Evaluation of Haemato-Biochemical Profiles of Broiler Chicken Supplemented with Dietary Minerals

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A B S T R A C T

Present study evaluated the haemato-biochemical profiles of broiler chicken supplemented with dietary minerals (Se+Zn+Cr) at Veterinary College, Anjora, Durg (Chhattisgarh). Day old Ross 308 broiler chicks (n=120) were equally divided into three groups with 40 chicks in each group. Group T1 was without any additional minerals supplementation (control); whereas, group T2 supplemented with minerals Se+Zn+Cr @ 0.15+25+0.30 ppm and group T3 supplemented with minerals Se+Zn+Cr @ 0.20+50+0.50 ppm, respectively. Dietary minerals supplementation affected serum cholesterol level significantly (P<0.05). Serum total cholesterol level was observed to be 191.73±1.6, 117.1±3.36 and 108.93±1.71 mg/dl (P<0.05) in T1, T2 and T3 groups, respectively. On the other hand, serum HDL-cholesterol levels in T1, T2 and T3 groups were 65.53±1.14, 76.53±1.71 and 89.56±1.94 mg/dl (P<0.05), respectively. In T3 group, Hb% increased significantly (P<0.05) as compared to T1 and T2 groups (9.81±0.03 vs. 9.00±0.27 and 9.65±0.65 g/dl), but heterophil count (37.00±0.70 vs. 39.25±0.47 and 39.00±0.40%) and H/L ratio (0.70±0.03 vs. 0.77±0.02 and 0.76±0.01) decreased in T3 group as compared to T1 and T2 groups, respectively (P<0.05). However, PCV%, monocyte, eosinophil and the basophil counts were similar among the groups. Taken together, it may be concluded that mineral supplementation (Se+Zn+Cr @ 0.20+50+0.50 ppm) reduced serum total cholesterol, increased HDL-Cholesterol and affected certain hematological parameters in broiler chickens.

Keywords
Dietary minerals, Haemato-biochemical, Broiler chickens

Introduction
In India, though the poultry industry is exploited to its maximum potential, today facing series of problems like disease outbreaks, climatic stress including high feed cost. In the present scenario there is continuous increase in rampant use of antibiotics in the poultry industry to overcome such disease problem and to increase disease resistance as well as optimum growth (Marshall and Levy, 2011). However, this preventive health management practice in food animals and birds has resulted antibiotic-resistant in the human populations (Phillips et al., 2004). Thus appropriate alternatives for
antibiotics could maintain accessible markets for poultry products (Marshall and Levy, 2011). Minerals, particularly trace minerals like Selenium, Zinc and Chromium play vital role in various metabolic, enzymatic and biochemical reactions and ultimately improves disease resistance leading to better growth as well as improved meat quality, and thus gaining more importance as dietary growth promoters (Suksombat and Kanchanatawee, 2005; Fawzy et al., 2016).

These minerals (Se, Zn and Cr) also play vital role to regulate antioxidant system of living tissues and there by reduces oxidative stress and protect the cells membrane phospholipids from lipid peroxidation (Fawzy et al., 2016; Haq et al., 2016). Thus, the present experiment was designed to evaluate the haematological and biochemical parameters of broiler chicken supplemented at two levels of dietary Selenium, Zinc and Chromium in combination.

Materials and Methods

Experimental birds and management

The present study was conducted on 120 day old unsexed Ross 308 broiler chicks at Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Anjora, Durg (Chhattisgarh) during summer (April to May, 2008). Chicks were divided into 3 groups and each with 4 replicates (10 chicks/ replicate) and the design was completely randomized design (CRD). Experimental birds were reared in deep litter system of housing under standard management practices and were vaccinated for poultry diseases (Marek’s and New castle disease) as per the guidelines for commercial broilers. Diet for the birds was formulated for different growth phase such as starter phase (0-14 day), grower phase (15-28 days) and finisher phase (29-42 days) using feed ingredients like maize, soybean meal, deoiled rice bran and fishmeal, dicalcium phosphate and limestone powder. The diet for starter, grower and finisher phase consisted of 23, 21.5 and 20% crude protein and 2900, 3000 and 3100 kcal metabolizable energy per kg, respectively (Table 1). The dietary minerals treatment (Sodium selenite, Chromium picolinate, Zinc-methionine) were offered in two different levels. Group T1 was without any additional supplementation of minerals; whereas, Group T2 and T3 were supplemented with minerals (Se+Zn+Cr) @ 0.15+25+0.30 ppm and @ 0.20+50+0.50 ppm, respectively.

