Journal of
Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12184

ORIGINAL ARTICLE

Comparison of plasma vitamin A and E, copper and zinc levels in free-ranging and captive greater flamingos (*Phoenicopterus roseus*) and their relation to pododermatitis

F. Wyss[1], P. Wolf[2], C. Wenker[3], S. Hoby[3], V. Schumacher[1], A. Béchet[4], N. Robert[3] and A. Liesegang[5]

1 Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
2 Institute of Animal Nutrition, University of Veterinary Medicine Hannover, Hannover, Germany
3 Zoo Basel, Basel, Switzerland
4 La Tour du Valat, Centre de recherche pour la conservation des zones humides méditerranéennes, le Sambuc, France, and
5 Institute of Animal Nutrition, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Summary

Pododermatitis is a worldwide problem in captive flamingos. Studies in domestic poultry showed that nutrition is a possible influencing factor for pododermatitis. Vitamin A and E, copper and zinc levels were analysed in two different diets (diet 1 = in-house mix and diet 2 = commercial diet) and in plasma of captive greater flamingos fed these diets and compared to those of free-ranging greater flamingos. Results were analysed with respect to type and severity of foot lesions of the individuals from the different groups. Juvenile and subadult/adult captive flamingos on diet 1 showed various types and severities of foot lesions, whereas no foot lesions were found at the time of blood sampling in juvenile captive flamingos on diet 2. Juvenile captive flamingos on diet 1 had significantly lower plasma zinc levels than juvenile captive flamingos on diet 2 and juvenile free-ranging flamingos; data were also lower than reference ranges for flamingos, poultry and cranes. There were no significant differences in plasma vitamin A, vitamin E, copper or zinc levels between animals with different types of foot lesions or with different severity scores. Shortly after the change to diet 2 (fed to juvenile captive flamingos that did not show any foot lesion), the flooring of the outdoor water pools was covered with fine granular sand. Because both factors (nutrition and flooring) were changed during the same evaluation period, it cannot be concluded which factor contributed in what extent to the reduction of foot lesions. While it is assumed that low plasma zinc levels identified in the group of juvenile captive flamingos on diet 1 were not directly responsible for foot lesions observed in these animals, they may have played a role in altering the skin integrity of the feet and predisposing them to pododermatitis.

Keywords pododermatitis, nutrition, zinc, greater flamingo, *Phoenicopterus ruber roseus*

Correspondence F. Wyss, Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland. Tel: +41 44 635 83 45; Fax: +41 44 635 89 20; E-mail: fwyss@vetclinics.uzh.ch

Received: 24 April 2013; accepted: 18 February 2014

Introduction

Around 5500 greater flamingos (*Phoenicopterus roseus*) are kept in more than 130 institutions around the world, with the majority being kept in Europe (ISIS, 2013). A review of flamingo necropsy reports performed between 2000 and 2011 by the Institute of Animal Pathology, University of Bern, Switzerland, revealed that pododermatitis was the most frequent lesion in captive flamingos with a prevalence of 95%, either as the reason for euthanasia or as a secondary finding to other pathologies (F. Wyss, unpublished observation). A survey within the European and North American flamingo population confirmed that 100% of captive flamingos have varying degrees of skin changes on their foot soles (Nielsen et al., 2010).

The influence of several nutritional factors on foot problems is well known in domestic poultry (Wahlström et al., 2001; Nagaraj et al., 2007; Kamphues et al., 2011; Youssef et al., 2012). Several vitamins and minerals are known for their direct or indirect impact on skin and especially foot pad health. For example, higher water intake and excretion and subsequent increased litter moisture due to feeding of soybean meal extract (high potassium levels) are considered as indirect effects (Kamphues et al., 2011; Youssef et al., 2011). Vitamin A is known to play an important role in epithelial proliferation and keratinization in humans (Ryan and Goldsmith, 1996). Hyperkeratosis of the avian integument is a clinical sign of vitamin A deficiency, but also vitamin A excess (Harrison and McDonald, 2006). Obesity and inactivity, combined with a weakened skin caused by vitamin A deficiency, is suspected to lead to erosions and ulcers of the plantar foot (Schmidt and Lightfoot, 2006). Vitamin E (alpha-tocopherol) plays a role in stabilizing cell membranes by deactivating free radicals with its antioxidant properties. Vitamin E is used for treating different types of skin disorders in mammals (Davenport and Reinhart, 2000), and cellulitis in broilers was reduced when supplementing with a combination of vitamin E and complexed zinc (Downs et al., 2000). Copper is essential for the production of mature collagen and elastin as well as hair maturation (Ryan and Goldsmith, 1996). Clinical manifestations of copper deficiency are hair and skin changes, especially depigmentation of the hair coat, follicular hyperkeratosis and inelastic skin in humans (Ryan and Goldsmith, 1996; Davenport and Reinhart, 2000). Zinc is a component of a number of metalloenzymes, and a supply of dietary zinc is needed for the maintenance of epidermal integrity (Davenport and Reinhart, 2000). Treatment with oral zinc ameliorated the clinical signs in dogs with periocular crusts and parakeratosis (White et al., 2001), and deficiencies of zinc and biotin have been associated with hyperkeratosis in birds (Harrison and McDonald, 2006). Feed supplementation of turkeys, housed on dry litter, with biotin and zinc reduced development and severity of foot lesions significantly; this effect was not seen when turkeys were housed on wet litter (Youssef et al., 2012). On the contrary, Zn-methionine and Zn-lysine supplemented to broilers did not show an influence on foot pad quality (Hess et al., 2001) and a 50% increased supplementation of zinc and manganese did not show any effect on the foot condition of single comb white leghorn layers (Burger et al., 1984).

