INSIGHTS ON PSITTACINE NUTRITION THROUGH THE STUDY OF FREE-LIVING CHICKS

A Dissertation

by

JUAN CORNEJO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Veterinary Microbiology

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Approved by:

Co-Chairs of Committee,	Donald J. Brightsmith
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ABSTRACT

Insights on Psittacine Nutrition through the Study of Free-living Chicks.
(May 2012)
Juan Cornejo, B.S., Universidad de Navarra
Co-Chairs of Committee: Dr. Donald J. Brightsmith
Dr. Christopher A. Bailey

The Psittacidae is one of the most endangered families of birds in the world. Knowledge of its nutrition is important for understanding their survival and productivity in the wild, as well as for their adequate husbandry in captivity. Hand-rearing is a common practice for this group. However, research on their requirements is limited. Analysis of the crop content of chicks can provide new insights into psittacine nutrition, but it is limited by the small sizes of samples which can be obtained. We sampled the crops from free-living chicks of scarlet macaws and red-and-green macaws from southeastern Peru, Cuban parrots from the Bahamas, lilac-crowned parrots from northwestern Mexico, and thick-billed parrots from northern Mexico. The predicted metabolizable energy, protein, fat, minerals, profile of essential amino acids and profile of fatty acids of the crop samples, as well as from 15 commercial hand-rearing formulas, were analyzed and contrasted. Near Infrared Spectroscopy was shown to be a valid technique for the nondestructive, low cost prediction of a variety of nutritional attributes of crop samples as small as 0.5 g dry weight, expanding the possibilities of wild animal nutrition research. The diets of the five studied species presented remarkable similarities and common patterns. The predicted dietary metabolizable energy and fat concentrations were particularly similar among species, the thick-billed parrot being the one with the most unique nutrient profile. The fatty acid profile of the crop contents differed markedly among genera, with the thick-billed parrot closer to the macaws than to the parrots. In comparison with the crop samples, the hand feeding formulas presented lower fat, Mg, arginine, and valine concentrations. The wide variation in nutrients suggests that there is not yet a consensus among manufacturers concerning the correct nutrition for growing psittacines. It is suggested that a single formulation could be used to hand-rear macaws and parrots from half its nesting time to fledging, and further research should focus on their nutrition during the first half. Our results suggest that manufacturers should evaluate if increasing the concentrations of crude fat, Mg, arginine, and valine in commercial formulas enhances psittacine chick growth and health.

DEDICATION

A mi madre, por haberme enseñado a observar y apreciar la naturaleza.

A mi padre, por ser el ejemplo a seguir.

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CHAPTER I
INTRODUCTION

General background

Macaws and other members of the Psittacidae family have been bred in captivity for more than 3000 years (1). In part due to their popularity as pets, they have become the most endangered order of birds in the world (over 25% of the species are listed as threatened and an additional 11% as near-threatened (2). Knowledge of nutrition is important for the adequate husbandry of the birds kept as pets, and for the efficient propagation of individuals kept in zoological collections for their ex situ conservation (3-6). It is also needed for understanding survival and productivity (6, 7), and it is therefore critical to implement adequate conservation strategies (3, 8, 9). There is an increasing volume of research on the nutritional requirements for growth and maintenance of psittacines (4, 10-22), but malnutrition is still one of the main issues in the care and propagation of this group (4, 5, 23-25) and providing nutritionally adequate diets must be a primary concern

Most psittacines consume plant-based diets and are classified as herbivores (26). Studies of the diet of free ranging Neotropical psittacines are scarce (9, 27-34). While it is possible to identify the main food sources of adult parrots through field observations (9, 35), field conditions usually make it very difficult to determine the less common items in the diet (36). In addition, parrot's extensive food manipulation and processing makes it challenging to quantify the exact proportion of each food type consumed (8, 15, 34). As a result it is not possible to determine nutritional content of wild adult diets.

This dissertation follows the style of The Journal of Nutrition.

Parrots feed their chicks an undigested regurgitate which the chicks hold in their crop before passing to the stomach for digestion. Analysis of these chick crop contents provides the opportunity to determine the composition of the chick diets as fed, unaffected by differential digestion (36-39). A variety of techniques exist to collect samples directly from the crops and stomachs of individual birds (36, 37). Unfortunately, the small size of the individual samples greatly limits the traditional wet lab analyses which can be done. Pooling samples of similar characteristics is often necessary to achieve the minimum sample sizes needed for analysis but this reduces statistical power needed to address ecological hypotheses (40, 41). Through crop sampling is also possible to directly determine each food type (32, 34, 35, 40) and the nutritional composition of the diet as a whole (40, 41).

To date there are very few studies that have looked at the nutritional composition of free ranging parrot chicks diet (9, 40, 41). Research into the nutrition of parent-fed chicks has been published for only two species, the scarlet macaw (*Ara macao*) from southeastern Peru (40, 42), and the kakapo (*Strigops habroptila*) from New Zealand (41). Given the diversity of food habits and ecology among parrot species it is impossible to generalize from these scant observations to the nutritional requirements of the family.

The overall goal of this dissertation is to use information from five species of free ranging (wild) psittacine chicks to provide novel information on psittacine nutrition which can be used to improve the hand-rearing of psittacines.

Chick diet and hand feeding

Hand rearing is a common practice for the propagation of psittacines, both for the pet market (43-45) and for conservation aviculture (46-50). Accurate information on the nutritional requirements of growing animals is essential for the formulation of captive rearing diets (51). However, research on the nutrition of growing psittacines is limited and the nutritional requirements for the growth of psittacine chicks are not well understood (12, 22, 52-56). As a result, nutritional imbalances resulting in problems such as stunted development, rickets, and vitamin deficiencies (5, 13, 57, 58) have been common, and still occur for some species (4). Generally hand fed parrots grow slower than parent fed (56, 59, 60), and present a delayed fractional grow rate (% increase in body weight per day) (3, 61). In the absence of further research, or comprehensive data on the nutrient composition of the diets of wild psittacines, nutritional prescriptions for their maintenance and growth are generally extrapolated from dietary recommendations for poultry (62) and modified based on experience rather than on scientific study (4). However, psittacines are not closely related to poultry which have been artificially selected for multiple generations and differ both developmentally (63) and ecologically (64). Therefore, it is questionable if the available poultry data adequately model the dietary requirements of psittacine chicks.

Hand feeding diets for psittacines were traditionally home-made recipes which required extensive preparation (3, 44, 45, 55, 58, 65) but now there is a wide array of commercially available products that require minimal preparation (4, 48). These products are intended to be used without supplementation and fulfill the nutritional requirements of most species. In a nutritional analysis of 11 commercial hand feeding products Wolf and Kamphues (56) found great differences in the nutritional content, with an average ME concentration of 15.2 MJ/kg DM (13.0-16.8), crude protein 14.4 g/MJ ME and crude fat 7.38 g/MJ ME. When compared with the nutritional requirements of budgerigars *(Melopsittacus undulatus)* and lovebirds *(Agapornis sp.)*(52), they found a number of formulas with insufficient concentrations of the sulphur amino acids methionine and cystine and others with apparently excessive calcium concentrations.

Diet texture

Parrots feed their chicks a regurgitated coarse mix of foods. Preliminary data from 31 crop contents of scarlet macaws (*Ara macao*) chicks in Peru (40), age 28-60 days, found that the largest food particles averaged 9.0 x 4.5 mm, and there was little variation with chick age. Feeding whole grain to young chickens has been associated with a more muscular gizzard and less occurrence of proventricular hypertrophy (66, 67). Greater development of the gastrointestinal tract suggests that feed may be retained in the upper digestive tract for a longer period allowing for increased enzymatic digestion and digestive efficiency (66, 68, 69). Captive psittacines are usually hand fed diets of very small particle size (finely ground). When attempting to hand feed a coarse texture similar to the regurgitate fed by their parents (40), the mortality of newly hatched chicks increased and created problems with the food passage time in older chicks (58). The capacity of a formula to maintain the solids in suspension is another important factor because separation of ingredients at the mixing dish will result in nutritional inconsistencies of the formula. If this situation occurs in the chick's crop, the solids will settle while the liquid is absorbed, making it more difficult to pass, and leading to dehydration and crop stasis.

Nutritional requirements for growth

Nutrients in the diet supply energy to fuel metabolism and the precursors for the synthesis of structural and functional macromolecules. The quantitative and qualitative aspects of nutrient requirements are well understood for commercial poultry species (62), but information about the requirements for wild avian species is very limited (6, 26).

Energy

The metabolizable energy (ME) is the amount of food energy that becomes available to the bird when nutrients such as amino acids, carbohydrates and lipids, are oxidized during metabolism. Knowledge of energy requirements is very important because birds usually eat the quantity of food needed to satisfy their energy needs (4, 26). The amount of food required depends upon the density of metabolizable energy in the diet and its digestibility (26). Thus, when provided low energy density diets (e.g., high-fiber), animals increase the amount consumed but decrease total intake when given high energy diets (e.g., high-fat). Growing birds need energy for basal requirements, thermoregulation, physical activity, and growth. Kamphues and Wolf (70) measured the rate of protein and lipid gain in growing budgerigars (177 mg/d and 160 mg/d, respectively) and lovebirds (153 mg/d and 153 mg/d, respectively). Correcting these rates for the cost of deposition (52 kJ/g) gives the additional energy needed for growth at 17.5 kJ/g for budgerigars and 15.9 kJ/g for lovebirds. The relative amount of energy needed for growth is based upon the fractional growth rate (26). In altricial nestlings, the proportion of energy requirement partitioned to growth changes with age, being proportionally highest at the beginning when percent weight gain is the highest and thermoregulation and activity are minimal (26). Earle and Clarke (15) reported that the peak energy provisioned to parent fed budgerigar chicks was maximal about a week before fledging at 28 kJ/chick/day. Birds in the order Psittaciformes are among the slowest growing of altricial species but they also develop endothermy at an early age (71, 72). Thus, their energy requirements are likely to be more similar to precocial species than to highly altricial species like Passeriformes, which grow faster and thermoregulate later. The data suggest that this is the case, as the content of crops of free ranging kakapo (*Strigops habroptila*) chicks contained 7.7 MJ ME/kg (41), although optimal growth of chickens 0 to 12 weeks of age is achieved when offering a diet with 11.9 MJ ME/kg (62). However, according to Wolf and Kamphues (56) the energy density of hand rearing formulas for parrots varies widely (13.0-16.8 MJ ME/kg) and consistently exceeds estimated needs.

Protein and amino acids

Proteins are the primary constituents of animal tissues and are also important as enzymes, hormones, and membrane components (6). While crude protein is commonly reported in nutritional studies, its interpretation without considering the amino acid (AA) profile can be misleading, as birds don't have a crude protein requirement *per se* (62). An essential level of protein must be included in the diet to meet nitrogen requirements of the animal, but the requirements depend on protein digestibility and the relative concentrations of the AA.

In growing birds the main fate of the dietary protein is for tissue accretion and, in smaller proportion, for maintenance (26). The relative requirements for growth are highest at hatch and decrease over time, as the chicks' fractional growth rate slows. The suggested requirements of crude protein for 0-12 weeks leghorn chickens in a corn-soybean meal diet (11.9 MJ ME/kg) is 16-18% DM (62). However, because of the higher fractional growth rate of psittacine birds (due to their altricial mode of development), an increase in the total amino acid requirements might be expected. A study of the crop content of free ranging kakapo chicks found 8.4% DM protein in crop samples (7.7 MJ ME/kg)(41) while scarlet macaws chicks in Peru had 23.5% DM protein in their crop contents (40).

Traditionally the AA can be divided into essential amino acids (EAA), whose carbon skeletons cannot be synthesized at all by the body or are synthesized in insufficient quantities to meet cellular requirements, and non-essential amino acids (NEAA) which can be synthesized *de novo*. Although all 20 protein-forming α-amino acids are physiologically essential and should be considered when formulating diets (73-75), only 12 amino acids (arginine, isoleucine, leucine, lysine, methionine, phenylalanine, valine, tryptophan, threonine, glycine, histidine, and proline) are considered essential for birds (62, 74, 76, 77). Amino acid requirements have been studied extensively in commercial fowl (62, 77, 78). However, psittacine AA requirements have received limited attention, with most of the studies focused on adult maintenance requirements (15, 17, 19, 79). The AA dietary requirements depend on the concentrations of the EAAs relative to an animal's needs (80), and will be driven by the concentration of the limiting EAA. The AA balance needed for growth is a close reflection of the profile of AA incorporated into tissue protein (26). The amino acid composition of the tissues of budgerigars is very similar to that of chickens (81), so the balance of AA required may be similar among a broad range of avian taxa (4). Differences in the concentration and balance of AA among species would be driven by the different developmental patterns and fractional growth rates (26). Experiments with captive cockatiels have shown that optimum growth is achieved feeding a diet with 20% DM crude protein (1.0% methionine + cysteine, 1.5% lysine, 14.6 MJ ME/kg) (53). A diet with 13.2% protein (0.65% lysine, 0.78% methionine + cysteine, 13.4 MJ ME/kg) supported maximal growth in growing budgerigar chicks (82).

Fat and fatty acids

Dietary lipids supply energy, essential fatty acids (FA), vitamin transportation, and pigments. Fatty acids have remarkably varied roles in animal physiology. Fatty acids can be saturated (SFA) if all the carbons of the tail are saturated with hydrogen atoms, or unsaturated if they contain one or more double bonds. Depending on the number of double bonds, FA can be monounsaturated (MUFAs), or polyunsaturated (PUFAs). Linoleic acid (C_{18}:2 *n6*) and α-linolenic acid (C_{18}:3 *n3*) are considered essential nutrients because they have double bonds beyond carbon 9 and birds lack the desaturases needed to produce them. These two FA and their derivatives are referred to as the *n-6* and *n-3* families of FA. Arachidonic (C_{20}:4 *n6)* and docosahexaonic acid (C_{22}:6 *n3)* cannot be synthesized by carnivorous mammals or fish from linoleic and linolenic precursors, and it is unknown if birds have the capacity to synthesize them. (83).

Linoleic acid is the only essential FA for which dietary requirements have been demonstrated in poultry (1% DM [11.93 MJ ME/kg] for 0-12 weeks leghorn chickens) (62). The diet of the Hyacinth macaw (*Anodorhynchus hyacinthinus*) and the Lear's macaw (*A. leari*) in Brazil contains predominantly SFA (3). A study of the crop content of free ranging kakapo (*Strigops habroptila*) chicks found 7.8% DM FA (41).

Macro and micro minerals

At least 13 minerals are required for the optimal health and productivity of birds (26). Minerals that serve structural or osmotic functions are required in relatively large amounts in the diet and are referred as the macrominerals: Ca, P, Na, K, Cl and Mg. Minerals that are required at relatively low dietary concentrations are referred as trace minerals: Cu, I, Fe, Mn, S, Se and Zn.

Beyond calcium, the requirement of psittacine birds for other minerals is unknown (4). Calcium is a vital component of bone and body fluid, and is important for chick growth (13). The requirement of calcium for growth has been determined empirically for poultry; it decreases from 1.0% to 0.8% between 0 and 8 weeks showing that the requirement is higher early in life when the growth rate is highest, and decreases in the adult bird. Previous research on scarlet macaws chicks crop content found 1.4% Ca DM (40). Phosphorus is an important constituent of bone, proteins, carbohydrates and lipid complexes (13). Evaluation of the Ca:P ratio in the diet is important as excess P can inhibit the uptake of calcium and result in bone growth abnormalities especially in growing animals (6, 62, 84). To a lesser extent surplus Ca reduces P uptake (6, 85). In leghorn chickens, the Ca to non-phytate P ratio increases from 2.2 to 2.7 between 0 and 8 weeks (62), while in the scarlet macaw crops was found to be 2.9 (40). The rate of skeletal growth of altricial hatchings is considerably faster than that of precocial birds, but the requirement for Ca has not been investigated. Presumably the combination of a faster growth rate and lower calcification of the skeleton at hatching cause altricial species to have greater requirements than precocial species (26).

Study species and sites

Five different Neotropical parrot species of three genera and from three countries were used in this study.

Cuban parrot (Amazona leucocephala bahamensis)

This subspecies breeds in the Caribbean pine forests of Abaco island [annual rainfall of 1,544 mm, monthly temperature means 21 – 27°C (86, 87)]. It is known to feed on 24 plant species (9, 88). During the breeding season their diet is predominately composed of Caribbean pine seeds and cones (*Pinus caribaea*), poisonwood fruits (*Metopium toxiferum*) and wild guava (*Tetrazygia bicolor*). According to extrapolations from observations of the feeding habits during the nesting season, the Cuban parrots feed their chicks a diet containing 22.4% DM protein, 21.3% DM fat, and 10.1% DM ash (9).

Thick-billed parrot (*Rhynchopsitta pachyrhyncha*)

This species nests in the conifer forest of Sierra Madre Occidental of western Mexico above 2,000 m elevation [annual rainfall 400 to 1,100 mm (89)]. This species nests during the peak in production of pine-seeds (90). The quantitative analysis of 102 crops of 64 Thick-billed parrot nestlings in 35 nests (27) showed that the chicks were fed pine seeds (86% by weight), bark (9%), acorns (4%), insects (< 1%) and pine needles (< 1%).

Lilac-crowned parrot (*Amazona finschi*)

This species is endemic to the tropical dry deciduous and semi-deciduous forest of the Pacific coast of Mexico (91) [average annual precipitation 748 mm (92)]. This species feeds on more than 33 different plants during the year (7).

They exhibit high flexibility in their diet, being able to adjust for temporary variations in the food resources (93). Their diet is composed of 82% seed, 9% fruits, 7% insect larva, and 3% bromeliad stems (7).

Scarlet macaw

This species was studied at the Tambopata Research Center (TRC). TRC is located in the lowland forests of south-eastern Peru. The center lies at the boundary between tropical moist and subtropical wet forest at an elevation of 250 m, and receives 3,200 mm of rain per year. This species feeds on 73 food species in the Amazonian rainforest of Peru (35, 94), 43 food species in tropical forest of Costa Rica (95) and 15 plant species during the breeding season in Belize (34). Crop samples from this species have been studied for nutritional content in Peru (40), and species composition in Belize (34). The samples contained a mixture of seeds, fruit, flowers, tree bark, insects, and in the case of Peru, soil from river edge “clay licks” (96, 97).

Red-and-green macaw (*Ara chloropterus*): This species was studied at the Tambopata Research Center (TRC). TRC is located in the lowland forests of south-eastern Peru. In the Amazonian rainforest of Peru it is reported to feed on > 56 food species (35, 94) and use clay licks .

Research objectives

To provide novel information on psittacine nutrition and generate suggestions that help improve the hand rearing of this group.

Specific objectives:

- Assess the feasibility of NIRS as a technique for the non-destructive, low cost prediction of nutritional composition of very small samples of parrot crop contents.

- Document and compare the concentrations of predicted metabolizable energy, crude protein, amino acid profiles, crude fat, FA profiles, fiber, and minerals of the crop samples of chicks from five free-ranging Neotropical parrot species.
- Document the nutritional content and physical characteristics of the main commercial parrot hand feeding products available in the U.S., and compare them with the crop samples of five free-ranging Neotropical parrot species.

CHAPTER II

PREDICTION OF THE NUTRITIONAL COMPOSITION OF THE CROP CONTENTS OF FREE-LIVING SCARLET MACAW CHICKS BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY*

Synopsis

It is difficult to determine with accuracy the nutrition of bird diets through observation and analysis of dietary items. Collection of the ingested material from the birds provides an alternative but it is often limited by the small sizes of samples which can be obtained. We tested the efficacy of near infrared reflectance spectroscopy (NIRS) to assess the nutritional composition of very small samples of growing parrot crop content. We used 30 samples of the crop content of free-living scarlet macaw (*Ara macao*) chicks. Samples were scanned with a Near-infrared Reflectance Analyzer, and later analyzed by traditional wet lab methods for Crude Protein/N, Fat, Ash, Neutral Detergent Fiber, P, K, Ca, Mg, Cu, Zn, and S. A calibration model was developed using principal components analysis. Coefficients of determination in the calibration (R^2) and standard errors of cross-validation (SECV) for most of the nutrients showed a good performance (average R^2 of 0.91 ± 0.11 SD, n = 10) when excluding Zn (R^2 of 0.15, SECV = 25.37). These results establish NIRS as a valid technique for the nondestructive, low cost prediction of a variety of nutritional attributes of avian crop contents as small as 0.5 g dry weight. The use of NIRS expands the possibilities of wild animal nutrition research.

Introduction

Knowledge of avian nutrition, as a component of both their ecology and management, is central to understanding the survival and productivity of the wild populations (6), and has a direct applications in *ex situ* husbandry (98). Through foraging observations it is often possible to identify species' main food sources, but it is difficult to determine the less common items in the diet and quantify the exact proportion of each food type consumed (8, 36). As a result, it is usually impossible to determine the nutritional content of diets through just observation and analysis of dietary items. However, a variety of techniques exist to collect samples directly from the crops and stomachs of individual birds (36, 37). Unfortunately, the small size of the individual samples greatly limits the traditional wet lab analyses which can be done. Pooling samples of similar characteristics is often necessary to achieve the minimum sample sizes needed for analysis but this reduces statistical power needed to address ecological hypotheses (40, 41).

Despite the high percentage of psittacine taxa threatened (99) there is a dearth of studies regarding the nutrition of free ranging psittacines (4). This is in part because the bird's extensive food manipulation and processing makes it very difficult to gather quantitative food intake data. Parrots feed their chicks undigested regurgitate, so tube sampling the crop provides the opportunity to determine the composition of the diet as fed and unaffected by differential digestion (37, 40). However, individual crop samples are usually less than 1.5 grams dry weight while analyses such as proximal and mineral composition commonly require 2.5 grams of sample each.

Near Infrared Reflectance Spectroscopy (NIRS) is an indirect method that estimates chemical composition by comparing spectra of samples with known composition to spectra of samples with unknown composition (100, 101). Near-infrared radiation (750 - 2500 nm) is absorbed mainly by organic bonds (102). The frequencies which match the vibrational waves of these bonds are absorbed, whereas other frequencies are reflected or transmitted resulting in NIR spectra which contain detail on the chemical composition of the material (100). The advantages of NIRS are that it is nondestructive, has high precision, produces no wastes, requires no costly reagents, needs minimal sample preparation and a requires very small sample size (103).

