The bioavailability of zinc from inorganic and organic sources in broiler chickens as affected by addition of phytase

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ABSTRACT

The effect of adding phytase to the diet on the bioavailability of zinc from organic and inorganic sources was studied in an experiment carried out on 560 broiler chickens aged from 4 to 28 days. Phytase was either added (750 FTU/kg) or not to a low-Zn wheat-soyabean diet. Simultaneously, gradient levels of zinc (0, 10, 20 or 40 mg kg) from fodder ZnSO₄ · H₂O or an organic complex of Zn (Zn chelated with amino acids, ZnAA) were added to the diets without and with phytase.

The bioavailability of zinc from ZnAA, calculated using multiple linear regression, was generally higher than from ZnSO₄, especially when the diet was not supplemented with phytase. The relative bioavailability of Zn from ZnAA (Zn from ZnSO₄ – 100%) in phytase-supplemented diets was: 103 (for body weight gain), 104 (for feed conversion), 114 (for Zn content in dried tibiae bones), 114 (for Zn content in tibiae ash), and 109% (for total amount of Zn in tibiae bone). In the diets that were not phytase-supplemented, the corresponding values were: 121, 116, 139, 142 and 117%, respectively.

KEY WORDS: broiler chickens, zinc, phytic acid, phytase

INTRODUCTION

Following reports indicating that trace minerals in organic compounds (bioplexes) have a higher value than inorganic forms, interest in using bioplexes as a source of microelements for farm animals has increased in recent years (Świątkiewicz and Koreleski, 1998).
In a previous experiment (Świątkiewicz et al., 2000), it was found that for broiler chickens, the bioavailability of zinc from an organic complex with amino acids (ZnAA) was higher than from inorganic zinc sulphate (ZnSO₄). The use of ZnAA as a Zn source had a positive effect on broiler performance, Zn balance, and increased the content of this microelement in the tibia.

Phytic acid is a naturally occurring organic complex found in plants. About 50-70% P present in feedstuffs of plant origin used in mixtures for poultry is in phytate form, unavailable for non-ruminant animals (Reddy et al., 1982). Phytic acid, as a reactive anion, forms insoluble salts with zinc and other divalent and trivalent cations (Sebastian et al., 1998). These complexes are indigestible for non-ruminant animals and lower utilization of microelements bound in phytate form (Brink et al., 1991; Sandberg and Svandberg, 1991; Zhou et al., 1992; Fisher and Giroux, 1993). It is well known that use of phytase, an enzyme that hydrolyzes phytate complexes, increases the availability of trace minerals (Sebastian et al., 1998).

The aim of this experiment was to evaluate the bioavailability of zinc from inorganic and organic sources for broiler chickens using diets with and without phytase.

MATERIAL AND METHODS

In the experiment, inorganic, feed-grade ZnSO₄·H₂O, and organic, Zn complex with amino acids (ZnAA) were used as a source of zinc. In the ZnAA preparation, Zn was bound with a total of 17 amino acids (including 10 essential) from a soya-bean protein hydrolyzate.

The experiment was carried out on 560 male broiler Starbro chickens from 4 to 28 days of age. The chickens were caged on a wire-mesh floor, with free access to feed and water. From days 1 to 3 of age the broilers were fed diets not supplemented with zinc or phytase.

At the age of 4 days the chickens were assigned to 14 groups with 5 replicates of 8 birds each. The experimental diets were formulated by supplementing a wheat-soyabean meal mixture (Table 1) with graded levels (0, 10, 20 or 40 mg/kg) of zinc from either ZnSO₄ or ZnAA, with (750 FTU/kg) or without phytase. The diet was formulated to meet the nutritional requirements of chickens.

