural RRR diastereoisomer, this oil product is a mixture of the alpha-, beta-, gamma- and delta-tocopherols. Currently used primarily as food antioxidant, it may be of interest in supplying a mixture of tocopherols to approximate those available to the animals in their natural habitat.

2. a-Tocopherol, in oil form is available both as natural source (d, or RRR) or synthetic (dl or all-rac). Its use in animal feeds is limited because it is not stable, especially when added to commercial feeds. It is used in injectable preparations and other special applications.

3. a Tocopheryl acetate, in oil form, available both as natural source and synthetic.

The above oil forms can be formulated into solid products.

II. Water-Dispersible

The most common product consists of the a-tocopheryl acetate formulated in solid form with gelatin. It is available

commercially both as the natural and the synthetic forms. Micelle formulations of the oil forms are currently being evaluated. It is important to differentiate the water-dispersible from the water-soluble TPGS below, because their absorption characteristics in some species (elephants and black rhinoceros for example) are markedly different.

III. Water-soluble

TPGS (d-a-tocopheryl polyethylene glycol 1000 succinate) is an ester of the natural form. A waxy solid, it forms clear solutions at concentrations up to 20% (6). TPGS was shown to be absorbed well by elephants and rhinoceros, while the fat-soluble and water-dispersible forms were very poorly absorbed.

In selecting a commercial vitamin E form, it is very important to consider its absorption and bioavailability for the supplemented animals. This is of particular importance for animals having low circulating levels or showing signs of deficiency despite supplementation with the commonly used form dl-a-tocopheryl acetate. For elephants and black rhinoceros, the TPGS is the preferred form. The importance of TPGS in other species is being investigated.

## STABILITY

Two aspects of stability are of interest, namely stability of vitamin E naturally occurring in feeds and stability of commercial forms.

The vitamin E content of feeds is highly variable and, more important, processing and storage causes large losses as illustrated in the following examples (7, 12, 15).

1. The a-tocopherol in green grasses decreases dramatically (up to 90%) from early stages to maturity.
2. Even simple processing of forage may cause significant losses. Drying of alfalfa causes losses up to 60%; artificial air drying causes less loss than sun curing. Storage of alfalfa hay for 12 weeks reduced vitamin E content by 73%. For both forage and grains, high moisture storage, ensiling or preservation with organic acids may cause total loss of tocopherols.

The stability of commercial products can be summarized as follows: the tocopherol forms are generally unstable, especially in vitamin-mineral premixes, basemixes or complete feeds. The esters such as the acetate are substantially more stable than the tocopherols. TPGS, as an ester, is believed to be stable in the solid form. As a solution, it should be refrigerated or preserved with a small amount of ethanol (5% of the total solution by volume). For all forms, stability is reduced by high temperature and relative humidity, presence of some minerals and other factors.

Because the content of feeds varies widely and processing, preservation or storage can cause total loss of vitamin E, the values shown in nutritional tables should be used with extreme care and allowance made for potential losses.

## REQUIREMENTS

Information on the requirements of some species is limited and the recommended allowances for others (horses for example) have

been revised (13, 15) or are the subject of considerable debate. For captive wild animals, information is even more limited. A reasonable approach, followed by some scientists, is to try to achieve in captive wild animals similar circulating levels as those observed in their natural habitat. It is of interest to consider, however, whether a higher circulating level is advisable for captive animals in order to increase their ability to overcome environmental stress and enhance their immune system (12, 14).

The proposed allowances for vitamin E vary considerably and for some species are in the range of .3 to 2.5 IU/kg body weight. At least part of the variation is the result of differences in the absorption and utilization of various forms by different species.

In practical supplementation, the total IU added to the diet is not meaningful without consideration of the absorption and utilization characteristics of the form used by the supplemented animals. This is very well illustrated by the case studies below.

CASE STUDY I: VITAMIN E NUTRITION OF ELEPHANTS

This study comprised two trials and was conducted at the Denver Zoological Gardens under the supervision of Dr. R. Cambre. Serum samples were assayed for a-tocopherol by the laboratory of Dr. R. Sokol at the University of Colorado School of Medicine. The objective was to determine the effectiveness of several vitamin E forms in elevating the circulating levels.

TRIAL 1

Two female Asian elephants (estimated ages of 34 and 31 years and weights 3264 and 4855 kg) were fed diets supplemented with the vitamin E forms in the sequence shown below (washout periods were allowed between forms and levels):

| SUPPLEMENTAL VITAMIN E | DOSE, IU/KG BW | COMMENT |
|---|---|---|
| Baseline (dl-a-tocopheryl acetate) | 1.8 | Basal feed level for whole trial |
| TPGS | 4.8 | Water-Soluble form |
| d-a-tocopherol, dil. with corn oil | 5.1 | Tocopherol in oil |
| d-a-tocopherol | 5.1 | Not diluted |
| d-a-tocopherol | 10.2 | As above at 2x level |
| d-a-tocopheryl acetate | 30.0 | Acetate in oil form, high dose |

The results showed that TPGS was by far the most effective form by increasing the circulating levels from the baseline of .11 to over .40 mcg/ml within five days of dosing. In contrast, the other forms tested, even at 6 times the dose, produced very small or no measurable increases in the circulating levels of vitamin E indicating very poor absorption.

TRIAL 2

The objective was to confirm the results of trial 1 and to determine whether very high levels of the tocopherol or a water-dispersible form of its acetate ester, added to the feed, would be

effective in elevating the circulating levels. One Asian (female 25 years old, 2895 kg) and 3 African elephants (male 5 years old, 811 kg; female 3 years old, 650 kg; female 3 years old, 547 kg) were assigned to the following protocol:

| DAYS | SUPPLEMENTAL VITAMIN E | | COMMENTS |
|---|---|---|---|
| 1-4 | Baseline (dl-a-tocopheryl acetate) | | basal feed for whole trial |
| 15-40 | d-a-tocopheryl acetate | 62 IU/kg BW | Water dispersible solid, high dose |
| 41-47 | None | | Washout |
| 48-68 | TPGS | 6.6 IU/kg BW | Water solution 20% |
| 69-85 | None | | Washout |
| 86-106 | d-a-tocopherol | 62 IU/kg BW | Oil form, high dose |
| 107-120 | None | | Washout |

The results are summarized in Fig. 3 and confirm that, as in Trial 1, the water soluble for TPGS was very effective in rapidly increasing the circulating levels from the baseline of .05 mcg/ml to .50 within 2 days and to over 1.0 mcg/ml within 20 days. In contrast, the acetate at almost 6x higher dose, produced a very small increase compared with TPGS. The tocopherol form also in the same very high dose as the acetate was much less effective than TPGS. The tocopherol appeared to be more effective than the acetate form, but due to the high residual levels from TPGS, it is difficult to draw final conclusions.

The two trials showed conclusively that TPGS was effective in rapidly increasing the circulating levels of vitamin E in elephants. The fat-soluble and water-dispersible forms tested produced very small increases suggesting that the elephants absorb these forms very poorly.

CASE STUDY II: VITAMIN E NUTRITION OF BLACK RHINOCEROS (PRELIMINARY RESULTS)

This research was conducted at the Miami Metro Zoo under the supervision of Dr. S. Citino. Two black rhinoceros (one male 12 years old and one female 16 years old) were placed on the following protocol:

| Week | Supplemental Vitamin E | | Comments |
|---|---|---|---|
| 1 | dl-a-tocopheryl acetate, | 2,000 IU/day | Fed for months before start of trial |
| 2-4 | d-a-tocopheryl acetate, | 2,100 IU/day | Water dispersible, used as solid |
| 5 | d-a-tocopheryl acetate, | 31,500 IU/day | As above, 30x higher dose |
| 6 | none | | Washout |
| 7-8 | TPGS | 2,100 IU/day | Water solution 20% |
| 9 | TPGS | 5,250 IU/day | As above, 2.5x higher dose |
| 10 | none | | Washout |

The animals were receiving before and during the trial a diet supplemented with 3,600 IU dl-a-tocopheryl acetate per kg. Blood samples were collected and plasma a-tocopherol was assayed by the laboratory of Dr. R Sokol at the University of Colorado School of Medicine. The preliminary results are shown in Fig. 4.

These results, which are similar to those obtained with elephants, show that the water-soluble form, TPGS, increased the circulating levels several fold while the fat-soluble or water-dispersible forms of the dl- and d-a-tocopheryl acetate respectively, caused a much smaller increase. It is important to note that even at the very large amount of 31,500 IU/day the apparent increase in circulating levels was very small compared to the increase produced by a 15-fold lower dose of TPGS.

These results demonstrate the usefulness of TPGS in meeting the needs of black rhinoceros. They also demonstrate the critical importance of the vitamin E form and the risks involved in basing the supplementation on the IU value alone.

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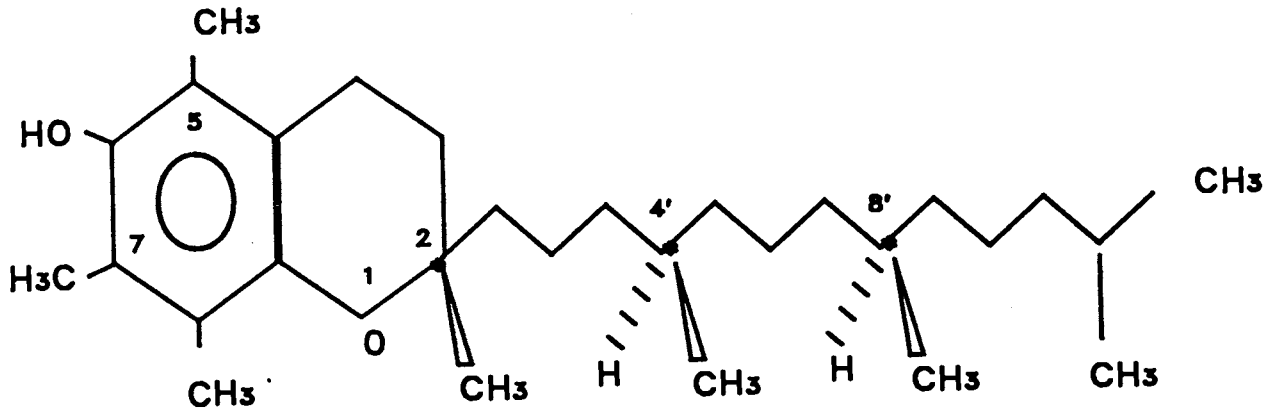
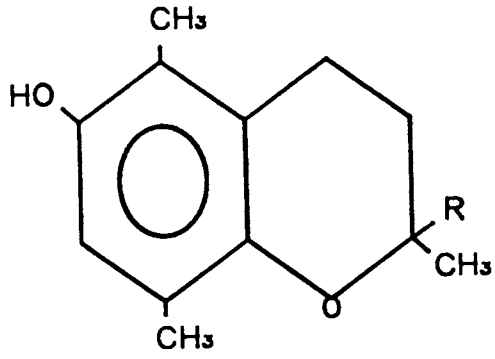
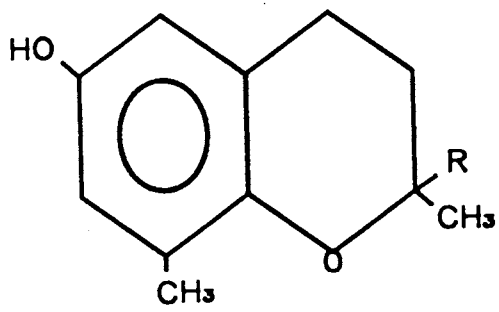
alpha–Tocopherol



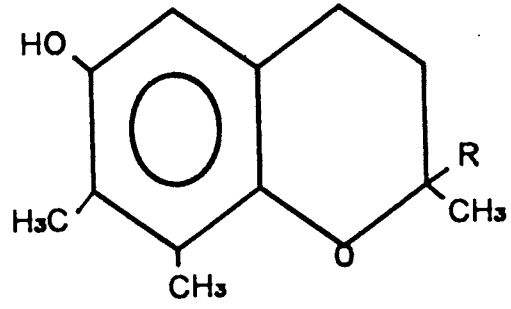

beta–Tocopherol




delta–Tocopherol




gamma–Tocopherol




R: Side chain identical for all tocopherols.

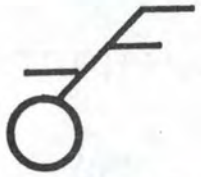
Figure 1

## Stereoisomers of a–Tocopherol

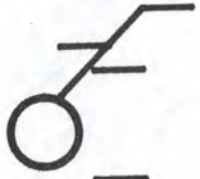
**Natural**

2R, 4'R, 8'R

**Synthetic**



2S, 4'R, 8'R



2R, 4'S, 8'R

2R, 4'R, 8'S

2R, 4'S, 8'S

2S, 4'S, 8'R

2S, 4'R, 8'S

2S, 4'S, 8'S

Figure 2

# STUDIES ON THE VITAMIN E NUTRITION OF ELEPHANTS

## DENVER ZOOLOGICAL GARDENS - TRIAL 2

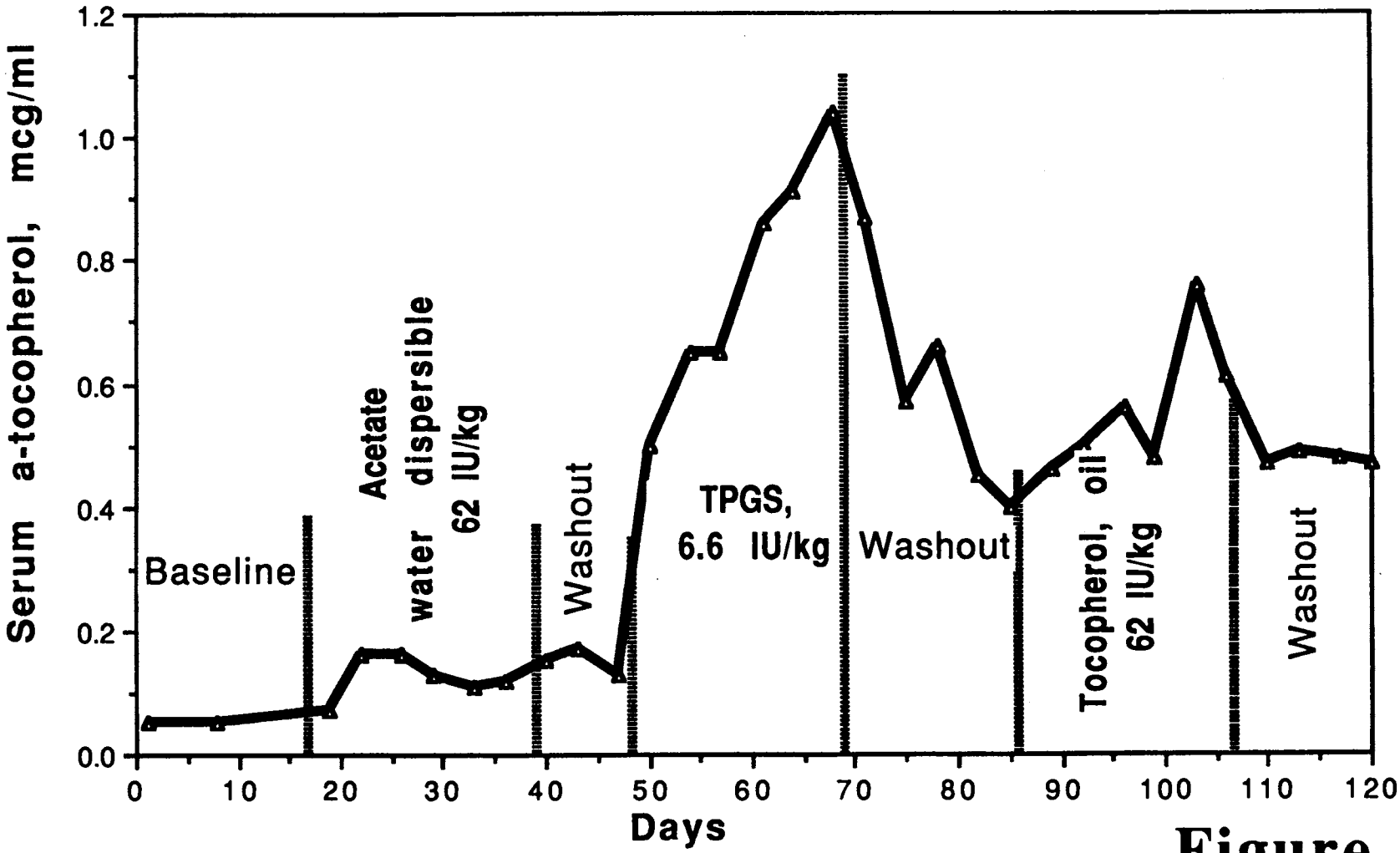



**Figure 3**

## STUDY ON THE VITAMIN E NUTRITION OF THE BLACK RHINO
## MIAMI METRO ZOO

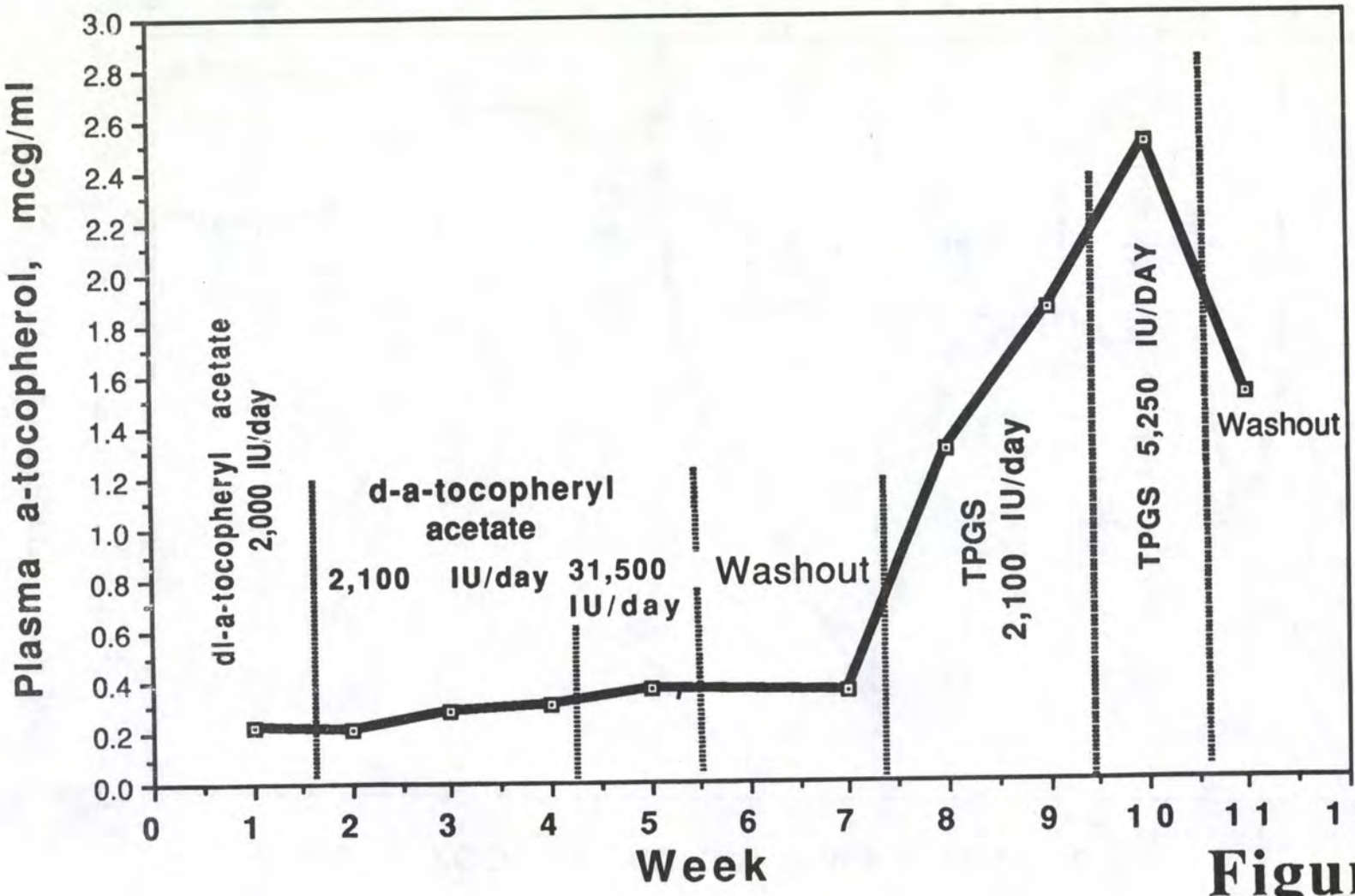



**Figure 4**

# NUTRITIONAL STUDIES WITH THE GREEN IGUANA

Mary E. Allen Ph.D.[1], Olav T. Oftedal Ph.D.[1], David J. Baer M.S.[1] and Dagmar I. Werner Ph.D.[2]

[1] Department of Zoological Research, National Zoological Park, Smithsonian Institution, Washington, DC 20008

[2] Pro Iguana Verde Foundation, Apartado 1501, Heredia 3000, Costa Rica

## INTRODUCTION

Although green iguanas have been maintained in captivity in zoos and by private owners for many years, relatively little is known about their nutritional requirements. This became especially clear when a large scale iguana production and reintroduction project was initiated in Panama in 1983. Early attempts at rearing hatchlings on diets of fruit, vegetables and local greens resulted in poor growth. Preliminary studies conducted by Dr. Dagmar Werner demonstrated that use of ingredients such as meat and bone meal could lead to a marked increase in growth. A collaborative nutritional research program was established in 1986 between the iguana management project and the Department of Zoological Research, National Zoological Park. This report provides some preliminary results of some of these research efforts.