NIRS is widely accepted for compositional and functional analyses in agriculture and manufacturing (104, 105). It is widely used to predict a variety of nutrients in leaves, grasses and grains (106, 107), including amino acids (108), tannins and alkaloids (109), and mineral elements (110, 111). It has been applied in the study of the foraging ecology and nutrition of several wild and domestic herbivorous mammals through the analysis of their diets, excreta and esophageal extrusa (112-114). In avian nutritional studies it only has been used to predict the nutritional composition of feeds and excreta (115-118). In this study we evaluated NIRS as a tool to assess the nutritional composition of small dietary samples collected from wild avian species using crop content samples from wild scarlet macaw chicks.

Methods

Sample description

This study analyzed the crop contents samples from free-living scarlet macaw (*Ara macao*) chicks previously collected in Brightsmith, McDonald et al. (40). Samples were collected during the 2005 breeding season at the Tambopata Research Center in the lowland forests of southeastern Peru (13º 07′ S, 69º 36′ W; 250 masl). Crop contents were sampled following Enkerlin-Hoeflich et al. (37). In this technique, the bird is hand restrained, the crop is massaged, a flexible and lubricated plastic tube is inserted in to the crop through the esophagus, the crop contents pushed up in to the tube, and the tube removed. A total of 48 individual samples were obtained from 10 chicks found in seven nests (average dry weight per sample 1.5 ± 0.9 g). Due to the small samples 13 of the samples were analyzed as independent samples, while the remaining 35 were grouped in 17 composite samples for analysis (average dry weight 2.5 ± 1.6 g). Composite samples were created by combining samples from chicks in the same nest collected on the same day or from chicks of similar age. During preprocess examination of the crop samples it was determined that they contained seeds, wood/bark, fruit pulp, insect larvae, and 19 % of them contained clay (40). All samples were placed in refrigeration at 4°C within 30 minutes of collection. The samples were dried to a constant weight in an oven at approximately 55°C (105), ground to a fine powder (< 1 mm particle size), and stored in airtight containers until analysis.

Standard nutritional analysis

Proximate laboratory analyses were performed at the Palmer Research Center at the University of Alaska. Crude protein was calculated using the Dumas method (105) in a LECO CHN-1000 analyzer for carbon, hydrogen and nitrogen. Crude fat was calculated using the ether extraction method (119). Concentrations of Ca, K, P, Mg, Zn, Cu, and S, were determined by mass spectroscopy (102) after wet ashing (120). Neutral detergent fiber (NDF) was calculated by Van Soest's detergent analysis system (121). Ash was calculated by heating the sample to 550 ^{o}C for 12 hrs. All results are presented on a dry matter basis (105).

Collection of spectral data

Each sample was scanned once with a Perten DA 7200 IR spectrometer (Perten Instruments AB, Sweden). A mirror module was used to accommodate the small sample. The window is made of sapphire, with a surface area of 25 cm^2, with a 256 pixel Indium-Gallium-Arsenide (InGaAs) detector operating in the wavelength range 900-1700 nm. The spectra were stored in optical sensitivity units log (1/R), where R represents the percent of energy reflected (Figure1).

Calibration set and model development

The multi-variant chemometrics package ‘Unscrambler’ (CAMO Software Inc., Woodbridge, USA) was used to process the spectral data from the samples. An independent calibration model for each nutritional attribute was developed through Principal Component Analysis (*PCA*) (122, 123). To evaluate the predictive power of the chosen model we determined the coefficients of determination (R^2) for each nutritional attribute, as a measure of the proportion of variability explained by the regression model.

Validation set

Due to the reduced sample size, for validation of the calibration accuracy we used the cross-validation method with samples from the initial data set (101), avoiding the need to set aside samples for a validation set. The pooled residuals of each prediction (standard error of cross-validation, *SECV*) were calculated to evaluate the precision of the chosen equations for each nutritional attribute.

Results

The NIRS equations for all nutrients except Zn showed strong predictive power. The R^2 between the NIRS predictions and laboratory analyses were above 0.85 for all nutritional variables tested, except Cu (R^2 = 0.63) and Zn (R^2 = 0.15) (Table 1).

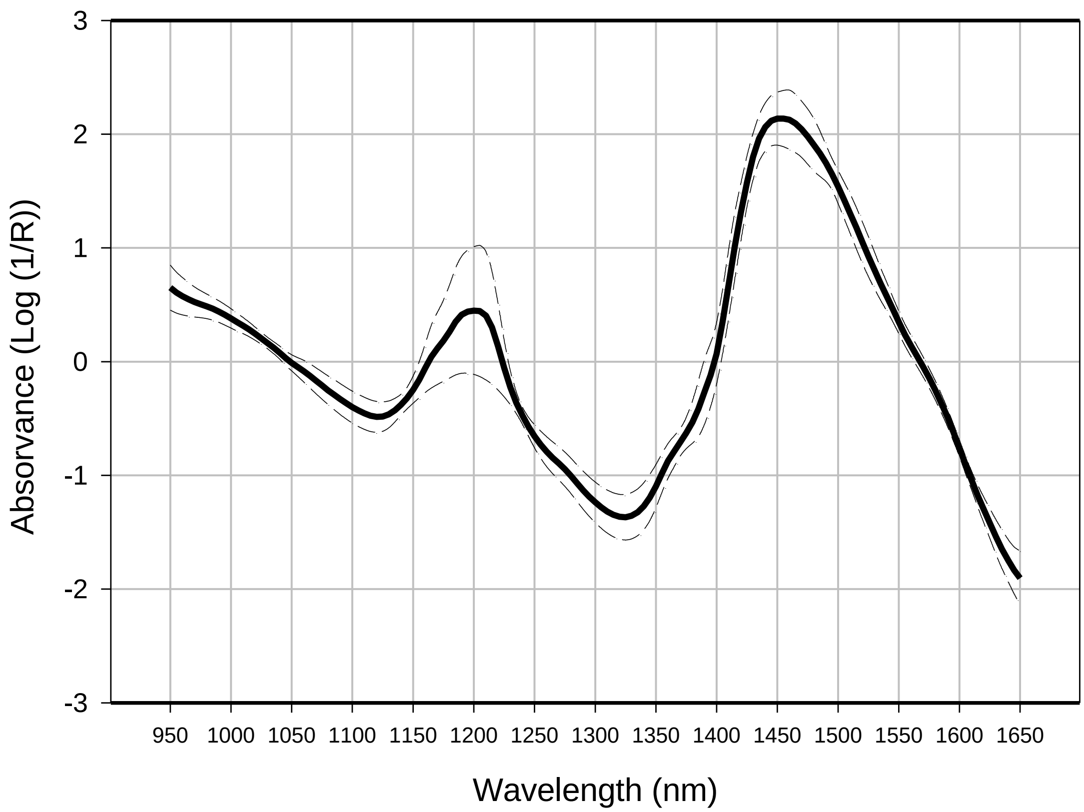

Fig. 1. Near infrared reflectance spectra (950 - 1650 nm) of crop samples from free-ranging scarlet macaws (*Ara macao*) in southeastern Peru.

Discussion

Our study found that NIRS accurately predicted the nutrient contents of the crop contents of scarlet macaw nestlings for 10 of 11 nutrients tested. These results mirror those achieved with commercial plant species such as rice (124) and oats (125), as well as wild plants consumed by mountain gorillas (*Gorilla beringei*) (113), African ungulates (126), and wombats (*Lasiorhinus krefftii*) (112).

Minerals are usually not well predicted by NIRS, unless they are part of organic complexes or chelates, or if concentrations are correlated with other constituents of the sample (109, 111). However, 6 of 7 minerals in our study showed good correlations, and only Zn showed less than 0.60 R^2 suggesting that most of the minerals are bound organically.

Table 1. Nutritional values and calibration equation performance for crop samples from free ranging scarlet macaw (*Ara macao*) chicks collected in southeastern Peru. NDF = Neutral detergent fiber.

	NDF %	Ash %	Fat %	Prot. %	P %	K %	Ca %	Mg %	Cu ppm	Zn ppm	S %
Average	42.8	7.15	19.0	17.3	0.34	0.92	0.88	0.29	14.4	39.8	0.31
SD	26.46	5.71	13.4	11.8	0.22	0.50	1.06	0.28	4.99	10.7	0.45
R^2	0.86	1.00	0.92	0.93	0.93	0.91	0.98	0.99	0.63	0.15	0.99
SECV	10.5	0.35	3.99	3.28	0.06	0.15	0.13	0.02	3.81	25.4	0.03
N	30	19	24	31	29	24	22	28	21	27	19

Our results show NIRS is a valid technique for the nondestructive, low cost prediction of the nutritional composition of avian crop contents. NIRS can be used with samples as small as 0.5 g dry weight, expanding the possibilities of research in the nutrition of parrots and other animals where only small samples are available. In our research we are using NIRS to increase the amount of ecologically relevant information from our samples by 1) scanning individual small samples with NIRS (as small as 0.5 g), 2) combining samples with similar spectra into composite samples large enough for traditional laboratory analysis, 3) scanning these composite samples with NIRS, and 4) conducting laboratory analyses on these composite samples. We can then use the lab analyses on the composite samples to create the NIRS calibration curves and predict the nutritional content of the individual small samples. In this way we can look at the samples in individual scales, and test for nutritional differences among nest mates, habitats, times of day collected, chick age, etc. with much finer resolution than possible without the NIRS. Further studies should explore the possibilities of using NIRS to identify the actual ingredients consumed by the birds (114). Determining the key food resources on which avian species depend will help in understanding their ecology and developing better management and conservation strategies.

CHAPTER III

PREDICTED METABOLIZABLE ENERGY DENSITY AND AMINO ACID PROFILE OF THE CROP CONTENTS OF FREE-LIVING SCARLET MACAW CHICKS (*Ara macao*)*

Synopsis

Hand-rearing of neonates is a common practice for the propagation of psittacines. However, nutritional requirements for their growth and development are not well understood and malnutrition is common. We analyzed the amino acid (AA) profile of the crop contents of 19 free-living scarlet macaw (*Ara macao*) chicks, 19 to 59 days old. Predicted metabolizable energy (PME) density was 16.9 MJ/kg DM, and true protein (total AA protein) 8.3 g/MJ PME. Crude protein (CP) was 10 g/MJ PME, lower than the requirements of 0 to 12 wk old leghorn chicks. The mean concentrations of leucine, isoleucine, threonine, lysine, and methionine on a PME basis were below the minimum requirements of 0 to 12 wk leghorn-type chicks. The calculated PME density of the samples did not vary with age. However there was a significant negative correlation between the average age of the chicks and the lysine concentration. We conclude that the lower CP and amino acid densities in parrot chick crop contents, compared with poultry, could result from a combination of: 1) differences in the essential amino acid composition of the body tissues, 2) adaptations which allow the birds to grow on low protein food sources, and 3) suboptimal nutrition of these free ranging chicks.

Introduction

Despite the increasing volume of research on the nutritional requirements of psittacines (4, 17, 54), malnutrition is still a major concerns for the care and propagation of this group (4, 24). Studies of the diet and nutrition of free ranging psittacines are scarce (8, 9, 34, 41, 127-130), in part because the bird's extensive food manipulation and processing make it very difficult to gather quantitative food intake data.

Hand rearing of neonates is a common practice for the propagation of psittacines, both for the pet industry (44) and for conservation aviculture (46, 48, 50). However the nutritional requirements for the growth and development of neonates are not yet well understood (4, 54, 56, 79), and imbalances are still common (4). Only a few studies have looked at the nutritional content of diets consumed by parent-fed psittacines chicks (40, 41), and the diversity of food habits and ecology among psittacines makes it tenuous to extrapolate from these limited studies to requirements for the Family as a whole.

In growing birds dietary protein is used for tissue accretion and maintenance. The amino acid (AA) balance needed for growth closely mirrors the AA composition in tissues (26). The tissue AA composition of different species is relatively similar, so the difference in AA requirements is mainly driven by the different fractional growth rates (26). Amino acid requirements have been studied extensively in commercial fowl (62, 77, 78). However, psittacines have received limited attention with most studies focused on adult maintenance requirements (15, 17, 19, 79) and only a few data available on growth requirements (4, 54, 56, 79). In the absence of controlled studies of requirements or comprehensive data on the diets of wild birds, nutritional prescriptions for psittacine growth and maintenance are generally extrapolated from dietary recommendations for poultry (4). However, psittacines are not closely related to poultry and differ both developmentally (63) and ecologically

(64), so it is questionable if the available data adequately model their dietary requirements.

The metabolizable energy (ME) densities of diets are the primary factor which determines the amount of food an animal will consume (26). Expressing nutrient concentrations on a per energy basis allows for more meaningful comparison among diets even when the ingested amounts are not known (26). Brightsmith et al. (40) studied the nutritional content of 30 crop samples of scarlet macaw chicks 28 to 60 days post hatch at the same study site. Crude protein, crude fat and mineral concentrations were reported, but neither the AA profile or the ME were described.

The present study provides the first estimates of the ME density and AA profile of free ranging Neotropical parrot chicks. The objectives are (1) to characterize the AA profile and ME concentration of the crop content of wild scarlet macaw (*Ara macao*) chicks, and (2) to compare the AA and ME levels of the crop contents with nutritional information from other psittacines and the domestic chicken.

Methods

Crop samples

We collected crop contents from free-living scarlet macaw chicks 19 to 59 days post hatch from Tambopata Research Center in the lowland forests of south-eastern Peru (13º 07′ S, 69º 36′ W; 250 m elevation). In this region, parrots and macaws consume a diverse mixture of seeds, fruit, flowers, tree bark and soil from river edge “clay licks” (96). Once every 7 to 10 days, crop contents were collected from chicks in nests of wild macaws following the procedures in Enkerlin-Hoeflich et al. (37). Samples were placed in refrigeration at 4°C within 30 minutes of collection. In the 2006 breeding season a total of 38 samples were collected from 10 chicks (mean dry weight per sample 2.4 ± 2.3 g). During the 2008 breeding season a total of 18 samples were obtained from 9 chicks (mean dry weight per sample 1.2 ± 1.1 g). All sampled chicks appeared in good health and fledged at appropriate ages for the species (61). Due to the small quantity of each sample, we pooled samples for analysis. For 2006 a total of 15 composite samples were created by combining samples from chicks in the same nest collected on the same day or from chicks of similar age. The 2008 samples were scanned with a near infrared reflectance spectroscope (Perten DA 7200 IR, Perten Instruments AB, Sweden, for more details see Cornejo et al. (131), and pooling was done according to the similarity of their spectra.

Chemical analysis

Samples were freeze-dried and ground. The crude nutrients were analyzed at the Palmer Research Center at the University of Alaska. N was determined by the Kjeldahl method, crude fat was calculated using the ether extraction method (119), NDF was calculated by Van Soest's detergent analysis system (121), and ash by high temperature ashing (105). True protein was determined as the total AA concentration (132), and crude protein by multiplying total N by a 6.25 factor (133). Soluble carbohydrates were calculated by difference following the formula: % soluble carbohydrates = 100 – % crude protein – % crude fat – % ash – % NDF. True protein, crude protein (CP), and AA concentration are presented as g/MJ predicted metabolizable energy (PME) for comparison among diets and species. PME values of the crop contents of the scarlet macaws and the kakapos, as well as the diet of leghorn chickens, budgerigars (*Melopsittacus undulatus*) and lovebirds (*Agapornis* spp.) were calculated using the formula PME (kJ/100 g DM) = (18.4 x CP) + (36.4 x crude fat) + (16.7 x soluble carbohydrates) (21, 62). Average true and crude protein level are also presented as % DM.

Macaws at the study site feed soil to their chicks (40) but the amount of soil varied among samples (40) and the soil may have led to inflated NDF values due to filtration issues (121). In order to calculate PME using the equation above, we corrected the NDF values for each sample by subtracting the estimated amount of soil ash from the original NDF value. The percent soil ash in each sample was estimated as the total ash minus the average ash content from samples known to contain no soil (6.2 ± 1.4 %, N = 8). We took into consideration that the average ash content of 6.2% is similar to the average ash for natural foods consumed by scarlet macaws in Peru (5.7 ± 3.4 %, N = 17, Brightsmith, unpublished data).

Complete AA analysis was performed in the Amino Acid Laboratory of the Department of Molecular Biosciences, School of Veterinary Medicine, UC Davis. The majority of the essential AAs were analyzed using the Association of Official Analytical Chemists (AOAC) modified method 994.12 (105). Protein hydrolysate was prepared by treating 10-mg finely grinded sample with 2.0 ml of 6 N HCl in a 10 ml evacuated ampule (Wheaton Prescored Gold-Brand, Fisher Scientific Cat No. 12-009-38) for 24 h at 110°C. After flash evaporation using nitrogen gas, the dried residue was dissolved in Biocrom loading buffer. Aliquots were analyzed by ion-exchange chromatography using an LKB Biochrom 30 automatic amino acid analyzer. Methionine (Met) and cystine (Cys) were analyzed separately (AOAC Method 994.12). After performing acid oxidation and subsequent hydrolysis with 6 N HCl, Cys and Met were determined by measuring cysteic acid and methionine sulfone using a Biochrom 30 amino acid analyzer. Tryptophan (Trp) was determined by AOAC method 988.15. After alkali (LiOH) hydrolysis, the quantification was performed using the Biochrom amino acid analyzer. As part of the QA/QC procedure, 1000 nmol/ml of norleucine was included in 6 N HCl as internal standard and casein powder of known AA value was used as reference sample to evaluate the hydrolysis and the chromatography procedures. To determine the reproducibility of the assay, four replicates were assayed using a casein control sample. On average, 99.2% of the sample was recovered, and the CV of the means was below 5% for most of the AAs, except for serine (Ser) and Proline (Pro) (6%), Glycine (Gly) (8%), aspartic acid (Asp) (9%) and Cys (15%).

Published requirements and crop content of other species

For comparison with our results we compiled the published CP and AA requirements for optimal growth of leghorn chickens age 0 to 6 and 6 to 12 weeks (62), broiler chicks 0 to 3 wk old (134, 135) and 3 to 8 wk old (136), and budgerigars and lovebirds estimated using the factorial method (56). We compared our average protein and AA concentrations from crop samples with the pooled crop contents of 15 free ranging kakapo (*Strigops habroptila*) chicks from 10 nests (age 10 to 43 days). The kakapos are phylogenetically far from the macaws (64) and very specialized herbivores, who feed their chicks almost exclusively rimu fruits (*Dacrydium cupressinum*), but it is the only other species of psittacine in which AA profile of the crop contents of parent-reared chicks have been published (41).

Statistical analysis

Mann-Whitney U tests were used to compare the levels of total protein and AAs in the scarlet macaw crop samples between years, and to compare the concentration of ash with the findings of a previous study. One-sample t-test was used to compare the crop nutrient levels with the published requirements for poultry and other psittacine species, and with the average levels found in the kakapo crop contents (41, 54, 56, 62). Linear correlation was used to determine the relation between the average age of the chicks in each sample with the concentration of PME, protein and AAs. Linear correlation was also used to look at the relation between the concentration of protein and of each AA. Statistical tests were conducted by using JMP software (version 8.02; SAS Institute Inc., Cary, NC) with $\alpha = 0.05$. Data are presented as mean ± standard deviation (minimum – maximum).

Results

Predicted metabolizable energy

The scarlet macaw chick crop contents contained, on average, 16.3 ± 4.3% DM (10.4-23.8) crude protein, 22.0± 5.6% DM crude fat, and 37.7± 17.3% DM soluble carbohydrates. The PME density was 16.9 ± 2.6 MJ/kg DM (10.2-19.7). Energy density was not significantly different from commercial diets offered to leghorn chicks ($p > 0.05$) (62), nor the average of 11 parrot hand feeding formulas (16.2 MJ PME/kg, $t = 2.60$, $df = 14$, $p < 0.05$) (56), but was significantly higher than the 7.7 MJ PME/kg received by kakapo chicks ($t = 5.56$, $df = 14$, $p < 0.001$) (41).

Crude and true protein

The mean true protein content, calculated as the sum of the AAs (105), was 13.6 ± 3.9% DM (7.5-20.6), and 8.3 ± 2.9 g/MJ PME (4.1-13.7, N = 15 pooled samples) (Table 2), with no significant differences between years ($U = 10.0$, $p > 0.05$). The CP content was 10.0 ± 3.5 g/MJ PME (5.8-16.9, N = 15 pooled samples). The mean CP content, on a PME basis, was lower than the requirements of 0 to 6 wk old leghorn chicks (16.6 g/MJ PME; $t = 7.33$, $df = 14$, $p < 0.001$), and 6 to 12 wk old leghorn chicks (14.7 g/MJ PME; $t = 5.23$, $df = 14$, $p < 0.001$) (62). It was not different than the requirements of budgerigars (10.2 g/MJ ME; $t = 0.24$, $df = 14$, $p > 0.05$) and lovebirds (9.5 g/MJ ME; $t = 0.54$, $df = 14$, $p > 0.05$) (56), nor the concentration found in the crop of wild kakapo chicks (10.9 g/MJ PME, $t = 1.01$, $df = 14$, $p > 0.05$) (41).

Amino acid profile

The concentrations of the AAs in the crop contents of the scarlet macaws did not differ significantly between years except for Cys, which was 0.25 g/MJ PME in 2006 and 0.05 g/MJ PME in 2008 ($U = 0.00$, $P < 0.01$). As a result,

Table 2. Crude protein and amino acid composition in g/MJ PME of crop contents of free-living scarlet macaw chicks (*Ara macao*), crop contents of kakapo (*Strigops habroptilus)* chicks, and the nutritional requirements of budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis* spp.), and leghorn chickens, (*Gallus gallus*).

