The effects of feeding high concentrations of cholecalciferol, phytase, or their combination on broiler chicks fed various concentrations of nonphytate phosphorus

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SUMMARY

Two experiments were conducted to investigate the effects of feeding high concentrations of cholecalciferol (D₃), phytase, or their combination on BW gain, FE, tibia ash, and total tract P retention in broiler chicks fed various concentrations of nonphytate P (nPP). In the first experiment, we compared dietary nPP concentrations (0.20, 0.26, 0.33, 0.39, and 0.45%) with supplemental D₃ (0, 7,500, and 15,000 IU/kg) for 5- to 23-d-old Ross 308 chicks. In experiment 2, we compared dietary nPP concentrations (0.15, 0.25, 0.35, and 0.45%) with concentrations of D₃ (0 or 7,500 IU/kg) and phytase (0 or 1,000 phytase units/kg) for 4- to 18-d-old Ross 308 chicks. The supplementation of D₃ resulted in improved FE and total tract P retention in experiment 1, but did not have a positive effect on any of the parameters tested in experiment 2. The addition of higher concentrations of D₃ to broiler diets resulted in improved P retention in experiment 1, but did not affect the performance of chicks in either experiment.

Key words: broiler, cholecalciferol, nonphytate phosphorus, phytase

DESCRIPTION OF PROBLEM

To meet the nonphytate P (nPP) requirement of the broiler chick for growth, many producers supplement an inorganic P source, such as dicalcium phosphate or rock phosphate. This practice is expensive and results in greater excretion of P that can lead to increased runoff and possible incidence of eutrophication [1–5]. The supplementation of phytase (PHY), vitamin D₃ or its metabolites 1,25-dihydroxycholecalciferol and 1α-hydroxycholecalciferol (D₃), or the combination of both PHY and D₃ increased P retention, resulting in reduced P excretion as well as cost savings resulting from decreased supplementation of inorganic P sources [1, 5–10]. Nawaz et al. [11] documented a significant (P ≤ 0.05) improvement in BW gain and feed intake with supplementation of D₃ at concentrations ranging from 200 to 3,000 IU/kg to diets with adequate dietary nPP. Baker et al. [12] demonstrated improved tibia ash percentage with supplementation of D₃ at 50,000 IU/kg to diets with nPP concentrations of 0.10 or 0.45%. Therefore, D₃
Table 1. Composition of the experimental diets (experiment 1)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dietary nPP,1 %</th>
<th>0.20</th>
<th>0.27</th>
<th>0.33</th>
<th>0.39</th>
<th>0.45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
<td>54.69</td>
<td>54.46</td>
<td>54.24</td>
<td>54.01</td>
<td>53.74</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
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<td>37.50</td>
<td>37.50</td>
<td>37.50</td>
<td>37.50</td>
<td>37.50</td>
</tr>
<tr>
<td>Animal-vegetable blend oil</td>
<td></td>
<td>4.11</td>
<td>4.20</td>
<td>4.29</td>
<td>4.38</td>
<td>4.49</td>
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<tr>
<td>Limestone</td>
<td></td>
<td>1.85</td>
<td>1.65</td>
<td>1.44</td>
<td>1.25</td>
<td>1.06</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td></td>
<td>0.40</td>
<td>0.74</td>
<td>1.08</td>
<td>1.42</td>
<td>1.75</td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Poultry VTM 882</td>
<td></td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td></td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Choline chloride</td>
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<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td></td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Calculated composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td>21.70</td>
<td>21.70</td>
<td>21.70</td>
<td>21.70</td>
<td>21.70</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
<td></td>
<td>3,200</td>
<td>3,200</td>
<td>3,200</td>
<td>3,200</td>
<td>3,200</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Total P</td>
<td></td>
<td>0.46</td>
<td>0.52</td>
<td>0.58</td>
<td>0.65</td>
<td>0.71</td>
</tr>
<tr>
<td>nPP</td>
<td></td>
<td>0.20</td>
<td>0.27</td>
<td>0.33</td>
<td>0.39</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Analyzed composition</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total P</td>
<td></td>
<td>0.61</td>
<td>0.67</td>
<td>0.79</td>
<td>0.79</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1nPP = nonphytate P. Each diet was replicated with the addition of 7,500 or 15,000 IU/kg of vitamin D₃.
2DSM Nutritional Products (Ames, IA). The vitamin and mineral mix provided 2,750 IU/kg of vitamin D₃ to each diet. The vitamin and mineral mix provided the following (per kilogram of premix): selenium, 40 ppm; vitamin A, 1,320,000 IU; vitamin E, 2,860 IU; menadione, 176 mg; vitamin B₁₂, 1.87 mg; biotin, 6.6 mg; choline, 71,500 mg; folic acid, 220 mg; niacin, 6,600 mg; pantothenic acid, 1,760 mg; pyridoxine, 176 mg; riboflavin, 880 mg; thiamine, 220 mg.

