Calcium and vitamin D status of wild psittacines: a study of the sulphur-crested cockatoo (*Cacatua galerita*), long-billed corella (*Cacatua tenuirostris*) and grey parrot (*Psittacus erithacus*).

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Abstract

Serum calcium, phosphorous and vitamin D status were evaluated for wild psittacines, including the Australian sulphur crested cockatoo (*Cacatua galerita*) and long-billed corella (*Cacatua tenuirostris*) with comparisons made to wild caught grey parrots (*Psittacus erithacus*) that had been maintained in captivity on a seed-based diet for a period of 12 months. Mean ionised calcium (1.08 mmol/L, *C. galerita*; 1.01 mmol/L, *C. tenuirostris*; 0.99 mmol/L, *P. erithacus*) expressed as a percent of total calcium (2.04 mmol/L, *C. galerita*; 2.26 mmol/L, *C. tenuirostris*; 1.98 mmol/L, *P. erithacus*) ranged from 43% (*C. tenuirostris*) to 47% (*Psittacus erithacus*) to 53% (*C. galerita*). Mean phosphorous values ranged from 0.79 mmol/L (*C. galerita*) to 2.41 mmol/L (*C. tenuirostris*), with Ca:P ratios ranging from 1.06 (*C. tenuirostris*) to 2.76 (*C. galerita*). Vitamin D (25-OHD) status varied taxonomically ranging from 11.1 nmol/L in *P. erithacus* to 46.43 nmol/L in *C. tenuirostris* (mean 23.33 nmol/L, *C. galerita*; 35.36 nmol/L, *C. tenuirostris*; 21.22 nmol/L, *P. erithacus*). Serum albumin ranged from 6 g/L (*C. tenuirostris*) to 18.04 g/L (*P. erithacus*) with mean values varying taxonomically (12.33 g/L, *C. galerita*; 9.9 g/L *C. tenuirostris*; 16.9 g/L *P. erithacus*). There was marginal variation in mean protein levels of the two Australian species (29 g/L, *C. galerita*; 26.5 g/L *C. tenuirostris*). There was no correlation between total and ionised calcium in *C. tenuirostris* with weak correlations between in *C. galerita* (0.66) *P. erithacus* (0.49) not considered to be statistically significant. There were no significant correlations between albumin and either ionised calcium or total calcium but there was a negative correlation (-0.7) between albumin and ionic calcium when expressed as a percent of total calcium for the Australian species. An even stronger correlation was evident between vitamin D and ionised calcium when expressed as a percent of total calcium (-0.98) in *C. tenuirostris*.

Introduction

Imbalances in dietary calcium, phosphorous and vitamin D are problematic for a number of pet and aviary birds. Hypocalcaemia is characterised by seizures in adult birds with osteodystrophy common in young chicks fed a diet deficient in calcium and vitamin D (Harcourt-Brown, 2003), and is evident in many birds confined to seed-based diets that are imbalanced with regard to calcium and phosphorus. Insufficient UV-B lighting is also correlated with vitamin D deficiencies

(Bernard et al, 1989), which is particularly problematic for birds maintained indoors. In contrast, hypocalcaemia is clinically characterised by soft tissue calcification radiographically, as well as evidence of renal disease, with the parathyroid glands of *Psittacula psittacula* also undergoing degenerative changes (Swarup et al, 1986).

The African grey parrot (*Psittacus erithacus*) is particularly susceptible to calcium deficiency (Hochleithner, 1989; Rosskopf et al, 1985). However, these symptoms can be modified with changes in dietary calcium and vitamin D_3 (Stanford, 2003a,b). In contrast, Australian psittacines such as the budgerigar (*Melopsittacus undulatus*) are vulnerable to excesses of dietary calcium, with recent investigations indicating that calcium levels exceeding 0.7% are toxic for budgerigars (Roset et al, 2000) and blue and gold macaws (Phalen, pers. comm.).

In order to make definitive diagnoses of calcium and vitamin D status of birds in a clinical context, it is necessary to establish baseline data from wild healthy birds. This study compares data from wild Australian and African psittacines with previously published data on captive birds.