Item	Scarlet macaw [1]			Kakapo [2]	Budgerigar[3]	Lovebird [3]	Leghorn chickens[4]	
							0-6 wk	6-12 wk
	g/MJ PME	SD	range	g/MJ PME	g/MJ ME	g/MJ ME	g/MJ PME	g/MJ PME
Crude protein	9.99	3.49	5.81-16.9	10.9	10.2	9.48	16.6[*]	14.7[*]
Arginine	0.88	0.32	0.43-1.49	1.30[*]	0.52[#]	0.46[#]	0.92	0.77
Leucine	0.60	0.22	0.31-1.01	0.82[*]			1.01[*]	0.78[*]
Valine	0.53	0.21	0.22-0.90	0.63[*]			0.57	0.48
Phenylalanine	0.41	0.15	0.18-0.63	0.50[*]			0.54[*]	0.41
Phe + Tyr	0.69	0.24	0.34-1.08	0.83			0.92	0.77
Isoleucine	0.36	0.14	0.18-0.61	0.51[*]			0.55[*]	0.46[*]
Threonine	0.36	0.14	0.17-0.61	0.38			0.63[*]	0.53[*]
Lysine	0.36	0.15	0.20-0.72	0.61[*]	0.33	0.32	0.78[*]	0.55[*]
Methionine	0.17	0.06	0.10-0.31	0.24[*]			0.28[*]	0.23[*]
Met + Cys	0.39[6]	0.14	0.21-0.72	0.51	0.50	0.37	0.57	0.48
Tryptophan	0.08	0.04	0.03-0.14					
Proline	0.42	0.15	0.18-0.68	0.54[*]			0.16[#]	0.13[#5]
Glycine	0.40	0.15	0.19-0.67	0.55[*]				
Histidine	0.23	0.08	0.12-0.36	0.38[*]			0.24	0.20
Glutamic acid	1.32	0.48	0.65-2.24	1.81				

Table 2. Continued

Item	Scarlet macaws [1]			Kakapo [2]	Budgerigar[3]	Lovebird [3]	Leghorn chickens[4] 0-6 wk	Leghorn chickens[4] 6-12 wk
	g/MJ PME	SD	range	g/MJ PME	g/MJ ME	g/MJ ME	g/MJ PME	g/MJ PME
Aspartic acid	0.73	0.35	0.39-1.33	1.23				
Alanine	0.47	0.17	0.24-0.82	0.58				
Serine	0.43	0.16	0.22-0.67	0.54				
Gly + Ser	0.83	0.30	0.41-1.34	1.09			0.65	0.53
Tyrosine	0.28	0.09	0.16-0.48	0.33				
Cystine	0.216	0.11	0.04-0.42	0.26				
Hydroxyproline	0.02	0.03	0.00-0.10					
Citruline	0.02	0.02	0.00-0.07					

[1] Chick crop contents, *n* = 15, 3-8 wk old, 16.87 ± 2.62 MJ PME/kg, total AA protein = 8.29 g/MJ PME
[2] (41) mean chick crop contents. 7.67 MJ PME/kg
[3] (56) minimum growing requirements. 16.22 MJ ME/kg
[4] (62) 11.93 MJ PME/kg

[5] (137)
[6] Significant differences between years (U = 0.00, $p < 0.001$). Cys average 2006 = 0.25 g/MJ PME, 2008 = 0.05 g/MJ PME
[*] Values significantly higher than the scarlet macaw crops ($p < 0.05$)
[#] Values significantly lower than the scarlet macaw crops ($p < 0.05$)

values for all AAs are combined across years for the remaining analyses. The AAs present in the highest concentrations were glutaminc acid (Glu 1.32 g per MJ ME), arginine (Arg 0.88 g), aspartic (Asp 0.73 g), and leucine (Leu 0.60 g) (Table 2). There was a strong positive correlation ($p < 0.001$) between the concentration of each essential amino acid (EAA) and the total protein content (as g per MJ PME). The mean concentrations of five EAAs were below the minimum requirements established for leghorn-type chicks aged 6 to 12 wk: Leu (76% of recommended), isoleucine (Ile 79%), threonine (Thr 69%), Lys (65%) and Met (75%). In addition phenylalanine was also below the minimum requirement for leghorn-type chickens 0 to 6 wk old (75%) (62) (Table 2). None of the EAAs were found in concentrations below the minimum requirements of budgerigars and lovebirds (56). The kakapo chick crops had higher concentrations of all the EAAs except for Thr (41).

Age variations

There was no significant correlation between the age of the chicks and the PME of the samples, the total protein content as percent DM or the protein in g/MJ PME ($R < 0.24$, $p > 0.05$, $N = 12$). However, a significant negative correlation was found between the age of the chicks and the concentration of Lys ($R = 0.56$, $p = 0.005$, $N = 12$) and Met ($R = 0.34$, $p = 0.044$, $N = 12$).

Discussion

The PME density of the regurgitate fed to the scarlet macaw chicks was equivalent to a starter poultry feed (62), and higher than the low quality food used by the kakapo to feed their chicks (41). The daily energy requirements of growing birds change as a function of weight gain rate and body composition (26, 138).As anticipated, the PME density of the diet fed to the scarlet macaw chicks did not change with age(139).

Our samples contained 16.3% CP on a DM basis. A previous study of scarlet macaw chick crop samples from the 2005 breeding season in the same study site found 23.5 ± 5.6% CP (DM basis) (40). We suspect that the difference was not due to annual variations in the composition of available food items, as the weather patterns did not differ greatly between years (Brightsmith, unpublished data). One possible explanation is that the samples from 2005 had lower clay content, which increased the relative concentration of CP on a DM basis. This is supported by the lower ash concentration (DM basis) of the 2005 samples vs. 2006 and 2008 samples (11.7 ± 8.7%, Brightsmith, unpublished data, vs. 26.3 ± 18.9%, $U = 5.84$, $p < 0.05$, respectively). It was not possible to calculate PME of the diet in that original study however, so we can't determine if there is also a difference on an energy density basis.

The CP values found in the scarlet macaw crop samples were lower than expected based on the estimated requirements of leghorn chickens (62), but in line with the requirements estimated for cockatiels and lovebirds (56) and the concentrations found in kakapo chicks (41). We expected that macaw chick dietary protein levels would be higher than those of poultry due to their altricial development and higher growth rate (4). However, birds don't have a CP requirement per se; instead the requirements depend on protein quality, i.e. the concentrations of EAAs and protein digestibility (26, 80). The complexity of the AAs interrelationships makes it difficult to define a protein requirement for any species, and the evaluations should be limited to comparing typical protein intake values among species (25).

The wild diet seems to contain a moderate level of non-protein nitrogen, as indicated by the difference between the crude protein (10.0 g/MJ ME) and the total AA concentration (8.3 g/MJ ME). The CP estimation obtained by multiplying N by the widely used value of 6.25 (133) is 20% higher than the total AA concentration. If summed AA are an accurate reflection of CP, this suggests that the appropriate N:protein conversion factor for true protein would be closer to 5.20, in the range of the 5.18 to 5.46 as proposed for nuts and seeds (140).

The concentrations of half of the EAA found didn't fulfill the requirements of growing 6 week old leghorn chicks. Normally, the profile of EAAs required by birds corresponds to that found in the body tissues (141). The lower densities in the diet of the scarlet macaw compared with the requirements of poultry could be driven by differences in the EAAs composition of the body tissues compared with precocial birds (142, 143). However, the bodies of adult budgerigars and chickens have similar AA profiles (81), and there is no reason to believe that body of scarlet macaws should have a significantly different composition. Deviations from a one-to-one relationship between body and required dietary EAAs can be caused by (a) different turnover rates of individual tissue proteins (77), (b) different digestibilities and efficiencies of reutilization of the EAAs (144), or (c) alternative metabolic fates of different EAAs (145, 146). More studies of the AA metabolism of the scarlet macaw are needed to better understand the apparently low concentrations of EAAs in the diets found here.

We found a 38% decrease in Lys from age 19 through 59 days post hatch. As the chick grows, protein gain as a percentage of total body weight gain decreases, as do the requirements for muscle accretion (62, 77, 136). Around the fourth week post hatch, when the chick starts to grow the flight feathers (147), the AA composition of the body protein changes, decreasing the relative concentration of total Lys and increasing Cys (138, 148). If this relationship holds for scarlet macaws as well, this mechanism could help explain the decrease in Lys found here.

In summary, our research suggests that young scarlet macaws at our site are being fed a diet with a PME protein and EAA concentration similar to the requirements estimated for other psittacines, but lower than the requirements for poultry. Here we discuss two possible explanations for this finding. One possibility is that the birds evolved to raise chicks on low protein food sources.

Adaptations to low protein food sources include low endogenous protein losses and low protein maintenance requirements. In psittacines, these mechanism have been found in strict frugivores including Pesquet's parrots (*Psittrichas fulgidus*) (17), rainbow lorikeets (*Trichoglossus haematodus*) (149), and kakapos. Adult kakapos are known to subsist during the nonbreeding period on a diet of 3.7% DM CP (150). They feed their chicks a diet almost exclusively of *Dacrydium cupressinum* fruit, which is relatively high in indigestible matter and low in CP (10.9 g/MJ PME, 8.4% DM) and other essential nutrients (Table 2) (41). Future research may look at the nitrogen balance of the scarlet macaws, to determine if their physiology is adapted to a low protein diet.

A second possible explanation for the low protein and EAAs concentrations is that the chick diets we studied are not sufficient to promote optimal growth. Experiments with cockatiels have shown that optimum growth is achieved at 20% DM CP and 0.8% DM Lys. However it was not until diets were below 10% DM CP that permanent damage or mortality occurred (54). The macaw chicks at our site fledge successfully and the populations are apparently not decreasing (Brightsmith, unpublished data). However, chick growth rate does not reach the maximum possible for the species as evidenced by the higher growth rates found in other wild populations (93) and hand-raised birds (3, 61, 151). Previous work in this population suggested that the concentration of Na in the chicks' diet may be deficient (40). Our current study suggests that several EAA may also be acting as growth-limiting factors, as has been proposed for other free-ranging parrots (71, 93, 152). As a result, the amino acid profiles presented here should be used with caution when formulating hand-rearing diets for macaws and other psittacines.

CHAPTER IV

FATTY ACID PROFILES OF CROP CONTENTS OF FREE-LIVING PSITTACINES AND IMPLICATIONS FOR HAND-FEEDING

Synopsis

Research on psittacine nutrition is limited and the chick's requirements are poorly understood. Although crude fat is recognized as an important energy component in the formulation of parrot hand-rearing products, fatty acid (FA) profiles have received little attention. To better understand the natural nutrition of psittacines chicks, we analyzed the FA profiles of the crop contents of free-living scarlet macaws (*Ara macao*), red-and-green macaws (*A. chloropterus*),Cuban parrots (*Amazona leucocephala bahamensis*), lilac-crowned parrots (*A. finschi*), and thick-billed parrots (*Rhynchopsitta pachyrhyncha*). We also analyzed 15 commercially available hand-feeding formulas for parrots. The total FA concentration of the crop samples ranged from 12 to 21% dry matter (DM), and in all cases, values were higher than the average in the hand-feeding formulas. The profiles of all crop samples and formulas were dominated by long-chain FA. For both crop samples and formulas the saturated fatty acids (SFA) were dominated by palmitic and stearic acid, the monounsaturated fatty acids (MUFA) by oleic acid, and the polyunsaturated fatty acids (PUFA) by linoleic acid. PUFA were largely dominated by the n6 family, both in the crop samples (7-108:1), and the formulas (15:1). Manufacturers should evaluate if the following profiles improves the performance of their formulas: at least 12% DM long-chain FA and ~20-30% SFA for all species, with the diets for *Ara* spp. and *Rynchopsitta* sp. containing 10-25% MUFA and 55-70% PUFA, and the diets for *Amazona* spp. containing 25-40% MUFA, and ~40% PUFA.

Introduction

The hand-rearing of psittacine chicks is commonly undertaken in the pet-trade (65) and for conservation (46, 48). However, the nutritional requirements of psittacine chicks are poorly understood (4). Nutritional imbalances have been common, and stunted development, rickets, and vitamin deficiencies still occur for some species (4, 5). Hand-feeding diets have generally been extrapolated from dietary recommendations for poultry (62) and modified based on trial and error rather than on scientific study (4).

In recent years there has been a considerable increase in research on the nutritional requirements of psittacines (4, 17, 54); however, the difficulty of gathering quantitative food intake data has prevented more studies of the nutrition of free ranging-psittacines, and most have focused on Austral-Asian species (8, 9, 34, 41, 127-130), The nutritional composition of the diets consumed by parent-fed scarlet macaws (*Ara macao*) from southeastern Peru has been the focus of previous studies (40, 42), but fatty acid (FA) profiles have not been reported. To our knowledge, only one previous study has detailed the FA profile of the diet consumed by a parent-fed psittacines chicks, the kakapo (*Strigops habroptila*) from New Zealand (41).

Crude fat is recognized as an important energy component in the formulation of parrot hand-rearing products, yet FA profiles have received little study. FA are central constituents of dietary lipids as providers of energy, regulators of cell membrane integrity, and precursors of signaling molecules (153), however the individual FA requirements and the ideal FA profile are unknown not only for psittacines but for nearly all birds (4). In poultry nutrition, linoleic and α-linolenic acids are recognized as metabolically essential FA (EFA), serving as precursors of other (n-6) and (n-3) PUFA respectively (154). Deficiency of EFA in chicks causes retarded growth and reduces resistance to diseases (155). Some PUFA derived from the EFA are transformed into eicosanoids that play key roles in the development and immunological responses of growing chicks (154). A diet high in (n-3) PUFA results in the reduction of the anti-oxidative status of broiler chickens (156). The intake ratio of (n-6) to (n-3) PUFA influences the types and amounts of eicosanoids produced in mammalian inflammatory and immune cells (157). A balanced intake of (n-6) and (n-3) PUFA is recommended for poultry in order to maintain the full spectrum of ecoisanoid effects in the body (62). Poultry growth requirements for linoleic acid are satisfied by feeding 1% dry matter (DM) (62), and excessive amounts may result in nutritional encephalomalacia (158). There is no known specific avian dietary requirement for α-linolenic acid (155), although high dietary intake has been suggested as a protective measure against the development of nutritional encephalomalacia in chickens (158) and atherosclerosis in parrots (159).

To gain a better understanding of parrot chick nutrition, we determined the FA compositions of the crop contents from chicks of five free-living Neotropical psittacine species. We also determined the FA composition of 15 commercial hand-feeding formulas. We compared FA concentrations for free-living psittacines and hand-feeding formulas with regard to general profiles, chain length, degree of saturation, and balance between (n-6) and (n-3) FA.

Methods

Crop samples

We collected crop contents from five species of free-living parrots: scarlet macaw (*Ara macao*) and red-and-green macaw (*A. chloropterus*) from the Tambopata Research Center in the lowland forests of southeastern Peru,Cuban parrot (*Amazona leucocephala bahamensis*) from the Abaco National Park on the Abaco Island of the Bahamas, lilac-crowned parrot (*A. finschi*) from the Chamela-Cuixmala Biosphere Reserve in northwest Mexico, and the thick-billed parrot (*Rhynchopsitta pachyrhyncha*) from the north of Mexico (Table 3). The scarlet macaw and the red-and-green macaw are reported to feed on 55 and 51 plant species respectively in the Amazonian rainforest of Peru (160), while the diet of the lilac-crowned parrot comprises 33 plant species in the tropical dry forest of Mexico (27). The breeding diet of both the Cuban parrot (J. Cornejo pers. obs.) and the thick-billed parrot (27) consists of 9 plant species.

Samples were collected following the procedures in Enkerlin-Hoeflich et al. (37) and placed in refrigeration at 4°C within 30 minutes of collection, and frozen at -4°C until analysis. All sampled chicks appeared in good health and fledged at appropriate ages for the species (61, 93). Because of the small size of each sample, we pooled samples for analysis (Table 3). Composite samples were created by combining samples collected from chicks in the same nest on the same day or from chicks of the same species in the same season.

Sample preparation and analysis

Samples were freeze-dried and ground with a mortar and pestle to a fine powder. FA profiles were determined at the Comparative Animal Research Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University. The dried samples were extracted using a modification of the Folch method (105) and total lipid concentration determined gravimetrically (161). Total lipid FA methyl esters were prepared and then fractionated by thin-layer chromatography on Silica Gel-G coated glass plates according to Bauer et al. (162). Samples were recovered using a 50:50 (v/v) mixture of hexane:dietyl

Table 3. Parrot crop samples used for this study. Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*),Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

Species	Source	Breeding season	# chicks	Average age in days (range)	# nests	# original samples	Original samples mean dry weight in g (SD)	# pooled samples
Scarlet macaw	Southeastern Peru	2006	9	42 (26-59)	6	20	4.08 (2.10)	12
		2008	9	63 (20-86)	6	26	1.01 (0.76)	3
Red-and-green macaw	Southeastern Peru	2009	1	64 (32-96)	1	10	1.76 (1.20)	3
		2011	4	46 (27-74)	2	14	1.63 (1.84)	4
Cuban parrot	Abaco Island, Bahamas	2010	27	23 (14-37)	17	35	0.73 (0.45)	5
Lilac-crowned parrot	Western Mexico	2010	15	41 (27-60)	7	44	0.82 (0.46)	6
Thick-billed parrot	Northern Mexico	2010	13	55 (52-58)	8	13	0.70 (0.34)	2

ether, and FA profiles were determined using an OmegawaxTM 320 fused silica capillary as described previously (161).

FA content is presented on a DM basis as well as on a metabolizable energy (ME) basis. Predicted metabolizable energy (PME) values of the crop contents were calculated using the formula PME (kJ/100 g DM) = (18.4 x crude protein) + (36.4 x crude fat) + (16.7 x soluble carbohydrates) (21, 62). The crude nutrients were analyzed at the Palmer Research Center at the University of Alaska. N was determined by the Kjeldahl method, crude fat was calculated using the ether extraction method (119), neutral detergent fiber (NDF) was calculated by Van Soest's detergent analysis (121), and ash by high temperature incineration (105). Crude protein was determined by multiplying total N by a 6.25 factor (133). Soluble carbohydrates were calculated by difference following the formula: % soluble carbohydrates = 100 – % crude protein – % crude fat – % ash – % NDF. Total FA are presented as g/MJ predicted metabolizable energy for comparison among species.

We compared the results with the FA profiles of 15 commercial parrot hand-feeding formulas from 10 different manufacturers (for more details on these formulas, see Chapter V of this dissertation), the previously reported composition of the crop of free-living kakapos chicks (41), the most common food items in the diet of the hyacinth macaw (*Anodrorhynchus hyacinthinus*) and the Lear's macaw (*Anodrorhynchus leari*) (130), the commercial poultry food (163-165), and the most common oils used in animal food industry (166).

Results

Fatty acid profiles

The mean percent FA of the crop contents ranged from 15 to 32% DM (9 to 15 g/MJ ME). In all cases, mean total FA of the five free-living Neotropical psittacines were higher that determined in the kakapo chicks' diet (41), and in the average for hand-feeding formulas (Table 4). Highest concentrations of total

Table 4. Total fatty acid (FA) and crude fat content in crop contents from the five studied free-living psittacine species, the kakapo (41), 15 commercial hand-feeding formulas (Chapter V), and the preferred food of the free-ranging hyacinth macaw (130). Data expressed as average ± SD (range). Values in % of dry matter and in g/MJ metabolizable energy. Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*),Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

	Scarlet macaw (n = 14)	Red-and-green macaw (n = 5)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)	Acuri and Bocaiuva
Crude fat (%DM)	21.6 ± 6.42 (9.53-29.42)	37.7 ± 10.4 (24.1-36.8)	30.5 ± 1.46 (29.2-32.5)	33.8 ± 8.9 (22.5-47.5)	41.4 ± 1.56 (40.3-42.5)	-	11.6 ± 4.61 (7.34-23.6)	
Crude fat (g/MJ ME)	12.4 ± 3.10 (6.34-17.7)	18.6 ± 4.06 (13.0-23.8)	15.9 ± 0.57 (15.2-16.5)	15.3 ± 2.86 (11.5-19.4)	18.6 ± 4.06 (13.0-23.8)	-	6.73 ± 2.10 (4.55-11.5)	
Total FA (% DM)	15.0 ± 5.17 (7.30-24.7)	30.9 ± 5.51 (25.4-36.8)	19.0 ± 2.74 (16.0-21.0)	24.8 ± 4.22 (19.1-29.9)	31.8 ± 0.56 (31.2-32.3)	7.81 ± 0.11 (7.73-7.89)	10.4 ± 4.05 (5.90-22.0)	(60.7-66.4)
Total FA (g/MJ ME)	8.72 ± 3.07 (5.87-15.8)	15.3 ± 1.81 (13.3-17.5)	9.91 ± 1.40 (8.29-11.3)	11.3 ± 1.21 (9.78-12.7)	14.2 ± 0.24 (14.1-14.4)	10.2 ± 0.32 (9.96-10.4)	6.05 ± 1.92 (3.62-10.7)	

FA were presented by the thick-billed parrot, the lilac-crowned parrot, and the red-and-green macaw exceeding even the high-end of the range of total FA for the 15 formulas (Table 4). All but one formula were below the mean total FA concentrations found in the scarlet macaw and in the Cuban parrot. By comparison, the main food plants of the hyacinth macaw had total FA concentrations of 61-66% DM (130), far higher than that presented by the five free-ranging Neotropical psittacines analyzed or the hand-rearing formulas (Table 4).

The profile of all crop samples was dominated by long-chain FA (14-22 carbons, Table 5), with average long-chain FA values ranging from 96% total FA in the Cuban parrot to 99.9% in the red-and-green macaw. No short-chain FA (less than 6 carbons) were found in any of the crop samples. Medium-chain FA (6-12 carbons) and very-long-chain (> 22 carbons) FA were also largely absent from the crop samples (Table 5), the only exception being the Cuban parrot with 4% very-long-chain of total FA in samples (Table 5). The hand-feeding formulas were also dominated by long-chain FA (> 81% FA, Table 5), did not contain short-chain FA, had very small amounts of very-long-chain FA (< 0.8%), and only three products contained medium–chain FA (10-18%). As for the other psittacine species analyzed, the main food plants of the hyacinth macaw (16) were predominantly long-chain FA (58-70%), though medium-chain FA occurred in a greater proportion (30-42%) than that found in the other free-living psittacines or in formulas (Table 5).

Parrot crop samples differed in saturation profiles by species. Crop contents of the thick-billed parrot, red-and-green macaw, and scarlet macaw were dominated by PUFA with mean values of 58-68% PUFA (Table 6). By comparison, saturation profiles of crop contents of the lilac-crowned parrot were more equivalent, and in the Cuban parrot MUFA and PUFA were found in similar proportions of around 40% each (Table 6). Saturated FA SFA and MUFA were found in similar proportions within the crop samples of each psittacine species, except in the red-and-green macaw where concentration of SFA was double MUFA, and in the Cuban parrot where SFA was half the concentration of MUFA (Table 6). By comparison, crop contents of the kakapo had higher mean and range values of SFA and MUFA concentrations, with far lower concentrations of PUFA (12) compared to the five Neotropical psittacines (Table 6).

