The Effect of Vitamin D₃ on Resistance to Stress-Related Infection in an Experimental Model of Turkey Osteomyelitis Complex


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ABSTRACT Male turkeys immunosuppressed by injection with dexamethasone (DEX) were given supplemental vitamin D₃ in their drinking water in two experiments. In Experiment 1, vitamin D₃ was supplemented at a dosage of either 2,064 IU/kg (low level) or 4,128 IU/kg (high level) in drinking water provided ad libitum only from Days 1 through 5 after hatch. In Experiment 2, vitamin D₃ was provided at the low dosage for the first 5 d after hatch, followed by treatment with the high dosage for 12 h before and 12 h after each stressful event, which included weekly weighings and two DEX treatments. In both experiments, at 5 wk of age half of the birds were given intramuscular injections of 2 mg/kg DEX on 3 alternating d. In Experiment 1, 100 cfu of Escherichia coli was inoculated into the left thoracic airsac at the time of the third DEX injection. All mortalities were examined, and 10 birds per pen were necropsied 2 wk after treatment and examined for lesions of airsacculitis and turkey osteomyelitis complex (TOC). Four birds per pen were bled before necropsy, and white blood cell total counts, differential white blood cell counts, and clinical chemistry values were determined. In Experiment 2, healthy surviving birds were grown for an additional 5-wk period, after which the DEX-treated birds were given a second series of DEX injections and were bled and necropsied 2 wk later. There were no significant effects of vitamin D₃ treatment in combined general linear models analysis of Experiment 1; however, when birds not treated with DEX or E. coli were compared with those treated with both DEX and E. coli, supplementation with the low level of vitamin D₃ significantly decreased TOC incidence. There were no significant effects of vitamin D₃ treatment in birds treated with DEX at 5 wk of age in Experiment 2. However, when surviving birds were given a second DEX treatment at 12 wk, vitamin D₃ treatment resulted in significantly lower incidence of mortality, TOC, green liver, isolation of bacteria from tissues, and lower airsacculitis scores and heterophil-to-lymphocyte ratios than controls. Vitamin D₃ also improved BW, relative weights of the liver and heart, and serum levels of glucose and alanine aminotransferase (ALT) of birds receiving two treatments with DEX. The ability of vitamin D₃ supplementation to protect turkeys from the immunosuppressive effects of multiple DEX treatments emphasizes the role of vitamin D₃ as a prohormone that affects health and disease resistance in turkeys.

INTRODUCTION

Processed turkey carcasses that outwardly appear to be healthy and wholesome can contain lesions including arthritis or synovitis, abscesses in the soft tissues of the leg and breast, and osteomyelitis. This condition has been termed turkey osteomyelitis complex (TOC), and the USDA Food Safety Inspection Service has established a mandated inspection program to remove these carcasses from the food chain (Cook, 1988). The inspection program is based on the observation that birds having these lesions usually have green livers. Turkeys with green livers are removed from the processing line, and the joints, muscles, and bones of these birds are cut in 10 places to expose the lesions. However, only about half of the birds with green livers have any other lesions. This procedure results in the downgrading of many wholesome birds and is costly to the turkey industry. This inefficient inspection process is justified by the Food Safety Inspection Service because there is no other reliable way to detect these unwholesome lesions, and because the lesions often con-
with studies have employed concurrent air-sac inoculation increased disease resistance in male turkeys but not in
birds with green livers (McCaskey, 1989; Clark et al., 1991; Barnes et al., 1993; Mutalib et al., 1996). However, the
disease process that results in these condemnations can escalate and result in serious and costly effects on produc-
tion before the turkeys go to market. These birds generally become lame and eventually die, usually late in the grow-
out when maximum expense has been put into their produc-
tion. Thus, production losses due to TOC can be as much of a problem as are the lesions occurring in pro-
cessed carcasses.

