A total of 280 unsexed broiler chicks were distributed randomly in seven dietary treatments (D). The diets were D1: basal diet + mineral mixture without Zn; D2: D1 + 15ppm inorganic Zn; D3: D1 + 15ppm organic Zn; D4: D1 + 7.5ppm organic Zn; D5: D1 + 3.0ppm nano Zn; D6: D1 + 0.06ppm nano Zn; D7: D1 + 0.03ppm nano Zn. Blood samples were collected from four birds of each group on 21st and 42nd day of the study. The different serum biochemical indices were spectrophotometrically measured using the chemical kits prepared by Crest Biosystems. The lowest serum cholesterol was found in group D3 which differed significantly (P<0.05) from D1 and D2. The highest level of SGPT was found in D7 which varied significantly (P<0.05) from D1, D2 and D3 and the lowest level of SGPT was observed in group D4 which varied significantly (P<0.05) with all the other treatments except D2. The highest average level of ALP was observed in treatment D5 which varied significantly (P<0.05) with all the other treatments except D2 whereas its lowest level was observed to be present in D1 which was significantly (P<0.05) different from all the remaining dietary groups. Organic and nano zinc supplemented @ 15 ppm and 0.06 ppm, respectively to the basal diet altered the serum cholesterol, SGPT and ALP levels significantly (P<0.05).

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MATERIALS AND METHODS

A total number of two hundred eighty day old unsexed broiler chicks were weighed, wing banded and distributed randomly into seven dietary treatment groups with two replicates having 20 chicks in each replicate. Seven dietary treatments (D) were: D1: basal diet + mineral mixture without any zinc; D2: D1 + 50% of Zn present in basal diet through inorganic zinc (ZnSO4) [15ppm]; D3: D1 + 50% of Zn present in basal diet through organic zinc (Zn-Met) [15ppm]; D4: D1 + 25% of Zn present in basal diet through organic zinc (Zn-Met) [7.5ppm]; D5: D1 + nano ZnO @ 1/1000th of Zn content in basal diet [0.33ppm]; D6: D1 + nano ZnO @ 1/500th of Zn content in basal diet [0.06ppm]; D7: D1 + nano ZnO @ 1/1000th of Zn content in basal diet [0.03ppm]. Basal diet for broiler starter and finisher was prepared as per BIS (1992) specification. The broilers were reared in deep litter system and fed with ad libitum feed and water for 42 days. Experimental feed samples were analyzed for proximate composition according to the AOAC (Association of official Analytical Chemists, 2000). The ingredient and proximate composition of the experimental basal diet for the birds is presented in Table 1. Zinc concentration of the basal diet was estimated to be 30 ppm by atomic absorption spectrophotometer.

Blood samples were collected from four birds of each dietary group on 21st and 42nd day of the experiment. Birds were bled by wing vein puncture using sterile syringes and needles and 3 ml of blood was collected using a 24 gauge needle with different blood metabolites in broiler chickens.

INTRODUCTION

Zinc (Zn) is an indispensable trace mineral for the over all functioning of the animal body (McDowell, L.R., 2003). It influences the immune system, nucleic acid synthesis, cell proliferation, protein synthesis, and enzymic activities in the living system (Ferreira et al., 2002). NRC (1994) estimated the Zn requirement for broiler chickens to be 40 mg/kg in the diet. Supplementation of Zn in its inorganic or chelated form marginally improved the cholesterol, glucose, albumin and total protein levels whereas significantly reduced the level of SGOT and SGPT (Shamsudeen, P. and Shrivastava, H. P., 2013). Herzig et al. (2009) also observed a significant decrease of plasma cholesterol when broilers were fed with high amounts of zinc in diet. In recent years, the use of nano-scale materials as nanoparticles of oxidized elements like zinc oxide nanoparticles (ZnO NPs) are being used in different fields of applied sciences (Handy et al., 2008). ZnO NPs can be effectively used as feed additive in the poultry diets. Due to their extreme small size and unique physical properties, the nanoparticles are likely to be different when compared to the inorganic and organic forms of zinc. Nanoparticles can effectively fulfill the mineral requirements in the animal body, promote growth rate and feed efficiency (Oberdörster, G. et al., 2005). As a feed additive, these are expected to have better bioavailability, small dose rate and stable interaction with other components.