Crop samples fatty acid profiles of the scarlet macaw, the Cuban parrot and the lilac-crowned parrot were within the range of values for hand-rearing formulas (Figure 2). Hand-feeding formulas differed widely in saturation profiles, but none of the products analyzed displayed a profile as low in MUFA as that of the thick-billed parrot and the green-wing macaw (Figure 2). Compared with the five Neotropical psittacine species analyzed, the diet of kakapo chicks (41) presented a FA profile lower in PUFA and higher in SFA (Figure 2). The mean concentration of PUFA for the kakapo (41) was also much lower than that in any of the hand-feeding formulas (Figure 2). In general, most of the oils used in the food industry (166) had FA profiles much lower in PUFA than that for free-living psittacines or in hand-feeding formulas (Figure 2). Of these, only soy, corn and cotton seed presented profiles within the range of that for free-living psittacines and formulas (Figure 2). The main food plant species for the lear's and hyacinth macaw (130) also had FA profiles with far lower concentrations of PUFA and higher concentrations of SFA than that for the other free-living psittacines and the formulas (Figure 2).

Saturated fatty acids

Palmitic (C16:0) and stearic acid (C18:0) dominated the SFA from all parrot species and from the hand-feeding formulas (Table 7). Palmitic acid was the most common SFA in scarlet macaws (52% SFA), red-and-green macaws (33%),Cuban parrot (52%), and in all the commercial formulas (41-79%) except one (29%; Table 7). Stearic acid dominated the SFA from lilac-crowned parrots (52%) and thick-billed parrot (69%; Table 7).

Table 5. Fatty acid (FA) profile, according to chain length, in crop contents from the five free-living psittacine species, the kakapo (41), 15 commercial hand-feeding formulas (Chapter V), and the preferred food of adult free-ranging hyacinth macaws (130). Profile data are presented as percentage of total fatty acid, mean ± standard deviation (minimum – maximum). Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Abaco parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).* No short-chain FA were found in any of the crop samples.

	Scarlet macaw (n = 15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)	Acuri and Bocaiuva
Medium-chain FA	1.37 ± 2.00 (0.00-5.64)	0.00 ± 0.00	0.13 ± 0.18 (0.00-0.39)	0.16 ± 0.15 (0.00-0.39)	0.00 ± 0.00	5.76 ± 1.90 (4.42-7.11)	2.68 ± 5.78 (0.00-18.3)	(29.6-42.4)
Long-chain FA	98.6 ± 1.99 (94.4-100)	99.9 ± 0.17 (99.6-100)	95.6 ± 1.60 (93.6-97.6)	99.6 ± 0.20 (99.4-99.9)	99.8 ± 0.27 (99.5-100)	94.2 ± 1.86 (92.9-95.5)	97.0 ± 5.71 (81.8-100)	(57.6-70.4)
Very -long-chain FA	0.08 ± 0.08 (0.00-0.23)	0.08 ± 0.14 (0.00-0.40)	4.28 ± 1.69 (2.12-5.95)	0.21 ± 0.08 (0.10-0.29)	0.02 ± 0.04 (0.00-0.07)	0.03 ± 0.04 (0.00-0.05)	0.32 ± 01.8 (0.00-0.81)	(0.00-0.00)

Table 6. Fatty acid profiles by degree of saturation of crop content samples from five free-living psittacine species, the kakapo (41), and the average of 15 commercial hand-feeding formulas (Chapter V). Data presented as percentage of total fatty acid, mean ± standard deviation (minimum – maximum). Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

	Scarlet macaw (n = 15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
SFA	21.1 ± 8.10 (11.6-35.0)	26.0 ± 7.03 (13.3-36.5)	20.2 ± 3.03 (16.8-23.2)	32.1 ± 9.22 (21.6-43.5)	17.8 ± 3.53 (15.7-21.8)	41.8 ± 1.10 (41.1-42.6)	21.5 ± 9.03 (10.4-41.2)
MUFA	20.6 ± 3.35 (14.2-27.3)	15.2 ± 10.8 (6.10-35.3)	39.9 ± 5.88 (30.4-45.9)	27.3 ± 10.4 (15.1-39.4)	13.8 ± 2.58 (11.0-16.1)	35.3 ± 0.56 (34.9-35.7)	28.2 ± 9.22 (19.9-50.8)
PUFA	58.3 ± 8.46 (42.9-71.0)	58.7 ± 13.3 (28.2-68.3)	38.6 ± 8.37 (29.8-50.7)	40.7 ± 9.22 (22.6-49.3)	68.3 ± 1.64 (66.7-70.0)	22.9 ± 0.54 (22.5-23.3)	45.6 ± 10.7 (31.6-68.9)

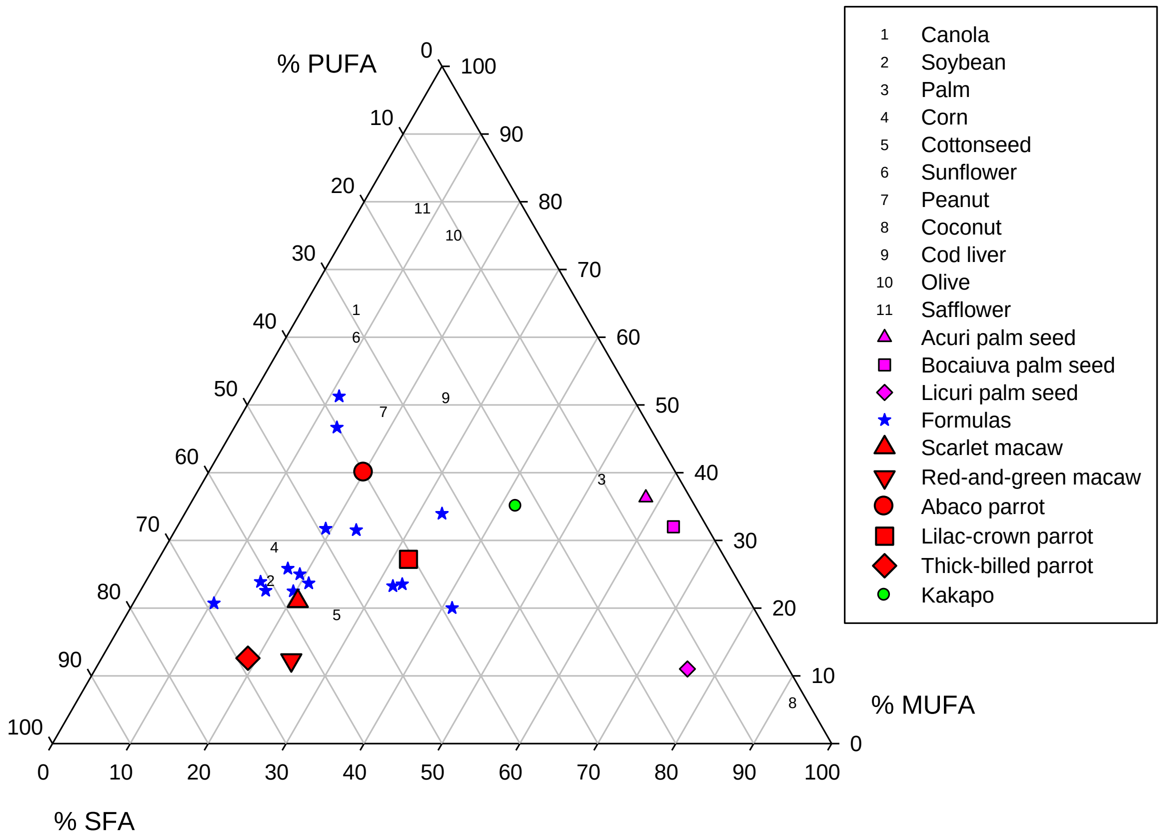

Fig. 2. Fatty acid profile of crop content samples of six psittacine species [this study and (41)], 15 commercial hand-feeding formulas, 11 different commercially available oils (166), the palm fruits that constitute the main foods of the hyacinth and Lear's macaws (130), and the average of three maintenance poultry feeds (163-165). Data presented as percentage of total fatty acids. Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

Table 7. Saturated fatty acid composition of crop content samples from the five studied free-living psittacine species, the kakapo (41), the average of 15 commercial hand-feeding formulas (Chapter V), and the preferred food of the free-ranging hyacinth macaw (130). Data presented as percentage of total fatty acid, mean ± standard deviation (minimum - maximum). Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*), kakapo (*Strigops habroptila*).

	Scarlet macaw (n =15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
C8:0	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	2.93 ± 0.05 (2.89-2.96)	0.00 ± 0.00 (0.00-0.00)
C10:0	0.02 ± 0.03 (0.00-0.10)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.01 ± 0.01 (0.00-0.04)	0.00 ± 0.00 (0.00-0.00)	0.90 ± 0.41 (0.61-1.19)	0.07 ± 0.27 (0.00-1.05)
C12:0	1.35 ± 1.99 (0.00-5.64)	0.00 ± 0.00 (0.00-0.00)	0.12 ± 0.18 (0.00-0.38)	0.16 ± 0.16 (0.00-0.39)	0.00 ± 0.00 (0.00-0.00)	1.94 ± 1.53 (0.86-3.02)	2.59 ± 5.63 (0.00-18.15)
C14:0	4.16 ± 5.13 (0.35-14.07)	0.76 ± 0.50 (0.00-1.45)	0.88 ± 0.11 (0.70-0.98)	0.25 ± 0.14 (0.13-0.52)	0.02 ± 0.03 (0.00-0.05)	0.87 ± 0.01 (0.86-0.88)	1.37 ± 2.42 (0.00-7.77)
C15:0	0.07 ± 0.17 (0.00-0.56)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.04 ± 0.9 (0.00-0.31)

Table 7. Continued.

	Scarlet macaw (n =15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
C16:0	11.1 ± 4.26 (6.79-23.7)	11.1 ± 8.84 (5.42-32.2)	10.4 ± 0.96 (8.86-11.4)	12.1 ± 1.31 (9.92-13.8)	3.98 ± 0.51 (3.41-4.39)	29.8 ± 0.43 (29.5-30.1)	12.8 ± 3.23 (6.78-19.4)
C17:0	0.06 ± 0.04 (0.00-0.09)	0.04 ± 0.05 (0.00-0.12)	0.08 ± 0.09 (0.00-0.21)	0.10 ± 0.04 (0.04-0.16)	0.10 ± 0.09 (0.00-0.18)	0.38 ± 0.02 (0.36-0.39)	0.10 ± 0.18 (0.00-0.65)
C18:0	2.58 ± 1.05 (0.76-4.67)	6.50 ± 3.90 (1.63-13.25)	5.23 ± 2.10 (3.00-7.55)	16.8 ± 10.1 (4.63-30.6)	11.6 ± 3.38 (9.10-15.5)	4.27 ± 0.18 (4.14-4.40)	3.90 ± 1.72 (2.19-9.03)
C20:0	1.50 ± 1.21 (0.37-4.00)	7.31 ± 6.19 (0.38-18.14)	0.67 ± 0.29 (0.36-1.08)	2.03 ± 1.5 (0.58-4.37)	1.93 ± 0.23 (1.72-2.18)	0.51 ± 0.03 (0.49-0.53)	0.31 ± 0.09 (0.18-0.50)
C22:0	0.22 ± 0.20 (0.00-0.63)	0.25 ± 0.17 (0.00-0.44)	2.37 ± 1.13 (0.88-3.99)	0.48 ± 0.38 (0.14-1.19)	0.10 ± 0.08 (0.00-0.15)	0.21 ± 0.15 (0.10-0.31)	0.12 ± 0.21 (0.00-0.65)
C24:0	0.06 ± 0.07 (0.00-0.23)	0.06 ± 0.14 (0.00-0.40)	0.45 ± 0.19 (0.23-0.70)	0.21 ± 0.08 (0.10-0.29)	0.02 ± 0.04 (0.00-0.07)	0.03 ± 0.04 (0.00-0.05)	0.15 ± 0.20 (0.00-0.80)

Table 8. Monounsaturated fatty acid composition of crop content samples from the five studied free-living psittacine species, the kakapo (41), and the average of 15 commercial hand-feeding formulas (Chapter V). Data presented as percentage of total fatty acid, mean ± standard deviation (minimum – maximum). Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

	Scarlet macaw (n =15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
C14:1n5	0.01 ± 0.03 (0.00-0.10)	0.00 ± 0.00 (0.00-0.00)	0.09 ± 0.13 (0.00-0.31)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.03 ± 0.10 (0.00-0.39)
C16:1	0.26 ± 0.16 (0.06-0.66)	0.25 ± 0.30 (0.09-0.98)	0.69 ± 0.20 (0.47-0.87)	0.46 ± 0.21 (0.26-0.83)	3.98 ± 0.51 (3.41-4.39)	0.33 ± 0.01 (0.32-0.34)	0.55 ± 0.55 (0.00-1.94)
C17:1	0.01 ± 0.02 (0.00-0.06)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)
C18:1n9	13.0 ± 5.23 (6.09-25.8)	9.74 ± 9.26 (2.64-31.0)	24.7 ± 2.23 (23.0-28.5)	19.1 ± 9.79 (10.7-32.8)	8.25 ± 1.31 (7.07-9.67)	34.7 ± 0.56 (34.3-35.1)	30.0 ± 9.09 (19.2-49.7)
C18:1n7	1.84 ± 2.42 (0.00-9.68)	1.36 ±1.12 (0.54-3.80)	8.37 ± 3.76 (3.93-11.54)	7.20 ± 7.06 (1.13-20.63)	1.17 ± 0.58 (0.53-1.66)	0.00 ± 0.00 (0.00-0.00)	0.00 ± 0.00 (0.00-0.00)

Table 8. Continued.

	Scarlet macaw (n =15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
C20:1	5.34 ± 3.70	3.85 ± 5.57	1.19 ± 0.57	0.49 ± 0.09	4.26 ± 0.90	0.00 ± 0.00	0.30 ± 0.16
	(1.04-12.8)	(0.30-17.5)	(0.60-1.92)	(0.38-0.59)	(3.28-5.06)	(0.00-0.00)	(0.13-0.65)
C22:1	0.14 ± 0.11	0.00 ± 0.00	1.04 ± 0.55	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.01	0.17 ± 0.19
	(0.00-0.34)	(0.00-0.00)	(0.31-1.85)	(0.00-0.00)	(0.00-0.00)	(0.25-0.27)	(0.00-0.72)
C24:1	0.02 ± 0.04	0.00 ± 0.00	3.78 ± 1.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.12
	(0.00-0.10)	(0.00-0.00)	(1.87-5.39)	(0.00-0.00)	(0.00-0.00)	(0.00-0.00)	(0.00-0.40)

Monounsaturated fatty acids

MUFA were dominated by oleic acid (C18:1n9) in all species (54-70% MUFA), as well as in the all hand-feeding formulas (91-99%, Table 8).

Polyunsaturated fatty acids

Linoleic acid (LA, C18:2n6) was the most common EFA found in the crop samples (67-99%) as well as in the hand-feeding formulas (76-99%, Table 9) Gamma linolenic acid (GLA, 18:3n6) and decosahexaenoic acid (DHA, 22:6n3) were present in significant amounts only in the Cuban parrot samples, in which they were the second (18%) and third (5%) most common PUFA respectively (Table 9). The PUFA of all species were largely dominated by the (n-6) family, with an average (n-6):(n-3) ratio 36:1 (range: 7-108, Table 10). The commercial hand-feeding formulas had a ratio of 3-92:1 (Table 10).

Discussion

The FA profile of the crop content of free-living psittacine chicks differed markedly among genera, with the *Rhynchopsitta sp*. closer to the *Ara* spp. than to the *Amazona* spp*.*

The wide variation in the FA profile of the analyzed hand-feeding formulas suggests that there is not yet a consensus among manufacturers concerning the correct lipid nutrition for growing psittacines. Supplementing the average hand-feeding formula with soybean or cottonseed oil would create a FA profile more closely resembling that of the FA profile of the scarlet macaw. Likewise, supplementation with peanut butter can result in a diet more similar to that naturally fed to Cuban parrot chicks, and supplementing with coconut oil more similar to that of the lilac-crowned parrot. None of the commercial hand-feeding formulas had a FA profile similar to that found in the crops of the green-wing macaws or the thick-billed parrots.

Table 9. Polyunsaturated fatty acid composition of crop content samples from the five studied free-living psittacine species, the kakapo (41), and the average of 15 commercial hand-feeding formulas (Chapter V). Data presented as percentage of total fatty acid, mean ± standard deviation (minimum – maximum). Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

	Scarlet macaw (n = 15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
C18:2n6	45.6 ± 13.6	56.0 ± 13.0	25.8 ± 7.81	31.9 ± 9.17	67.7 ± 1.53	6.93 ± 0.86	44.3 ± 9.87
LA	(22.8-64.9)	(25.9-66.5)	(16.5-36.5)	(19.9-43.5)	(66.2-69.3)	(6.3-7.5)	(29.0-68.2)
C18:3 n6	0.00 ± 0.00	0.00 ± 0.00	7.11 ± 3.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
GLA	(0.00-0.00)	(0.00-0.00)	(2.92-12.3)	(0.00-0.00)	(0.00-0.00)	(0.00-0.00)	(0.00-0.00
C18:3n3	11.4 ± 9.38	2.24 ±1.55	0.88 ± 0.46	8.70 ± 6.55	0.65 ± 0.14	15.9 ± 1.40	5.03 ± 3.45
ALA	(1.52-37.4)	(0.85-4.79)	(0.43-1.52)	(2.03-18.7)	(0.49-0.75)	(15.0-16.9)	(0.74-15.0)
C20:2n6 Eicosadienoic acid	1.23 ± 0.75	0.00 ± 0.00	0.55 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.11
	(0.18-2.40)	(0.00-0.00)	(0.30-0.93)	(0.00-0.00)	(0.00-0.00)	(0.00-0.00	(0.00-0.44)
C20:3n6	0.01 ± 0.02	0.00 ± 0.00	0.99 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
DGLA	(0.00-0.08)	(0.00-0.00)	(0.38-1.85)	(0.00-0.00)	(0.00-0.00)	(0.00-0.00	(0.00-0.00
C20:4n6	0.01 ± 0.02	0.00 ± 0.00	0.80 ± 0.63	0.02 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.13
AA	(0.00-0.10)	(0.00-0.00)	(0.13-1.73)	(0.00-0.10)	(0.00-0.00)	(0.00-0.00	(0.00-0.39)

Table 9. Continued.

	Scarlet macaw (n =15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
C20:5 n3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.31
EPA	(0.00-0.00)	(0.00-0.00)	(0.00-0.00	(0.00-0.00)	(0.00-0.00)	(0.00-0.00	(0.00-1.21)
C22:4n6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.07
Adrenic acid	(0.00-0.00)	(0.00-0.00)	(0.00-0.00	(0.00-0.09)	(0.00-0.00)	(0.00-0.00	(0.00-0.24)
C22:6n3	0.04 ± 0.06	0.00 ± 0.00	2.40 ± 1.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.24
DHA	(0.00-0.19)	(0.00-0.00)	(0.66-4.41)	(0.00-0.00)	(0.00-0.00)	(0.00-0.00	(0.00-0.78)

Table 10. n6 and n3 families of polyunsaturated fatty acids and their ratio in the crop content from the five studied free-living psittacine species, the kakapo (41), and the average of 15 commercial hand-feeding formulas (Chapter V). Data presented as percentage of total fatty acid, mean ± standard deviation (minimum – maximum). Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha)*, kakapo (*Strigops habroptila).*

	Scarlet macaw (n = 15)	Red-and-green macaw (n = 7)	Cuban parrot (n = 5)	Lilac-crowned amazon (n = 6)	Thick-billed parrot (n = 2)	Kakapo (n = 2)	Commercial formulas (n = 15)
(n-6)	46.9 ± 14.2	56.5 ± 12.4	35.3 ± 10.3	32.0 ± 9.12	67.7 ± 1.52	6.93 ± 0.86	44.4 ± 9.91
	(23.0-66.7)	(27.3-66.5)	(23.9-49.7)	(20.0-43.5)	(66.2-69.3)	(6.32-7.54)	(29.0-68.2)
(n-3)	11.5 ± 9.39	2.24 ± 1.55	3.28 ± 1.96	8.70 ± 6.44	0.65 ± 0.14	15.9 ± 1.40	5.24 ± 3.40
	(1.52-37.5)	(0.85-4.79)	(1.09-5.93)	(2.03-18.72)	(0.49-0.75)	(15.0-17.0)	(0.74-15.0)
(n-6):(n-3)	10.1 ± 12.6	35.2 ± 19.1	18.2 ± 17.4	7.05 ± 7.03	107.7 ± 23.6	0.44 ± 0.09	15.5 ± 22.3
	(0.66-43.9)	(13.2-60.7)	(4.04-45.4)	(1.35-20.3)	(92.1-134)	(0.37-0.50)	(3.10-92.4)

In the United States, poultry food is typically formulated with a commercial feed grade animal-vegetable blend of fat (Chapter V). This source of fat is dominated by MUFA (49%) and SFA (41%), with small amounts of PUFA (9%) (62). The (n-6):(n-3) ratio of three commercial poultry diets has been found to range from 5.9 to 6.5 (163-165).

The kakapo feeds its chicks almost exclusively high lipid rimu fruits (*Dacroydium cupressinum*)(167). FA profile of two pooled samples from chicks (13 samples from 10 chicks 10-43 days old, on six nests) (41) presented important contrasts with the studied crop samples (Tables 4-10). The kakapo crop samples had a 7.8% FA in DM basis. The profile was dominated by SFA and MUFA (42 and 35% respectively), with higher values of SFA and lower PUFA than any of the other five species. Oleic acid comprised more than 98% of the total MUFA, and α-linolenic (ALA, C18:3n3) dominated the PUFA (70% total PUFA). Unlike the studied species, the PUFA were dominated by the (n-3) family [(n-6):(n-3) ratio 0.4:1].

The hyacinth macaw and the Lear's macaw have both a specialized diet on the high-fat endocarp of palm fruits (> 60% DM) (3). The hyacinth macaw feeds almost exclusively on the fruits of the acuri (*Scheelea phalerata*) and the bocaiuva (*Acrocomia aculeate*), while the Lear's macaw specializes on the fruit of the licuri (*Syagrus coronata*) (3). The FA profiles of all these fruits are dominated by SFA (more than 55%), and the PUFA levels are relatively low (less than 15%) (130). The acuri FA profile is dominated by medium-chain FA (130). These two parrot species are known for their dietary specialization, and the diversity of food habits and ecology among psittacines prevents the extrapolation to the rest of the family.