Calcium

The majority of the body's calcium (99%) is located in the skeleton with the remaining 1% important in cellular metabolism, blood clotting, enzyme activation, neuromuscular action (Soares, 1995a). The average chick increases body weight 15-fold from hatching to three weeks of age and must accrete 350 mg Ca/kg body weight daily (Soares, 1987). Calcium exists as three fractions in the avian blood: the ionised salt, calcium bound to proteins (mainly albumin) and as complexed calcium bound to a variety of anions (citrate, carbonate and phosphate). While calcium status is often determined from blood concentrations of total calcium, there is some conjecture about the use of total calcium to determine calcium status (Hollis et al, 1997) as a large component (up to 30%) is bound to albumin (Hochleithner, 1997) and is biologically inactive. Therefore, while fluctuations in protein levels or disease state can influence total calcium measurements (Stanford 2003b) any increase would not be considered to have a pathophysiological significance. Serum albumin levels in laying females may rise by up to 100% (Bentley, 1988) and can inflate total calcium results while not impacting on the biologically active ionised calcium but delays of up to 72 hours before processing samples stored in heparin do not significantly impact on results (Stanford, 2003c). Positive correlations between albumin and total protein reported in African greys are not seen in *Amazona* spp (Lumeij, 1990).

In contrast, ionic calcium is maintained by a cascade of regulatory processes and is more likely to be influenced by dietary aspects. Since calcium is absorbed in the ionic form, factors that reduce the concentration of ionic calcium (oxalate, Figures 1 and 2, phytate, excessive sulphate) reduce the uptake (Nelson and Kirby, 1987; Poneros-Schneier and Erdman, 1989). While measurement of the biologically active form (ionised calcium) is the preferred method, there is no published data from wild psittacines with studies confined to captive birds maintained on varying intakes of calcium (Stanford, 2003b).

Phosphorous

Approximately 80% of the body's phosphorous is located in the skeleton with the remaining 20% in nucleotides (ATP), nucleic acids, phospholipids, phosphorylated compounds necessary for metabolism. Phosphorous bound as phytate is nutritionally unavailable in the absence of phytase enzymes. While phytic acid is present in the aleuron and pericarp portion of cereal grains such as barley, rice and wheat (O'Dell et al, 1972) and the germ of corn grains, phytases are only rich in wheat bran so much of the phosphorous in the other grains may have limited nutritional availability. While the ratio of Ca:P in bone is approximately 2:1m optimal dietary ratios recommended for poultry range from 1:1 up to 4:1 in laying hens (NRC, 1994). The inhibitory effect of excess calcium on phosphorous absorbance is linear (Soares, 1995b) so dietary excesses of either calcium or vitamin D_3 may result in calcium/phosphorous imbalances.

Vitamin D

Vitamin D is a group of sterol compounds produced from the provitamins ergosterol in plants and 7-dehydrocholesterol in animals. UV-B radiation spontaneously cleaves the β ring of the sterols to form ergocalciferol (D_2) and 7-dehydrocholesterol (D_3). The term vitamin D is appropriate for all steroids having cholecalciferol biological activity. Vitamin D is efficiently absorbed along with fat from the upper small intestine and fat malabsorption problems due to pancreatic insufficiency or lack of bile salt production decreases vitamin D absorption.

Vitamin D is necessary for the uptake of calcium but dietary excesses can result in hypocalcaemia. There is a lesser tendency for vitamin D_2 forms to cause hypocalcaemia compared to vitamin D_3 in chicks (Rambeck et al, 1984), which may be attributed to metabolic differences whereby vitamin D_2 is deactivated via C-24 hydroxylation in contrast to deactivation of vitamin D_3, which involves metabolic steps subsequent to C-24 hydroxylation (Horst et al, 1986). However, vitamin D_2 is poorly utilised by poultry (Tsang et al 1988), with plasma turnover rate of D_2 in male chickens 1.5 times faster than D_3 and turnover of D_2 metabolites 11-33 times faster than D_3 metabolites (Hoy et al 1988).

