A SPOONFUL OF FORMULA: HAND-REARING AND METABOLIC BONE DISEASE OF ROSEATE SPOONBILLS (*PLATALEA AJAJA*) AT ZOO MIAMI

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Introduction

From 2021-2023, 6 Roseate Spoonbill *(Platalea ajaja)* chicks were hatched from eggs and hand-reared at Zoo Miami. A new hand-rearing protocol was developed based on protocols from 4 other institutions and adjusted over the course of hand-rearing at Zoo Miami. Previous formulas were labor intensive to make and involved discarding 30-50% of the recipe due to the inability to strain this portion. As a result, significant loss of nutrients was possible with a final product potentially differing from batch to batch. A simpler recipe was developed for ease and consistency and this was successful in rearing 1 chick in 2021 and 2 chicks in 2022. However, in 2023, all 3 spoonbill chicks presented with extreme metabolic bone disease (MBD) around the time of weaning and were euthanized between 15 and 26 d of age. This paper describes the Zoo Miami formulation and the growth outcomes and explores the nutritional aspects of the 2023 clinical cases.

Materials & Methods

The Zoo Miami Spoonbill formula (Table 1) was designed to minimize labor and waste and to ensure a consistent formula product. The formula is compared to previous formulas in Table 2. Whole fish (often requiring portions like heads, tails or fins removed) was replaced with pre-processed commercial fish filet. This also removed any concern about thiaminases which may persist in the gut of whole fish, thus eliminating any need to supplement additional thiamin.

Commercial raw meat mixes contain connective tissue that is not possible to strain and increase food safety concerns due to the need to thaw for processing. Raw meat mixes were replaced by a high protein, high fat fish-based dry product: Mazuri Amphibian & Carnivore Gel 5ME0 (Mazuri Exotic Animal Nutrition, St. Louis, MO 63166). Hardboiled egg was incorporated to balance the fat level. Commercial dry flamingo biscuit (Mazuri Flamingo Complete 5644) was also included, similar to other formulas, but limited to restrict carbohydrate level. Wild diets are expected to be high in protein and fat but very low in carbohydrates.

Expected analysis of the Zoo Miami Spoonbill formula (based on ingredients) is shown in Table 3. This formula was expected to meet requirements based on other institutions' formulas and targets derived from poultry and carnivores (NRC Poultry 1994, NRC Dogs & Cats 2006). The Zoo Miami spoonbill formula was sent for direct proximate, mineral, and vitamin analyses after chick concerns arose.

Calcium carbonate ($CaCO_3$) was supplemented in 3 out of 4 of the previous formulas, resulting in calcium levels above 2%. Although critical for growth, excessive calcium may be associated with bone and leg development issues. High calcium may also interfere with fat absorption, potentially impacting fat-soluble vitamins (Stevens *et al.*, 1983). Flamingo hand-rearing formulas at 1.2-1.9%

calcium have been successful so this was selected as an appropriate target range and is consistent with the spoonbill formula from institution 3.

Vitamin E supplementation is recommended for fish-based diets on the premise that frozen thawed fish is deficient in vitamin E. All spoonbill formulas contain a portion of commercial nutritionally complete raw meat or biscuit already supplemented with vitamin E. Over-supplementation of vitamin E is associated with coagulopathies in pelicans due to interference with other fat-soluble vitamins (Treiber *et al.*, 2017).

All spoonbill formula feeding protocols involved dilutions to the base formula that changed over the course of weaning. This could significantly impact the calories and nutrients provided, especially when feeding a restricted volume. Dilution was simplified in the Zoo Miami protocol to be diluted 1:1 w:w with distilled water on days 1-3 (giving 9.8% DM) and 3:1 w:w with distilled water on day 4 to weaning (14.6% DM). Rather than diluting in future years, the recipe should be adapted to reflect the desired dry matter level. This will eliminate mathematical errors and confusion.