Table 2. Composition of the experimental diets (experiment 2)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dietary nPP,1 %</th>
<th>0.15</th>
<th>0.25</th>
<th>0.35</th>
<th>0.45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
<td>56.42</td>
<td>55.97</td>
<td>55.57</td>
<td>55.19</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td></td>
<td>37.43</td>
<td>37.50</td>
<td>37.50</td>
<td>37.50</td>
</tr>
<tr>
<td>Animal-vegetable blend oil</td>
<td></td>
<td>2.50</td>
<td>2.65</td>
<td>2.82</td>
<td>2.97</td>
</tr>
<tr>
<td>Limestone</td>
<td></td>
<td>1.99</td>
<td>1.69</td>
<td>1.39</td>
<td>1.07</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<td>0.14</td>
<td>0.67</td>
<td>1.20</td>
<td>1.75</td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Poultry VTM 882</td>
<td></td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td></td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Choline chloride</td>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td></td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Calculated composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td>21.80</td>
<td>21.80</td>
<td>21.80</td>
<td>21.80</td>
</tr>
<tr>
<td>ME, kcal/kg</td>
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<td>3,115</td>
<td>3,115</td>
<td>3,115</td>
<td>3,115</td>
</tr>
<tr>
<td>Ca</td>
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<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Total P</td>
<td></td>
<td>0.42</td>
<td>0.51</td>
<td>0.61</td>
<td>0.71</td>
</tr>
<tr>
<td>nPP</td>
<td></td>
<td>0.15</td>
<td>0.25</td>
<td>0.35</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Analyzed composition</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total P</td>
<td></td>
<td>0.53</td>
<td>0.60</td>
<td>0.66</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1nPP = nonphytate P. Each diet was replicated with the addition of 7,500 IU/kg of vitamin D₃, 1,000 phytase units (FTU)/kg of phytase, or their combination.
2DSM Nutritional Products (Ames, IA). The vitamin and mineral mix provided 2,750 IU/kg of vitamin D₃ to each diet. The vitamin and mineral mix provided the following (per kilogram of premix): selenium, 40 ppm; vitamin A, 1,320,000 IU; vitamin E, 2,860 IU; menadione, 176 mg; vitamin B₁₂, 1.87 mg; biotin, 6.6 mg; choline, 71,500 mg; folic acid, 220 mg; niacin, 6,600 mg; pantothenic acid, 1,760 mg; pyridoxine, 176 mg; riboflavin, 880 mg; thiamine, 220 mg.
has the potential to improve broiler chick performance when supplemented to corn-soybean meal diets with reduced dietary nPP.

With these benefits in mind, the objective of the current study was to determine the effects of feeding higher than commercial concentrations of cholecalciferol to growing broiler chicks fed various concentrations of nPP on bird performance, tibia ash, and P retention. A second experiment followed the first to determine the effects of high cholecalciferol feeding in combination with PHY on bird performance, tibia ash, and P retention.