Once formed, provitamin D_3 undergoes a thermally induced isomerisation to the active form of vitamin D_3 that takes two to three days to reach completion (McDowell, 2000). As there is a fourfold increase in the circulating concentration of vitamin D_3 that peaks at around 30 hours postradiation (Tian et al, 1994), it is important to compare captive birds that have been sampled at similar times of the day, under similar lighting conditions.

Although vitamin D requirements have yet to be established for pet and aviary birds, few commercial products fall below those for poultry (200 IU D_3/kg) or turkeys (900 IU/kg), Table 1. Excess vitamin D can cause calcification, nephrosis and gout (Highfill, 1998) and cockatiels seem to be particularly susceptible to high vitamin D_3. Other studies indicate that high dietary vitamin D_3 (4000 IU vitamin D_3/kg) is associated with rapid decline in both reproduction and overall health of cockatiels in the second year of breeding season (Brue, 1994; Brue et al, 1998). High mortality has also been reported in budgies fed game bird starter (3300 IU/kg vitamin D_3, 1% Ca) with metastatic calcification of soft tissues (Roset et al, 2000). Metastatic mineralisation in blue and gold macaws on a diet ranging from 1-4000 IU/kg vitamin D_3 (Takeshita et al, 1986) has been reported with nestlings more susceptible to metastatic mineralisation and nephrocalcinosis than other species (Roset et al, 2000).

While most animals and humans do not have a nutritional requirement for vitamin D when sufficient sunlight is available, this is not the case for poultry. Stanford (2003a) also reports a requirement for UV-B radiation in the presence of adequate vitamin D_3 for African greys but extensive studies are yet to be done on other psittacines. Since many species are more sensitive to vitamin D_3 intoxication than poultry (Roset et al, 2000) it is important to determine optimal vitamin D status from a number of different bird species. Vitamin D status is best evaluated from blood samples as, once vitamin D_3 is formed, it is transported in the blood with only minimal concentrations stored in the liver.

Methodology

Nutritional status of three wild psittacine species was evaluated. Blood samples were taken into heparinised tubes from the long-billed corella (*Cacatua tenuirostris*) in January 2003 and the sulphur-crested cockatoo (*Cacatua galerita*) in July 2003 from the north-east of Victoria. Blood samples were also collected from African grey parrots (*Psittacus erithacus*) in September, 12 months after transfer from the wild to captivity and maintained on a seed-based diet. Nutritional status was determined for protein, albumin, total calcium, ionised calcium, phosphorous, and 25-OHD (vitamin D_3).

Results

Summary data are presented in Table 2. Serum calcium and phosphorous concentrations of birds in this study compared favourably with data from other psittacines that range from 1.9-8.08 mmol/L calcium and 0.94-2.91 mmol/L phosphorous (Clubb et al, 1990/1991; Drew et al, 1993; Candeletta et al, 1993). While there was little taxonomic variation in ionic calcium, there was greater variability in serum vitamin D concentrations with more similarity between *Cacatua galerita* and *Psittacus erithacus* than between the two Australian species from the same area.

There were no strong correlations between albumin and either ionised calcium or total calcium levels in the Australian birds, indicating that albumin is not a reliable indicator of either total or ionised calcium levels. However, there were strong negative correlations between ionised calcium as a percent of total calcium and albumin (-0.7) as well as vitamin D (-0.98) in *Cacatua tenuirostris*. There were no correlations between vitamin D and either total/ionised calcium or phosphorous in any of the species evaluated in this study.

Discussion

While previous studies indicate a correlation between total calcium and serum albumin, similar relationships were not detected here. Although there was little taxonomic variation in either the total or ionised content, there was greater variation in albumin levels with highest mean value detected in *Psittacus erithacus* and lowest in *Cacatua tenuirostris*. It would therefore appear that relationships between total calcium and serum albumin are not indicative of true calcium status.

