

# Nutrient Requirements

## Adult Cockatiels (*Nymphicus hollandicus*) at Maintenance Are More Sensitive to Diets Containing Excess Vitamin A Than to Vitamin A-Deficient Diets<sup>1,2</sup>

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**ABSTRACT** The purpose of this experiment was to examine the physiological responses of adult cockatiels at maintenance to dietary vitamin A (VA) concentrations, and to identify concentrations associated with deficiency and toxicity. Adult cockatiels at maintenance ( $n = 22$ , 2–3 y of age) were fed a diet of 0, 600, 3000 or 30,000  $\mu\text{g}$  VA/kg (0, 2000, 10,000 or 100,000 IU), and monitored for signs of VA deficiency or toxicity for up to 706 d. The analyzed diet concentrations were 0, 835, 2815 and 24,549  $\mu\text{g}/\text{kg}$ , respectively. After 269 d, birds fed the 30,000  $\mu\text{g}/\text{kg}$  VA diet had greater plasma retinal concentrations, markedly intensified vocalization patterns, pancreatitis and multifocal accumulation of lymphocytes in the lamina propria of the duodenum compared to birds fed the 600  $\mu\text{g}/\text{kg}$  diet ( $P < 0.05$ ). The 3000  $\mu\text{g}/\text{kg}$  VA diet induced increased plasma retinol, splenic hemosiderosis and altered vocalization patterns ( $P < 0.05$ ), although not as striking as those induced by the 30,000  $\mu\text{g}/\text{kg}$  VA diet. The secondary antibody response was reduced after 225 d and vocalization patterns were altered in birds fed 0  $\mu\text{g}/\text{kg}$  VA ( $P < 0.05$ ), but after almost 2 y there were no changes in body condition, plasma retinol, organ pathology or classical signs of deficiency such as squamous metaplasia of nasal epithelia. Thus, adult cockatiels at maintenance were more susceptible to VA toxicity than to VA deficiency and concentrations  $\geq 3000$   $\mu\text{g}$  VA/kg diet can cause toxicity. It is possible that disturbances in VA nutrition contribute to the widespread incidence of behavioral problems reported in companion birds. *J. Nutr.* 133: 1898–1902, 2003.

**KEY WORDS:** • cockatiel • deficiency • requirement • toxicity • vitamin A

Vitamin A (VA) is an essential micronutrient involved in vision, reproduction, immunity, membrane integrity, growth and embryogenesis. In avian diet formulations, VA can be one of the most challenging nutrients because the range between deficiency and excess is the most narrow of any of the vitamins and the amount found in foods is extremely variable (1,2). Furthermore VA is very heat and light sensitive, and diet processing and storage are associated with a loss of VA activity. For these reasons, various commercial diets may have either deficient or excess concentrations of VA.

The minimum VA requirements for growth and egg production of chickens, turkeys, domestic ducks and Japanese quail have been determined (3), although the requirements of poultry at maintenance (adult, nonreproductive, healthy, non-geriatric animals) are unknown. Virtually no research has been done to determine the dietary concentrations of VA that are required by, or are toxic to, companion avian species, so data from poultry serve as the basis for estimating the needs of companion birds. Most companion avian species differ from

poultry species by being granivorous instead of omnivorous and having an altricial instead of a precocial mode of development. Consequently, extrapolations from poultry to companion avian species are potentially erroneous because of substantial differences between digestive physiology, reproductive performance and rates of embryonic and posthatch development. Additionally, the goals for poultry production (e.g., fast growth rate, maximal egg production) differ markedly from those for companion species (e.g., optimal body condition and feathering, maximal disease resistance and longevity). Because the VA requirements of companion birds are not known and grains used in their diets are very low in VA, feed manufacturers supplement VA to pelleted diets and many, but not all, seed mixtures. Thus, VA concentrations in commercial diets range from potentially deficient to potentially toxic. Characterization of the response of companion birds to excessive and deficient VA concentrations is needed for accurate diagnoses of VA malnutrition.

