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Original Study

Blood Plasma Mineral Concentrations in Captive Lesser Flamingos (*Phoeniconaias minor*) of a German Zoological Collection

Christoph Leineweber, Lukas Reese, Marco Roller, and Rachel E. Marschang

Abstract: Lesser flamingos (*Phoeniconaias minor*) are a common species in zoological collections; however, little is known about their plasma mineral concentrations. For this reason, plasma concentrations of selected minerals (calcium, cobalt, copper, iodine, iron, potassium, magnesium, manganese, molybdenum, sodium, inorganic phosphorus, selenium, and zinc) were measured in lesser flamingos ($n = 84$) from a zoological collection after the breeding season and before their molt. Differences between sexes were only found for cobalt, which was significantly ($P = 0.004$) lower in female flamingos. Significant positive correlations between the plasma mineral concentration and the age of the individuals were found for copper ($r = 0.36$, $P = 0.001$) and zinc ($r = 0.27$, $P = 0.014$), while negative correlations were found for iodine ($r = -0.30$, $P = 0.006$) and selenium ($r = -0.24$, $P = 0.030$). The results showed that the sex of the flamingos had a limited effect on the plasma mineral concentrations after the breeding season. The age of the individual influenced some minerals, although all flamingos were fed the same diet. Mineral concentrations in the blood reflect the current intake and the minerals currently circulating in the body.

Key words: copper, flamingos, iodine, ICP-MS, minerals, zinc, avian

INTRODUCTION

Lesser flamingos (*Phoeniconaias minor*) are found in alkaline lakes in sub-Saharan Africa and western India.[1] The species is listed as near threatened with a decreasing population trend due to habitat loss, human activity, and environmental pollution from industry and mining.[1] They are the smallest species of flamingos with a weight between 1.2 and 2.7 kg and a body size of 80–90 cm.[2] The main food source of lesser flamingos is spirulina algae, which causes the characteristic pink coloration of their plumage.[2] Other algae, including *Synechocystis minuscula*, *Synechococcus pevalekii*, *Synechococcus elongatus*, *Monoraphidium minutum*, *Oscillatoria* spp., and *Lyngbya* spp., as well as small invertebrates like copepods (*Paradiaptomus africanus*), diatoms (*Navicula* sp.), and rotifers (*Brachionus* sp.) in small amounts and alkaliphilic cyanobacteria (*Arthrospira fusiformis, A. maxima*) are also consumed.[2] Because of the way they filter their nutrients in flat water, flamingos in managed care are generally fed crumbled commercial feeds with fine particle sizes and a high-protein content for free-choice feeding during the day.[3]

The mineral concentrations in the blood reflect the current intake, with the feed and the circulating concentrations in the body. Studies in birds have shown that plasma and serum concentrations of calcium (Ca), copper (Cu), iron (Fe), inorganic phosphorus (P), selenium (Se), and zinc (Zn) are influenced by dietary intake.[4–9] However, it should be noted that blood concentrations of Ca, magnesium (Mg), and P are kept relatively constant in the blood by hormones such as parathyroid hormone and vitamin D.[10–13] Blood concentrations of electrolytes, such as sodium (Na) and potassium (K), are also relatively constant, including in animal species from saline habitats through salt glands, and are more likely influenced by diseases, such as renal insufficiency, heat stress, alkalosis, or acidosis.[6,11] The trace minerals cobalt (Co), Cu, Fe, iodine (I), manganese (Mn), Se,

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and Zn are also important for animal health. Cobalt is a component of vitamin B12 and is also important in erythropoiesis.[11] Copper is also necessary for erythropoiesis as well as for plumage pigmentation in some birds.[11] Iron is a component of hemoglobin and myoglobin and is essential for oxygen transport in the body.[11,14] Iodine is important for the normal thyroid function.[11] Magnesium is an enzyme co-factor and is important for tendon and bone structure.[11] Selenium is an important antioxidant and both Se deficiency and Se excess can trigger clinical disease.[11,14] Zinc is important for cell metabolism, the immune system, and bone and cartilage development;[11,14] however, increased concentrations can also lead to intoxications, which are more frequently observed in psittacine birds than in other birds.[15,16] In greater flamingos (*Phoenicopterus roseus*), a Zn deficiency may predispose animals to pododermatitis, which is often a problem in managed flamingos.[9]