## BACKGROUND: IGUANA REARING AND REINTRODUCTION IN CENTRAL AMERICA

The Iguana Management Project (IMP) began in 1983 at the Smithsonian Tropical Research Institute in the Republic of Panama with funding from the W. Alton Jones Foundation. An objective of this five-year project was the development of the green iguana as a renewable food resource for local peoples. Iguana production provides an alternative use of forest resources, and encourages reforestation of denuded areas. Another benefit is the reintroduction of green iguanas into areas where they had been formerly abundant, but are now extinct or severely depleted due to habitat destruction and over-hunting. By involving local personnel and establishing goals that are of direct benefit to local populations, the green iguana project has gained both political acceptance and worldwide attention (eg. Chapin 1986, Kohn 1989).

The production of large numbers of healthy iguanas is of course a primary objective. The production colony was established from an initial capture of 400 hatchlings in 1983 as well as from eggs laid in 1984 by wild females that were induced to lay in specially constructed "artificial" chambers. By 1988 the colony numbered over 8,000 iguanas, most of which were produced by the successful captive breeding program. Successful large scale production has hinged upon a thorough understanding of iguana biology as well as trial and error in cage design, diet, animal density, nesting substrate and incubation conditions.

Captive-reared iguanas that have reached an appropriate size (approximately 90 grams) are released into suitable habitats as part of the reintroduction project. Suitable sites are small forested areas on farms, private property or reserves. Each release is coordinated with local authorities and the release becomes a community ceremony. The villagers come to view the released iguanas as their own resource which must be guarded against poaching. Survival appears to be very high. For example, after the first release of 450 tattooed iguanas in 1985, 250 were relocated and identified in 1986. In coordination with the reintroduction efforts, educational programs have been instituted to teach the campesinos about iguana rearing and habitat management. The importance of trees as shelter and food for iguanas is stressed. Tree-planting projects have been initiated using 15 species that can supply iguana food (and habitat), fruit, fuel, timber and living fence posts. Reforestation of areas previously denuded by slash and burn agriculture is an ultimate goal of the project (Werner 1989).

Local villagers and campesinos are taught to maintain feeding stations as a source of supplemental food for the released animals. A manufactured, meal-type diet that was developed for animals in the production colony is supplied in the feeding stations. Interestingly, wild iguanas, with no prior experience with manufactured diets, have been observed feeding on the meal-type diets at the stations.

From 1983 to 1988, the project was located at Summit Gardens, between Gamboa and Panama City. With the growing political turmoil in Panama and the end of funding from the W. Alton Jones Foundation, the project relocated to Costa Rica. About 2400 adult iguanas were transported to Costa Rica in 1988, where productivity remains high, yielding about 17,000 hatchlings in 1990. Reorganized as the Pro Iguana Verde Foundation, the project receives support both from the international community and from the Costa Rican government. In 1990 more than 16,000 iguanas will be released at various sites in Panama and Costa Rica.

## THE COMPOSITION OF FOODS EATEN BY IGUANAS

The first step in developing a stock diet for iguana rearing is the establishment of target nutrient levels. We were not certain of the levels of protein, fiber or other constituents typically encountered by iguanas foraging in the wild. Although available time did not permit a detailed study of iguana feeding behavior, project personnel have spent a considerable amount of time searching for and watching reintroduced iguanas. Therefore an informal and preliminary survey was made of the plants that iguanas are believed to eat at the release sites, and 18 samples of these plants and plant parts were collected for analysis. An additional 17 samples were obtained that represented plant parts that were abundant but that the iguanas did not seem to eat. The collection sites were generally areas that had been heavily used by livestock, and the plants collected include a number of introduced species; hence these data cannot be considered representative of foods eaten by indigenous iguanas in undisturbed conditions.

There was considerable variation in the composition of plant

parts selected, as well as those apparently avoided (Table 1). Many factors undoubtedly play a role in iguana food choices, including physical characteristics of the plants, accessibility and secondary plant compounds (toxins). However many of the plants eaten by iguanas were rather high in protein (mean = 21%), while many of those not eaten were considerably lower in protein (mean = 12%). In part this difference reflects the fact that iguanas were usually reported to eat young leaves, rather than mature leaves that are often high in fiber and low in protein. These results supported our initial choice of a 20% protein level in the stock diet, and suggested that iguanas may be able to tolerate quite high fiber levels (Table 1).

PROTEIN AND GROWTH

Originally, captive iguanas were fed rice, some natural vegetation, and fruits and vegetables purchased from local markets. This proved to be unsatisfactory since the animals did not thrive. It was assumed that the poor condition of iguanas could be attributed, at least in part, to inadequate intake of nutrients, especially protein. Most produce items available in human markets are low in dry matter (high in water), but even if expressed on a dry matter basis many of these items are low in protein and fiber (Table 2). For example, a typical 'reptile salad' prepared in zoos for feeding herbivorous reptiles may contain only 12% protein on a dry matter basis, even though it contains some dry dog food, egg, kale and green beans in addition to such items as fruits, carrot and sweet potato.

In preliminary trials, iguanas were offered dietary supplements of high protein content, such as meat and bone meal, fish meal or soy bean meal. The animals supplemented with meat and bone meal grew much faster than did animals on the original diet.
The economic feasibility of iguana rearing and release depends on rapid growth of hatchlings to a large enough size that predation is reduced after release. The survival rate of wild, hatchling iguanas is estimated to be only 5% during their first year of life. Their small size at hatching (10-11 grams) makes them particularly vulnerable to predators. Rapid growth is essential to the rearing program in order to reduce the duration of time from hatching to release and thus to minimize food and labor costs. It was clear that more careful nutritional studies would need to be undertaken to determine the optimum level of dietary protein for maximal growth.

We hypothesized that dietary protein concentration would significantly influence growth rates if levels were beneath the requirements of hatchlings, but that increasing protein content at higher levels would have little benefit. Using locally available feedstuffs, we formulated a basal meal-type diet which included vitamins and minerals at levels above those typically used in domestic animal rations. Given the compositional data on plants apparently eaten by free-ranging iguanas (see above), we selected 20% (as fed basis, or 22% on a dry matter basis) as the median protein level, and compared growth rates on this diet to those of iguanas fed lower and higher protein levels. In one study, hatchlings fed a formulated diet containing 25% protein for three

months weighed on average 60 grams (n=30), as compared to 46 grams (n=30) for animals on a 20% protein diet, and 18 grams (n=30) for animals on a 15% protein diet. Data from an additional study measuring the effects on iguana growth of diets containing 25%, 27.5% and 30% crude protein are presently being evaluated.

Given the growth-promoting effect of 25% dietary protein, the stock ration used in Costa Rica has been changed to this level. Both growth rates and breeding success remain high. In a colony of research animals at the National Zoo, some of the 25 iguanas that have been maintained for 4 years predominantly on a 25% protein diet developed evidence of renal gout and soft tissue mineralization. One theory is that high dietary protein levels may predispose some reptiles to gout. It is not clear if this is in fact the case, and in a study of the effect of dietary protein (15, 22.5 or 30% crude protein) on nitrogen excretion, no effect could be seen on serum uric acid levels. Of course animals fed diets higher in nitrogen excreted more nitrogen, and a greater percentage of the nitrogen in the excreta (feces plus renal waste) appeared to be uric acid. Further study is needed, both of the responses of older iguanas to dietary protein levels, and of the laboratory methodologies used in measuring uric acid in mixed excreta.

FIBER UTILIZATION

The finding that iguanas appear to consume plants that may be quite high in fiber content (Table 1) suggests that iguanas may be able to adapt to rather high-fiber diets. In many herbivores, however, high-fiber diets can limit food intake through effects on the gastrointestinal tract. Limitations on food intake may reduce growth rate and the efficiency of feed utilization. Three meal-type diets were formulated that differed in fiber content: a low fiber diet (14.7% Neutral detergent fiber (NDF) and 7.6% acid-detergent fiber (ADF)), a medium fiber diet (20.9% NDF, 11.9% ADF) and a relatively high fiber diet (27.1% NDF, 16.2% ADF). In a cross-over design 21 animals were fed each of these diets in sequence (Baer et al. 1989). On the higher fiber diet animals had lower intake, lower dry matter digestibility, reduced growth rate and required more feed per g weight gain (Table 3). While high fiber plant materials may be important to the survival of iguanas in the wild, it appears that maximal growth is not achieved on a high fiber diet.

NUTRITIONAL ASPECTS OF EGG PRODUCTION

Success of a long-term breeding program depends on sustained productivity of reproductive females. Much of the early effort at the iguana project was involved with testing of incubation conditions and techniques (Werner and Miller 1984, Werner 1988). Artificial nesting chambers were designed that succeeded in inducing egg deposition by wild females. Eggs from these free-ranging iguanas were artificially incubated to obtain initial stock for the colony. More recent efforts have been dedicated to simplification of the incubation procedures such that it is now adaptable to village conditions, and campesinos in the field have been very successful at egg incubation. The current hatching rate of artificially incubated eggs is close to 95%.

A number of studies have been initiated to examine nutritional aspects of egg production and incubation. For example, the chemical composition of eggs laid by females in cages of high male density (and thus much male-male antagonism) were compared to those laid by females in cages of low male density, as well as to those laid by free-ranging females. No difference could be found in the proximate or mineral composition of these eggs, although in a preliminary study a few pooled egg samples from wild females appeared to be higher in vitamins A and E than were those of the captive animals. In the future we intend to test the effect of dietary levels of fat-soluble vitamins on egg mass, composition, and hatchability, and on hatchling survival and growth. We have also initiated studies of the effects of incubation conditions on body mass and composition of hatchlings, and are interested in the efficiency of conversion of energy and nutrients in the egg to tissues of the hatchlings. In the first weeks after hatching, the hatchling must rely on stored energy and nutrients in the yolk sac before it begins to feed on its own.

IGUANAS IN ZOOS

While most of the research being conducted is directed at practical concerns associated with large-scale breeding, rearing and reintroduction of green iguanas in Central America, the information gained on nutritional requirements of iguanas can be used to evaluate and improve diets of iguanas and similar herbivorous reptiles in zoos. However there is one key difference between iguanas in the tropics, and captive animals in north temperate zoos: in Costa Rica and Panama the iguanas are outside and can regularly bask in solar radiation, whereas farther north cold weather during much of the year does not permit animals to be kept outdoors. The sun provides a spectrum of radiation, including ultraviolet light of the wavelengths (ca. 290 to 315 nanometers) required for biological synthesis of vitamin D. Given the limited UV output of most artificial lights, it may be necessary to provide a dietary source of vitamin D to iguanas in zoos, even if this is not necessary in outdoor housing conditions.

A study was initiated of the effects of various artificial light sources on growth and vitamin D status of green iguanas at the National Zoo. An experimental bulb with enhanced UV output was tested, but seemed to depress food intake and growth of animals fed a diet devoid in vitamin D-3 (Allen 1988). It is possible that these animals were exposed to excessive UV radiation, especially at shorter wavelengths. The animals exposed to the high UV bulb were found to contain very high plasma levels (351 ± 29 sem nanogram/ml) of a key vitamin D metabolite (25-hydroxy vitamin D-3). The levels of this metabolite were significantly lower for animals kept under cool white fluorescent bulbs or "black lights" (mean values of 237 and 228 nanogram/ml, respectively). Plasma samples obtained several months after the end of this study showed a reduction of this metabolite despite the fact that vitamin D-3 had been added to the diet. The practical significance of these findings is not clear, however. In humans and some lab animals, the levels of 25-hydroxy D-3 observed with the experimental UV bulb would be considered indicative of vitamin D toxicity, but normal levels for

an iguana are not known. Further study is needed to resolve the role of light sources and the appropriate levels of vitamin D to include in the diet. It is intriguing that the pathological mineralization of soft tissues seen in some iguanas on necropsy bears a resemblance to the effects of vitamin D toxicity (R. Montali, personal communication), but a relationship to either diet composition or light source has yet to be confirmed.

## CONCLUSION

The iguana production project of the Pro Iguana Verde Foundation presents a unique opportunity for research on the nutritional requirements of an herbivorous reptile. Nutritional research has become a top priority for the project because of the demonstrated importance of nutrition to growth and performance of the animals. The few studies mentioned in the present report provide valuable information, but are only a beginning of the research that needs to be done to develop diets that are optimal with respect to growth, reproduction and disease resistance.

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Table 1. Composition of some plants that are available to released iguanas in Panama.

| Species | Plant Part | Dry Matter % | Crude Protein % | Crude Fat % | NDF % | ADF % |
|---|---|---|---|---|---|---|
| | | | (Dry matter basis) | | | |
| **Plants eaten** | | | | | | |
| Vigna sinensis | L | 11.9 | 33.3 | 3.4 | 54.9 | 36.7 |
| Annona muricata | YL | 20.1 | 16.2 | 8.8 | 47.4 | 34.4 |
| Hibiscus sp. | F | 14.5 | 12.8 | 5.1 | 34.4 | 16.4 |
| Erythrina fusca | YL | 19.5 | 28.2 | 4.1 | 43.9 | 20.3 |
| **Plants not eaten** | | | | | | |
| Mangifera indica | ML | 43.0 | 7.5 | 3.5 | 57.0 | 53.7 |
| Byrsonima sp. | F | 33.7 | 7.8 | 12.6 | 45.1 | 39.6 |
| Bursera simabura | ML | 23.1 | 14.2 | 3.7 | 62.0 | 44.2 |
| Manihot esculenta | ML | 34.3 | 17.1 | 15.5 | 46.1 | 23.6 |

Plant part abbreviations as follows: YL, young leaves; ML, mature leaves; L, leaves (young and old not apparent); F, fruit.

Table 2. Composition of market produce that is commonly fed to green iguanas in zoos.

| Plant parts | Dry Matter % | Crude Protein % | Crude Fat % | NDF % | ADF % |
|---|---|---|---|---|---|
| | | (Dry matter basis) | | | |
| Kale | 12.6 | 24.6 | 3.8 | 22.8 | 15.9 |
| Romaine | 7.5 | 16.0 | 3.0 | 19.0 | 8.0 |
| Cabbage | 8.9 | 14.7 | 3.9 | 20.6 | 21.9 |
| Green bean | 10.7 | 17.9 | 1.8 | 28.0 | 25.1 |
| Apple | 16.1 | 1.2 | 2.2 | 11.1 | 7.7 |
| Orange | 13.3 | 7.1 | 0.9 | 10.4 | 10.6 |
| Grape | 18.7 | 3.4 | 1.9 | 2.7 | 2.2 |

Table 3. Effect of dietary fiber on growth and food intake of green iguanas (n=21)[1].

| | Growth Rate (g/day) | Dry Matter Intake (g/day) | Dry Matter Digestibility (%) | Feed/gain Ratio |
|---|---|---|---|---|
| Low fiber | 2.35[a] | 5.60[b] | 65.8[a] | 3.58[a] |
| Medium fiber | 2.23[a] | 6.71[a] | 62.2[a,b] | 3.93[a,b] |
| High fiber | 1.43[b] | 5.31[b] | 57.8[b] | 6.37[b] |

[1] Based on 21 animals each of which was fed each diet for about 3 months. A summary of the experimental conditions and partial results was reported by Baer et al. (1989). In a column, numbers with the same superscripts are not statistically different ($p<0.05$, Tukey's test)

# PRINCIPLES OF FRESH WATER FISH NUTRITION

Laura Jane Stewart
Nutritionist
San Antonio Zoological Park and Aquarium
San Antonio, Texas 78212

Aquariums and zoos in the United States are facing the same challenges today the mammal and bird collections faced twenty years ago; the challenge of changing from an exhibit oriented collection to that of a conservation oriented collection. As aquarium technology continues to progress in providing an environment which allows the animals to live their normal life span, the focus on nutrition becomes increasingly important. Delicate species of fish are now surviving long enough in captivity to develop nutritional deficiencies, which in the past went unnoticed. Proper nutrition of exotic fishes becomes even more important as greater emphasis is placed on breeding as well as display.

Unfortunately, tropical fish nutrition is in its infancy. However, just as all the other areas of zoo nutrition has its foundation on domestic animal research, tropical fish nutrition can use information gained from the latest aquaculture research.

While fish farming has been practiced for centuries, fish nutrition has evolved from an art to a science in the past twenty years with the development of intensive aquaculture practices. Species under intensive cultivation worldwide include channel catfish, penaeid (marine) shrimp, carp, eel, tilapia, salmon, and lobster.

## PRINCIPLES OF FISH NUTRITION

Many are surprised to learn that fish have the same nutrient requirements as land animals. They must have water (of course), a source of energy, protein, vitamins, and minerals. These nutrients can be provided in a variety of forms ranging from live feeder fish, freshly prepared foods such as ground fish and vegetables, to commercially prepared dry foods. No matter what form of food is offered, the diet must meet the animal's total nutrient requirements for optimum health and successful reproduction.

### Protein

Fish utilize much higher levels of protein in their diet than land animals. The explanation for this lies in the fact that fish require less energy for protein synthesis than warm blooded animals. Table 1 shows that broiler chickens consume 3.4 times the energy a catfish must consume, but only 1.6 times the protein. A typical poultry ration will contain 18-23% protein. Protein requirements of young fish vary from 31-38% for common carp, Cyprinus carpio, (Ogino and Saito, 1970; Takeuchi et al., 1979) to 56% for tilapia fry, Tilapia aurea, (Winfree and Stickney, 1981).

Work by Mangalik (1986) shows that the protein/energy ratio of catfish changes little with growth. The actual amount of protein per kcal of digestible energy (DE) consumed for maximum growth in channel catfish decreased only a slight amount from 0.10 g for a 3-g fish to 0.09 g for a 200-g fish. Table 2 shows the decrease in daily protein consumption as size of the fish increased.

As with land animals, it would be more accurate to express protein requirements as requirements for the same ten essential amino acids. Quantitative requirements have been determined so far in carp, japanese eel, channel catfish, tilapia,and chinook salmon (Table 3).

While supplementing diets with crystalline amino acids is standard practice in commercial poultry diets, research with fish supplementation has had mixed results (NRC,1983). Murai (1985) found young carp fed once a day excreted up to 40% of the free amino acids fed. Until more data is available, amino acid supplementation of tropical fish diets would not be practical at this time.

Energy

Fish require far less energy for protein synthesis than farm animals (Lovell, 1979) (Table 4). They do not have to maintain a constant body temperature, they exert less energy maneuvering in water than land animals, and they excrete ammonia, which requires less energy in protein catabolism than uric acid or urea (NRC,1983).