When formulating diets it is not enough to provide a source of dietary fat in hand-feeding formulas, rather, the FA profile should also be considered. Unpublished experiments supplementing the hand-feeding diet of 10 parrot species from days 0 to 7 with a modified blend containing added (n-3) FA in the form of algal-based DHA (Trevera®, Novus International, St. Charles, MO) resulted in a significantly greater immune response compared with the control birds, measured through a PHA skin test.

Although it is not possible to determine the parrots' nutrient requirements based solely on diets from free-living parrots, our data suggest that a single formulation may not be ideal for hand-rearing all parrot species, and the diet for *Ara* spp. and *Rynchopsitta* sp. should be different from the diet for *Amazona* spp. Experimental studies should evaluate if replicating the FA profile found in the wild diets improves the performance of their formulas. Suggested profiles are: at least 12% DM long-chain FA and ~20-30% SFA (61 < 16:0 + 18:0 < 90% SFA), with the diets for *Ara* spp. and *Rynchopsitta* sp. containing 10-25% MUFA (18:1n9 ~60% MUFA) and 55-70% PUFA (78 < 18:2n6 < 99% PUFA), and the diets for *Amazona* spp. containing 25-40% MUFA (62 < 18:1n9 < 70% MUFA), and ~40% PUFA (67 < 18:2n6 < 70% PUFA). The suggested importance of a low (n-6):(n-3) FA ratio in parrots' diets (24, 159) is not supported by the analysis of the crop contents of free-living psittacine chicks, and deserves further investigation.

CHAPTER V
NUTRITIONAL AND PHYSICAL CHARACTERISTICS OF COMMERCIAL HAND-FEEDING FORMULAS FOR PARROTS

Synopsis

Hand-rearing is a common practice for the propagation of psittacines, however, research on their nutrition is limited and the chicks' requirements are not well understood. We analyzed the nutrient composition and physical characteristics of 15 commercially available parrot hand-feeding formulas. Formulas were compared with the average nutritional content of the crops of free-living scarlet macaw (*Ara macao*) chicks. When the formulas were prepared by diluting with warm water (1:5), two maintained less than 85% of solids in suspension after 5 min, and only 50% maintained more than 90% of solids in suspension after 15 min. On average the formulas had a similar predicted metabolizable energy density as wild macaw crop samples. The concentration of crude protein in all the formulas was higher than that of the crop sample average, while the crude fat in all formulas was lower than the average crop samples. More than 50% of the formulas had concentrations of K, Mg and Mn less than the crop sample average, and Ca and Na concentrations below the requirements established for 6-12 wk old leghorn chickens. For > 45% of the formulas the concentrations of arginine, leucine and methionine + cystine were below the requirements of 6-12 wk leghorns. When commercial formulas were prepared according to the manufacturer's instructions, nutritional differences among them were greatly magnified. Overall, the inconsistency in the nutrient concentrations among the formulas suggests that there is no consensus among manufacturers of the correct nutrition for growing psittacines and the industry could benefit from continued research in this area.

Introduction

Hand-rearing is a common practice for the propagation of psittacines, both for the pet market (65) and for conservation (46, 48). However, research on psittacine nutrition is limited and the requirements of growing chicks are not well understood (4). Nutritional recommendations for optimal growth are generally extrapolated from dietary requirements for domestic poultry (62) and modified empirically rather than based on scientific study (4). As a result, nutritional imbalances resulting in problems such as stunted development, rickets, and vitamin deficiencies are common (4, 5). Hand-feeding diets for psittacines were traditionally home-made recipes which required elaborate preparation (3, 44, 45, 55, 58) but now there are a wide array of commercially available formulas that require minimal preparation. These formulas are intended to be used without supplementation and fulfill the nutritional requirements of most psittacine species.

The main goal of hand-feeding is to provide the chick with the adequate nutrition that allows it to achieve optimum growth and development. Commercial hand feeding formulas are supplied as dry powder that is reconstituted with warm water and fed as a suspension. The capacity of any formula to maintain the solids in suspension is important in order to avoid the unintentional selection of ingredients from the mixing dish, and the consequent nutritional imbalance. Also it is critical to avoid the separation of the formula in the chick’s crop, as separation allows the liquid to be absorbed rapidly, which can increase solids passage time and lead to fermentation, crop impaction and associated health problems (168).The dilution factor of the formula as fed is important, as formulas need to have enough water to pass quickly through the digestive tract, yet have enough solids to provide adequate nutrient density. To investigate the current “state of the art” in growing parrot nutrition, we analyzed the physical characteristics and nutrient composition of 15 commercial hand-feeding formulas for parrots available in the USA and compared them with the nutritional requirements of leghorn chickens and the composition of the crop contents of free-living scarlet macaws (*Ara macao*) in Peru.

Methods

We examined a total of 15 commercial parrot hand-feeding formulas from 10 different manufacturers (Table 11), representing the main products available in the US market. Samples were purchased from local vendors and kept in closed containers and refrigerated at 4°C until analysis.

The nutrient concentrations of the commercial formulas were compared with the nutrient densities from crop contents of free-living scarlet macaw chicks collected during the 2006 and 2008 breeding season at Tambopata Research Center in southeastern Peru. Crop contents were collected following Enkerlin-Hoeflich et al. (37) from 20 free-living chicks from 14 nests. The average age of the chicks at sampling was 42 days (range 19–59). A total of 117 crop samples were collected, and pooled into 21 combined samples for analysis. Formulas were compared with the amino acid data of the same crop samples already published (42). Although the nutrition of wild birds may have deficiencies (93), the observed fractional growth rates of the scarlet macaw chicks at the Tambopata Research Center (61) are not below those of hand-reared chicks (3), which suggests that the nutrition provided by the parents is adequate for the chicks' growth and development. Formulas were also compared with the nutritional requirements of growing leghorn chickens (62).

The proximate (crude protein, crude fat, ash) and neutral detergent fiber (NDF) analyses were conducted at the Palmer Research Center, University of Alaska. Nitrogen was calculated using the Dumas method (105), crude fat using the ether extraction method, and concentrations of Ca, K, P, Mg, Fe, Na, Zn, Cu, and S determined by mass spectroscopy (102). Soluble carbohydrates were calculated by difference following the formula % soluble carbohydrates = 100 – % crude protein – % crude fat – % ash – % NDF. Complete amino acid (AA)

Table 11. Commercial parrot hand-feeding formulas analyzed and preparation dilutions recommended by the manufacturers.

Manufacturer	Product	% solids 1 week	% solids 6 weeks
Hagen[1]	Tropican Breeding Mash B-2262	20	20
Harrison's Birds Foods[2]	Neonate formula	33	-
Harrison's Birds Foods[2]	Juvenile formula	33	33
Kaytee[3]	Exact Hand-Feeding Formula - Macaw	27.5	30
Kaytee[3]	Exact Hand-Feeding Baby Bird	27.5	30
Lafeber Company[4]	Nutri-Start Baby Bird Formula	25	25
Scenic Bird Food[5]	High Energy Hand-Feeding	25	25
Mazuri[6]	Hi energy formula 5D1W	10	30
Mazuri[6]	Hand-Feeding formula 5TMX	10	30
Pretty bird[7]	Handrearing 19/15	20	30
Pretty bird[7]	Handrearing 19/8	20	28
Roudybush[8]	Formula 3/Optimum Handfeeding Diet	10	30
Ziegler Bros.[9]	Hand-feeding formula	30	40
ZuPreem[10]	Embrace Plus	25	33
ZuPreem[10]	Embrace Hand-Feeding Formula	25	33

[1]Mansfield, MA, [2] Delray Beach, FL, [3] Chilton, WI, [4]Cornell, IL, [5] Plymouth, MN, [6] Saint Louis, MO, [7] Stacy, MN, [8] Woodland, CA, [9] Gardners, PA, [10] Mission, KS

analysis was performed in the Amino Acid Laboratory at UC Davis using the modified AOAC methods 994.12 and 988.15, using an automatic amino acid analyzer [for more details refer to (42)]. True protein was calculated as the sum of the AAs. The predicted metabolic energy was calculated with the following formula: PME (KJ/100 g DM) = (18.4 x % crude protein) + (36.4 x % crude fat) + (16.7 x % soluble carbohydrates) (21, 62).

The capacity of the commercial formulas to remain in suspension was assessed by preparing it with distilled water at 70^{o}C in a 100 ml glass graduated cylinder, and according to each manufacturer recommended dilution for a 1 week old chick. Measurements of the water/solid interface were taken at 5, 15 and 30 min after preparation. The particle size distribution was assessed using a nest of three test sieves arranged in order of descending sieve mesh size (1.0 mm, 0.5 mm and 0.25 mm). A 20 g dry sample was placed on the top sieve and the nest of sieves were manually shaken until the weight of material in the sieves stabilized (169).

The volume of formula needed to fulfill the estimated daily energy requirements of chicks of different ages was calculated for each formula. We followed the manufacturer's recommendations to determine the energy densities of the hand-feeding formulas prepared for hand-feeding scarlet macaws of seven days and six weeks of age (Table 11). The energy requirements of the chicks will depend of their basal metabolic rate, growth, activity, and thermoregulation needs (26). There is no model to predict the daily ME requirement of parrots, but based on the work with altricial species (170) we estimated that a 1 and a 6 weeks old scarlet macaw will need 2.5 and 2.0 times, respectively the ME requirements of an adult psittacine of the same weight; 86 and 837 g body weight respectively (BW)(61). The adult psittacine ME was estimated with the formula ME = 0.647 x $BW^{0.73}$ MJ/day (4). Resulting ME for 1 week = 0.27 MJ/day, for 6 weeks = 1.14 MJ/day.

Results

Physical characteristics

When prepared according to the manufacturer's directions for feeding one week old chicks, almost half of the formulas (seven out of 15) maintained 100% of solids in suspension after 30 minutes. Three formulas showed a 10% decrease in the maintained solids in suspension after 15 min and a 20% decrease after 30 min (Figure 3). On average 2.2 ± 0.4% of the particles were bigger than 1.0 mm, and 54 ± 10.7% were smaller than 0.25 mm.

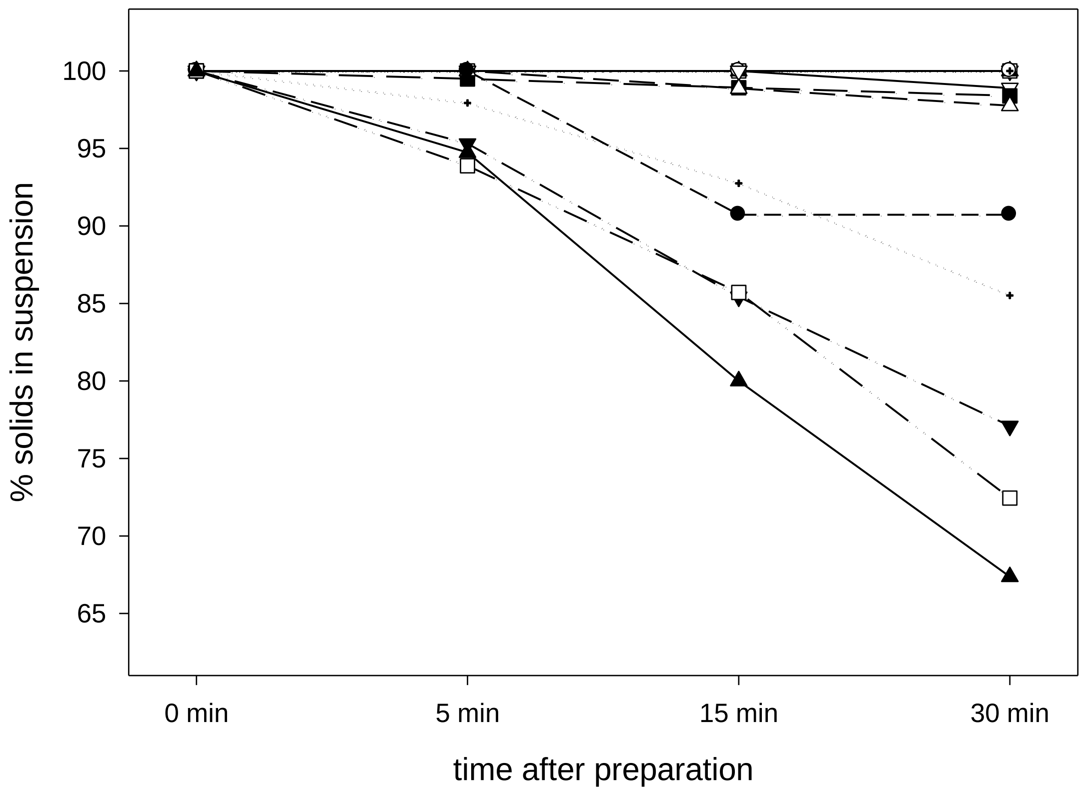

Fig. 3. Percentage of solids in suspension of 15 commercial parrot hand-feeding formulas prepared according to the manufacturer's directions for 1 wk old bird.

Nutritional characteristics

Because of the quantitative and qualitative variability observed in the crop samples, we consider that the complete nutrition of the free-ranging chicks is not achieved at each feeding, but through multiple feeding episodes. For this reason, the crop samples average (and its 95% CI) was used for comparison with the formulas.

The hand-feeding formulas had a similar energy density compared with the free-living macaw crop samples: only two of 15 formulas fell outside the 95% CI for the crop samples (Table 12, Figure 4). In the formulas, the main source of ME is carbohydrates (49.5 ± 8.7%), followed by crude protein (26.1 ± 4.4%) and crude fat (24.4 ±7.6%), while in the crop samples fat is the main ME source (47.0 ± 11.8%), followed by carbohydrates (34.9±16.1%) and crude protein (18.1± 5.2%, Table 12, Figure 4). The concentration of crude protein in all the formulas except one was above the 95% CI of the concentration found in the crop samples, while the crude fat in all formulas was lower than the 95% CI of the average crop samples (Table 12, Figure 4).

The Ca level in13% of the products was below the 95% CI of the crop samples, and in 73% of the cases it was below the requirements for 6-12 wk old leghorn chickens (62). The Ca:P ratio in 93% of the formulas was below the 95% CI of the crop samples and the requirements of 6-12 wk old leghorn chickens (Table 12, Figure 5). The Na concentration in one of the formulas was below the 95% CI of the crop samples, however, in 87% of the formulas it was below the requirement for 6-12 wk leghorn chickens (Table12, Figure 5). The K concentration in 73% of the formulas was below the 95% CI of the crop samples (Table 12, Figure 5). The Na:K ratio of one product was below the 95% CI of the crop samples, but 87% of the formulas had a ratio lower than the requirements of 6-12 wk old leghorn chickens (Table 12, Figure 5). All formulas had Mg and Mn concentrations below the 95% CI of the crop samples, and two of the sampled formulas had a Mn level lower that the requirements of 6-12 wk old leghorn chickens (Table 12, Figures 5 and 6).

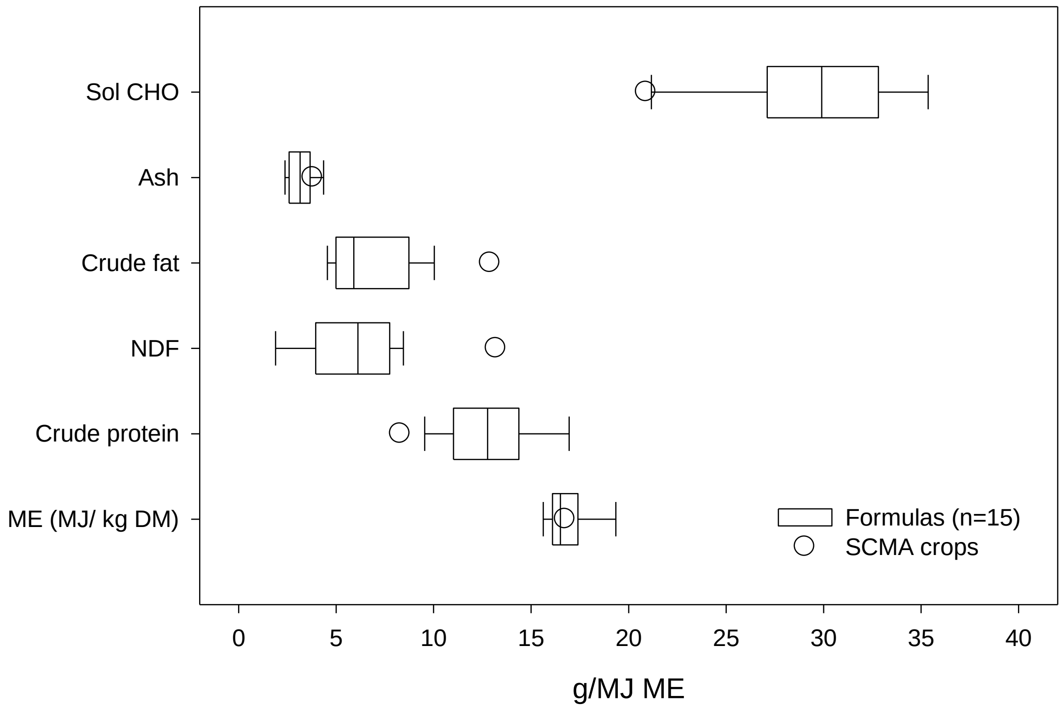

Fig. 4. Proximate analysis of 15 commercial parrot hand-feeding formulas (box plots whiskers representing the 5th and 95th percentiles), compared with the average concentrations found in the crop content of free-living scarlet macaw (SCMA) (*Ara macao*) chicks from southeastern Peru (white dots, n = 21 composite samples). Sol CHO = soluble carbohydrates, NDF = neutral detergent fiber, ME = metabolic energy.

Table 12. Macronutrients and mineral analysis of 15 commercial parrot hand-feeding formulas and the crop of free-living scarlet macaw (*Ara macao*) chicks from southeastern Peru, compared with the nutritional requirements of growing leghorn chickens. CP = crude protein, CF = crude fat, Sol. CHO = soluble carbohydrates, NDF = neutral detergent fiber.

		Hand-feeding formulas (n = 15)					Scarlet macaw crop content (n = 21)			Leghorn requirements[a]
Nutrient	unit	average	SD	min	max	CV	average	95% CI		6-12 wk
ME[b]	MJ/kg DM	16.9	1.3	15.5	20.6	7.6	16.7	15.8	17.7	-
CP	g/MJ ME	14.2	2.4	10.0	18.8	16.9	9.8	8.3	11.3	14.7
CF	g/MJ ME	6.7	2.1	4.5	11.4	31.3	12.9	11.5	14.4	-
Ash	g/MJ ME	3.2	0.71	2.2	4.6	22.0	3.8	3.5	4.1	-
Sol. CHO[c]	g/MJ ME	29.6[d]	5.2	14.2	35.7	17.7	20.9	16.6	25.1	-
NDF[e]	g/MJ ME	5.6	2.3	1.6	9.1	40.6	13.2	16.6	28.4	-
P	g/MJ ME	0.36[d]	0.09	0.21	0.48	25.5	0.20	0.16	0.23	0.33
K	g/MJ ME	0.37	0.12	0.14	0.54	32.6	0.56	0.48	0.64	0.23
Ca	g/MJ ME	0.61	0.16	0.25	0.84	26.3	0.57	0.44	0.59	0.75
Ca:P	ratio	1.7	0.43	1.1	2.9	25.0	2.9	2.5	3.3	2.5
Mg	g/MJ ME	0.08	0.03	0.02	0.13	41.0	0.20	0.17	0.23	0.05

Table 12. Continued.

Nutrient	unit	Hand-feeding formulas (n = 15)					Scarlet macaw crop content (n = 21)			Leghorn requirements[a]
		average	SD	min	max	CV	average	95% CI		6-12 wk
Na:K	ratio	0.30[d]	0.26	0.03	0.92	85.2	0.07	0.03	0.12	0.5[f]
Na	mg/MJ ME	93.8[d]	55.2	8.1	198	58.8	39.2	20.2	58.2	140
Cu	mg/MJ ME	1.5[d]	2.3	0.24	9.7	151	0.80	0.73	0.86	0.37
Zn	mg/MJ ME	8.5[d]	6.0	2.0	22.8	70.6	3.7	3.0	4.3	3.3
Mn	mg/MJ ME	7.4	7.1	1.5	25.2	95.7	43.0	30.6	55.4	2.8
Fe	mg/MJ ME	10.1	7.5	1.1	23.6	74.2	-	-	-	5.6
S	g/MJ ME	0.19	0.05	0.13	0.27	23.8	0.16	0.12	0.19	-

[a]: (62) 11.9 MJ/kg DM

[b]: ME = metabolic energy

[c]: CHO = carbohydrates

[d]: above the 95% CI of the scarlet macaw crop content

[e]: NDF = neutral detergent fiber

[f]: (171)

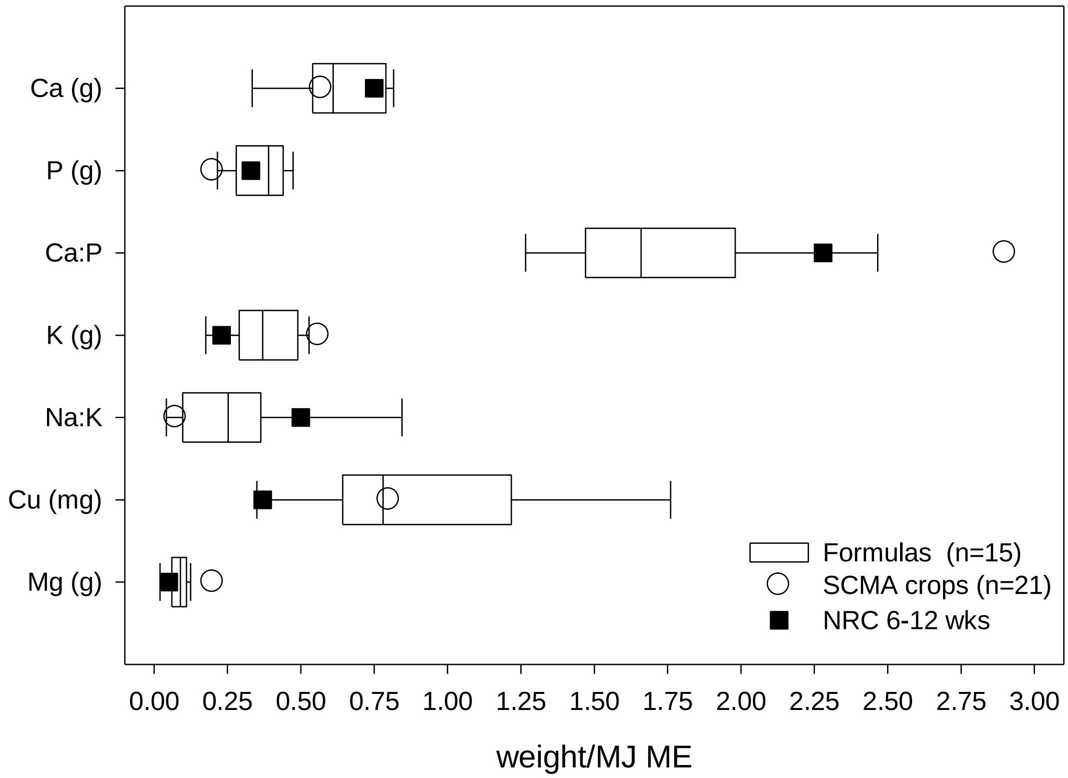

Fig. 5. Mineral concentrations of 15 commercial parrot hand-feeding formulas(box plots whiskers representing the 5th and 95th percentiles) compared with the average concentrations found in the crop content of free-living scarlet macaw (SCMA) (*Ara macao*) chicks from southeastern Peru (white dots, n = 21 composite samples), and the nutritional requirements for growing leghorn chickens [(62), black squares].