Of note, different minerals like Ca, Cu, Fe, Mn, P, and Zn also influence one another in their intestinal absorption from feed; an excess of 1 mineral can lead to a deficiency of others.[11,14] Physiological conditions like age, breeding status, molting, excretion, and diurnal fluctuations can also influence the mineral content in the blood of birds.[17–21]

In flamingos, little is known about mineral concentrations in the blood, although Zn, Cu, and Se have been measured in blood samples from managed populations of lesser and greater flamingos using flame atomic absorption spectrophotometry.[9,22] In a previous study, plasma mineral concentrations of Ca, chloride, Cu, Fe, K, Mg, Mn, Na, P, Se, and Zn were measured using spectrometry and inductively coupled plasma mass spectrometry (ICP-MS) in a managed flock of greater flamingos in another zoological collection in Germany.[6] To the authors' knowledge, there are no available studies on plasma Co, I, and Mo in flamingos at this time.

The aim of this study was to measure plasma concentrations of selected minerals and electrolytes in a managed collection of lesser flamingos and to evaluate the possible effects of sex and age of the flamingos on the measured values. We hypothesize that sex and age would have significant effects on some of the measured minerals in the blood.

MATERIALS AND METHODS

Animals

In this study, 89 lesser flamingos (47 males, 42 females) were sampled. The approximate age of the animals was between 1 and 34 years, with a mean $\pm$ standard deviation (SD) of 14 $\pm$ 12 years (n = 72). Unfortunately, there was no confirmed date or place of birth available for many birds other than the date of acquisition. An exact age structure and history of the origin of the group can, therefore, not be specified. After years of keeping this species without breeding, the zoological collection has developed a successful breeding program in recent years due to optimizing husbandry and equalizing the sex ratio.[23] In summer, from April to October, the group was housed in an outdoor enclosure of 215 m^2 containing a natural watercourse of 75 m^2 at a zoo in southern Germany. During the winter, between November and March, the flamingos were kept in an indoor enclosure of 100 m^2 with a water basin. During the breeding season (January to June), the flamingos were fed a commercial pelleted diet for breeding flamingos (Lundi Flamingo Premium, Hof Bremehr GmbH & Co KG, Verl, Germany), while in the non-breeding season, they were fed a maintenance diet (Lundi Flamingo Regular, Hof Bremehr GmbH & Co KG) (Table 1).

Sampling began at 9:00 AM during annual health evaluations in July. The routine examination of the flamingos included a general health check with an inspection of the eyes, oral cavity, plumage, and large joints of the limbs. To assess the body condition, the nutritional status was evaluated by palpation of the pectoral musculature. Individual animals were identified by leg bands (EAZA rings), feather sampling was carried out in unsexed specimens, and photo documentation of the palmar foot was done for pododermatitis screening. All flamingos were classified as clinically healthy. Eight of the examined animals had no signs of pododermatitis (grade 0), 76 animals had mild pododermatitis (55 grade 1 and 21 grade 2), and 5 had moderate pododermatitis (4 grade 3, 1 grade 4).[24]

Sample collection and analysis

Blood samples were collected from the right jugular vein with 3-mL syringes using a Luer system (Henke-Ject, Henke-Sass, Wolf GmbH, Tuttlingen, Germany) and 23-G needle (Berpu blue, B. Braun Deutschland GmbH & Co. KG, Melsungen, Germany). The whole blood was directly transferred into lithium heparin tubes (Micro sample tube lithium heparin, 1.3 mL; SARSTEDT AG & Co. KG, Nürnbrecht, Germany), centrifuged at 5000*g* for 10 minutes (Hettich EBA 3S, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany), and pipetted into a tube with no anticoagulant. All samples were processed within 2 hours of blood collection. The plasma samples were shipped cooled ($< 8°C$ [$< 46.4°F$]) and protected from light in microtubes with screw caps

Table 1. Labeled compositions of the diets fed to the lesser flamingos (*Phoeniconaias minor*) in a zoological collection in Germany during and after breeding season.