The purpose of this experiment was to characterize the physiological responses of one species of companion birds, the cockatiel (*Nymphicus hollandicus*), to various dietary VA concentrations, and to provide rough guidelines for the approximate dietary VA concentration resulting in deficiency and toxicity. Cockatiels were chosen as a reference species based on their altricial development pattern, small size and experimental malleability. Additionally, cockatiels are an extremely common companion bird and represent the family Psittacidae,

<sup>1</sup> Portions of these data were previously presented [Koutsos, E. A. & Klasing, K. K., Vitamin A nutrition of cockatiels, in Proceedings of the 4th Comparative Nutrition Society Symposia, Antwerp, Belgium, p. 59].

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which includes parrots, lorikeets, lovebirds, cockatoos and many other popular companion birds.

## MATERIALS AND METHODS

The UC Davis Animal Care and Use Committee approved all protocols. Adult female cockatiels (*Nymphicus hollandicus*, 2–3 y of age,  $n = 22$ ) that were not reproductively active were individually housed ( $30 \times 30 \times 60$ -cm wire cages), at  $24^\circ\text{C}$ , with a 12-h light/dark lighting cycle. Before this experiment, birds were fed a commercial pelleted diet (Roudybush Maintenance; Roudybush Inc., Sacramento, CA) that was formulated to contain  $2363 \mu\text{g VA/kg}$  diet. Birds were randomly assigned to one of four dietary treatments (Table 1), containing 0 ( $n = 6$ ), 600 ( $n = 5$ ), 3000 ( $n = 5$ ) or 30,000 ( $n = 6$ )  $\mu\text{g VA/kg}$  diet (0, 2000, 10,000, or 100,000 IU/kg VA; supplied as retinyl palmitate, containing 75,000 mg retinol/kg; Sigma, St. Louis, MO). Diets were prepared as previously described (4) and the basal diet had no detectable VA or carotenoids. Birds were offered ad libitum access to feed and water.

Birds were monitored for signs of VA deficiency or toxicity by a variety of variables, including monthly assessment of body weight change (body weight at each time point – initial body weight) and general physical appearance. Physical appearance (i.e., body condition) was evaluated subjectively by examining the integument (feather presence and integrity) and by palpating the pectoral muscle to determine the extent of musculature surrounding the keel.

Plasma retinol was assessed after monthly jugular venipuncture and liver retinol was assessed from samples collected at necropsy. The capacity to mount a primary and secondary antibody response was assessed after subcutaneous vaccination of 0.25 mL of sheep red blood cells (SRBC, 2% v/v) on d 211, 218 and 225. At 9 d after the first and last injections (d 211 for primary response, d 234 for secondary response), antibody titers were measured by agglutination procedures as previously described (5). Additionally, bimonthly assessments of oral/nasal epithelium cell types were made by analysis of nasal flushes, obtained by flushing the nasal cavity with 3 mL sterile saline then collecting oral cavity exudates. Subsequently, exudates were centrifuged (5 min at  $800 \times g$ ), concentrated onto slides (Cytospin 2;

Shandon Lipshaw, Pittsburgh, PA), stained with Wright's stain and evaluated microscopically for the presence of basal epithelial cells (ciliated or nonciliated), squamous cells (keratinized or nonkeratinized) and mucin-containing cells.

As the experiment progressed, we noted diet-dependent changes in behavior during handling for nasal flushes, so vocalization analysis was performed on experimental d 135, 163, 198, 449 and 623. Birds were individually placed in a cage in a soundproof room, and after a 2-min acclimation period, birds were held gently while vocalizations were recorded (SpectraPLUS; Pioneer Hill Software, Poulsbo, WA) for 2 min. Recordings were analyzed for the number of vocalizations within a 2-min period, the number within each 30-s interval, the average length of each vocalization and the peak frequency, amplitude and power of vocalizations.

After 269 d of consuming experimental diets, birds fed 30,000  $\mu\text{g VA}$  showed poor body condition, and therefore all birds fed 30,000  $\mu\text{g VA}$  as well as two birds fed 3000  $\mu\text{g VA}$  and two birds fed 600  $\mu\text{g VA}$  were killed by  $\text{CO}_2$  inhalation, after which necropsies were performed. Remaining birds ( $n = 3$  each for 3000  $\mu\text{g VA}$  and 600  $\mu\text{g VA}$ ;  $n = 6$  for 0  $\mu\text{g VA}$ ) were fed dietary treatments for a total of 706 d, at which time they were killed and necropsied. Liver, spleen, kidney, eye, trachea, syrinx, oropharynx, pancreas, bursa, duodenum, sinus and brain were fixed in 10% buffered neutral formalin and processed for histopathology. Birds were coded so that the treatment group was not known during evaluation.

