

Original Study

Squamous Metaplasia Associated With Hypovitaminosis A of the Crop and Salivary Glands in Captive Falcons in the United Arab Emirates

Peter A. McKinney, Ulrich Wernery, Johan Forsman, Joerg Kinne, Barbara Arca-Ruibal, Peter Wencel, Sunita Joseph, Preethamole Varghese, and Jyothi Thyagarajan

Abstract: Twenty falcons exhibiting tongue swelling, oropharyngeal pustules, and crop mucin gland impactions were presented for veterinary care to the Al Aseefa Falcon Clinic in Dubai, United Arab Emirates, from October to January 2018–2021. Squamous metaplasia was confirmed in 2 euthanized falcons from this group. A study was undertaken to assess circulating concentrations of serum retinol in this group of diseased falcons ($n = 20$) compared with the values of a clinically healthy group ($n = 20$). Blood samples were collected and analyzed for concentrations of serum retinol. Comparison of serum retinol concentrations between the 2 groups showed a significant difference ($P < 0.001$), with clinically normal falcons having a median (25–75%) range of 1.43 (1.34–1.66) $\mu\text{g/mL}$ and clinically abnormal falcons 0.17 (0.11–0.36) $\mu\text{g/mL}$. The significant difference in the serum retinol concentrations between groups provided strong evidence that the squamous metaplasia was associated with hypovitaminosis A. These results demonstrate that circulating serum retinol concentrations may be useful for assessing and diagnosing hypovitaminosis A in cases exhibiting distinctive lesions in the tongue, salivary glands, crop, and oropharynx.

Key words: falcon, hypovitaminosis A, vitamin A, avian, retinol, squamous metaplasia, crop, salivary glands

INTRODUCTION

Vitamin A is a fat-soluble vitamin and an essential nutrient for animals. Dietary vitamin A is available as preformed vitamin A (retinol and retinyl esters) and provitamin A carotenoids (beta-carotene and alpha-carotene). In the 1920s, it was first established that a diet deficient in vitamins A, C, and D could induce changes, at the time referred to as keratinization, to the epithelial lining of the larynx, trachea, and ducts of the Meibomian, submaxillary, sublingual, and parotid glands of rats.¹ Further studies showed that vitamin A deficiency alone was enough to induce these changes, which are now referred to as squamous

metaplasia.² In birds, vitamin A is known to play an important role in maintaining healthy and intact epithelial cells within the digestive, respiratory, urinary, and reproductive tracts.^{2–6} In addition, vitamin A deficiency in birds has been reported to lead to issues with embryogenesis and growth, carbohydrate metabolism, vision, immunity, and reproduction.^{2,5,7,8} Vitamin A requirements vary by avian species. For example, granivorous species derive vitamin A from beta-carotene in grain-based diets that, after ingestion, is converted to retinol.⁵ In contrast, birds of prey, such as falcons, may ingest vitamin A as retinol and retinol esters from their prey.⁹ A previous study investigating the concentrations of retinol and its esters in the plasma of 5 species of nestlings and 3 species of adult free-ranging birds of prey found that median plasma retinol concentrations in both adult and nestlings varied greatly between species, possibly as a result of different nutritional strategies.¹⁰ Unfortunately, evidence-based reference values for plasma or serum retinol concentrations for birds of prey do not exist. However, these data are needed to characterize the

From the Al Aseefa Falcon Hospital, Nad Al Shiba 1, Dubai, United Arab Emirates (McKinney, Arca-Ruibal, Wencel); the Central Veterinary Research Laboratory, Zabeel Dubai, United Arab Emirates (Wernery, Kinne, Joseph, Varghese, Thyagarajan); and Distriktsveterinärerna, Vargarda, Herrgard 244791, Vargarda, Sweden (Forsman).

Corresponding Author: Peter A. McKinney, birdvetmckinney@gmail.com

variability of retinol plasma concentrations across species and to determine the specific requirements for falcons.

Falconry in the United Arab Emirates (UAE) is an ancient tradition. Captive falcons used for hunting and racing are typically fed whole-carcass quail or pigeon with organs, skin, and bones. The training, hunting, and racing seasons last from September through March, and some falcons are fed a diet of unsupplemented, boneless pigeon muscle during this period. Between 2018 and 2021, an increased number of falcons were presented to Al Aseefa Falcon Hospital, Dubai, with tongue swellings, impactions of the crop mucin glands, salivary gland swellings, and oropharyngeal raised pustules suggestive of hypovitaminosis A.