Institutions 1, 2, and 3 weaned spoonbill chicks starting as early as 7 d and completing by 15 d. This weaning was abrupt (institutions 1 & 2) or introduced solids gradually (institution 3). Institution 4 had a much later weaning period around 1 month of age or later. Zoo Miami elected to begin weaning at 12 d and reflect a gradual weaning schedule similar to institution 3 (Table 4). Feeding amount from other protocols was based on % bodyweight (%BW) and adjusted based on target weight gains per day (also on %BW). With variability in formulas and dilutions, feeding on this volume basis could be misleading. Some recommendations included feeding over 100%BW per day with meals exceeding 20%BW. Following these recommendations appeared to result in the death of one spoonbill chick at Zoo Miami (21B071) at 6 d so meal size was restricted to <20%BW per meal not to exceed 100%BW per day with an initial target of 15%BW per meal 6x daily.

Spoonbill chicks 23B063, 23B066, and 23B068 presented initially with foot curling which was wrapped overnight to encourage proper posture. Birds were just beginning to sit upright. After fractures were discovered in the first bird, the remaining birds were radiographed and bone-thinning was apparent. 22B066 survived the longest and was treated with physical therapy and supplemental calcium (100 mg calcium gluconate subcutaneously) but eventually was unable to stand due to a fracture. Birds were euthanized when fractures were discovered. Blood samples were collected in the days prior to and at the time of euthanasia and tissue samples collected at necropsy and sent out for nutrient status and histopathology.

Results & Discussion

Due to dilution of the formulas, actual dry matter as offered to chicks from all institutions ranged from 9.9% (1-3 d) to 25% (from as early as 2 d). All formulas achieved ~20% DM or higher by 10 d. The Miami protocol resulted in the lowest DM achieving only 14.6% from 3 d to weaning. The Miami formula had the highest kcal/g DM somewhat balancing the kcal offered. Overall, institution 1 had a much higher initial feeding rate which likely explains the higher growth rates observed (Figure 1).

Growth curves for 14 hand-reared spoonbills from 3 institutions and 6 hand-reared spoonbills from Zoo Miami were fit by a Gompertz curve (Figure 1). Data were weighted by % error of the residual to increase the importance of early growth. The resulting fit predicted an asymptotic mature weight of 1355 g which is consistent with the median weight for adult spoonbills in ZIMS. Despite weighting, early growth (0-3 d) was underestimated.

Daily weight gain recommendations for spoonbill chicks ranged from 10 to 40%BW per day. High growth rates early in hand-rearing may predispose to developmental orthopedic disease. Per ZIMS the average overall growth rate during the first month was 31.7 g/d (this is likely an overestimate early on and an underestimate per later growth). From 7-21 d the Gompertz curve is nearly linear, showing a growth rate of 48 g/d for hand-reared birds during this period of maximal growth. Compared to ZIMS weight ranges at 30 d (600-1200 g), most hand-reared birds at this age were on the high end of this range or significantly heavier (Figure 1). This could suggest that hand-rearing growth rate tends to be more rapid that parent reared. Contrastingly, parent reared birds reported by Hudson *et al.* appeared to be heavier at 14 d than hand-reared chicks from institution 3, 4, and Zoo Miami but similar to institution 1 (1997).

Growth curves for 5 Roseate Spoonbills hand-reared at Zoo Miami were almost identical (Figure 1). These birds were intermediate in growth rate between birds from institution 1 and institution 4 and very similar in early growth to institution 3 as well as the Gompertz best-fit curve. 22B071 grew much slower. This bird had to be assist-hatched and was delayed throughout the growth phase including delayed weaning starting at 18 d. Regardless this bird was overall successful and following weaning caught up in growth by 30 d of age. Based on these comparisons, rapid growth does not appear to be the primary factor in the MBD observed at Zoo Miami in 2023.

Extrapolating from reported formulas, feeding amounts and observed growth rates of hand-reared Roseate Spoonbills from institutions 1, 3, 4, and Zoo Miami an energy requirement for growth was estimated empirically (Figure 2):