Our research has focused on discovering why a small population of turkeys consistently develop these lesions, what causes the occasional escalation into an important field production problem, and what can be done to pre-
vent the disease. The TOC lesions have been associated with a number of opportunistic bacteria, mainly Staphylo-
coccus aureus and Escherichia coli (Clark et al., 1991; Bayyari et al., 1994; Droual et al., 1996; Mutalib et al., 1996). Our
studies have led us to believe that immune dysfunction caused by genetic variation in the stress response of indi-
vidual birds is far more important to the etiology of TOC than is the virulence of any specific pathogen (Bayyari et al., 1997a,b; Huff et al., 1998, 1999a,b,c). We have de-
veloped an experimental model for reproducing all of the lesions that define TOC, which exploits the immuno-
suppressive effects of a synthetic glucocorticoid, dexametha-
sone (DEX) (Huff et al., 1998). Dexamethasone has been
shown to induce cell-mediated immunosuppression and to decrease resistance to infection in avian species (Long
ethasone has also had wide application in studying transport stress and shipping fever in cattle (Roth and
Flaming, 1990). Our ability to reproduce all of the lesions of TOC using a 2 mg/kg BW DEX injection suggests that
individual variation in the stress response may be involved in the etiology of the disease. Previous studies have
suggested that DEX treatment at 0.5 mg/kg BW increased disease resistance in male turkeys but not in female turkeys (Huff et al., 1999a). Whereas previous studies have employed concurrent air-sac inoculation with E. coli to induce respiratory disease and TOC lesions, we have observed that treatment with DEX alone, in the absence of bacterial inoculation, can result in the same
disease process (Huff et al., 1999 b,c).

In a previous trial with DEX immunosuppression and E. coli challenge, we treated all of the poultws with commer-
cial liquid vitamin D3 (High-D 2X3) in the drinking water

\[ \text{VITAMIN D3 AFFECTS DISEASE RESISTANCE IN TURKEYS} \]

\[ \text{for the first 5 d of life in order to prevent field rickets, which had affected 90\% of the previous turkey grow-out in our facility (Huff et al., 1999e). In that trial, we had a very low incidence of TOC compared with previous trials, although mortality and airsacculitis scores were high (Huff et al., 1999a). The purpose of the present study was to experimentally determine the effects of vitamin D3 supplesmmentation on the incidence of TOC lesions in birds immuno-

MATERIALS AND METHODS

Experiment 1. Six hundred day-old male poults were wing-banded and evenly divided into 24 randomly as-
signed floor pens with 25 birds per pen under continuous incandescent lighting. They were treated from Days 1
through 5 with a low-level dosage of vitamin D3 (.025
oz./128 gal; 2,064 IU/L), a high-level dosage (0.5 oz./128
gal; 4,128 IU/L), or with no vitamin D3 (High D3) in their
drinking water, which was made available ad libitum. These levels were recommended by the manufacturer, with the higher level recommended on the packaging label for birds under stress. Fresh solutions were prepared daily. All poultws were maintained on a standard turkey starter diet that met or exceeded the nutrient requirements established by the NRC (1994) and provided 2,204
IU vitamin D3/kg calculated to be fed in a vitamin premix. Individual bird weights and feed consumption were de-
termined weekly. At 5 wk of age, half of the birds were given three intramuscular injections of approximately 2
mg/kg BW DEX4 into a thigh muscle on 3 alternating d.
A 200 mg/mL stock solution was prepared in absolute ethanol. This solution was suspended in sterile normal saline as a 3 mg/mL suspension, and 0.5 mL was inocu-
lated into each bird, based on a mean BW. On the day of the third DEX injection, all birds were inoculated in the left cranial-thoracic air sac with either 200 µL sterile
tryptose phosphate broth (TPB) or with 200 µL TPB contain-
ing approximately 100 cfu of a nonmotile strain of E.
coli serotype O2. The inoculum was prepared by adding two full loops from an overnight culture on blood agar
to 100 mL TPB and incubating for 2.5 h in a 37 C shaking water bath. The culture was held overnight at 4 C while a standard plate count was made. The dilution was made and verified with a second plate count.

Morbidity, mortality, lameness, BW, and feed con-
sumption were monitored for 2 wk postinoculation. All
dead birds were collected twice daily and examined for
lesions of colibacillosis and TOC. At the end of 2 wk, a
blood sample was taken from the hearts of four birds from each pen. Ten birds per pen were then euthanized and scored for air sacculitis and for TOC lesions. The
following key, modified from that described by Piercy
and West (1976), was used to score lesions of airsacculitis or pericarditis observed in mortalities and at necropsy:
0, no inflammation; 1, opacity and thickening of the inocu-
lated air sac; 2, mild airsacculitis and mild pericarditis; 3,
moderate airsacculitis or pericarditis with spread to liver
or adominal cavity (perihepatitis or peritonitis); 4, severe