This study aimed to measure serum retinol concentrations in clinically healthy, untrained falcons fed a whole carcass diet and to compare this with serum retinol concentrations of diseased falcons under training and fed an unsupplemented pigeon meat diet. Finally, we attempt to relate retinol concentrations to clinical signs of squamous metaplasia and describe the typical oral lesions observed in falcons with this condition. We hypothesized that serum vitamin A concentration would be higher in clinically healthy versus pigeon-fed birds and that lesions would be consistent with squamous metaplasia.

MATERIALS AND METHODS

Animals

Two groups of falcons, group A and group B, were evaluated. Group A consisted of 20 diseased birds, and group B consisted of 20 clinically healthy birds that served as controls. Group B was comprised of 9 female and 11 male gyrfalcons (*Falco rusticolus*) that were imported to the UAE 1 week before sampling from a breeder based in the United Kingdom (UK). Group B falcons were between 5 and 8 months old, untrained, and presented with no clinical signs; thus, these birds were determined to be clinically healthy. Before their arrival in the UAE, group B falcons were fed a whole-carcass diet, including rats, whole chicken, day-old chicks, and quail. No vitamin supplement was added to the diet. All falcons in groups A and B underwent a basic visual examination of the oral cavity and endoscopic examination of the caudal thoracic air sac and crop. Group A consisted of 20 clinically affected falcons, 4 males and 16 females, of various species, including 1 female saker (*Falco cherrug*; n = 1), 5 female and 1 male gyrfalcon (n = 6), 3 female and 1 male peregrine (*Falco peregrinus*;

n = 5), and 2 male and 6 female gyrfalcon/peregrine hybrids (*Falco rusticolus* × *Falco peregrinus*; n = 8). The falcons in group A originated from breeding farms where they were fed a complete whole-carcass diet before importation to the UAE. All birds were between 5 and 8 months of age. Before commencing their training in September, the diet of all these falcons was changed from a whole-carcass diet to one exclusively of unsupplemented pigeon muscle. The falcons had been in training for at least 2 months and were presented to the Al Aseefa Falcon Hospital over a 3-month period between October and January, 2018–2021, with nonspecific signs of lethargy, reduced appetite, and weight loss. The lesions found in the tongue, oropharynx, salivary glands, and esophageal and crop mucin glands in falcons from group A were typical of those previously described for squamous metaplasia in chickens^{5,11} and turkeys.⁸

Clinical examination, blood sampling, and endoscopy

All falcons in groups A and B were fasted for 14 hours before examination. Anesthesia was induced with 5% isoflurane (Vet One, Boise, ID, USA) in 2 L/min oxygen administered by a face mask. Once anesthesia was induced, the isoflurane concentration was reduced to 2.5%. A blood sample (3 mL) was collected from each bird via the metatarsal vein and placed into a blood collection tube with no anticoagulant. Blood samples were centrifuged after collection at 9000g for 5 minutes. The serum was then aliquoted into Eppendorf microcentrifuge vials and stored in a light-proof container at −20°C (−4°F) and subsequently submitted for serum retinol analysis. Endoscopic examination of the esophagus and crop was performed with a 2.7-mm rigid endoscope (Karl Storz, Tuttlingen, Germany).

Necropsy and histopathology

Two falcons from group A exhibiting severe tongue swelling, oropharyngeal pustules, bilateral periorbital swelling, maxillary salivary gland swelling, and diffuse impaction of the crop mucin glands were euthanized with 2 mL intravenous pentobarbitone (Lethabarb, Virbac Milperra NSW, Australia) and submitted to the Central Veterinary Research Laboratory (Dubai, UAE) for routine necropsy and histological analysis.

Bacteriology

Bacterial cultures were performed with tissues collected from the maxillary salivary gland, oral pustules,