In the experiment, the weight of the birds and their feed intake were measured. These data were used to calculate body weight gain and feed conversion efficiency. On day 28, four broilers from every replicate were killed by cervical dislocation. Right tibiae bones were taken for chemical analysis (four bones from each subgroup were pooled in one sample). After removing all adhering tissue, the bones were defatted in petroleum ether in a Soxlet apparatus. After wet mineralization (using a mixture of HNO₃, HClO₄ and H₂SO₄), the Zn concentration in the samples
## TABLE 1

### Composition of basal diet, g kg⁻¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>541.4</td>
</tr>
<tr>
<td>Soyabean oilmeal</td>
<td>312</td>
</tr>
<tr>
<td>Rapeseed oilmeal</td>
<td>50</td>
</tr>
<tr>
<td>Rape seed oil</td>
<td>60</td>
</tr>
<tr>
<td>Limestone</td>
<td>11</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>17</td>
</tr>
<tr>
<td>NaCl</td>
<td>3</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.6</td>
</tr>
<tr>
<td>Vitamin-mineral premix (without Zn)*</td>
<td>5</td>
</tr>
</tbody>
</table>

### Content of nutrients in the diet

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein¹</td>
<td>223</td>
</tr>
<tr>
<td>Metabolizable energy², MJ/kg</td>
<td>12.24</td>
</tr>
<tr>
<td>Ca¹</td>
<td>9.2</td>
</tr>
<tr>
<td>Total P¹</td>
<td>8.1</td>
</tr>
<tr>
<td>Phytic P¹</td>
<td>3.5</td>
</tr>
<tr>
<td>Available P¹</td>
<td>4.6</td>
</tr>
<tr>
<td>Zn²</td>
<td>ppm 32</td>
</tr>
<tr>
<td>Zn¹</td>
<td>ppm 44</td>
</tr>
</tbody>
</table>

¹ analyzed
² calculated according to European Table of Energy Values for Poultry Feedstuffs (1989)
³ calculated according to Nutrient Requirements of Poultry (1996)

* supplied per 1 kg of diet: vit. A 12000 IU; vit. D₃ 3000 IU; mg: vit. E 20; vit. K₃ 3; vit. B₃ 2; vit. B₂ 6; vit. B₆ 2; vit. B₉ 0.015; biotin 0.05; folic acid 1; nicotinic acid 20; D-calcium panthotenate 12; choline chloride 200; Mn 65; Cu 6; Fe 40; J 0.5; Se 0.1; Co 0.2

The ash content in bones was analyzed after dry-ashing at 600°C for 6 h.

The nutrient composition of the basal diet was analyzed using standard methods (AOAC, 1990): total phosphorus, calorimetrically by a molybdenum-vanadate method (AOAC, 1990), phytic phosphorus, by the Frübeck et al. method (Frübeck et al., 1995), Ca and Zn, by flame atomic absorption spectrophotometry (AOAC, 1990).

Data were analyzed statistically; the significance of differences between means was determined by Duncan's multiple range test. The relative bioavailability of zinc from both studied sources was calculated using multiple linear regression (Wedekind et al., 1992) taking into account the following parameters: body weight gain, feed conversion, Zn content in dried, defatted tibiae bones, Zn content in tibiae ash, total amount of Zn in tibiae bone. All calculations were done using Statistica 5.0 PL software.
RESULTS

Analyses of performance results for experimental groups (Table 2) indicated significant differences between groups for body weight gains and feed conversion. The lowest body weight gain and worst feed conversion were noted in the group fed the diet without phytase and without added zinc and these values differed (P<0.01) from the results in all other groups. The best performance was found in chickens fed the diet supplemented with phytase and 40 mg/kg Zn from ZnAA. Broilers from different groups had similar feed intake, significant differences were found only between groups I and II (P<0.05).

