HEMATOLOGICAL AND IMMUNOLOGICAL VARIATIONS IN NAKED NECK FOWL SUPPLEMENTED WITH ORGANIC SOURCE OF IRON AND ZINC

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A study was conducted to evaluate the effect of organic source of Iron (Fe- Methionine) and Zinc (Zn- Methionine) in replacing their inorganic source on the hematological and immunological parameters of Naked neck fowl. The study was carried out for a period of 7 weeks in a total of 192 one week old Naked neck chicks. They were allotted to four different treatment groups (T1 to T4) of 48 chicks each, the treatments being further subdivided equally into four replicates consisting of 12 birds. Control diet (T1) was formulated by incorporating inorganic iron (FeSO₄) and zinc (ZnSO₄) according to BIS, (1992) specifications, i.e., T1 (control) with FeSO₄ (120 ppm/kg) and ZnSO₄ (60 ppm/kg). T2, T3 and T4 supplemented with 50%, 100% and 150% of the BIS specifications of iron and zinc, respectively from organic sources. Results showed that Fe- Methionine and Zn- Methionine significantly (P ≤ 0.05) improved hematology (TEC, PCV, serum cholesterol and Hb) and immune status (ND and IBD titers) in the birds. The supplementation of Fe- Methionine and Zn- Methionine did not significantly affect TLC. Thus it can be concluded that feeding of organic source (T2) of Fe and Zn (60 ppm/kg and 30 ppm/kg) improved the growth performance and can be used without any negative effects on performance.

Iron balance is rigorously controlled by the regulation of absorption. Recognition of zinc as an integral component of the enzyme carbonic anhydrase established the first biochemical role of zinc. Zinc is present in a wide variety of metabolic processes by virtue of its diverse catalytic roles in over 200 enzymes. Zinc enzymes are involved in the synthesis and or degradation of carbohydrates, lipids, proteins and nucleic acids and encompass all known classes of enzymes. Organically complexed trace minerals may provide alternative pathways for absorption, by decreasing mineral excretion because chelated minerals can be more effectively absorbed into the intestines than inorganic oxide and sulfate. The Naked neck birds seems to have an edge over their fully feathered counterparts as far as performance traits like BWG, feed efficiency etc are concerned especially under constant higher environmental temperatures i.e., >30°C. They are very good foragers and are immune to most diseases. Scientific studies have indicated that the naked neck gene (Na) improves breast size and reduces heat stress in chickens of non-broiler breeds which are homozygous for the trait. The present study was undertaken to study the effect of supplementing Fe-methionine and Zn-methionine at increasing levels on hematological and immunological parameters in Naked neck fowl.

MATERIAL AND METHODS

An experimental study of seven weeks (7-49 days of age) was conducted at the Department of Poultry Science, Veterinary
College, Bangalore, to evaluate the effect of supplementing organic source of Iron (Fe-methionine) and Zinc (Zn-methionine) on hematological and immunological parameters in Naked neck fowl.

**Experimental birds and their care:** 192 one week old straight run Naked neck chicks of uniform body weight were procured from the Department of Poultry Science, Veterinary College, Bangalore. The chicks were wing banded for identification, weighed and randomly distributed to four treatment groups with 48 birds in each treatment and each treatment having 4 replicates with 12 birds in each replicate. The birds were maintained in cages throughout the experimental period under standard managemental conditions. Birds were vaccinated against New Castle Disease (B1 strain) on the 7th and 21st days of age and Infectious Bursal Disease (IBD) (Intermediate strain) on 14th and 28th days of age. The diets were provided in linear feeders and potable water was provided in continuous channel to all the birds ad-libitum with free access to feed and water throughout the experimental period. All the methods regarding bird care in this experiment were approved by the Institution of Animal Ethical Committee of the University (KVAFSU, Bidar, Karnataka).