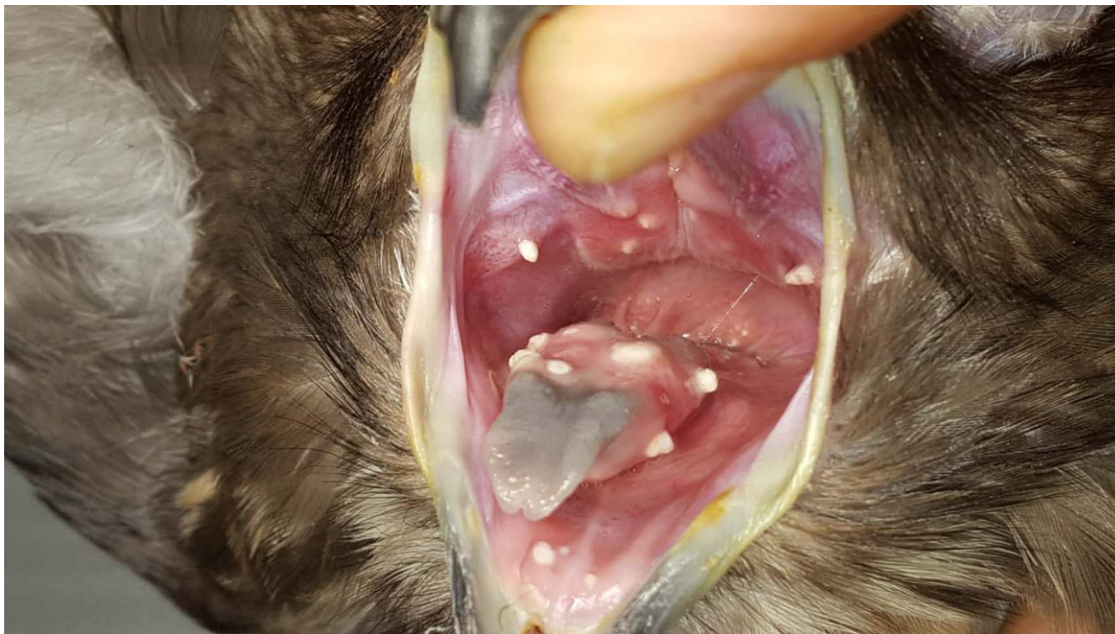


Figure 1. Nodules in the oral mucosa and tongue of a gyrfalcon (*Falco rusticolus*) with hypovitaminosis A.

and superior palate from the 2 euthanized falcons by directly plating samples onto 5% sheep blood agar (Neogen, Ayr, UK), brilliant green phenol red lactose sucrose agar (Merck, Rahway, NJ, USA), and nutrient agar (Oxoid, Basingstoke, Hampshire, UK). Plates were incubated at 37°C (98.6°F) for 24 hours. Subcultures were done to yield pure growth of bacterial strains, and these samples were further characterized by an analytical profile index system (API; bioMérieux, Durham, NC, USA) and Vitek 2 Compact system (bioMérieux). Antimicrobial sensitivity testing of these strains was performed according to the Kirby-Bauer disk diffusion method at the Central Veterinary Research Laboratory in Dubai.

Retinol assay

The serum samples were processed with the chromsystem kit (Chromosystems, Grafelfing, Bavaria, Germany), which consists of standard, control, internal standard, and precipitation reagent. Fifty microliters of the supernatant from each bird were injected into a high-performance liquid chromatography system with an ultraviolet detector. The instrument parameters were set at 1.5-mL/min flow rate of the column, 325 ultraviolet wavelength, and a run time of approximately 9 minutes. The chromatographic peaks were recorded for vitamin A level quantification.

Statistical analyses

The distribution of the retinol data was evaluated by the Shapiro-Wilk test, skewness, kurtosis, and q-q plots. The data did not meet the assumption of normality and were log transformed. A univariate general linear model was used to determine if sex, age, species, or group impacted serum retinol concentrations. If differences were found, a post-hoc Bonferroni test was done. SPSS 25.0 (IBM Statistics, Armonk, NY, USA) was used to analyze the data. A $P < 0.05$ was used to determine significance.

RESULTS

All group A falcons exhibited a range of clinical findings, including swelling of the tongue and mandibular and maxillary salivary glands. Oropharyngeal nodules ranged in size from < 1 to 5 mm in diameter (Fig 1). Thick yellow purulent material was manually expressed from swollen tongues and maxillary salivary glands and was submitted for microscopic examination. Endoscopic examination of the crop mucosa demonstrated nodule-like lesions in the area of the crop mucin and esophageal mucus glands (Fig 2). Microscopic examination of wet and air-dried smears revealed primarily squamous epithelial cells with few bacteria and a minimal number of inflammatory cells. The lack of inflammatory cells in the affected sites suggested a noninfectious etiology. Group B falcons had no evidence of visual lesions on the tongue or