Energy requirements of fish are usually determined through growth responses to various protein levels. This response is usually reported as the kcal digestible energy/ gram protein, or DE/P. A DE requirement of 8 to 9 kcal/g of protein is recommended for production diets of carp and channel catfish (NRC, 1983.)

As with birds and mammals, fish eat to satisfy their energy requirement. Both insufficient and excess energy levels in the diet will result in stunted growth. A diet with excessive levels of energy will cause protein deficiencies as the animal will not consume enough food to meet its protein requirement. All fish species are able to digest protein and lipids efficiently. Warm water species are able to digest carbohydrates well, but cold water species digest starches poorly. For example, the energy in uncooked cornstarch is 20% digestible by cold water rainbow trout, but channel catfish can digest 60 %.

Vitamins

Fish require the same fifteen vitamins as land animals, although not every species requires all fifteen (Lovell, 1989). Catfish do no appear to require inositol, but trout require all of the vitamins (NRC, 1983.). Some warm water fishes including most tilapia, appear to have some B-vitamin synthesis take place in the intestine. Vitamin requirements have been determined for only a few species (Table 5).

Deficiency symptoms for all of the vitamins include depressed appetite and lack of growth. Anemia, ataxia, abnormal skin pigmentation, hypersensitivity, fatty livers, and increased susceptibility to bacterial infections are common symptoms for

several vitamins. Clinical deficiency symptoms are difficult to produce for some vitamins. Table 6 includes a list of known vitamin deficiency symptoms.

Vitamin supplementation is very important for all fish maintained in a closed system aquarium or an intensively stocked pond. It is important to remember that not all commercial fish foods, either flaked, pelleted, or extruded, contain each of the essential vitamins. Both fresh and prepared foods can suffer from vitamin loss due to freezing, heat, or long term storage. Even feeding methods can affect vitamin loss. The water soluble vitamins can leach out of foods remaining in the water for some time before it is consumed.

Minerals

Fish are able to meet their mineral requirements by direct absorption through the gill membranes of fresh water fish or across the gut wall of saltwater fishes. Channel catfish can meet their requirement for calcium if the dissolved calcium ion concentration in the water is 5 mg/liter or higher (Lovell, 1978).

Fatty Acids and Sterols

While homeothermic animals utilize omega-6 (n-6) structured fatty acids, salmonids and other cold water species require omega-3 fatty acids (NRC, 1983.) It is assumed that the cold water species require a greater degree of unsaturated fatty acids to maintain cell permeability and flexibility at low temperatures. Warm water fishes such as tilapia require omega-6 fatty acids or a combination of the two (Kanazawa et al. 1980.)

Crustaceans require sterols in the diet to maintain health and optimum growth. Both panaeid shrimp and lobster require 0.5% cholesterol in the diet (Kanazawa, 1971.) (Castell et al., 1975.). Marine crustaceans also require high levels of lecithin. Panaeid shrimp showed improved growth with 1% lecithin added to the diet (Kanazawa, 1979.) and lobster show optimum growth with 7-8% lecithin added to the diet (Conklin et al., 1980.)

A new book by R. T. Lovell, Fish Nutrition and Feeding, is available from Van Nostrand Publisher. This is an excellent nutrition textbook as well an up to date reference book. Another book scheduled to be published late 1990 is Fish Medicine, edited by Michael Stoskopf and published by Harcourt, Brace, and Jovanovich. The author wishes to thank Dr. Lovell of Auburn University for his assistance as well as permission to use tables from his book.

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TABLE 1. COMPARISON OF DAILY CONSUMPTION PER KG BODYWEIGHT OF FEED, PROTEIN, AND ENERGY BETWEEN BROILER CHICKEN (0.8 KG) AND CHANNEL CATFISH (0.1 KG)[1]

| Animal | Feed (g) | Energy (kcal/ME)[2] | Protein (g) |
|---|---|---|---|
| Broiler chicken | 89 | 284 | 17.8 |
| Channel catfish | 30 | 83 | 10.8 |
| Ratio: chicken/catfish | 3/1 | 3.4/1 | 1.6/1 |

[1]Sources: NRC (1983, 1984).

[2]ME adjusted from DE for channel catfish.

TABLE 2. PROTEIN AND DIGESTIBLE ENERGY (DE) REQUIREMENTS BY VARIOUS SIZES OF CHANNEL CATFISH FOR MAXIMUM PROTEIN SYNTHESIS[1]

| Fish size (g) | Protein (g/100 g fish/day) | DE (kcal/100 g fish/day) | DE/Protein ratio (kcal/g) |
|---|---|---|---|
| 3 | 1.64 | 16.8 | 10.2 |
| 10 | 1.11 | 11.4 | 10.3 |
| 56 | 0.79 | 9.0 | 11.4 |
| 198 | 0.52 | 6.1 | 11.7 |
| 266 | 0.43 | 5.0 | 11.6 |

[1]Source: Mangalik (1986).

TABLE 3. ESSENTIAL AMINO ACIDS REQUIREMENTS OF SEVERAL FISHES, CHICKENS, AND SWINE (PERCENTAGE OF THE PROTEIN)

| Amino acid | Japanese eel[1] | Common carp[1] | Channel catfish[1] | Chinook salmon[1] | Tilapia nilotica[2] | Chicken[1] | Swine[1] |
|---|---|---|---|---|---|---|---|
| Arginine | 4.5 | 4.2 | 4.3 | 6.0 | 4.2 | 5.6 | 1.2 |
| Histidine | 2.1 | 2.1 | 1.5 | 1.8 | 1.7 | 1.4 | 1.2 |
| Isoleucine | 4.0 | 2.3 | 2.6 | 2.2 | 3.1 | 3.3 | 3.4 |
| Leucine | 5.3 | 3.4 | 3.5 | 3.9 | 3.4 | 5.6 | 3.7 |
| Lysine | 5.3 | 5.7 | 5.1 | 5.0 | 5.1 | 4.7 | 4.4 |
| Methionine + cystine | 5.0 | 3.1 | 2.3 | 4.0 | 3.2 | 3.3 | 2.3 |
| Phenylalanine + tyrosine | 5.8 | 6.5 | 5.0 | 5.1 | 5.5 | 5.6 | 4.4 |
| Threonine | 4.0 | 3.9 | 2.0 | 2.2 | 3.8 | 3.1 | 2.8 |
| Tryptophan | 1.1 | 0.8 | 0.5 | 0.5 | 1.0 | 0.9 | 0.8 |
| Valine | 4.0 | 3.6 | 3.0 | 3.2 | 2.8 | 3.4 | 3.2 |

[1]Source: NRC (1979, 1981, 1983, 1984).

[2]Source: Santiago (1985). The two values represent $X_1$ and $X_{max}$, where $X_{max}$ is the requirement for maximum growth and $X_1$ is the requirement for a level of growth within the 95% confidence range of maximum growth, determined by polynomial regression. The one value represents optimum requirement determined by broken line analysis.

TABLE 4. ENERGY REQUIREMENT FOR PROTEIN SYNTHESIS BY FISH AND WARMBLOODED FOOD ANIMALS[1]

| Food animal | Dietary protein (%) | Dietary ME (kcal/g) | Protein gain/Mcal ME consumed (g) |
|---|---|---|---|
| Channel catfish | 32 | 2.8[2] | 47 |
| Broiler chickens | 20 | 2.8 | 23 |
| Swine | 16 | 3.3[2] | 9 |
| Beef cattle | 11 | 2.6 | 6 |

[1]Source: NRC (1983).

[2]ME estimated from DE.

TABLE 5. MINIMUM VITAMIN REQUIREMENTS FOR GROWTH OF FISH (AMOUNT PER KG OF DIET)

| Vitamins | Units | Channel catfish[1] | Common carp[1] | Salmonids[2] |
|---|---|---|---|---|
| A | IU | 1000-2000 | R[3] | 2500 |
| D | IU | 500-1000 | - | 2400 |
| E | IU | 50 | R | 30 |
| Mezadione | mg | R | - | 10 |
| Thiamin | mg | 1 | 1 | 10 |
| Riboflavin | mg | 9 | 8 | 20 |
| Pyridoxine | mg | 3 | 6 | 10 |
| D-calcium pantothenate | mg | 20 | 30-50 | 40 |
| Niacin | mg | 14 | 28 | 150 |
| Folacin | mg | R | N[4] | 5 |
| $B_{12}$ | mg | R | N | 0.02 |
| Inositol | mg | N | 10 | 400 |
| Biotin | mg | R | R | 0.1 |
| Choline | mg | 500 | 4000 | 3000 |
| Ascorbic acid | mg | 60 | R | 100 |

[1]Source: NRC (1983) except vitamin E requirement for channel catfish which is from Wilson et al. (1984).

[2]Source: NRC (1981).

[3]"R" means essential but dietary requirement not determined.

[4]"N" means no dietary requirement found under defined experimental conditions.

TABLE 6. VITAMIN DEFICIENCY SIGNS IN FISH[1]

| Vitamin | Channel catfish | Trout and salmon | Common carp | Eel |
|---|---|---|---|---|
| A | Exophthalmos<br>Edema<br>Ascites | Exophthalmos<br>Eye lens displacement<br>Corneal and retinal degeneration<br>Ascites<br>Skin depigmentation | Depigmentation<br>Twisted opercula<br>Fin and skin hemorrhages | --[2] |
| D | Low bone ash | Impaired calcium homeostasis<br>Tetany of white skeletal muscle | --[2] | -- |
| E | Muscular dystrophy<br>Exudative diathesis<br>Skin depigmentation<br>Low hematocrit<br>Ceroidosis and hemosiderosis in visceral organs<br>Lipid peroxidation of liver microsomes<br>Erythrocyte hemolysis | Anemia<br>Variable sized, fragmented erythrocytes<br>Ascites<br>Muscular dystrophy<br>Lipid peroxidation of liver microsomes<br>Reduced immune responses<br>Skin depigmentation | Muscular dystrophy<br>Exophthalmos<br>Kidney, pancreas degeneration<br>Ceroids in visceral organs | Skin and fin hemorrages<br>Dermatitis |

Table 6. Continued

| Vitamin | Channel catfish | Trout and salmon | Common carp | Eel |
|---|---|---|---|---|
| K | Skin hemorrhages<br>Prolonged blood clotting time | Prolonged blood clotting<br>Anemia | -- | -- |
| Thiamin | Dark skin color<br>Loss of equilibrium<br>Hypersensitivity<br>Convulsions | Hyperirritability<br>Convulsions<br>Loss of equilibrium<br>Low transketolase activity | Fin congestion<br>Nervous disorders<br>Depigmentation<br>Subcutaneous hemorrhage | Trunk-winding<br>Subcutaneous hemorrhage<br>Congested fins |
| Riboflavin | Poor growth<br>Short, stubby body | Lens cataract<br>Adhesion of lens and cornea<br>Reduced activity of erythrocyte glutathione reductase<br>Dark pigmentation | Skin and fin hemorrhages<br>Hemorrhagic heart muscle<br>Anterior kidney necrosis | Dermatitis<br>Photophobia<br>Fin hemorrhage<br>Abdominal hemorrhage |
| Pyridoxine | Nervous disorders<br>Tetany<br>Greenish-blue coloration | Convulsions<br>Hyperirritability<br>Erratic, spiral swimming<br>Rapid breathing, gasping<br>Rapid onset of rigor mortis | Nervous disorders<br>Hemorrhage<br>Edema<br>Low hepato-pancreatic transferases<br>Dermatitis | Convulsions<br>Nervous disorders |

Table 6. Continued

| Vitamin | Channel catfish | Trout and salmon | Common carp | Eel |
|---|---|---|---|---|
| Pantothenic acid | Clubbed, exudate-covered gills<br>Anemia<br>Erosion of skin, barbels, lower jaw | Clubbed, exudate-covered gills<br>Anemia<br>Atrophied pancreatic acinar cells<br>Vacuoles and hyaline bodies in kidney tubules | Anemia<br>Exophthalmos | Dermatitis<br>Congested skin<br>Hemorrhagic skin<br>Abnormal swimming |
| Biotin | Skin depigmentation<br>Anemia<br>Reduced liver pyruvate carboxylase | Degeneration of gill lamellae<br>Skin lesions<br>Fatty liver<br>Reduced acetyl CoA and pyruvate carboxylase<br>Altered fatty acid synthesis<br>Degeneration of pancreatic acinar cells | Poor growth<br>Increased dermal mucous cells | Abnormal swimming<br>Dark skin |
| Niacin | Skin and fin lesions<br>Exophthalmos<br>Anemia<br>Deformed jaws | Skin and fin lesions<br>Colon lesions<br>Anemia<br>Photosensitivity<br>Sunburn | Skin hemorrhages | Poor swimming coordination<br>Dark color<br>Skin lesions<br>Anemia |

Table 6. Continued

| Vitamin | Channel catfish | Trout and salmon | Common carp | Eel |
|---|---|---|---|---|
| Folic acid | No deficiency found | Anemia<br>Pale gills<br>Large, segmented erythrocytes | No deficiency found | Poor growth<br>Dark coloration |
| $B_{12}$ | Reduced hematocrit | Anemia<br>Small, fragmented erythrocytes | No deficiency found | Poor growth |
| C | Lordosis<br>Scoliosis<br>Reduced bone collagen<br>Increased sensitivity to bacterial infection<br>Slow wound repair<br>Reduced hematocrit | Lordosis<br>Scoliosis<br>Hemorrhagic exophthalmos<br>Ascites<br>Reduced hematocrit<br>Deformed operculum<br>Abnormal histology of support cartilage in eye and gill | No deficiency, found | Fin and dermal hemorrhages<br>Lower jaw erosion |
| Choline | Fatty liver<br>Hemorrhagic kidney and intestine | Fatty liver | Fatty liver | White-grey intestine |
| Inositol | No deficiency found | Slow gastric emptying<br>Reduced cholinesterase and transaminase<br>Fatty liver<br>Decreased phosphotides | Skin lesions | White-grey intestine |

# THE IMPORTANCE OF MILK LIPASE FOR FAT DIGESTION IN THE NEONATE

Charlotte L. Kirk[1], David J. Baer[2], and Margit Hamosh[1]
[1]Georgetown University Medical Center
Washington D.C. 20007
[2]Allen and Baer Associates
Silver Spring, MD 20904

The hand raised neonate faces many difficulties, one of which concerns the adequacy of the selected milk or milk replacer. Many exotic species are raised on a domestic species' milk as a substitute. In other instances, the neonate may be raised on a milk substitute (formula). There are differences in the composition of milk as compared to a formula and these differences may have an effect on fat digestion in the infant. Digestive problems encountered when raising orphaned neonates may be related to the composition of the milk substitute.

The fat in milk or formula is the primary source of energy for the neonate of most species and a vector of absorption for many fat soluble vitamins. The digestion of fat (triglyceride hydrolysis) is carried out by the enzymes gastric lipase, pancreatic lipase, and if present, the milk lipase identified as bile salt stimulated lipase (BSSL). Some infants such as the human and the dog are born with pancreatic insufficiency (the pancreas is not fully functional at birth including lipase secretion), and must rely solely on gastric lipase or milk lipase (BSSL) for fat digestion. If the infant is raised on formula, there is no milk lipase present to aid in the digestion of fat, which may limit the extent of digestion and absorption of this crucial nutrient.

Milk lipase has recently been found to be present in the milks of the dog, ferret, gorilla, hooded seal, cat, black bear, and human. This lipase is characterized by secretion of the enzyme by the mammary gland into the milk, with optimum conditions for activity including a pH of 8.5 and the presence of bile salts (hence the name bile salt stimulated lipase). The primary function of this enzyme is triglyceride hydrolysis in the small intestine.

When the neonate ingests formula or milk, the hydrolysis of fat is initiated in the stomach by gastric lipase at a pH of approximately 5.4. After this initial hydrolysis, the milk containing bile salt stimulated lipase is hydrolyzed further in the small intestine at a higher pH of 8.5. The extent of hydrolysis is dependent upon the specific activity of the milk lipase in the particular species.

In vitro studies were conducted to simulate the digestion of milk and/or formula by the neonate of several species. Gastric biopsies were obtained from infants of the dog, ferret, and human as a source of gastric lipase in the assay. Biopsies were incubated with the milk of each species and also with an appropriate milk replacer. The initial conditions were optimal for gastric lipase activity, simulating conditions in the newborn stomach. After incubation of milk with the gastric biopsies, the

pH was raised from 5.4 to 8.5 and bile salts were added to activate the milk lipase. After incubation of formulas with gastric lipase, a source of milk lipase (skim portion of each species natural milk) was added, the pH was raised from 5.4 to 8.5 and bile salts were added. Hydrolysis was measured by titration of free fatty acids after digestion by gastric lipase alone and after the digestion by the combined action of gastric and milk lipase in both milk and in formula (when skim was added to formula as a source of milk lipase). Results are presented in table 1.

Triglyceride hydrolysis by the action of gastric lipase is similar in milk and formula in all species tested. Additional hydrolysis of fat by milk lipase (BSSL) after digestion with gastric lipase reached similar levels in milk and formula. Milk lipase (BSSL) can not hydrolyze fat without prior digestion by gastric lipase. It is important to note that the ferret, whose milk contains high levels of BSSL, achieved much higher levels of hydrolysis than the other species tested. Of considerable importance is the fact that fat hydrolysis of formulas would normally be accomplished only by gastric lipase, and the increase in fat hydrolysis obtained artificially in this experiment (by the addition of natural milk lipase) would not occur in the formula fed neonate. It is clear that without the milk lipase, BSSL, the infant raised on formula may be at a disadvantage with respect to digestion and absorption of the dietary fat.

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Table 1. Range of triglyceride hydrolysis in milk and formula

| SPECIES FORMULA | MILK OR LIPASE | % HYDROLYSIS GASTRIC LIPASE | % HYDROLYSIS BSSL (ALONE) | % HYDROLYSIS GASTRIC LIPASE + BSSL |
|---|---|---|---|---|
| Dog | milk | 1.0- 1.2 | 0.3-0.9 | 9.4-11.0 |
| Ferret | milk | 2.3- 3.5 | 0.4-1.4 | 34.0-48.2 |
| Human | milk | 0.2- 2.0 | 0.2-0.4 | 4.4- 4.5 |
| Gorilla | milk | 1.3 | NA | 2.0 |
| Dog | Esbilac | 0.7- 1.0 | 0.6-1.0 | 6.0- 9.9 |
| Ferret | KMR | 0.89-1.2 | 0.5-0.8 | 20.3-30.0 |
| Human | Similac | 1.2- 2.0 | 0.3-0.7 | 5.7- 7.7 |

# INVESTIGATIONS OF HEPATIC IRON LEVELS IN ZOO BIRDS

Ellen Dierenfeld, Ph.D. and Christine D. Sheppard, Ph.D.
New York Zoological Society
Bronx, New York 10460

## INTRODUCTION

Iron storage disease is a non-specific term used in reference to the accumulation of iron in tissues (notably liver) reported in a number of captive bird species including mynas (Lowenstine and Petrak, 1978; Randell et al., 1981; Gosselin and Kramer, 1983) birds of paradise (Assink and Frankenhuis, 1981; Frankenhuis et al., 1989), tanagers (Kincaid and Stoskopf, 1987) and others (Taylor, 1984; Ward et al., 1988). The cause of the condition is unknown, but has been attributed to both genetic and nutritional factors, thus may encompass different physiological mechanisms.

Primary hemochromatosis is an inherited metabolic disorder in which excess iron is absorbed from iron balanced diets, resulting in hepatocyte accumulation (Kincaid and Stoskopf, 1987; Monmaney, 1980). Similarity of mynahs and humans led Gosselin and Kramer (1983) to conclude that diet likely played a minor role in iron overload in this species. Other studies, however, (Taylor, 1984; Kincaid and Stoskopf, 1988) have shown distinct pathologies form those previously described, with more generalized accumulation of iron pigment in avian tissues (hemosiderosis) that is commonly associated with dietary iron excess (Brink et al., 1976). Dietary iron levels, quantified in these latter studies only, suggest that nutritional components are more likely responsible for hepatic iron overload than genetics in may zoo species.