The Zoo Basel has kept greater flamingos since 1933 and has a long history of flamingo research (Studer-Thiersch, 2000, 2005). Foot problems have already occurred for a period of time but were first examined comprehensively when the birds were moved to their new enclosure in 1991. A classification system for different foot lesions was used at that time, and when the examination was repeated in 1997/1998, lesions seemed to have decreased (Studer-Thiersch et al., 2005). The present study was initiated because since 2005, foot problems had again subjectively increased. In winter 2010/2011, the feeding regime was changed from an in-house food mix to a commercially available product. Shortly after the feeding change, the flooring of the outdoor water pools in the flamingo enclosure was covered with fine granular sand as a result of successful trials with small flamingo groups to reduce foot lesions (Wyss et al., 2014). The aim of this study was to test whether deficiencies of vitamin A or E, copper or zinc could be a contributing factor in the onset and development of pododermatitis in captive flamingos. Plasma levels of vitamin A, vitamin E, copper and zinc were determined from greater flamingos in the Zoo Basel and compared to levels of free-ranging greater flamingos from the Camargue (Southern France). Additionally, two different diets were analysed and the results were evaluated in relation to the blood analyses. No foot lesions were found in free-ranging flamingos (Wyss et al., 2013); therefore, they serve as a reference.

Materials and methods

Study population

The greater flamingo group of Zoo Basel consists of approximately 110 adult animals and their juveniles (20–25 each year). The age varies from a few months up to 56 years and the sex ratio is balanced. 60 captive greater flamingos at Zoo Basel were used in this study, among them 10 males, 16 females and 34 of unknown gender. Blood sampling and foot examination were performed during routine capture operations for feather clipping and ringing between November 2010 and August 2011. The captive study groups are described in Table 1. Typing of foot lesions and severity scoring were performed according to an established protocol (Nielsen et al., 2010; Wyss et al., 2013) and always carried out by the first author. Severity scoring was used to provide an overall evaluation of the feet independent of the type of lesion, from no lesion (=0), to mild, mild-moderate, moderate, moderate-severe and severe lesions (=5). In this study, none of the birds that received severity score 5 was selected. None of the captive flamingos (only free-ranging flamingos) was scored 0.

Blood from 12 three-month-old free-ranging greater flamingos from the Camargue, Southern France, was collected during yearly routine capture activities in July 2011 when juvenile birds were ringed, weighed and measured (Johnson and Cézilly, 2007). This operation was supervised by the Tour du

Table 1 Comparison of plasma levels (mean ± standard deviation) and corresponding dietary contents of vitamin A, vitamin E, copper and zinc from greater flamingos in relation to blood levels in flamingos and other bird species