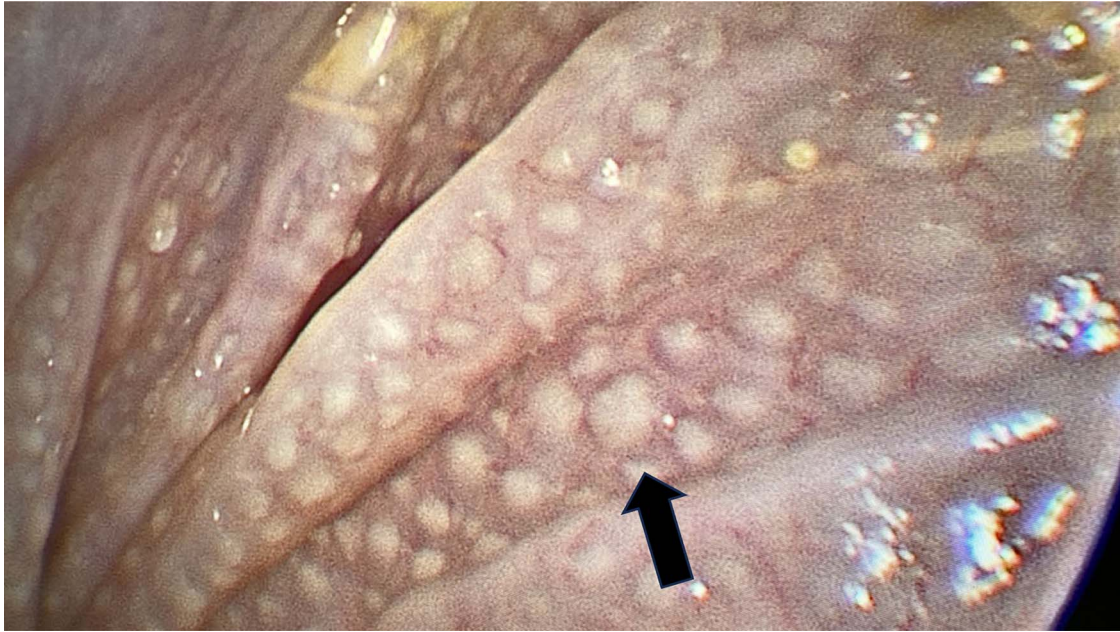


Figure 2. Endoscopic view of impacted crop mucin glands (arrow) of a saker falcon (*Falco cherrug*) with hypovitaminosis A.

oropharynx, nor were there any endoscopic changes to the crop mucosa.

Necropsy of the 2 euthanized falcons from group A revealed swollen maxillary salivary glands, tongues covered with a yellow film, and pustules in the oropharyngeal mucosa. Histopathology revealed extensive hyperplastic squamous metaplasia of the epithelium of the crop mucin glands and focal replacement of cuboidal or columnar epithelium by stratified squamous epithelium (Fig 3), indicative of hypovitaminosis A. *Staphylococcus aureus* and *Escherichia coli* were isolated from the oral cavity and crop lesions of the 2 euthanized falcons and were identified with a latex agglutination kit (Oxoid, Cheshire, UK) and the VITEK system, respectively.

There was a significant difference ($F = 42.9$, $P < 0.001$) in the retinol concentrations between groups A and B, with group B falcons having significantly higher (median $1.43 \mu\text{g/mL}$; 25–75% 1.34 – 1.66 ; minimum–maximum 1.06 – 1.8) retinol concentrations than group A falcons (median $0.171 \mu\text{g/mL}$; 25–75% 0.11 – 0.36 ; minimum–maximum 0.09 – 0.46) (Table 1). There were no significant differences in retinol concentrations by age ($F = 3.3$, $P = 0.075$), sex ($F = 1.6$, $P = 0.208$), or species ($F = 1.8$, $P = 0.168$).

DISCUSSION

Squamous metaplasia as a consequence of hypovitaminosis A has been reported in numerous avian species,

including chickens,^{12–14} turkeys,^{2,8} ducks,^{15,16} parrots,¹⁷ canaries,⁴ and penguins.¹⁸ However, while multiple nutritional deficiencies have been reported in raptors fed a nonsupplemented, meat-only diet,¹⁹ there have been no previous reports of squamous metaplasia associated with hypovitaminosis A in Falconiformes. Vitamin A is essential for the maintenance of secretory tissues, with deficiency leading to changes to mucus-producing cells in the digestive, reproductive, and respiratory systems.^{5,20,21} All falcons in Group A had tongue and salivary gland swellings, oropharyngeal pustules, and crop mucin gland changes, similar lesions to the lesions identified in the 2 euthanized falcons with squamous metaplasia confirmed on histological examination. All showed classic signs of squamous metaplasia reported in poultry where metaplastic changes affecting the salivary glands result in duct blockage and symmetrical swellings developing in the rostral choana, oropharynx, tongue, and mandibular and maxillary salivary glands. Squamous cell metaplasia of glands in the upper respiratory and digestive tracts is considered pathognomonic for hypovitaminosis A, with secondary bacterial infections a feature of the condition.^{5,8,12,13,22,23} The significant difference seen in serum retinol concentrations between the falcons of groups A and B provides strong evidence that the squamous metaplasia was associated with low serum retinol concentrations.