Reports of iron storage disease in captive avifauna have historically been based on qualitative and semi-quantitative assessments of liver iron deposition through histological examination of tissues. Few quantitative data on hepatic iron concentrations, particularly in relation to dietary iron levels, are available (Ward et al., 1988; Frankenhuis et al., 1989). Our studies were conducted to: 1) obtain baseline norms and ranges of total liver iron among various taxonomic groups of captive birds and 2) test the preliminary hypothesis that dietary iron level affects liver iron concentration in the Sturnidae. Hepatic iron levels in wild birds were measure whenever possible for comparison with captive specimens.

## MATERIALS AND METHODS

Liver Iron Content: All samples from captive birds were obtained from livers removed during necropsy. Total hepatic iron concentration was determined in liver specimens preserved in 10% buffered formalin. A 0.25-0.50g subsample of each liver was ashed overnight in a muffle furnace at 550 C, dissolved in 3ml 3N HCl, and diluted to 25ml with deionized water according to the methods of Parker (1963). Chemical analysis was performed on a Model 2280 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) equipped with an iron lamp, using analytical-grade acetylene.

The majority of samples were obtained from birds in the New

York Zoological Park collection that had died between 1984-1988; others were supplied from birds necropsied at the St. Louis Zoological Park (n=20), San Diego Zoo (n=7), and Parkwood Pet Clinic (n=10; Woodland Hills, CA). Sex, age at death, previous dietary were recorded for each sample as available.

An additional 14 livers were obtained from 11 bird species (4 Orders) which died in Papua New Guinea during a collection trip, August through September 1988. Formalinized tissues were imported and shipped under USDA permit #18956 (San Diego Wild Animal Park).

Feeding Trial: The European starling (Sturnis vulgaris) was selected as a representative model of the Sturnidae for use in feeding trials based on previous studies of iron metabolism (Gosselin et al., 1983). Prior to feeding trials, preliminary tests of analytical methods and age/sex variability among starlings were conducted. Livers were collected form identifiable juvenile (< 1 yr; n=3) and adult birds (> 1 yr; n=5) for iron analysis, as well as a separate group of 18 females and 16 males (all adults). Sex and age effects were examined using Student's t-test, with level of significance set at P= 0.05

Forty-four starlings were live-trapped and randomly assigned to 4 cages measuring approximately 4 x 3 x 2 m. Sexes and ages of the birds were unknown, although all were estimated to be > 1 yr of age. During an adaptation period of 25 days, the birds were fed a commercial pelleted diet (Turkey Starter, Blue Seal Feeds, Lawrence, MA) containing 137mg/kg Fe (all diet information on dry matter basis). Each group was switched to an experimental semipurified basal diet (Roudybush Feeds, Davis, CA) containing 40mg/kg Fe for 10 days, then fed treatment diets containing 40 (control), 200, 400 or 1500 mg/kg Fe as FeSO4 for 68 days. Intake was recorded daily, and deionized water was provided ad libitum throughout the feeding study.

At the completion of the trial, starlings were euthanized with CO2. Body mass, liver mass, and sex were recorded for each individual. Livers were placed into 10% buffered formalin, and iron concentration assayed as described previously.

RESULTS

Captive Survey: Means and ranges or iron concentrations in liver samples from 418 captive birds comprising 19 Orders and 63 Families are found in Table 1. Most birds (80%) had liver iron concentrations below 50ug/g (wet weight basis; 55.85ug = 1 mol). Orders (n=2) for which the mean liver iron concentration was > 100ug/g are detailed by Family in Table 2.

Sex differences in liver iron content were not significant (P> 0.05) for any of the captive bird comparisons (entire group, within Order, and within Family, total sample, n=191 females and n=163 males).

Liver iron concentrations in birds collected from Papua New Guinea are found in Table 3. No values were greater than 100ug/g, although hemosiderosis was reported as a microscopic finding in several of these livers (K. Osborn, San Diego Zool. Soc., pers. comm.).

Among the eight wild starlings examined initially, age had a

highly significant effect ($P < 0.001$) on liver iron concentration ($x = 1$ 10.55 $\pm$ 1.23 for juveniles [n=3] and 61.60 $\pm$ 8.82ug/g adults [n=5]. Sex also had a significant effect ($P < 0.01$) on liver iron measurements, with females averaging 41.12 $\pm$ 4.39ug/g (n=18) and males, 22.35 $\pm$ 4.90ug/g (n=16).

Feeding Trials: Body and liver measurements ($x \pm$ SEM) of European starlings fed the four experimental diets are found in Table 4. No significant differences were seen among dietary treatment groups for daily intake, total body mass, or liver iron concentration. Total liver mass of (both male and female) starlings fed diets containing 1500mg/kg Fe was greater than birds fed other dietary treatments. Subjective evidence of liver disease (mottling, discoloration) was seen only in birds in the highest dietary iron treatment group.

Male starlings tended to be larger ($x = 80.48 \pm 0.84$g) than females ($x = 75.06 \pm 1.19$g) across all treatment groups. Female starlings fed diets containing 400mg/kg Fe had the highest liver iron concentration (43.87ug/g) than any other treatment group regardless of sex; although liver iron content tended to increase with increasing dietary iron, the pattern of response appeared to differ by sex (see Figure 1).

## DISCUSSION

The majority of birds, both captive and wild, in the survey study showed liver iron concentrations between 20-40ug/g wet tissue, values 10-fold higher than those found in commercially-produced chicken livers measured during this study ($x = 3.4 \pm 0.2$; n=10). These values were, however, lower than those reported for domestic quail (males, 90 to 300ug/g; laying females, 120 to 500ug/g) fed iron deficient or normal rations for up to 4 months (Garcia et al., 1987). Interestingly, the exotic Galliformes in this study showed a similar wide range of hepatic iron content ($x = 20.0$ug/g, n=58, Phasianidae; $x = 390.8$ug/g, n=2, Cracidae) as domestic species.

Of 44 individuals whose Family mean liver iron content measured > 100ug/g, necropsy reports were available for 28 birds; hemochromatosis was a significant pathological finding in all but 8 of the reports. Among the 8 negative cases (5 Paradisaedae, 2 Meliphagidae, 1 Laniidae), only 3 birds, which showed high iron levels, but no hemochromatosis, (2 Meliphagidae, 1 Paradisaedae) were males held in captivity < 3 years; two of the three were wild caught in Papua New Guinea. Although a direct correlation between histologically scored iron deposition and total hepatic iron concentration has not been completed on these samples, this and other (Ward et al., 1988; Frankenhuis et al., 1989) evidence suggests a strong quantitative relationship.

The Sturnidae livers sampled (n=21) did not show a remarkable mean iron concentration (58.2ug/g) within the Passeriformes as might be expected from literature reports. Necropsy reports available for these starlings and mynahs, however, recorded hepatic iron deposition in 7 of 10 livers containing > 50ug/g; hemochromatosis was diagnosed in only 1 of 11 livers measuring < 50ug/g.

Captive birds of paradise (Paradisaedae) and toucans (Rhamphastidae), in particular, showed extremely high liver iron concentrations compared with most other groups, and particularly in comparison with the single value available (17.1ug/g) for a wild subadult (P. Ensley, San Diego Zool. Soc., pers. comm.) bird of paradise (see Table 3). These quantitative data confirm taxonomic patterns of accumulation of iron in livers reported for previously described captive avians.

Most of the wild birds (both starlings and those from Papua New Guinea) had liver iron values less than 40ug/g. Exceptions from Papua New Guinea included a female rufous monarch (Monarcha rubiensis), with a level > 3-fold that of the male (see Table 3). Sex differences in liver iron concentration from wild birds with information reported in domestic poultry (Balasch and Planas, 1972; Garcia et al., 1984), where estrogen has been shown to influence liver iron storage and possibly facilitate intestinal uptake. Non-laying females have higher liver iron stores (2-3x) than males; the laying process reduces those stores rapidly, but values recover if dietary levels are adequate (Garcia et al., 1986).

The sex effect observed with wild-caught birds, although limited, suggests a different iron metabolism from that in human hemochromatosis, where males show far more incidence of disease than females (Monmany, 1989). Possible reasons for a lack of sex effect in the captive survey include uncontrolled variables of captive management (diet, disease, length of time in captivity, age, reproductive status) which could not be obtained from available records. Furthermore, sex effects may have been obscured if captive males already had diet-induced excessive hepatic iron concentrations.

Dietary iron levels in our captive feeding trials were chosen to encompass both low normal (40mg/kg) and excessive (1500mg/kg) literature values, as well as intermediate amounts. The controlled feeding trials with starlings showed a trend toward increased liver iron and hepatomegaly with increased dietary iron (see Table 4), even over the short time period in which they were conducted (68 days). Other limitations of this study (escape of subjects resulting in reduced sample size, compounded by sex and age effects, and unknown previous histories of the birds) are recognized. Nonetheless, these feeding trial data strongly suggest diet as a component in liver iron accumulation in the Sturnidae, and warrant further study.

Dietary and management histories of birds in this report were not complete enough to establish a direct link between dietary and liver iron content in most instances. In general, however, carnivorous, piscivorous, and/or primarily granivorous birds had lower liver iron concentrations (< 50ug/g) than more frugivorous, insectivorous or omnivorous birds (>50ug/g) within the same Order. Zoo diets fed to the first group are often single ingredients (meat, fish) or complete mixes (pellets, seeds) which offer little opportunity for selective consumption. Moreover, in evaluating the iron content of these items (see Table 5), few products contained more than 200mg Fe/kg dry matter. Similarly, fruits and vegetables commonly used in mixtures fed to frugivorous and/or omnivorus birds (Table 5)rarely contained more than about 100mg/kg Fe. However,

the contribution of supplements used to balance diets should be evaluated carefully, as should the potential for selective feeding.

A total dietary iron content of 490mg/kg (reported as wet basis, but likely dry), of which commercial pellets containing 1230mg/kg Fe comprises 20% of intake, was reported in one study (Kincaid and Stoskopf, 1987). Taylor (1984) reported commercially-produced pellets (15-55mg/kg Fe) as the dietary staple for many zoo birds, with supplements providing 150-11,200mg/kg Fe added and consumed in unknown quantities. Another study (Assink and Frankenhuis, 1981) identified five avian foods containing 126-1650mg/kg iron (presumably reported on a dry basis), with a significant contribution of iron from the food mixer and the lime ($CaCO_3$) supplement. It is possible that in each case dietary iron content (or selective intake of a mixed diet) was excessive compared to established poultry requirements of 55-111 mg/kg dry matter (NRC, 1984). Controlled studies of total, as well as available, dietary iron in both captive and wild avian diets need to be conducted.

Iron requirements for exotic avian species may differ from those of domestics. Recommendations for domestic poultry were derived using plant iron sources (Davis et al., 1968). Commercial or user-formulated diets for zoo birds are often based heavily upon animal sources of iron (heme), rather than plant or insect sources (non-heme). Although heme iron has been shown to be up to 7 times more available for metabolism than non-heme iron in mammals, (Fairbanks et al., 1971) 90% absorption of iron from a plant-based (non-heme) diet was reported by Frankenhuis et al. (1989) in a single study conducted with birds of paradise. Thus, species, as well as iron source, variations must be taken into account.

Finally, nutrient interactions must be considered in evaluating iron metabolism of zoo avian species. Deficiencies of protein and specific amino acids, B vitamins, vitamin A and vitamin E have all been cited as contributing to excessive hepatic iron stores in other species (Moraal, 1977), as have mineral imbalances (Lowenstine and Petrak, 1978). Ascorbic acid from various sources (i.e. citrus fruit, green leafy plants) may enhance iron bioavailability (Turnbull, 1974; Monsen, 1982; Reinhold et al., 1986), but has not been demonstrated experimentally with zoo birds. A lack of natural mineral chelating agents (phytates, tannins) in zoo diets has also been suggested as a possible factor in iron overload observed in zoo species (Ward et al., 1988; Spelman et al., 1989), although phytate levels in natural feedstuffs do not appear to substantially interfere with iron uptake in domestic poultry (Davis et al., 1968).

## SUMMARY

Although substantial inferential evidence is currently available, long term (6 months to 2 years), age- and sex-controlled feeding trials are required to determine if hepatic iron overload, observed in many zoo species, results from chronic excessive dietary iron ingestion. Until mechanisms are understood, a prudent management strategy involves feeding captive avian species diets which contain no more than about 200 mg/kg (dry matter basis) available iron. Iron requirements for exotic avifauna may vary,

but until further research is conducted, dietary ranges should be considered similar to those of domestic poultry (NRC, 1984). This feeding regimen may be particularly relevant for those species previously shown susceptible to iron storage disease.

ACKNOWLEDGEMENTS

We especially appreciate the laboratory assistance of K. Harr, and computer input of M. Pini. Thanks to V. Chiappetta, K. McClave, E. Davis, J. McGuire, and K. Resnick for sample preparation and analysis. Necropsy specimens were collected primarily by T. McNamara and J. Tappe at NYZP; additional liver samples were sent by A. Worell, C. Kuehler, and R. Junge. Wild starlings were supplied by the St. Louis Zoo; we thank B. Bohmke. Experimental birds were cared for by B. Karp, K. Stein, and A. Zaremba; diets were formulated by T. Roudybush. We sincerely appreciate the encouragement and input of numerous zoo, university medical, and feed industry personnel in these studies, particularly B. Bohmke (St. Louis Zoological Society), C. Kuehler, P. Ensley, and K. Osborn (Zoological Society of San Diego), P. McGill-Harelstad and S. Crissey (Chicago Zoological Society), and D. Taylor (Hiram College). Funding was supplied by Mr. and Mrs. Charles A. Fritz III, and Mr. and Mrs. John Kasten.

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Table 1. Mean liver iron concentration (µg/g) measured in wet formalinized tissue from zoo avifauna.

| Order | n | Families | Mean | SEM[1] | Range |
|---|---|---|---|---|---|
| Anseriformes | 45 | 4 | 28.8 | 9.0 | 0.0 - 338.2 |
| Charadriiformes | 45 | 10 | 24.3 | 2.5 | 1.8 - 101.6 |
| Ciconiiformes | 23 | 4 | 26.9 | 6.9 | 2.0 - 138.0 |
| Columbiformes | 34 | 1 | 28.5 | 3.2 | 2.6 - 76.9 |
| Coraciiformes | 21 | 6 | 34.9 | 7.9 | 4.6 - 132.0 |
| Cuculiformes | 3 | 1 | 32.1 | 7.5 | 20.6 - 46.2 |
| Falconiformes | 2 | 2 | 19.2 | 6.5 | 12.7 - 25.7 |
| Galliformes | 60 | 2 | 32.3 | 10.5 | 1.0 - 540.0 |
| Gruiformes | 33 | 6 | 43.9 | 24.3 | 1.2 - 808.9 |
| Passeriformes | 86 | 16 | 109.4 | 13.6 | 0.0 - 509.7 |
| Pelecaniformes | 9 | 2 | 17.0 | 4.3 | 3.9 - 38.5 |
| Piciformes | 18 | 2 | 223.7 | 83.0 | 0.6- 1190.3 |
| Psittaciformes | 19 | 1 | 19.1 | 2.8 | 0.8 - 40.3 |
| Rheiformes | 3 | 1 | 9.1 | 4.0 | 4.4 - 17.1 |
| Sphenisciformes | 8 | 1 | 37.1 | 5.8 | 15.6 - 66.1 |
| Strigiformes | 2 | 1 | 28.5 | 7.8 | 20.7 - 36.2 |
| Struthioniformes | 1 | 1 | 14.6 | | |
| Tinamiformes | 4 | 1 | 3.6 | 2.0 | 0.0 - 9.5 |
| Trogoniformes | 2 | 1 | 54.7 | 6.4 | 48.0 - 61.3 |

[1]Standard error of the mean.

Table 2. Mean liver iron concentration (µg/g) measured in wet formalinized tissue from captive avian species.

| Order/Family | n | Mean | SEM[1] | Range |
|---|---|---|---|---|
| Passeriformes | | | | |
| Corvidae | 1 | ND[2] | | |
| Cotingidae | 2 | 124.9 | 32.3 | 92.7 - 157.0 |
| Emberizidae | 1 | 47.4 | | |
| Furnariidae | 8 | 30.4 | 9.5 | 2.9 - 80.3 |
| Irenidae | 3 | 79.1 | 42.4 | 28.5 - 163.3 |
| Laniidae | 2 | 201.4 | 179.1 | 22.7 - 380.0 |
| Meliphagidae | 6 | 143.3 | 47.3 | 4.1 - 320.9 |
| Muscicapidae | 13 | 81.0 | 26.2 | 8.2 - 298.2 |
| Paradisaedae | 17 | 240.2 | 17.1 | 8.2 - 509.7 |
| Pittidae | 3 | 48.9 | 8.6 | 32.0 - 63.1 |
| Ploceidae | 3 | 8.8 | 2.3 | 5.1 - 13.1 |
| Ptilonoryhnchidae | 1 | 263.2 | | |
| Pychonotidae | 2 | 71.4 | 67.9 | 3.6 - 139.1 |
| Sturnidae | 21 | 58.2 | 9.9 | 0.0 - 179.4 |
| Thraupinae | 2 | 212.4 | 127.3 | 85.5 - 339.3 |
| Vireonidae | 1 | 5.6 | | |
| Piciformes | | | | |
| Capitonidae | 6 | 82.9 | 39.2 | 0.6 - 254.5 |
| Rhamphastidae | 12 | 294.1 | 119.3 | 0.8 -1190.3 |

[1]Standard error of the mean.

[2]None detected.

Table 3. Liver iron concentrations (µg/g) measured in wet formalinized tissues collected from wild birds in Papua New Guinea (28 August through 16 September 1988).

| Species | Sex | Liver Iron (µg/g) |
|---|---|---|
| Metallic starling *Aplonis metallica* | NA[1] | 18.50 |
| Yellowbilled kingfisher *Halcyon forotoro* | NA | 17.39 |
| Paradise kingfisher *Tanysiptera* sp. | F | 30.00 |
| Rusty pitohuis *Pitohui ferrugineus* | M | 36.06 |
| Papuan frogmouth *Podargus papuensis* | NA | 20.78 |
| Hooded pitohuis *Pitohui dichrous* | M | 19.32 |
| Rufous-bellied kookaburra *Dacelo gaudichaud* | M | 32.15 |
| Stephen's ground dove *Chalcophaps stephani* | M<br>F | 9.56<br>10.71 |
| Rufous monarch *Monarcha rubiensis* | M<br>F | 19.31<br>70.28 |
| White-eared catbird *Ailuroedus buccoides* | M<br>F | 94.90<br>86.44 |
| King bird of paradise *Cicinnurus regius* | F | 17.71 |

[1]NA = Not available

Table 4. Body and liver measurements ($\bar{x} \pm SEM^1$) of European starlings (Sturnis vulgaris) fed diets containing different iron levels for 68 days.

| Diet Treatment | n | Daily Intake | Body Mass | Liver Mass | Liver Iron |
|---|---|---|---|---|---|
| | | (g) | (g) | (g) | (µg/g) |
| 40 mg/kg | 5 | 17.0 ±0.7 | 77.3 ±0.9 | 2.8 ±0.2 | 22.7 ±3.8 |
| 200 mg/kg | 9 | 17.1 ±0.7 | 76.6 ±2.0 | 3.0 ±0.1 | 27.9 ±3.0 |
| 400 mg/kg | 8 | 17.2 ±0.6 | 78.7 ±1.5 | 3.0 ±0.1 | 29.6 ±5.1 |
| 1500 mg/kg | 9 | 17.6 ±0.6 | 80.5 ±1.3 | 3.9 ±0.1 | 31.9 ±3.3 |

[1]Standard error of the mean.