\[ \text{\textsuperscript{1}I. D. Russell Company Laboratories, Longmont, CO 80501.} \]

\[ \text{\textsuperscript{2}Sigma Chemical Co., St. Louis, MO 63178-9916.} \]
fibrinous airsacculitis and severe pericarditis; 5, severe airsacculitis or pericarditis with spread to liver or abdominal cavity. Liver, heart, spleen, and bursa of Fabricius were weighed. The incidence of TOC was determined by subjecting each carcass to the standard 10-cut procedure used to detect TOC by processing plant inspectors. This procedure includes examining hip, knee, and wing joints, cutting the muscles of the thigh and leg, and cutting across the proximal tibiotarsus to inspect for inflammatory lesions. All TOC lesions, as well as liver and pericardium from every bird, were swabbed with sterile transport swabs and cultured on blood agar. Positive cultures were subcultured onto MacConkey agar and mannitol salt agar.

Data collected from mortalities and from necropsied birds were combined for analyses. All percentage data were subjected to arcsine transformation. Pen means for the effects of DEX, E. coli, and vitamin D₃ were analyzed by ANOVA as a 2 × 2 × 2 factorial arrangement using the general linear models (GLM) and least squares means procedures of SAS (SAS Institute, 1988). A P ≤ 0.05 was considered significant for main treatment effects and their interactions, unless otherwise noted.

Experiment 2

One hundred twenty male, day-old Nicholas poults were wing-banded and divided into eight randomly assigned pens of 15 birds per pen. The poults were maintained on the same diet as described in Experiment 1 under continuous incandescent lighting and were treated with either no vitamin D₃ or with the low level (2064 IU/L) added to drinking water, which was available ad libitum for the first 5 d. Because the high dose of vitamin D₃ was recommended by the manufacturer for use during periods of stress, the vitamin D₃-treated birds were also given the high dose of vitamin D₃ (4,128 IU/L) in their drinking water from 12 h before until 12 h after each stressful event, which included weekly weighings, and all DEX injections. Fresh solutions were prepared daily. At 5 wk of age, half of the birds from each treatment (four pens; n = 60) were given three injections of 2 mg/kg DEX as previously described, and the remaining four pens were untreated. In this study the birds were not challenged with E. coli. All mortalities were necropsied, and 2 wk later, a blood sample was taken from four birds per pen, and 10 birds per pen were necropsied as previously described. Birds that survived the first DEX treatment (n = 36 no DEX; n = 24 DEX) were grown in the same pens for an additional 5-wk period, after which time only the DEX-treated birds were given a second series of three injections of 2 mg/kg DEX. All mortalities were examined, and after 2 wk, all surviving birds were blood-sampled and necropsied as described for Experiment 1.

Total leukocyte counts and the proportions (%) among various leukocytes were determined using a Cell-Dyn 3500 blood analysis system that was standardized for analysis of turkey blood. Heterophil/lymphocyte ratios (H/L), an indicator of stress in birds (Gross and Siegel, 1983), were calculated. Serum was collected, and the clinical chemistry analyses were measured for serum levels of calcium, phosphorus, total protein, albumin, glucose, triglycerides, cholesterol, uric acid, and iron. The enzyme activities of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase, and creatine kinase (CK) were measured using an automated clinical chemistry analyzer.

Data collected from mortalities and from necropsied birds were combined for analyses. All percentage data were subjected to arcsine transformation. Pen means for effects of DEX and vitamin D₃ treatment were analyzed as a 2 × 2 factorial arrangement using the GLM and least squares means procedures of SAS software (SAS Institute, 1988). A probability (P ≤ 0.05) was considered significant.

RESULTS

Experiment 1

There were no significant vitamin D₃ effects on airsacculitis score, mortality, BW, relative organ weights, white blood cell differential counts, or most clinical chemistry analyses when vitamin D₃ was supplemented at either the low or high levels for the first 5 d after hatch (data not shown). Serum levels of uric acid were decreased by vitamin D₃ treatment (data not shown). There was no effect of vitamin D₃ supplementation on the incidence of TOC lesions in combined GLM analyses. However, when birds that were not treated with DEX or challenged with E. coli were compared only with those that were treated with DEX and challenged with E. coli, those supplemented with the low level of vitamin D₃ had a 20% incidence of TOC, whereas those birds not given supplemental vitamin D₃ had a 9% incidence of TOC (P = 0.02).