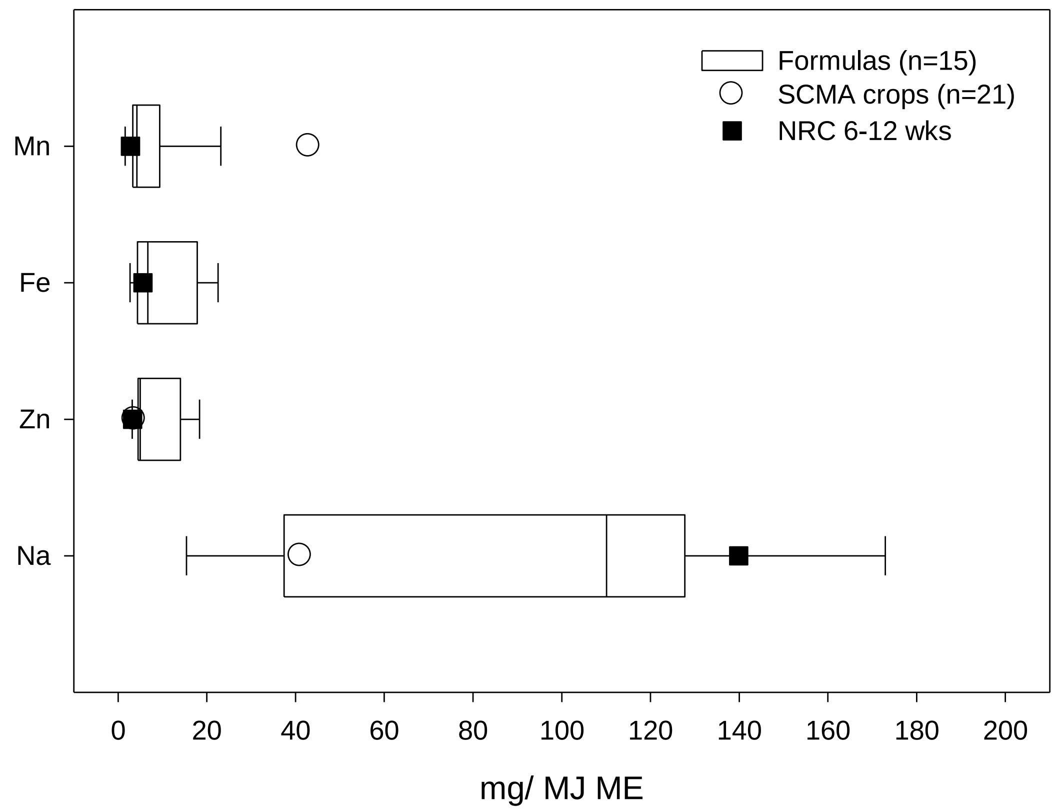

Fig. 6. Mineral concentrations of 15 commercial parrot hand-feeding formulas(box plots whiskers representing the 5th and 95th percentiles) compared with the average concentrations found in the crop content of free-living scarlet macaw (SCMA) (*Ara macao*) chicks from southeastern Peru (white dots, n = 21 composite samples), and the nutritional requirements for growing leghorn chickens [(62), black squares].

Table 13. Comparison of the true protein and essential amino acids profile in ME basis of 15 commercial parrot hand-feeding formulas, the crop content of 20 free-living scarlet macaw (*Ara macao*) chicks from southeastern Peru(42) (n = 15 composite samples), and the nutritional requirements of growing leghorn chickens. ME = metabolic energy, aver. = average.

	Hand-feeding formulas (n = 15)				Scarlet macaw crop content			Leghorn chickens [a]
g/MJ ME	aver.	SD	min	max	aver.	95% CI		6-12 wk
True protein	12.9[b]	2.6	8.2	18.3	8.3	6.7	9.9	-
Arginine	0.76	0.21	0.56	1.39	0.88	10.1	11.1	0.77
Leucine	0.86	0.22	0.60	1.29	0.60	6.9	7.4	0.78
Valine	0.56	0.11	0.34	0.72	0.53	5.9	6.6	0.48
Phenylalanine	0.66	0.13	0.42	0.87	0.41	4.7	5.1	0.41
Isoleucine	0.49	0.11	0.29	0.72	0.36	4.2	4.5	0.46
Threonine	0.60	0.15	0.25	0.84	0.36	4.2	4.5	0.53
Lysine	0.75	0.21	0.35	1.12	0.36	4.0	4.6	0.55
Methionine	0.42	0.21	0.16	1.04	0.17	2.0	2.3	0.23
Meth + Cys	0.52	0.23	0.27	1.19	0.39	0.9	1.1	0.48
Tryptophan	0.17	0.05	0.10	0.25	0.08	4.8	5.2	0.13
Proline	1.73	0.55	0.02	2.58	0.42	4.7	5.2	-
Glycine	0.76	0.22	0.31	1.26	0.40	4.7	4.9	-
Histidine	0.32	0.09	0.11	0.46	0.23	2.7	2.9	0.20

[a]: (62)

[b]: above the 95% CI of the scarlet macaw crop content

The arginine concentration in 40% and 67% of the formulas was below the 95% CI of the crop samples and the requirements of 6-12 week old leghorn chickens, respectively (Table 13, Figure 7). Almost half of the formulas had concentrations of leucine and methionine + cystine less than the requirements of 6-12 week old leghorn chickens(Table 13, Figure 7).

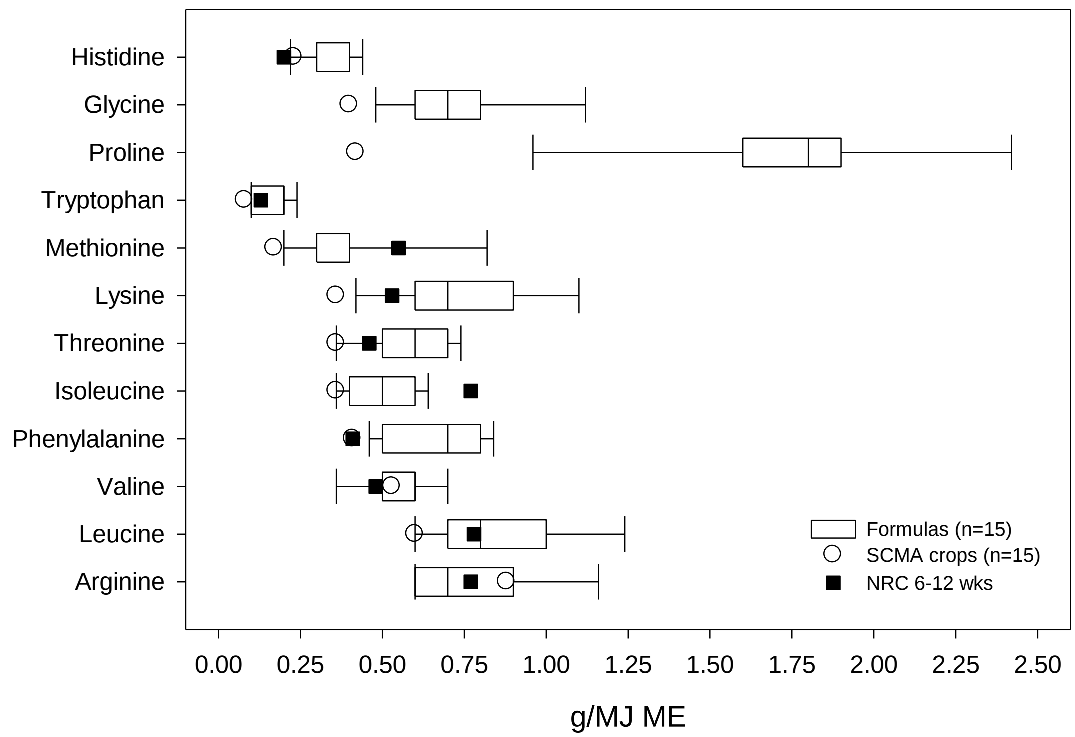

Fig. 7. Comparison of the essential amino acids profile (in ME basis) of 15 commercial parrot hand-feeding formulas (box plots whiskers representing the 5th and 95th percentiles), the crop content of free-living scarlet macaw (SCMA) (*Ara macao*) chicks from southeastern Peru (42) (white dots, n = 15 composite samples), and the nutritional requirements for growing leghorn chickens (62) (black squares).

Dilution effect

The ME density of the powdered formulas as sold varied between 15.5 and 20.6 MJ/kg DM (average = 16.9 MJ/kg, CV = 7.6%). When the formulas were prepared according to the manufacturer's instructions (Table 11) for a six week old chick, the variability in ME density increased by 2-fold (average = 4.96 MJ/kg wet basis (WB), CV = 15.7%, range = 3.30-6.49 MJ/kg WB, Table 14). However, when the formulas were prepared for a one week old chick, the variability increased by 5-fold (average = 3.87 MJ/kg WB, CV = 37.1%, range = 1.55-6.79 MJ/kg ME WB) (Table 14).

Table 14. Average concentration of nutrients in the hand-feeding formula (wet basis) offered to scarlet macaw (*Ara macao*) chicks at one (86 g) and six weeks (837 g) of age, when following the manufacture's preparation suggestions. Weight of chicks according to Vigo et al. (61). CV shown in brackets. Sol. CHO = soluble carbohydrates, NDF = neutral detergent fiber, ME = metabolic energy.

Dilution	ME MJ/kg	Crude protein %	NDF %	Crude fat %	Ash %	Sol. CHO %
1 wk	3.9 (37.1)	5.5(46.2)	2.1 (51.5)	2.7 (64.8)	1.2 (36.9)	11.2 (36.2)
6 wk	5.0 (15.7)	6.9(19.9)	2.9 (38.5)	3.2 (35.4)	1.6 (27.3)	15.2 (17.6)
	P %	K %	Ca %	Mg %		
1 wk	0.13 (37.8)	0.14 (45.7)	0.23 (38.6)	0.03 (55.2)		
6 wk	0.18 (28.3)	0.19 (38.1)	0.30 (31.7)	0.04 (38.0)		
	Na ppm	Cu ppm	Zn ppm	Mn ppm	Fe ppm	S %
1 wk	369.1 (79.8)	6.0 (152.7)	34.1 (74.6)	28.8 (101.8)	42.1 (84.5)	0.07 (36.3)
6 wk	437.2 (62.1)	8.1 (150.8)	42.7 (71.9)	39.1 (97.4)	52.3 (79.8)	0.10 (27.7)

Discussion

In the wild, parrot chicks are fed a coarse textured regurgitate of undigested food items (seeds, fruit, flowers, tree bark and soil from river edge "clay licks") (96, 97) by their parents (40). The largest food particles found in crop samples of scarlet macaws (age 13-77 days) was 9.0 x 3.9 mm (40). Attempts to hand-feed such coarse textured diets increases passage time and the mortality of young chicks (58). It is not yet known why parent-fed coarse textured diets are so readily accepted by chicks, while hand-fed course textured diets are not. It has been suggested that the parents may add enzymes (172) or probiotics (173, 174) that aids in the chicks' digestion. Alternatively, it could just be the gradual habituation of the chick's digestive system which allows them to process these coarser parental diets.

Most hand feeding formulas provide an energy density similar to that of the crop contents of the free-living scarlet macaws. However, the differences found in the proportions of fat, carbohydrates and protein could require dissimilar metabolic pathways, particularly at different stages of development. In some avian species, specific digestive enzymes become functional at different stages of growth (passeriformes (175), phoenicopteriformes (176) anseriformes (177), galliformes (178, 179), and there is increasing evidence that this could be the case in psittacines (172). The effectiveness of protease, carbohydrase, and lipase enzymes at different stages of growth may reflect adaptation to different primary diet ingredients (175, 180), and imply that optimal nutrition may be achieved by altering nutrient balance in hand-rearing diets correspondingly.

Previous studies of crop samples of scarlet macaw chicks in southeastern Peru identified low concentrations of protein and several amino acids compared to the requirements of poultry of similar age (42) (Table 12). Experiments show that adult cockatiels (*Nymphicus hollandicus*) are able to up-regulate enzymes for amino acid catabolism as well as mechanisms for nitrogen excretion when fed high protein diets up to 48 g/MJ ME (19). Growing Australian parakeets (*Melopsittacus undulatus*) fed protein levels from 9.9 to 18.7 g/MJ ME were found to have no increase in either their growth or in the plasma uric acid (82). Experimental studies should evaluate if the higher protein values or imbalanced amino acid ratios of some hand feeding formulas favor increased chick growth or cause damage through overloading the birds' excretory systems.

Low levels of arginine, leucine and methionine + cystine in most formulas could be acting as limiting factors for protein quality (74, 80) and thereby reducing overall growth and development. The low concentrations of some minerals, particularly those used as co-factors for enzymes (Mg, Mn), and/or those necessary for the maintenance of osmotic balance (Na, K), could slow the growth of parrot chicks and/or make them more susceptible to infections. If the mineral requirements of parrot chicks are similar to those of poultry, we would anticipate developmental problems when using some of the analyzed diets. Breeders and veterinarians should look for signs of deficiencies.

It is a common practice to supplement hand-feeding formulas with high nutrient density products like peanut butter, cereal flour, or vegetable oil (45). However, even if supplementing the formulas with ingredients containing similar energy density and lower protein:fat ratios will result in a pattern of energy source more similar to the crop contents of the free-living scarlet macaws, such manipulation should be done with precaution as it will alter also the proportion of all other nutrients and further deficiencies may appear.

The poor capacity of some of the formulas to maintain solids in suspension suggest that different density particles will settle out by weight, resulting in an altered nutrient composition. Moreover, when filled to capacity, crops usually take more than 30 minutes to empty (J. Cornejo pers. obs.), meaning that some formulas are at risk of separation while in the crop. Manufacturers should conduct additional research on this property of their formulas to help minimize digestion and nutritional inconsistency problems.

Formula preparation is critical for correct nutrition when using hand feeding formulas. When too diluted, formulas will not provide the needed nutrient density. When too dense, formulas will cause dehydration and digestion problems like crop impaction. When preparing formulas according to the manufacturer's instructions, nutritional differences among formulas are greatly magnified, especially for young chicks. Depending on the product used, a seven day old scarlet macaw chick weighing 86 g (61), would need to consume 40 to 174 ml of formula per day to fulfill its estimated energy needs (0.27 MJ/day). This is equivalent to 5 to 20 feedings daily (estimating a 10% BW crop capacity). At this age, most manufacturers and hand feeding recommendations call for 6-8 feedings per day, suggesting that chicks would be underfed using 40% of the formulas analyzed here. For a 42 day old scarlet macaw chick [837 g, (61)], the daily amount of formula needed would be between 175 and 345 ml, equivalent to filling the crop two to four times a day (daily energy need: 1.14 MJ/day) which is similar to the 3-4 times per day suggested by practical hand feeding guidelines (58, 168).

The different dilutions will also affect the total daily amount of each nutrient the birds are receiving. Wolf and Kamphues (56) estimated the protein requirement for growing budgerigars (*Melopsittacus undulatus*) to be 9.54 g/MJ ME, and of lovebirds (*Agapornis sp.*) 8.90 g/MJ ME. Assuming similar requirements, a seven day old scarlet macaw chick will need to be fed between 2.4 and 2.6 g of protein a day, and a six week old chick between 10.1 and 10.9 g of protein a day. Depending on which hand-feeding product is used, a seven day old chick being fed eight times a day 10% of its body weight will receive between 2 and 9 g/day (CV = 46%) of crude protein, and a six week old chick fed three times a day 10% of its body weight will be getting between 11 and 24 g/day (CV = 20%) of protein. If these requirements are correct, some products may be providing 2 to 3 times as much protein as required. The nutritional requirements of growing birds depends on their fractional growth rates which are highest during the first days after hatching and drop as the chicks age (26, 181). The use of overly diluted formulas likely underestimates the higher nutrient requirements during the first days of life. This could explain, in part, the delayed development found in the first weeks of hand-fed compared to parent raised parrot chicks (3, 59, 61).

Our findings suggest that manufacturers should investigate if increasing the concentrations of Na, K, Mg and Mn, as well as arginine, leucine and methionine + cystine would have a positive impact in the health and growth of parrot chicks.

A single formulation may not be ideal for hand-rearing parrot chicks from hatching to weaning. Instead, a series of age-specific diets may be more appropriate, as proposed by (182). Alternatively, two products may suffice: a lower nutrient density formula for older chicks and a higher nutrient density product for younger chicks. The two could be mixed to match the requirements of chicks as they mature.

The variation in nutrient densities of the formulas suggests that there is no consensus among manufacturers on the correct nutrition for growing psittacines. As a result, the industry as a whole could benefit from additional research.

CHAPTER VI
NUTRITION OF FREE-LIVING NEOTROPICAL PARROTS CHICKS, AND IMPLICATIONS FOR HAND-FEEDING FORMULAS

Synopsis

The Psittacidae is one of the most endangered families of birds in the world. Knowledge of its nutrition is important for understanding their survival and productivity in the wild, as well as for their adequate husbandry in captivity. Hand-rearing is a common practice for this group, however research on their nutrition is limited. We analyzed the predicted metabolizable energy, protein, fat, minerals and profile of essential amino acids of the crop samples content from free-living chicks of scarlet macaw and red-and-green macaws form southeastern Peru, Cuban parrots in Bahamas, lilac-crowned parrots from northwestern Mexico, and thick-billed parrots from northern Mexico, as well as 15 commercial hand-rearing formulas. Compared with the requirements of 6-12 wk leghorn chickens, all free-ranging parrot diets contained lower Ca and Na concentrations. In comparison with the crop samples, the hand feeding formulas presented lower fat, Mg, and arginine concentrations, as well as much higher levels of Ca and Zn. The nutrition of the different parrots presented important similarities and common patterns. The predicted dietary metabolizable energy and fat concentrations were particularly similar among species. Wider variations were found in the concentrations of Na and Fe, as well as the amino acid Arginine. The thick-billed parrot stood out for its diet higher in crude protein and low Na; the Cuban parrot for its high Na content diet. Nonetheless, the different parrot species displayed a remarkably similar nutritional profile in crop contents, considering their differences in habitat and ecology. Our data suggest that a single formulation could be used to hand-rear *Ara* and *Amazona* spp. of the studied ages. Experimental studies should evaluate if increasing the

concentration of crude fat, Mg, arginine, and valine enhances psittacine chick growth and health.

Introduction

Macaws and other members of the Psittacidae family have been breed in captivity for more than 3000 years (1). In part due to their popularity as pets, they have become the most endangered order of birds in the world [over 25% of the species are listed as threatened and an additional 11% as near-threatened (2)]. Knowledge of nutrition is important for the adequate husbandry of the birds kept as pets, and for the efficient propagation of individuals kept in zoological collections for their ex situ conservation (3-6). It is also needed for understanding survival and productivity (6, 7), and it is therefore critical to implement adequate conservation strategies (3, 8, 9).Hand rearing is a common practice for the propagation of psittacines, both for the pet industry (44) and for conservation aviculture (46, 48, 50). However, the nutritional requirements for growth and development of this group are not well understood (4, 54, 56, 79).Hand-feeding diets have generally been extrapolated from the nutritional requirements of growing poultry (62) and modified empirically rather than thru scientific study (4). Nutritional imbalances have been common, and stunting, rickets, and vitamin deficiencies still occur for some species (4, 5). Psittacines and poultry differ both developmentally (63) and ecologically (64), so it is questionable if the available poultry data adequately model growing psittacine dietary requirements.

There are very few studies looking into the nutrition of free ranging psittacines (8, 9, 34, 41, 127-130), in part because the birds' extensive food manipulation and processing behaviors make it very difficult to determine the ingested nutrition (36, 130). Research into the nutrition of parent-fed chicks has been published in only two species previously, the scarlet macaw (*Ara macao*) from south-eastern Peru (40, 42), and the kakapo (*Strigops habroptila*) from

New Zealand (41). The diversity of food habits and ecology among psittacines makes it tenuous to extrapolate the conclusions from these limited studies to the rest of the family.

The present study provides novel information on the nutrition of free-living Neotropical psittacines during its nesting period, and provides useful data for improving the hand rearing of this group. The objectives are (1) to characterize and compare the predicted metabolizable energy (PME), protein, fat, mineral, and the amino acid (AA)composition of the crop content of free-living chicks of scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), lilac-crowned parrot (*Amazona finschi*), Cuban parrot (*Amazona leucocephala bahamensis*), and thick-billed parrot (*Rhynchopsitta pachyrhyncha*), and (2) to compare the nutritional profile of the crop contents of the studied species with commercial hand feeding formulas manufactured in the US, and with the requirements of growing 8-12 week old leghorn chickens.

Methods

Crop sample collection

We collected crop contents from five species of free-living parrots: scarlet macaw and red-and-green macaw from the Tambopata Research Center in the Tambopata National Reserve in southeastern Peru (12°48'S; 69°18'W), Cuban parrot from Abaco National Park on Abaco Island, Bahamas (26°54'N; 77°25'W), lilac-crowned parrot from the Chamela-Cuixmala Biosphere Reserve (19°27'N; 104°59'W) in northwestern Mexico, and thick-billed parrot from northern Mexico (29°11'N; 108°29'W) (Table 15).

