NUTRIENT COMPOSITION OF WHOLE PREY COMMONLY FED TO REPTILES AND AMPHIBIANS

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INTRODUCTION

Nutrient composition data, including vitamin and mineral levels, are readily available for evaluation of fruits, vegetables, and other produce items utilized in zoo diets from human food tables (Watt and Merrill, 1963). Likewise, information from forages and grains used in animal diets has been compiled (NRC, 1982), and many commercial feed companies supply relatively complete nutrient specification data for manufactured or processed products fed to zoo animals. By contrast, whole prey items comprise one category of feedstuffs for which nutritional data are quite limited, or lacking entirely. The published literature that can be found on nutrient composition of meat or fish products focuses on species and/or portions for human consumption, such that information is often not directly applicable to feeding programs for captive animals. Even fewer data exist concerning the nutrients contained in insects used as food. This dearth of information can lead to inappropriate, unnecessary, or at times, detrimental vitamin and mineral supplementation regimens. Basic chemical composition information which can be applied to the feeding management of carnivorous reptiles and amphibians includes proximate composition (water, protein, fat, ash), vitamin, and mineral data. Existing data from our laboratory is summarized here, with implications for applied animal nutrition, improved animal health, and future research thrusts.

MATERIALS AND METHODS

Samples of whole prey were frozen, ground, and freeze-dried prior to analysis using AOAC methodologies for meat samples (Ellis, 1984). Moisture content was determined during freeze-drying, and total ash was calculated following incineration at 550°C overnight. Crude fat was determined by extraction with petroleum ether; crude protein was analyzed using a macro-Kjeldahl method with a copper catalyst, and calculated as total nitrogen X 6.25. While carbohydrate is negligible (except possibly from gut contents) in most of these samples, chitin in insects was assayed as neutral detergent fiber, a "dietary fiber" complex carbohydrate fraction (Pennino, et al., 1991).

Fat-soluble vitamins A and E were quantified by HPLC following hexane extraction of fresh samples, using procedures and equipment described previously (Dierenfeld, et al., 1991; Pennino, et al., 1991; Douglas, et al., 1994). Vitamin A activity was calculated as 0.3 μg retinol = 1 IU; vitamin E activity was calculated by summing α- and gamma- tocopherols, where 1 mg α-tocopherol = 1.1 (rodents) or 1.5 (fish and insects) IU and 1 mg gamma-tocopherol = 0.1 IU (Olson, 1984; Machlin, 1984).

Tissues were prepared for mineral (Ca, Cu, Fe, Mg, Mn, Zn) analyses according to standardized methodology for meat (Parker, 1963). Dried samples were reground in a food mill to ensure homogeneity, and duplicate subsamples (0.5 g) were ashed overnight at 550°C. The ash was dissolved in 3 ml 3N HCl and diluted to volume with double distilled water containing 1% lanthanum. When necessary, the samples were diluted further with 0.36N HCl to ensure output within analytical range. Each mineral was run individually on a Perkin-Elmer atomic absorption spectrophotometer (Model 3100), using the appropriate standard conditions and excitation lamps; 0.36N HCl solution (with 1% lanthanum) was used as a blank.

RESULTS AND DISCUSSION

All data are reported as mean ± standard deviation (SD). Table 1 lists proximate composition (water, crude protein, crude fat, and total ash) of whole fish (n=6 spp.), rodents (n=3 spp.), and poultry (n=2 spp.) used in captive feeding programs. Various ages, sexes, and diet treatments are combined for this summary. Similar data for insects and other invertebrates are found in Table 2.

Whole prey, containing 65-80% water, provide an excellent dietary source of water for carnivores, although limited data suggest that free-ranging whole prey may contain even more water (Robbins, 1993). In general, neonates contain more water than older age categories (Douglas, et al., 1994) and calorie concentration is less dense due to less fat. Species differences in body composition among fish, rodents, and poultry, however, are apparent from the data in Table 1. Capelin, mackerel, and whitebait are all examples of "low fat" fish, compared to other species of similar body size. Among rodents, guinea pigs contained considerably more fat than other species examined (Robbins, 1983), and chickens can be significantly fatter than quail raised as food sources. In previous studies, we found no significant differences in body composition among various age categories of rats used as feed, but young mice (fuzzies/crawlers) contained more fat than other age groups. These differences can have a significant effect upon total food and nutrient intake, and ultimately, economics of feeding programs as animals eat to fulfill caloric requirements.