### MATERIALS AND METHODS

#### General Procedure

All bird procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University. Two experiments were conducted with Ross 308 female chicks that were purchased from a local hatchery [13]. Chicks were maintained in raised wire battery pens (508 cm²/bird) with continuous light in an environmentally controlled room, where all chicks had access to supplemental heat begin-

#### Table 3. Performance, tibia ash, and total tract P retention (TTPR) of chicks fed various concentrations of vitamin D₃ and nonphytate P (nPP; experiment 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>df</th>
<th>BW gain, g/bird</th>
<th>G:F, g/kg</th>
<th>Tibia ash, g/tibia</th>
<th>Tibia ash, %</th>
<th>TTPR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>nPP, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>7</td>
<td>504c</td>
<td></td>
<td>0.525d</td>
<td>37.68d</td>
<td>52.59</td>
</tr>
<tr>
<td>0.26</td>
<td>7</td>
<td>577b</td>
<td></td>
<td>0.684c</td>
<td>41.68c</td>
<td>49.16</td>
</tr>
<tr>
<td>0.33</td>
<td>7</td>
<td>584b</td>
<td></td>
<td>0.798b</td>
<td>44.31b</td>
<td>55.83</td>
</tr>
<tr>
<td>0.39</td>
<td>7</td>
<td>623a</td>
<td>582</td>
<td>0.884a</td>
<td>46.16a</td>
<td>52.61</td>
</tr>
<tr>
<td>0.45</td>
<td>7</td>
<td>615a</td>
<td>552</td>
<td>0.901a</td>
<td>46.34a</td>
<td>48.99</td>
</tr>
<tr>
<td>D₃, IU/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7</td>
<td>579</td>
<td>519</td>
<td>0.747</td>
<td>43.22</td>
<td>48.34</td>
</tr>
<tr>
<td>7,500</td>
<td>7</td>
<td>579</td>
<td>524</td>
<td>0.754</td>
<td>43.37</td>
<td>53.71</td>
</tr>
<tr>
<td>15,000</td>
<td>7</td>
<td>584</td>
<td>526</td>
<td>0.775</td>
<td>43.10</td>
<td>53.46</td>
</tr>
<tr>
<td>nPP, %, + D₃, IU/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20 + 0</td>
<td>7</td>
<td>510</td>
<td>454a</td>
<td>0.486</td>
<td>36.62</td>
<td>55.49⁡&lt;sub&gt;xy&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.20 + 7,500</td>
<td>7</td>
<td>511</td>
<td>445⁡&lt;sub&gt;xy&lt;/sub&gt;</td>
<td>0.543</td>
<td>37.81</td>
<td>49.12⁡&lt;sub&gt;xy&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.26 + 7,500</td>
<td>7</td>
<td>575</td>
<td>526⁡&lt;sub&gt;xw&lt;/sub&gt;</td>
<td>0.670</td>
<td>41.70</td>
<td>40.37⁡&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.33 + 7,500</td>
<td>7</td>
<td>589</td>
<td>530⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.694</td>
<td>41.87</td>
<td>58.10⁡&lt;sub&gt;xy&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.39 + 7,500</td>
<td>7</td>
<td>602</td>
<td>543⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.820</td>
<td>45.16</td>
<td>52.18⁡&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.45 + 7,500</td>
<td>7</td>
<td>581</td>
<td>537⁡&lt;sub&gt;xv&lt;/sub&gt;</td>
<td>0.788</td>
<td>45.92⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>0.33 + 15,000</td>
<td>7</td>
<td>569</td>
<td>490⁡&lt;sub&gt;xy&lt;/sub&gt;</td>
<td>0.785</td>
<td>43.75</td>
<td>60.40⁡&lt;sub&gt;xv&lt;/sub&gt;</td>
</tr>
<tr>
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<td>611</td>
<td>542⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.865</td>
<td>45.86</td>
<td>42.10⁡&lt;sub&gt;x&lt;/sub&gt;</td>
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<tr>
<td>0.45 + 15,000</td>
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<td>629</td>
<td>616⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.876</td>
<td>46.48</td>
<td>60.10⁡&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.45 + 7,500</td>
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<td>629</td>
<td>587⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.913</td>
<td>46.14</td>
<td>55.63⁡&lt;sub&gt;x&lt;/sub&gt;</td>
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<tr>
<td>0.45 + 15,000</td>
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<td>616</td>
<td>550⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.893</td>
<td>46.78</td>
<td>51.57⁡&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.45 + 7,500</td>
<td>7</td>
<td>607</td>
<td>529⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.873</td>
<td>46.28</td>
<td>51.36⁡&lt;sub&gt;x&lt;/sub&gt;</td>
</tr>
<tr>
<td>0.45 + 15,000</td>
<td>7</td>
<td>623</td>
<td>576⁡&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.938</td>
<td>45.94</td>
<td>44.04⁡&lt;sub&gt;x&lt;/sub&gt;</td>
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<tr>
<td>Pooled SEM</td>
<td>34</td>
<td>40</td>
<td>0.063</td>
<td>1.50</td>
<td>5.39</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA P-value