The aim of this comparative study was to evaluate the effect of inorganic, organic and nano zinc supplemented diets on different blood metabolites in broiler chickens.
minimum disturbance. The blood samples after collection were transferred into sterile tubes without any addition of anticoagulant and kept for 3 hours in slanting position. Samples were centrifuged at 5000 rpm for 10 minutes at 4°C. Sera were collected by one ml auto-pipette. The collected sera samples were stored in deep freeze at -20°C in properly capped and labeled tubes for serum biochemical studies. Biochemical parameters viz., glucose, cholesterol, triglyceric acid, total protein, albumin (A), globulin (G), A/G ratio, urea, Blood urea nitrogen (BUN), Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxalate transaminase (SGOT), Alkaline phosphatase (ALP), calcium and phosphorus were estimated by atomic absorption spectrophotometry using the kits prepared by Crest Biosystems.

A significant variation in the serum cholesterol, SGPT and ALP levels of all the treated groups of broiler birds showed no significant variations. Parák and Straková (2011) reported non-significant levels of serum glucose, serum calcium and phosphorus while comparing feeding of inorganic with organic zinc in breeding cocks. Idowu et al. (2011) also reported that serum glucose levels between control and zinc-proteinate groups did not differ significantly. An experiment on feeding of organic zinc in broilers showed non-significant variation in serum albumin level Feng et al. (2010). In contrast to these results, Al-Daraji and Amen (2011) reported significantly (P<0.05) higher levels of serum cholesterol, serum calcium and phosphorus on increasing zinc concentration in the diet by addition of 100 mg pure zinc/ kg of diet than that of control (basal diet) in broiler breeders from 58-66 weeks of age.

In our study, the serum cholesterol, SGPT and ALP levels of all the treated groups of broiler birds differed significantly (P<0.05). A significant variation in the serum cholesterol level of broiler birds was found in case of zinc supplemented groups than the control. Herzig et al. (2009) proved that there was a significant decrease of plasma cholesterol when broilers were fed with high amounts of zinc in diet. It was also observed by Parák and Straková (2011) while comparing feeding of inorganic with organic zinc in breeding cocks. The plasma cholesterol levels were influenced by ZnO supplementation when fed 80 mg/day, either alone or in combination with copper or vitamins (Gensler et al., 2002). Zinc showed an antiatherogenic effect in hypercholesterolemic rabbits (Ren et al., 2006; Rashtchizadeh et al., 2008). Mice with zinc-deficient diets exhibited increased cholesterol and triglycerides levels in their blood plasma (Reiterer et al., 2005). Bolkent et al. (2006) proved the protective effect of zinc supplementation on lipid metabolism indices in laboratory rats. Zinc deficiency caused increased plasma lipid levels in LDL recipient mice. Our results along with the different research findings confirm the positive impact of zinc on lipid metabolism. Aksu et al. (2010) also reported decreased total and LDL cholesterol, and increased HDL cholesterol in the blood plasma of chickens, when the feed mixtures were supplemented with organic complexes of zinc, copper and manganese. However, Kucuk et al. (2008) reported no significant changes in the total cholesterol, triglycerides and glucose levels when supplementing 30 mg of Zn per 1 kg of a feed mixture. The serum SGPT level of organic zinc fed birds were found to be significantly higher than the inorganic zinc fed and control groups.

A significant difference in SGPT level was found in birds of D1 and D6, fed with two different levels of Zn-Met. Osman and Ragab (2007) also observed significant effects (P<0.05) of Zn-Met supplemented @ 30 ppm, 40 ppm and 50 ppm on serum activity of SGPT, SGOT and ALP.

### RESULTS

Serum biochemical parameters viz., glucose, cholesterol, triglyceric acid, total protein, albumin, globulin, urea, BUN, SGPT, SGOT, ALP, calcium and phosphorus of six week old broiler birds are presented in the Table 2. The levels of glucose, triglyceric acid, total protein, urea, albumin, globulin, BUN, SGPT, Ca and P in blood serum of the broiler birds varied insignificantly (P>0.05) between the treatments whereas, cholesterol, SGPT and ALP levels in the blood serum showed significant (P<0.05) differences. Graph 1. shows significant variations (P<0.05) in the serum cholesterol, SGPT and ALP profiles of broiler birds under different dietary treatments The highest level of serum cholesterol was observed in group D1 (98.27 ± 4.73 mg/dl) and it was found to be significantly different (P<0.05) from D2, D3 and D6. The lowest serum cholesterol was observed in group D6 (82.53 ± 4.69 mg/dl) which was observed to be differed significantly (P<0.05) from D5 and D8. The highest level of SGPT was found in treatment D1 (15.55 ± 1.34 U/L) which varied significantly (P<0.05) from D1, D2 and D6. The lowest level of SGPT was observed in group D1 (8.09 ± 0.79 U/L) which varied significantly (P<0.05) with all the other treatments except D3. The highest average level of ALP was observed in treatment D1 (134.21 ± 5.30 U/L) which varied significantly (P<0.05) with all the other treatments except D1 whereas its lowest level was observed to be present in treatment D6 which was significantly (P<0.05) different from all the remaining treatment groups.