The value of invertebrates, and particularly insects, as an economic source of high-quality protein (approximately 93% available, Finke, et al., 1989; Pennino, et al., 1991) is well known (Martin, et al., 1976; DeFoliart, et al., 1982; Redford and Dorea, 1984), ranging from 32% (earthworms) to >60% of dry matter (DM) in crickets and beetles, and up to 80% in cockroaches, dragonfly nymphs, and gypsy moths.

Crude fat content is quite variable in food insects, changing with reproductive state, season, life stage (age), or sex (Myers and Pedigo, 1977; Redford and Dorea, 1984; Mason, et al., 1990; Pennino, et al, 1991). Fat contains twice the calories of other nutrient categories (9 kcal/g), is highly digestible, and provides a major energy source to insectivorous species. Additionally, fat yields almost two times more metabolic water when digested, thus may provide a significant water supply to insectivores. Published values of crude fat content range from 10% in honeybees (*Apis mellifera*) to 58% (DM basis) in waxmoth larvae (*Galleria mellonella*) and dragonfly nymphs (species unknown). In general, nymph, larval, and alate stages have a higher fat content than other life stages of the same species. This fact may be nutritionally relevant for animal species which consume immature compared to adult sizes, but has not been investigated in detail.

Insects contain little simple carbohydrate such as sugars or starch, but may have a substantial (around 20% of DM) complex carbohydrate component in the form of chitin, which is a nitrogen-containing polysaccharide. Insectivores possessing chitinases (enzymes to break down chitin) may obtain energy from digestion of the exoskeleton (up to 20% digestibility values of chitin have been reported in small mammals; Allen and Oftedal, 1989). For animals not exhibiting chitinase activity, the dilution of high fat and high protein insect diets with indigestible "fiber" may be important to gut function by affecting passage, fecal bulk, or absorption of other nutrients. Specific examples of this effect in the zoo community can be found by the addition of chitin (15% of DM) to diets fed to ant- and termite-eating specialists, with dramatically improved stool quality (firmness) an immediate observation.

Energy content of whole prey can be estimated from proximate composition data (5.43 kcal/g protein and 9.11 kcal/g fat [if not determined experimentally] Robbins, 1983), and compared against calculated calorie requirements of various carnivores to determine total quantities of food required.

Fat-Soluble Vitamins

While cases of vitamin toxicities (Annis, 1993; Gosselin, et al., 1989) and deficiencies (Frye, 1984; Dierenfeld, 1989) have been reported among zoo carnivores, few data on vitamin content of whole prey are available to evaluate diet suitability. Table 3 contains calculated vitamin A and vitamin E concentrations in whole fish (7 spp.), rodents (3 spp.), and poultry (2 spp.) used as feed, while Table 4 contains similar information for insects and other invertebrates, along with carotenoid content of selected samples.

Vitamin A requirements established for wildlife and domestic species range from 3000 to 15,000 IU/kg dietary DM (Robbins, 1983), and presumed upper safe levels for domestic carnivores range from 33,000 (dog) to 100,000 (cat) IU/kg DM (NRC, 1987). No requirement data are available for reptiles and amphibians. Concentrations of vitamin A reported here (3900 in goldfish to >1,000,000 IU/kg in mice) demonstrate that whole vertebrate prey (fed presumably overly adequate diets) clearly met or greatly exceeded known dietary requirements for this nutrient without the need for any additional supplementation. Yet many commercial supplements fed to zoo- or privately-owned carnivores (and particularly piscivores) still contain unwarranted high levels of vitamin A. Vitamin A concentrations increase with age in mice and rats (Douglas, et al., 1994), as this fat-soluble vitamin is well-stored in the liver and adipose tissue.