Diet	Unit	Diet for breeding flamingos (Lundi Flamingo Premium; Hof Bremehr GmbH & Co KG, Verl, Germany) from January to June	Maintenance feed (Lundi Flamingo Regular; Hof Bremehr GmbH & Co KG) from July to December
Ingredients		Fishmeal, wheat, corn, alfalfa meal, minerals with sea salt, astaxanthin	Wheat, corn, fishmeal, alfalfa meal, minerals, beet pulp, prawns, algae, astaxanthin
Crude fiber	%	4.5	5.0
Crude protein	%	30.0	20.0
Crude fat	%	3.5	3.5
Crude ash	%	5.0	9.5
Calcium (Ca)	%	1.4	1.4
Phosphorus (P)	%	0.9	0.9
Ca/P ratio		1.56/1	1.56/1
Sodium (Na)	%	0.8	0.8
Vitamin A	IE/kg	12 000	10 000
Vitamin E	mg/kg	35	25
Vitamin D_3	IE/kg	1200	1000
Vitamin C	mg/kg	60	50

and rubber seals (Screw cap micro tubes, SARSTEDT AG & Co. KG) to the laboratory overnight. Five lipemic samples were excluded from the statistical analysis. Sodium, K, Ca, and P were measured as part of the clinical health check spectrophotometrically (Cobas 8000 analyzer series module c701, Roche Diagnostics, Mannheim, Germany) after arrival to the laboratory. Cobalt, Cu, Fe, I, Mg, Mn, Mo, Se, and Zn were stored frozen (−20°C [−4°F]) for 1 month and then measured from the 84 plasma samples. Measurements were carried out in triplicate of a 1:56 dilution with 1% nitric acid (HNO_3) solution using ICP-MS (ICPMS-2030, Shimadzu Germany GmbH, Duisburg, Germany).[25] The instrument was calibrated using the ClinCal serum Calibrator (RECIPE Chemicals + Instruments GmbH, Munich, Germany) and germanium and terbium in specific concentrations as internal standards.[25] The calculated mean value of the triplicate measurement for each element from each animal was used for the statistical analyses. All measured mineral concentrations were above the limit of quantification (LOQ) for each element as follows: Co (LOQ < 0.2 μg/L), Cu (LOQ < 0.01 mg/L), Fe (LOQ < 0.1 mg/L), I (LOQ < 1.0 μg/L), Mg (LOQ < 0.1 mg/L), Mn (LOQ < 1.0 μg/L), Mo (LOQ < 1.0 μg/L), Se (LOQ < 1.0 μg/L), and Zn (LOQ < 0.2 mg/L).

Statistical analysis

The statistical evaluation of the data was carried out using the statistical analysis software (SAS) OnDemand for academics (SAS Institute Inc, Cary, NC, USA) for the calculation of the mean, SD, median, and minimum and maximum values. The Anderson-Darling test with a P value of ≤ 0.3 was used to determine normal distribution. The recommendations of the American Society for Veterinary Clinical Pathology guidelines for reference intervals[26] and the Reference Value Advisor[27] were followed, and the nonparametric method was used for the calculation of the 95% reference intervals. Outliers were identified by the Tukey and Dixon-Reed tests but not were excluded from the calculation.[27] The differences between males and females were evaluated using Student's *t*-test in normally distributed datasets and Wilcoxon rank sum test for non-normally distributed parameters. The correlation between the age of the individuals and the plasma mineral concentration was calculated using Spearman's correlation coefficient. A statistical difference between the groups was considered significant at $P \leq 0.05$.