**Diets:** Basal diets were formulated using maize and soybean meals as per BIS (1992) standards. Starter mash was fed from 7 to 28 days and finisher from 29 to 56 days. The chicks in the control group (T1) received the basal diet formulated by incorporating corn, soya, inorganic iron (FeSO$_4$.7H$_2$O) and zinc (ZnSO$_4$.H$_2$O) as per BIS (1992) specifications (Fe 120ppm/kg and Zn 60ppm/kg) (Table-1). Inorganic Fe and Zn of the basal diet were replaced by Fe-methionine (12% Fe) and Zn-methionine (12% Zn) procured from Pristine Organics Private Ltd, Bangalore, at 50% (T2- Fe 60ppm/kg and Zn 30ppm/kg), 100% (T3- Fe 120ppm/kg and Zn 60ppm/kg) and 150% (T4- Fe 180ppm/kg and Zn 90ppm/kg) of BIS (1992) specifications.

**Hematological parameters:** Hematological parameters including total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), serum cholesterol and hemoglobin (Hb) concentration were evaluated as per standard procedures. The erythrocyte and leucocyte counts were determined using a flask method (dilution and counting blood cells using a Burker chamber); the hemoglobin concentration was determined by Sahli's method. The packed cell volume (PCV) was determined using the capillary microhaematocrit method.

**Immunological parameters:** Haemagglutination Inhibition (HI) titer against New Castle Disease: HI test was used to check the antibody titer against New Castle Disease. The micro-test method described by Allan and Gough was used for detection of HI titers from serum samples collected on 21st and 56th day post immunization of birds to assess the titers and their relation with the test feed. The HI test was done manually by β - procedure in ‘U’ bottom microplates using diluters, droppers and 4 Haemagglutination (HA) units of Newcastle Disease viral antigen. The titers were expressed as the reciprocal of highest dilution of serum showing the button formation.

**Antibody titer against IBD:** The serum antibodies against IBD vaccine were titered by using Poultry Diagnostic and Research Center (PDRC) indirect ELISA Kit. Each of the steps was followed as per the manufacturer’s instructions.

Each of the wells of antigen pre-coated plate provided in the kit was used for the test. 100 L each of the positive control serum and the negative control serum were added in duplicates to the respective control wells. Then, 100 L of each test serum sample diluted in the sample buffer were added in duplicates to corresponding wells of the plate (apart from the control wells) and incubated at 37°C for 1 hr. The plate was washed using the wash buffer provided in the kit. 100 L of mouse anti-chicken IgG conjugated with Horse Raddish Peroxidase (HRP) in wash buffer was added to each of the wells and incubated at 37°C for 1 hr. The plate was washed as aforementioned. 100L of freshly prepared chromogen-substrate solution containing OPD and 3% H$_2$O$_2$ as substrate (4L/ml of chromogen) were added to each of the wells and the plate was kept at room temperature for 15 min. Finally, 50 L of 2.5 N HCl was added to each of the wells to stop enzyme-substrate reaction. Absorbance values were read using the ELISA reader (Bio Rad) with an interference filter at 492 nm. Readings were taken after the wells with only substrate-chromogen and HCl were blanked to ‘zero’ at 492 nm.

**Heterophil and Lymphocyte ratio (H:L):** Blood smears were prepared from the fresh blood of slaughtered birds and stained with Giemsa stain. Heterophils and lymphocytes were counted...
Table-1. Composition of basal diet (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Broiler starter (%)</th>
<th>Broiler finisher (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow maize</td>
<td>56</td>
<td>63</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>*Mineral mixture</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salt</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>DL Methionine</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Vit AB2D3K</strong></td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>*<strong>Vit B complex</strong></td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Hepatocare</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Albac (Antibiotic)</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Coccidiostat</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Biobantix</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Provided per kilogram of feed
Calcium-1%, phosphorus-0.5%, copper-12 ppm, cobalt-60 ppm, iodine-1 mg, Mn-60 mg.
** Vit-AB2D3K: Per gram contains Vit A-82, 500 IU, Vit D3-12,000 IU, Vit B2-50 mg and Vit K-10 mg.
*** Vit B-complex: Per gram contains Vit B1-4 mg, Vit B6-8 mg, Vit B12-40 µg, Vit E-20 mg, Niacin -60 mg and calcium pantethenate-12.5 mg.