Effects of dietary vitamin A levels upon ultimate body composition of whole prey, and health of the secondary consumer, are currently under investigation. Apparent excessive levels in adult rodents, poultry, and some fish, with few (identified) accompanying clinical problems in carnivores consuming them may, in fact, suggest that vitamin A requirements of wildlife species are higher than those of domestic counterparts, or that animals have mechanisms for protection against vitamin A toxicity (Schweigert, et al., 1987). In nature, carnivores are not likely to limit themselves to any single species or age category of small vertebrates, and young are easier to catch. Thus, vitamin A toxicity from exclusive consumption of adults may not be a problem for free-ranging predators. However, excess vitamin A has been suggested as a relatively common cause of death in captive wildlife (Robbins, 1983), and adult rodents comprise the sole diet for a number of reptilian carnivores in zoos.

Furthermore, recent reports (Annis, 1993; Dierenfeld, et al., 1995) suggest that strict insectivores may, in fact, have low dietary requirements of this nutrient. Insects appear to be a poor dietary source of vitamin A, with concentrations ranging from 150 to <1000 IU/kg DM, substantially lower than requirements specified for domestic animals. Many of the invertebrates, however, contain carotenoids, with possible vitamin A precursor activity (Brush, 1990); carotenes have previously been detected in a number of insects (Nestler, et al., 1949) even when vitamin A (as retinol) was unquantifiable. Numerous species have the ability to convert carotenoid pigments (primarily ß-carotene) to active vitamin A, but the physiology of this has not been examined to any extent in insectivores. The lack of preformed vitamin A suggests other sources of this nutrient for obligate insectivores.

Taken together, these data indicate that more suitable diets for feeding prey species (with regard to vitamin A) may need to be developed, including detailed investigations of carotenoid metabolism in reptiles and amphibians.

Vitamin E levels measured in whole vertebrate prey also vary with species, age, and diet. Numerous reports support the need for supplementation of fish-based diets (Dierenfeld, 1989; Dierenfeld, et al., 1991; Dierenfeld and Traber, 1992); these data provide some baseline information for appropriate supplementation levels necessary to meet recommendations of 400 IU/kg DM (Englehardt and Geraci, 1978). Vitamin E concentrations in whole rodents have only recently been documented (Douglas, et al., 1994). No differences were seen among six age categories of mice, but neonatal rats contained excessive levels (>450 IU/kg) compared to other rodents. Whole rodents and poultry contained vitamin E levels within dietary requirements established for domestic carnivores (20-80 IU/kg), rats contained substantially more. The significance of this apparent difference in metabolism of vitamin E among rodent species remains to be evaluated, as does the influence of different dietary levels of this nutrient fed to prey species.

Most invertebrates sampled contained vitamin E concentrations within ranges for domestic carnivores, with the exception of one mealworm category and, notably, waxworms as well as commercially-available earthworms. Pennino, et al. (1991) reported that vitamin E concentrations could be altered in insects by altering dietary levels of this nutrient, therefore it is important to consider the diet of the invertebrates in formulating diets for captive animals. While the earthworm variation may have been due to differences in soil substrate (food), the waxworm value probably represents efficient body storage of this fat-soluble vitamin as the larvae do not normally consume food. While vitamin E deficiencies have been recognized in a number of zoo species (Dierenfeld, 1989), they have not been specifically documented in insectivores. Thus, insects commonly fed in zoos or private collections would appear to contain adequate concentrations of this nutrient.

Minerals

Calcium and magnesium requirements of growing mammals and birds (0.4-1.2%, and 0.04-0.1% of DM, respectively; Robbins, 1983) would be met by any of the whole vertebrate prey discussed and summarized in Table 5; no requirement data exist for reptiles or amphibians. Additionally, calcium (Ca) must be evaluated in relation to dietary phosphorus (P) and vitamin D. Although not reported here, whole vertebrate prey in general contain adequate Ca:P ratios (1:1-2:1) to meet dietary requirements of carnivores (Fowler, 1986).

Much of the existing literature on mineral content of insects (Table 6) has focussed on imbalances of Ca:P ratios in crickets or mealworms, along with subsequent health problems associated with calcium deficiency in species fed solely on these food items (Allen, et al., 1993). Supplementation of the diet fed to prey insect species has been shown to increase calcium content, and high-calcium cricket diets are available commercially. A simple mixture consisting of approximately 20% (by weight) powdered calcium carbonate, and 80% nutritionally-complete chick mash, with a final calcium content of 8% (DM) appears suitable for feeding crickets and Tenebrio larvae two days before they are fed out. Additionally, insects should be coated in calcium carbonate immediately prior to feeding to insectivores (if coated for more than 30 minutes, crickets may have opportunity to remove the calcium supplement through grooming).

