# Nutrient Composition Of American Flamingo Crop Milk

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Crop milk samples (30 mL) were collected from juvenile (6-7 wks old) American flamingos (*Phoenicopterus ruber ruber*, *n* = 14) in the Ria Lagartos Biosphere Reserve (El Cuyo, Mexico) on the northern coast of the Yucatan Peninsula. The samples were analyzed for dry matter, crude protein, fat, minerals (calcium, phosphorus, magnesium, manganese, sodium, potassium, iron, copper, manganese, molybdenum, zinc), vitamin A, vitamin E, lutein and zeaxanthin, beta-cryptoxanthin, echinenone, canthaxanthin, and beta-carotene. In addition, morphometric measurements and blood samples were taken at the time of sample collection. This information will allow us to learn more about crop milk and avian lactation, and, most practically, to aid in flamingo hand-rearing efforts.

**Key words: *Phoenicopterus ruber ruber*, carotenoids, holocrine secretion**

## INTRODUCTION

American flamingos (*Phoenicopterus ruber ruber*) occur throughout the coastal wetland system of the Yucatan Peninsula. One of the largest flamingo colonies nests in the Ria Lagartos Lagoon on the northeastern coast. These birds utilize the surrounding habitat, which includes local commercial salt production operations, for roosting and foraging throughout the year [Arengo and Baldassarre, 1998].

Flamingos support their young for up to six months with crop milk. Along with several species of pigeons and doves, and some penguins, flamingos share with mammals the ability to secrete milk for the nourishment of their young. Much like in mammals, the production of milk in birds is prolactin mediated. However, unlike mammal milk, which is an exocrine secretion from the female, milk in birds is a holocrine secretion produced by both the male and female in response to hormonal stimulation during brooding. Lipid droplets are incorporated into the tissue lining of the crop, which is followed by detachment of cells into the lumen of the crop and, thus, the production of crop "milk." Over time, crop milk production decreases and more of the adult diet is offered to the chicks until the point of self-feeding. Adaptively, it has been postulated that the secretion of crop milk has been suggested to allow birds to survive and reproduce in environments where food supplies may be irregular or lack the energy density to support growth of young. The nutrient content of crop milk has been described for several species of Columbidae but for few other bird species [Kirk-Baer, 1999; Kirk-Baer and Romo, 1997].

Flamingos are reported to lose plumage color during the period of crop milk production, and this has been attributed (anecdotally) to the crop milk containing a vitamin derivative provided by the adult birds [Kear, 1974]. Crop milk offered to flamingo chicks from free-ranging adults has been reported as bright red during the first few weeks of life, gradually fading thereafter. This bright red color was once attributed to the presence of blood in the secretion, however crop milk has not been observed to contain any red blood cells. The color has been attributed to canthaxanthin (0.4 mg 100 $mL^{-1}$) [Fox, 1979; van Bocxstaele, 1974].

Access to the large breeding colony in the Ria Lagartos Biosphere Reserve allows collection of crop milk samples from individual flamingo chicks prior to completely fledging from the crèche. The objective of this study is to determine the nutrient content of flamingo crop milk, with special emphasis on the vitamins and minerals present in the secretions.

## MATERIALS AND METHODS

The Ria Lagartos Lagoon is approximately 80 km long and 0.02-3.50 km wide. There are two openings to the Gulf of Mexico (near the towns San Felipe and Rio Lagartos). A detailed description of the habitat surrounding the lagoon is provided in Arengo and Baldassarre [Arengo and Baldassarre, 1998].

Unflighted chicks were herded from the open water and corralled along the shore of the lagoon. Chicks were captured from the group and leg bands were attached. Morphometric measurements were taken (body weight, tibio-tarsal length, wing length, etc.). Blood samples (2-5 mL) were taken for physiological measurements. Chicks weighing more than 1 kg with visibly full crops were physically restrained and a sample of crop contents (30 mL) was taken. Crop milk samples were obtained via a large gauge feeding tube inserted down the esophagus and into the crop. A 60 mL syringe was used to collect the sample and each sample was stored in a light protected container. The total time from first human interaction until the chicks were released from the holding corral was 4 h.

Blood and crop milk samples were immediately stored in a cooler. Blood samples were centrifuged to separate serum, and serum was incubated at 60°C for 3 h. Serum samples were sealed in light protected vials and frozen prior to transport back to the United States. Crop milk samples were acidified with 1M HCl to a pH of 6.5 before being frozen for transport back to the United States. Samples were stored in an ultralow freezer (-80°F) prior to analysis. Samples were homogenized (biohomogenizer) and duplicate samples (0.1 g) were taken for subsequent mineral analysis. The samples were digested in nitric acid (6 mL) in a CEM microwave digestion system (MDS). Digested samples were diluted to 60 mL with deionized water and then analyzed for mineral content using a Inductively Coupled Plasma (ICP) spectrophotometer according to AOAC methods [AOAC, 1996]. High performance liquid chromatography (HPLC) was used to determine crop milk vitamin and carotenoid concentrations following a hexane extraction [Stacewicz-Sapuntzakis et al., 1987]. Samples were analyzed for proximates (dry matter, crude protein, and fat) using wet chemistry techniques (Dairy One, Ithaca, NY).