Observations of the feeding habits of the scarlet macaw and the red-and-green macaw report that they feed on 55 and 51 plant species respectively, in the Amazonian rainforest of Peru. The diets are principally composed of seeds with lesser amounts of leaves, flowers, nectar, bark, and insects(160). The diet of the lilac-crowned parrot reportedly contains 33 plant species in the tropical dry

Table 15. Characteristics of the parrot crop samples include in this study. Scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

Species	Source	Breeding season	# chicks	Average age in days (range)	# nests	# original samples	Original samples mean dry weight in g (SD)	# pooled samples without soil	# pooled samples for amino acid analysis
Cuban parrot	Abaco Island, Bahamas	2010	27	23 (14-37)	17	35	0.73 (0.45)	5	5
Red-and-green macaw	Southeastern Peru	2008	1	53 (20-86)	1	9	0.86 (1.84)	1	2
Red-and-green macaw	Southeastern Peru	2009	1	64 (32-96)	1	10	1.76 (1.20)	2	2
Red-and-green macaw	Southeastern Peru	2011	4	46 (27-74)	2	14	1.63 (1.84)	2	3
Lilac-crowned parrot*	Western Mexico	2010	15	41 (27-60)	7	44	0.82 (0.46)	6	5
Scarlet macaw	Southeastern Peru	2006	9	32 (13-59)	6	33	2.78 (2.48)	2	13
Scarlet macaw	Southeastern Peru	2008	10	57 (20-88)	6	68	1.07 (1.84)	5	2
Scarlet macaw	Southeastern Peru	2010	4	54 (25-80)	3	11	2.24 (1.93)	3	-
Thick-billed parrot	Northern Mexico	2010	13	55 (52-58)	8	13	0.70 (0.34)	2	2

forest of Mexico (82% seeds, 9% fruits, 7% insect larvae and 3% bromeliad stems, according to 132 feeding bout observations)(7). Cuban parrots are known to feed on 24 plant species (9, 27, 88). Although during the breeding season it has been observed to use predominately Caribbean pine seeds and cones (*Pinus caribaea*), poisonwood fruits (*Metopium toxiferum*) and wild guava (*Tetrazygia bicolor*). The thick-billed parrot are known to feed primarily on immature and mature pine seeds of various species, and to lesser extent on acorns (*Quercus* spp.), alligator juniper berries (*Juniperus deppeana*), bark, nectar from agave flowers, and insects (27, 89). The quantitative analysis of 102 crops of 64 thick-billed parrot nestling in 35 nests analyzed in 1996-1997 showed that the chicks were fed three or four species of pine seeds, bark, acorns, insects and pine needles. The pine seeds made 87% of the diet by weight (27). In addition, both macaws have been documented consuming clay from river edge “clay licks” and feeding it to the chicks (96).There are reports of the lilac-crowned parrot and the thick-billed parrots practicing geophagy outside the breeding season (183).

Samples were collected from chicks’ crops following Enkerlin-Hoeflich et al. (37), placed in refrigeration at 4°C within 30 minutes of collection, and then frozen at -4°C until analysis. All sampled chicks appeared in good health and fledged at appropriate ages for the species (9, 27, 61, 93). Because of the small size of each crop sample collected from each bird, we pooled samples for analysis (Table 15). Composite samples were created by combining samples collected from chicks in the same nest on the same day or from chicks of the same age in the same season. Scarlet macaw 2008 samples were scanned with a near infrared reflectance spectroscope (Perten DA 7200 IR, Perten Instruments AB, Sweden, for more details see Cornejo et al. (131), and pooling was done according to the similarity of their spectra.

Chemical analysis

Samples were freeze-dried and ground. The crude protein, crude fat, ash, neutral detergent fiber (NDF), and mineral analyses were conducted at the Palmer Research Center at the University of Alaska. Nitrogen was determined by the Kjeldahl method, crude fat was calculated using the ether extraction method (119), NDF was calculated by Van Soest's detergent analysis system (121), and ash by high temperature ashing (105). Concentrations of Ca, K, P, Mg, Fe, Na, Zn, Cu, and S were determined by mass spectroscopy (102). Crude protein was calculated by multiplying total N by a 6.25 factor (133). Soluble carbohydrates were calculated by difference following the formula: % soluble carbohydrates = 100 – % crude protein – % crude fat – % ash – % NDF. Predicted metabolizable energy was calculated using the formula PME (kJ/100 g DM) = (18.4 x % CP) + (36.4 x % crude fat) + (16.7 x % soluble carbohydrates) (21, 62).

The soil present in the *Ara* spp. samples led to inflated NDF values due to filtration issues (121) and prevented their use in the calculation of PME. Considering that scarlet macaw samples known to contain no soil had an ash content of 6.2 ± 1.4% DM [N = 8 (42)], and that ash for natural foods consumed by scarlet macaws in Peru is on average 5.7 ± 3.4% (N = 17, Brightsmith, unpublished data), we considered those samples from *Ara* spp. with an ash content > 9% DM (6.2 + 2 x SD) to contain clay, and therefore were excluded from the study.

Complete AA analysis was performed in the Amino Acid Laboratory at UC Davis using the modified AOAC methods 994.12 and 988.15, using an automatic amino acid analyzer (for more details of the methods refer to (42). True protein was calculated as the sum of the AAs.

Proximate analyses are presented as weight/MJ PME for comparison among species and with hand-feeding formulae. AA are presented as % of the true protein to compare among species and with the hand-feeding formulas, and as g/MJ PME to compare with poultry requirements. Average values are presented as: X ± SD (min-max), and coefficient of variation (CV). The metabolizable energy density of diets is the primary factor which determines the amount of food an animal will consume (26). Expressing nutrient concentrations on a per energy basis allows for more meaningful comparison among diets even when the ingested amounts are not known (26).

Published references

The nutrient concentrations of the crop samples were compared with those of 15 commercial hand feeding formulas (Chapter V), and with the nutritional requirements of 6-12 wk leghorn chickens (62). The AA profile of the scarlet macaw samples were previously published on a PME basis (Chapter III); we used them as comparison after converting them to % of true protein.

Total daily nutrient intake

The energy requirements of the chicks will depend on their basal metabolic rate, growth, activity, and thermoregulation needs (26). There is no model to predict the daily metabolic energy needs of parrots, but based on the work with other altricial species (170) we estimated that the studied psittacine chick, 2/3 through its nesting period, will need 2 times the metabolizable energy requirements of an adult of the same weight [$ME = 0.647 \times BW^{0.73}$ MJ/day (4)]. We calculated the total daily intake for each nutrient dividing the daily energy requirements of each species by the energy density of each diet. Average body weight of the chicks of different species were obtained from: scarlet macaw (61), red-and-green macaw (Brightsmith, unpublished data), lilac-crowned parrot (93), Cuban parrot (Stahala, unpublished data), and thick-billed parrot (Cruz-Nieto, unpublished data) (Table 15).

Results

Crop sample contents

Fifty percent of the collected red-and-green macaw samples contained soil [42.7 ± 34.8 % DM (5.86-86.4)], as did 58% of the scarlet macaw samples [26.6 ± 15.6 % DM (4.20-53.5)], so were excluded from the study except for the AA analysis. None of the samples from other species contained any detectable amount of soil. The macaws' crop samples contained more than 10 different plant species, but it was not possible to identify them. Nine different species of plant were identified in the crop of the Cuban parrot and eight in the lilac-crowned parrot's. All the thick-billed parrot crops contained pine seeds of two species, 71% contained bark, and 7% contained insect's larva. Bark and insects were present also in the samples taken from all other species.

Nutritional characteristics

The mean PME ranged from 16.8 MJ/kg for the scarlet macaw to 22.5 MJ/kg for the thick-billed parrot (CV = 11.3%) (Table 16, Figure 8). The PME of most the hand feeding formulas was below that of the parrot crop contents (15.2-20.4 MJ/kg). All species had a similar crude protein concentration, ranging from 11.5 to 14.5 g/MJ ME, except for the thick-billed parrot that had a higher concentration (17.8 g/MJ PME) (Table 16, Figure 9). Most of the hand-feeding formulas had crude protein concentrations greater than the average of the crop samples of all the species, except for the thick-billed parrot. Only the thick-billed crop samples had crude protein above the requirements for 6-12 wk old chickens. The mean concentration of crude fat of the crop contents was very similar, ranging from 15.3 to 18.5 g/MJ PME (Table 16, Figure 10). All the formulas had a crude fat concentration below the crop sample averages for all species. The proportions of fat, protein and ash + carbohydrates of the crop samples of *Amazona* spp. and *Ara* spp. occupy a similar and narrow nutritional

Table 16. Predicted metabolizable energy (PME) and proximate analysis of crop contents from the five studied free-living psittacine species compared with the analysis of 15 commercial hand-feeding formulas (Chapter V), and the nutritional requirements of 6-12 wk leghorn chicken (11.9 MJ/kg) (62). Data expressed as average ± SD (range). Species include: scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

	Cuban parrot (n = 5)	Red-and-green macaw (n = 5)	Lilac-crowned parrot (n = 6)	Scarlet macaw (n = 10)	Thick-billed parrot (n = 2)	Hand-feeding formulas (n = 15)	Leghorn chicken 6-12 weeks
ME (MJ/kg)	19.2 ± 0.45 (18.6-19.6)	19.5 ± 1.36 (18.4-21.8)	21.7 ± 1.74 (24.4-29.4)	16.8 ± 3.38 (10.2-22.0)	22.5 ± 0.91 (21.8-23.1)	(15.2-20.4)	
Crude protein (g DM/MJ PME)	13.1 ± 1.53 (11.0-15.1)	11.5 ± 1.59 (9.38-13.7)	12.4 ± 2.46 (10.2-16.9)	12.6 ± 2.83 (9.00-18.0)	17.8 ± 2.87 (15.7-19.8)	(10.2-18.9)	14.9
Crude fat (g DM/MJ PME)	15.9 ± 0.57 (15.2-16.5)	17.2 ± 2.87 (12.1-21.0)	15.4 ± 2.86 (11.5-19.4)	17.0 ± 3.67 (12.1-22.1)	18.5 ± 1.45 (17.5-19.5)	(4.55-11.5)	-
CHO (g DM/MJ PME)	20.6 ± 1.67 (19.4-23.4)	19.2 ± 6.22 (10.7-26.1)	16.8 ± 4.90 (9.7-23.2)	29.0 ± 13.4 (13.7-58.9)	5.29 ± 2.64 (3.42-7.15)	(15.5-40.6)	-
Ash (g DM/MJ PME)	2.58 ± 0.44 (2.21-3.23)	3.57 ± 0.39 (3.15-4.17)	1.80 ± 0.33 (1.36-2.29)	3.58 ± 1.28 (1.48-5.10)	3.02 ± 0.59 (2.61-3.44)	(2.03-5.11)	-

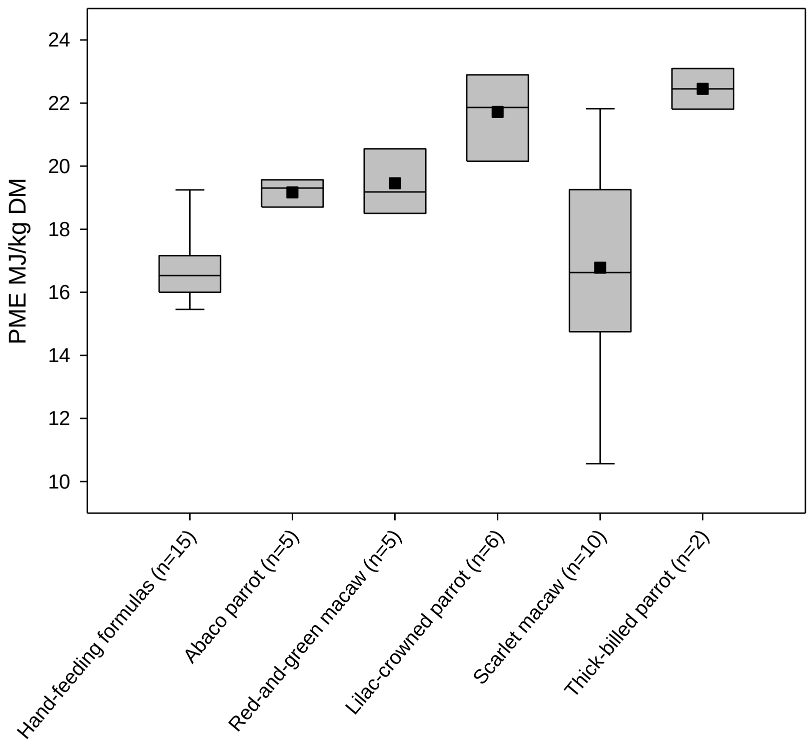

Fig. 8. Predicted metabolizable energy (PME) in the crop samples from free-living chicks of five different parrot species and commercial parrot hand-feeding formulas (Chapter V). Box plots presenting mean (squares), median (horizontal line) and 5th and 95th percentile (whiskers) of the data from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

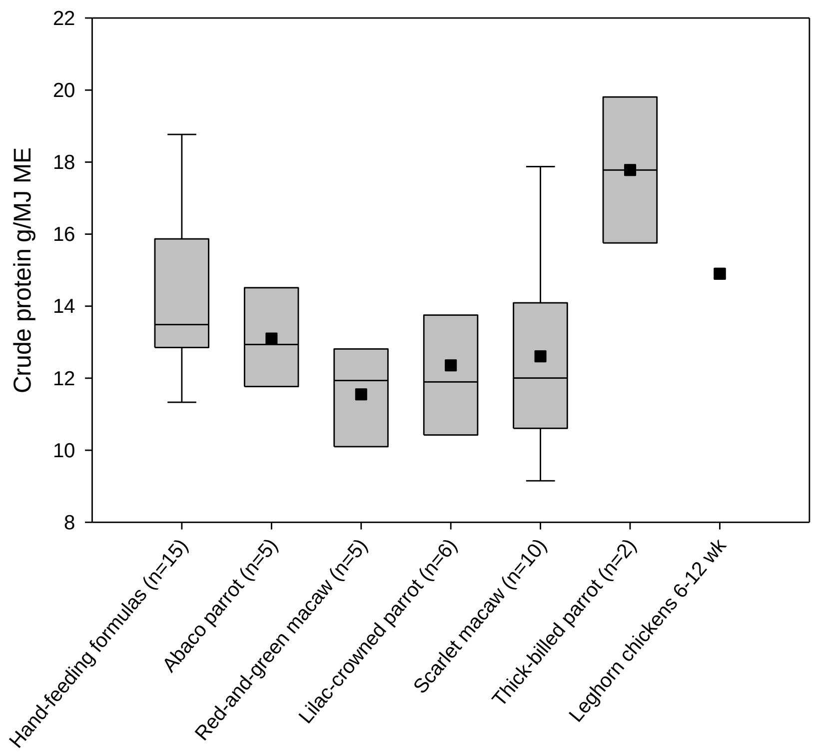

Fig. 9. Crude protein in the crop samples from free-living chicks of five different parrot species, commercial parrot hand-feeding formulas (Chapter V), and the requirements of 6-12 wk leghorn chickens (62). Box plots presenting mean (squares), median (horizontal line) and 5th and 95th percentile (whiskers) of the data from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

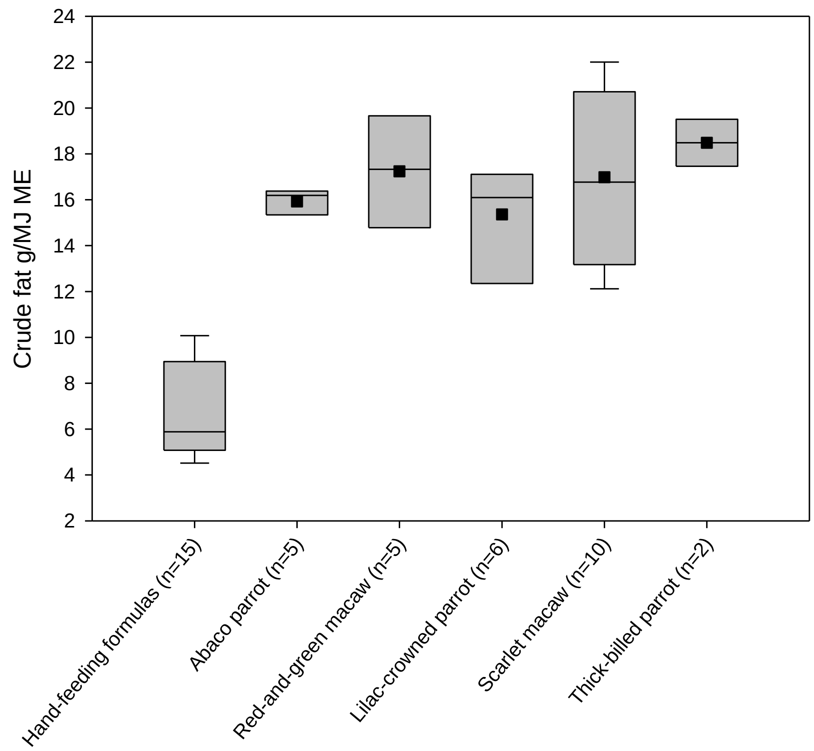

Fig. 10. Crude fat in the crop samples from free-living chicks of five different parrot species and commercial parrot hand-feeding formulas (Chapter V). Box plots presenting mean (squares), median (horizontal line) and 5th and 95th percentile (whiskers) of the data from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

space, with the thick-billed parrot the species most different from the others (Figure 11). The hand-feeding formulas occupy, with only one exception, a wider but also well-defined nutritional space, which differs from the crop samples by having a lower proportion of fat and higher carbohydrate content.

Mean Ca concentrations in the crop samples varied between 0.18 g/MJ PME for the lilac-crowned parrot and 0.56 g/MJ PME for the scarlet macaw (Table 17, Figure 12). Mean P ranged between 0.16 g/MJ PME for lilac-crowned parrot and 0.44 g/MJ PME for the thick-billed parrot (Table 17).The Ca:P ratio varied between 0.87 and 2.62; the Cuban parrot had the lowest concentration and both *Ara* spp. a ratio > 2.0 (Table 17, Figure 13). The Na concentration in the crop samples ranged widely between 3.91 mg/MJ PME for the thick-billed parrot and 72.8 mg/MJ PME for the Cuban parrot (Table 17, Figure 14). The K concentration had the smallest variation among species (0.34-0.47 g/MJ ME, CV = 15.2) (Table 17). The Na:K proportion ranged between 0.01 for the thick-billed parrot and 0.17for the Cuban parrot (Table 17). The Fe concentration of the *Ara* spp. crop contents was more than 10-fold higher than for the *Amazona* spp. and the thick-billed parrot (Table 17).The majority of hand feeding formulas had higher Ca and Na levels than the crop samples. The majority of hand feeding formulas also had higher P and Zn levels than any of the species' crop samples, with the exception of the thick-billed parrot. The majority of hand-feeding products had lower concentrations of Mg that any of the studied species' crop samples. Most of the hand-feeding formulas had a lower level of Fe than the crop samples, except for the lilac-crown parrot.

The profile of essential amino acids (EAA) as % of true protein was dominated in all species by arginine (average 13.9% of true protein);especially in the thick-billed parrot and the Cuban parrot, where it was more than 3 times higher than any of the other essential amino acids (Table 18). Proline was the only AA found in all hand-feeding formulas at a higher concentration than in the crop samples. Most of the formulas had concentrations of arginine and valine

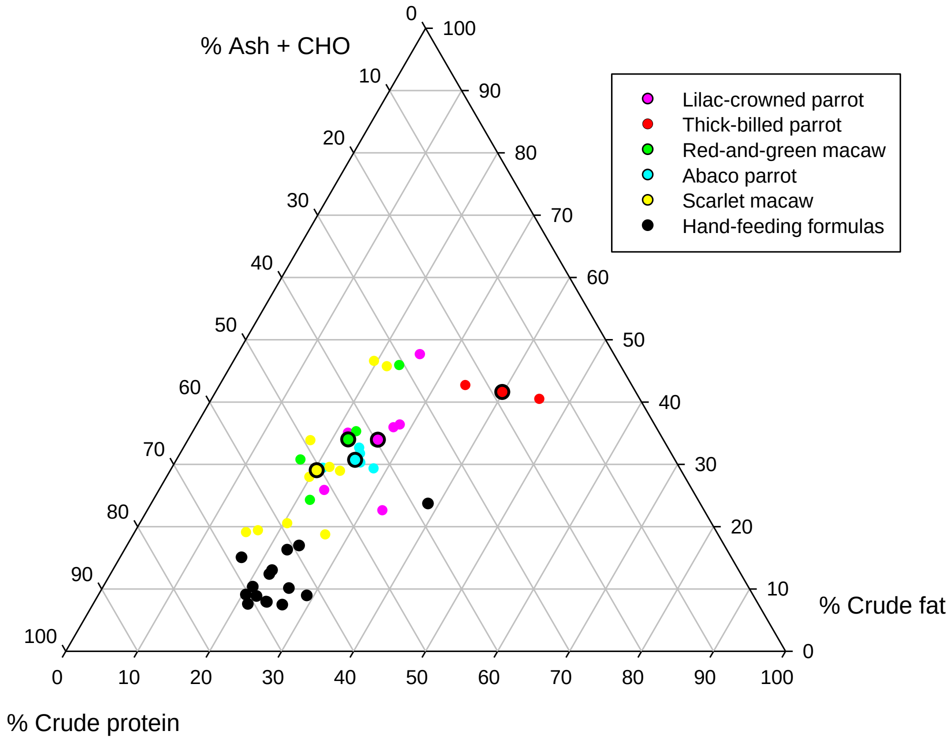

Fig. 11. Relative proportions of crude protein, fat and carbohydrates (CHO) + ash per unit of ME, of the crop samples from free-living chicks of five different parrot species (color dots), and 15 commercial parrot hand-feeding formulas (black dots)(Chapter V). Circled dots represent averages for each species: scarlet macaw (*Ara macao,* n = 10), red-and-green macaw (*Ara chloropterus,* n = 5), Cuban parrot (*Amazona leucocephala bahamensis,* n = 5), lilac-crowned parrot (*Amazona finschi,* n = 6), thick-billed parrot (*Rhynchopsitta pachyrhyncha,* n = 2).