	Free-ranging group	Captive Group A	Captive Group B	Captive Group C	Reference Flamingo (Ward et al., 2001)	Reference Flamingo (Benato et al., 2013)*	Reference Chicken (Puls, 1994a,b)	Reference Amazon Parrots (Osofsky et al., 2001)
Age	3 months	5–7 months	Subadult/adult	3 months				Adult
Blood (n)	12 (10)†	32 (12)†	9 (4)†	20		21		45
Sampling time point	July 2011	Nov-Jan 2011/12	Nov-Jan 2011/12	Aug 2011				
Vitamin A (mg/dl)	0.059 ± 0.009^{ab}	0.063 ± 0.015^{a}	0.066 ± 0.013^{a}	0.051 ± 0.010^{b}		0.034–0.058	0.030–0.080	-
Vitamin E (mg/dl)	0.74 ± 0.22^{a}	0.89 ± 0.29^{ab}	1.72 ± 0.41^{c}	1.16 ± 0.30^{bc}		0.92–1.64	0.10–0.35	-
Copper (μg/dl)	32.5 ± 2.4^{a}	38.1 ± 22.0^{a}	55.4 ± 8.0^{b}	44.0 ± 5.1^{b}		29.4–41.3	8.0–50.0	8.0–21.0
Zinc (μg/dl)	177.9 ± 23.7^{a}	125.9 ± 40.0^{b}	173.0 ± 20.6^{ab}	208.6 ± 32.6^{a}		172.0–232.7	145.0–340.0	130.0–254.0
Diet (in dm)	Natural diet	Diet 1‡	Diet 1‡	Diet 2‡	Crop milk			
Vitamin A (IE/kg)	n.d.	14 737	14 737	18 502	16 697 –19 085			
Vitamin E (mg/kg)	n.d.	143	143	173	8.1–28.2			
Copper (mg/kg)	n.d.	16.5	16.5	11.2	-			
Zinc (mg/kg)	n.d.	145	145	95	8.4–27.3			
Calcium (g/kg)	n.d.	14.6	14.6	8.6	0.8–7			

n.d., not determined.

[a,b,c]Within each row, means with different lower-case superscript differ significantly ($p < 0.05$) from each other.

*Converted levels from male greater flamingos; levels from female greater flamingos did not differ significantly (Benato et al., 2013).

†Pooled samples were used to analyse copper and zinc levels in the free-ranging and captive groups A and B; number without brackets is sample size of vitamin A and E, number in brackets is sample size of copper and zinc.

‡Diet 1: self-mixed diet, diet 2: commercially available diet ('Flamingo floating' pellets, Versele-Laga, Deinze, Belgium).

Valat Research Center, France, and took place at the Etang du Fangassier breeding site (43°25′N, 4°38′E).

Enclosures

The enclosure in Zoo Basel is designed for a group of 100–110 adult flamingos with their juveniles and measures about 2000 m^2 including 425 m^2 of shallow water ponds with concrete flooring (max. depth: 25 cm), 135 m^2 of deep water ponds with a natural mud ground (max. depth: 200 cm), two breeding islands (25 and 35 m^2), grass resting sites and marsh area. The winter house is integrated in the enclosure, measures 94 m^2 and has a concrete floor. One half is covered with black rubber mats designed for cattle that offer sufficient resting space for all birds, and the other half consists of two water ponds with concrete floor (25 and 10 m^2, max. depth: 40 cm). In between the evaluation of groups A and B (November-January 2010/2011) and group C (August 2011), the concrete flooring of the outdoor water ponds was covered with fine granular sand due to good results on foot health in preliminary trials with small flamingo groups (Wyss et al., 2014).

Blood plasma analysis

Animals were manually restrained and approximately 0.5 ml of blood was taken from the right jugular vein at the base of the neck with a 22-G needle and a 2.5-ml syringe. Animals were not fasted before blood sampling. Lithium–heparin tubes for plasma were filled immediately after blood sampling and processed using current methods. Plasma samples were frozen at −20 °C for 1–5 days prior to shipping and were shipped on dry ice.

Vitamins A and E, copper and zinc levels in blood plasma were analysed at the Institute of Animal Nutrition in Hannover (Bischofsholer Damm 15, D-30173 Hannover, Germany). For the measurement of copper and zinc, samples were dissolved in distilled water (1:10) and analysed by atom absorption spectrometry (Unicam Solaar M; Unicam, Dreieich, Germany). For the determination of vitamin A and E, 1 ml of plasma was added to 5 ml methanolic KOH 40% and saponified for 1 h. Afterwards, 20 ml of a mixture of ethanol and water (1:4) was added. After the above-mentioned alkaline saponification, the sample was extracted with 10 ml toluol-hexane (1:1). The extract was evaporated and dissolved in methanol (shaken four times) and then the vitamin A and E levels were measured by HPLC (high-pressure liquid chromatography, 610 Series; Unicam, Kassel, Deutschland). Vitamin A and E were analysed separately for each animal, but from some animals not enough blood could be taken and, therefore, analyses of copper and zinc were performed with pooled samples from 2 to 4 birds.