## RESULTS

Analysis of blood samples and the majority of morphometric measurements are still in process. Chicks sampled in 1999 were denoted with a post-script of "99" after they band number, whereas those from 2000 were denoted with a post-script of "00." Chicks were sampled at a younger age during the summer of 1999 than the summer of 2000. Average body weight for the birds measured in 1999 was slightly less than the body weights of those measured in 2000 (1.7 $\pm$ 0.2 kg vs. 2.2 $\pm$ 0.4 kg). Proximate and mineral analyses (Table 1) varied widely. Mean dry matter of the samples was 14.8 $\pm$ 4.4%. Dry matter of the samples taken in 2000 appeared greater on average than those collected in 1999 (16.8 $\pm$ 4.0 vs. 11.2 $\pm$ 1.2%). Mean crude protein and fat were 34.9 $\pm$ 8.8% and 57.5 $\pm$ 13.2%, respectively, on a dry matter basis (DMB). Calcium was implausibly low, even though the samples were analyzed twice (0.19 $\pm$ 0.23% DMB). Sodium was the most abundant mineral (2.0 $\pm$ 0.4% DMB). Copper, manganese, and molybdenum were present in concentrations less than 10 ppm (DMB) in the analyzed samples. Vitamins A (17665 $\pm$ 1083 IU $kg^{-1}$ DMB) and E (22.2 $\pm$ 12.2 IU $kg^{-1}$; Table 2) varied widely as well. Analyzed carotenoids ranged from beta-cryptoxanthin (5.0 $\pm$ 3.3 mg $kg^{-1}$ DMB) to canthaxanthin (29.7 $\pm$ 11.1 mg $kg^{-1}$ DMB).

## DISCUSSION

Flamingo milk contains the same macronutrients as mammalian milk, with the exception of carbohydrate. Moisture content of flamingo crop milk resembles the milk of primates (85-90%) or most artiodactyls (75-80%) [Robbins, 1993]. Reported DM of pigeon crop milk (24-37%) is slightly higher than flamingo crop milk values [Kirk-Baer and Thomas, 1996]. Whereas pigeon crop milk samples were collected over a period of 4 days (out of a 10-14 day period of crop milk production), the flamingo crop milk samples represent a single point in time during a longer "lactation" period (up to 6 months). Fat decreased (53-38%) and crude protein increased (58-65%) over the first four days of lactation in pigeons [Kirk-Baer and Thomas, 1996]. Fat content of the flamingo crop milk appeared close to the high end of reported pigeon crop milk, however protein content appeared lower in the flamingo milk than the pigeon milk. Based on a possible 24-wk "lactation" period, the samples would be expected to (1) represent an early time point during the entire period, and (2) change with time based on seasonality of the diet and development of the chicks.

Flamingo milk samples appeared low in Ca compared to values reported for pigeons (0.2 vs 0.8% DM), however the variation associated with the mean was great [Kirk-Baer, 1999]. The observed mean calcium to phosphorous ratio in the flamingo crop milk was 0.3:1. Again, this is seemingly implausible. Sodium content of the flamingo crop milk appeared slightly greater than the values reported for pigeon crop milk (2.0 vs. 0.5%), however this may be expected due to observations that these flamingos forage primarily in local artificial commercial salt production ponds and in the Ria Lagartos lagoon (also a saline habitat) [Arengo and Baldassarre, 1998].

Carotenoids are dietary pigments in part responsible for imparting coloration to birds (in conjunction with melanin, porphyrin, etc.). Carotenoids are synthesized by plants. Animals that feed extensively on plants (insects, mollusks, crustaceans, fish, etc.) can concentrate and further metabolize the carotenoids they consume, providing a rich source for animals that, in turn, feed on them. Of the two forms of carotenoids, free and esterified, free form carotenoids are more easily digestible. Carotenoids can be divided into two categories: carotenes (alpha- and beta-carotene) and xanthophylls (lutein, zeaxanthin, beta-cryptoxanthin, echinenone, canthaxanthin, astaxanthin, etc.). Even though birds do not appear to have a nutritional requirement for carotenoids (as long as adequate vitamin A is provided), they are necessary for communication of reproductive fitness.

Flamingos deposit carotenoid pigments (canthaxanthin and astaxanthin) in their feathers to provide their characteristic pink coloration. However, flamingos do not readily utilize many dietary xanthophylls, including astaxanthin. They do effectively absorb and utilize beta-carotene as a precursor for some of those pigments [Fox, 1979]. Canthaxanthin can be synthesized from beta-carotene or absorbed from the diet (although it is not produced in high concentrations by most plants, thus most likely not abundant in the diet). Astaxathin is not well absorbed from the diet and these birds synthesize it from beta-carotene and canthaxanthin. Birds fed astaxanthin as their sole dietary pigment appear very poorly colored [Klasing, 1998]. Feeding canthaxanthin at about 25 mg $kg^{-1}$ DM can supply most of the pigmentation needs for captive flamingos, but dose-response relationships have not been established [Fox, 1979]. In addition, dietary carotenoid requirements may not always be predictable from the color of feathers or the spectrum of tissue carotenoids [Dierenfeld and Sheppard, 1996]. The canthaxanthin levels measured in flamingo crop milk on average exceeded the levels needed to maintain color in captive adults. Astaxanthin in the crop milk was not analyzed, but is listed as one of the primary carotenoids present in many of the proposed diet items of these birds [Klasing, 1998]. Although not quantified, astaxanthin appeared to contribute significantly to the carotenoid content of the crop milk samples (Stacewicz-Sapuntzakis, pers. comm.).