The higher vitamin D concentrations in *Cacatua tenuirostris* may have been a result of higher insolation and variation in oestrogen levels as these samples were collected in the middle of summer, while those of *C. galerita* were collected in the middle of winter. The diet of *C. tenuirostris* also varies from that of *C. galerita* in that it contains a predominance of onion weed corms but it is unlikely that these would provide significant amounts of provitamin D.

Given the susceptibility of *P. erithacus* to calcium deficiency, one may expect significant variation in the nutritional parameters compared to the Australian counterparts. However, there is no data from this study that clearly explains these differences. Studies of African greys in captivity indicate a wider variation in vitamin D status, with birds maintained on seed and housed outdoors having higher mean vitamin D levels (116.52 nmol/L ± 126.7), compared to those housed indoors on either seed (37.91 nmol/L ± 73.74 SD) or formulated products (HBD high potency, 72.79 nmol/L ± 100.41; Stanford, 2003a,b,c). Given that the vitamin D levels of birds maintained indoors on seed-based diets is marginally higher than values from wild birds, it is possible that activity of the parathyroid gland is more important in regulating calcium uptake than dietary parameters and UV-B lighting alone.

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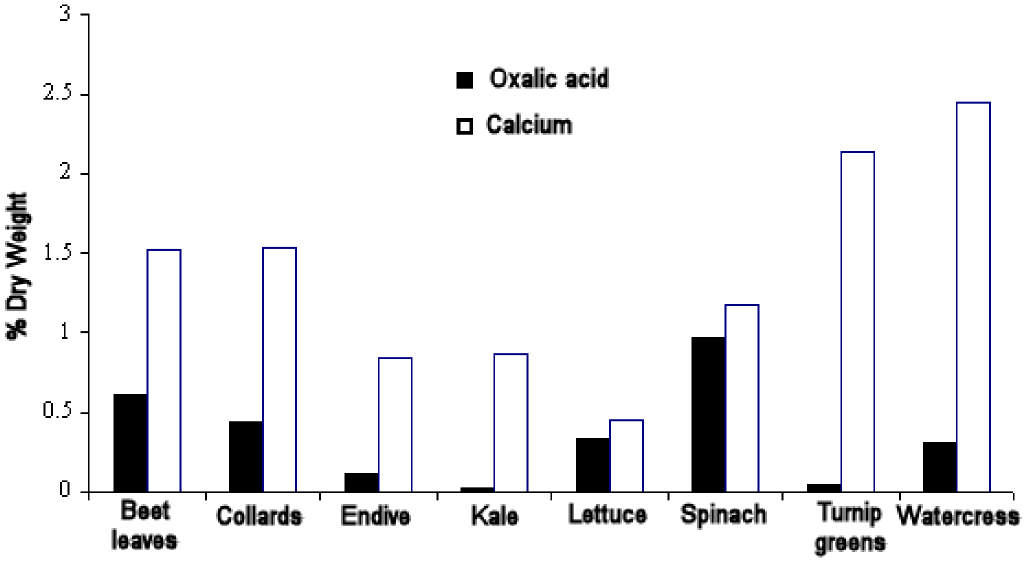

Figure 1. Oxalic acid and calcium content of vegetable greens commonly fed to birds.