Table 5. Iron content (dry matter basis) of typical foods used in diets for zoo birds.[1]

| Food | Iron (mg/kg) | Food | Iron (mg/kg) |
|---|---|---|---|
| **MEAT PRODUCTS** | | **WHOLE FISH** | |
| Horsemeat | 179.0 | Capelin | 143.7 |
| Chicken flesh | 48.1 | Herring | 44.5 |
| Whole quail | 114.6 | Talapia | 61.8 |
| Whole chicken | 146.6 | Goldfish | 54.5 |
| Veal | 73.7 | Brook trout | 78.3 |
| Adult rat | 171.2 | Smelt | 38.1 |
| Adult mouse | 239.0 | Mackeral | 90.0 |
| Mouse pup | 125.3-217.0 | Sardines | 60.0 |
| Heart, beef | 125.0 | **INSECTS** | |
| Heart, chicken | 126.9 | | |
| Liver, beef | 216.7 | Crickets | 32.0 |
| Liver, chicken | 207.9 | Crickets dusted with limestone | 41.0 |
| **EGGS** | | Mealworms | 94.8 |
| Chicken, hard-cooked w/o shell | 81.9 | **FRUITS** | |
| | | Apple | 18.8 |
| **VEGETABLES** | | Banana | 28.8 |
| | | Cantelope | 44.4 |
| Beans, green | 80.0 | Figs | 50.0 |
| Broccoli | 100.0 | Grapes | 22.2 |
| Carrots | 58.3 | Mango | 22.2 |
| Corn | 18.9 | Papaya | 27.3 |
| Kale | 150.0 | Pear | 17.6 |
| Peas | 86.4 | Orange | 28.6 |
| Potatoes | 30.0 | Raisins | 42.6 |
| Spinach | 310.0 | | |
| Yam | 23.1 | | |
| **SEEDS** | | | |
| Millet | 79.0 | | |
| Peanut | 23.2 | | |
| Pumpkin | 161.2 | Poultry iron requirements: | |
| Sesame | 153.2 | 55 - 111 mg/kg diet dry | |
| Sunflower | 74.7 | matter (NRC, 1984). | |

[1]All values from Composition of Foods, USDA Agr. Handbook #8, Washington, DC, 1963, or New York Zoological Society, unpublished data.

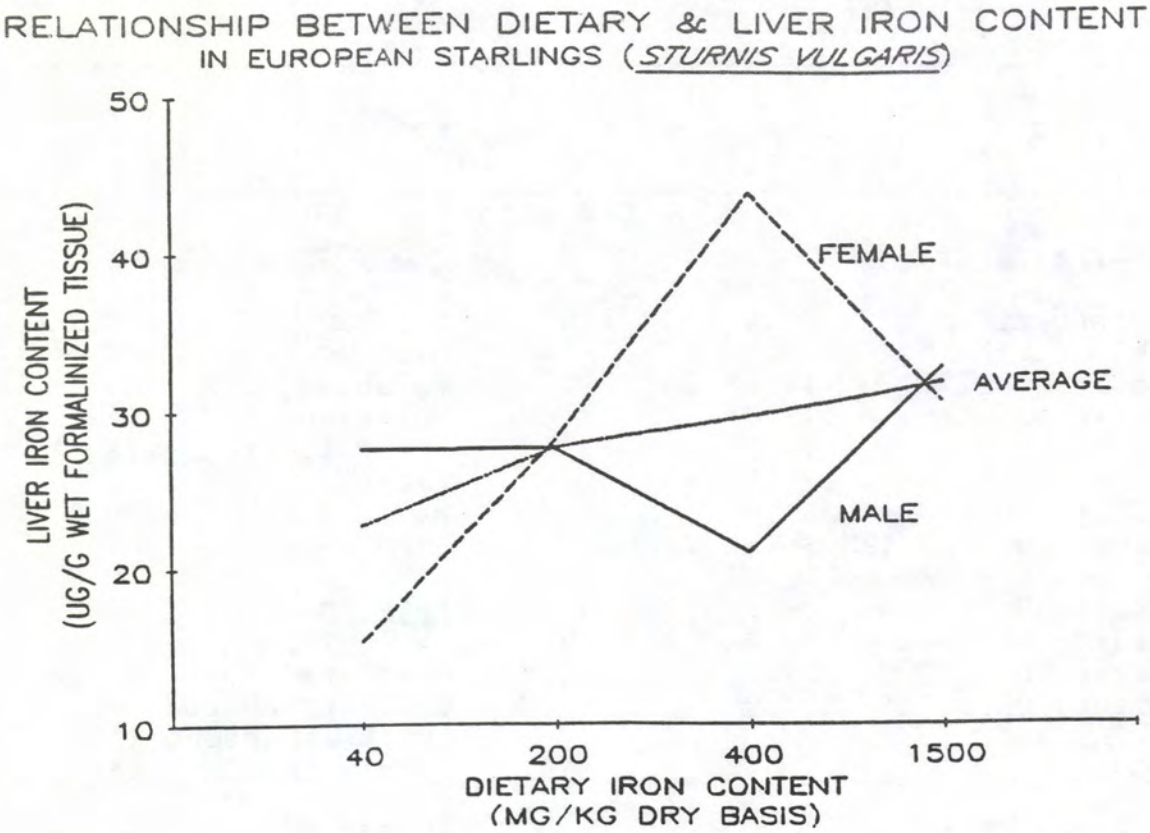
RELATIONSHIP BETWEEN DIETARY & LIVER IRON CONTENT
IN EUROPEAN STARLINGS (STURNIS VULGARIS)
LIVER IRON CONTENT
(UG/G WET FORMALINIZED TISSUE)
50
40
30
20
10
FEMALE
AVERAGE
MALE
40
200
400
1500
DIETARY IRON CONTENT
(MG/KG DRY BASIS)

Figure 1.

# EFFECT OF AGE ON DIGESTIBILITY IN EXOTIC RUMINANTS

Ann M. Trusk[1], Michael R. Murphy Ph.D.[1],
Susan Crissey Ph.D.[2], Ann Petric[2]

[1]University of Illinois
Animal Science Department
Urbana, IL 61801

[2]Brookfield Zoo
Brookfield, IL 60513

## INTRODUCTION

Ruminants can be classified into three overlapping feeding types: concentrate selectors (CS), intermediate feeders (IM), and grazers (GR) (Hofmann, 1989; Figure 1). Diet selectivity is an important criterion for animal classification. Animals consuming a diet consisting primarily of fruits, tubers, seeds, and 30 to 40% leaves, buds, blossoms,and young shoots are considered CS. More than 60% of the diet of IM contains leaves, shoots, blossoms, and other plant parts with decreasing amounts of fruits, tubers and other reserve organs of the plant. Grazers, or bulk and roughage eaters, consume a predominance of grasses, stems, leaves, and buds (Langer, 1984).

Digestive tract morphology of the prehensile organs (mouth opening, lips, tongue, hard palate, teeth), salivary glands, forestomach, omasum, abomasum, and hindgut differs among the three groups (Figure 2). The mouth opening of CS is large, allowing for sideways stripping of twigs or gnawing on fruit; whereas, that of GR is small, preventing grass losses during plucking. Concentrate selectors (IM) have lips with more serous labial glands than those of GR who have many more mucous labial glands. The lining of the mouth and tongue covering of CS is comprised of thinner less cornified stratified squamous epithelia while that of the GR is more cornified with fewer, more rigid buccal papillae. The tongue of CS has a short torus and a long free mobile portion with a soft tip important in selecting foliage. Grazers have a more cornified tongue with a long torus used to tear and shred grass. The hard palate of the CS is less cornified with a more extensively developed submucosal vascular layer (Hofmann, 1988). The incisors and cheek teeth of CS are more delicately built with sharper ridges and tubercles which are needed for puncturing seed coats; whereas, the teeth of GR are more adapted to the grinding of fibrous food.

The salivary glands of CS contain more saliva producing tissue than those of GR. Concentrate selectors have large parotid glands

(0.18 - 0.25% of body weight) and a larger seromucous sublingual gland. The glands produce mainly a serous secretion. The parotid glands of the GR are 1/4 to 1/5th of that of the CS (0.05 -0.07% of body weight) and they have a smaller seromucous sublingual gland. These glands produce predominantly a mucous secretion. Intermediate feeders are in between CS and GR with parotid glands that constitute 0.08 -0.15% of their body weight (Saber and Hofmann, 1984). Concentrate selectors have larger salivary glands and produce more saliva than GR, possibly as a result of a need for increased buffering due to high fermentation rates in the rumen. Increased production of saliva may have a diluting effect on the rumen, facilitating higher rates of passage and fluid turnover. Concentrate selectors have a denser, more evenly distributed rumen papillation. Increased surface area may allow increased absorption of VFA and less danger of ruminal acidosis. Grazers, on the other hand, may have smaller glands with less saliva production to maintain a steady state in their large reticulo-rumen with long food retention times and slow fluid turnover necessary for adequate fiber digestion. Also, excess saliva may help wash much of the plant cell contents down the ventricular or esophageal groove of CS, reducing their fermentation in the rumen. Another possibility is that more serous saliva production may be an adaptation to overcome plants' chemical defenses. Plants produce phenolic compounds that form insoluble complexes with protein (tanning effect). Excess serous saliva may bind the insoluble tannin-protein complex which can then be dissolved in the acidic environment of the abomasum (Provenza and Malechek, 1984).

Differences in the reticulo-rumen and omasum of these animals may explain why CS have higher rates of passage and fluid turnover rates than GR. The forestomach of CS is smaller in weight and capacity, with less subdivision and a larger reticulo-omasal orifice compared to GR. Even papillation, especially of the dorsal ruminal wall of CS, facilitates greater rates of absorption of VFA formed in larger quantities on a concentrate diet (Figure 3). The ruminal pillar musculature of CS is weak and the mucosal coat is papillated and less cornified (Figure 4). Ingesta is not stratified; short feeding and ruminating periods alternate frequently during several diurnal periods. Their reticulum is relatively larger with crests that are lower, less subdivided, and heavily studded with sharp cornified papillae. The reticulo-omasal orifice is more effectively barred by long clawlike papillae and is wider. The omasum is small with few laminae and long clawlike papillae.

Grazers have a ruminal pillar musculature that is thick and powerful. Extensive cornification may be necessary with ingestion of high fiber diets (Figure 4). The omasum of GR is capacious and extensively subdivided with larger surfaces on both sides of numerous omasal laminae of various sizes. The omasum of GR is an

important site of water and electrolyte absorption, which is facilitated by the large surface area of the many laminae.

The abomasum of CS is smaller with a mucosa 100% thicker than that of the GR. According to Axmacher (1987) the proportion of HCl producing cells is 20% of the mucosal tissue in all ruminants irrespective of feeding type. Consequently, CS have more parietal cells and more HCl per unit area. Concentrate selectors may need this increased amount of HCl to: 1) solubilize nutrients escaping ruminal fermentation via the ventricular or esophageal groove; 2) kill/disrupt ruminal micro-organism; 3) provide a suitable pH for pepsin activity; 4) dissolve Ca-P plant salts and complexes (abundant in foliage); 5) breakup tannin-protein complexes; and 6) macerate and breakdown hemicellulose bonds of dicot ingesta escaping ruminal fermentation (Ulyatt et al. 1975).

Digestion in the reticulo-rumen of CS is predominately amylolytic compared to that of GR which is predominately cellulolytic. The rate of cellulose digestion is lowest in CS irrespective of body weight (Boomker, 1981; Figure 5). As a result structural carbohydrates make up a higher proportion of the food that escapes ruminal degradation in CS and IM than in GR; thus, the distal fermentation chamber and colon must be more highly developed.

Two striking differences in the hindgut of CS and the GR are the ratio of small intestine length to large intestine length (CS 65-73:27-35 and in the GR 82:18-20) and the ratio of relative capacity of the wide cecocolon portion to that of the reticulo-rumen or distal fermentation chamber to proximate fermentation chamber (CS 1:6-10, IM 1:9-14, GR 1:15-30). It is apparent from these ratios that the major site of fiber digestion makes up a larger proportion of the digestive tract of each feeding type. The percentage of entire intestine length that is spiral colon decreases from CS to IM to GR. The CS have a spiral colon that is longer, allowing longer retention times for cellulolysis and providing sufficient surface and time for water and electrolyte absorption (the omasum of CS does not have as large a surface area for absorption as that of GR).

Longevity is important in zoos where the primary objective is to maintain exotic species for educational and breeding programs. It has been assumed that digestion in old animals may be compromised by the effects of age on dentition and metabolic rate, but quantitative data are limiting. Literature on the effect of age on domestic animal nutrition is scarce due to intense culling practices. For example, the average age of a dairy cow in the United States is 4 1/2 years. Available data is limited to comparing very young growing animals to mature animals of 2 years or older. Studies involving Merino sheep indicate that weaners have higher intakes relative to live weight than mature sheep at least 2 years of age across a wide range of diet digestibilities

(Arnold 1966, Egan and Doyle 1982). Differences in intake were greater between young and old on highly digestible diets than on diets of low digestibility. Greater consumption of a high quality diet by young sheep may indicate a reduced demand for energy by older sheep.

Selective feeding in the wild probably masks differences in wild ruminants' ability to digest plant cell walls. However, in a zoo these animals do not have access to the same selection of plant material. According to Prins et al. (1983), most ruminants in zoos are able to digest 50% or more of the plant cell fraction. Intermediate feeders and GR (including African buffalo, banteng, American bison, fallow deer and moose) are able to digest greater than 80% of the potentially available cell wall material. Concentrate selectors (including kudu and okapi) have low digestibility coefficients for plant cell walls. Foose (1982) has reported fiber digestibilities for African buffalo and eland to be 65% and 50% respectively.

Our experiment was designed to study the effects of aging on nutrient digestibility in banteng and African forest buffalo. The study also included common eland though no age comparison could be made within this species. Banteng can be found grazing in swamp forests with brush, bamboo jungles, light forests and grasslands with scattered woodland. The African forest buffalo prefers pastures of grasses such as Cynodon or stargrass and in the dry season swamp vegetation. Small herbaceous plants, Combietum woodlands and early flushes of grasses are the favored diet of the common eland during the wet season while they feed on pods of various acacia trees and tubers during the dry season (geographic locations figures 6 and 7).

## MATERIALS AND METHODS

Two adult male banteng (aged 6 and 10 years) a 17 year old female, two adult female African forest buffalo (aged 3 and 4 years) two old African forest buffalo (female aged 9 and a male aged 17), and an adult male common eland (age 9) were included in the study. All animals were fed a high protein low fiber zoo ration pellet labelled with Yb as a digestibility marker (Table 1). In addition, the banteng and African forest buffalo were fed timothy hay at forage to pellet ratios of 2.5:1 and 4:1. The common eland was fed timothy and alfalfa hay at a 1:1 ratio with a forage to pellet ratio of 2:1 (Tables 2 and 3). The experimental period was 10 days in length; 7 days adaptation and 3 days of fecal collection. Hay samples were taken periodically during the experimental period and pellet orts were collected. Orts and feces were sampled, frozen and later composited by animal. The samples were dried at 55Ĉ and ground through a 2 mm screen in a Wiley mill. They were analyzed for DM, N, and ether extract (EE) (Association of Official Analytical Chemists, 1975); and neutral

detergent fiber (NDF) and acid detergent fiber (ADF) (Goering and Van Soest, 1970). Feed and feces were analyzed for Yb (Ellis and Lascano, 1982) using an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, Norwalk, CT). Prior to reading on the atomic absorption spectrophotometer Yb was leached from the ashed residue of the feed and fecal samples with a mixture of 1 N nitric acid and 1 N hydrochloric acid. Interfering elements in the fecal matrix were addressed by spiking samples with 0, 1, 2, 4, and 8 ppm Yb (Aldrich Chemical Company, Inc. Milwaukee WI).

RESULTS

Dry matter intakes varied little during the study. While dry matter digestibilities did not differ between young and old banteng they were higher for the young buffalo. In addition, NDF, ADF, and EE digestibilities all were higher for the younger animals of both species. Crude protein digestibilities were higher for the older animals of both species (Table 4). The NDF digestibilities correspond well with those reported earlier by Foose (1982) for animals consuming similar diets. Lower fiber digestibilities for the common eland compared to the banteng and African forest buffalo, may be explained by their classification into different feeding types. The common eland can be classified as an IM whereas banteng and African forest buffalo can be classified as GR which have more cellulolytic digestion than the IM (Boomker, 1981).

Body weights of any of the animals could not be measured; however, known weight ranges are: African forest buffalo, 270 - 320 kg; banteng, 400 - 800 kg; eland, 400 - 942 kg (Hall and Underwood, 1984). Also, due to limited sample size, assessing differences between young and old groups by statistical analysis was not possible.

DISCUSSION

Ruminants can be grouped into CS, IM, or GR based on their digestive tract morphology. Nutritionists can use these differences in digestive tract morphology to formulate appropriate diets in captivity. The digestibility study conducted at Brookfield Zoo has shown a small numerical trend towards decreased fiber and fat digestibility and an increased crude protein digestibility in older animals. However, again due to the small sample size preventing statistical analysis, more studies need to be conducted to determine the significance of these results. Selective feeding was not evident in this study since all animals were fed to minimize refusals. This was necessary because behavior patterns prevented orts collection.

It is important to keep in mind the possibility of interfering elements in the fecal matrix when using Yb as a digestibility marker. Problems with absorption readings can be addressed by either collecting feces before the marker is fed to used to develop

standard curves or by spiking samples with known amounts of Yb as was done in this study (Hatfield et al., 1990).