As vitamin A is only acquired by birds through dietary intake, it is proposed that the hypovitaminosis A

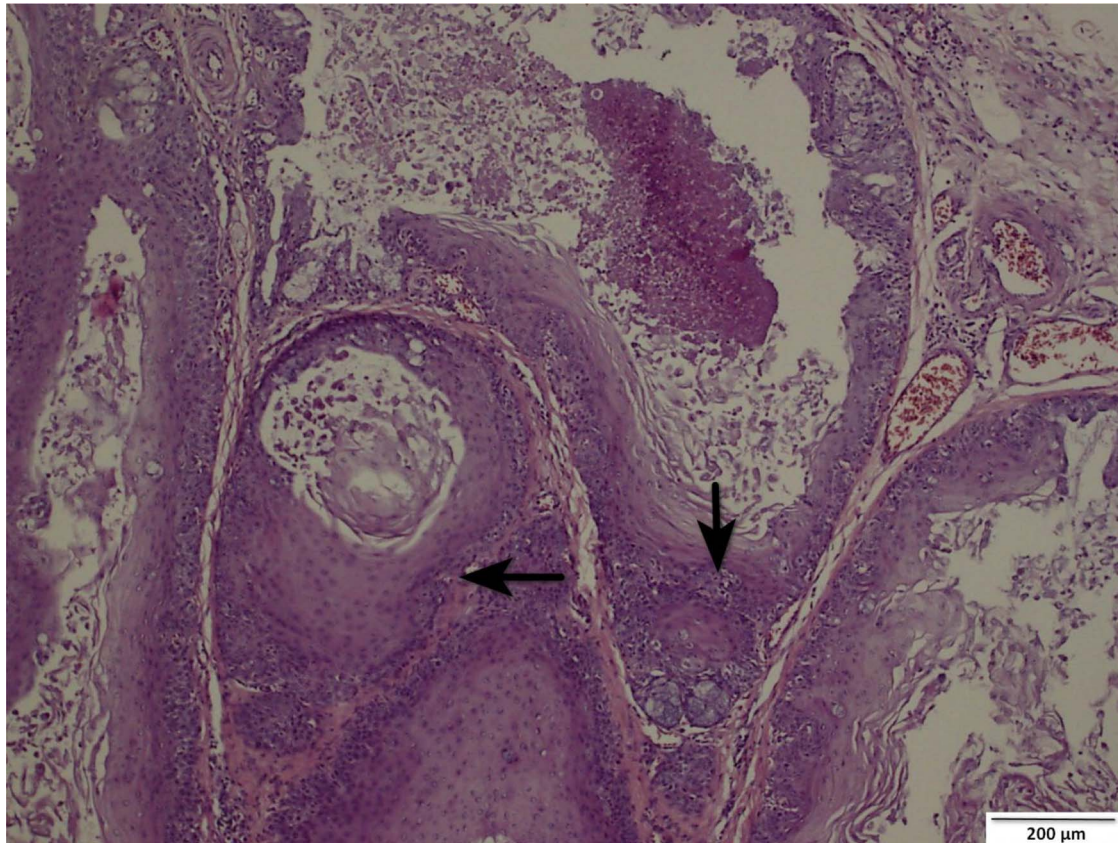


Figure 3. Extensive hyperplastic squamous metaplasia (arrows) of the oral mucosal epithelium in a saker falcon (*Falco cherrug*). Hematoxylin and eosin stain; bar = 200 μ m, $\times 100$.

associated with squamous metaplasia was due to the marked dietary differences between the 2 groups of falcons. In group A, the falcons had been fed an unsupplemented, boneless pigeon meat diet for at least 3 months before the veterinary examination. Lean meat is considered an incomplete diet for falcons and should only be fed for very short periods of time, as it is nutritionally unbalanced and requires vitamin supplementation.²⁴ Feeding liver and skin is considered essential in falconry birds and helps to ensure that birds of prey receive essential fat-soluble vitamins, but this is not a common practice in modern Arabian falconry. Moreover, it is not only birds of prey that may fall victim to a lack of supplementation of meat-only diets because exotic felids in the UAE have also been reported to have lower serum concentrations of vitamin A when fed such diets.²⁵ In addition to vitamin A, it is likely that other vitamins and minerals are deficient in a meat-only diet. This study focused on measuring serum retinol concentrations with the assumption that the diet was chiefly responsible for the low concentrations measured. However, nonnutritional factors such as concurrent infections,

