The effects of 25-hydroxy-cholecalciferol and a phytase on ileal utilization of P in laying hens fed basal diets low in phosphorus content (3 g/kg feed) were studied in a cage trial over 4 weeks. The hens were fed mash diets based on maize and soybean meal. 128 laying hens (“ISA Brown”, 22 weeks of age) were allocated at random to 8 treatments with two hens per cage. Two different basal diets were prepared with 300 and 2500 IU of vitamin D₃ (ROVIMIX® D3-500) per kg feed, respectively. Beside the control treatments, the animals received the basal diets supplemented with 69 µg 25-hydroxy-cholecalciferol (Hy•D®) per kg feed, 450 U phytase (RONOZYME® P5000) per kg feed or a combination of both products, respectively. At levels of 300 IU vitamin D₃ per kg a significant (p<0.05) improvement of the ileal utilization of phosphorus was obtained for the inclusion of Hy•D® (+59%), RONOZYME® P5000 (+88%) and the combination of both products (+121%) compared to the control treatment. The highest effect was found for the combination of Hy•D® and RONOZYME® P5000. At 2500 IU vitamin D₃ per kg feed, ileal P utilization was numerically improved by about +15% over all treatments compared to the control.

Keywords: 25-hydroxy-cholecalciferol; phytase; laying hens; phosphorus utilization; laying performance

Introduction

Most of the phosphorus in plant sources is bound in a phytate complex, which makes it relatively unavailable for poultry, because birds lack endogenous phytase and thus are not able to degrade phytate effectively (Nelson, 1976). The aim of the present trial was to study the effects of a combination of Hy•D® (25-OH-D₃), a metabolite of Vitamin D₃, with a phytase derived from Peniophora lycii at two different basal supplementation levels of vitamin D₃, on the performance of laying hens and the ileal utilization of phosphorus fed low phosphorus diets based on maize and soybean meal.

Materials and methods

128 Isa Brown laying hens, 22 weeks of age, were randomly allocated to eight treatments with 8 replicates per treatment. The laying hens were housed battery cages with two hens per cage in an environmentally controlled room at a room temperature of 16°C. Experimental diets and tap water were made available for ad libitum consumption. The hens were allocated to one of the two different

Effects of 25-hydroxy-cholecalciferol and a peniophora lycii phytase on the ileal utilization of phosphorus in laying hens fed basal diets low in phosphorus and with different levels of vitamin D₃

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basal diets supplemented with either 300 and 2500 IU of vitamin D₃ and moreover to three different
treatments supplemented with 69 µg 25-OH-D₃ (ROVIMIX® Hy•D®), 450 U RONOZYME® P 5000
CT and a combination of both products per kg, respectively. The feed was provided as mash. In a 7-
days pre-experimental period the laying hens were fed a low phosphorus basal diet (Table 1) which
was also used in the balance trial period over the following four weeks. The basal mash was
formulated based on maize (72.1 %) and soybean meal (17.3 %) as main ingredients. Titanium dioxide
(TiO₂) was added to the feed as indigestible marker at a concentration of 1000 mg/kg feed. Feed
consumption was determined weekly. Daily feed intake was calculated. Number of broken eggs was
noted for each group. Eggs were collected daily. Once a week, the collected eggs were weighted per
group. The laying performance (egg weight, laying rate and rate of broken eggs) was calculated per
group. At 27 weeks of age blood samples from each hen were taken from Vena jugularis. In the
plasma the concentrations of inorganic phosphate were determined. At the end of the trial (week 27),
the hens were sacrificed and the contents of the terminal part of the ileum, defined as from 17 to 2 cm
before the ileo-caecal junction, were sampled, pooled for two hens per cage, freeze-dried, and ground
for chemical analysis. Feed and chime samples were analyzed for dry matter, TiO₂, and P. The
apparent ileal utilization of P was calculated. The analyses of the nutrient content in the samples were
performed according to standard methods (VDLUFA 1997) (Table 1). TiO₂, and total P were
determined by ICP according to DIN EN ISO 11885:1997 (DIN EN ISO 1998) after H₂SO₄ / Na₂SO₄
mineralization. Phytate was determined colorimetrically as released P after extraction, elution and wet
digestion with HNO₃/H₂SO₄ (AOAC 1990). In the blood plasma, the concentration of inorganic
phosphate was determined with a HITACHII 912 automatic analyzer according to the methods
described by Henry (1974) and the concentration of 25-OH-D₃ according to Gössl et al. (2004).

For the statistical evaluation a one-factorial analysis of variance (factor: treatment) was carried out
using the software "Stat Box Pro", version 5.0 (Grimmer soft 1995). Where significant treatment
effects (p < 0.05) were indicated, the differences among treatment means were analyzed with the
Newman-Keuls test.