Table 17. Macro and micro mineral analysis of crop contents from the five studied free-living psittacine species, from 15 commercial hand-feeding formulas (Chapter V), and the requirements of 6-12 wk leghorn chickens (11.9 MJ/kg)(62). Data expressed as average ± SD (range). Species include scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

	Cuban parrot (n = 5)	Red-and-green macaw	Lilac-crowned parrot (n = 6)	Scarlet macaw	Thick-billed parrot (n = 2)	Hand-feeding formulas (n = 15)	Leghorn chickens 6-12 wk
Ca (g/MJ PME)	0.24 ± 0.15 (0.11-0.48)	0.56 ± 0.18 (0.31-0.82) n = 5	0.18 ± 0.06 (0.11-0.28)	0.50 ± 0.31 (0.12-1.13) n = 10	0.43 ± 0.26 (0.24-0.61)	0.25-0.83	0.75
P (g/MJ PME)	0.26 ± 0.04 (0.22-0.32)	0.24 ± 0.02 (0.21-0.26) n = 5	0.16 ± 0.02 (0.14-0.19)	0.25 ± 0.09 (0.13-0.40) n = 10	0.44 ± 0.03 (0.42-0.46)	0.21-0.47	0.33
Ca:P	0.87 ± 0.44 (0.42-1.50)	2.33 ± 1.30 (0.71-4.34) n = 10	1.12 ± 0.44 (1.92-0.69)	2.62± 1.02 (0.38-4.19) n = 28	0.95 ± 0.54 (0.57-1.33)	1.11-2.88	2.29
Na (mg/MJ PME)	72.8 ± 12.9 (55.7-87.5)	13.2 ± 7.18 (6.32-22.3) n = 5	15.4 ± 7.00 (9.71-27.4)	11.1 ± 14.8 (0.84-48.4) n = 9	3.91 ± 1.68 (2.72-5.10)	8.10-198	140
K (g/MJ PME)	0.44 ± 0.03 (0.41-0.48)	0.34 ± 0.07 (0.25-0.44) n = 5	0.35 ± 0.07 (0.26-0.45)	0.47 ± 0.11 (0.38-0.65) n = 10	0.37 ± 0.05 (0.33-0.40)	0.14-0.54	0.23
Na:K	0.17 ± 0.02 (0.14-0.19)	0.06 ± 0.04 (0.01-0.17) n = 10	0.04 ± 0.02 (0.02-0.07)	0.07 ± 0.10 (0.003-0.45) n = 28	0.010 ± 0.003 (0.008-0.013)	0.03-0.92	0.60
Cu (mg/MJ PME)	0.91 ± 0.16 (0.70-1.15)	0.69 ± 0.09 (0.57-0.81) n = 5	0.44 ± 0.07 (0.36-0.56)	0.80 ± 0.19 (0.62-1.26) n = 10	0.92 ± 0.02 (0.91-0.93)	0.24-9.67	0.37

Table 17. Continued.

	Cuban parrot (n = 5)	Red-and-green macaw	Lilac-crowned parrot (n = 6)	Scarlet macaw	Thick-billed parrot (n = 2)	Hand-feeding formulas (n = 15)	Leghorn chickens 6-12 wk
Zn (mg/MJ PME)	4.09 ± 0.73 (2.81-4.53)	2.16 ± 0.34 (1.70-2.51) n = 5	1.71 ± 0.23 (1.49-2.04)	2.50 ± 0.59 (1.67-3.49) n = 10	5.09 ± 0.39 (4.81-5.36)	2.02-22.8	3.27
Mg (g/MJ PME)	0.15 ± 0.02 (0.13-0.17)	0.16 0.02 (0.13-0.18) n =5	0.10 ± 0.01 (0.08-0.12)	0.17 ± 0.05 (0.13-0.25) n = 10	0.20 ± 0.001 (0.20-0.20)	0.02-0.13	0.05
Fe (mg MJ PME)	14.3 ± 8.38 (5.62-24.8)	48.8 ± 31.0 (17.5-101) n = 5	2.06 ± 0.54 (2.01-2.43)	28.8 ± 35.1 (3.48-106.6) n = 10	6.62 ± 0.20 (6.48=6.79)	1.13-23.8	5.60

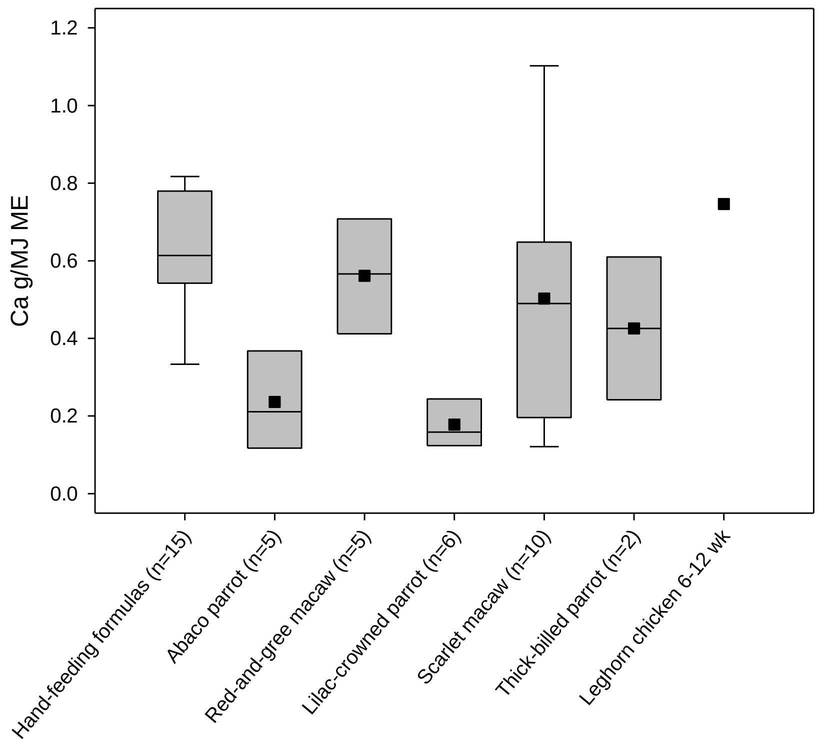

Fig. 12. Calcium concentration in the crop samples from free-living chicks of five different parrot species, commercial parrot hand-feeding formulas (Chapter V), and the requirements of 6-12 wk leghorn chickens (62). Box plots presenting mean (squares), median (horizontal line) and 5^{th} and 95^{th} percentile (whiskers) of the data, from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

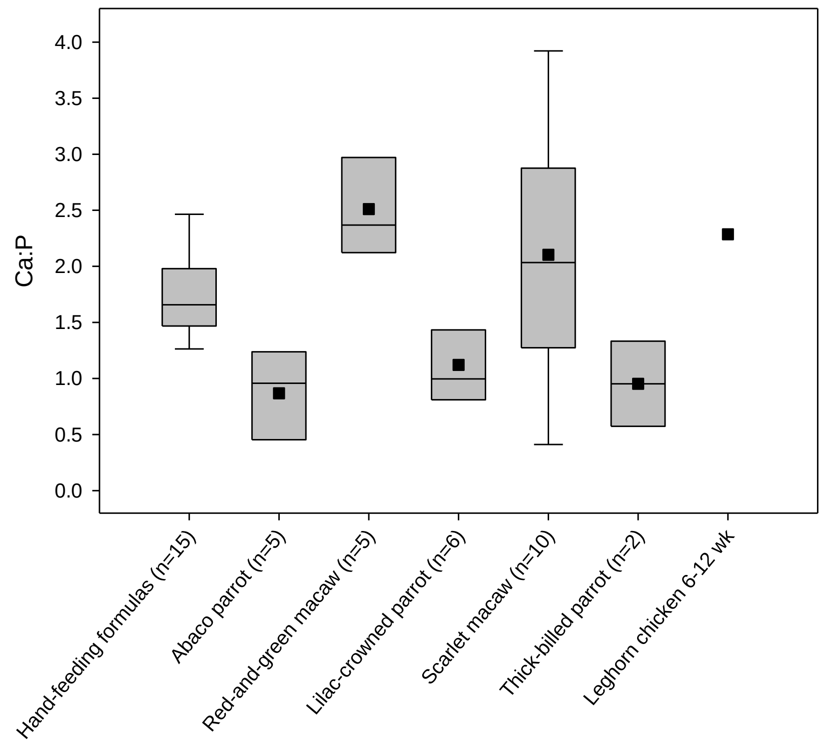

Fig. 13. Calcium:Phosphorus ratio in the crop samples from free-living chicks of five different parrot species, commercial parrot hand-feeding formulas (Chapter V), and the requirements of 6-12 wk leghorn chickens (62). Box plots presenting mean (squares), median (horizontal line) and 5th and 95th percentile (whiskers) of the data, from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

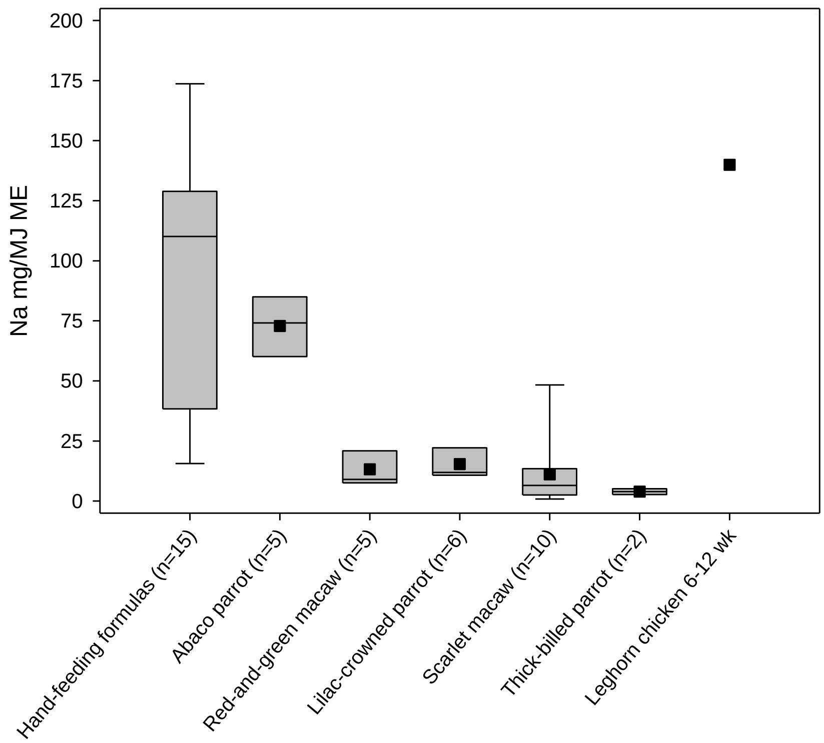

Fig. 14. Sodium concentration in the crop samples from free-living chicks of five different parrot species, commercial parrot hand-feeding formulas (Chapter V), and the requirements of 6-12 wk leghorn chickens (62). Box plots presenting mean (squares), median (horizontal line) and 5th and 95th percentile (whiskers) of the data from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*).

Table 18. Essential amino acid concentration, as % of true protein, of the crop samples from free-living chick of five different parrot species (scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*), and comparison with the range found in commercial parrot hand-feeding formulas (Chapter V).

	Crop content of chicks of five parrot species			Hand-feeding formulas (n = 15)
% true protein	Average ± SD	Range	CV	Range
Arginine	13.9 ± 4.80	9.37-20.6	34.6	0.87-3.19
Leucine	6.93 ± 0.86	6.13-8.31	12.4	5.31-8.94
Valine	5.33 ± 0.81	4.23-6.27	15.2	3.86-4.89
Phenylalanine	4.66 ± 0.72	3.42-5.22	15.4	4.76-5.70
Isoleucine	3.86 ± 0.76	2.90-4.47	19.6	3.29-4.46
Threonine	3.72 ± 0.70	2.85-4.36	18.8	3.13-6.01
Lysine	3.85 ± 1.12	2.12-5.19	29.1	4.29-7.13
Methionine	1.80 ± 0.27	1.39-2.11	15.2	1.71-8.18
Meth + Cys	3.85 ± 0.68	3.17-4.91	17.7	2.20-9.31
Tryptophan	1.13 ± 0.48	0.56-1.89	42.8	0.82-1.79
Proline	4.87 ± 0.36	4.53-5.41	7.38	0.21-21.3
Glycine	4.32 ± 0.77	3.04-5.00	17.8	3.83-6.87
Histidine	2.57 ± 0.30	2.05-2.81	11.6	0.87-3.19

lower than those found in the crop samples. Except for arginine, lysine and tryptophan, the intraspecific variation was small (CV < 20%). When comparing on a PME basis, most of the formulas match or exceed the concentrations of all essential AA found in the crops, except for arginine (Table 18). The requirements of 6-12 wk leghorn chickens fell below the concentrations found in several of the parrots' crops, mainly arginine and proline, but also valine, phenylalanine, and histidine.

Metabolic energy needs and total nutrient intake

According to the energy content of their diets, at the average sampling age the different studied species will need to consume between 7.7 % (scarlet macaw) and 10 % (Cuban parrot) of its body weight in food daily (Table 19).

Discussion

The diets of the different parrots presented important similarities and common patterns. All studied species presented similar PME density, similar fat concentration, and a higher proportion of fat than crude protein. Crude fat was the nutrient with the smallest variation among the study species. The variations in crude protein were greater, but the AA profiles were remarkably similar.

Preliminary data from the thick-billed parrot shows it has the most specialized diet, and the most unique nutrient profile of the studied species. It nests in temperate forest, in contrast with the tropical habitats of the *Ara* and *Amazona* spp. In growing birds, dietary protein is used for tissue accretion and maintenance, with the AA balance needed for growth closely mirroring the composition in tissues (26). The lower protein and EAA concentrations of the

Table 19. Sampled chick characteristics, predicted metabolizable energy (PME) needs, food energy density, and calculated daily food intake from scarlet macaw (*Ara macao*), red-and-green macaw (*Ara chloropterus*), Cuban parrot (*Amazona leucocephala bahamensis*), lilac-crowned parrot (*Amazona finschi*), thick-billed parrot (*Rhynchopsitta pachyrhyncha*)

	Average age (days)	Nesting period	Body weight (g)	PME (MJ/day)	Food energy density (MJ/kg)	Daily intake to meet PME requirements (g)	Daily intake as % BW
Cuban parrot	23	60[a]	200[a]	0.40	19.2	20.8	10.4
Red-and-green macaw	55	93[b]	1161[b]	1.44	19.5	74.0	6.37
Lilac-crowned parrot	41	64[c]	306[c]	0.54	21.7	25.1	8.21
Scarlet macaw	51	86[d]	996[d]	1.29	16.8	76.8	7.71
Thick-billed parrot	55	62[e]	320[e]	0.56	22.5	25.0	7.82

a (Stahala, unpublished data)

b (Brightsmith, unpublished data)

c (93)

d (61)

e (27)

thick-billed parrot may reflect a lower growth rate, or a higher thermoregulatory expenditure.

Sodium (Na) is an essential nutrient for vertebrates, required for the regulation of blood pressure, the conduction of nerve impulses, and muscle contraction (184). It may also be needed for processing secondary plant compounds that serve as toxic deterrents to vertebrate herbivores (185). Its deficiency can result in dehydration, poor growth and weakness (6). Na is typically low in tropical soils (186), and it is not required by most plants, resulting in very low concentrations in most plants (6). Coastal areas are usually Na replete, while continental areas are usually Na depleted (6). The Cuban parrot crop Na concentration was 19 times that of the thick-billed parrot, but still fell below the requirement for 6-12 wk leghorn chickens (62), suggesting that the studied species are able to conserve Na better than poultry.

The effectiveness of the different digestive enzymes may reflect adaptation to different primary diet ingredients (175, 180). Therefore, the differences in proportions of fat, carbohydrates and protein in the diet could require different metabolic pathways (187), which implies that appropriate nutrient balance in hand-rearing diets is necessary for optimal nutrition of the different species.

The nutrition of wild birds may have deficiencies and limitations (188). However, the growth rate of nesting parrots can adapt to fluctuations in food resources (93, 152), and even in years of food shortage there is little variation in the fledging's weight (93, 152). The observed fractional growth rates of the scarlet macaw chicks at the Tambopata Research Center (61) are not below those of hand-reared chicks (3), suggesting that the parents provided nutrition is adequate for the chicks' growth and development. All the sample sites have been the focus of long term monitoring studies, with no evidence of severe nutritional deficiencies in the chicks or nutrition related population declines (27, 61, 88, 189).

According to the contents of the crops, the diet of all studied psittacines contained concentrations of Ca, Na, threonine and lysine below the requirements of leghorn chickens of similar age. In addition, several diets were below the poultry requirements for P, Zn, Fe, and in all the EAA except for proline and arginine. According to these results, poultry do not provide the ideal model for psittacine nutrition, as was already suggested. Also one must consider that poultry not only are bred selectively to maximize growth, but also the requirements are set to obtain the maximal muscle deposition while minimizing food consumption (62).

Our data suggest that a single formulation could be used to hand-rear *Ara* and *Amazona* spp. of the studied ages. Low levels of essential amino acids could be acting as limiting factors for protein quality (74, 80), thereby reducing overall growth and development. Experimental studies should evaluate if increasing the concentration of crude fat, Mg, arginine, and valine enhance psittacine chick growth and health.

CHAPTER VII
CONCLUSIONS AND RECOMMENDATIONS

The research presented in this dissertation provides new insights on the diet and nutrition of Neotropical psittacines. This information helps us understand the feeding ecology of this group of birds, and is of direct application to the nutrition of birds in captivity. Natural diets do not always provide ideal nutrition, preventing the determination of the parrots' nutrient requirements based solely on diets from free-living chicks, however the information generated provides a baseline for further research.

- Chapter II showed that near-infrared (NIR) spectroscopy is a valid technique for the nondestructive, low cost prediction of the nutritional composition of avian crop contents. It expands the possibilities of research in the nutrition of parrots and other animals where only small samples are available.
- Chapter III suggests that young scarlet macaws at Tambopata Research Center are being fed a diet with a predicted metabolic energy (PME), protein and essential amino acid concentration similar to the requirements estimated for other psittacines, but lower than the requirements for poultry. As anticipated, the PME density of the diet fed to the scarlet macaw chicks did not change within the age of the studied chicks.
- Chapter IV showed that the fatty acid profile of the crop content of free-living psittacine chicks differed markedly among genera, with the *Rhynchopsitta* sp. closer to the *Ara* spp. than to the *Amazona* spp. Hand-feeding formulas differed widely in saturation profiles, and none them resembled the profile found in the thick-billed parrot or the red-and-green macaw.
- Chapter V showed that the commercial hand-feeding formulas differed from each other in their physical and nutritional characteristics, showing the lack of consensus among manufacturers concerning the correct nutrition for growing

psittacines. Low levels in some formulas of nutrients such as crude fat, K, Mg, arginine, and leucine, could be limiting growth rates and increasing disease susceptibility. Manufacturers' feeding recommendations likely underestimate the higher requirements of chicks during the first days of life.

- Chapter VI showed that the diets of the five studied species presented remarkable and important similarities and common patterns, the thick-billed parrot being the one with the most unique nutrient profile. It confirmed that poultry do not provide the ideal model for psittacine nutrition, and suggested the evaluation of several changes in the formulation of hand-feeding diets for this group of birds.

The similarity of nutritional profiles achieved by the different species is noteworthy, considering their diversity in habitats and food sources. The scarlet macaw samples displayed much higher variability in the concentrations of almost every nutrient compared with the other species, probably a reflection of a more generalist diet, and of the pooled samples (made by combining crop samples of similar NIR spectra). However, the average concentration of most nutrients was very similar to other species. This suggests that the adult birds apply selective feeding (190) to regulate the nutrition provided to their chicks, towards a determined nutritional target (191).

Because of the intra-specific quantitative and qualitative variability in nutrient content observed in the crop samples, we consider that complete nutrition is not achieved in each meal, but is achieved through complimentary feeding events. In this way, the parents feed a mixture of foods in which nutrients deficient in one food are supplied by another. Dietary complementation allows an animal to consume a nutritionally balanced diet when no one food fulfills all its nutrient requirements or when supplies of an optimal food are inadequate (190). An example of apparent dietary complementation was found in the thick-billed parrot, which feeds its chicks with pine seeds and tree bark.

Analysis of the main pine seeds (*Pinus arizonica*) found in the crop of the thick-billed parrots revealed that the crop contents had a concentration of Ca 30 times greater than the pine seeds alone, and a concentration of Na 5 times higher. Considering their specialized diet, it is reasonable to think that the bark is the main source of these minerals. Similar complementary feeding has been reported for the White-winged Crossbill, which occur in a similar habitat and share a diet based on pine seeds complemented with bark (192, 193). Therefore, we consider the best estimate of the complete nutrition to be the average of the crop samples for each species.

The direct measurement of nutrition through the sampling and analysis of crop contents proved to be a valid method to determine the diet and nutritional intake of chicks of different genera. Through the sampling of the Cuban parrot crop contents it has been possible to determine the parents' foraging habits and the diet provided to the chicks, with more accuracy and precision than through the direct observation of the parental feeding bouts (9). The extrapolations from observations of the feeding habits underestimated the protein fraction of the diet, and missed their preference for ripe over unripe fruits of the poison wood, as well as the ingestion of insect larva.

Due to limitations of the sampling technique, the studied chicks were relatively old, and their nutrition does not necessarily represent that of chicks during the first weeks of age. However, current formulations may oversimplify chicks changing nutritional requirements during growth. Younger birds present a higher fractional growth rate, and therefore higher nutrient requirements. As a result, age related changes in parrot nutritional requirements deserve further research.

Given the high concentrations of crude fat, Mg, arginine, and valine found in wild diets, future research should evaluate if increasing the concentration of these nutrients in hand-feeding formulas enhances psittacine chick growth and health.

Likewise, the presentation and delivery of the hand-feeding formulas should be considered further. Current hand-feeding practices are limited by the capacity of the chicks to digest the fine powder formulas, limiting the total daily nutrition provided, which could prevent the chicks from achieving their full growth potential.

Because of its high fat concentration, a hand-feeding formula that replicates the nutrition of the free-living parrot chicks may not be best produced as a dry powder. Instead it may need to be manufactured in a concentrated wet basis to be diluted according to the chick's age. Such a formula would need to be preserved under refrigeration or with high levels of antioxidants to minimize fat oxidation. Furthermore, by presenting it in two nutrient dilutions, a lower nutrient density formula for older chicks and a higher nutrient density product for younger chicks, these could be mixed to match the requirements of chicks as they mature.

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