Effects of Supplemental Zinc on Growth, Serum Glucose, Cholesterol, Enzymes and Minerals in Broilers

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Abstract: The effects of zinc supplementation on growth and serum parameters were investigated in Ross PM3 broiler chicks. 60 day old chicks were assigned to four treatments of 0, 20, 40, 80 ppm supplemental zinc. Blood from wing veins was analyzed for serum glucose, cholesterol, enzymes and minerals. Zinc supplementation had no significant effect on live weight and 80 ppm zinc lowered the serum glucose concentrations. Cholesterol levels slightly decreased in zinc supplemented groups. Serum alanine aminotransferase activity remained unchanged but aspartate aminotransferase activity was increased and alkaline phosphatase activity was decreased in zinc supplemented chicks. Gamma-glutamyltransferase activity was also decreased in all supplemented groups. Significant reductions occurred in serum calcium, magnesium and inorganic phosphorus levels. None of the supplemental zinc levels had significant effect on iron and copper concentrations.

Key words: Zinc, growth, serum parameters, broilers

Introduction
It is well documented that zinc (Zn) is an essential trace element for the growth and development of plants, animals and human being, needed for various physiological functions including bone formation, host defence, sexual maturity, reproduction and tissue growth (McDowell, 1992). Zinc is commonly added to all formulated animal diets. Mohanna and Nys (1996) reported that under normal commercial dietary condition, 94 % of the ingested zinc is excreted due to high amount of zinc ingested and low utilization of the element. They also indicated the risk of soil phytotoxicity, which may result from manure of poultry that contain the large amounts of zinc higher than plant requirements. Although the zinc requirement of chicks varies depending upon the nutrient contents as well as protein sources in the diet, Dewar and Downie (1984) reported that the zinc requirement of broiler for maximal live weight was 18 mg/kg diet, and 24 mg/kg diet for maximal zinc concentration in blood serum. It was reported that 40 mg Zn/kg diet is optimal for growth of chicks (McDowell, 1992). Using data from a single sampling time to determine the effects of supplementation may lead to disparity. This study was designed to investigate the effects of the moderate levels of supplemental zinc on growth and serum constituents and interaction of zinc with some minerals in broilers on practical diet at repeated sampling times.

Materials and Methods
Sixty day old Ross PM3 broiler chicks were assigned to four treatments. Chicks, 15 in each group, were kept in separated pens on floor and maintained on a 24 h constant light schedule. Stainless-steel feeders and plastic waterers were used. Chicks received 0 (control), 20, 40 and 80 ppm supplemental zinc in reagent grade of ZnSO₄·7H₂O (Berkö Ilac ve Kimya San. Ltd. Şti., İstanbul, Turkey) in drinking water containing 0.048 ppm zinc and fed on commercial diet (Table I) containing 108 mgZn/kg. Body weight of chicks was measured and blood was collected from wing veins at days 20 and 35. Sera were stored at -20°C until analysis. after separation by centrifugation at 3000 rpm for 10 minutes following one hour incubation at 37°C.

Serum glucose, cholesterol, calcium (Ca), inorganic phosphorus (P), magnesium (Mg) concentrations and aspartate aminotransferase (AST), alanine aminotransferase (ALT) gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) activities were determined spectrophotometrically using commercial kits. Serum iron concentrations were measured using Beckman 2100 A atomic absorption spectrophotometer (AAS) as described by Fairbanks and Klee (1994) and serum copper (Anonymous, 1983), water and diet zinc concentrations (AOAC, 1998) were determined by SP9 Series Pye Unicam AAS. Statistical analyses were performed using SPSS 9.0 software (Anonymous, 1998). The data were subjected to variance analysis (General Linear Model). When significant F values were obtained, comparisons were made by New Duncan's multiple range test (DMR) and all data were expressed as mean ± SEM.