The data given in Table 2 show that there were no significant differences in body weight gain, feed conversion, and feed intake between chickens fed the diet with addition of zinc in inorganic form (ZnSO\(_4\)) and those receiving the diet with ZnAA. However, in groups supplemented with ZnAA, the birds gained 0.8% more weight and had a 0.8% better feed conversion ratio. The addition of phytase significantly influenced broiler performance (P<0.01). Birds that received the diet enriched with this enzyme had a 2.3% higher body weight gain and 2.1% better feed conversion than birds fed mixture without phytase. Similarly, the level of added Zn had a significant effect on broiler performance (P<0.01). The lowest body weight gain was noted in chickens fed the diet without supplemented Zn, and the addition 10, 20 and 40 mg/kg of this microelement increased body weight by 7.7, 10.5, and 11.4%, respectively. For feed conversion these values were 8.6, 10.7 and 11.8%, respectively. In case of feed intake, no significant effect of the experimental factors (phytase supplement, source of Zn, and level of Zn addition) was found.

There were some significant differences between experimental groups in the zinc content of crude ash in tibiae bones (Table 3). The lowest content of Zn in dried, defatted tibiae bones and in bone ash, total Zn in tibiae, and level of ash in this bone were noted in the group fed the diet without addition of zinc and phytase. Supplementing the enzyme to the diet without adding Zn increased all of the above characteristics by 15.2 (P<0.01), 9.2 (P<0.01), 18.2 (P<0.01) and 5.5% (P<0.05), respectively. Similarly, Zn added in even the lowest amounts, both in the form of ZnSO\(_4\) and ZnAA (groups III and IV), had a positive effect on zinc and ash content in the bones. The highest level of all of the estimated parameters in the tibiae was found in the group fed the diet with addition of phytase and 40 ppm Zn from ZnAA.

The zinc source had no significant effect on the content of analyzed components in tibiae (Table 3). However, the levels of Zn in dried, defatted bones, in tibiae ash, and the total amount of Zn were 1.8, 1.6 and 2.6% higher in chickens fed diets supplemented with ZnAA than with ZnSO\(_4\). Phytase addition increased the level of Zn in dried bones by 4.0%, in tibiae ash by 2.1%, and the total amount
<table>
<thead>
<tr>
<th>Number of group</th>
<th>Phytase addition 750 FTU/kg of diet</th>
<th>Zinc supplement to the diet, mg/kg</th>
<th>Body weight gain, g</th>
<th>Feed conversion kg feed/kg BWG</th>
<th>Feed intake g/bird</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>703&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.94&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1364&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>769&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1412&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt; - 10</td>
<td>779&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1375&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt; - 10</td>
<td>800&lt;sup&gt;c&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.71&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1367&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt; - 20</td>
<td>803&lt;sup&gt;c&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.71&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1371&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt; - 20</td>
<td>816&lt;sup&gt;e&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1369&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt; - 40</td>
<td>810&lt;sup&gt;c&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1365&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt; - 40</td>
<td>822&lt;sup&gt;e&lt;/sup&gt;E</td>
<td>1.66&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1366&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>ZnAA - 10</td>
<td>790&lt;sup&gt;c&lt;/sup&gt;B&lt;sup&gt;C&lt;/sup&gt;D</td>
<td>1.73&lt;sup&gt;abc&lt;/sup&gt;C</td>
<td>1360&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>ZnAA - 10</td>
<td>801&lt;sup&gt;c&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.70&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1365&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>ZnAA - 20</td>
<td>815&lt;sup&gt;c&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1376&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>ZnAA - 20</td>
<td>817&lt;sup&gt;c&lt;/sup&gt;D&lt;sup&gt;E&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;bc&lt;/sup&gt;AB</td>
<td>1368&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>ZnAA - 40</td>
<td>823&lt;sup&gt;e&lt;/sup&gt;E</td>
<td>1.66&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1369&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>ZnAA - 40</td>
<td>823&lt;sup&gt;e&lt;/sup&gt;E</td>
<td>1.66&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1366&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>13.6</td>
<td>0.06</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