Estimation of haemato-biochemical parameters

Blood samples were collected from wing vein on 42nd day in non heparinised and clean test tubes from three birds randomly selected from each replicate (12 from each treatment) for estimation of biochemical profiles. Serum was separated and analysed on the same day for alkaline phosphatase (Bernet, 1974), albumin, cholesterol (Allain et al., 1974) and HDL-cholesterol (Lopes-Virella et al., 1977) in semi-automated analyzer using diagnostic kits (Bayer Autopk biochemistry kits- Baroda).

On the other hand, blood samples were collected from wing vein in heparinised vials (Heparin @10 IU/ ml of blood) on 42 day of experiment for haematological studies. The haematological observations were recorded in 3 birds randomly selected from each replicate (12 from each treatment).

Haemoglobin was estimated by cyanmethemoglobin method using Drabkin’s solution (Dacie and Lewis, 1968). Packed cell volume (PCV) was determined by micro haematocrit method and red and white blood cells were determined by using haemocytometer method as described by Jain (1986).
Statistical analysis

For interpretation of the result the data were presented as mean and standard error of mean. The data was analysed by one-way analysis of variance with general linear model. Duncan multiple range test was used as post hoc test to compare all pair wise mean differences and was considered as significant when \( P \leq 0.05 \). All the statistical analysis were carried out using SPSS software package (SPSS ver. 10.0, USA).

Results and Discussion

Biochemical changes

There was significant (\( P<0.05 \)) reduction in the serum total cholesterol level in mineral supplemented groups (\( T_2 \) and \( T_3 \)) as compared to control group (\( T_1 \)). Moreover, comparatively the effect was significantly higher in \( T_3 \) than \( T_2 \) treatment group (108.93±1.71 vs. 117.1±3.36 mg/dl, \( P<0.05 \)) indicated that groups supplemented with higher dose had significantly low concentration (Table 2). On the other hand, though serum HDL-cholesterol level differed significantly (\( P<0.05 \)) among the groups, the trend was reverse. The serum HDL-cholesterol level was observed to be lower in non-mineral supplemented group (\( T_1 \)) and increased in \( T_2 \) and \( T_3 \) mineral supplemented groups (65.53±1.14, 76.53±1.71 and 89.56±1.94 mg/dl, respectively).

The results were in accordance with the finding of other studies where the dietary minerals were supplemented either separately or in combination (Kroliczewska et al., 2004; El-Hommosany, 2008; Tawfeek et al., 2014; Fawzy et al., 2016). Dietary Cr at different levels decreased the serum total cholesterol; whereas, increased HDL- cholesterol level in broiler chicken (Kroliczewska et al., 2004 and El-Hommosany, 2008; Tawfeek et al., 2014). However, Tawfeek et al., (2014) observed insignificant reduction of cholesterol level in chicken supplemented with dietary combination of Zn + Se which is contrary to our result. Moreover, Fawzy et al., (2016) observed reduction of serum total cholesterol and elevation of HDL- cholesterol in broiler chicken supplemented with combined Zn+Se, but the results were inconsistent when such minerals were supplemented separately. The decreased total cholesterol level might be associated with improved hepatic transport ability of cholesterol and its improved catabolism due to the increasing activity of lipoprotein lipase in plasma by the trace minerals supplemented in the diet (Vincent, 2001). Further, elevated circulating HDL-cholesterol may be the one of the reason of lower serum total cholesterol, as HDL-cholesterol helps in transportation of cholesterol from tissue to liver (Ismail et al., 2013). Additionally, lower serum total cholesterol might be attributed to decrease lipolysis of body lipids due to higher intake and improved feed efficiency (Doneria et al., 2017).