RESULTS

The calculated plasma mineral concentrations for all flamingos are shown in Table 2. A sex-specific difference was only found for Co, which was significantly lower in female flamingos ($P = 0.004$; Table 2). The Spearman's correlation coefficient showed significant positive correlations between plasma mineral concentrations and age for Cu (r = 0.36, $P = 0.001$) and Zn (r = 0.27, $P = 0.014$; 0.27) and significant negative correlations between mineral concentration and age for I (r = −0.30, $P = 0.006$) and Se (r = −0.24, $P = 0.030$).

DISCUSSION

The present study provides a comprehensive evaluation of mineral concentrations in the blood of lesser

Table 2. Calculated reference intervals for plasma mineral concentrations of captive lesser flamingos (*Phoeniconaias minor*) (n = 84) in a zoological collection in Germany.

Analyte	Unit	Sex	Mean	Median	SD	Minimum	Maximum	10th percentile	90th percentile	LRL by 2.5 percentile (90% CI)	URL by 97.5 percentile (90% CI)	D	*P* value
Potassium (K)	mmol/L	All	2.58	2.50	0.64	1.80	7.00	2.00	3.10	1.80 (1.80–1.90)	3.70 (3.20–7.00)	NG	< 0.001
Sodium (Na)	mmol/L	All	157.63	157.00	3.37	147.00	166.00	154.00	162.00	151.00 (147.00–153.00)	164.00 (163.90–166.00)	NG	0.067
Inorganic phosphorus (P)	mmol/L	All	0.79	0.80	0.32	0.30	1.60	0.40	1.20	0.30 (0.30–0.30)	1.40 (1.40–1.60)	NG	0.044
Total calcium (Ca)	mmol/L	All	3.19	3.10	0.39	2.50	4.60	2.80	3.70	2.60 (2.50–2.70)	4.30 (4.00–4.60)	NG	< 0.001
Cobalt (Co)	nmol/L	All	83.64	80.09	28.46	35.63	149.32	51.58	124.55	40.72 (35.63–45.81)	144.23 (135.74–149.32)	NG	0.009
		Male	92.31	91.88	29.64	44.46	149.32	58.20	129.81	30.54 (28.85–45.81)	222.28 (142.53–234.16)	NG	0.295
		Female	74.29	69.23	24.19	35.63	139.99	44.80	109.10	35.63 (35.63–42.42)	139.14 (113.69–140.83)	NG	0.186
Copper (Cu)	µmol/L	All	5.23	4.40	2.33	2.35	13.03	3.14	8.01	3.14 (3.14–3.14)	12.56 (9.42–12.56)	NG	< 0.001
Iodine (I)	nmol/L	All	1195.40	1142.60	395.80	449.95	2891.96	765.15	1615.40	494.08 (449.95–712.35)	2070.08 (1937.69–2891.96)	NG	0.029
Iron (Fe)	µmol/L	All	68.18	63.54	24.45	18.08	138.01	41.17	100.78	28.64 (17.90–35.80)	132.46 (119.93–137.83)	NG	0.002
Magnesium (Mg)	mmol/L	All	1.26	1.19	0.24	0.84	2.21	1.02	1.56	0.94 (0.84–1.00)	1.99 (1.70–2.21)	NG	< 0.001
Manganese (Mn)	nmol/L	All	964.58	680.75	845.05	123.77	4131.85	327.64	1678.22	212.96 (123.77–282.13)	3904.33 (2930.52–4131.85)	NG	< 0.001
Molybdenum (Mo)	nmol/L	All	240.28	190.74	209.16	60.24	1271.61	86.09	395.03	67.75 (60.45–75.05)	1233.04 (543.03–1271.61)	NG	< 0.001
Selenium (Se)	µmol/L	All	4.01	3.94	1.23	1.64	7.10	2.61	5.84	1.68 (1.64–2.05)	6.98 (6.15–7.10)	NG	0.155
Zinc (Zn)	µmol/L	All	55.98	53.24	18.56	26.32	122.40	36.87	81.86	30.60 (26.01–35.19)	108.63 (93.33–122.40)	NG	< 0.001

Abbreviations: LRL, lower reference limit; URL, upper reference limit; CI, confidence interval; D, distribution; G, Gaussian distribution; NG, non-Gaussian distribution; *P* value of the Anderson-Darling test for Gaussian distribution.