Experiment 2

Vitamin D₃ supplementation did not result in significant differences in mortality, airsacculitis, TOC or green liver incidence, isolation of bacteria from tissues, hematology values, or body and organ weights of birds treated with DEX at 5 wk of age and necropsied 2 wk later (data not shown). However, D₃ treatment did significantly affect disease resistance and performance when the same birds at 12 wk of age were treated with a second series of DEX injections 5 wk after full recovery from the first DEX treatment (2X DEX). The 2X DEX-treated turkeys given supplemental vitamin D₃ had a significantly lower incidence of mortality, TOC, green liver, and isolation of bacteria from tissues, as well as lower airsacculitis or pericarditis scores, than turkeys given two DEX treatments but not given supplemental D₃ (Table 1). There was an antagonistic interaction between DEX and vitamin

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8Abbott Diagnostics, Abbott Park, IL 60064.
9Express Plus, Ciba-Corning Diagnostics Corp., Medfield, MA 02052.
VITAMIN D3 AFFECTS DISEASE RESISTANCE IN TURKEYS

TABLE 1. Effect of vitamin D3 (Vit D3) supplementation on the incidence of mortality (Mort), turkey osteomylitis complex (TOC), green liver (GL), bacterial recovery from lesions (Bact), and airsacculitis or pericarditis (AS) scores in 12-wk-old turkeys after a second treatment with dexamethasone (2X DEX)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>No DEX</th>
<th>With 2X DEX</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Vit D3 (n = 19)</td>
<td>Vit D3 (n = 17)</td>
<td></td>
</tr>
<tr>
<td>% Mort</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1.0</td>
</tr>
<tr>
<td>% TOC</td>
<td>5 ± 5</td>
<td>0 ± 0</td>
<td>0.6</td>
</tr>
<tr>
<td>% GL</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1.0</td>
</tr>
<tr>
<td>% Bact</td>
<td>5 ± 5</td>
<td>0 ± 0</td>
<td>0.6</td>
</tr>
<tr>
<td>AS score</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1.0</td>
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</tbody>
</table>

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<thead>
<tr>
<th></th>
<th>No DEX</th>
<th>With 2X DEX</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>No Vit D3 (n = 11)</td>
<td>Vit D3 (n = 13)</td>
<td></td>
</tr>
<tr>
<td>% Mort</td>
<td>73 ± 14</td>
<td>23 ± 12</td>
<td>0.0001</td>
</tr>
<tr>
<td>% TOC</td>
<td>64 ± 15</td>
<td>15 ± 1</td>
<td>0.001</td>
</tr>
<tr>
<td>% GL</td>
<td>64 ± 15</td>
<td>8 ± 8</td>
<td>0.0001</td>
</tr>
<tr>
<td>% Bact</td>
<td>36 ± 15</td>
<td>0 ± 0</td>
<td>0.0029</td>
</tr>
<tr>
<td>AS score</td>
<td>1.91 ± 0.56</td>
<td>0.54 ± 0.33</td>
<td>0.0002</td>
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</tbody>
</table>

\(^1\)Data represent the mean and SEM of two pens of birds that survived the first DEX treatment. Data include all mortalities and birds necropsied 2 wk after the second DEX treatment.

D\(_3\) affecting all five parameters of disease resistance, which indicated a sparing effect of vitamin D\(_3\) supplementation on the effects of DEX. Vitamin D\(_3\) supplementation had no effect on the total leukocyte counts, H/L, or differential white blood cell percentages of birds that were not treated with DEX (Table 2). However, DEX-treated birds given vitamin D\(_3\) supplementation had significantly lower H/L and percentages of heterophils, monocytes, and basophils and higher percentages of lymphocytes compared with 2X DEX-treated birds not given supplemental D\(_3\). There were antagonistic interactions between DEX and vitamin D\(_3\) affecting total leukocyte counts, H/L, and percentages of heterophils, lymphocytes, monocytes, and basophils. As previously reported, DEX treatment decreased BW and the relative weight of the bursa of Fabricius and increased relative weights of the liver, heart, and spleen (data not shown). Vitamin D\(_3\) supplementation of 2X DEX-treated birds resulted in higher BW (P ≤ 0.0001) (Table 3). There were significant antagonistic interactions between DEX treatment and vitamin D\(_3\) affecting BW and relative heart weights. There was a synergistic interaction affecting relative spleen weights. Vitamin D\(_3\) treatment had no significant effects on serum levels of calcium, phosphorus, total protein, albumin, triglycerides, cholesterol, iron (data not shown), or alkaline phosphatase. Vitamin D\(_3\) increased levels of ALT, aspartate aminotransferase, and CK in birds not treated with DEX (Table 4). In 2X DEX-treated birds, vitamin D\(_3\) supplementation resulted in higher serum levels of glucose and ALT and lower levels of uric acid. There was an antagonistic interaction between DEX and vitamin D\(_3\) affecting glucose and CK and a synergistic interaction affecting uric acid (Table 4).