The activity of enzyme alkaline phosphatase did not differ statistically (\( P>0.05 \)) in the mineral supplemented groups (\( T_2 \) and \( T_3 \)) as compared to non-supplemented group (\( T_1 \)). The results also revealed no significant difference in the concentration of albumin in the groups supplemented with minerals in group \( T_2 \) and \( T_3 \) (Table 3). In a similar line, other studies also observed non-significant effect of trace minerals supplemented either individually or separately on alkaline phosphatase activity (Fawzy et al., 2016) and serum albumen (El-Hommosany, 2008; Ismail et al., 2013) in broilers.

However, Fawzy et al., (2016) reported non-significant effect of dietary Zn and Se on serum albumen level when supplemented separately, but significant effect in combined form.
Table.1 Ingredient and chemical composition of the experimental diet

| Feed Ingredient                  | Starter (0-14 d) | Grower (14-28 d) | Finisher (29-42 d) |
|----------------------------------|------------------|-------------------|--------------------|
|                                  | T₁               | T₂               | T₃                 | T₁               | T₂               | T₃     | T₁               | T₂               | T₃     |
| Yellow maize                     | 53.0             | 53.0             | 53.0               | 56.40            | 56.40            | 56.40  | 59.7             | 59.7             | 59.7   |
| Deoiled soybean meal             | 36.40            | 36.40            | 36.40              | 33.40            | 33.40            | 33.40  | 26.3             | 26.3             | 26.3   |
| Deoiled rice bran                | 2.50             | 2.5              | 2.50               | 0.0              | 0.0              | 0.0    | 0.0              | 0.0              | 0.0    |
| Fish meal                        | 2                | 2                | 2                  | 2                | 2                | 2      | 5                | 5                | 5      |
| Soyabean oil                     | 2.40             | 2.40             | 2.40               | 4.60             | 4.60             | 4.60   | 5.5              | 5.5              | 5.5    |
| Di calcium phosphate             | 1.70             | 1.70             | 1.70               | 1.60             | 1.60             | 1.60   | 1.30             | 1.30             | 1.30   |
| Limestone powder                 | 0.70             | 0.70             | 0.70               | 0.70             | 0.70             | 0.70   | 0.70             | 0.70             | 0.70   |
| DL-methionine                    | 0.28             | 0.28             | 0.28               | 0.26             | 0.26             | 0.26   | 0.22             | 0.22             | 0.22   |
| Lysine                           | 0.02             | 0.02             | 0.02               | 0.0              | 0.0              | 0.0    | 0.17             | 0.17             | 0.17   |
| Sodium bicarbonate               | 0.17             | 0.17             | 0.17               | 0.16             | 0.16             | 0.16   | 0.23             | 0.23             | 0.23   |
| Common salt                      | 0.28             | 0.28             | 0.28               | 0.33             | 0.33             | 0.33   | 0.26             | 0.26             | 0.26   |
| ¹Se (ppm)                        | -                | 0.15             | 0.20               | -                | 0.15             | 0.20   | -                | 0.15             | 0.20   |
| ²Zn (ppm)                        | -                | 25               | 50                 | -                | 25               | 50     | -                | 25               | 50     |
| ³Cr (ppm)                        | -                | 0.30             | 0.50               | -                | 0.30             | 0.50   | -                | 0.30             | 0.50   |
| CP (%)                           | 22.95            | 22.95            | 22.95              | 21.46            | 21.46            | 21.46  | 19.76            | 19.76            | 19.76  |
| ME kcal/kg                       | 2900             | 2900             | 2900               | 3000             | 3000             | 3000   | 3100             | 3100             | 3100   |
| Ca (%)                           | 1                | 1                | 1                  | 1                | 1                | 1      | 1                | 1                | 1      |
| Available P (%)                  | 0.5              | 0.5              | 0.5                | 0.5              | 0.5              | 0.5    | 0.5              | 0.5              | 0.5    |