Diet analyses

Diet 1 is the previous, in-house food mix (fed to groups A and B) and was composed of 50% of a pelleted complete diet for lying hens (Legehennenwürfel 3960; Provimi Kliba AG, Kaiseraugst, Switzerland), 30% cereal (corn, wheat and oat flakes), 10% shredded shrimps, 5% supplement for carnivores (Zusatz für Fleischfresser 3750, Provimi Kliba AG), 5% mineral for cattle and 0.05% canthaxanthin and biotin. This was replaced by the commercially available diet 2, 'Flamingo floating' pellets (Versele-Laga, Deinze, Belgium) and fed to group C. 'Flamingo floating' pellets are an extruded complete diet for flamingos and ibises and primarily contains wheat, fish meal and soya beans. Proximate nutrient analysis of both feed was performed in the Institute of Animal Nutrition (Institute of Animal Nutrition, University of Zurich, Zurich, Switzerland), and vitamin A, vitamin E, zinc and copper levels were determined at the Institute of Animal Nutrition in Hannover. For measurement of copper and zinc levels in the feedstuffs, 1 g of the sample was dissolved in 15 ml of the ashing mixture (1000 ml nitric acid 65% and 250 ml perchloric acid 70%). After ashing, the sample was cooked with 5 ml hydrochloric acid and added into a volumetric flask. In 1 ml of this solution, copper and zinc levels were measured after acid hydrolysis by atom absorption spectrometry. For determination of vitamins A and E, 1 g of the feedstuff was added to 1 ml ascorbic acid 15%, 2 ml ethanol 98% and 1 ml methanol 98%. The solution was saponified with 1 ml KOH (10 M) for 30 min in a 60 °C water bath. Afterwards, the samples were shaken five times with 3 ml hexane and centrifuged at 3500 RMP/min. The hexane phase was added to a 25-ml volumetric flask and the sample was dried at 40 °C. The residue was dissolved in 1000 μl HPLC-eluent, filtrated (syringe filters of 2 μm) and measured by HPLC.

Statistical analyses

Differences in plasma vitamin A and E, copper and zinc levels between the different groups were assessed using Kruskal–Wallis one-way ANOVA and the Kruskal–Wallis multiple-comparison z-value test (Dunn's test). Statistics were performed in NCSS 2007 (www.ncss.com). The level of statistical significance was 0.05.

Results

Diet 1 (fed to groups A and B) was lower in vitamin A and vitamin E than diet 2 (fed to group C), but higher in copper, zinc and calcium (Table 1). The vitamin A and E contents of both diets were higher than recommended for maintenance in poultry (6000–8000 IU vitamin A/kg, 30–50 mg vitamin E/kg, NRC, 1994). Likewise, the minerals copper, zinc and calcium of both diets exceeded usual requirements of poultry in maintenance and the recommendations given by NRC (1994).

Captive juvenile flamingos on diet 2 (group C) had significantly lower plasma vitamin A levels than the captive groups on diet 1 (group A and B) ($p = 0.002$). However, this was not significantly different from the free-ranging group. Plasma vitamin E levels were lowest in free-ranging birds followed by captive juvenile flamingos on diet 1 (group A). Captive subadults/adults on diet 1 (group B) and captive juveniles on diet 2 (group C) had significantly higher plasma vitamin E levels than the free-ranging reference ($p < 0.001$). The free-ranging group and the captive juveniles on diet 1 (group A) had significantly lower plasma copper levels than the captive subadults/adults (group B) on diet 1 and the captive juveniles on diet 2 (group C) ($p < 0.001$). Plasma zinc levels of captive juveniles on diet 1 (group A) were significantly lower than those of the free-ranging group or the captive juveniles on diet 2 (group C) ($p < 0.001$), but did only numerically differ from the captive subadults/adults on diet 1 (group B) (Table 1). Plasma zinc levels of captive juvenile flamingos from group A were also lower than reference ranges of greater flamingos, chickens and Amazon parrots, whereas all other flamingo groups were within these reference ranges (Puls, 1994a; Osofsky et al., 2001; Benato et al., 2013).

Various degrees and types of foot lesions were found at the time of blood withdrawal in captive flamingos of groups A and B, but none in captive flamingos of group C and in free-ranging juvenile flamingos (Table 2). All juveniles of group C were checked again 3 months later (November 2011) at the age of 5 months and only in two (out of 20) juveniles, minimal cracks, so mild that they were not even scored, were found (severity score 0); all other 18 animals did not have any lesions. Concerning birds with foot lesions, no major differences were seen comparing different categories of severity scores or different types of lesions with plasma vitamin A and E, copper or zinc levels (Table 2).