$$Kcal/d = 0.45*(Bodyweight_g)+22.06 \qquad (r=0.931)$$

Expected adult energy requirement is ~300 kcal/d, which is achieved in growing chicks at approximately 619 g bodyweight or 3 wk of age. Most chicks were weaned onto an approximately adult diet. The weaning diet was 50% Milliken small carnivore and 50% chopped capelin (28% fat, 1.0% Ca, 1.0% P, 17800 IU/kg vitamin A, 225 ppm vitamin E, 1622 IU/kg vitamin D3). Towards the end of weaning, Mazuri Flamingo Complete 5644 and Breeder 5645 pellets were sprinkled on the water to encourage self-feeding. This diet meets expected requirements although Ca and vitamin D3 are lower than the formula while fat and vitamin E are higher. Previous studies have implicated capelin in MBD in piscivorous birds due to low vitamin D levels (Horgan *et al.*, 2021; Slifka *et al.*, 2001). However, MBD was already present at early weaning when formula was still the primary dietary component so it is unlikely that the weaning diet was responsible for MBD, though possibly nutrient changes, the stress of weaning, and the period of most rapid growth all contributed to the ultimate failure.

Necropsy results from the 3 Miami chicks were consistent with metabolic bone disease (MBD), including deformity or fractures of legs, ribs and wings (Horgan *et al.*, 2021). 23B066 and 23B068 also showed circulating indicators of inflammation. Values for nutrient content of blood and tissue samples are reported in Table 5.

Blood ionized calcium levels were within the expected range so initially MBD was not suspected. Plasma total calcium levels at 8.7-9.3 mg/dL were also considered within the expected range. However, multiple studies in growing domestic chickens suggest that calcium levels below 10 or 11 mg/dL are associated with high incidence of MBD (Aburto *et al.*, 1998; Edwards *et al.*, 1994; Mitchell *et al.*, 1997; Stevens *et al.*, 1983). Nevertheless, higher levels of circulating calcium alone do not preclude MBD.

Besides calcium, the primary culprit for MBD is vitamin D3, a key factor in calcium uptake and metabolism. Incidence of MBD in 16 d chickens was shown to be inversely proportional to dietary vitamin D levels (Aburto *et al.*, 1998). Ultraviolet light (UVB) is also an important source of activating vitamin D3 in birds and has been shown to decrease the incidence of MBD in young domestic chickens by as much as 80% in the absence of dietary vitamin D3 (Edwards *et al.*, 1994). High incidence (14.3%) of MBD in mixed species aviary chicks was decreased to 3.2% by provision of UVB lamps and associated with an increase in plasma 25(OH)D3 (Drake *et al.* 2017). In the absence of UVB, young domestic chickens required 800 IU D3/kg feed to minimize the incidence of MBD (Edwards *et al.*, 1994). This is consistent with the NRC recommendation for vitamin D3 in diets for growing turkeys (1100 IU/kg feed) and quail (750 IU/kg; NRC 1994). All Zoo Miami spoonbill chicks received 30 min of sunlight daily. All spoonbill formulas were formulated to exceed the expected requirements for vitamin D3 with the Zoo Miami formula the highest. In fact, this was a potential concern that vitamin D3 might be too high. Based on USDA food database and manufacturers' expected values, each individual ingredient in the Zoo Miami formula at the volumes fed would have been expected to provide between 80 and 270% of the total vitamin D3 requirement (Table 1). Analysis exceeded the expectation with over 10,000 IU/kg vitamin D3 in the formula, which is extremely high. Nevertheless, circulating levels of vitamin D3 and 25(OH)D3 from the spoonbill chicks suggest that vitamin D was deficient as is supported by the clinical presentation.

Vitamin A requirement for growing chicks is 1500 IU/d (NRC Poultry) and deficiency may be noted in chicks around week 2 of age when stores become depleted. Deficiency manifests initially as ataxia or inability to stand (Edwards *et al.*, 1994; Erasmus *et al.*, 1960; Johnson *et al.*, 1948); however, impacts on bone mineralization are associated with vitamin A toxicity rather than deficiency. The liver vitamin A values in the Miami spoonbill chicks appeared to be in the normal range (Table 5) with one chick on the low end of the normal spectrum. Much higher liver vitamin A levels (>300 ug/g) are observed in growing domestic chickens consuming high dietary vitamin A (e.g. 44,000 IU/kg) and associated with increased incidence of MBD (Stevens *et al.*, 1983; Aburto *et al.*, 1998). The Miami spoonbill formula contained ~10,000 IU/kg, which is within the expected appropriate range. Vitamin A can inhibit bone mineralization and may also compete for absorption with dietary fat and fat-soluble vitamins (Hymoller *et al.*, 2016; Stevens *et al.*, 1983). The natural diet for spoonbills is much higher in fat than the omnivorous diet of poultry so they might be expected to have higher ability to absorb fat - and potentially fat-soluble vitamins.