**Retinol analysis.** All chemicals were purchased from Sigma or Fisher Scientific (Springfield, NJ), unless otherwise noted. Briefly, under amber light and/or in amber tubes, liver sections were homogenized in phosphate-buffered saline (PBS) at a ratio of  $\sim 2$  parts tissue: 1 part PBS (w/v). A 1-g sample of tissue homogenate or 300–500  $\mu\text{L}$  plasma was used to extract retinol. Samples were first vortexed with methanolic potassium hydroxide [5% KOH in methanol (w/v),  $1 \times$  sample volume; methanol]. Butanol:acetonitrile (1:1 v/v; butanol) was added at twice the sample volume, then samples were vortexed vigorously for 5 min. Hexane:chloroform (2:1 v/v) was then added at  $1 \times$  sample volume; samples were again vortexed for 5 min and then  $\text{K}_2\text{HPO}_4$  (saturated solution of  $\text{K}_2\text{HPO}_4$  in deionized  $\text{H}_2\text{O}$ ) was added at  $0.1 \times$  sample volume. Samples were vortexed vigorously for 5 min, then centrifuged ( $5000 \times g$ ) for 15 min. The top organic phase was removed, dried under  $\text{N}_2$  and frozen. Before HPLC analysis, samples were reconstituted into the mobile phase consisting of 75% methanol, 12.5% distilled deionized water, 9% acetonitrile, 3% tetrahydrofuran, 0.5% isopropanol, and 0.46 g ammonium acetate/L. The mobile phase was maintained at pH 7.15 with glacial acetic acid.

HPLC analysis was performed by use of a C18 reverse-phase column (5  $\mu\text{m}$ , 300 nm, 4.6 mm I.D.  $\times$  250 mm L; Vydac 201TP54, Hesperia CA) and a high performance guard column (5  $\mu\text{m}$ ; Vydac 201GD54T). The isocratic mobile phase (described above) was maintained at a flow rate of 1.0 mL/min (Waters 510 pump, Waters Associates, Milford MA), and automated injections (Waters WISP 712) of 75  $\mu\text{L}$  were made. A UV/visible detector (Waters 484) monitored at 234 nm, and peak identification and quantitation (Waters Millennium software) were made by comparing samples to a purified all-trans retinol standard. Three injections of the retinol standard at different volumes were made before each sample set, and standard curves were generated by use of linear regression.

The analysis of dietary retinol concentrations was kindly provided by Roche Vitamins (Parsippany, NJ). Each diet was analyzed in duplicate, and identification of calculated diet concentrations was not supplied to the laboratory. Diets were analyzed to contain the following (mean  $\pm$  SD): 0  $\mu\text{g}$  diet, no detectable retinol; 600  $\mu\text{g}$  diet,  $835 \pm 36 \mu\text{g/kg}$ ; 3000  $\mu\text{g}$  diet,  $2815 \pm 70 \mu\text{g/kg}$ ; 30,000  $\mu\text{g}$  diet,  $24,549 \pm 397 \mu\text{g/kg}$ .

**Statistical analysis.** Data were analyzed by a general linear model (JMP; SAS Institute, Cary, NC). Those data collected at a single time (antibody titers, liver retinol) were analyzed by one-way ANOVA for the main effect of the dietary treatment. Data collected at multiple times (body weight change, plasma retinol, nasal epithelial flushes, vocalizations) were analyzed by two-way ANOVA for the main effects of the dietary treatment, days fed the dietary treatment and their interactions. Additionally, this model nested individual

TABLE 1

Composition of the basal diet fed to adult female cockatiels at maintenance<sup>1</sup>

Ingredient	g/kg diet
Rice flour	470
Soybean meal	380
Cellulose	65.3
Vegetable oil	50
Dicalcium phosphate	25
Calcium carbonate	9
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	5.5
NaCl	5.5
D,L-Methionine	3
Vitamin mix <sup>2</sup>	2.5
Mineral mix <sup>2</sup>	2.5
Choline	2
Threonine	1
$\text{Na}_2\text{CO}_3$	0.9

<sup>1</sup> Adult female cockatiels ( $n = 22$ , 2–3 y of age, not reproductively active) at maintenance were offered ad libitum access to diet.