Table-2. Effect of supplementing iron- and zinc-methionine on the hematological parameters in Naked neck fowl.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Level (ppm/kg)</th>
<th>HEMATOLOGICAL PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Zn</td>
</tr>
<tr>
<td>T1</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>T2</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>T3</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>T4</td>
<td>180</td>
<td>90</td>
</tr>
</tbody>
</table>

Means bearing different superscripts in a column differ significantly (P ≤ 0.05).

Table-3. Effect of supplementing iron- and zinc-methionine on the immunological parameters in Naked neck fowl.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Level (ppm/kg)</th>
<th>IMMUNOLOGICAL PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Zn</td>
</tr>
<tr>
<td></td>
<td>1st week</td>
<td>2nd week</td>
</tr>
<tr>
<td>T1</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>T2</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>T3</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>T4</td>
<td>180</td>
<td>90</td>
</tr>
</tbody>
</table>

Means bearing different superscripts in a column differ significantly (P ≤ 0.05).

Statistical analysis: All the data pertaining to various parameters obtained during the experiment were analyzed statistically by ANOVA using GRAPHPAD PRISM 5.01. The significant mean differences between treatments were determined at P ≤ 0.05 using Tukey's Multiple Comparison Test.

RESULTS AND DISCUSSION

Hematological parameters: The Hematological parameters of Naked neck fowl supplemented with inorganic and organic Iron and Zinc are presented in Table-2.

Total erythrocyte count (TEC): The lowest mean TEC (3.19 X10^6/mm^3) was observed in T1, highest mean TEC (3.79 X10^6/mm^3) was observed in T3. The lowest mean TEC observed in
T1 was significant compared to T2, T3 and T4. However, among organic Fe and Zn fed groups, TEC was non-significant. The TEC in the present study showed significant difference between inorganic source of Fe and Zn fed groups and the different groups fed with different concentrations of organic source of Fe and Zn. This difference may be attributed to the consequence of better bioavailability of organic Fe and Zn when compared to their inorganic counterparts and the fact that iron is involved in erythropoiesis. The finding of the present study was in agreement with that of Vladimir et al. who found that broiler chickens fed with dietary supplementation of inorganic Cu (5mg/kg), Zn (50mg/kg), Fe (50mg/kg), Mn (50mg/kg) and sodium selenite (0.3mg/kg) and 50% of the inorganic source in bioplexed form produced the same effect on RBC count. The results of the present study is in agreement with that of Milanovic et al. who found that addition of organic iron supplements increased the RBC count during 21 days of the experiment. The present findings were in contrary to the findings of Devrim et al. who found that there was no significant effect of different levels and sources of Zn, Cu and Mn on RBC count.

**Total leucocyte count (TLC):** The lowest mean TLC (7.42 X10³/mm³) was recorded in T2 and T4. The highest mean TLC (7.48 X10³/mm³) was recorded in T1. However, there was no significant difference in TLC between inorganic (T1) and organic Fe and Zn (T2, T3 and T4) fed groups. The TLC observed in the present study showed non-significant difference in naked neck fowl fed diets supplemented with inorganic and organic Fe and Zn in different concentrations. The finding of the present study was in agreement with that of Devrim et al. who reported that broiler chickens supplemented with different levels of Zinc, Copper and Manganese in organic and inorganic form had no significant effect on WBC count. The present finding is in agreement with that of Vladimir et al. who found that broiler chickens fed with dietary supplementation of inorganic Cu (5mg/kg), Zn (50mg/kg), Fe (50mg/kg), Mn (50mg/kg) and sodium selenite (0.3mg/kg) and 50% of the inorganic source in bioplexed form had the same effect on WBC count.