Despite the Zoo Miami spoonbill formula being lower in vitamin E than previous formulas, liver vitamin E content was well above expected levels from other species (Ilynia *et al.*, 2014). Pelicans with similar levels of liver vitamin E were at risk for coagulopathies (Treiber *et al.*, 2017), which occur when vitamin E interferes with the action and uptake of another fat-soluble vitamin, vitamin K. Vitamin E has also been shown to ameliorate the effect of high dietary vitamin A in chicks (McCuaig & Motzok, 1970). No coagulopathies were observed in the Miami spoonbill chicks; however, high vitamin E levels could potentially compete with vitamin D for uptake (Hymoller *et al.*, 2016).

Conclusions

The apparent cause for the 2023 MBD cases is vitamin D deficiency despite an expected high level of vitamin D3 in the formula. This most likely demonstrates a congenital dysfunction in vitamin D absorption or metabolism. Another chick from the same batch of eggs was hand-reared at a different institution and also presented with MBD (personal communication) while chicks at Zoo Miami were successful in 2021 and 2022 on the same formula and protocol, including only 30 min. per day of sunlight/UVB exposure. MBD might have been precipitated from multiple concurrent risk factors including congenital vulnerability, rapid growth, low UVB exposure, and interference with vitamin D absorption from other fat-soluble vitamins or dietary fat.

Important considerations for future hand-rearing cases:

1) Ionized calcium levels may not be useful in predicting MBD in young growing birds.
2) Plasma calcium level below 10 mg/dL in young growing birds may indicate increased risk for MBD.
3) Vitamin E level of formula should be considered carefully before supplementing to avoid excess. Attention to the ratios of fat-soluble vitamins may help to optimize formulas (Hymoller *et al.*, 2016).
4) Chicks should be regularly exposed to UVB during growth to bolster vitamin D from diet, especially when dietary vitamin D is not ensured.
5) Waiting to until 21 d to begin weaning may avoid the period of most rapid growth, reducing stressors during a critical window.

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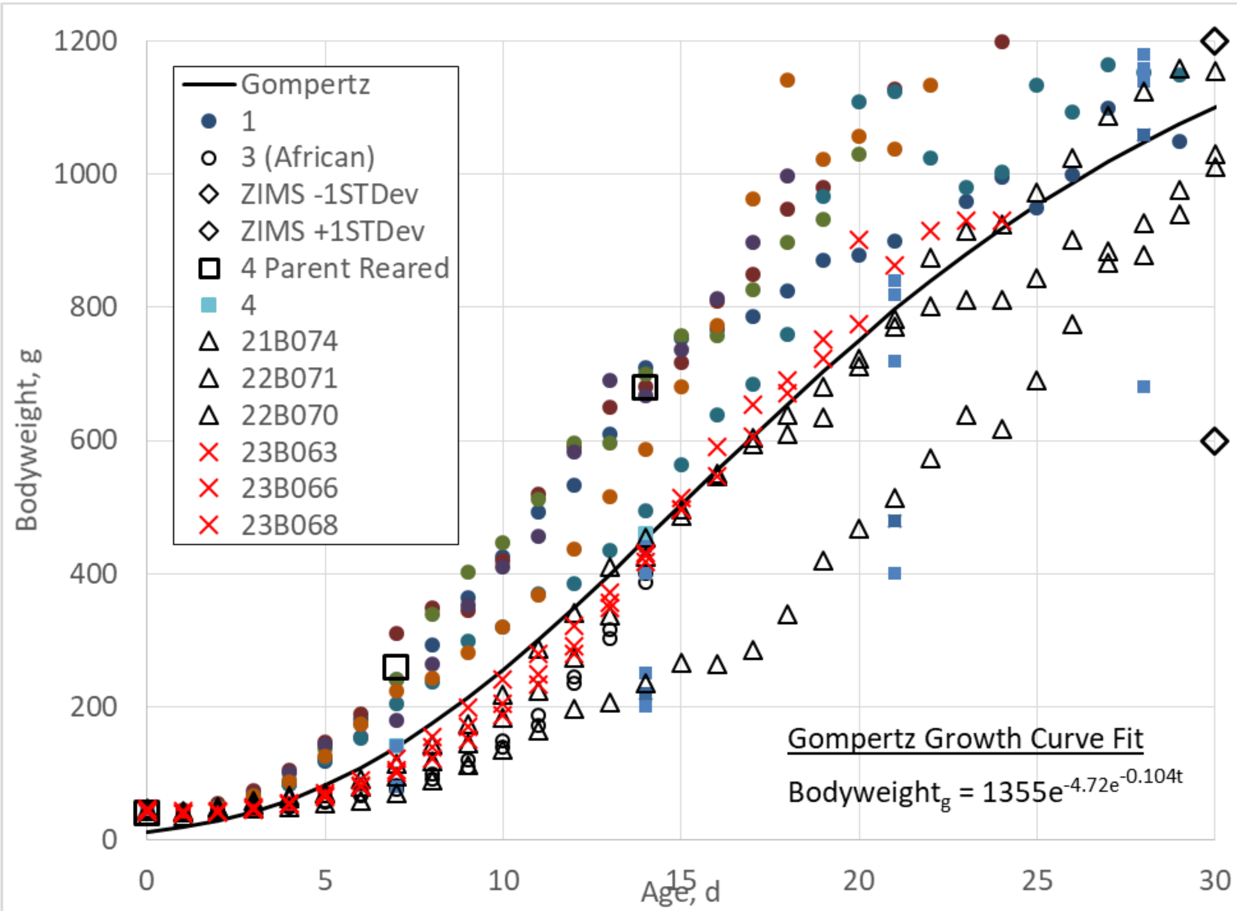