D₃ | 2  | 0.7579 | 0.7852 | 0.2147 | 0.7820 | 0.0002 |

nPP | 4  | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0012 |

nPP × D₃ | 8  | 0.5388 | 0.0029 | 0.5252 | 0.3078 | <0.0001 |

Values within each individual main effect column with no common superscript are significantly different (P ≤ 0.05).

Values with an interaction column with no common superscript are significantly different (P ≤ 0.05).

Data are means of 6 replicate groups of 5 chicks per treatment during the period 5 to 23 d after hatching; average initial BW of 100 g.

Cholecalciferol.
ning at 35°C on day of hatch and decreasing 2°C every week. Chicks were fed a standard corn-soybean meal diet that met or exceeded NRC [14] recommendations before being placed on experimental diets at either 4 or 5 d of age. All chicks were weighed, sorted, wing banded, and randomly allotted to dietary treatments. Feed and water were provided ad libitum throughout the experimental period. Individual BW were determined at the beginning and at the end of the experimental period to determine BW gain. Chicks were monitored twice daily, with mortalities and cull chicks removed from the pens as they occurred. Mortalities or cull chicks were weighed and recorded before disposal. Feed intake was determined by the difference in feed offered and feed refused calculated on a pen basis for the duration of the experimental period. Mortality-corrected FE was determined as the ratio of pen weight gain plus BW gained before mortality divided by the feed intake over the experimental period. Excreta samples (approximately 250 g/experimental unit) were collected over the final 2 d of the experimental period to determine total tract P retention (TTPR) using TiO₂ as an inert dietary marker [15, 16].

At the end of the experiment, chicks were euthanized by CO₂ asphyxiation and the right tibiae from all chicks were collected and pooled by experimental unit for tibia ash determination [17, 18]. Statistical analysis was conducted using the GLM function of SAS [19]. Statistical significance was assigned at $P \leq 0.05$.

**Experimental Diets**

The experiment diets are provided in Table 1 and 2. The D₃ supplement premix used in both experiments was guaranteed to contain 500,000 IU/g, and additions were based on the guaranteed content. All supplemental D₃ additions were made on top of a complete vitamin and mineral premix that contained 2,750 IU of D₃/kg of diet. The PHY used in experiment 2 was an *Escherichia coli*-derived PHY and was added according to guaranteed minimum activity.

![Figure 1](https://academic.oup.com/japr/article-abstract/21/3/579/724700/21March2019)

**Figure 1.** The interactive effect of nonphytate P (nPP) × vitamin D₃ on total tract P retention (experiment 1; pooled SEM = 2.200). C-SBM = corn and soybean meal diet.
Experiment 1