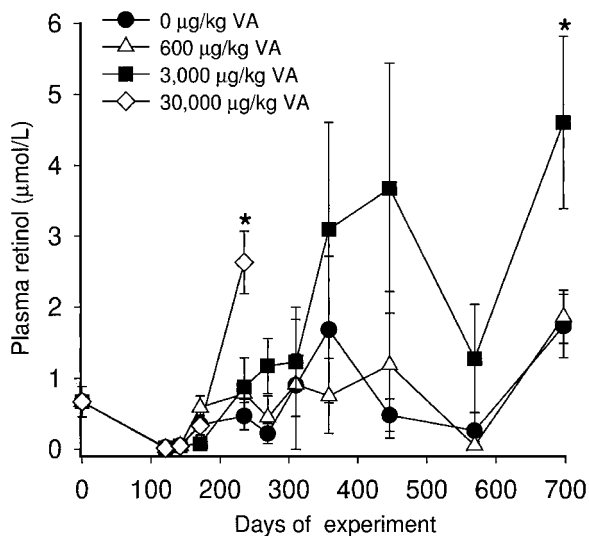
<sup>2</sup> Vitamin mix supplied (per kg final diet): thiamin HCl, 13.3 mg; riboflavin, 15 mg; Ca-D-pantothenate, 20 mg; niacin, 50 mg; pyridoxine HCl, 7.8 mg; folic acid, 6 mg; biotin, 0.5 mg; cholecalciferol, 7.65  $\mu\text{g}$ ; DL- $\alpha$ -tocopherol acid succinate, 24.2 mg; ascorbate, 50 mg; B-12, 20  $\mu\text{g}$ ; menadione sodium bisulfate, 4.5 mg. Mineral mix supplied (per kg final diet): KCl, 1 g;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 350 mg;  $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$ , 150 mg;  $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ , 500 mg;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 30 mg;  $\text{Na}_2\text{SeO}_3$ , 0.2 mg;  $\text{KIO}_4$ , 2.0 mg;  $\text{CoCl}_2$ , 1.7 mg;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 123 mg;  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 8.3 mg.

bird observations within the dietary treatment, which accounts for repeated observations on a single individual. When appropriate, means comparisons were made by preplanned orthogonal contrasts in which the 0, 3000 and 30,000  $\mu\text{g}/\text{kg}$  treatments were individually compared to the 600  $\mu\text{g}/\text{kg}$  treatment. Histopathology data were scored for the presence or absence of pathology and then values were analyzed by chi-square for the main effect of the dietary treatment. Finally, liver and plasma retinol data were also analyzed by linear regression to determine the correlation between diet and liver or plasma retinol concentrations. For all statistical analyses, significance was set at  $P < 0.05$ .

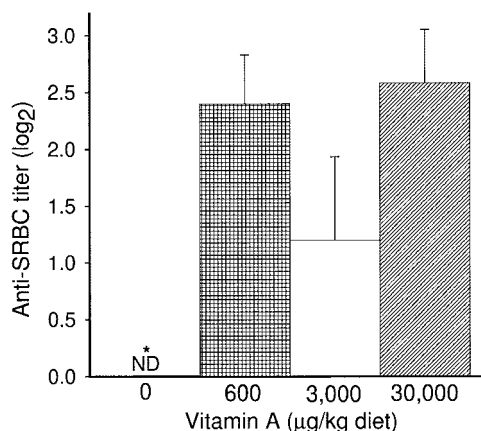
## RESULTS

**Body weight and condition.** After consuming experimental diets for 269 d, birds fed 30,000  $\mu\text{g}/\text{kg}$  VA had reduced body condition, based on reduced feather quality and atrophy of the pectoral muscle, although the body weight resulting from the dietary treatment did not change ( $P = 0.30$ ). At experimental d 697, cockatiels fed 0 and 3000  $\mu\text{g}/\text{kg}$  VA had body weights ( $P = 0.86$ ) and conditions that were indistinguishable from birds fed 600  $\mu\text{g}/\text{kg}$ . A significant day effect ( $P < 0.01$ ) indicated that body weights fluctuated throughout the experiment. Overall, changes in body weight for birds fed 0, 3000 or 30,000  $\mu\text{g}/\text{kg}$  VA did not differ from those of birds fed 600  $\mu\text{g}/\text{kg}$  ( $P > 0.1$ ).