### DISCUSSION

The serum glucose, total protein, urea, blood urea nitrogen, albumin, globulin, albumin and globulin ratio, SGOT, calcium and phosphorus levels of all the treated groups of broiler birds showed no significant variations. Parák and Straková (2011) reported non-significant levels of serum glucose, serum calcium and phosphorus while comparing feeding of inorganic with organic zinc in breeding cocks. Idowu et al. (2011) also reported that serum glucose levels between control and zinc-proteinate groups did not differ significantly. An experiment on feeding of organic zinc in broilers showed non-significant variation in serum albumin level Feng et al. (2010). In contrast to these results, Al-Daraji and Amen (2011) reported significantly (P<0.05) higher levels of serum cholesterol, serum calcium and phosphorus on increasing zinc concentration in the diet by addition of 100 mg pure zinc/ kg of diet than that of control (basal diet) in broiler breeders from 58-66 weeks of age.

In our study, the serum cholesterol, SGPT and ALP levels of all the treated groups of broiler birds differed significantly (P<0.05). A significant variation in the serum cholesterol level of broiler birds was found in case of zinc supplemented groups than the control. Herzig et al. (2009) proved that there was a significant decrease of plasma cholesterol when broilers were fed with high amounts of zinc in diet. It was also observed by Parák and Straková (2011) while comparing feeding of inorganic with organic zinc in breeding cocks. The plasma cholesterol levels were influenced by ZnO supplementation when fed 80 mg/day, either alone or in combination with copper or vitamins (Gensler et al., 2002). Zinc showed an antiatherogenic effect in hypercholesterolemic rabbits (Ren et al., 2006; Rashtchizadeh et al., 2008). Mice with zinc-deficient diets exhibited increased cholesterol and triglycerides levels in their blood plasma (Reiterer et al., 2005). Bolkent et al. (2006) proved the protective effect of zinc supplementation on lipid metabolism indices in laboratory rats. Zinc deficiency caused increased plasma lipid levels in LDL recipient mice. Our results along with the different research findings confirm the positive impact of zinc on lipid metabolism. Aksu et al. (2010) also reported decreased total and LDL cholesterol, and increased HDL cholesterol in the blood plasma of chickens, when the feed mixtures were supplemented with organic complexes of zinc, copper and manganese. However, Kucuk et al. (2008) reported no significant changes in the total cholesterol, triglycerides and glucose levels when supplementing 30 mg of Zn per 1 kg of a feed mixture. The serum SGPT level of organic zinc fed birds were found to be significantly higher than the inorganic zinc fed and control groups.