DISCUSSION

These studies suggest that vitamin D\(_3\) supplementation of turkey diets results in increased resistance to opportu-
nistic bacterial infection resulting from DEX treatment and that supplementation with vitamin D₃ may affect stress-related immunosuppression, particularly in birds subjected to multiple stressors over time. Although there was little effect of vitamin D₃ supplementation in birds given only one series of DEX injections at 5 wk of age, surviving birds that were given a second series of injections at 12 wk of age had less mortality, lower airsacculitis scores, and decreased incidence of TOC, green liver, and a higher TOC incidence, because TOC lesions generally do not develop until at least 6 d postchallenge (Huff et al., 1998). It is not clear from these studies whether the differences between experiments in this study were due primarily to the effects of the second DEX treatment, or whether they were the result of the age difference of the birds that were given the second DEX treatment.

Vitamin D₃ supplementation had no significant effects on total or differential white blood cell counts of un-stressed turkeys. However, vitamin supplementation significantly decreased percentages of heterophils, monocytes, and basophils and increased percentage of lymphocytes in 2X DEX-treated birds. These changes tended to bring these values back toward those of the un-stressed birds. Of particular importance is the ability of vitamin D₃ to normalize H/L, which is an accepted indicator of physiological stress in birds (Gross and Siegel, 1983). The same normalizing effect of vitamin D₃ supplementation was observed by analysis of several of the clinical chemis-

**Table 3.** Effect of vitamin D₃ (Vit D₃) supplementation on BW and relative weights (percentage BW) of liver, heart, spleen, and bursa of Fabricius of 12-wk-old turkeys after a second treatment with dexamethasone (2X DEX)

<table>
<thead>
<tr>
<th></th>
<th>No Vit D₃</th>
<th>Vit D₃</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 17)</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>11,872 ± 367</td>
<td>12,419 ± 224</td>
<td>0.1824</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>1.33 ± 0.06</td>
<td>1.24 ± 0.05</td>
<td>0.5262</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>0.40 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.4344</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.07 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.3321</td>
</tr>
<tr>
<td>Bursa (g)</td>
<td>0.05 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.9476</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>No Vit D₃</td>
<td>Vit D₃</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>(n = 11)</td>
<td>(n = 13)</td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>5,729 ± 365</td>
<td>7,498 ± 277</td>
<td>0.0005</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>3.25 ± 0.24</td>
<td>2.22 ± 0.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>0.64 ± 0.04</td>
<td>0.51 ± 0.02</td>
<td>0.0002</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.11 ± 0.02</td>
<td>0.15 ± 0.03</td>
<td>0.0686</td>
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<tr>
<td>Bursa (g)</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.1337</td>
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</table>

1Data represent the mean and SEM of two pens of birds that survived the first DEX treatment. Data include all mortalities and birds necropsied 2 wk after the second DEX treatment.

**Table 4.** Effect of vitamin D₃ (Vit D₃) supplementation on serum levels of glucose, uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and creatine kinase (CK) in blood of surviving turkeys drawn 2 wk after a second treatment with dexamethasone (2X DEX)