Experiment 1 was conducted to determine the effects of feeding high concentrations of D₃ to growing chicks fed various concentrations of nPP on bird performance, P utilization, and body P status. The experiment consisted of a 5 × 3 factorial arrangement of treatments in a completely randomized design with 5 dietary concentrations of nPP (0.20, 0.26, 0.33, 0.39, and 0.45%) and 3 concentrations of D₃ (0, 7,500, and 15,000 IU/kg), which were selected based on results observed in the literature [20–23]. In total, 450 chicks were randomly allotted to 90 experimental units in groups of 5 chicks. Chicks were selected from an initial pool of 500 chicks, using BW as the determining factor. Body weights of selected birds ranged from 81 to 112 g at the beginning of the experimental period, with an average pen weight of 100 g. Each of the 15 treatments was replicated 6 times, resulting in 30 birds per treatment. The experimental diets were fed for the 18-d period (d 5 to 23).

Experiment 2

Experiment 2 was conducted to determine the effects of feeding high concentrations of D₃ and PHY to growing chicks fed various concentrations of nPP on bird performance, tibia ash, and TTPR. The study was arranged as a 4 × 2 × 2 factorial in a completely randomized design with 4 dietary concentrations of nPP (0.15, 0.25, 0.35, and 0.45%), 2 concentrations of PHY (0 or 1,000 FTU/kg), and 2 concentrations of D₃ (0 or 7,500 IU/kg), which were selected based on the results from experiment 1. In total, 480 chicks were randomly allotted to 96 experimental units in groups of 5 chicks. Chicks were selected from an initial pool of 525 chicks, using BW as the determining factor. Body weights of selected birds ranged from 60 to 125 g at the beginning of the experimental period, with a pen average of 88 g. Each of the 16 treatments was replicated 6 times, resulting in 30 birds per treatment. The experimental diets were fed for a 15-d period (d 4 to 18).

RESULTS AND DISCUSSION

Experiment 1

Supplementation of D₃ has been shown to improve BW gain, feed intake, FE, tibia ash, and TTPR in birds fed diets both adequate and deficient in nPP [1, 7, 8, 11, 12, 24–28]. In this experiment, cholecalciferol was not effective in improving BW gain or tibia ash (Table 3). A significant \( P \leq 0.05 \) nPP × D₃ interaction for FE and TTPR occurred, whereas a main effect of nPP resulted in a significant \( P \leq 0.05 \) improvement in BW gain, tibia ash weight, and tibia ash percentage. As expected, BW gain and tibia ash improved as dietary nPP increased because of the increased P in the diet. Supplementation of D₃ (at either level) improved FE with increasing concentrations of nPP until FE peaked at 0.39% dietary nPP, whereas FE peaked at 0.26% dietary nPP for the nonsupplemented diets. Results of the current study are in contrast to those of Rama Rao et al. [1] and Biehl and Baker [8].
who noted a significant \( (P \leq 0.05) \) improvement in FE when 3,600 IU/kg of D\(_3\) was supplemented to 0.27\% dietary nPP and when 1,000 IU/kg of D\(_3\) was supplemented to 0.14\% dietary nPP, respectively.

A significant \( (P \leq 0.05) \) nPP \( \times \) D\(_3\) interaction occurred for TTPR (Figure 1). Although there were differences in the interaction of TTPR across the various nPP concentrations, no clear pattern could be determined. It was evident that at least over some nPP concentrations, D\(_3\) increased the TTPR of the birds. These findings are similar to those reported in the literature [1, 5–7] in that supplementation of D\(_3\) or its metabolite (1,25-dihydroxycholecalciferol) improved P retention. The mechanism for this response has 2 possible explanations: first, D\(_3\) can act on the cells of the intestine to upregulate mRNA expression of the Na-coupled P cotransporter, thus improving P absorption [6, 29]; second, D\(_3\) can act directly on the phytate compound to release the bound P, resulting in a greater supply of P for the body [12]. In past research, cholecalciferol improved performance, tibia ash, and TTPR, although its effectiveness in the current study was observed only through interactive effects on TTPR and did not affect BW gain, FE, or tibia ash. Although D\(_3\) increased TTPR, this

Table 5. Tibia ash and total tract P retention (TTPR) of chicks fed various concentrations of vitamin D\(_3\), phytase, and nonphytate P (nPP; experiment 2)