**Plasma and liver retinol.** After consuming experimental diets for 235 d, birds fed 30,000  $\mu\text{g}/\text{kg}$  VA had greater plasma retinol concentrations than birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.05$ , Fig. 1). After 697 d, birds fed 3000  $\mu\text{g}/\text{kg}$  VA had greater plasma retinol concentrations than birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.01$ ). Plasma retinol concentrations did not differ between ( $P < 0.05$ ) birds fed 0 or 600  $\mu\text{g}/\text{kg}$  VA at any time during the trial. Based on the regression model (calculated by use of final plasma retinol concentrations for each treatment group), plasma retinol concentrations increased in a diet-dependent manner [ $\text{plasma retinol (mmol/L)} = 0.112 + (1.96 \times \text{diet VA})$ ;  $R^2 = 0.60$ ;  $P < 0.001$ ]. Liver retinol increased with increasing dietary VA concentration (0  $\mu\text{g}/\text{kg}$  VA at d 706 =  $19.0 \pm 5.4$ ; 600  $\mu\text{g}/\text{kg}$  VA at d 706 =  $34.3 \pm 12.2$ ; 3000



**FIGURE 1** Effect of dietary vitamin A concentration ( $\mu\text{g}/\text{kg}$ ) on plasma retinol concentration ( $\mu\text{mol}/\text{L}$ ) of cockatiels. Birds fed the 30,000  $\mu\text{g}/\text{kg}$  VA diet were removed from the study on d 269. Values are means  $\pm$  SEM,  $n = 5$  or 6. \*Different from birds fed 600  $\mu\text{g}/\text{kg}$  VA,  $P < 0.05$ .



**FIGURE 2** Effect of dietary vitamin A concentration ( $\mu\text{g}/\text{kg}$ ) on secondary antibody responses of cockatiels. Birds were vaccinated subcutaneously on d 211, 218 and 225 with 0.25 mL of 2% sheep red blood cells (SRBC) and antibody titers were measured 9 d after the final injection. Values are means  $\pm$  SEM,  $n = 5$  or 6. \*Different from birds fed 600  $\mu\text{g}$  VA/kg,  $P < 0.05$ . ND, not detectable.

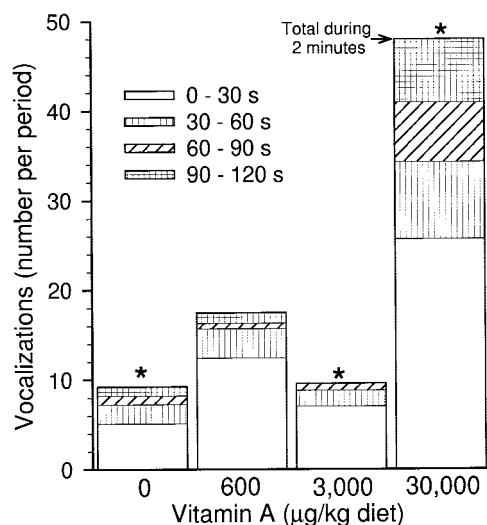
$\mu\text{g}/\text{kg}$  VA at d 706 =  $48.2 \pm 14.1$ ; 30,000  $\mu\text{g}/\text{kg}$  VA at d 269 =  $61.3 \pm 20.5$  mmol/kg), as demonstrated by linear regression [ $\text{liver retinol (mmol/kg)} = 27.77 + 0.18 \times \text{diet VA}$ ;  $R^2 = 0.28$ ,  $P < 0.02$ ].