Results and discussion

Table 2 shows the determined concentrations of the products in feed. Both, phytase activity and
concentration of vitamin D₃ were in general in accordance with the target values, whereas the
analyzed concentration of 25-OH-D₃ was higher than intended. In Tables 3a and b the results on
laying performance are listed for both basal supplementation levels of vitamin D₃. No significant
differences among the treatments were noted. These findings could be related to the short observation
period and the low number of replicates. However, positive trends for all treatments additionally
supplemented with Hy•D®, Peniophora lycii phytase and for the combination of both were noted.

At low supplementation of vitamin D₃ (300 IU/feed) the apparent ileal utilization of P was
significantly increased from 25.2% (control), to 40% (Hy•D), 47.5% (phytase) and 55.8% (combination of Hy•D® with phytase) (Figure 1a). At the high supplementation level of vitamin D₃ (2500 IU/feed) the apparent ileal utilization of P was numerically increased by about 15% for all
treatments compared to the un-supplemented control (Figure 1b). The determined concentrations of P,
in plasma are shown in Figure 2a and b. No significant differences were found. However, at 300 IU
vitamin D₃/kg, the numerically highest concentration was found for the combination of Hy•D® with
Peniophora lycii phytase. The concentrations of 25-OH-D₃ were dose-dependently increased at both
supplementation levels of vitamin D₃ (Figure 3a and b).

It can be concluded that the supplementation of Hy•D and Peniophora lycii phytase in combination to
a low-P basal diet at two basal supplementation levels of vitamin D₃ resulted in beneficial effects on
the apparent ileal utilization of P in laying hens. At levels of 300 IU vitamin D₃ per kg a significant
(p<0.05) improvement of the ileal utilization of phosphorus was obtained for the inclusion of Hy•D®
(+59%), RONOZYME® P5000 (+88%) and the combination of both products (+121%) compared to
the control treatment. The highest effect was found for the combination of Hy•D® and RONOZYME®
P5000. At 2500 IU vitamin D₃ per kg feed, ileal P utilization was numerically improved by about
+15% over all treatments compared to the control.
### Table 1  Composition of the basal diet.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>72.1</td>
</tr>
<tr>
<td>Soybean meal (50 % CP)</td>
<td>17.3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>1.00</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.20</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>1.50</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>6.70</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.10</td>
</tr>
<tr>
<td>Premix without D₃</td>
<td>1.00</td>
</tr>
<tr>
<td>Titane oxyde</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**Ingredients (%):**
- Crude protein (g/kg): 143
- Calcium (g/kg): 32.3
- Total P (g/kg): 3.0
- Non-phytic acid P (g/kg): 1.1

**Calculated content:**
- ME₃ (MJ/kg): 12.1
- Lysine (%): 0.7
- Methionine + Cystine (%): 0.7

### Table 2 Analyzed activities of Peniophora lycii phytase and concentrations of 25-OH-D₃ and vitamin D₃ in feed samples

<table>
<thead>
<tr>
<th>Product</th>
<th>Control D₃ 300</th>
<th>Hy•D* 69 µg/kg</th>
<th>RONOZYME® P 5000 CT</th>
<th>Hy•D* 69 µg/kg</th>
<th>Vitamin D₃ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>-</td>
<td>450 U/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### a) Daily feed intake and laying performance of hens fed basal diets supplemented with 300 IU of vitamin D₃ or 2500 IU of vitamin D₃, respectively.

<table>
<thead>
<tr>
<th>Product</th>
<th>Control D₃ 300</th>
<th>Hy•D* 69 µg/kg</th>
<th>RONOZYME® P 5000 CT</th>
<th>Hy•D* 69 µg/kg</th>
<th>Vitamin D₃ (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g/day and hen)</td>
<td>104.7 ± 7.81</td>
<td>109.6 ± 7.49</td>
<td>110.0 ± 5.40</td>
<td>112.9 ± 3.08</td>
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</tr>
<tr>
<td>Egg weight (g)</td>
<td>57.4 ± 2.02</td>
<td>60.2 ± 3.27</td>
<td>57.8 ± 2.73</td>
<td>59.2 ± 4.10</td>
<td></td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>72.8 ± 12.4</td>
<td>85.1 ± 8.4</td>
<td>79.7 ± 10.7</td>
<td>81.7 ± 8.4</td>
<td></td>
</tr>
<tr>
<td>Broken eggs (%)</td>
<td>0.6 ± 1.8</td>
<td>0.2 ± 0.6</td>
<td>0.6 ± 1.3</td>
<td>0.9 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

**There were no significant differences between treatments.**
Figure 1a Ileal utilization of P at 300 IU vit. D3/kg.  Figure 1b Ileal utilization of P at 2500 IU vit. D3/kg.

Figure 2a Concentration of Pi (%) in the plasma of laying hens at 300 IU vit. D3/kg.  Figure 2b Concentration of Pi (%) in the plasma of laying hens at 2500 IU vit. D3/kg.

Figure 3a Conc. of 25-OH-D3 in the plasma of laying hens at 300 IU vit. D3/kg.  Figure 3b Conc. of 25-OH-D3 in the plasma of laying hens at 2500 IU vit. D3/kg.

References


GRIMMERSOFT (1995) StatBoxPro, Version 5.0, Manuel d'utilisation