Table 2 Plasma vitamin A, vitamin E, copper and zinc levels (mean ± standard deviation) of the free-ranging group and categories of captive flamingos with different severity foot lesions scores and with different types of foot lesions

	Diet	n*	Vitamin A (mg/dl)	Vitamin E (mg/dl)	Copper (μg/dl)	Zinc (μg/dl)
Without lesions						
Free-ranging	-	12 (10)	0.059 ± 0.009	0.74 ± 0.22	32.5 ± 2.4	177.9 ± 23.7
Captive (group C)	2	20 (20)	0.051 ± 0.010	1.16 ± 0.30	44.0 ± 5.1	208.6 ± 32.6
With lesions: severity						
Grade 1	1	8 (5)	0.069 ± 0.016	1.17 ± 0.47	28.3 ± 8.5	108.3 ± 22.6
Grade 2	1	15 (11)	0.055 ± 0.015	0.96 ± 0.42	36.8 ± 10.0	136.9 ± 40.3
Grade 3	1	16 (5)	0.069 ± 0.011	1.19 ± 0.51	58.7 ± 29.1	154.9 ± 47.6
Grade 4	1	2 (1)	0.065 ± 0.001	0.70 ± 0.20	51.9	145
With lesions: type of lesion						
FSs 1 + 2	1	41 (15)	0.064 ± 0.014	1.08 ± 0.48	42.2 ± 21.1	136.9 ± 40.9
FSs 2	1	36 (14)	0.064 ± 0.015	1.03 ± 0.45	44.4 ± 21.3	138.1 ± 40.9
NLs 1 + 2	1	37 (14)	0.065 ± 0.013	1.04 ± 0.39	41.0 ± 22.0	135.5 ± 42.4
NLs 2	1	19 (11)	0.062 ± 0.012	1.01 ± 0.36	39.0 ± 20.3	123.1 ± 43.5
PGs 1 + 2	1	19 (6)	0.066 ± 0.013	1.19 ± 0.59	61.2 ± 23.0	158.2 ± 42.8
PGs 2	1	18 (5)	0.065 ± 0.013	1.24 ± 0.57	63.0 ± 23.9	177.9 ± 23.7

FSs 1, fissures less than 2 mm; FSs 2, fissures deeper than 2 mm; NLs 1, nodular lesions without exposed necrotic tissue; NLs 2, nodular lesions with exposed necrotic tissue; PGs 1, papillomatous growths, finger-like proliferations; PGs 2, papillomatous growths, clusters or lumps of proliferations (Nielsen et al., 2010).

Typing of foot lesions and severity scoring were performed by the first author according to an established protocol (Nielsen et al., 2010; Wyss et al., 2013).

*Pooled samples were used to analyse copper and zinc levels; number without brackets is sample size of vitamin A and E, number in brackets is sample size of copper and zinc.

Discussion

The results show that significantly low plasma zinc levels occurred in flamingos of group A (juvenile captive flamingos). As foot lesions were found in this group, this could indicate that low plasma zinc levels contribute to the problem.

Blood sampling was performed at different time points throughout the year (groups A and B: November to January; group C: August). Therefore, a seasonal variation of blood mineral and vitamin levels cannot be excluded. However, captive flamingos were always fed with the same diet (diet 1 for groups A and B, diet 2 for group C, respectively). One should note that juvenile captive flamingos on diet 2 (group C, without foot lesions) were only 2.5 months old at the time that blood sampling and foot scoring was performed, as compared to juvenile captive flamingos on diet 1 (group A, with foot lesions), which were 5–7 months old.

Two factors (nutrition and flooring) that may influence foot lesions in flamingos were changed during the same evaluation period; it is therefore impossible to decide whether either factor contributed to the disease.

Vitamin A deficiency as well as vitamin A excess causes hyperkeratosis, which may lead to erosions or ulcers in birds (Harrison and McDonald, 2006). In our study, vitamin A probably did not have a direct effect on pododermatitis, because none of the captive groups differed significantly in plasma vitamin A level from the free-ranging reference group, although flamingos on diet 2 (group C, without foot lesions) had significantly lower plasma vitamin A levels than both other captive groups (groups A and B, with foot lesions) in spite of higher dietary vitamin A levels. Furthermore, all flamingo groups were within the reference range of vitamin A levels of chicken (Puls, 1994b) and within or above those of greater flamingos (Benato et al., 2013). Vitamin E and copper deficiency are suspected to predispose to skin disease (Ryan and Goldsmith, 1996; Davenport and Reinhart, 2000). Significantly lower vitamin E and copper levels were present in juvenile flamingos on diet 1 (group A, with foot lesions) compared to those on diet 2 (without foot lesions), but levels of the free-ranging reference group were even lower. Plasma copper levels were within or above reference ranges of greater flamingos, chickens or Amazon parrots (Puls, 1994a; Osofsky et al., 2001; Benato et al., 2013). Therefore, the measured values are not considered as indicative of vitamin E or copper deficiency.

