Nutrient Requirements

Adult Cockatiels (Nymphicus hollandicus) at Maintenance Are More Sensitive to Diets Containing Excess Vitamin A Than to Vitamin A–Deficient Diets1,2

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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 μg VA/kg (0, 2000, 10,000 or 100,000 i.u.), 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 μg/kg, respectively. After 269 d, birds fed the 30,000 μg/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 μg/kg diet (P < 0.05). The 3000 μg/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 μg/kg VA diet. The secondary antibody response was reduced after 225 d and vocalization patterns were altered in birds fed 0 μg/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 ≥3000 μg 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,
VITAMIN A IN ADULT COCKATIELS

MATERIALS AND METHODS

The UC Davis Animal Care and Use Committee approved all
depotocols. Adult female cockatiels (Nymphicus hollandicus, 2–3 y of
age, n = 22) that were not reproductively active were individually
housed (30 × 30 × 60-cm wire cages), at 24°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 μg 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) μg VA/kg diet (0, 2000, 10,000, or 100,000 μg/kg VA;
supplied as retinyl palmitate, containing 75,000 mg retinol/kg; Sigma,
St. Louis, MO). Diets were prepared as previously described (4) and
supplied as retinyl palmitate, containing 75,000 mg retinol/kg; Sigma,
St. Louis, MO). Diets were 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 condi-
tion) was evaluated subjectively by examining the integument
(brush 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 days after the first
and last injections (d 211 for primary response, d 234 for secondary
response), antibody titer were measured by agglutination procedures
as previously described (5). Additionally, bimonthly assessments of
oral/nasal epithelium cell types were made by analysis of nasal
epithelium cell types were made by analysis of nasal
flushes, which includes parrots, lori keets, lovebirds, cockatooos
and many other popular companion birds.

Drinking from nutri tion by on March 17, 2009

TABLE 1
Composition of the basal diet fed to adult female
cockatiels at maintenance1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet</th>
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<tbody>
<tr>
<td>Rice flour</td>
<td>470</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>380</td>
</tr>
<tr>
<td>Cellulose</td>
<td>65.3</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>254</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>9</td>
</tr>
<tr>
<td>MgSO4 · 7H2O</td>
<td>5.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.5</td>
</tr>
<tr>
<td>d,L-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin mix2</td>
<td>2.5</td>
</tr>
<tr>
<td>Mineral mix2</td>
<td>2.5</td>
</tr>
<tr>
<td>Choline</td>
<td>2</td>
</tr>
<tr>
<td>Threonine</td>
<td>1</td>
</tr>
<tr>
<td>Na2CO3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

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

2 Vitamin mix supplied (per kg final diet): thiamin HCl, 13.3 mg;
riboflavin, 15 mg; Ca-pantothenate, 20 mg; niacin, 50 mg; pyridoxine
HCl, 7.8 mg; folacin, 6 mg; biotin, 0.5 mg; cholecalciferol, 7.65 μg;
d,α-tocopheryl acid succinate, 24.2 mg; ascorbate, 50 mg; B-12, 20
μg; menadione sodium bisulfate, 4.5 mg. Mineral mix supplied (per kg
final diet): KCl, 1 g; MnSO4 · H2O, 350 mg; ZnSO4 · 7H2O, 150 mg;
FeSO4 · 7H2O, 500 mg; CuSO4 · 5H2O, 30 mg; Na2SeO3, 0.2 mg;
KIO4, 2.0 mg; CoCl2, 1.7 mg; MgSO4 · 7H2O, 123 mg; Na2MoO4 ·
2H2O, 8.3 mg.

which includes parrots, lori keets, lovebirds, cockatooos
and many other popular companion birds.

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, amplitu-
de and power of vocalizations.