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Table 1. Herbivore pellet

| Ingredient | Percentage by weight |
|---|---|
| alfalfa meal, dehydrated | 31.9 |
| wheat middlings | 29.8 |
| corn grain | 19.0 |
| soybean meal | 11.3 |
| cane molasses | 5.0 |
| soybean oil | 1.0 |
| mono-dical phosphate | 1.0 |
| salt | 0.6 |
| mineral premix | 0.1 |
| vitamin premix | 0.2 |
| Mold-Chek (or equivalent) | 0.1 |
| | 100.0 |

Table 2. Nutrient composition of pellet and hays

| Ingredient | Pellet | Timothy hay | Alfalfa hay |
|---|---|---|---|
| Dry matter % | 93.02 | 91.72 | 91.13 |
| Crude protein % | 20.83 | 10.07 | 23.65 |
| Neutral detergent fiber % | 34.95 | 68.31 | 47.86 |
| Acid detergent fiber % | 18.15 | 41.88 | 36.21 |
| Ether extract % | 4.9 | 2.91 | 3.25 |

Table 3. Nutrient composition of final diets

| Ingredient | Banteng | African forest buffalo | Eland |
|---|---|---|---|
| Dry matter % | 92.50 | 92.39 | 91.72 |
| Crude protein % | 13.30 | 12.55 | 19.12 |
| Neutral detergent fiber % | 57.08 | 61.50 | 50.03 |
| Acid detergent fiber % | 35.63 | 36.08 | 31.92 |
| Ether extract | 3.55 | 3.35 | 3.68 |

Table 4. Dry matter intake and total tract nutrient digestibility

| | Species | | | | | | |
|---|---|---|---|---|---|---|---|
| | Banteng | | | African forest buffalo | | | Eland |
| | Old | Young | | Old | | Young | Young |
| | 1 | 2 | SD[a] | 2 | SD | 1 pair | 1 |
| Item | | | | | | | |
| Dry matter intake kg/d | 3.90 | 9.53 | 0.92 | 7.63 | 1.59 | 7.61 | 9.2 |
| Digestibility percentage | | | | | | | |
| Dry matter | 63.74 | 63.70 | 5.81 | 64.27 | 2.08 | 66.57 | 68.22 |
| Crude protein | 71.90 | 69.98 | 7.93 | 70.36 | 1.87 | 66.57 | 77.56 |
| NDF | 55.99 | 57.01 | 6.95 | 59.37 | 1.20 | 64.15 | 56.64 |
| ADF | 51.84 | 52.76 | 4.82 | 52.19 | 4.49 | 54.25 | 52.22 |
| EE | 57.76 | 68.05 | 7.47 | 69.95 | 0.35 | 72.72 | 74.74 |

[a]Sample standard deviation

Figure 1 African ruminant feeding types (Hofmann, 1973)

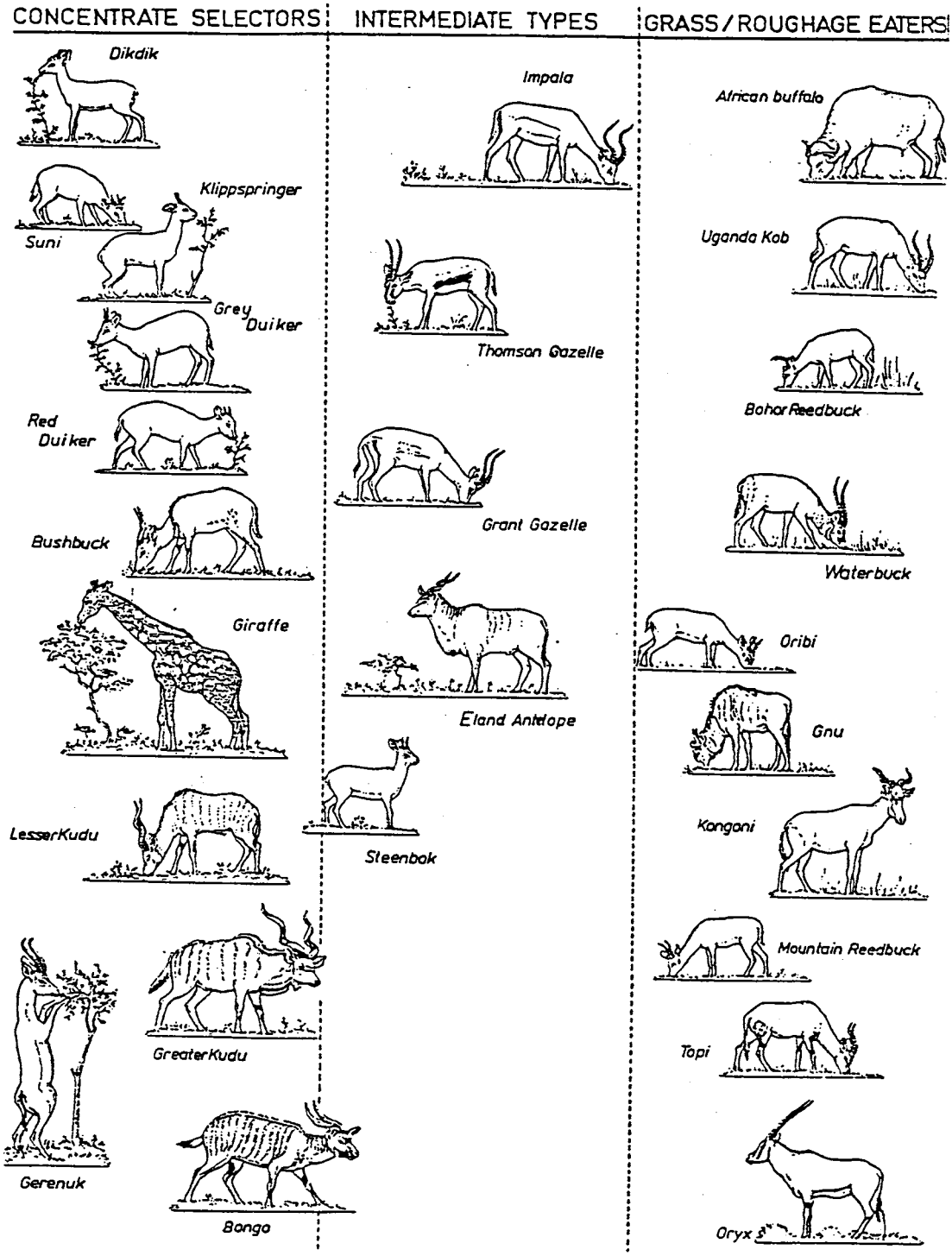


Figure 2 Morphophysiological characteristics common to all ruminants belonging to concentrate selectors and grazers (Hofmann, 1985)

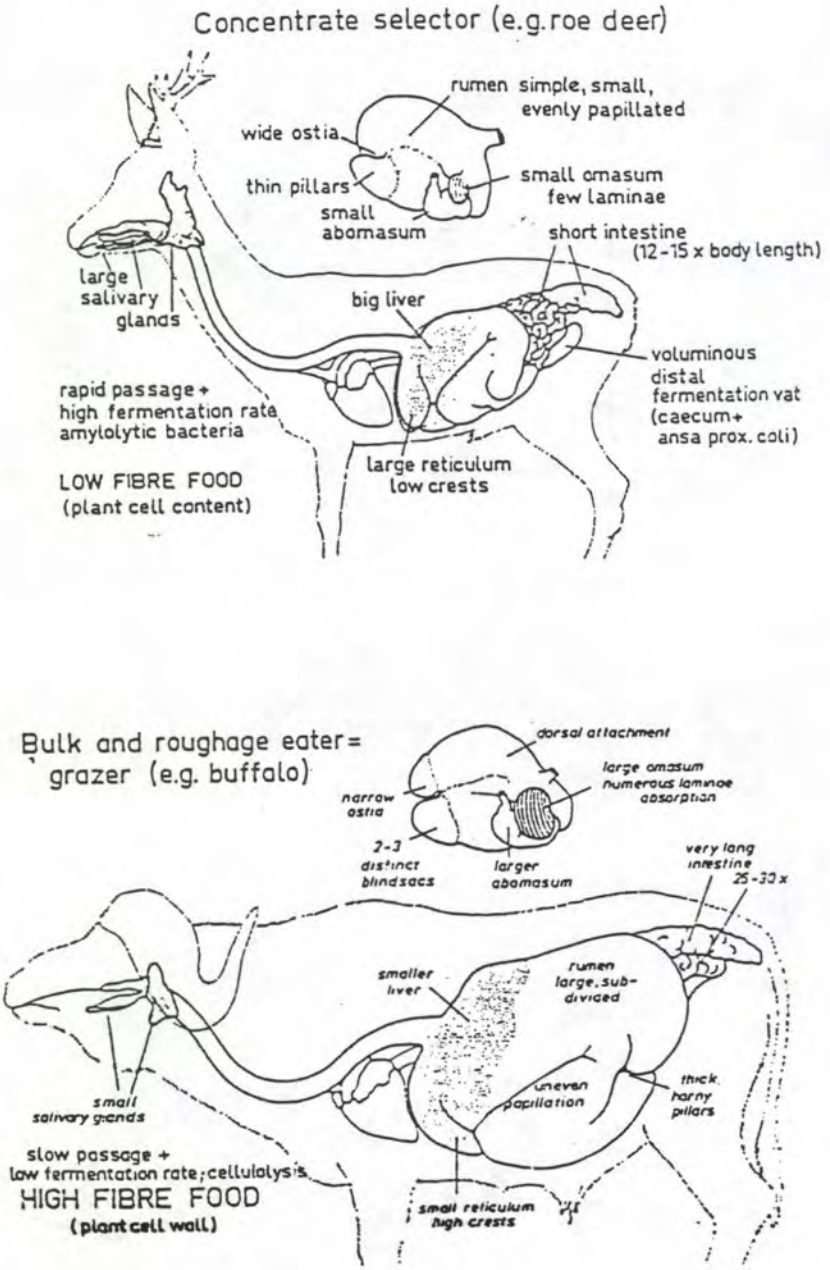


Figure 3 Center of dorsal wall of rumen (Hofmann, 1982)

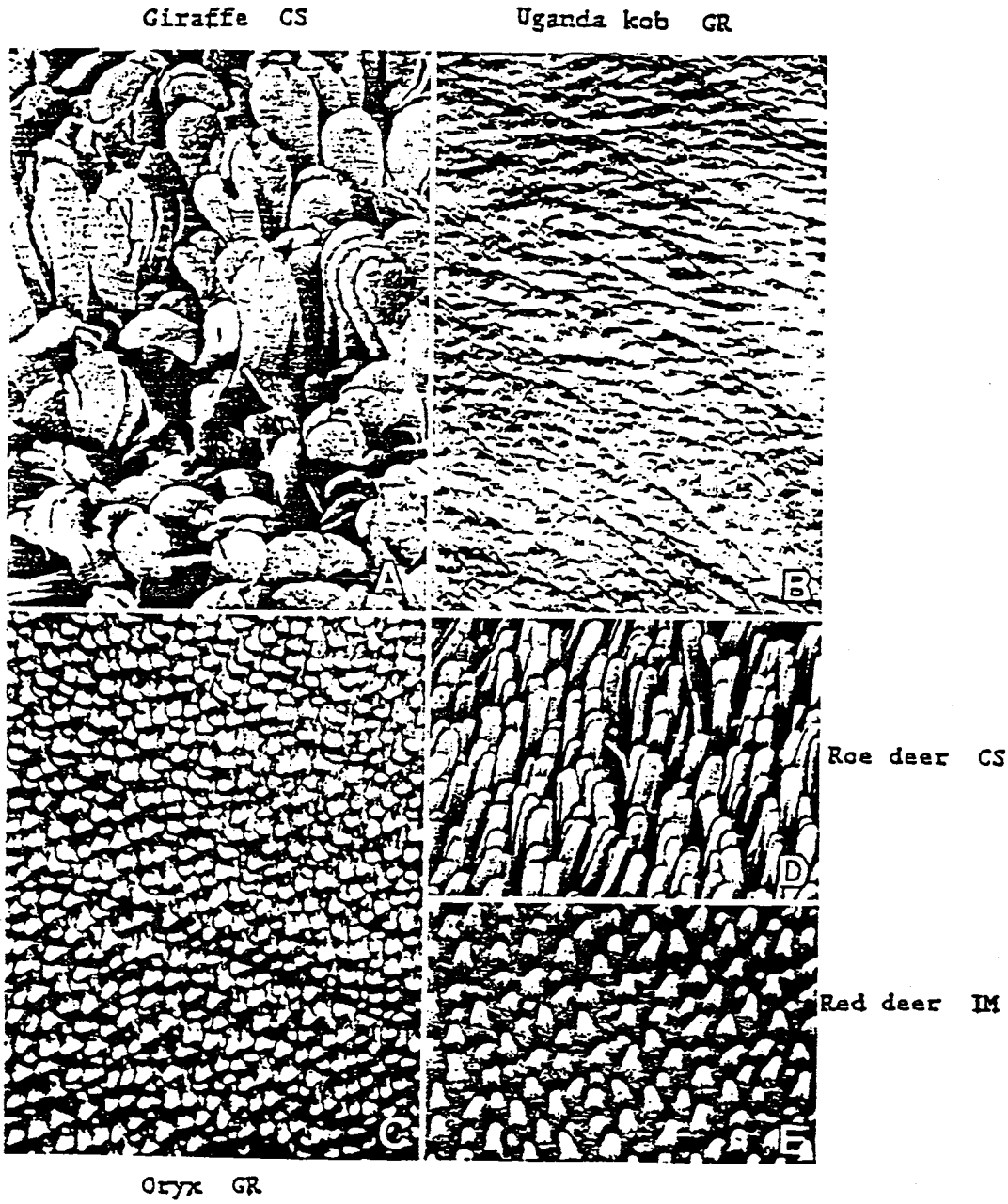
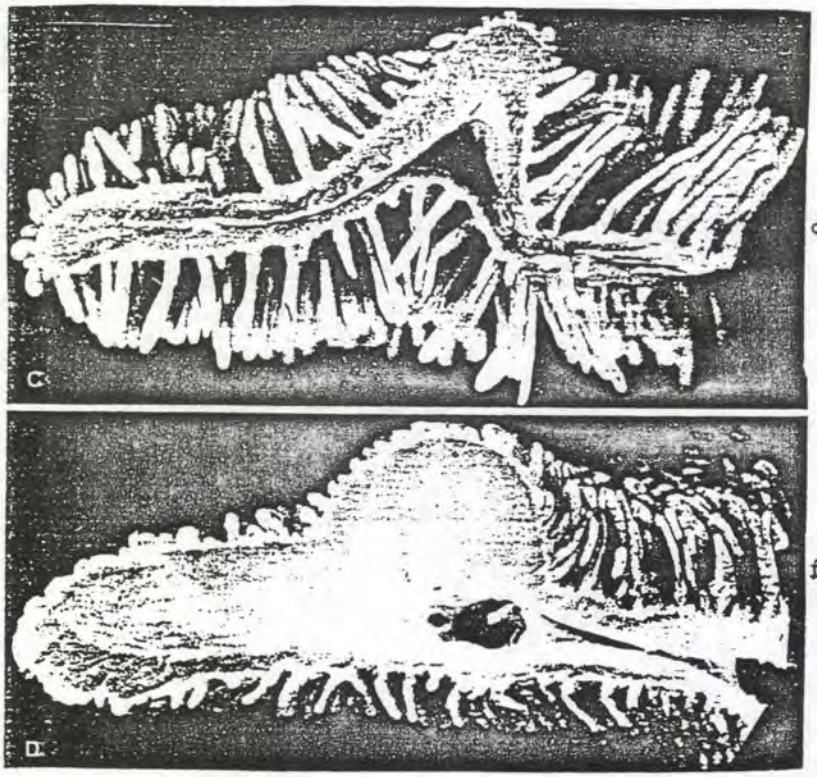


Figure 4 Muscle fiber development of the internal oblique (Hofmann, 1988)

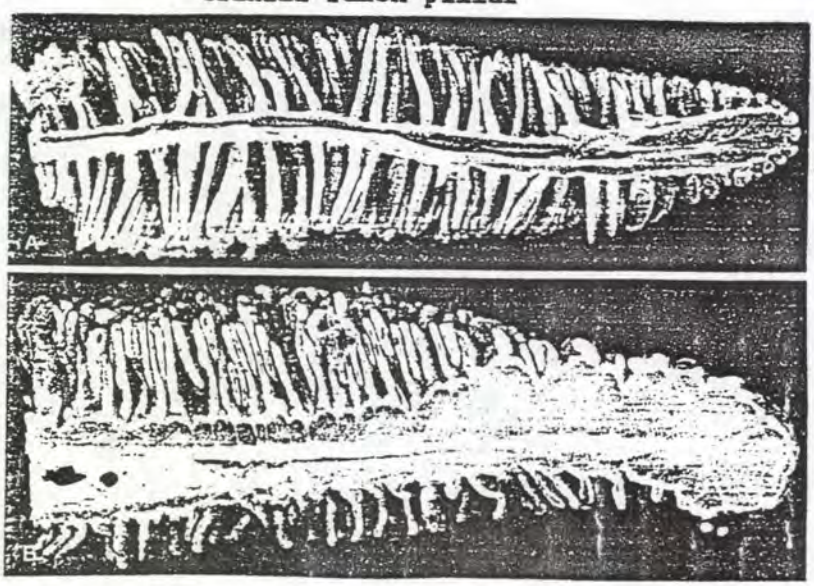
caudal ruminal pillar



duikar CS

fat-tailed sheep GR

cranial rumen pillar



duiker CS

fat-tailed sheep GR

Figure 5 Rate of in vitro cellulolysis obtained by incubating rumen fluid inocula from various ruminants with excess substrate (ground filter paper) for 20h (Boomker, 1981)

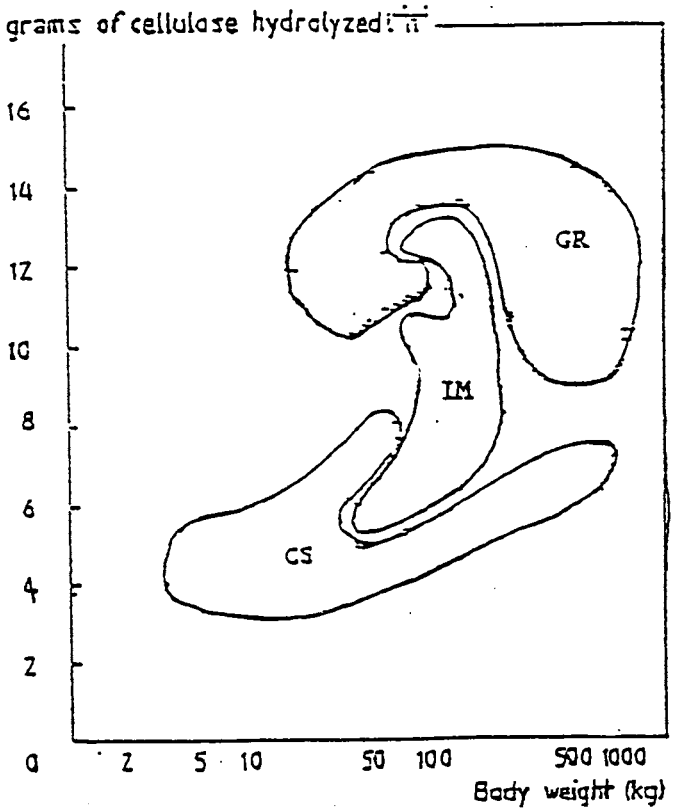
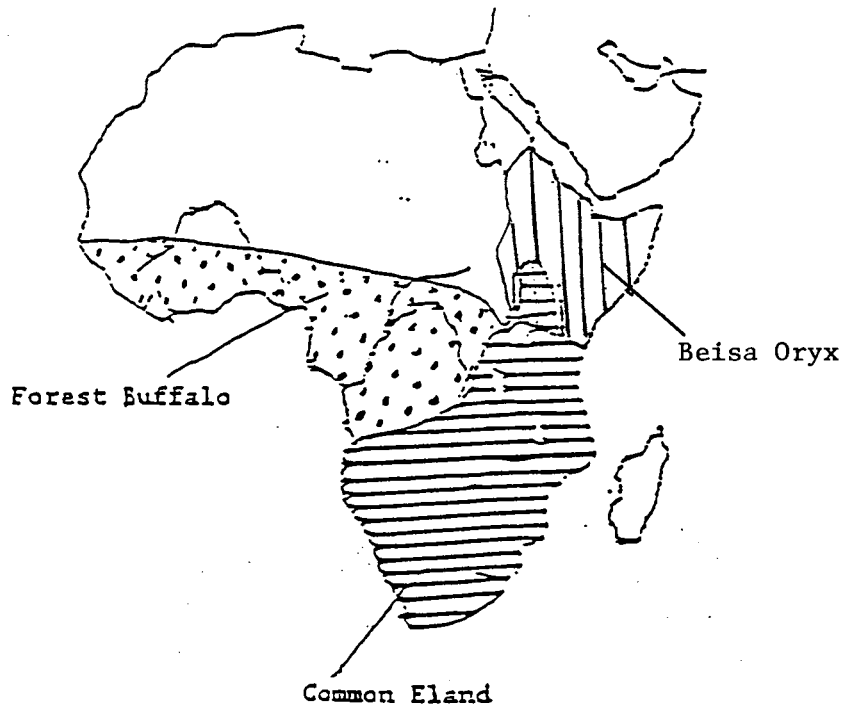


Figure 6 Geographic location of banteng (Lekagul and McNeely, 1977)

Figure 7 Geographic location of African forest buffalo and common eland (Kingdon, 1982)



# THE ROLE OF FIBER IN NATURAL AND MANUFACTURED DIETS FED TO RED HOWLER MONKEYS (ALOUATTA SENICULUS)

Susan D. Crissey, Ph.D.,
Chicago Zoological Society,
Brookfield Zoo, Brookfield IL 60513.

Mark S. Edwards,
National Zoological Park,
Washington, D.C. 20008
and Michigan State University,
E. Lansing, MI 48824

Olav T. Oftedal, Ph.D.,
Julie A. Currier,
R. Rudran, Ph.D.,
National Zoological Park,
Washington, D.C. 20008

This project was sponsored by the Smithsonian Institution, IESP.

## Objective

When establishing captive diets for red howler monkeys, the composition of the animal's natural diet may be of particular importance. The captive diet should not only include the quantities of nutrients which meet the animal's probable nutrient requirements but, also, should try to reflect the gross nutrient composition of the natural diet.

A series of studies designed to investigate a) the role of fiber in the natural diet of the red howler monkey, b) the red howler's digestive morphology, and c) the possible effect of dietary fiber on food passage rates are ongoing. Preliminary results are presented in this paper.