**Packed cell volume (PCV %):** The lowest mean PCV (25.75%) was recorded in T1, whereas, the highest PCV (29.00%) was recorded in T4. The highest PCV recorded in T4 significantly (P ≤ 0.05) differs with the lowest PCV recorded in T1. Among the organic Fe and Zn fed groups, the higher level fed Fe and Zn (T3 and T4) are non-significantly comparable with each other and there was significant difference between T4 and T2. The results of the present study revealed a significant difference in PCV values between T1 and T4, whereas the PCV value was highest in organic fed groups which may be because of the increase in plasma Fe level due to increased bioavailability. This was found similar to the findings of Milanovic et al. who reported that inclusion of organic iron supplements increased the PCV during 21 days of the experiment. Devrim et al. also observed a significant increase in PCV in Ross-308 broiler chickens fed 13mg/kg Zn, 2.5mg/kg Cu and 20 mg/kg Mn in organic form with that of those fed inorganic form.

**Serum cholesterol:** The lowest mean serum cholesterol content (98.88 mg/dl) was recorded in T2, whereas, the highest (146 mg/dl) serum cholesterol content was recorded in T1. Among the organic source fed Fe and Zn groups, the highest serum cholesterol content (118.4 mg/dl) was recorded in T3 and significantly differ from T2 and T4. However, there was no significant difference between T2 and T4. There was significant (P ≤ 0.05) difference in serum cholesterol content between T1 and T2, T3 and T4.

The present study revealed a significant difference in the serum cholesterol content between the groups fed with inorganic source of Fe and Zn and the groups fed with organic source of Fe and Zn. The present finding was in agreement with that of Devrim et al. who found that cholesterol level significantly decreased with increase in the level of organic minerals in the diet of Ross-308 broiler chickens. The higher level of serum zinc might have decreased the serum cholesterol. However, the present findings were in contrary with that of Osman and Ragab who reported that there was no significant effect on serum cholesterol in broiler chicks supplemented with varied levels of Zn methionine.

**Hemoglobin (Hb %):** The lowest mean Hb level (8.34%) was recorded in T1, whereas, the highest Hb level (9.51%) was recorded in T4. The highest Hb% recorded in group fed T4 was non-significantly comparable with T3 and the lowest Hb% recorded in T1 was non-significantly comparable with T2. Among the groups fed organic Fe and Zn, the Hb% was increased as the level of organic Fe and Zn increased in the diet. However, there was no significant difference in Hb%
between organic Fe and Zn fed T3 and T4 and similarly between T2 and T3 groups. The result of present study showed that there was significant difference in Hb% between the inorganic Fe and Zn fed group and the groups fed higher (100 and 150%) levels of organic Fe and Zn. This may be attributed to the bioavailability of Fe which in turn increases the plasma Fe level which has an influence on the hemoglobin concentrations. The result of the present study is in agreement with that of Vahl and Klooster\textsuperscript{17} who found that haemoglobin level increased linearly with increase in dietary iron content. The result of the present study is also in line with Milanovic et al.\textsuperscript{13} and Devrim et al.\textsuperscript{15} who observed that organic Fe and organic Zn, Cu and Mn increased the haemoglobin level, respectively. The result of the present study is in agreement with that of Vladimir et al.\textsuperscript{13} who found that diet with inorganic Cu, Zn, Fe, Mn and sodium selenite and 50\% of the inorganic source in bioplexed form produced the same effect on haemoglobin level.

**Immunological parameters:** The Immunological parameters of Naked neck fowl supplemented with inorganic and organic Iron and Zinc are presented in Table-3.

**HI titer against New Castle Disease:** The lowest mean HI titer value (152 units) at the end of first week was recorded in T1 and the highest (P ≤ 0.05) mean HI titer value (320 units) was recorded in T3. Among the organic Fe and Zn fed groups, the highest mean HI titer value (320 units) was recorded in T4. However, the non- significant difference in HI titer value was observed between T3 and T4 groups.