pretraining liver reserves of retinol, exercise intensity, malabsorption, protein malnutrition, and stress may also affect serum retinol concentrations and their depletion. Although group B falcons were considered clinically healthy captive-bred falcons, ideally, serum retinol concentrations from wild falcons would be a more accurate assessment of healthy vitamin A retinol status. Most vitamin A reserves are in the liver, but serum concentrations can reflect a deficiency when liver reserves are depleted. Measurement of vitamin A retinol liver reserves via a liver biopsy would provide greater insight into how quickly such reserves can be depleted in trained falcons.^{26–28} However, such techniques were impractical in a clinical setting as part of the pretraining veterinary assessment. Ideally, serum retinol concentrations should be examined from each falcon at the commencement of training and repeated after several months of fitness training to determine whether any bird is at risk of developing disease before clinical signs become apparent. In poultry, adult birds can be fed a vitamin A deficient diet for 2–5 months before signs of deficiency become apparent, with the timespan to clinical

Table 1. Serum retinol concentrations of clinically abnormal (Group A) and clinically normal (Group B) falcons. Falcons are classified based on age, sex, and species.

Group	Hospital number	Species	Age	Sex	µg retinol/mL
A	8980	GxP	Juvenile	Female	0.09
A	8351	G	Juvenile	Female	0.09
A	3431	P	Juvenile	Female	0.09
A	8853	G	Juvenile	Female	0.09
A	3444	P	Juvenile	Male	0.11
A	2786	GxP	Adult	Male	0.11
A	8750	P	Juvenile	Female	0.11
A	3158	P	Juvenile	Female	0.14
A	8852	GxP	Juvenile	Female	0.14
A	8844	GxP	Juvenile	Female	0.14
A	8826	G	Juvenile	Female	0.20
A	8782	S	Juvenile	Female	0.20
A	8850	G	Juvenile	Female	0.23
A	9053	GxP	Juvenile	Female	0.32
A	3287	GxP	Adult	Male	0.34
A	8749	P	Juvenile	Female	0.37
A	8878	G	Juvenile	Female	0.40
A	8819	G	Juvenile	Male	0.43
A	9054	GxP	Juvenile	Female	0.46
A	6859	GxP	Adult	Female	0.46
B	7574	G	Juvenile	Male	1.06
B	7599	G	Juvenile	Male	1.15
B	7603	G	Juvenile	Male	1.29
B	7610	G	Juvenile	Female	1.32
B	7617	G	Juvenile	Female	1.35
B	7587	G	Juvenile	Male	1.35
B	7591	G	Juvenile	Male	1.37
B	7620	G	Juvenile	Female	1.37
B	7614	G	Juvenile	Female	1.43
B	7680	G	Juvenile	Male	1.43
B	7619	G	Juvenile	Female	1.43
B	7583	G	Juvenile	Male	1.46
B	7608	G	Juvenile	Female	1.52
B	7579	G	Juvenile	Male	1.63
B	7604	G	Juvenile	Male	1.66
B	7621	G	Juvenile	Female	1.66
B	7578	G	Juvenile	Male	1.69
B	7622	G	Juvenile	Female	1.72
B	7613	G	Juvenile	Female	1.72
B	7597	G	Juvenile	Male	1.80

Abbreviations: G, gyrfalcon (*Falco rusticolus*); GxP, gyrfalcon (*Falco rusticolus*) and peregrine (*Falco peregrinus*) hybrid; P, peregrine falcon (*Falco peregrinus*); S, saker falcon (*Falco cherrug*).

signs influenced by hepatic retinol reserves.^{14,29} In turkeys, it has been reported that Vitamin A concentrations were depleted after just 5 weeks of deficient diets, with clinical lesions in the esophagus and oropharynx evident at 4 weeks.^{8,12} Falconry birds are trained to high levels of fitness, and the intense training the birds undertake would be expected to increase their nutritional requirements for vitamin A and potentially

decrease vitamin A reserves faster than in sedentary birds such as poultry. The earliest lesions identified in group A were identified by endoscopic examination of the esophagus and crop mucin glands. Likewise, in poultry, the mucus glands of the alimentary tract present as the first signs of hypovitaminosis A. The progression of the condition in the falcons is similar to that described in poultry.^{8,12,13}

Bacteria isolated from oral lesions of the oropharynx, tongue, and salivary glands from the 2 falcons submitted for necropsy in this study were *S. aureus* and *E. coli*. These likely represent opportunistic bacteria that invaded damaged epithelial tissues. Secondary infections are a likely consequence of the breakdown of the mucous membranes, allowing pathogens to invade, along with immunosuppression, which is a feature of vitamin A deficiency in birds.^{30–33} Falcons with similar clinical presentations have been reported in cases of pseudomoniasis, trichomoniasis, and candidiasis.^{14,34}