## Fiber as a Dietary Component

There is currently no known requirement for fiber in the diets of primates. However, the presence of fiber in the diet of the red howler monkey may be very important. Dietary fiber influences a number of factors which include food passage rate and digestibility of nutrients (Cummings, 1978).

Dietary fiber, in general, can be thought of as a group of food constituents that are resistant to digestion by animal enzymes. Some types of fiber can be fermented by microorganisms in the GI-tract of animals. Degradation will vary with fiber's composition and physical properties (Akin, 1989). Fermentation may enable the animal to utilize fiber by utilizing some of the fermentation products such as volatile fatty acids. Generally, as a plant ages, lignification and other factors promote the development of a non-fermentable fraction. (Van Soest, 1978). The extent of fiber fermentation depends on the GI-tract microbial population, which in turn is dependant on the retention time of

food particles in the GI-tract. The activities and products of the fermentation are influenced by the composition of the microbial population. These microbial species are influenced by the substrates available for fermentation and their retention with digesta in the GI tract. The fermentation may be limited by rapid passage which would increase the loss of microbes and limit the time for significant degradation of the fiber. The capacity of the GI tract also may influence passage rate with an increased capacity providing decreased passage rate.

Dietary fiber can be measured by a number of methods. Constituents measured in the detergent fiber determination include: Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin. Generally, NDF includes cellulose, hemicellulose, and lignin, where as ADF includes only cellulose and lignin (Bailey et al., 1978). Thus hemicellulose may be calculated as NDF minus ADF, and cellulose is ADF minus lignin.

The Role of Fiber in the Natural Diet of the Red Howler Monkey

In August, 1987, a field study was initiated in the central llanos of Venezuela. One of the objectives of this feeding ecology study was to determine the feeding habits of free-ranging howlers by direct observation. Additionally, samples of the plants/plant parts which howlers used as food items were collected and analyzed for nutrient composition. Focal animal observations of feeding duration with two howler social groups were conducted monthly for a period of 1 year. Each animal in a group was observed for a total of 12 hours per month. The contribution of the fiber component to the red howler diet was determine.

Observations showed that, of the time red howlers spent feeding, about 46% was spent consuming mature leaves while only 2.79% was spent with young leaves and 4.53% with new leaves (Table 1). Overall, leaves comprised 53.3% of the diet as calculated by time spent feeding. In addition, fruit comprised 28.32%, flowers 16.29% and pulvinus (swelling at leaf base) 2.1%.

Mean concentration (percent of dry matter) of the fiber component of items selected for food by the howlers are presented in Table 2. Fiber components reported are: neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and lignin. It can be seen that the food plants consumed by red howler monkeys have a wide range of fiber levels. However, when the fiber content of the food items are weighted by the time spent feeding on an individual item, the data are much more meaningful. Table 3 shows the fiber concentrations of the red howler diet as calculated by weighted means. These levels of fiber in the diet appear high when compared to fiber components of some common feedstuffs (National Academy Sciences, 1971) (Table 4). Thus theoretically, in order to achieve gross fiber levels of the wild diet in a captive red howler diet, one would consider feeding alfalfa hay.

The Red Howler's Digestive Morphology

We have established that the red howler is highly folivorous and the fiber components of its diet are relatively high. To

formulate an appropriate captive diet for the red howler, it is important to examine both the fiber components in the natural diet and gastro-intestinal tract morphology.

The gastro-intestinal tracts of two adult (1.1) red howler monkeys were opportunistically collected from presumably healthy animals struck by automobiles. The Gi-tracts were measured and fill capacity was determined by filling the tract with water expanded to the extent of "normal" gut fill. Normal expansion was determined from gut measurements taken before gut was emptied.

Digestive tract measurements of red howlers are compared (Table 5) to data collected on mantled howlers and black-handed spider monkeys by Milton (1981) according to length by width. These data show some similarities. When compared to the somewhat larger animals, red howlers appear to have an intermediate sized stomach, slightly larger small intestines, a smaller cecum and larger large intestines.

A comparison of the gut fill capacity of some domestic species (Maynard et al., 1979) to the red howler (Table 6) shows that, as a percentage of body weight, the red howler has a) a stomach capacity near that of the pig; b) a small intestine capacity similar to the human and pig; c) a cecum capacity like to the horse; d) a colon capacity similar to the pig; and e) a total gut capacity less than the horse yet larger than the pig. When the capacity of the cecum alone (2.7 as a % of body weight) is compared to a number of small mammals (Robbins, 1983), the red howler appears similar to the beaver (2.1%), rabbit (1.8 - 2.5%) and rat (1.8 - 2.4%).

These comparisons show that not only is the red howler a "behavioral folivore" (Milton, 1981) but it is also an "anatomical folivore" (Milton, 1981).

The Possible Effect of Dietary Fiber on Digesta Passage Rates

The purpose of these trials was to examine digesta passage in red howler monkeys when fed diets of varying levels of fiber. For this portion of the project, one of the groups observed as free-ranging was brought into captivity and successfully maintained for two years. Passage rates were determined by feeding plastic beads as markers in either banana or soaked monkey biscuit and determining marker appearance in the feces.

Passage rate studies were begun with the adult male, the sub-adult male and an individual (captive pet) sub-adult female. Six trials were conducted: three using leaf-eater monkey biscuit (11% ADF) (Trial 1) and 3 using ficus leaves (Trial 2). Each was used as a sole dietary item. The animals were fed ad libitum on these diets for the duration of each trial. Results from Trials 1 and 2 showed a remarkably delayed time of first marker appearance (19 - 24 hours). See Tables 7 and 8. Times of last appearance, or over 95% marker recovery, were as long as 9.6. days.

Milton (1981) determined time of first appearance for mantled howlers and spider monkeys. Her howler data appear to be in agreement with the red howler first appearance data (Table 9). However, Milton estimated that the diet took as long as 72 hours to

pass through the GI-tract of the mantled howler. From these studies, it was determined that 50% of the markers were passed 54 hours and last appearance occurred closer to 134 hours for the red howler.

In Conclusion

The red howler has evolved as both an anatomical and behavioral folivore. As such, it consumes a large portion of its diet as leaves. The majority of diet studies with howler monkeys (Alouatta sp.) have been with mantled howlers (A. villosa) (Milton, 1981, Smith, 1977, Altmann, 1959, and Carpenter, 1934). It has been observed that they, too, consume the majority of their diet as leaves (Smith, 1977).

Milton (1978) concluded that mantled howlers consume leaves of high nutritional quality with less than 5% of feeding time spent on mature leaves. In contrast, the majority of leaves consumed by red howlers are mature leaves containing relatively high levels of fiber. Milton also concluded that mantled howlers were "behavioral folivores" and not "anatomical folivores." It appears from this study that red howlers are both anatomical and behavioral folivores, consuming highly fibrous diets and possessing increased digestive capacity of the lower gut, not unlike the horse.

Passage rate data suggest that there are similarities between red howlers and mantled howlers. It appears that red howlers may process high fiber levels in the diet via a relatively slow digesta passage rate.

Further investigation into the effects of fiber on passage rate and digestibility in the red howler is needed. Available data on natural feeding behavior, digestive morphology and digesta passage times suggest that the red howler may be well adapted to a high fiber diet. This data may have implications for diets fed to captive red howlers and to folivorous primates in general.

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TABLE 1. PERCENT DISTRIBUTION OF TIME SPENT FEEDING -- RED HOWLER MONKEYS

| PLANT PART | % TIME |
|---|---|
| Leaves - mature | 45.98 |
| Leaves - young | 2.79 |
| Leaves - new | 4.53 |
| Flowers | 14.65 |
| Flower buds | 1.64 |
| Fruit - unripe | 12.68 |
| Fruit - ripe | 15.65 |
| Pulvinus | 2.10 |

TABLE 2. RED HOWLER MONKEY FOOD PLANT FIBER CONCENTRATIONS[1]

| FIBER CONTENT | RANGE | MEAN | SD |
|---|---|---|---|
| ADF | 8.2 - 55.5 | 37.72 | 11.78 |
| AL | 1.6 - 40.0 | 19.07 | 8.96 |
| CELLULOSE | 5.0 - 30.2 | 17.72 | 5.30 |
| NDF | 5.8 - 74.1 | 55.91 | 10.80 |

[1]All values reported as percent on a dry matter basis

ADF = Acid Detergent Fiber

AL = Acid Lignin

NDF = Neutral Detergent Fiber

TABLE 3. WEIGHTED MEAN FIBER CONCENTRATIONS[1]

| ANNUAL AVERAGE | |
|---|---|
| | WEIGHTED MEAN |
| ADF | 37.6 |
| AL | 18.2 |
| CELLULOSE | 18.0 |
| NDF | 56.9 |

[1]Percentages reported as percent on a Dry Matter Basis

TABLE 4. FOODSTUFFS AND FOOD PLANT FIBER CONCENTRATIONS[1]

| | ALFALFA HAY [2] | PEANUT HULLS [2] | PRIMATE BISCUIT [3] | ANNUAL MEAN |
|---|---|---|---|---|
| ADF | 40.0 | 59.0 | 4.9 | 37.55 |
| AL | 13.0 | 21.0 | 1.3 | 18.20 |
| CELLULOSE | 26.0 | 36.0 | 3.6 | 18.00 |
| NDF | 51.0 | 67.0 | 14.3 | 56.90 |

[1]Percentages reported as percent on a Dry Matter Basis

[2]National Academy Science, 1971

[3]As analyzed by Michigan State University Comparative Nutrition Laboratory

TABLE 5. DIGESTIVE TRACT MEASUREMENTS OF HOWLERS AND SPIDER MONKEYS

| SPECIES | STOMACH L / W | SMALL INTESTINE L / W | CECUM L / W | LARGE INTESTINE L / W |
|---|---|---|---|---|
| Mantled Howler* (adult - wt. 7-9 Kg) | 21/9 | 126/2 | 18/6.5 | 43/3.5 |
| Black-handed Spider* (adult - wt. 7-9 Kg) | 15/9 | 90-120/1.5 | 22/6.5 | 33/1.5 |
| Red Howler (adult - wt. 5-7 Kg) | 19.5/9 | 117/2.5 | 11.5/8 | 66/3 |

L = length in CM

W = width in CM

*from Milton, 1981

TABLE 6. ESTIMATED CAPACITY OF DIGESTIVE TRACT OF VARIOUS SPECIES AS A PERCENTAGE OF BODY WEIGHT*

| SPECIES | TOTAL STOMACH | SMALL INTESTINE | CECUM | LARGE INTESTINE | TOTAL |
|---|---|---|---|---|---|
| HUMAN | 1 | 5 | -- | 1 | 7 |
| PIG | 4 | 5 | 0.5 | 5 | 14.5 |
| HORSE | 2 | 6 | 3 | 9 | 20 |
| SHEEP | 25 | 7 | 1 | 4 | 37 |
| RED HOWLER | 5 | 5 | 3 | 5 | 18 |

*Calculated from Maynard & Loosli, 1979, Animal Nutrition

TABLE 7. MEAN MARKER RECOVERIES FROM RED HOWLER MONKEYS FED *FICUS PERTUS* LEAVES (ADF 38%) OR COMMERCIAL MONKEY BISCUIT ADF-11 IN HOURS

| | FIRST APPEARANCE | | 50% RECOVERY | | TOTAL* RECOVERY | |
|---|---|---|---|---|---|---|
| | FICUS | BISCUIT | FICUS | BISCUIT | FICUS | BISCUIT |
| CHRIS | 20 | 19 | 45 | 60 | >160 | 140 |
| MORI | 23 | 20 | 63 | 38 | 162 | 101 |
| ROMEO | 20 | 23 | 125 | 63 | >230 | 162 |
| X | 21 | 21 | 78 | 54 | >184 | 134 |

*Over 90% of the markers recovered.

TABLE 8. MEAN MARKER RECOVERIES FROM RED HOWLER MONKEYS[1], MANTLED HOWLER MONKEYS AND SPIDER MONKEYS[2] IN HOURS

| | FIRST APPEARANCE | 50% RECOVERY | TOTAL RECOVERY |
|---|---|---|---|
| RED HOWLERS | 21 | 54 | 134 |
| MANTLED HOWLERS | 20.4 | * | * |
| SPIDER MONKEYS | 4.4 | * | ** |

[1]RED HOWLER MARKER RECOVERIES FED 11% ADF BISCUITS

[2]MILTON, K. 1981. AM. NAT. VOL. 117, PP. 496-505.

*MANTLED HOWLERS: ESTIMATE BULK OF DIET THROUGH IN 30 HOURS; AS LONG AS 72 HOURS.

**SPIDER MONKEYS: ESTIMATE BULK OF DIET THROUGH IN 8 HOURS; AS LONG AS 24 HOURS.

# THE RED PANDA SSP DIET EVALUATION PROJECT

K. Fulton[1], M. Roberts[2], M. Allen[3], D. Baer[3], O. Oftedal[2] and S. Crissey[4]

[1]The Baltimore Zoo, Baltimore, MD 21217
[2]The National Zoological Park, Washington, D.C. 20008
[3]Allen and Baer Associates, Inc., Silver Spring, MD
[4]The Brookfield Zoo, Brookfield, IL 60513

## INTRODUCTION

Red pandas (Ailurus fulgens), native to the deciduous and bamboo forests of Nepal, have adapted to a unique ecological niche. These animals are almost exclusively herbivorous, feeding primarily on bamboo (Yonzon and Hunter, 1989) yet possess a straight carnivore gut, completely lacking any type of fermentation vat such as a caecum. The red panda's ability to cope with fibrous plant materials as a sole food source, while lacking a digestive system able to process these materials, makes the red panda a unique animal to manage in captivity and an interesting subject for nutritional research.

Diets commonly fed in captivity include bamboo in limited quantities with an equal, if not larger, percentage of the diet consisting of gruel. Gruel composition varies among zoos with variability in ingredients and quantities of ingredients at 10 surveyed institutions. There were concerns as to the variation in nutrient composition and the possible ramifications especially with respect to reproduction. These concerns were discussed at the red panda Species Survival Plan (SSP) meeting at the American Association of Zoological Parks and Aquariums convention in September of 1987. The red panda SSP diet evaluation project was initiated in 1987 with the agreement of SSP member institutions to gather information on diets consumed by red pandas in their collection.

## OBJECTIVES

I. The short-term objectives of this project were to identify diets consumed by red pandas in 16 zoological institutions, establish target dietary nutrient levels, and reformulate diets to meet the established nutrient levels.

II. The long-term objective of the project was to eventually convert all red pandas in the North American SSP to a nutritionally complete, commercially available feed, which meets the established nutrient levels for red panda diets. In addition, digestion and transit time studies were conducted on red pandas consuming this commercially available feed item.

## METHODS

I. Each institutions was mailed data collection forms and protocols. Food intake by each animal was measured for a minimum of three days. Samples of food items with unknown nutrient content, including bamboo, were collected at all participating zoos. Proximate analyses of these food items and computer analysis

of the diets was performed.

Information used to establish target nutrient levels included the natural history of the red panda, wild feeding behavior, dietary requirements of animals of similar body size, gut morphology, food preference, and requirements of domestic animals such as the dog and pig. Diets were then reformulated to bring the nutrient intakes in line with the established target nutrient levels.

Past studies with the red panda indicated that mixing a fiber source (such as alfalfa meal) in to gruel may decrease palatability (Warnell, 1989). The fiber product chosen to be incorporated into the gruel was Marion Zoological's Primate Leafeater Apple Fiber Biscuit (AFB) (Table 1). This product was palatable to a variety of species, contained adequate nutrient levels (including fiber) and was commercially available to all regions of the country.

Table 1. Marion Zoological's primate leaf eater diet with apple fiber included, high fiber monkey biscuit.

| Guaranteed Analysis: | |
|---|---|
| Crude Protein, min. | 23.0% |
| Crude Fat, min. | 4.5% |
| ADF, max. | 16.0% |
| ADF, min. | 13.0% |
| Ash, max. | 5.0% |

In April of 1989, a digestion and transit time study was conducted using 2.2 animals that had been fed only the apple fiber biscuit (AFB). Its purpose was to determine the extent to which the red panda was able to digest the apple fiber biscuit. Data was available from other studies (Warnell, 1988) to compare the digestibilities of bamboo and gruel to the apple fiber biscuits.

RESULTS and DISCUSSION

I. Previous research (Warnell, 1989) indicated that 10% ADF (acid detergent fiber) was essential for proper stool formation. Because there is little digesta mixing in the gut of the red panda it was important to include fiber in the diet. Without this level as a minimum, tails may frequently get matted with feces. One of the our major concerns in reformulation was adequate fiber intake. From survey of feeding information, it was apparent that some zoos had unlimited supplies of bamboo while others had access to little or none.

Recognizing the empirical establishment of physiological nutrient requirements of red pandas would involve a very detailed study with many animals, it was not deemed practical to attempt such a study at this time. It is also important to note that the intake information accumulated by Allen and Baer provided important baseline data on the nutrient tolerances already present for these animals. Table 2 lists the target nutrient levels established for red pandas.

Table 2. Target nutrient levels for red pandas

| Nutrient | Recommended minimum level (dry basis) |
|---|---|
| Crude protein | 18% |
| Ether extract | 5% |
| Acid detergent fiber (ADF) | 10% |
| Calcium (Ca) | 0.75% |
| Phosphorous (P) | 0.60% |
| Sodium (Na) | 0.15% |
| Potassium (K) | 0.65% |
| Magnesium (Mg) | 0.10% |
| Iron (Fe) | 100 ppm |
| Copper (Cu) | 8 ppm |
| Manganese (Mn) | 40 ppm |
| Selenium (Se) | 0.18 ppm |
| Zinc (Zn) | 50 ppm |
| Thiamin | 2.5 ppm |
| Riboflavin | 5.0 ppm |
| Vitamin $B_6$ | 2.0 ppm |
| Vitamin $B_{12}$ | 30 ppb |
| Niacin | 30 ppm |
| Folate | 600 ppb |
| Biotin | 100 ppb |
| Choline | 1250 ppm |
| Pantothenate | 15 ppm |
| Vitamin A | 8000 IU/kg |
| Vitamin E | 220 IU/kg |
| Vitamin D | 800 IU/kg |
| Linoleic acid | 1.0% |

Some of the major changes in the proposed reformulations were decreasing the fat levels in the gruel portion of the diet, increasing the fiber levels and decreasing the amounts of fruits and vegetables offered.

II. In order to test the possibility of converting animals to an all biscuit diet three institutions (with a total of 5 males and 4 females) agreed to attempt a conversion of their red pandas to the biscuit diet. The product proved palatable and the conversions went well, therefore this feed appeared to be appropriate as a sole food source. The diet revisions provided to the participating institutions included the AFB as a portion of the gruel. Each institution was provided with their nutrient analyses and a written summary of excesses and or deficiencies of their diet. They also received step by step suggestions for the proposed dietary modifications. Tables 1-10 in appendix A summarize results.

The dry matter, crude protein and gross energy digestibility values of the AFB (Table 3) were intermediate to the gruel and bamboo trials. To maintain condition, red pandas would need to consume 30% more AFB (on a dry matter basis) than if it fed on gruel exclusively. Likewise, animals consuming only bamboo would need to consume 31% more bamboo than an animal fed only AFB.

Transit times (TT) of the AFB (6.7 hr) were intermediate to the bamboo (5.0 hr) and gruel (9.4 hr) (Table 4). Presumably, the more rapid the passage rate the lower the digestibility values. This observation is consistent with the digestibility values reported for this trial. Total mean retention times (TMRT) were longest for the AFB trial. This result may be due in part to individual animal variation or differences in collecting procedures.

Table 3. Digestibility comparisons of three diets fed to red pandas.

| Diet | Apparent digestibilities (%) | | |
|---|---|---|---|
| | DM[2] | CP[3] | GE[4] |
| Gruel[1] (n=5) | 84.7 | 83.5 | 87.0 |
| AFB (n=4) | 54.7 | 72.0 | 58.3 |
| Bamboo[1] (n=4) | 24.0 | 50.4 | 30.3 |

[1]Data from Warnell, 1988.
[2]Dry matter basis
[3]Crude protein
[4]Gross energy

Table 4. Comparison of transit times (TT) and total mean retention times (TMRT) of three diets fed to red pandas.

| Diet | TT | TMRT |
|---|---|---|
| Bamboo[1] | 5.0 hr | 5.7 hr |
| AFB | 6.7 hr | 17.4 hr |
| Gruel[1] | 9.4 hr | 9.7 hr |

[1]Data from Warnell, 1988.