After 269 d of consuming experimental diets, birds fed 30,000 μg
VA showed poor body condition, and therefore all birds fed 30,000
μg VA as well as two birds fed 3000 μg VA and two birds fed 600 μg
VA were killed by CO2 inhalation, after which necropsies were per-
fomed. Remaining birds (n = 3 each for 3000 μg VA and 600 μg
VA; n = 6 for 0 μg 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 homog-

enized in phosphate-buffered saline (PBS) at a ratio of ~2 parts
tissue: 1 part PBS (w/v). A 1-g sample of tissue homogenate or
300–500 μL plasma was used to extract retinol. Samples were first
vortexed with methanol-potassium hydroxide [5% KOH in meth-

anol (w/v), 1× 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× sample volume; samples were again vortexed for
5 min and then K2HPO4 (saturated solution of K2HPO4 in deionized
H2O) was added at 0.1× sample volume. Samples were vortexed
vigorously for 5 min, then centrifuged (5000 × g) for 15 min. The
top organic phase was removed, dried under N2, 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 ace-
tate/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 μm, 300 nm, 4.6 mm I.D. × 250 mm L; Vydac 210TP54,
Hesperia CA) and a high performance guard column (5 μm; Vydac
210GD34T). The isocratic mobile phase (described above) was main-
tained at a flow rate of 1.0 mL/min (Waters 510 pump, Waters
Associates, Milford MA), and automated injections (Waters WISP
712) of 75 μL were made. A UV/visible detector (Waters 484)
monitored at 234 nm, and peak identification and quantitation (Wa-
ters 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 diet retinol concentrations was kindly provided
by Roché 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 fol-
lowing (mean ± SD): 0 μg diet, no detectable retinol; 600 μg diet,
835 ± 36 μg/kg; 3000 μg diet, 2815 ± 70 μg/kg; 30,000 μg,
24,549 ± 397 μg/kg.

Statistical analysis. Data were analyzed by a general linear model
(JMP, SAS Institute, Cary, NC). Those data collected at a single
time (antibody titer, liver retinol) were analyzed by one-way
ANOVA for the main effect of the dietary treatment. Data collected
during 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 treat-
ment and their interactions. Additionally, this model nested individual
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 μg/kg treatment were individually compared to the 600 μg/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 μg/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 μg/kg VA had body weights (\( P = 0.86 \)) and conditions that were indistinguishable from birds fed 600 μg/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 μg/kg VA did not differ from those of birds fed 600 μg/kg (\( P > 0.11 \)).

**Plasma and liver retinol.** After consuming experimental diets for 235 d, birds fed 30,000 μg/kg VA had greater plasma retinol concentrations than birds fed 600 μg/kg VA (\( P < 0.05 \), Fig. 1). After 697 d, birds fed 3000 μg/kg VA had greater plasma retinol concentrations than birds fed 600 μg/kg VA (\( P < 0.01 \)). Plasma retinol concentrations did not differ between (\( P > 0.05 \)) birds fed 0 or 600 μg/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 [plasma retinol (mmol/L) = 0.112 + (1.96 × diet VA); \( R^2 = 0.60 \); \( P < 0.001 \)]. Liver retinol increased with increasing dietary VA concentration (0 μg/kg VA at d 706 = 19.0 ± 5.4; 600 μg/kg VA at d 706 = 34.3 ± 12.2; 3000 μg/kg VA at d 706 = 48.2 ± 14.1; 30,000 μg/kg VA at d 269 = 61.3 ± 20.5 mmol/kg), as demonstrated by linear regression [liver retinol (mmol/kg) = 27.77 + 0.18 × 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 μg/kg VA had reduced secondary anti-SRBC antibody titers compared to birds fed 600 μg/kg VA (\( P < 0.05 \), Fig. 2), whereas secondary anti-SRBC antibody titers of birds fed 3000 or 30,000 μg/kg VA did not differ from those fed 600 μg/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 μg/kg VA than for those fed 600 μg/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 μg/kg VA than in birds fed 600 μg/kg VA (\( P < 0.01 \), Fig. 3). This difference occurred within the first min and the last 30 s of a 2-min observation period (\( P < 0.05 \)). In contrast, birds fed 0 or 3000 μg/kg VA had fewer numbers of vocalizations compared to birds fed 600 μg/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 μg/kg VA compared to that in birds fed 600 μg/kg VA (\( P < 0.01 \)). The peak frequency (Hz) of vocalizations (Table 2) was reduced in birds fed 0 (\( P < 0.01 \)) or 3000 μg/kg VA (\( P < 0.02 \)) compared to that in birds fed 600 μg/kg VA, and the peak amplitude (dB) and total power (dB) were reduced in birds fed 0 μg/kg VA compared to that in birds fed 600 μg/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 μg/kg VA groups on d 706. Birds fed 30,000 μg/kg VA were more likely to have pancreatitis than were birds fed 600 or 3000 μg/kg VA (\( P < 0.01 \)). Additionally, increases in multifocal accumulations of lymphocytes within the duodenal lamina propria were greater in birds fed 30,000 μg/kg VA than in birds fed 600 or 3000 μg/kg VA (\( P < 0.05 \)). No significant differences in pathology of other tissues were noted. After