Means for main experimental factors
- phytase addition to the diet
  - - 789 1.74 1369 3
  - + 801 1.70 1373 1.5
- zinc source
  - ZnSO<sub>4</sub> 805 1.70 1369 2
  - ZnAA 812 1.69 1367 1
- level of zinc addition, mg/kg
  - 0 736<sup>a</sup>A 1.89<sup>c</sup>C 1388 5
  - 10 792<sup>b</sup>B 1.73<sup>bc</sup>H 1367 2
  - 20 813<sup>c</sup>C 1.69<sup>bc</sup>A 1371 0.5
  - 40 820<sup>c</sup>C 1.68<sup>bc</sup>A 1367 2.5

effect of
- phytase ** ** NS
- source NS NS NS
- level ** ** NS
- interaction ** NS

<sup>a,b,c,d,e,f</sup> - P<0.05; <sup>A,B,C,D,E</sup> - P<0.01; ** - P<0.01; * - P<0.05; NS - P>0.05

1 interactions between following experimental factors: phytase supplement - level of zinc addition (P<0.01) and phytase supplement - zinc source (P<0.01)
2 interactions between following experimental factors: phytase supplement - level of zinc addition (P<0.01) and phytase supplement - zinc source (P<0.01)
### TABLE 3

#### Content of zinc and crude ash in broilers tibiae bones

<table>
<thead>
<tr>
<th>Number of group</th>
<th>Phytase addition 750 FTU/kg of diet</th>
<th>Zinc supplement to the diet mg/kg</th>
<th>Zinc content in dry, fat extracted tibiae bones ppm</th>
<th>Crude ash content in dry, fat extracted tibiae bones %</th>
<th>Content of Zn in crude ash ppm</th>
<th>Total amount of Zn in tibiae bone µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>148A</td>
<td>38.7A</td>
<td>381A</td>
<td>468A</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>170AB</td>
<td>40.8AB</td>
<td>416B</td>
<td>555B</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>ZnSO₄ - 10</td>
<td>182C</td>
<td>41.0BC</td>
<td>444BC</td>
<td>595BC</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>ZnSO₄ - 10</td>
<td>189CD</td>
<td>40.7AB</td>
<td>465AC</td>
<td>658AB</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>ZnSO₄ - 20</td>
<td>187CD</td>
<td>40.8AB</td>
<td>458AC</td>
<td>655AC</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>ZnSO₄ - 20</td>
<td>185C</td>
<td>40.7AB</td>
<td>455AC</td>
<td>721AC</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>ZnSO₄ - 40</td>
<td>185C</td>
<td>40.7AB</td>
<td>454AC</td>
<td>725AC</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>ZnSO₄ - 40</td>
<td>201DE</td>
<td>42.6AC</td>
<td>472AC</td>
<td>774AC</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>ZnAA - 10</td>
<td>188CD</td>
<td>40.2AB</td>
<td>468AC</td>
<td>626AC</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>ZnAA - 10</td>
<td>186C</td>
<td>40.3AB</td>
<td>461AC</td>
<td>653AC</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>ZnAA - 20</td>
<td>192CD</td>
<td>41.6AC</td>
<td>461AC</td>
<td>685AC</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>ZnAA - 20</td>
<td>184C</td>
<td>40.1AB</td>
<td>459AC</td>
<td>733AC</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>ZnAA - 40</td>
<td>191CD</td>
<td>40.8AB</td>
<td>470AC</td>
<td>748AC</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>ZnAA - 40</td>
<td>208E</td>
<td>43.5ED</td>
<td>477BC</td>
<td>791BC</td>
</tr>
</tbody>
</table>

| SEM             | 7.4                                 | 0.9                              | 10.1                                          | 27.5                                          |

Means for main experimental factors:

- **Phytase addition**
  - -
  - +

- **Zinc source**
  - ZnSO₄
  - ZnAA

- **Level of zinc addition, mg/kg**
  - 0
  - 10
  - 20
  - 40

- **Effect of**
  - Phytase
  - Zinc source
  - Level
  - Interaction

- **SEM**
  - 7.4

- **Means**
  - 40.5
  - 41.3
  - 41.1
  - 41.1

- **Crude ash content**
  - 449
  - 458
  - 458
  - 466

- **Total amount of Zn**
  - 643
  - 698
  - 688
  - 706

- **SEM**
  - 27.5

- **Phytase addition**
  - NS
  - *P<0.05
  - **P<0.01

- **Zinc source**
  - NS
  - NS
  - NS

- **Level**
  - NS
  - NS
  - NS

- **Interaction**
  - NS
  - NS
  - NS

---

Interactions between following experimental factors:

- Phytase addition - Level of zinc addition (P<0.01)
- Phytase addition - Zn source (P<0.05)
- Phytase addition - Level of zinc addition (P<0.05)

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Significance levels:

- *P<0.05
- **P<0.01
- NS - P<0.05

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Notes:

- a, b, c, d, e, f, g, h, i - P<0.05
- A, B, C, D, E, F, G, H - P<0.01

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*Interpretation and analysis should be based on the statistical significance levels presented.*
of Zn in tibiae by 8.5% (P<0.01). The level of zinc supplemented to the diet had a significant effect on these characteristics. The addition of 10, 20 and 40 ppm of Zn, in comparison with groups not supplemented with zinc, increased; the zinc content in tibiae rose by 17.3, 17.8 and 23.6%; the level of crude ash by 2.0, 2.5 and 5.3%; the Zn content in crude ash by 15.0, 14.8 and 17.3%, and total amount of zinc in tibiae bone by 23.7, 36.5 and 48.4%, respectively.

The relative bioavailability of zinc from ZnAA measured by all of the evaluated parameters was higher than from ZnSO$_4$, especially when chickens were fed the diet without added phytase (Table 4). The bioavailability of ZnAA was 21 and 3% higher than that of ZnSO$_4$ for body weight gains (in diets without and with phytase, respectively); feed conversion, 16 and 4%; Zn content in tibiae, 39 and 14%; Zn level in crude ash, 42 and 14%; total amount of this microelement in the tibia, 17 and 9%, respectively.

**DISCUSSION**

The results reported in this paper confirm the conclusion based on the results of an earlier experiment (Świątkiewicz et al., 2000) that supplementing diets with a higher level of zinc has a positive effect on chicken performance and Zn content in tibiae. In both experiments, the highest body weight gain and the best feed conversion ratio were noted in chickens fed the diet with the highest zinc level (40 ppm). Similarly as in the investigation cited above, the present results indicate that the organic zinc complex with amino acids has a higher feeding value for broiler chickens in comparison with an inorganic source (ZnSO$_4$).

The main aim of this paper was to evaluate the effect of phytic acid on utilization of zinc in broiler chicken diets supplemented with organic and inorganic sources of this element. The properties of phytic acid as an antinutritive factor, which can decrease the utilization of microelements, including Zn, in monogastric animals have been known from many years (O'Dell and Savage, 1960; Likuski and Forbes, 1964; O'Dell et al., 1964; Oberleas et al., 1966). Also in this experiment, the negative effect of phytic acid on utilization of zinc was seen. The addition of phytase, an enzyme hydrolyzing phytic acid to inositol and inorganic phosphates, releasing microelements from phytates, significantly improved body weight gain, feed conversion ratio and increased the Zn content in tibiae. The positive effect of phytase was more pronounced when chickens were fed the diet with a low level of zinc (without Zn supplementation). In this case phytase increased body weight gain by 9.4% and improved feed conversion by 5.3%. With the graded increase of dietary Zn supplementation this tendency declined.