Flamingos have been observed to lose plumage color during the period of crop milk secretion [Kear, 1974]. This may be more a result of the incorporation of carotenoid pigments into crop milk preferentially over continued deposition in the feathers (rather than a mobilization of pigments already incorporated in the feathers). Skin, egg yolk, and adipose tissue color in chickens reflects the types and levels of carotenoids consumed. Even when consuming a diet rich in carotenoids, poultry will divert the majority to yolk production, and other tissues will lose color due to not receiving a maintenance level amount of pigment [Klasing, 1998]. The rate of loss appears tissue specific, based on the rate of cell turnover in the area. Observations of adult birds from the crèche were not recorded.

## CONCLUSIONS

1. Flamingo crop milk is a rich source of macronutrients for growing chicks.
2. Crop milk sampled from the flamingo chicks at the Ria Lagartos Reserve contained carotenoid levels observed to be appropriate to maintain adequate pigmentation in adult flamingos.
3. The relationship between dietary pigment levels and observed color in flamingos is a complex relationship, thus more research is needed to examine these interactions.
4. Additional work is needed to more adequately delineate the nutrient content of flamingo crop milk and make applications to captive hand-rearing diets.

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**TABLE 1. Nutrient content of flamingo crop milk on a dry matter basis[1]**

| Band Number | DM % | CP % | Fat % | Ca % | P % | Mg % | K % | Na % | Fe ppm | Zn ppm |
|---|---|---|---|---|---|---|---|---|---|---|
| FWCD-99 | 11.7 | 31.0 | 59.7 | 0.09 | 0.5 | 0.0 | 0.4 | 2.1 | 188 | 17.1 |
| FWJP-99 | 9.0 | 46.6 | 29.5 | 0.7 | 0.71 | 0.1 | 0.6 | 1.6 | 611 | 22.2 |
| FZAX-99 | 11.6 | 30.1 | 68.7 | 0.09 | 0.61 | 0.09 | 0.26 | 1.9 | 155 | 17.2 |
| FXHV-99 | 11.0 | 38.8 | 49.6 | 0.09 | 0.63 | 0.0 | 0.27 | 2.05 | 164 | 27.3 |
| FZFZ-99 | 12.6 | 24.0 | 74.9 | 0.08 | 0.56 | 0.0 | 0.24 | 1.46 | 103 | 7.9 |
| DZJF-00 | 22.7 | 22.7 | 73.6 | - | - | - | - | - | - | - |
| DFHF-00 | 14.1 | 38.8 | 48.4 | 0.14 | 0.58 | 0.01 | 0.54 | 2.34 | 210 | 8.4 |
| DFHL-00 | 11.1 | 50.9 | 45.1 | 0.14 | 0.58 | 0.01 | 0.58 | 2.73 | 603 | 12.4 |
| DZLL-00 | 13.7 | 36.9 | 58.2 | - | - | - | - | - | - | - |
| DFNF-00 | 16.2 | 34.6 | 59.5 | - | - | - | - | - | - | - |
| DFNV-00 | 22.3 | 29.9 | 68.6 | - | - | - | - | - | - | - |
| DXPT-00 | 19.8 | 28.1 | 63.9 | - | - | - | - | - | - | - |
| DFVS-00 | 18.8 | 29.1 | 64.8 | - | - | - | - | - | - | - |
| DFLV-00 | 12.6 | 47.4 | 40.6 | - | - | - | - | - | - | - |

[1](-) indicates sample values not available.

**TABLE 2. Vitamin and carotenoid content of flamingo crop milk on a dry matter basis[1]**

| Band Number | Vitamin A | Vitamin E | Lutein / Zeaxanthin | Beta – cryptoxanthin | Echinenone | Canthaxanthin | Beta-carotene |
|---|---|---|---|---|---|---|---|
| | IU $kg^{-1}$ | IU $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ |
| FWCD-99 | 16697.5 | 17.8 | 3.4 | 4.3 | 5.9 | 15.9 | 6.8 |
| FWJP-99 | - | 12.1 | - | 1.0 | 16.8 | 37.8 | 11.2 |
| FZAX-99 | 16959.1 | 25.3 | 9.9 | 1.8 | 9.0 | 27.8 | 22.6 |
| FXHV-99 | 19085.0 | 13.7 | 3.4 | 2.8 | 11.8 | 23.6 | 11.3 |
| FZFZ-99 | 17920.0 | 42.1 | 3.3 | 1.7 | 7.4 | 43.6 | 10.3 |

[1](-) indicates sample values not available.