These data support those of earlier studies, in that calcium content, in general, is quite low in food invertebrates. A notable exception, however, were the pinhead crickets which (while quite variable) had an average calcium concentration >2.0% of DM. Additionally, earthworms were a good source of calcium for reptiles and amphibians. Composition of both prey items is likely highly dependent on substrate on which they are raised and/or fed. All invertebrates examined would meet dietary magnesium requirements of domestic carnivores.

Trace element requirements of mammalian carnivores (Cu, 5.0- 7.3 mg/kg; Fe, 60.0-100.0 mg/kg; Mn, 5.0-10.0 mg/kg; Zn, 30.0-50.0 mg/kg; Robbins, 1983) would generally be met by rodents analyzed in these studies (Table 5), with the exception of copper and manganese. Juvenile rodents appear not to provide adequate dietary manganese, and considerable diet effects upon ultimate mineral composition of whole prey are apparent. Whole poultry and fish (see also Bernard and

Ullrey, 1989) species appear to contain inadequate levels of dietary Cu, and possibly Mn. Although studies of selected mineral (Cu, Zn, Ca, Na, K) requirements for raising crickets (McFarlane, 1991), and the mineral composition (Fe, Ca, Mg, Na, K) of June beetles has been reported (Keeler and Studier, 1992), the mineral content of insects in general has not been summarized, nor has insectivore nutrition research concentrated on the links between diet, substrate, mineral interactions, and subsequent whole body composition for other mineral nutrients apart from Ca and P. Table 6 contains trace element values measured for selected insects in our laboratory. Copper, Mn, and Zn needs would appear to be met by these food items; iron content was most variable, ranging from possibly deficient in mealworms to overly excessive in earthworms, although availability of the iron was not measured.

This area (trace mineral nutrition) is one which deserves much further attention, particularly as we strive to characterize and optimize diets for wildlife species. Trace mineral requirements for most non-domestic species have simply not been determined; furthermore, the influence of diets upon ultimate nutrient composition of prey items should be investigated in more detail as a variable which can certainly be controlled in managed feeding programs. Clearly such interactions should be considered integral in the development of optimal diets for whole prey as food for other species, and must be considered when evaluating nutritional adequacy of carnivore diets.

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Table 1. Proximate Composition of Selected Whole Prey Food Items[a,b]

SPECIES	WATER %	CRUDE PROTEIN % of dry matter	CRUDE FAT % of dry matter	TOTAL ASH % of dry matter
FISH				
Capelin (n=27)	80.3±2.0	69.3±6.2	16.5±5.6	10.1±2.3
Herring (n=25)	71.5±4.4	59.7±10.0	31.4±10.8	7.9±2.0
Mackerel (n=7)	73.7±3.4	67.7±7.4	15.9±11.0	10.5±2.0
Rainbow trout (n=12)	74.7±2.7	64.0±9.2	22.6±5.3	12.4±2.7
Spearing (n=6)	72.9±3.7	53.2±8.5	32.3±6.5	8.4±2.5
Whitebait (n=5)	79.1±3.7	68.7±9.8	18.4±7.6	10.6±2.0
RODENTS				
Mice (n=36) (6 ages, ♂,♀)	71.3±6.8	52.6±9.6	30.1±14.0	11.8±4.4
(3 diets, ♀) (n=9)	67.9±2.0	33.0±10.6	21.3±7.5	10.6±1.6
Rats (n=22) (6 ages, ♂,♀)	71.0±5.5	56.4±5.8	27.6±8.0	13.1±5.9
(2 diets, ♂) (n=6)	65.2±2.0	32.2±5.7	34.6±6.6	8.0±2.0
Guinea pigs (2 diets, ♂)	68.7±1.4	23.1±4.3	50.2±3.5	9.3±1.3
POULTRY				
Quail (n=18) (3 diets, ♂,♀)	67.0±3.1	47.4±7.1	25.8±10.0	10.3±1.8
Chicken (n=6) (2 diets, ♂)	68.6±1.4	27.9±3.9	48.7±5.4	9.2±2.5

[a] mean ± SD; all values except water on a dry basis

[b] summarized from Dierenfeld, et al., 1991; Douglas, et al., 1994; and Clum, et al., unpubl. data.