**Antibody response.** Dietary VA concentration had no impact on the primary antibody response to SRBC ( $P = 0.7$ , data not shown). After 269 d, birds fed 0  $\mu\text{g}/\text{kg}$  VA had reduced secondary anti-SRBC antibody titers compared to birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.05$ , Fig. 2), whereas secondary anti-SRBC antibody titers of birds fed 3000 or 30,000  $\mu\text{g}/\text{kg}$  VA did not differ from birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P > 0.1$ ).

**Cytological evaluation of nasal flushes.** There was no consistent effect of dietary VA concentration on the percentage of any cell type throughout the duration of the trial. However, on d 490, the percentage of keratinized squamous cells was greater for birds fed 3000  $\mu\text{g}/\text{kg}$  VA than for those fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.05$ , data not shown).

**Vocalization analysis.** The total number of vocalizations provoked by physical restraint was much greater in birds fed 30,000  $\mu\text{g}/\text{kg}$  VA than in birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.01$ , Fig. 3). This difference occurred within the 1st min and the last 30 s of a 2-min observation period ( $P < 0.05$ ). In contrast, birds fed 0 or 3000  $\mu\text{g}/\text{kg}$  VA had fewer numbers of vocalizations compared to birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.03$ ), primarily in the first 30 s ( $P < 0.05$ ). The average length of vocalizations was reduced in birds fed 0 or 3000  $\mu\text{g}/\text{kg}$  VA compared to that in birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.01$ ). The peak frequency (Hz) of vocalizations (Table 2) was reduced in birds fed 0 ( $P < 0.01$ ) or 30,000  $\mu\text{g}/\text{kg}$  VA ( $P < 0.02$ ) compared to that in birds fed 600  $\mu\text{g}/\text{kg}$  VA, and the peak amplitude (dB) and total power (dB) were reduced in birds fed 0  $\mu\text{g}/\text{kg}$  VA compared to that in birds fed 600  $\mu\text{g}/\text{kg}$  VA ( $P < 0.01$ ).

**Histopathology.** Necropsies were completed on birds from all treatment groups on d 269 and on birds in the 0, 600 and 3000  $\mu\text{g}/\text{kg}$  VA groups on d 706. Birds fed 30,000  $\mu\text{g}/\text{kg}$  VA were more likely to have pancreatitis than were birds fed 600 or 3000  $\mu\text{g}/\text{kg}$  VA ( $P < 0.01$ ). Additionally, increases in multifocal accumulations of lymphocytes within the duodenal lamina propria were greater in birds fed 30,000  $\mu\text{g}/\text{kg}$  VA than in birds fed 600 or 3000  $\mu\text{g}/\text{kg}$  VA ( $P < 0.05$ ). No significant differences in pathology of other tissues were noted. After



**FIGURE 3** Effect of dietary vitamin A concentration ( $\mu\text{g}/\text{kg}$ ) on the mean number of vocalizations of cockatiels ( $n = 5$  or  $6$ ). Birds were restrained and vocalizations were recorded during four successive 30-s intervals. \*Different from birds fed  $600 \mu\text{g}/\text{kg}$  VA,  $P < 0.05$ .

697 d, birds fed the  $3000 \mu\text{g}/\text{kg}$  VA diet had increased incidence of splenic hemosiderosis compared to that in birds fed  $600 \mu\text{g}/\text{kg}$  VA ( $P < 0.03$ ), although there were no other differences between birds fed  $600 \mu\text{g}/\text{kg}$  VA and those fed  $0$  or  $3000 \mu\text{g}/\text{kg}$  VA.

## DISCUSSION

Seed mixtures commonly fed to companion birds have very low concentrations of VA ( $<30 \mu\text{g}/\text{kg}$ ) and birds maintained on such diets often develop VA-deficiency symptoms. VA is commonly supplemented through the water supplied or by addition to the diet. Many commercially available supplements prescribe levels of supplementation that result in very high levels of intake, often equivalent to  $7500$ – $30,000 \mu\text{g}/\text{kg}$  diet. Because the actual requirement for VA of companion birds is not known, and because VA is labile during processing and storage, most commercially available pelleted diets are overformulated (e.g.,  $1500$ – $6000 \mu\text{g}/\text{kg}$  diet) relative to realistic estimates of the requirement for maintenance of  $600 \mu\text{g}/\text{kg}$  or below. Consequently we chose to test a wide range of dietary VA concentrations that represent the extremes that companion birds experience. Because estimates of VA requirements are influenced by long-lasting storage pools, we fed these diets for almost 2 y.