In group A, the characteristic lesions and low serum retinol concentrations compared with group B made squamous metaplasia induced by hypovitaminosis A the most probable diagnosis. Further evidence for this diagnosis was that the most severely affected falcons had the lowest retinol concentrations. Plasma retinol concentrations have been reported for some other raptor species (white-tailed sea eagle [*Haliaeetus albicilla*]; osprey, [*Pandion haliaetus*]; northern goshawk, [*Accipiter gentilis*]), with results confirming that there are species-specific differences in plasma vitamin A concentrations, possibly due to differences in nutrition.¹⁰ Significant variations in retinol concentrations have also been reported for different species of parrots.³⁵ Falconiformes have higher retinol concentrations than mammals, and this may represent an adaption to a carnivorous diet with a high supply of vitamin A.⁹ While the current study had limited numbers of falcons ($n = 20$) to establish meaningful reference data for vitamin A concentrations in clinically healthy falcons, it does provide baseline data for these species.

Based on these results, the authors conclude that the primary condition of squamous metaplasia described in the affected falcons resulted from low dietary vitamin A. Clinicians treating such cases should provide vitamin A as part of the treatment protocol. Further, we suggest that the practice of feeding a diet of unsupplemented pigeon meat is detrimental to the nutritional status of hunting falcons because of their intensive training programs. The condition can likely be prevented by feeding liver, which is an excellent source of vitamin A. There is currently insufficient data available

to characterize the vitamin A requirements for Falconiformes and the degradation rate for retinol reserves in intensely trained birds; therefore, it is important to educate falconers on the benefits of feeding a more balanced whole-carcass diet to maintain the health of falconry birds in the UAE.

Acknowledgments: We thank HH Sheikh Mohammad bin Rashid al Maktoum, HH Sheikh Hamdan bin Mohammad al Maktoum, and the falconers of Dubai UAE. We also thank Dr Sandra Wilsher for assistance in this study.