Table 2. Proximate Composition of Selected Invertebrate Prey Items Fed to Reptiles and Amphibians[a]

SPECIES	WATER %	CRUDE PROTEIN % of dry matter	CRUDE FAT % of dry matter	TOTAL ASH % of dry matter
Mealworm *Tenebrio molitor* (6)	62.9±3.6	51.8±5.3	31.1±3.9	4.3±3.7
Super mealworm *Xophobas morio* (6)	57.0±1.4	42.9±1.4	40.8±2.3	3.5±0.6
Mighty mealworm *Tenebrio molitor* (6)	58.3±0.6	48.0±0.9	40.3±0.7	3.2±0.7
Waxworm *Galleria mellonella* (6)	61.9±2.1	41.1±2.8	51.4±5.4	3.3±1.0
Cricket *Acheta domesticus* (6)	73.2±1.9	64.1±2.2	22.8±1.5	5.1±1.4
Pinhead cricket (6)	62.9±11.2	55.1±3.4	9.8±1.4	11.2±8.2
Fruit fly (3) *Drosophila melanogaster*	67.2±4.3	56.3±0.8	16.7±2.1	5.3±0.8
Dung Beetle (4)	53.3±1.4	62.6±3.4	12.3±1.7	3.1±0.8
Earthworm (6) *Lumbricus terrestris* (Commercial)	75.8±4.8	50.4±9.4	10.6±1.7	24.9±11.4
Earthworm (6) (Wild-caught)	74.5±2.0	32.2±6.6	2.6±1.0	45.7±6.6

[a] mean ± SD; all values except water on a dry basis

Table 3. Vitamins A and E Measured in Whole Vertebrate Prey[a,b]

SPECIES	n	VITAMIN A (IU/g DM)	VITAMIN E (IU/kg DM)
FISH			
Capelin	27	33.3±21.3	152.3±90.3
Goldfish	3	3.9±0.4	45.2±1.2
Herring	25	22.9±14.9	93.3±63.6
Mackerel	7	234.8±143.7	166.4±32.0
Rainbow trout	12	26.7±24.1	313.8±104.4
Spearing	6	21.7±13.3	236.6±183.5
Tilapia	5	10.5±2.0	490.0±134.0
RODENTS			
Mice			
(6 ages, ♂,♀)	36	91.0±120.0	43.8±13.4
(3 diets, ♀)	9	1114.0±567.5	74.4±20.7
Rats			
(6 ages, ♂,♀)	22	128.5±170.7	11.8±169.2
(2 diets, ♂)	6	58.8±24.8	141.7±90.9
Guinea pigs (2 diets, ♂)	6	16.5±45.8	24.1± 6.5
POULTRY			
Quail (3 diets, ♂,♀)	18	90.1±60.0	44.7±21.3
Chickens (2 diets, ♂)	6	35.6±10.4	51.3±14.7

[a] mean ± SD

[b] summarized from Dierenfeld, et al., 1991; Douglas et al., 1994; and Clum, et al., unpubl. data.

Table 4. Vitamins A[a], E[b], and Carotenoids in Whole Invertebrates Consumed by Reptiles and Amphibians[c]

SPECIES	n	Vitamin A (IU/kg DM)	Vitamin E (IU/kg DM)	Carotenoids Identified
Snail	4	317±569	NA[d]	ß-carotene, lutein zeaxanthin
Mealworm *Tenebrio molitor*	6	811±324	30±3	None
Super mealworm *Xophobas morio*	6	972±570	32±6	NA
Mighty mealworm *Tenebrio molitor*	6	161± 91	15±3	NA
Waxworm *Galleria mellonella*	5	150±160	509±232	lutein, zeaxanthin
Grasshopper	4	1034±1327	NA	ß-carotene, lutein, canthaxanthin
Cricket *Acheta domesticus*	6	811±849	81±41	ß-carotene
Pinhead cricket	6	628±600	80±46	
Fruitfly *Drosophilus melanogaster*	3	0	16±7	
Dung beetle	4	192±171	57±35	phytofluene, carotenes
Earthworm *Lumbricus terrestris* (Commercial)	6	328±171	229±82	none
Earthworm (Wild-caught)	6	2400±279	70±12	

[a] vitamin A Activity (IU/kg) = (Retinol/0.3) x 1000
[b] vitamin E activity (IU/kg) = [(1.5)(α-tocopherol) + ((0.15)(gamma-tocopherol)]
[c] mean ± SD
[d] NA= not available.