At the end of third week, similar trend in mean HI titer values were observed as observed at the end of second week. The lowest mean HI titer value (176 units) was recorded in T1 and the highest (P ≤ 0.05) mean HI titer value (352 units) was recorded in T3. Among the organic Fe and Zn fed groups, the highest mean HI titer value (352 units) recorded in T3 was non- significantly comparable with that of T4 and significantly (P ≤ 0.05) comparable with T2. However, HI titer in T1 was non- significantly comparable with T2. Immunological studies using New Castle Disease vaccine showed significantly higher HI titer values at 1\textsuperscript{st} and 3\textsuperscript{rd} week of age in T3 when compared to T1. This result was similar to the findings of Hudson et al.\textsuperscript{18} and Moghaddam and Jahanian\textsuperscript{19} who reported that supplemental zinc when provided as zinc amino acid form had significant increase in antibody titers for new castle disease vaccine. The present results were also in accordance with Vladimir et al.\textsuperscript{13} who found that 50\% of the inorganic source of minerals Cu (5mg/kg), Zn (50mg/kg), Fe (50mg/kg), Mn (50mg/kg) and sodium selenite (0.3mg/kg) in bioplexed form produced the same effect on HI titer against New Castle Disease.

The results of present study were contrary to Yang et al.\textsuperscript{20} who found that supplementation of basal broiler diets with graded levels of trace minerals had no significant effect on New Castle Disease virus specific antibodies titer.

**Antibody titer against IBD:** The lowest mean antibody titer value (125.3 units) at the end of second week was recorded in T1 and the highest (P ≤ 0.05) mean antibody titer value (323 units) was recorded in T3. Among the organic Fe and Zn fed groups, the highest mean antibody titer value (323 units) recorded was statistically similar with T2 and T4. However, the significant difference (P ≤ 0.05) in antibody titer value was observed between control group (T1) and the groups fed organic Fe and Zn (T2, T3 and T4). At the end of fourth week, analysis of variance revealed non-significant effect of treatment groups on the antibody titer value against infectious bursal disease. Among various treatment groups, highest antibody titer value (316.8 units) was recorded in T3 as against the lowest value (171.5 units) recorded in T1. Immunological studies using IBD vaccine showed significantly higher antibody titer values at 2\textsuperscript{nd} week of age in groups fed organic Fe and Zn when compared to control group supplemented with inorganic Fe and Zn. Zinc is essential for thymulin, a thymic hormone that regulates T- lymphocyte maturation and activation of B-lymphocytes by T-helper cells which might have improved the antibody production.

This result of present study was in agreement with Moghaddam and Jahanian\textsuperscript{18} who observed that Zinc-methionine increased the antibody titer against infectious bursal disease virus at 12\textsuperscript{th} day post inoculation in broiler chicks. The present results were also in accordance with Vladimir et al.\textsuperscript{13} who found that 50\% of the inorganic source of minerals Cu (5mg/kg), Zn (50mg/kg), Fe (50mg/kg), Mn (50mg/kg) and sodium selenite (0.3mg/kg) in bioplexed form produced the same effect on antibody titer against IBD. The antibody titer values at fourth week revealed a non significant difference between the treatment groups and a decreasing trend in the titer values in all the treatments compared to values of third week. The present results are in contrary with Milanovic et al.\textsuperscript{14} who found
that addition of organic iron supplements increased titers of antibodies against infectious bursal disease on 35th day in Arbor acres chicken.

**Heterophil and Lymphocyte ratios (H:L):** Analysis of variance revealed non-significant effect of treatment groups on heterophil and lymphocyte ratio. Among various treatment groups, highest H:L value (0.376) was recorded in T4 group as against the lowest value (0.345) recorded in T1.

The result of the present study revealed that there was no significant difference in H:L in naked neck fowl fed with inorganic and organic Fe and Zn in different concentrations. The present findings are in accordance with that of Devrim et al\(^3\), Vladimir et al\(^3\) who found that there was no significant effect of inorganic and organic minerals on H:L.

The present finding was in contrary with that of Shyam et al\(^3\) who found that Zn at 40 ppm had a perceptible decline in the ratio between heterophil and lymphocyte which was significantly better than at lower levels (0, 10 and 20 ppm). The present finding was also in contrary with that of Moghaddam and Jahanian\(^9\) who observed that dietary supplementation with Zinc-methionine partially in place of inorganic zinc source, decreased heterophil and lymphocyte ratio.

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**REFERENCES**