Table 3. Comparison of selected previously published reference intervals for captive and free-ranging flamingos.

Analytes	Unit	Leineweber et al 2022[6] (*Phoenicopterus roseus*) a, c (data 2019)	Benato et al 2013[22] (*Phoeniconaias minor)* a, c	Peinado et al 1992[29] (*Phoeniconaias minor*), a, c	Benato et al 2013[22] left (*Phoenicopterus roseus*), a, m, c right (*Phoenicopterus roseus*), a, f, c	
Potassium	mmol/L	2.83 ± 0.5	na	na	na	na
Sodium	mmol/L	151.3 ± 2.46	na	na	na	na
Chloride	mmol/L	112.7 ± 3.37	na	119.4 ± 14.4	na	na
Inorganic phosphorus	mmol/L	1.38 ± 0.5	na	na	na	na
Total calcium	mmol/L	3.11 ± 0.24	na	na	na	na
Copper	µmol/L	2.69 ± 1.32	2.45 ± 1.96	na	5.57 ± 1.30	5.65 ± 1.53
Iron	µmol/L	44.84 ± 18.49	na	na	na	na
Magnesium	mmol/L	0.88 ± 0.12	na	na	na	na
Manganese	nmol/L	606.70 ± 268.70	na	na	na	na
Selenium	µmol/L	3.20 ± 0.60	0.45 ± 0.29	na	2.74 ± 0.43	2.54 ± 0.70
Zinc	µmol/L	42.14 ± 10.35	24.49 ± 6.72	na	30.96 ± 6.49	33.11 ± 6.28

Abbreviations: na, not analyzed; j, juvenile; a, adult; m, male; f, female; c, captive; fr, free-ranging.

Table 3. Extended.

Analytes	Wyss et al 2014[9] left (*Phoenicopterus roseus*), j, fr right (*Phoenicopterus roseus*), a, c (group B)		Peinado et al 1992[29] (*Phoenicopterus roseus*), a, c	Amat et al 2007[4] (*Phoenicopterus roseus*), j, fr	Schlegel et al 2015[30] (*Phoenicopterus roseus*), a, c (Dezember)	Amirad-Triquet et al 1991[28] (*Phoenicopterus roseus*), j, fr
Potassium	na	na	3.2 ± 0.5	na	3.28	na
Sodium	na	na	160.0 ± 8.0	na	160.8	na
Chloride	na	na	115.7 ± 6.6	na	na	na
Inorganic phosphorus	na	na	12.6 ± 0.9	2.2 ± 1.1	1.53	na
Total calcium	na	na	3.4 ± 0.6	3.6 ± 2.6	2.90	na
Copper	5.10 ± 0.38	8.70 ± 1.26	na	na	5.18	3.85 ± 1.21
Iron	na	na		na	18.26	na
Magnesium	na	na	1.0 ± 0.1	0.7 ± 0.3	1.08	na
Manganese	na	na	na	na	na	na
Selenium	na	na	na	na	9.91	na
Zinc	27.22 ± 3.63	26.47 ± 3.15	na	na	36.72	> 48.61

flamingos and establishes new reference intervals for Co, Fe, I, Mg, Mn, and Mo for this species. Compared with the values for Cu, Zn, and Se reported in an earlier study of lesser flamingos in human care,[22] the values for all 3 minerals found in the present study were distinctly higher (Table 3). Several mineral values also differ from those previously reported from greater flamingos (Table 3).[1,6,9,22,28–30]