Results
The results are summarised in Table 2. Zinc supplementation had no significant effect on live weight throughout the experiment, however it was slightly increased in all treatment groups on day 35. Serum glucose concentrations did not change in 20 and 40 ppm zinc supplemented groups at both sampling times, but 80 ppm zinc supplementation significantly lowered (p<0.001) serum glucose concentration throughout the experiment. Serum cholesterol levels slightly decreased in all treatment groups at both sampling times, but a significant decrease (p<0.05) was observed only in 40 ppm zinc supplemented group on day 35. Zinc supplementation had no significant effect on serum ALT activity. On the other hand there was an increase in AST activity (p<0.05) and decrease in ALP activity (p<0.05 in 20 ppm zinc supplemented group at the first sampling time but no significant differences was observed thereafter. All levels of additional zinc reduced the serum GGT activity overall the experiment. The magnitude of the reduction was higher (p<0.001) at the first sampling time than the second one (p<0.05). Significant reductions occurred in serum calcium (p<0.01) and magnesium (p<0.001) concentrations on day 20, but a fluctuation in results was observed on day 35. Zinc supplementation did not influence the inorganic phosphorus...
Table 1: Ingredients of diet (0, 20, 40 and 80 ppm Zinc supplemented) fed to Broilers

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
<th>Calculated Nutritional Values</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>55.562</td>
<td>Crude protein</td>
<td>20.500</td>
</tr>
<tr>
<td>Full fat soya</td>
<td>20.000</td>
<td>Digestible crude protein</td>
<td>15.500</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>12.710</td>
<td>ME (kcal/kg)</td>
<td>3000.018</td>
</tr>
<tr>
<td>Meat-bone meal</td>
<td>5.354</td>
<td>Crude cellulose</td>
<td>3.655</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>3.000</td>
<td>Crude fat</td>
<td>7.813</td>
</tr>
<tr>
<td>Fish oil</td>
<td>1.093</td>
<td>Crude ash</td>
<td>6.910</td>
</tr>
<tr>
<td>Fish meal</td>
<td>1.000</td>
<td>Ca</td>
<td>1.000</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>0.500</td>
<td></td>
<td>0.708</td>
</tr>
<tr>
<td>Salt</td>
<td>0.350</td>
<td>Digestible P</td>
<td>0.334</td>
</tr>
<tr>
<td>Vitamin-mineral mix*</td>
<td>0.250</td>
<td>Methionine</td>
<td>0.440</td>
</tr>
<tr>
<td>Antibacterial-anticoccidial</td>
<td>0.100</td>
<td>Sine</td>
<td>0.367</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.083</td>
<td>Lysine</td>
<td>1.306</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>Na</td>
<td>2.026</td>
</tr>
</tbody>
</table>

**Analyzed Nutritional Values**

| Crude protein | 21.27  |
| Crude cellulose| 5.06   |
| Crude ash     | 6.30   |
| Ca            | 1.00   |
| P             | 1.20   |
| Zn (mg/kg)    | 1.09  |

* Vitamin-mineral premix provided per kg of the diet: vitamin A, 15,000 IU; vitamin D3, 2000 IU; vitamin E, 20 mg; vitamin K3, 5 mg; vitamin B1, 2.5 mg; vitamin B2, 7.5 mg; vitamin B6, 5 mg; vitamin B12, 0.002 mg; folic acid, 0.75 mg; calcium pantothenate, 10 mg; ascorbic acid, 50 mg; monosodium phosphate, 100 mg; choline chloride, 400 mg; niacinamide, 25 mg; D-biotin, 0.06 mg; manganese, 50 mg; iron, 40 mg; zinc, 80 mg; copper, 5 mg; iodine, 0.4 mg; selenium, 0.15 mg; colidat 0.1 mg; antioxidants, 10 mg.

Table 2: Mean values of live weight and serum parameters of 0, 20, 40 and 80 ppm Zinc supplemented Broilers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Supplemental Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>and sampling days</td>
</tr>
<tr>
<td>Live Weight (g)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>466.4±7.42</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>185.7±4.36</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>131.6±6.33</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>128.67±7.19</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>10.9±3.08</td>
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<tr>
<td>GGT (U/L)</td>
<td>20</td>
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<td></td>
<td>n</td>
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<td></td>
<td>15</td>
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<tr>
<td></td>
<td>28.47±2.72</td>
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<tr>
<td>ALP (U/L)</td>
<td>20</td>
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<td></td>
<td>n</td>
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<tr>
<td></td>
<td>15</td>
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<tr>
<td></td>
<td>34.18±5.10</td>
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<tr>
<td>Calcium (mg/dl)</td>
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</tr>
<tr>
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<td>n</td>
</tr>
<tr>
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<td>15</td>
</tr>
<tr>
<td></td>
<td>9.23±1.57</td>
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<tr>
<td>Phosphorus (mg/dl)</td>
<td>20</td>
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<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>11.80±0.80</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>14.62±0.93</td>
</tr>
<tr>
<td>Ibron (µg/ml)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.26±0.32</td>
</tr>
<tr>
<td>Copper (µg/ml)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.31±0.08</td>
</tr>
</tbody>
</table>