<table>
<thead>
<tr>
<th></th>
<th>No Vit D₃</th>
<th>Vit D₃</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 6)</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>261.5 ± 6.8</td>
<td>259.3 ± 4.9</td>
<td>0.82</td>
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<tr>
<td>UA (mg/dL)</td>
<td>4.25 ± 0.21</td>
<td>4.23 ± 0.33</td>
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<tr>
<td>ALT (U/L)</td>
<td>3.97 ± 0.43</td>
<td>5.45 ± 0.43</td>
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<tr>
<td>AST (U/L)</td>
<td>356.9 ± 14.7</td>
<td>419.2 ± 24.0</td>
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<tr>
<td>AP (U/L)</td>
<td>75.3 ± 6.9</td>
<td>89.0 ± 15.9</td>
<td>0.79</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>11,450 ± 1,188</td>
<td>19,057 ± 3,379</td>
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<tr>
<td></td>
<td>No Vit D₃</td>
<td>Vit D₃</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>(n = 2)</td>
<td>(n = 6)</td>
<td></td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>195.0 ± 18.0</td>
<td>248.2 ± 5.8</td>
<td>0.0005</td>
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<tr>
<td>UA (mg/dL)</td>
<td>3.85 ± 1.5</td>
<td>0.98 ± 0.03</td>
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<tr>
<td>ALT (U/L)</td>
<td>3.05 ± 0.75</td>
<td>5.20 ± 0.4</td>
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<tr>
<td>AST (U/L)</td>
<td>224.4 ± 5.0</td>
<td>280.4 ± 13.0</td>
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<tr>
<td>AP (U/L)</td>
<td>24.0 ± 5.0</td>
<td>133.3 ± 51.3</td>
<td>0.1</td>
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<tr>
<td>CK (U/L)</td>
<td>1,305 ± 65</td>
<td>2,855 ± 417</td>
<td>0.71</td>
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1Data represent the mean and SEM of serum samples from birds in duplicate pens.
try values of stressed birds and in its ability to provide protection against DEX-induced weight loss and enlargement of liver and spleen. It should be noted that the birds in this study were in blacked-out houses under incandescent bulbs for 24 h/d. This environment, although common in commercial turkey production, in effect eliminated vitamin D synthesis from exposure to ultraviolet light and may also have affected immune responses through disruption of circadian rhythm (Kirby and Froman, 1991). Although vitamin D has historically been valued in poultry nutrition for its role in calcium and phosphorus homeostasis, the results of this study support its role as a homeostatic modulator of diverse physiological and immunological functions in turkeys.

The vitamin D₃ preparation used in this study is claimed, according to its package label, to improve poult response to stress when used at the high dosage for the first 5 d posthatch. Vitamin D₂ (cholecalciferol) is considered to be biologically inactive, and its in vivo effects are attributed to its various metabolites (DeLuca, 1988; Crowle and Ross, 1990). The most active metabolite of vitamin D (1,25(OH)₂D₃) has been shown to have immunoregulatory and immunomodulatory activity and directly affects all cells of the mononuclear lineage (Reinhardt and Hustmyer, 1987; Rigby, 1988; Manolagas et al., 1989; Muller and Bendtzen, 1996). This metabolite inhibits growth of Mycobacteria tuberculosis in cultured human monocytes and macrophages (Crowle et al., 1987; Crowle and Ross, 1990) and improves resistance to tuberculosis in a murine model (McMurray et al., 1990). Macrophages from vitamin D₃-deficient mice function abnormally, and their function can be restored both in vitro and in vivo by treatment with 1,25(OH)₂D₃ (Reichel et al., 1985).

Natural suppressor cells develop in normal bone marrow and the spleen in response to various stressors, including radiation, graft versus host disease, and cancer, and are reported to secrete soluble suppressor factors that block interleukin-2 synthesis and inhibit interleukin-2-dependent T cell proliferation, even in the presence of excess interleukin-2 (Moore et al., 1992). Suppressor macrophages are induced in the spleens of mice treated with acute cold stress, and the generation of these cells is mediated by the action of glucocorticoids (Kizaki et al., 1996a,b). Bone marrow suppressor cells have also been induced in mice injected with lipopolysaccharide (Holda, 1992). A precursor form of 1,25(OH)₂D₃, 1 alpha-hydroxyvitamin D₃, was shown to restore both cellular and humoral immune responses to normal levels in mice that had been subjected to restraint stress (Ohsugi et al., 1985). In this series of studies it was concluded that 1 alpha-hydroxyvitamin D₃ was able either to enhance or suppress immune responses through the activation of helper and suppressor cells and that this effect was dependent on the magnitude of the immune response (Komori et al., 1985). Deficiency of serum levels of 1,25(OH)₂D₃ has been associated with advanced disease and high levels of tumor necrosis factor (TNFα) in AIDS patients (Haug et al., 1998a), suggesting that vitamin D₃ metabolism may be an important feature of AIDS pathogenesis. Decreased macrophage bactericidal activity has been implicated as the responsible effector mechanism (Haug et al., 1998b).