Figure 1. Growth curves for Spoonbills hand-reared at 3 institutions and at Zoo Miami successfully (21B074, 22B070, 22B071) or with metabolic bone disease (23B063, 23B066 23B068). Values are compared to parent-reared birds (Hudson *et al.*, 1997; black squares) and the typical range of birds reported in ZIMS at 30 d of age (black diamonds; includes hand- and parent-reared birds). All data are for Roseate Spoonbills except for 2 birds from institution 3 which were African Spoonbills (*Platalea alba*).

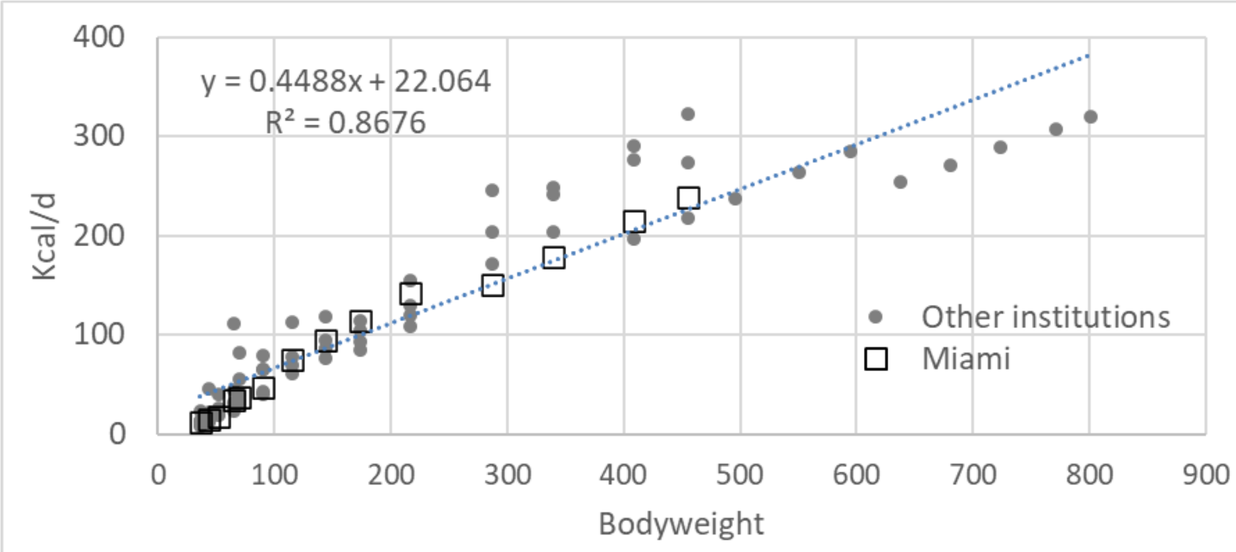

Figure 2. Linear fit to estimated kcal/d offered to hand-reared spoonbills at 4 institutions. Kcal/d offered estimated based on % bodyweight fed, formula kcal/g and dilution schedule.