Juvenile captive flamingos on diet 1 (group A, with foot lesions) had significantly lower plasma zinc levels compared to juvenile captive flamingos on diet 2 (group C, without foot lesions) and juvenile free-ranging flamingos and were also lower than reference ranges of greater flamingos, chickens and Amazon parrots (Puls, 1994a; Osofsky et al., 2001; Benato et al., 2013). However, foot lesions were also present in group B (subadult/adult captive flamingos), which did not have low plasma zinc levels, and in relation to lesion scores/types, no differences in plasma zinc levels were detected. These latter findings indicate that the link between zinc status and pododermatitis is not a direct causative one; low plasma zinc levels may rather be a contributing or predisposing factor (especially for juveniles). It also needs to be noted that normal plasma zinc levels might be measured even if a diet deficient in zinc is fed, as shown in horses (Wichert et al., 2002). Hyperkeratosis was associated with zinc deficiency in birds (Harrison and McDonald, 2006), and turkeys supplemented with zinc showed a lower incidence of foot pad dermatitis if kept on dry substrate (Youssef et al., 2012).

Juvenile flamingos in 2011, which were fed with diet 2, started to eat on their own at an age of 3 weeks, much earlier than the juveniles in 2010 (and the years before), which were still fed regularly by their parents up to an age of 5–6 months. Free-ranging flamingos in the Camargue are usually fed by their parents until fledging, which occurs at the age of about 10 weeks (Johnson and Cézilly, 2007). It is possible that captive juveniles eating by themselves ingested and absorbed more zinc even with a lower amount in the diet than juveniles, which were fed by their parents with crop milk. More evidence for this hypothesis is given by the fact that crop milk of American flamingos contained almost six times less zinc (7.9–27.3 mg/kg) than diet 2 analysed in our study (Ward et al., 2001).

Apart from low zinc availability, either due to low absolute dietary levels or forms comparatively unsuitable for absorption, interactions with various other dietary components, in particular vitamin A, calcium, copper and phytate, can reduce zinc availability. Zinc absorption is positively associated with vitamin A metabolism due to a vitamin A-dependent synthesis of a protein in the ileum, which is involved in zinc absorption (Christian and West, 1998). The diet fed to juveniles with low plasma zinc levels (diet 1) had a 40% higher calcium content compared with the diet fed to juveniles with normal or high plasma zinc levels. High dietary calcium was suspected to reduce zinc absorption due to a zinc–calcium interaction the mechanism of which is not yet clear (Wood and Zheng, 1997); however, this hypothesis is not supported by newer literature (Takasugi et al., 2007). Both diets had a much higher calcium content than various natural food items of free-ranging flamingos; for example, dried *Spirulina* sp. contain 1.3 g/kg calcium, *Artemia salina* contain 0.7 g/kg calcium, Shrimp-*Penaeidae* and *Panalidae* contain 2.2 g/kg calcium (all in dry matter) (Dierenfeld et al., 2000). Potentially, dietary Ca levels in the flamingo diets used here could be reduced during the non-breeding season.

Diet 1, fed to juveniles with low plasma zinc levels, had a higher copper content than diet 2. Zinc absorption can also be reduced due to high copper content of the diet by inducing metallothionein in the intestinal epithelium. Metallothionein binds zinc and thus prevents its binding to the cysteine-rich intestinal protein and subsequently the transport to the blood (Klasing, 1998). An interaction of these two minerals could therefore be possible and have led to reduced zinc absorption in juveniles with low plasma zinc levels.

Phytates form strong and insoluble complexes with cations such as zinc and are present in foods like cereals, corn and rice (Lönnerdal, 2000). Diet 1, fed to juveniles with low plasma zinc levels, consisted of 80% pellets and cereals. The commercially available diet 2 is produced with 40–45% of wheat and 7.5–15% of wheat flour (maximal 60% wheat products) mixed with other ingredients (G. Werquin, personal communication). Diet 2 seems to contain lower amount of cereals than diet 1 and therefore may have reduced phytate content.