A significant difference in SGPT level was found in birds of D1 and D6, fed with two different levels of Zn-Met. Osman and Ragab (2007) also observed significant effects (P<0.05) of Zn-Met supplemented @ 30 ppm, 40 ppm and 50 ppm on serum activity of SGPT, SGOT and ALP.
Serum biochemical profile of the broiler birds under different dietary treatments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>256.29±15.37</td>
<td>250.95±4.75</td>
<td>244.60±4.91</td>
<td>252.70±9.02</td>
<td>247.01±5.61</td>
<td>241.26±8.41</td>
<td>253.18±5.89</td>
<td>0.877</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>98.27±4.73</td>
<td>89.09±3.48</td>
<td>83.06±1.92</td>
<td>89.84±2.52</td>
<td>83.65±1.44</td>
<td>82.53±4.69</td>
<td>93.71±2.24</td>
<td>0.017</td>
</tr>
<tr>
<td>Triglyceric acid (mg/dl)</td>
<td>69.33±1.70</td>
<td>65.90±2.87</td>
<td>62.45±1.93</td>
<td>67.53±2.45</td>
<td>64.75±3.10</td>
<td>63.10±3.38</td>
<td>67.53±4.06</td>
<td>0.609</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>2.68±0.16</td>
<td>2.76±0.05</td>
<td>3.15±0.20</td>
<td>3.10±0.22</td>
<td>2.95±0.06</td>
<td>3.20±0.06</td>
<td>3.03±0.09</td>
<td>0.996</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.80±0.04</td>
<td>1.87±0.09</td>
<td>1.90±0.07</td>
<td>2.13±0.08</td>
<td>1.77±0.14</td>
<td>2.00±0.06</td>
<td>1.95±0.04</td>
<td>0.081</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>0.87±0.19</td>
<td>0.89±0.12</td>
<td>1.25±0.17</td>
<td>0.97±0.17</td>
<td>1.17±0.09</td>
<td>1.20±0.09</td>
<td>1.08±0.09</td>
<td>0.344</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.25±0.76</td>
<td>0.23±0.53</td>
<td>0.16±0.20</td>
<td>0.23±0.31</td>
<td>0.15±0.26</td>
<td>0.17±0.17</td>
<td>0.15±0.16</td>
<td>0.458</td>
</tr>
<tr>
<td>Urea (mg %)</td>
<td>8.41±0.25</td>
<td>8.65±0.10</td>
<td>8.67±0.27</td>
<td>8.63±0.36</td>
<td>8.51±0.18</td>
<td>9.13±0.25</td>
<td>8.68±0.16</td>
<td>0.497</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>1.96±0.16</td>
<td>0.20±0.38</td>
<td>2.34±0.08</td>
<td>2.13±0.20</td>
<td>2.24±0.14</td>
<td>2.27±0.12</td>
<td>2.04±0.13</td>
<td>0.791</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>8.09±0.79</td>
<td>10.02±0.58</td>
<td>15.22±1.01</td>
<td>11.38±0.29</td>
<td>13.40±0.66</td>
<td>13.49±0.80</td>
<td>15.55±1.34</td>
<td>1.76E-05</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>102.72±1.84</td>
<td>106.88±1.62</td>
<td>108.85±3.37</td>
<td>105.07±2.16</td>
<td>102.14±2.30</td>
<td>102.40±2.61</td>
<td>101.40±1.89</td>
<td>0.119</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>88.51±2.80</td>
<td>109.01±2.08</td>
<td>120.41±3.02</td>
<td>107.79±3.52</td>
<td>134.21±5.30</td>
<td>130.04±3.97</td>
<td>120.84±3.97</td>
<td>9.54E-08</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>7.82±0.24</td>
<td>8.41±0.57</td>
<td>9.89±0.32</td>
<td>8.64±0.49</td>
<td>8.34±0.58</td>
<td>8.59±0.33</td>
<td>8.08±0.81</td>
<td>0.177</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>3.37±0.13</td>
<td>4.17±0.33</td>
<td>4.26±0.43</td>
<td>4.02±0.16</td>
<td>3.84±0.31</td>
<td>3.33±0.06</td>
<td>3.61±0.16</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Values bearing different superscripts in a row differ significantly (P<0.05).

The increase in ALP level on zinc supplementation might be due to increase in corticosteroid hormone secretion, epinephrine and nor-epinephrine (Al-Duraji and Amen, 2011). In contrast to this, non-significant level of serum ALP in organic zinc fed groups was reported by Parák and Straková (2011). As far as groups of zinc nanoparticles fed birds are considered, a significant difference was observed (P<0.05) in the levels of serum cholesterol, SGPT and ALP than the inorganic and organic zinc supplemented groups.

Ahmadi (2009) also observed different serum biochemical parameters of broiler chicks such as SGPT, SGOT, ALP, total protein, albumin, gamma-globulin, triglyceride, and cholesterol were significantly affected (P<0.05) when their diets were supplemented with silver nano particles. In his report it was mentioned that these effects might be related to oxidative stress that caused peroxidation of fat and release of free radicals in the body. These reasons can be possibly assumed to be responsible for the significant differences in the levels of different serum metabolites of nano zinc fed birds.

CONCLUSION

Zinc plays an important role in improving different serum metabolite levels. ZnO NPs and Zn-Met were found to be more effective when compared to ZnSO₄ on the serum biochemical indices. It can be concluded that organic and nano zinc supplemented @ 15 ppm and 0.06 ppm, respectively to the basal diet of broiler birds altered the serum cholesterol, SGPT and ALP levels significantly (P<0.05) when compared to its inorganic form. Further study is needed to explore its metabolic pathway, absorption and assimilation mechanism and ideal dose rate to supplement in poultry rations.

Acknowledgment

This study was conducted under All India Co-ordinate Research Project (AICRP) on “Improvement of feed resources and nutrient utilization in raising animal production” of ICAR. Authors are highly obliged for getting all the financial support for the research work. Inorganic zinc (ZnSO₄) and organic zinc (Zn-Met), used in the present study were facilitated by Dr. N.K.S. Gowda, Principle Scientist, National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore, India and ZnO-NPs were provided by Dr. P. Swain, Principle Scientist, Central Institute of Freshwater Aquaculture, Bhubaneswar, India.

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