The results reported in this paper are similar to those of Yi et al. (1996). They observed that adding phytase to a low-zinc diet, through improvement of zinc availa-
TABLE 4

Relative bioavailability of zinc from ZnAA in comparison to ZnSO₄, calculated using broilers performance and content of Zn in tibiae bones (multiple linear regression)

<table>
<thead>
<tr>
<th>Zinc source</th>
<th>Body weight gain</th>
<th>Feed conversion</th>
<th>Zn content in dry, fat extracted tibiae bones</th>
<th>Zn content in tibiae bones ash</th>
<th>Total amount of Zn in tibiae bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>phytase addition</td>
<td>phytase addition</td>
<td>phytase addition</td>
<td>phytase addition</td>
<td>phytase addition</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ZnAA</td>
<td>121</td>
<td>103</td>
<td>116</td>
<td>114</td>
<td>117</td>
</tr>
</tbody>
</table>

Multiple linear regression equations used for above calculations:

Body weight gain (Y) without phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 747.04 + 1.3887 (+/-0.267196)X₁ + 1.6780 (+/-0.266278)X₂, \quad R = 0.75 \]

Body weight gain (Y) with phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 785.08 + 0.7919 (+/-0.188826)X₁ + 0.8156 (+/-0.188876)X₂, \quad R = 0.63 \]

Feed conversion (Y) without phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 1.836741 - 0.003272 (+/-0.000621)X₁ - 0.003794 (+/-0.000619)X₂, \quad R = 0.74 \]

Feed conversion (Y) with phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 1.767259 - 0.002282 (+/-0.000485)X₁ - 0.002367 (+/-0.000485)X₂, \quad R = 0.68 \]

Content of Zn in dry, fat extracted tibiae bones (Y) without phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 169.27 + 0.3820 (+/-0.132384)X₁ + 0.5315 (+/-0.131929)X₂, \quad R = 0.56 \]

Content of Zn in dry, fat extracted tibiae bones (Y) with phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 174.50 + 0.4967 (+/-0.096755)X₁ + 0.5681 (+/-0.096781)X₂, \quad R = 0.73 \]

Content of Zn in tibiae bones ash (Y) without phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 423.43 + 0.7464 (+/-0.275973)X₁ + 1.0570 (+/-0.274845)X₂, \quad R = 0.53 \]

Content of Zn in tibiae bones ash (Y) with phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 438.10 + 0.6844 (+/-0.216557)X₁ + 0.7838 (+/-0.216615)X₂, \quad R = 0.53 \]

Total amount of Zn in tibiae bone (Y) without phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 530.65 + 3.7957 (+/-0.42349)X₁ + 4.4188 (+/-0.422034)X₂, \quad R = 0.89 \]

Total amount of Zn in tibiae bone (Y) with phytase in the mixture, for supplemental Zn intake from ZnSO₄(X₁) and ZnAA(X₂):
\[ Y = 596.50 + 3.5523 (+/-0.398756)X₁ + 3.8703 (+/-0.398862)X₂, \quad R = 0.88 \]
bility, had a positive effect on body weight gain and feed intake and increased the Zn content in chick tibiae bones. It was found that in the range that phytase was added (150-600 units/kg), every 100 units of this enzyme released about 0.9 mg of Zn from 1 kg of diet. Biehl et al. (1995) showed that addition of 1200 units of phytase/kg diet containing 41 ppm Zn, increased body weight gain of chickens by about 20% and raised the level of Zn in tibiae bones by 55%. When a low-Zn diet was used (13 ppm of Zn), phytase improved body weight gains more effectively (by 25%).

In contrast with the presented results, Mohanna and Nys (1999) did not observe any effect of phytase supplementation of a maize-soyabean mixture (37 ppm of Zn) on the performance of chickens from 5 to 21 days of life. This lack of response to phytase in broiler growth probably resulted from a lower level of phytic acid in the diet (0.67-1.10%) in comparison with the present experiment (1.24%). However, in agreement with the described study, the authors cited above, using Zn content in tibiae bones as a criterion, demonstrated that hydrolysis of phytate by phytase had positive effect on Zn availability when its level was lower than 60 ppm. When zinc was added at a higher level, the positive influence of phytase markedly declined.