Table 1. Zoo Miami Spoonbill formula recipe.

Ingredient	% As fed	Vitamin D3 IU/kg As fed[1]	Vitamin D3 IU at 14 d[2]
Tilapia Filet	14.3	1240	36
Egg, Hardboiled	14.3	870	26
Mazuri Amphibian & Carnivore Gel 5ME0 powder	7.1	5975	87
Mazuri Flamingo Complete BITS 5Z50	7.1	4450	57
Water	57.2	0	0

[1]Values from Mazuri product sheet (www.mazuri.com) and USDA food database (fdc.nal.usda.gov)
[2]Expected contribution to total vitamin D3 consumed from each food item. Expected requirement at 14 d of age (~206 g formula consumed) is 32 IU vitamin D3 (to achieve 800 IU/kg feed DM).

Table 2. Comparison of Roseate Spoonbill (*Platalea ajaja*) hand-rearing formulas and the Zoo Miami Spoonbill formula recipe. All values are % of recipe on a Dry Matter basis.

	Institutions				
Food item	**1**	**2**	**3**	**4**	**Miami**
Water (% as fed)	75.0	75.0	78.6	80.2	80.5
Fish[1]	21.1	23.2	11.2	9.0	16.2
Shrimp (peeled)				11.2	
Hardboiled Egg					18.7
Commercial Raw Meat Mix[2]	45.5	45.7	21.3	21.2	
Commercial Dry Biscuit[3]	27.5	27.6	67.2	56.5	65.7
Calcium Carbonate	3.5	3.5		0.8	

[1]Reported products include: Lake smelt, capelin, tilapia filet
[2]Reported products include: Nebraska BOP, Zoo Carnivore Diet 5, Nebraska Premium Feline, Milliken Small Carnivore
[2]Reported products include: Mazuri Flamingo Breeder 5645, Mazuri Flamingo Pellets (not specified), Mazuri Flamingo Complete Bits 5Z50, Proprietary Flamingo Pellet, Science Diet Canine Maintenance and Mazuri Amphibian & Carnivore Gel 5ME0

Table 3. Comparison of key nutrients across spoonbill formulas on a dry matter basis. All formulas appear to meet nutrient targets. Values estimated from ingredients except where indicated[1].

	Institution					
	1	**2**	**3**	**4**	**Miami**	**Target[2]**
Energy, kcal/kg	4013	4141	3814	3575	4375	
Protein, %	47	49	32	43	54[1]	25-30
Fat, %	17	18	13	13	15[1]	9
Carbohydrate (by diff), %	22	22	47	33	26	
Ca, %	2.95	2.95	1.66	2.36	1.85[1]	1.2-1.9
P, %	1.12	1.17	1.17	1.16	1.47[1]	0.4-0.8
K, %	1.03	1.11	0.83	0.96	0.92[1]	0.25-0.60
Na, %	0.54	0.61	0.27	0.74	0.51[1]	0.15-0.25
Mg, ppm	1428	1494	1773	2190	1400[1]	60-80
Fe, ppm	224	240	285	239	297[1]	80
Zn, ppm	87	84	118	102	172[1]	40-75
Cu, ppm	10	10	12	21	12[1]	5
Thiamin, ppm	1210	13	153	16	18	1 to 5
Riboflavin, ppm	5	5	17	11	18	4
Niacin, ppm	36	36	44	37	173	27-60
Vitamin B6, ppm	803	6	19	16	16	4 to 6
Vitamin A, IU/kg	12205	12257	15357	16659	10301[1]	1500-5000
Vitamin E, ppm	1792	189	265	177	172[1]	20-54
Vitamin K, ppb	1775	1782	550	1637	2014	500
Vitamin D3, IU/kg	1740	1748	2752	2606	10402[1]	200-500
Fiber (NDF), %	4	4	16	9	6	

[1]Values from direct analysis. Vitamin A is reported as retinol only, although beta-carotene was also high (10,050 IU/kg).