Adult cockatiels at maintenance were much more susceptible to dietary VA toxicity than to dietary VA restriction. In fact, birds fed  $30,000 \mu\text{g}/\text{kg}$  VA developed dramatic changes in vocalization patterns and sufficient deterioration in feather quality and breast muscle mass that euthanasia and necropsy was warranted after  $<1$  y. In contrast, birds fed  $0 \mu\text{g}/\text{kg}$  VA for almost 2 y did not show clinically identifiable signs of VA deficiency. The cockatiels used in this experiment previously had been fed a VA-adequate diet (based on successful growth and breeding of this colony for  $>20$  y) formulated to contain  $2363 \mu\text{g}/\text{kg}$  VA. Presumably liver VA stores were sufficient to provide VA to the birds for the entire trial. In fact after almost 2 y of consuming a diet with no detectable VA, cockatiels had liver retinol concentrations of  $\sim 19.0 \pm 5.4 \text{ mmol}/\text{kg}$ . In other parrot species, squamous metaplasia (a common symptom of VA deficiency) was associated with liver retinol concentra-

tions  $< 7.3 \text{ mmol}/\text{kg}$  (6), suggesting that further depletion of the cockatiels in the current study would have been required to achieve deficiency. These data indicate that cockatiels are very adept at storing VA for use in times of low dietary VA and conserve their VA efficiently. Considering their wild-type diet, which is seed-based and would likely contain low concentrations of preformed VA (1,7), this type of adaptation and efficient storage of VA is not surprising. However, birds fed  $0 \mu\text{g}/\text{kg}$  VA were unable to mount a secondary antibody response to SRBC, which is also seen in chickens fed a VA-deficient diet (8,9). Thus, changes in immunocompetence were a better indication of diminishing VA status in cockatiels than changes in body condition, plasma retinol, organ pathology or classical signs of deficiency such as squamous metaplasia of nasal epithelia. We did not observe decreased antibody responses in cockatiels fed  $30,000 \mu\text{g}/\text{kg}$  VA, although chickens fed extremely high concentrations of dietary VA ( $>900,000 \mu\text{g}/\text{kg}$ ) had reduced antibody titers, accompanied by signs of acute toxicity (8).

In general plasma retinol does not change markedly with marginal VA status, and reduced concentrations are not seen until animals reach a severe deficiency (10). Our results showed that birds fed  $0 \mu\text{g}$  VA for almost 2 y did not have reduced plasma retinol concentrations, suggesting that plasma retinol values are not a reliable predictor of dietary VA deficiency; although the low number of birds used in this study may have made small differences undetectable. In contrast, plasma retinol concentrations were increased ( $P < 0.05$ ) in birds fed  $30,000 \mu\text{g}/\text{kg}$  VA (235 d) and  $3000 \mu\text{g}/\text{kg}$  (697 d), suggesting that high plasma retinol concentrations are diagnostic of excessive or toxic dietary VA concentrations.

Signs of VA toxicity included poor feathering, pancreatitis, multifocal accumulations of lymphocytes within the duodenal lamina propria and hyperexcitability. In particular, birds developed highly exaggerated vocalizations in response to restraint. There are several potential mechanisms for this behavioral response. First, impaired production of corticosteroids and resulting impaired stress tolerance may contribute to behavioral changes (11). Second, changes in vocalizations may be a result of the pathology of tissues associated with vocalizations, such as specific regions of the brain (12), respiratory and tracheal muscles (13) or the syrinx (14), although we did not observe changes in the epithelium of the syrinx or consistent pathologies in muscle or brain. Third, VA toxicosis in humans has been implicated in idiopathic intracranial hypertension, resulting in headaches, papilledema and elevated intracranial pressure, without evidence of intracranial lesions

**TABLE 2**

*Effect of dietary vitamin A levels on the peak frequency, peak amplitude and total power of vocalizations of adult female cockatiels at maintenance<sup>1</sup>*