It has been reported that the molar ratio of phytic acid:Zn in the diet is a more precise index for evaluation of Zn availability than only the phytic acid content (Morris and Ellis, 1980; Lo et al., 1981; Oberleas and Harland, 1981). If the value of above ratio exceeds 15-20, Zn retention could be decreased and negative effects of Zn deficiency on growth and metabolic processes in monogastric animals can develop (Oberleas and Harland, 1981). Further experiments showed that another accurate index for evaluation of Zn availability is the molar ratio of phytic acid x Ca:Zn (Fordyce et al., 1987); the value of this index should not be higher than 3.5.

In our experiment, the molar ratios of phytic acid:Zn and phytic acid x Ca:Zn in the basal diet (without added zinc) were 27.8 and 6.4, respectively. Both values markedly exceeded critical values, above which symptoms of zinc deficiency can be observed. This explains the significant positive reaction in body weight gain, feed conversion and Zn content in the tibiae of chickens after adding phytase as well as zinc to the diet. Both of these supplements decreased molar ratios, phytase by hydrolysis of phytate bonds, zinc by increasing the total amount of Zn in the diet. Addition of 10, 20 and 40 ppm of Zn decreased the molar ratio of phytic acid:Zn to 22.7, 19.1, 14.6 and phytic acid x Ca:Zn to 5.2, 4.4 and 3.4, respectively. This was probably the reason for the lower positive effect of the phytase supplement plus higher zinc supplementation on zinc availability.

In Tables 2 and 3 it is shown that there were significant interactions between the addition of phytase and the form of supplemental zinc in body weight gain, feed conversion ratio and Zn content in the tibiae of chickens. The relative Zn bioavailability from organic and inorganic forms (Table 4) confirm these interactions – in diets not supplemented with phytase, the bioavailability of ZnAA was
markedly higher than that of ZnSO₄. Addition of phytase to the diet significantly decreased the relative bioavailability Zn from ZnAA (in comparison to ZnSO₄). These results pointed out that the main reason for the higher nutritive value of organic than inorganic Zn compounds is that microelements in organic forms are considerably better protected from forming indigestible complexes with phytic acid (phytates) than those in inorganic form.

CONCLUSIONS

It can be concluded that supplementation of the diet with zinc positively affects production parameters and Zn content in the tibiae of broiler chickens. Supplementation of the diet with phytase, an enzyme hydrolyzing phytate bonds, also improves broiler performance and increased the Zn content in tibiae. The bioavailability of Zn from ZnAA is higher than from ZnSO₄, especially when the diet is not supplemented with phytase.

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STRESZCZENIE

Wpływ fitazy na dostępność cynku ze związków nieorganicznych i organicznych u kurcząt brojlerów

W doświadczeniu wykonanym na 560 kurczątach brojlerach, od 4 do 28 dnia życia, badano wpływ dodatku fitazy do paszy na bioprzyswajalność cynku ze źródeł nieorganicznych i organicznych. Do mieszanki paszowej o małej zawartości Zn (z premiksem bez Zn) nie dodawano lub dodawano fitazę w ilości 750 jednostek aktywności (FTU)/kg. Jednocześnie do obydwóch rodzajów dawek dodano 0, 10, 20 lub 40 ppm Zn w postaci ZnSO₄·H₂O lub organicznego kompleksu Zn z aminokwasami (ZnAA).

Bioprzyswajalność Zn z ZnAA, liczona przy użyciu równania wielokrotnej regresji liniowej, była wyższa niż ZnSO₄, szczególnie gdy nie stosowano dodatku fitazy. Względna bioprzyswajalność Zn z ZnAA (ZnSO₄ - 100%) w mieszankach z udziałem fitazy wynosiła, %: 103 (dla przyrostów masy ciała), 104 (dla wykorzystania paszy), 114 (dla zawartości Zn w wysuszonych kościach piszczałowych), 114 (dla poziomu Zn w popielu kości piszczałowych) i 109 (dla całkowitej ilości Zn w kości piszczałowej). W przypadku kiedy nie wprowadzono fitazy do mieszanki paszowej odpowiednie wartości wyniosły: 121, 116, 139, 142 i 117%.