![FIGURE 1](https://example.com/figure1.png) **FIGURE 1** Effect of dietary vitamin A concentration (μg/kg) on plasma retinol concentration (μmol/L) of cockatiels. Birds fed the 30,000 μg/kg VA diet were removed from the study on d 269. Values are means ± SEM, \( n = 5 \) or 6. *Different from birds fed 600 μg/kg VA, \( P < 0.05 \).

![FIGURE 2](https://example.com/figure2.png) **FIGURE 2** Effect of dietary vitamin A concentration (μg/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 ± SEM, \( n = 5 \) or 6. *Different from birds fed 600 μg/kg VA, \( P < 0.05 \). ND, not detectable.
697 d, birds fed the 3000 μg/kg VA diet had increased incidence of splenic hemosiderosis compared to that in birds fed 600 μg/kg VA (P < 0.03), although there were no other differences between birds fed 600 μg/kg VA and those fed 0 or 3000 μg/kg VA.

DISCUSSION

Seed mixtures commonly fed to companion birds have very low concentrations of VA (<30 μg/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 μg/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 μg/kg diet) relative to realistic estimates of the requirement for maintenance of 600 μg/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 μg/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 μg/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 μg/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 ~19.0 ± 5.4 mmol/kg. In other parrot species, squamous metaplasia (a common symptom of VA deficiency) was associated with liver retinol concentra-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Diet vitamin A</th>
<th>Peak frequency</th>
<th>Peak amplitude</th>
<th>Total power</th>
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<td>μg/kg diet</td>
<td>Hz</td>
<td>dB</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1827.5 ± 14.6*</td>
<td>−20.9 ± 0.4*</td>
<td>−14.0 ± 0.4*</td>
</tr>
<tr>
<td>600</td>
<td>2956.9 ± 58.7</td>
<td>−33.4 ± 1.1</td>
<td>−22.5 ± 0.6</td>
</tr>
<tr>
<td>3000</td>
<td>3212.8 ± 93.6</td>
<td>−30.5 ± 0.8</td>
<td>−18.8 ± 0.7</td>
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<tr>
<td>30,000</td>
<td>2263.4 ± 39.5*</td>
<td>−31.2 ± 0.5</td>
<td>−21.0 ± 0.3</td>
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</tbody>
</table>

* Different from the 600 μg/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 μg/kg VA had liver retinol concentrations similar to those of birds fed 30,000 μg/kg VA, and plasma retinol concentrations of birds fed 3000 μg/kg VA for 697 d surpassed that of birds fed 30,000 μg/kg VA for 235 d. Furthermore, birds fed 3000 μg/kg VA had a greater incidence of splenic hemosiderosis and differences in vocalization compared to those of birds fed 600 μg/kg VA. Although the behavioral changes caused by 3000 μg/kg VA were subtle compared to those caused by 30,000 μg/kg VA, it is likely that the 3000 μg/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'-dioxxygenase mRNA and can use β-carotene as a source of VA (unpublished data). Thus, it may be prudent to supplement diets with a combination of retinol and β-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 μg/kg VA approached the plasma and liver retinol concentrations of birds that had an identifiable VA toxicity, indicating that dietary VA concentrations ≥ 3000 μg/kg VA may cause toxicity. Therefore, optimal dietary VA concentrations for adult cockatiels appear to be <3000 μg VA/kg diet (diet analyzed to contain 2815 μg VA/kg diet), whereas diets formulated to contain 600 μg VA/kg diet (diet analyzed to contain 835 μg/kg diet) appear to meet all VA needs at maintenance.

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LITERATURE CITED