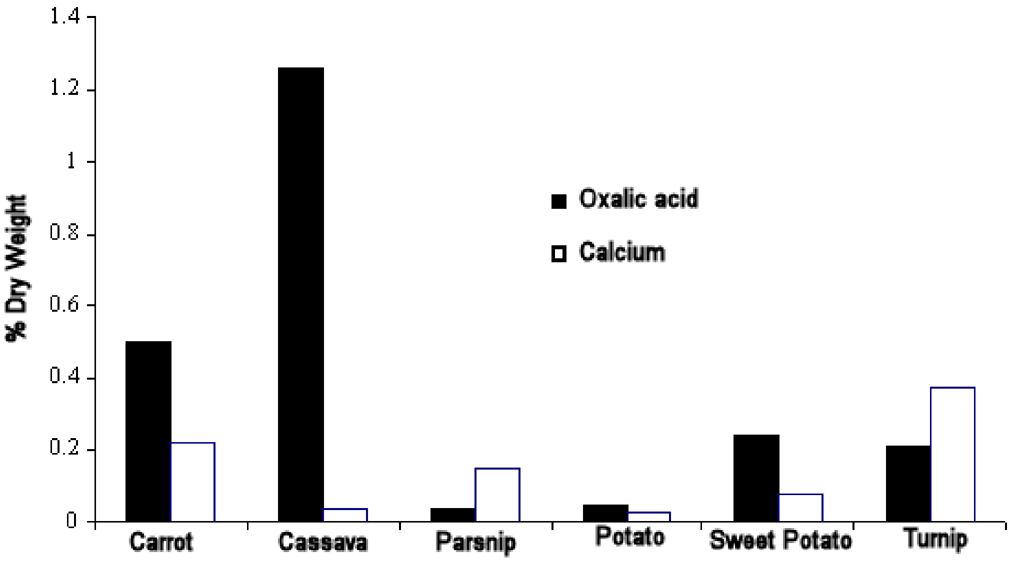

Figure 2. Oxalic acid and calcium content of tuberous vegetables commonly fed to birds.

Product	Manufacturer	Vitamin D_3 (IU/kg)	Calcium (%)
Avipels	Blue Seal	4170	1.11
Bird of Paradise	Zeigler	3970	1.34
Bird of Prey (frozen)	Animal Spectrum	3470	1.05
Chick Starter	Blue Seal	4000	1.19
Crane	Mazuri	10830	2.62
Exotic Game Bird	Mazuri	2500	0.89
Flamingo	Mazuri	6670	1.72
HPC	HBD International	**150**	0.69
Nutribird Parrot	Nutribird	1200	0.9
Palm Cockatoo	SSP	1900	1.1
Psittacine Breeder	Roudybush	1560	1
Psittacine Handfeeder	Roudybush	1560	1
Psittacine Maintenance	Roudybush	**890**	0.44
Scenic Bird	Marion	1600	1.2
Poultry	NRC	**200**	0.99
Turkey	NRC	**900**	0.5

Table 1. Vitamin D and calcium content of various commercial bird foods.

	Protein g/L	**Albumin g/L**	**Ionised Ca mmol/L**	**pH**	**Total Calcium mmol/L**	**Ionised/Total Ca %**	**P mmol/L**	**Total Ca:P**	**OHD nmol/L**
Long-billed Corella	26.5 ± 5.02	9.9 ± 1.66	1.01 ± 0.06	7.71	2.26 ± 0.2	43.35 ± 2.95	2.41 ± 0.91	1.06 ± 0.37	35.36 ± 6.41
Cacatua tenuirostris	(20-38)	(6-12)	(0.92-1.13)	(7.29-8.15)	(1.79-2.43)	(39.75-46.01)	(1.25-4.57)	(0.39-1.78)	(24.71-46.43)
(Count)	10	10	12	12	10	4	10	10	20
Sulphur-crested Cockatoo	29 ± 3.03	12.33 ± 1.77	1.08 ± 0.04	7.37 ± 0.07	2.04 ± 0.16	52.80 ± 3.41	0.79 ± 0.25	2.76 ± 0.65	23.33 ± 3.59
Cacatua galerita	(25-33)	(10-15)	(1.04-1.14)	(7.29-7.46)	(1.85-2.24)	(47.77-56.77)	(0.63-1.28)	(1.56-3.52)	(19.3-29.5)
(Count)	6	6	5	5	6	5	6	6	6
African grey Parrot		16.99 ± 1.05	0.99 ± 0.07		1.98 ± 0.48				21.22 ± 10.12
Psittacus erithacus		(15.94-18.04)	(0.92-1.06)		(1.5-2.46)				(11.1-31.34)
(Count)		20	20		20				20

Table 2. Nutritional status of wild psittacines. Mean values ± SD, with ranges in parentheses.