REFERENCES

1. Mori S. The changes in the para-ocular glands which follow the administration of diets low in fat-soluble A; with notes of the effect of the same diets on the salivary glands and the mucosa of the larynx and trachea. *Bull Johns Hopkins Hosp.* 1922;357.
2. Aye PP, Morishita TY, Saif YM, et al. Induction of vitamin A deficiency in turkey breeding stock. *Poult Sci.* 2000;31:71–73.
3. Chandra RK, Vyas D. Vitamin A, immunocompetence and infection. *Food Nut Bull.* 1989;11:12–19.
4. Zwijnenberg RJ, Zwart P. Squamous metaplasia in the salivary glands of canaries (a case report). *Vet Q.* 1994; 16:60–61.
5. Klasing KC. Nutritional diseases. In: DE Swayne, ed. *Diseases of Poultry* 13th edition. Ames, IA: John Wiley and Sons Inc.; 2013:1205–1232.
6. Fan X, Liu S, Liu G, et al. Vitamin A deficiency impairs mucin expression and suppresses the mucosal immune function of the respiratory tract in chicks. *Plos One.* 2015;30:e0139131.
7. Nockels CF, Philips RW. Influence of vitamin A deficiency on tissue glycogen metabolism in growing chickens. *Poult Sci.* 1971;1:174–481.
8. Cortes PL, Tiwary AK, Puschner B, et al. Vitamin A deficiency in turkey poults. *J Vet Diagn Invest.* 2006; 18:489–494.
9. Schweigert FJ, Uehlein-Harrell S, Hegal GV, et al. Vitamin A (retinol and retinyl esters), alpha-tocopherol and lipid levels in captive wild mammals and birds. *J Vet Med.* 1991;38:35–42.
10. Muller K, Raila J, Altenkamp R, et al. Concentrations of retinol, 3, 4-didehydroretinol, and retinyl esters in plasma of free-ranging birds of prey. *J Anim Phys Anim Nutrition.* 2011;96:1044–1053.
11. Seifried O. Studies on A-avitaminosis in chickens; lesions of respiratory tract and their relation to some infectious diseases. *J Exp Med.* 1930;52:519.
12. Jungherr E. Nasal histopathology and liver storage in subtotal vitamin A deficiency of chickens. *Storrs Agri Exp Station Bull.* 1943;250:5–36.
13. Jungherr E. A-hypovitaminosis in commercial poultry flocks on the basis of nasal histopathology. *Poult Sci.* 1945;24:213–256.
14. Konstantinov A. A contribution to the pathomorphology of vitamin A deficiency in chickens. *Zbl Vet Med A.* 1972;19:407–416.
15. Prabhu SN, Gangwar NK, Kumar R, et al. Squamous metaplasia of oesophageal mucous glands in a non-descript duck. *Indian J Poultry Sci.* 2015;50:229–230.
16. Honour SM, Trudeau S, Kennedy S, et al. Experimental vitamin A deficiency in mallards (*Anas platyrhynchos*): lesions and tissue vitamin A levels. *J Wildl Dis.* 1995;31:277–288.
17. Dorrestein GM, Swart S, Vander Hage MH, et al. Metaplastic alterations in the salivary glands of parrots in relation to liver vitamin A levels. *Proc 1st Intl Conf Zoo Avian Med.* 1987;169–174.
18. Naylor AD, Romain P, Cole G, et al. Suspected hypovitaminosis A-associated salt gland adenitis in northern rockhopper penguins (*Eudyptes moseleyi*). *J Zoo Wildl Med.* 2018;4:420–428.
19. Graham DL. Malnutrition in captive birds of prey. In: Page LA, ed. *Wildlife Diseases*. Boston, MA: Springer; 1976:89–94.
20. Manna B, Lund M, Ashbaugh P, et al. Effect of retinoic acid on mucin gene expression in rat airways *in vitro*. *Biochem J.* 1994;297:309–313.
21. Tei M, Spurr-Michaud SJ, Tisdale AS, et al. Vitamin A deficiency alters the expression of mucin genes by rat ocular surface epithelium. *Invest Ophthalmol Vis Sci.* 2000;4:82–88.
22. Macwhirter P. Malnutrition. In: Ritchie BW, Harrison GJ, and Harrison LR, eds. *Avian Medicine Principles and Applications*. Lake Worth, FL: Wingers Publishing, Inc; 1994:852–853.
23. Villazana-Espinoza ET, Hatch AL, Tsin AT. Effect of light exposure on the accumulation and depletion of retinyl ester in the chicken retina. *Exp Eye Res.* 2006; 83:871–876.
24. Fox N. Managing a breeding program: 2.10 food supply, storage, vitamins, and food-borne diseases. In: Fox N, ed. *Understanding the Bird of Prey*. Surrey, UK: Hancock House Publishers Ltd. Zero Ave; 1995:76–78.
25. Kaiser C, Wernery U, Kinne J, et al. The role of copper and vitamin A deficiencies leading to neurological signs in captive cheetahs (*Acinonyx jubatus*) and lions (*Panthera leo*) in the United Arab Emirates. *Food Nutr Sci.* 2014;20:1978–1990.
26. Kollias GV. Liver biopsy techniques in avian clinical practice. *Vet Clin North Am Small Anim Pract.* 1984; 14:287–298.
27. Underwood BA, Loerch JD, Lewis KC. Effects of dietary vitamin A deficiency, retinoic acid and protein quantity and quality on serially obtained plasma and liver levels of vitamin A in rats. *J Nutr.* 1979;109:796–806.
28. Zebisch K, Krautwald-Junghanns ME, Willuhn J. Ultrasound-guided liver biopsy in birds. *Vet Radiol Ultrasound.* 2004;45:241–246.
29. Leeson S. Vitamin deficiencies in poultry. The Merck Veterinary Manual. Updated April 2023. Accessed March 1, 2022. <https://www.msdvetmanual.com/poultry/nutrition-and-management-poultry/vitamin-deficiencies-in-poultry>

30. Bang BG, Bang FB, Ford MA. Lymphocyte depression induced in chickens on diets deficient in vitamin A and other components. *Am J Pathol.* 1972;68:147–162.
31. Freidman A, Meidovsky A, Leitner G, et al. Decreased resistance and immune response to *Escherichia coli* infection in chicks with low or high intakes of vitamin A. *J Nutr.* 1991;121:395–400.
32. Sijtsma SR, Rombout JH, Dohmen MJ, et al. Effect of vitamin A deficiency on the activity of macrophages in Newcastle disease virus-infected chicken. *Vet Immunopathol.* 1991;28:17–27.
33. Dalloul RA, Lillehoj HS, Shellem TA, et al. Effect of vitamin A deficiency on host intestinal immune response to *Eimeria acervulina* in broiler chickens. *Poult Sci.* 2002;8:1509–1515.
34. Samour JH. *Pseudomonas aeruginosa* stomatitis as a sequel to trichomoniasis in captive saker falcons (*Falco cherrug*). *J Avian Med Surg.* 2000;14:113–117.
35. Torresgrossa A, Puschner B, Tell L, et al. Circulating concentrations of vitamins A and E in captive psittacine birds. *J Avian Med Surg.* 2005;19:225–229.