Mean values in the same row with different superscripts differ significantly. * p<0.05, ** p<0.01, *** p<0.001.
concentrations at the first sampling time however, a
decrease (p < 0.01) was determined on day 35. None of
the supplemental zinc levels had significant effect on
serum iron and copper concentrations.

**Discussion**

Mohanna and Nys (1999) reported increased body weight gain
and food intake until the total dietary zinc content raised to 46
mg/kg and they found no further responses at higher zinc
concentrations. In the present study, chicks in control or
treatment groups were neither depleted before the experiment
nor raised on purified or semi-purified diet and zinc intake of
the chicks was highly over the recommended level of 40 mg
Zn/kg diet for broiler chicks even in the lowest supplemental
Zinc level. It is possible that chicks, which are depleted or
on purified and semi-purified diets may well respond to zinc
supplementation. Cao et al. (2000) reported decreased feed
intake and daily weight gain in chicks given 600 mg Zn/kg
while dietary zinc concentration up to 400 mg/kg had no
effect on food intake and growth. In the present experiment, lowered glucose level may either partly result from the depressed pancreatic enzyme activities by excess zinc or increased zinc uptake of pancreas with dietary zinc concentration (Lü and Combs, 1988) because of the putative effect of zinc on insulin metabolism (Keen and
Graham, 1989) indicating the increased glucose utilization.
The relationship between dietary zinc and plasma cholesterol
homeostasis is not well characterized. Zinc supplementation
did not influence serum cholesterol in chicks (Lü and
Combs, 1988). In contrast, reduced serum total cholesterol
concentrations in the present study was in agreement with the
work of Boukaiba et al. (1993). Rupic et al. (1997) found a
decrease in the activity of both AST and ALT in zinc depleted
pigs but observed no correlation between serum zinc. In the
present study, the AST activity increased significantly in only
20 ppm zinc supplemented group on day 20, while ALT
activity was not elevated, as stated in the study of Lü and
Combs (1988), which indicates no liver damage. In contrast
to these findings, decreased GGT activity in all zinc
supplemented chicks with the most pronounced decreases on
the first sampling day is consistent with the findings of Rupic
et al. (1997) who reported a negative correlation between
GGT activity and serum zinc level. A study conducted in our
laboratory (Uyanik et al., 2000) showed that serum zinc levels
were higher in 20 and 40 ppm zinc supplemented chicks.
Mohanna and Nys (1999) found no effects of 10, 25 or 40
mg/kg supplemental zinc as sulphate on ALP activity as in this
study, which confirms that a severe zinc deficiency is
necessary to affect the ALP activity (Mohanna and Nys,
1999).

Zinc homeostasis is regulated by a zinc binding protein,
metallothionein (high zinc intake induce intestinal and liver
metallothionein synthesis (Cao et al., 2000) which is
associated with zinc absorption. The reductions in serum
calcium, inorganic phosphorus and magnesium concentrations
in this experiment may be due to the diminished absorption of
these elements because of the competition for similar binding
sites (Prasad, 1985) or incorporation of them into skeletal
tissue or both.

High levels of zinc intake depresses copper absorption and iron
uptake (Underwood, 1977). Tissue contents of copper and
magnesium (Brcich and Sullivan, 1976) were affected by
deficiency, tissue iron and copper (Gibson et al., 1986) were
affected by excess zinc in chickens. In contrast, zinc
supplementation had no significant effect on serum copper
and iron levels in this study. In chick studies, Stahl et al.
(1989) showed that moderate amounts of zinc (100 mg
Zn/kg) had no effect on soft tissue accumulation of copper
and iron. In conclusion, since moderate levels of zinc
supplementation did not improve growth but altered some
serum parameters, no further zinc supplementation is of value
in broilers under the practical dietary conditions.

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