Table 5. Mineral Composition of Selected Whole Vertebrate Prey[a,b]

SPECIES	n	Ca %	Mg %	Cu	Fe	Mn	Zn
					mg/kg dry matter		
FISH							
Capelin	13	1.8±0.3	0.1±0.03	2.8±1.0	75.0±35.3	2.4±0.7	57.2±7.7
Herring	3	2.5±1.2	0.1±0.03	2.3±1.0	98.2±27.6	7.5±3.4	61.1±27.5
Mackerel	3	2.4±1.4	0.1±0.03	1.0±0.7	158.2±16.3	3.0±0.7	44.4±20.0
Trout	18	2.2±0.5	0.1±0.03	4.8±1.1	55.3±12.0	4.0±0.6	86.3±13.5
Spearing	2	2.9±0.5	0.1±0.01	2.8±0.3	95.4±46.7	6.5±2.3	80.1±37.4
RODENTS[c]							
Mice	30	1.7±0.5	0.1±0.02	13.1±5.3	156±31.2	6.4±5.3	68.9±13.2
	9	3.7±0.8	0.5±0.1	3.9±0.6	102±22.9	5.6±1.6	52.3± 9.1
Rats	30	2.4±0.9	0.1±0.03	20.4±23.1	227±89.8	13.8±12.0	87.3±18.2
	6	2.7±0.7	0.3±0.03	1.5±0.4	50.5±9.5	3.0±0.6	36.8±11.7
Guinea pigs	6	3.0±0.4	0.7±0.1	5.6±2.9	56.4±6.6	6.5±0.6	46.4±26.1
POULTRY[c]							
Quail	18	3.8±0.7	0.5±0.2	2.9±0.8	71.4±30.5	6.6±2.8	56.6±14.8
Chicken	6	2.5±0.3	0.5±0.1	2.6±0.1	7.6±10.2	11.2±1.2	74.1±21.1

[a] mean ± SD; all values on a dry basis
[b] summarized from Clum, et al. and Dierenfeld, et al., unpubl. data.
[c] diets, sexes, and groups as in Table 3.

Table 6. Mineral Composition of Selected Whole Invertebrate Prey[a] Species Fed to Reptiles and Amphibians

SPECIES	n	Ca %	Mg %	Cu	Fe	Mn	Zn
					mg/kg dry matter		
Mealworm	6	0.1±0.1	0.3±0.02	17.7±4.7	39.7±19.2	6.8±4.2	131±7
Mighty mealworm	6	0.03±.01	0.2±0.01	14.9±1.5	25.9±16.0	5.6±0.2	83±4
Super mealworm	6	0.12±0.2	0.2±0.02	13.9±3.1	50.3±6.5	1.5±0.6	88±4
Waxworm	6	0.06±0.01	0.1±0.01	3.1±1.3	77.3±13.1	3.3±0.8	79±6
Cricket	6	0.21±0.03	0.1±0.01	8.5±1.0	112.3±58.1	30±5	186±16
Pinhead cricket	6	2.1 ± 2.7	0.2±0.06	10.4±1.1	224±91	56±22	157±20
Fruit fly	6	0.2±0.02	0.1±0.03	7.3±3.6	473±41	16.1±2.7	158±82
Dung beetle	6	0.1±0.01	0.1±0.01	20.7±5.3	238±51	61.1±25.4	128±8
Earthworm (Commercial)	6	1.2±0.03	0.2±0.03	8.1±1.7	5802±2324	113±51	231±56
Earthworm (Wild caught)	6	1.0±0.3	0.3±0.03	32.9±18	11087±2938	199±45	271±89

[a] mean ± SD; all values on a dry basis