Conclusion

It is suggested that zinc absorption in juvenile captive flamingos on diet 1 (group A) was reduced, and that the resulting significant low plasma zinc levels could play a role in the development of pododermatitis in this study group. It seems that low plasma zinc levels itself do not provoke foot lesions, but could weaken the skin and predispose the feet to develop foot lesions due to other factors.

With only two diets examined, this study does not allow making feeding recommendations of a specific diet for captive flamingos, but it suggests that ensuring appropriate circulating zinc levels may be beneficial to flamingos to prevent the development of pododermatitis. When attempting to ensure such levels, it is not only the dietary zinc concentration that needs to be considered, but also other factors such as vitamin A, copper, calcium or phytate content, as well as the age of the juveniles at which they start to feed on their own. The values determined in this study can serve as reference data for future flamingo blood evaluations.

Acknowledgements

The authors thank Dr. Guy Werquin from Versele Laga for his helpful information about the content of ‘Flamingo Floating Pellets’ (diet 2 used in our study), Prof. Marcus G. Doherr for the statistical analyses, Prof. Marcus Clauss for comments on the manuscript, and the Swiss Association of Zoo-, Wild- and Pet Animal Medicine (SVWZH) for financial support.

References

Benato, L.; Rice, C.; Wernery, U.; McKeown, S.; Bailey, T., 2013: Serum concentrations of vitamins and trace elements in clincally healthy greater flamingos (*Phoeniconaias Phoenicopterus rubeus*) and lesser flamingos (*Phoeniconaias minor*). *Journal of Zoo and Wildlife Medicine* **44**, 245–250.

Burger, R. A.; Atuahene, Y. O.; Arscott, G. H., 1984: Effect of several dermatitis preventing agents on foot pad dermatitis in dwarf and normal sized single comb white Leghorn layers. *Poultry Science* **63**, 997–1002.

Christian, P.; West, K. P., 1998: Interactions between zinc and vitamin A: an update. *American Journal of Clinical Nutrition* **68**, 435–441.

Davenport, G. M.; Reinhart, G. A., 2000: Overview: the impact of nutrition on skin and hair coat. Recent advances in canine nutrition and feline nutrition. *IAMS Nutrition Symposium Proceedings*, Chicago, pp. 3–21.

Dierenfeld, E. S.; Sheppard, C. D.; McDonald, D. L., 2000: Nutrition. In: C. Brown, C. E. King (eds), *Flamingo Husbandry Guidelines*, AZA/EAZA, Dallas/Rotterdam, pp. 109–128.

Downs, K. M.; Hess, J. B.; Macklin, K. S.; Norton, R. A., 2000: Dietary zinc complexes and vitamin E for reducing cellulitis incidence in broilers. *Journal of Applied Poultry Research* **9**, 319–323.

Harrison, G. J.; McDonald, D., 2006: Nutritional considerations: section II: nutritional disorders. In: G. J. Harrison, T. L. Lightfoot (eds), *Clinical Avian Medicine*. Spix Publishing, Palm Beach, pp. 108–140.

Hess, J. B.; Bilgili, S. F.; Parson, A. M.; Downs, K. M., 2001: Influence of completed zinc products on live performance and carcass grade of broilers. *Journal of Applied Animal Research* **19**, 49–60.

ISIS, 2013: International species information system. www.isis.org (last accessed 8, April 2013).

Johnson, A. R.; Cézilly, F., 2007: *The Greater Flamingo*. T & AD Poyser, London.

Kamphues, J.; Youssef, I. M. I.; Abd El-Wahab, A.; Üffing, B.; Witte, M.; Tost, M., 2011: Einflüsse der Fütterung und Haltung auf die Fussballengesundheit bei Hühnern und Puten. *Übersichten zur Tierernährung* **39**, 147–195.

Klasing, K. C., 1998: *Comparative Avian Nutrition*. Cab International, Michigan.

Lönnerdal, B., 2000: Dietary factors influencing zinc absorption. *Journal of Nutrition* **130**, 1378–1383.

Nagaraj, M.; Wilson, C. A. P.; Saenmahayak, B.; Hess, J. B.; Bilgili, S. F., 2007:

Evaluation of feed-grade enzyme in broiler diets to reduce pododermatitis. *Journal of Applied Poultry Research* **16**, 52–61.

Nielsen, A. M. V.; Nielsen, S. S.; King, C. E.; Bertelsen, M. F., 2010: Classification and prevalence of foot lesions in captive flamingos (Phoenicopteridae). *Journal of Zoo and Wildlife Medicine* **41**, 44–49.