Based on these digestibility data, proximate analyses of the apple fiber biscuit, and the criteria for selection of target nutrient levels, the use of the apple fiber biscuit as a complete feed for red pandas appears to be an appropriate recommendation. It had also been shown that red pandas could be adapted to consuming a dry biscuit. If a complete conversion to a standardized, nutritionally complete dry diet could be accomplished, the potential error that comes with mixing several different ingredients (as in gruel) would be eliminated. In addition, if all zoos fed a standard diet, animals that were transferred between

zoos would no longer have to adjust to a new diet. A standard diet would also limit the potential problems caused by low availability of bamboo at some institutions. Finally, this conversion to a standard diet would provide a standard baseline from which other management considerations could be evaluated.

It is critical to note that each participating institution was encouraged to monitor their animals very closely. This included obtaining regular weights from all of their animals, preferably at least one time a week, as well as accurately measuring food intake.

## CONCLUSION

This Red Panda SSP nutrition project was probably one of, if not the most comprehensive nutrition projects undertaken by an SSP. The project analyzed and reformulated the total diets of red pandas in 15 institutions. Nutrition is only one component in the management success of propagating red pandas. The success of this program, in terms of reproduction is still left to be objectively evaluated. In many instances, improvement in the level of nutrients provided in the diets will be the first step toward that goal of improved reproduction. Hopefully, this project might serve as a model for other institutions to systematically gather data, upon which to base important nutrition and management decisions.

## ACKNOWLEDGEMENTS

We would like to extend our thanks to all institutions participating in this project, especially the Knoxville Zoo for allowing us to perform digestion and transit time studies with their animals.

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## APPENDIX A

Table 1. Dry matter, crude protein, and fiber composition (% of dry matter) of bamboo.

| Zoo | Dry matter[1] | Crude protein | CWC[2] | LC[3] |
|---|---|---|---|---|
| Baltimore Zoo | 53.8 | 15.5 | 63.7 | 38.4 |
| Bronx | NA | 13.5 | 65.6 | NA |
| Buffalo Zoo | NF | NF | NF | NF |
| Calgary Zoo | 54.9 | 15.0 | 42.1 | 38.1 |
| Columbus Zoo | 57.0 | 17.5 | 58.3 | 38.6 |
| Dallas Zoo | 56.8 | 18.7 | 63.9 | 37.1 |
| Kansas City Zoo | 43.4 | 15.1 | 67.1 | 39.4 |
| Knoxville Zoo | 37.8 | 17.0 | 69.2 | 35.9 |
| Los Angeles Zoo | 45.4 | 15.0 | 72.2 | 31.0 |
| Minnesota Zoo | 22.1 | 20.0 | 64.6 | 30.3 |
| National Zoo | 42.2 | 16.5 | 60.0 | 41.8 |
| Rio Grande Zoo | 94.8 | 12.2 | 63.1 | 40.7 |
| Santa Barbara Zoo | 53.9 | 13.6 | 71.2 | 36.6 |
| Washington Park Zoo | 51.3 | 15.1 | 65.6 | 31.6 |
| Woodland Park Zoo | 96.3 | 7.1 | 37.8 | 25.7 |
| Mean: | 54.6 | 15.1 | 61.7 | 35.8 |
| Range (low): | 22.1 | 7.1 | 37.8 | 25.7 |
| Range (high): | 96.3 | 20.0 | 72.2 | 40.7 |

[1]As fed
[2]Cell wall constituents (=NDF, fiber fraction)
[3]Lignocellulose (=ADF, fiber fraction)
NA=not available, NF=not fed

Table 2. Mineral Composition (% of dry matter) of bamboo.

| Zoo | Calcium | Phos-phorous | Sodium |
|---|---|---|---|
| Baltimore Zoo | .34 | .13 | .004 |
| Bronx | .53 | .14 | NA |
| Buffalo Zoo | NF | NF | NF |
| Calgary Zoo | .73 | .66 | .055 |
| Columbus Zoo | .79 | .13 | .008 |
| Dallas Zoo | .89 | .13 | .020 |
| Kansas City Zoo | .40 | .19 | .011 |
| Knoxville Zoo | .68 | .19 | .005 |
| Los Angeles Zoo | .30 | .18 | .046 |
| Minnesota Zoo | .44 | .36 | .065 |
| National Zoo | .83 | .14 | .000 |
| Rio Grande Zoo | .52 | .14 | .106 |
| Santa Barbara Zoo | .78 | .15 | .016 |
| Washington Park Zoo | .37 | .15 | .041 |
| Woodland Park Zoo | .27 | .07 | .104 |
| Mean: | .56 | .19 | .030 |
| Range (low): | .27 | .07 | .000 |
| Range (high): | .89 | .66 | .106 |

Table 3. Dry matter, crude protein, crude fat and fiber composition (% of dry matter) of red panda gruel.

| Zoo | Dry matter[1] | Crude protein | Crude fat | CWC[2] |
|---|---|---|---|---|
| Baltimore Zoo | 22.3 | 22.1 | 10.2 | 7.0 |
| Bronx | NA | 14.4 | 6.0 | 4.2 |
| Buffalo Zoo | 17.5 | 15.3 | 8.8 | 21.8 |
| Calgary Zoo | 21.4 | 13.8 | 11.2 | 5.4 |
| Columbus Zoo | 22.4 | 20.5 | 10.8 | 9.9 |
| Columbus Zoo | 24.1 | 22.1 | 11.8 | 9.7 |
| Dallas Zoo | 33.4 | 14.3 | 8.8 | 10.4 |
| Folsom Children's | 44.7 | 15.1 | 12.1 | 17.2 |
| Kansas City Zoo | 48.3 | 23.2 | 4.1 | 11.4 |
| Knoxville Zoo | 18.6 | 23.7 | 5.3 | 1.5 |
| Los Angeles Zoo | 24.3 | 25.0 | 12.8 | 0.9 |
| Minnesota Zoo | 25.9 | 20.5 | 9.1 | 7.0 |
| National Zoo | 34.4 | 18.2 | 14.8 | 2.5 |
| Rio Grande Zoo | 31.4 | 30.8 | 9.9 | 1.7 |
| Santa Barbara Zoo | 27.3 | 21.3 | 13.9 | 8.2 |
| Washington Park Zoo | 26.1 | 12.8 | 13.3 | 13.0 |
| Woodland Park Zoo | 18.6 | 22.9 | 1.0 | 15.1 |
| Mean: | 26.4 | 20.1 | 9.5 | 8.1 |
| Range (low): | 17.5 | 12.8 | 1.0 | 0.9 |
| Range (high): | 48.3 | 30.8 | 14.8 | 21.8 |

[1] As fed
[2] Cell wall constituents (=NDF, fiber fraction)
NA = Not available

Table 4. Mineral composition (% of dry matter) of red panda gruel.

| Zoo | Calcium | Phos-phorous | Sodium |
|---|---|---|---|
| Baltimore Zoo | 0.60 | .51 | .130 |
| Bronx | 0.71 | .28 | NA |
| Buffalo Zoo | 0.45 | .66 | .370 |
| Calgary Zoo | 0.75 | .62 | .140 |
| Columbus Zoo | 0.90 | .58 | .150 |
| Columbus Zoo | 0.97 | .63 | .160 |
| Dallas Zoo | 0.53 | .44 | .119 |
| Folsom Children's | 1.51 | .85 | .159 |
| Kansas City Zoo | 1.19 | .85 | .412 |
| Knoxville Zoo | 0.99 | .91 | .416 |
| Los Angeles Zoo | 0.66 | .54 | .160 |
| Minnesota Zoo | 0.67 | .58 | .096 |
| National Zoo | 0.64 | .62 | .117 |
| Rio Grande Zoo | 0.75 | .62 | .140 |
| Santa Barbara Zoo | 0.62 | .54 | .107 |
| Washington Park Zoo | 0.66 | .44 | .088 |
| Woodland Park Zoo | 1.75 | .91 | .290 |
| Mean: | 0.80 | .60 | .190 |
| Range (low): | 0.45 | .28 | .088 |
| Range (high): | 1.75 | .91 | .416 |

Table 5. Vitamin composition (% of dry matter) of red panda gruel.

| Zoo | Thiamin (ppm) | Ribo-flavin (ppm) | Vitamin E (IU/kg) |
|---|---|---|---|
| Baltimore Zoo | 9.3 | 13.8 | 17 |
| Bronx | NA | NA | 25 |
| Buffalo Zoo | 7.3 | 10.5 | 5 |
| Calgary Zoo | 15.7 | 20.5 | 16 |
| Columbus Zoo | 12.7 | 17.3 | 11 |
| Columbus Zoo | 14.0 | 18.9 | 13 |
| Dallas Zoo | 4.4 | 6.2 | 64 |
| Folsom Children's | 4.1 | 7.1 | 16 |
| Kansas City Zoo | 6.6 | 9.2 | 3 |
| Knoxville Zoo | 22.0 | 28.9 | 21 |
| Los Angeles Zoo | 12.3 | 16.3 | 7 |
| Minnesota Zoo | 16.0 | 22.3 | 13 |
| National Zoo | 15.2 | 22.6 | 4 |
| Rio Grande Zoo | 18.2 | 19.7 | 17 |
| Santa Barbara Zoo | 7.6 | 8.8 | 77 |
| Washington Park Zoo | 8.9 | 1.9 | 50 |
| Woodland Park Zoo | 12.1 | 21.0 | 117 |
| Mean: | 12.2 | 15.9 | 29 |
| Range (low): | 4.4 | 1.9 | 3 |
| Range (high): | 22.0 | 28.9 | 117 |

Table 6. Dry matter, crude protein, crude fat and fiber composition (% of dry matter) of the CONSUMED DIETS.

| Zoo | Dry matter[1] | Crude protein | Ether extract | LC[2] |
|---|---|---|---|---|
| Baltimore Zoo | 28.4 | 19.7 | 6.4 | 15.7 |
| Buffalo Zoo | 17.4 | 14.6 | 8.4 | 8.7 |
| Calgary Zoo | 29.7 | 13.6 | 7.7 | 16.7 |
| Columbus Zoo | 23.0 | 17.4 | 9.2 | 5.8 |
| Columbus Zoo | 21.4 | 11.6 | 6.0 | 4.9 |
| Columbus Zoo | 23.0 | 10.1 | 4.9 | 5.2 |
| Columbus Zoo | 22.1 | 14.2 | 7.5 | 5.5 |
| Columbus Zoo | 23.1 | 12.7 | 6.1 | 7.0 |
| Dallas Zoo | 37.7 | 15.5 | 6.4 | 12.0 |
| Folsom Children's | 44.7 | 15.1 | 12.1 | 9.3 |
| Kansas City Zoo | 44.7 | 21.9 | 3.2 | 6.7 |
| Knoxville Zoo | 24.5 | 13.8 | 2.0 | 15.0 |
| Knoxville Zoo | 25.3 | 13.9 | 1.8 | 16.7 |
| Knoxville Zoo | 24.5 | 14.4 | 2.0 | 15.9 |
| Knoxville Zoo | 24.5 | 14.7 | 2.0 | 15.8 |
| Los Angeles Zoo | 24.9 | 21.8 | 9.8 | 2.3 |
| Minnesota Zoo | 25.2 | 20.4 | 7.7 | 6.3 |
| National Zoo | 32.9 | 16.6 | 12.0 | 4.4 |
| Rio Grande Zoo | 41.3 | 10.3 | 3.3 | 25.1 |
| Santa Barbara Zoo | 28.2 | 16.5 | 8.7 | 10.2 |
| Washington Park Zoo | 32.1 | 13.7 | 8.2 | 15.4 |
| Woodland Park Zoo | 45.3 | 9.1 | 1.8 | 19.6 |
| Mean: | 28.5 | 15.1 | 7.8 | 14.7 |
| Range (low): | 17.4 | 9.1 | 1.8 | 2.3 |
| Range (high): | 45.3 | 21.9 | 12.1 | 25.1 |

[1]As fed
[2]Lignocellulose (=ADF, fiber fraction)

Table 7. Dry matter, crude protein, crude fat and fiber composition (% of dry matter) of the PROPOSED REVISED DIETS.

| Zoo | Dry matter[1] | Crude protein | Ether extract | LC[2] |
|---|---|---|---|---|
| Baltimore Zoo | 29.2 | 20.7 | 3.0 | 15.7 |
| Buffalo Zoo | 23.2 | 25.1 | 3.8 | 11.1 |
| Calgary Zoo | 28.7 | 19.8 | 4.2 | 18.3 |
| Columbus Zoo | 33.1 | 28.1 | 4.2 | 9.7 |
| Columbus Zoo | 27.4 | 29.5 | 4.5 | 7.2 |
| Columbus Zoo | 34.9 | 27.9 | 4.2 | 10.0 |
| Columbus Zoo | 31.1 | 28.2 | 4.3 | 7.8 |
| Columbus Zoo | 37.5 | 27.4 | 4.0 | 12.3 |
| Dallas Zoo | 49.1 | 25.1 | 4.2 | 15.0 |
| Folsom Children's | 44.0 | 25.8 | 3.8 | 11.6 |
| Kansas City Zoo | 77.0 | 25.8 | 3.9 | 15.0 |
| Knoxville Zoo | 26.1 | 22.8 | 2.0 | 23.1 |
| Knoxville Zoo | 33.4 | 24.1 | 2.6 | 20.3 |
| Knoxville Zoo | 27.2 | 22.1 | 2.0 | 24.8 |
| Knoxville Zoo | 69.6 | 24.8 | 3.2 | 18.6 |
| Los Angeles Zoo | 27.1 | 28.0 | 5.0 | 10.0 |
| Minnesota Zoo | 25.1 | 26.3 | 3.7 | 15.4 |
| National Zoo | 25.0 | 24.1 | 3.9 | 15.8 |
| Rio Grande Zoo | 51.4 | 23.4 | 4.1 | 16.8 |
| Santa Barbara Zoo | 43.2 | 25.2 | 3.6 | 16.8 |
| Washington Park Zoo | 32.4 | 20.0 | 2.9 | 17.3 |
| Woodland Park Zoo | 35.6 | 21.4 | 3.0 | 15.8 |
| Mean: | 36.5 | 24.7 | 3.5 | 15.1 |
| Range (low): | 23.2 | 19.8 | 2.0 | 7.2 |
| Range (high): | 77.0 | 28.2 | 5.0 | 24.8 |

[1]As fed
[2]Lignocellulose (=ADF, fiber fraction)

Table 8. Mineral composition (% of dry matter) of CONSUMED red panda diet.

| Zoo | Calcium | Phos-phorus | Sodium |
|---|---|---|---|
| Baltimore Zoo | 0.50 | .37 | .081 |
| Buffalo Zoo | 0.43 | .63 | .350 |
| Calgary Zoo | 0.92 | .71 | .100 |
| Columbus Zoo | 0.60 | .38 | .090 |
| Columbus Zoo | 0.52 | .31 | .070 |
| Columbus Zoo | 0.39 | .25 | .051 |
| Columbus Zoo | 0.75 | .48 | .227 |
| Columbus Zoo | 0.47 | .30 | .067 |
| Dallas Zoo | 0.63 | .35 | .090 |
| Folsom Children's | 1.51 | .85 | .159 |
| Kansas City Zoo | 1.07 | .77 | .376 |
| Knoxville Zoo | 0.53 | .32 | .101 |
| Knoxville Zoo | 0.58 | .35 | .115 |
| Knoxville Zoo | 0.57 | .34 | .108 |
| Knoxville Zoo | 0.54 | .30 | .089 |
| Los Angeles Zoo | 0.61 | .48 | .145 |
| Minnesota Zoo | 0.63 | .55 | .088 |
| National Zoo | 0.60 | .52 | .090 |
| Rio Grande Zoo | 0.38 | .14 | .020 |
| Santa Barbara Zoo | 0.58 | .37 | .069 |
| Washington Park Zoo | 0.55 | .33 | .069 |
| Woodland Park Zoo | 0.48 | .21 | .060 |
| Mean: | 0.58 | .40 | .110 |
| Range (low): | 0.38 | .14 | .020 |
| Range (high): | 1.51 | .85 | .376 |

Table 9. Mineral composition (% of dry matter) of PROPOSED REVISED red panda DIET.

| Zoo | Calcium | Phos-phorus | Sodium |
|---|---|---|---|
| Baltimore Zoo | 0.88 | .59 | .150 |
| Buffalo Zoo | 1.01 | .68 | .250 |
| Calgary Zoo | 1.04 | .75 | .220 |
| Columbus Zoo | 1.26 | .83 | .340 |
| Columbus Zoo | 1.40 | .92 | .370 |
| Columbus Zoo | 1.25 | .81 | .340 |
| Columbus Zoo | 1.27 | .84 | .340 |
| Columbus Zoo | 1.20 | .76 | .320 |
| Dallas Zoo | 1.08 | .62 | .200 |
| Folsom Children's | 1.06 | .66 | .298 |
| Kansas City Zoo | 1.10 | .66 | .290 |
| Knoxville Zoo | 1.10 | .67 | .150 |
| Knoxville Zoo | 1.10 | .67 | .200 |
| Knoxville Zoo | 1.10 | .61 | .130 |
| Knoxville Zoo | 1.00 | .58 | .240 |
| Los Angeles Zoo | 1.01 | .68 | .250 |
| Minnesota Zoo | 1.10 | .66 | .280 |
| National Zoo | 1.00 | .65 | .270 |
| Rio Grande Zoo | 0.99 | .59 | .200 |
| Santa Barbara Zoo | 1.10 | .62 | .270 |
| Washington Park Zoo | 1.00 | .72 | .170 |
| Woodland Park Zoo | 0.91 | .54 | .250 |
| Mean: | 1.09 | .68 | .240 |
| Range (low): | 0.88 | .54 | .130 |
| Range (high): | 1.40 | .92 | .370 |

Table 10. Summary of daily dry matter (DM) intake.

| Zoo | DM intake g/animal | Gruel (%) | Bamboo (%) | Other (%) |
|---|---|---|---|---|
| Baltimore Zoo | 205 | 63 | 37 | 0 |
| Buffalo Zoo | NA | NA | NA | NA |
| Calgary Zoo | 257 | 49 | 44 | 7 |
| Columbus Zoo | 342 | 56 | 5 | 39 |
| Columbus Zoo | 274 | 44 | 11 | 45 |
| Columbus Zoo | 198 | 31 | 8 | 61 |
| Columbus Zoo | 488 | 74 | 3 | 23 |
| Columbus Zoo | 198 | 46 | 5 | 49 |
| Dallas Zoo | 263 | 72 | 28 | 0 |
| Folsom Children's | 273 | 100 | 0 | 0 |
| Kansas City Zoo | 65 | 75 | 5 | 20 |
| Knoxville Zoo | 156 | 24 | 42 | 34 |
| Knoxville Zoo | 162 | 27 | 44 | 29 |
| Knoxville Zoo | 184 | 26 | 44 | 30 |
| Knoxville Zoo | 163 | 21 | 47 | 32 |
| Los Angeles Zoo | 180 | 74 | 3 | 23 |
| Minnesota Zoo | 101 | 85 | 15 | 0 |
| National Zoo | 188 | 80 | 10 | 10 |
| Rio Grande Zoo | 58 | 6 | 61 | 33 |
| Santa Barbara Zoo | 182 | 60 | 26 | 14 |
| Washington Park Zoo | 236 | 62 | 38 | 0 |
| Woodland Park Zoo | 404 | 16 | 71 | 13 |
| Mean: | 215 | 50 | 27 | 23 |
| Range (low): | 58 | 6 | 3 | 0 |
| Range (high): | 488 | 100 | 71 | 61 |