It is difficult to establish uniform reference intervals for flamingos because several factors influence plasma mineral concentrations. Several studies in birds have shown that the dietary intake of minerals and other nutrients with the feed affects the measured values of those nutrients in the blood.[4–9,31] For example, in wild Andean flamingo chicks (*Phoenicoparrus andinus*), a study showed that the serum concentrations of total protein, cholesterol, and Ca were affected by differences in diet, while P concentrations were not.[4] While greater and lesser flamingos occur together in some areas of Africa, the 2 species have different diet and feeding behaviors because greater flamingos tend to feed more on crustaceans and small invertebrates in the mud below the water,[2,32] while lesser flamingos filter algae in flat water. Differences in feed composition can also influence plasma mineral values in animals under human care.[6,9] A study evaluating managed greater flamingos found significant differences in Zn and Cu in the blood depending on the diet,[9] while in another study, Ca, Cu, Fe, Mg, Mn, Na, and Se blood concentrations were significantly influenced by a dietary change in the same collection.[6] In the present study, detailed information on the mineral content of the feed, water, or environment was not available, but could all influence plasma mineral concentrations.

It is not only the diet that affects plasma mineral values but also the sex of the animals, their age, and, in this context, the season of blood sampling. The concentrations of minerals like Ca, Mg, and P remain relatively constant in the blood due to hormonal influences, including from parathyroid hormone and vitamin D; however, they can change in response to physiological changes in requirements (eg, increased Ca requirements during oviposition in females or during bone growth in juveniles).[10–13,33] During oviposition, sex hormones like estrogen and estradiol moderate the mobilization of Ca from the bones.[10,33] Other minerals are also released during the course of bone remodeling, leading to changes in plasma concentrations of other minerals as well.[17,34] Some minerals are also stored in the egg and the eggshell, leading to significant differences in plasma concentrations for a variety of minerals in males and females during egg production.[17,34] In the present study, which was carried out outside of the laying phase, only Co was significantly different between males and females.

The age of the individuals was only associated with minor changes in plasma concentrations of select minerals, with Cu and Zn increasing and I and Se decreasing with age. It is likely that there are greater differences between young animals and adults during the first few months of age. However, the youngest animals tested in the present study were just over 1 year old and had been eating the same diet as the adults for some time. Lesser flamingos do not achieve their adult plumage until they are 3 to 4 years old and do not breed until they are 6 years of age.[2] Other seasonal influences, such as molting, can play a role in plasma mineral concentrations, as has been documented for Zn in waterfowl.[8] Diurnal fluctuations can also play a role in Cu and Zn concentrations in psittacine birds.[20] To minimize these fluctuations, the samples in this study were all collected in the morning on the same day after the breeding season and before molting.

Sample storage, transport, and treatment can also affect measured concentrations of specific analytes. The plasma samples tested were all centrifuged $<$ 2 hours after collection, shipped cooled and light protected, and were analyzed after 1 month of freezing in microtubes with screw caps and rubber seals to prevent dehydration. A study in human blood and plasma showed that trace elements are stable in frozen (-20°C; -4°F) samples for 1 year.[35] Another important consideration is sample quality. In psittacine bird plasma, hemolysis has been shown to influence the mineral concentrations measured by spectrophotometry, especially the Fe concentration.[20] In flamingos, the reddish coloration of the plasma due to the carotenoids fed must also be taken into account.[36] In a previous study in greater flamingos,[6] the ICP-MS method used in this study was significantly less sensitive to optical alterations of the sample than spectrophotometry. Lipemic samples were excluded from the evaluation to avoid possible influences of sample quality on the measurements, especially on the spectrophotometric measurement of Ca, P, Na, and K.

The results of the present study provide plasma mineral and electrolyte reference intervals from a large population of managed lesser flamingos. The sex and age of the flamingos outside the breeding season and in birds over 1 year had no significant influence on most of the measured plasma minerals. The values compiled here can serve as a basis for future studies of flamingos, both in captivity and in the wild, and as the basis for a useful monitoring tool to evaluate the nutritional status and diets of managed flamingos.

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Conflicts of Interest: Two of the authors (CL and REM) are employed by a commercial laboratory. This employment had no influence on the study.

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