NRC (National Research Council) 1994: *Nutrient Requirements of Poultry*, 9th revised edn. National Academy of Sciences, Washington, DC.

Osofsky, A.; Jowett, P. L. H.; Hosgood, G.; Tully, T. N., 2001: Determination of normal blood concentrations of lead, zinc, copper, and iron in hispaniolan Amazon parrots (*Amazona ventralis*). *Journal of Avian Medicine and Surgery* **15**, 31–36.

Puls, R., 1994a: *Mineral Levels in Animal Health. Diagnostic Data*. Sherpa International, Clearbrook, Canada.

Puls, R., 1994b: *Vitamin Levels in Animal Health. Diagnostic Data and Bibliographies*. Sherpa International, Clearbrook, Canada.

Ryan, A. S.; Goldsmith, L. A., 1996: Nutrition and the skin. *Clinics in Dermatology* **14**, 389–406.

Schmidt, R. E.; Lightfoot, T. L., 2006: Integument. In: G. J. Harrison, T. L. Lightfoot (eds), *Clinical Avian Medicine*. Spix Publishing, Palm Beach, pp. 395–410.

Studer-Thiersch, A., 2000: What 19 years of observation on captive Greater flamingos suggest about adaptation to breeding under irregular conditions. *Waterbirds* **23**, 150–159.

Studer-Thiersch, A., 2005: Behavioural considerations in flamingo enclosure design. EAZA Bird TAGs Meeting, Heidelberg.

Studer-Thiersch, A.; Von Houwald, F.; Heldstab, A., 2005: Some remarks on foot infections in flamingos. EAZA Bird TAGs Meeting, Heidelberg.

Takasugi, S.; Matsui, T.; Omori, H.; Yano, H., 2007: Excess calcium increases bone zinc concentration without affecting zinc absorption in rats. *Biological Trace Element Research* **116**, 311–320.

Wahlström, A.; Tauson, R.; Elwinger, K., 2001: Plumage condition and health of aviary-kept hens fed mash or crumbled pellets. *Poultry Science* **80**, 266–271.

Ward, A. M.; Hunt, A.; Maslanka, M.; Brown, C., 2001: Nutrient composition of American flamingo crop milk. *Proceedings of the American Zoo and Aquarium Association (AZA) Nutrition Advisory Group*, Lake Buena Vista, pp. 189–194.

White, S.; Bourdeau, P.; Rosychuk, R.; Cohen, B.; Bonenberger, T.; Fieseler, K.; Ihrke, P.; Chapman, P.; Schultheiss, P.; Zur, G.; Cannon, A.; Outerbridge, C., 2001: Zinc-responsive dermatosis in dogs: 41 cases and literature review. *Veterinary Dermatology* **12**, 101–109.

Wichert, B.; Frank, T.; Kienzle, E., 2002: Zinc, copper and selenium intake and status of horses in Bavaria. *Journal of Nutrition* **132**, 1776–1777.

Wood, R. J.; Zheng, J. J., 1997: High dietary calcium intakes reduce zinc absorption and balance in humans. *American Journal of Clinical Nutrition* **65**, 1803–1809.

Wyss, F.; Wenker, C.; Hoby, S.; Gardelli, B.; Studer-Thiersch, A.; Von Houwald, F.; Schumacher, V.; Clauss, M.; Doherr, M. G.; Häfeli, W.; Furrer, S.; Béchet, A.; Robert, N., 2013: Factors influencing the onset and progression of pododermatitis in captive flamingos (Phoenicopteridae). *Schweizer Archiv für Tierheilkunde (SAT)* **155**, 497–503.

Wyss, F.; Wenker, C.; Hoby, S.; Von Houwald, F.; Schumacher, V.; Doherr, M. G.; Robert, N., 2014: The effect of fine granular sand on pododermatitis of captive greater flamingos (*Phoenicopterus roseus*). *Animal Welfare* **23**, 57–61.

Youssef, I. M. I.; Beineke, A.; Rohn, K.; Kamphues, J., 2011: Effects of high dietary levels of soybean meal and its constituents (potassium, oligosaccharides) on foot pad dermatitis in growing turkeys housed on dry and wet litter. *Archives of Animal Nutrition* **65**, 148–162.

Youssef, I. M. I.; Beineke, A.; Rohn, K.; Kamphues, J., 2012: Influences of increased levels of biotin, zinc or mannan oligosaccharides in the diet on foot pad dermatitis in growing turkeys housed on dry and wet litter. *Journal of Animal Physiology and Animal Nutrition* **96**, 747–761.