<table>
<thead>
<tr>
<th>Item</th>
<th>Tibia ash, g/tibia</th>
<th>Tibia ash, %</th>
<th>TTPR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>nPP, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>0.289</td>
<td>36.42</td>
<td>62.06</td>
</tr>
<tr>
<td>0.25</td>
<td>0.393</td>
<td>41.72</td>
<td>56.71</td>
</tr>
<tr>
<td>0.35</td>
<td>0.474</td>
<td>45.19</td>
<td>55.43</td>
</tr>
<tr>
<td>0.45</td>
<td>0.519</td>
<td>46.88</td>
<td>42.56</td>
</tr>
<tr>
<td>D(_3),(^2) IU/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.417</td>
<td>42.50</td>
<td>54.31</td>
</tr>
<tr>
<td>7,500</td>
<td>0.421</td>
<td>42.60</td>
<td>54.07</td>
</tr>
<tr>
<td>Phytase, FTU/(^3)kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.382</td>
<td>40.80</td>
<td>52.77</td>
</tr>
<tr>
<td>1,000</td>
<td>0.456</td>
<td>44.30</td>
<td>55.61</td>
</tr>
<tr>
<td>nPP, % + phytase, FTU/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15 + 0</td>
<td>0.247(^c)</td>
<td>33.40(^c)</td>
<td>—</td>
</tr>
<tr>
<td>0.15 + 1,000</td>
<td>0.330(^b)</td>
<td>39.44(^b)</td>
<td>—</td>
</tr>
<tr>
<td>0.25 + 0</td>
<td>0.338(^a)</td>
<td>39.61(^a)</td>
<td>—</td>
</tr>
<tr>
<td>0.25 + 1,000</td>
<td>0.449(^a)</td>
<td>43.82(^a)</td>
<td>—</td>
</tr>
<tr>
<td>0.35 + 0</td>
<td>0.431(^a)</td>
<td>43.83(^a)</td>
<td>—</td>
</tr>
<tr>
<td>0.35 + 1,000</td>
<td>0.517(^a)</td>
<td>46.54(^a)</td>
<td>—</td>
</tr>
<tr>
<td>0.45 + 0</td>
<td>0.512(^a)</td>
<td>46.36(^a)</td>
<td>—</td>
</tr>
<tr>
<td>0.45 + 1,000</td>
<td>0.526(^a)</td>
<td>47.40(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.048</td>
<td>1.73</td>
<td>3.29</td>
</tr>
</tbody>
</table>

ANOVA \( P \)-value

<table>
<thead>
<tr>
<th>Item</th>
<th>DF</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D(_3)</td>
<td>1</td>
<td>0.6601</td>
</tr>
<tr>
<td>nPP</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phytase</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>nPP ( \times ) D(_3)</td>
<td>3</td>
<td>0.7889</td>
</tr>
<tr>
<td>nPP ( \times ) PHY</td>
<td>3</td>
<td>0.0051</td>
</tr>
<tr>
<td>D(_3) ( \times ) PHY</td>
<td>1</td>
<td>0.2322</td>
</tr>
<tr>
<td>nPP ( \times ) D(_3) ( \times ) PHY</td>
<td>3</td>
<td>0.6049</td>
</tr>
</tbody>
</table>

\( ^a\)Values within a column with no common superscript are significantly different \( (P \leq 0.05) \).

\( ^b\)Values with an interaction column with no common superscript are significantly different \( (P \leq 0.05) \).

\(^1\)Data are means of 6 replicate groups of 5 chicks per treatment during the period from 4 to 18 d after hatching; average initial BW of 88 g.

\(^2\)Cholecalciferol.