Diet vitamin A	Peak frequency	Peak amplitude	Total power
$\mu\text{g}/\text{kg}$ diet	Hz	dB	
0	$1827.5 \pm 14.6^*$	$-20.9 \pm 0.4^*$	$-14.0 \pm 0.4^*$
600	$2956.9 \pm 58.7$	$-33.4 \pm 1.1$	$-22.5 \pm 0.6$
3000	$3212.8 \pm 93.6$	$-30.5 \pm 0.8$	$-18.8 \pm 0.7$
30,000	$2263.4 \pm 39.5^*$	$-31.2 \pm 0.5$	$-21.0 \pm 0.3$

<sup>1</sup> Birds were removed from their cages and restrained for 2 min, during which time vocalizations were recorded. Values are means  $\pm$  SEM,  $n = 5$  or  $6$ .

\* Different from the  $600 \mu\text{g}/\text{kg}$  treatment,  $P < 0.05$ .

(15,16), bone and joint pain and "psychiatric" symptoms (17). Finally, it is also possible that alterations in vocalization patterns with VA malnutrition are adaptive and not pathological. Several nutritional conditions, including amino acid imbalances and calcium deficiencies, result in behavioral changes that alter food selection and serve to improve the nutrient balance of the diet (2). The exact mechanism by which dietary VA concentrations alter cockatiel vocalization patterns is unclear, and warrants further investigation, given that it is possible that disturbances in VA nutrition contribute to the widespread incidence of behavioral problems reported in companion birds.

Birds fed 3000  $\mu\text{g}/\text{kg}$  VA had liver retinol concentrations similar to those of birds fed 30,000  $\mu\text{g}/\text{kg}$  VA, and plasma retinol concentrations of birds fed 3000  $\mu\text{g}/\text{kg}$  VA for 697 d surpassed that of birds fed 30,000  $\mu\text{g}/\text{kg}$  VA for 235 d. Furthermore, birds fed 3000  $\mu\text{g}/\text{kg}$  VA had a greater incidence of splenic hemosiderosis and differences in vocalization compared to those of birds fed 600  $\mu\text{g}/\text{kg}$  VA. Although the behavioral changes caused by 3000  $\mu\text{g}/\text{kg}$  VA were subtle compared to those caused by 30,000  $\mu\text{g}/\text{kg}$  VA, it is likely that the 3000  $\mu\text{g}/\text{kg}$  VA diet is marginally toxic. If this is the case, commercial vitamin supplements and some commercial pelleted feeds have excessive concentrations of vitamin A. Cockatiel chicks express 15,15'-dioxygenase mRNA and can use  $\beta$ -carotene as a source of VA (unpublished data). Thus, it may be prudent to supplement diets with a combination of retinol and  $\beta$ -carotene to avoid VA toxicity, yet ensure a margin of safety to circumvent deficiencies arising from uncertainty in requirements and the instability of retinol during diet storage.

In conclusion, adult female cockatiels at maintenance are more susceptible to VA toxicity than to VA deficiency. Clinically, this observation is very important because anecdotal evidence often suggests that Psittacines are most likely to be VA deficient. In the case of adults, VA toxicity may be more prevalent than currently reported, given that vitamin supplements and commercially available diets often contain very high concentrations of VA. Assuming that cockatiels were previously fed diets containing adequate dietary VA concentrations, producing a VA deficiency would presumably require accelerated losses, such as by egg production, or very long term (>2 y) dietary depletion. Additionally, cockatiels fed 3000  $\mu\text{g}/\text{kg}$  VA approached the plasma and liver retinol concentrations of birds that had an identifiable VA toxicity, indicating that dietary VA concentrations  $\geq$  3000  $\mu\text{g}/\text{kg}$  VA may cause toxicity. Therefore, optimal dietary VA concentrations for

adult cockatiels appear to be <3000  $\mu\text{g}$  VA/kg diet (diet analyzed to contain 2815  $\mu\text{g}$  VA/kg diet), whereas diets formulated to contain 600  $\mu\text{g}$  VA/kg diet (diet analyzed to contain 835  $\mu\text{g}/\text{kg}$  diet) appear to meet all VA needs at maintenance.

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