It has become apparent that 1,25(OH)₂D₃ can stimulate and suppress immune responses, depending on the circumstances, but that deficiencies are usually associated with decreased rather than enhanced immunity (Manolagas et al., 1989). In studies with female broiler chickens, vitamin D deficiency has been shown to decrease thymus weights, cutaneous basophil hypersensitivity response, and phagocytosis by sephadex-elicited abdominal macrophages (Aslam et al., 1998). Addition of 20 µg/kg supplemental 1,25(OH)₂D₃ to turkey diets has been shown to increase antibody response to sheep erythrocytes, increase phagocytosis by sephadex-elicited abdominal macrophages, and increase the percentage of tumor cells killed by lipopolysaccharide-stimulated abdominal macrophages (Garlich et al., 1992).

The ability of vitamin D supplementation to modulate the immune response of stressed turkeys may reflect enhancement or suppression of many different immune functions. We have shown that the immunosuppression induced by DEX results in decreased bactericidal activity of peripheral blood glass-adherent mononuclear cells and that antibacterial activity is significantly lower in males than in females (Huff et al., 1999d). Because TOC is primarily a problem in male turkeys (Huff et al., 1999a), we hypothesize that defective macrophage function, possibly related to the effects of vitamin D modulation of the stress response (Puchacz et al., 1996), may be involved in its pathogenesis. Whether or not any individual bird has sufficient vitamin D for growth and homeostasis may be related not only to the composition of its diet and exposure to ultraviolet light but also to the level of stressors in its environment and its genetic inheritance, which may affect both the perception of stressors and the physiological responses to stressors.

Chicks selected for a high level of tibial dyschondroplasia (TD) have been shown to have a subnormal ability to metabolize vitamin D (Mitchell et al., 1997; Xu et al., 1997), which has been inconsistently related to the number of intestinal and growth plate vitamin D receptors (Soares et al., 1990; Mitchell et al., 1997). Turkay osteomyelitis complex has been often, but inconclusively, associated with TD (Nairn, 1973; Wyers et al., 1991; Rath et al., 1994); however, the incidence of TD is usually much higher. This association between TD and TOC may be due to the wide-ranging physiological effects of vitamin D and the commonality of dietary factors, environmental stressors, and genetics within a group of turkeys. Differences in vitamin D receptor phenotypes, rather than the numbers of these receptors, may affect the incidence of both diseases, whereas the birds that develop TOC may reflect a subgroup with an abnormally high stress response. In avian species, there are large individual differences as well as sex-related differences in the stress response (Silverin, 1998). We have previously hypothesized that the development of TOC is related to genetically determined differences in the response of male turkeys.
to the stressors of commercial turkey production (Huff et al., 1998; 1999a).

In a previous study in which turkeys were experimentally challenged with *E. coli*, the turkeys that lost the most BW had the highest cutaneous basophil hypersensitivity responses, and there were significantly higher first week mortality and air sacculitis scores in the strain of birds having the highest response (Bayyari et al., 1997c). We have also reported that turkeys that developed TOC had higher antibody responses to sheep red blood cells (Bayyari et al., 1997a). Gross et al. (1980) found that a strong humoral immune response is associated with decreased resistance to both *E. coli* and *S. aureus* and suggested that birds with average immune responses were better able to resist infection than birds with either very high or very low responses. In the current study, vitamin D₃ may function by modulating various immune responses toward this ideal. The genetic selection of turkeys for increased growth rate appears to have been accompanied by changes in the immune response (Bayyari et al., 1997b). Vitamin D receptor polymorphisms have been found to be associated with gender-related differences in growth rate in humans (Suarez et al., 1997, 1998), as well as with susceptibility to a number of infectious diseases (Hill, 1998).

In the present study, vitamin D supplementation of repeatedly stressed turkeys greatly increased disease resistance and improved BW, relative weights of liver and heart, differential white blood cell counts, and several serum chemistry values. The ability of supplemental vitamin D₃ to affect changes in the physiology and disease resistance of stressed birds supports the current conceptual view that vitamin D₃ is a prohormone involved in homeostasis of diverse biological systems (DeLuca, 1992; DeLuca and Zierold, 1998; Jones et al., 1998) and that individual variation in the ability to metabolize or bind its metabolites may affect health and disease resistance in turkeys.

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