\(^3\)FTU = phytase units.
Experiment 2

The combination of supplemental D₃ and PHY has been shown to improve performance, tibia ash, and TTTR in several experiments [5, 9, 10, 25]. In contrast to results of the previous studies, results of the current study suggest that D₃ did not improve BW gain, FE, tibia ash, or TTTR (Tables 4 and 5). A significant 3-way interaction between nPP × D₃ × PHY was noted. Significant main effects of dietary nPP and PHY were observed on BW gain, FE, tibia ash, and TTTR. In general, performance significantly improved as PHY was supplemented at 1,000 FTU/kg or as dietary nPP increased. These findings are similar to previously published reports [30, 31].

A significant (P < 0.006) nPP × PHY interaction occurred for both tibia ash weight and percentage in comparison with diets containing higher amounts of nPP. Results of the current study are similar to those in the literature [31, 32]. A significant 3-way nPP × PHY × D₃ interaction occurred in TTTR (Figure 2); this was due to a large reduction in P retention for the nonsupplemented 0.45% nPP diet, and no current explanation is available regarding why this value was so small (28.24%). These results are in contrast to those of Qian [9], who noted a significant improvement in TTTR when D₃ (2,640 or 26,400 IU/kg), PHY (0 to 900 FTU/kg), or their combination was fed with 0.27% nPP to chicks from d 1 to 21. In the current study, D₃ did not improve performance, tibia ash, or TTTR. The supplementation of PHY had beneficial effects on all parameters tested in the current study. In this study, increased concentrations of D₃ supplemented to an adequate diet did not improve the performance of broiler chicks fed low-nPP diets.

CONCLUSIONS AND APPLICATIONS

1. The supplementation of higher concentrations of D₃ to broiler diets already adequate in vitamin D was able to improve...
TTPR, although this was not evident in either experiment.
2. High supplemental D₃ was not effective in improving performance in either experiment.
3. Phytase supplementation increased performance of the chicks.
4. Birds supplemented with a combination of PHY and D₃ did not perform significantly better than birds supplemented with PHY alone.

REFERENCES AND NOTES
13. Welps Hatchery, Bancroft, IA.
16. For TTPR determination, excreta were weighed and then dried for 5 d at 65°C, after which the dry excreta and experimental diet samples were weighed and ground to pass through a 1-mm screen. The dry samples, 0.3 g of excreta and 0.8 g of feed, were digested with 0.8 g of Na₂SO₄ (anhydrous) in 5 mL of H₂SO₄ on a heat block at 105°C for 72 h. Once cooled, the samples were diluted to 50 mL with distilled water in a 50-mL glass volumetric flask, and then transferred to 50-mL conical tubes for storage. Phosphorus percentage of the experimental unit (EU) sample was determined in triplicate via a 1:1:3 mixture of molybdovanadate solution, sample, and distilled water in a 96-well plate, with an incubation period of 30 min, before being read at a wavelength of 400 nm using a Synergy 4 Hybrid Multi-Mode Microplate Reader (BioTek, Winooski, VT). Titanium dioxide values were determined in triplicate in a 96-well plate by a 20:1 concentration solution of EU sample to H₂O₂, with a 30-min incubation period, before being read at a wavelength of 410 nm using a Synergy 4 Hybrid Multi-Mode Microplate Reader [15]. The TTPR values were determined by the following equation: \( \frac{[P_{\text{die}} - P_{\text{fai}}]}{\left(\frac{T_{\text{die}}}{T_{\text{fai}}}\right)/P_{\text{die}}} \times 100 = \text{TTPR percentage} \), where \( P_{\text{die}} \) is the percentage of P in the diet, \( P_{\text{fai}} \) is the percentage of P in the EU fecal sample, \( T_{\text{die}} \) is the percentage of Ti in the diet, and \( T_{\text{fai}} \) is the percentage of Ti in the EU fecal sample.
18. Briefly, bones were pooled by EU, autoclaved (121°C at 0.124 MPa for 30 min), and processed to remove any adhering tissue and cartilage caps before the bones were dried at 100°C for 72 h. The dried bones were weighed before they were dry-ashed for 24 h at 600°C in a muffle furnace. The remaining ash was weighed to determine tibia ash, expressed as grams of total ash per tibia and as a percentage of dry bone.