[2]Targets based on poultry and carnivores as the closest model species (NRC Poultry 1994, NRC Dogs & Cats 2006)

Table 4. Weaning schedule for Roseate Spoonbill chicks at Zoo Miami. Weaning was expected to begin at 12 d; however, 22B071 was delayed to 18 d. Weaning was adjusted according to the individual bodyweight. Formula and solids were distributed across 4 feedings per day.

Day of Weaning		1	2	3	4	5	6	7	8	9	10
Spoonbill Formula*	%BW/d	75	75	75	50	50	50	25	25	25	0
Solids[1,2]		50% of remaining kcal as Commercial meat mix 50% of remaining kcal as chopped, whole fish									100% solids

[1]For Zoo Miami solids were Milliken Small Carnivore and capelin

[2]Kcal/d estimated empirically from hand-rearing examples to be: $Kcal/d = 0.45*Bodyweight_g)+22$

Table 5. Blood and tissue values for 3 spoonbill chicks euthanized at 15 d (23B063), 21 d (23B068) and 26 d (23B066) of age due to metabolic bone disease (MBD). Values are compared to published values for young domestic chickens, avian species and carnivores.

	23B063	23B066	23B068	Deficient range	Normal values[5]	Population	Reference
					15-22	15 d chickens	Warren *et al.*, 2020
				3.7 (15% MBD)	5.7 (3% MBD)	Mixed aviary	Drake *et al.*, 2017
				ND (MBD)	21-35	3 wk chickens	Hughes *et al.*, 1977
25-OH D3, ng/mL	7.3	3.9	7.9	3.4-13.3 (MBD)	6.1-9.2 (<5% MBD)	16 d chickens	Mitchell *et al.*, 1997
					48-78	11-25 d chickens	Sakkas *et al.*, 2019
					5.2-51	Adult parrots	Howard *et al.*, 2004
					52, 59	Adult mink	Hymoller *et al.*,
Vitamin D3, ng/mL	2.0	<1.5	<1.5		7.3-11		Warren *et al.*, 2020
Vitamin E, liver ug/g dry	519	360	337		45-120	Poultry	MSU[3]
					131-193	Carnivores	Ilyina *et al.*, 2014
					14	16 d chickens	Aburto *et al.*, 1998
Vitamin A, liver ug/g dry	40.3	60.4	5.3	<0.9[2] (ataxia)	>1.4[2]	3-6 wk chickens	Erasmus *et al.*, 1960
					>4.9[2]	4 wk chickens	Harms *et al.*, 1959
				2-3[2] (death)		2-8 wk chickens	Johnson *et al.*, 1948
Zinc, umol/L	1.6	2.1	1.1		0.8-3.0	Birds	LADDL[6]
				8.0 (MBD)	11.0-11.8[4]	16 d chickens	Mitchell *et al.*, 1997
Ca, mg/dL	8.7	8.9	9.3/8.8	9.2 (0.6% Ca)	10.4	26 d chicken	Stevens *et al.*, 1983
				6.7-10.7 (MBD)	10.6, 11.4, 11.0-11.1	16 d chickens	Edwards *et al.*, 1994
P, mg/dL	6.1	8.2[1]	6.3/6.0				
Ionized Ca, mmol/L	1.3	1.16	1.22		1.29-1.47	15 d chickens	Warren *et al.*, 2020
					0.82-1.30	Adult parrots	Howard *et al.*, 2004

[1]May be elevated due to sample hemolysis

[2]Values reported as wet liver. Dry matter content of liver was assumed to be 30% for purposes of comparison

[3]Michigan State Diagnostic Laboratory, 4125 Beaumont Road, MI 48910-8104

[4]Plasma Ca levels as high as 12 mg/dL did not preclude a high incidence of MBD in chicks in other trials depending on dietary treatment

[5]Values were considered normal if dietary levels met NRC requirements and/or if health was reported normal

[6]Lousiana Animal Disease Diagnostic Laboratory, River Road Room 1043, Baton Rouge, LA 70803