¹Sodium selenite, ²Zinc-methionine, ³chromium picolinate
Table 2: Effect of dietary mineral supplementation on biochemical Constituents of broilers chicken

| Particulars                     | T1              | T2              | T3              |
|---------------------------------|-----------------|-----------------|-----------------|
| Cholesterol (mg/dl)             | 191.73±1.6      | 117.1b±3.36     | 108.93a±1.71    |
| Alkaline Phosphatase (U/L)      | 512.66±4.4      | 507±9.21        | 521.35±0.62     |
| Albumin (mg/dl)                 | 1.53±0.02       | 1.63±0.03       | 1.54±0.02       |
| HDL-Cholesterol (mg/dl)         | 65.53±1.14      | 76.53b±1.71     | 89.56±1.94      |

T1: Se: 0, Zn:0, Cr:0 (ppm); T2: Se:0.15, Zn:25, Cr:0.3 (ppm); T3: Se:0.2, Zn:50, Cr:0.5 (ppm); Means with different superscripts (a, b, c) differed statistically (P≤0.05)

Table 3: Effect of dietary mineral supplementation on hematological Parameters of broilers chicken

| Particulars | T1              | T2              | T3              |
|-------------|-----------------|-----------------|-----------------|
| Hb (g/dl)   | 9.00±0.27       | 9.65±0.65       | 9.81b±0.03      |
| PCV (%)     | 29.50±1.19      | 28.75±1.10      | 28.33±0.96      |
| Heterophil  | 39.25b±0.47     | 39.00b±0.40     | 37.00±0.70      |
| Lymphocyte  | 50.75a±1.03     | 50.71±0.62      | 52.75±1.10      |
| Monocyte    | 8.01±0.40       | 8.00±0.40       | 8.00±0.40       |
| Eosinophil  | 2.12±0.40       | 2.25±0.25       | 2.15±0.25       |
| Basophil    | 0               | 0               | 0               |
| H/L ratio   | 0.77b±0.02      | 0.76b±0.01      | 0.70±0.03       |

T1: Se: 0, Zn: 0, Cr: 0 (ppm); T2: Se: 0.15, Zn: 25, Cr: 0.3 (ppm); T3: Se: 0.2, Zn: 50, Cr: 0.5 (ppm); Means with different superscripts (a, b) differed statistically (P≤0.05)

The disparity of results in different study may be due to different dose of mineral or their organic or inorganic form in which supplemented to the chickens (Suksombat and Kanchanatawee, 2005).

Hematological changes

In the present study, haemoglobin concentration increased significantly (P<0.05) in T3 as compared to T1 and T2 groups. However, there was (P<0.05) significant reduction of heterophil count in T3 group as compared to T1 and T2 groups. Additionally, H/L ratio in group T3 was observed to be significantly lower than the T1 and T2 groups (P<0.05). These results indicated that at lower dose of minerals the effect was non-significant on haemoglobin concentration, heterophil and H/L ratio. On the other hand, PCV%, monocyte, eosinophil and the basophil counts does not differ statistically due to supplementation of dietary minerals in combination (Zn+Se+Cr).

The results are more or less similar to that of previous experiments involving supplemental Zn, Se and Cr either separately or in combination on Hb%, heterophils and H/L ratio (Toghyani et al., 2007; Biswas et al., 2011; Ihsan et al., 2012; Fawzy et al., 2016). However, the results reported by several authors are inconsistent owing to variation of dose and the form of the minerals fed to the birds (Biswas et al., 2011; Ihsan et al., 2012; Fawzy et al., 2016). Further, Toghyani et al., (2007) reported that haemoglobin concentration increased by Cr
supplementation; while, heterophil to lymphocyte ratio decreased. The heterophil to lymphocyte ratio has been accepted as a reliable index of determining stress in poultry. The increased lymphocyte count and decrease H/L ratio might be attributed to decreased glucocorticoid secretion (Ebrahimzadeh et al., 2012).

Based on the present findings, it may be concluded that mineral supplementation in the ratio of Se 0.20+Zn 50+Cr 0.50 ppm reduced serum total cholesterol level while increased serum HDL-Cholesterol in broiler chickens. Further, mineral supplementation (Se 0.20+Zn 50+Cr 0.50 ppm) affected certain hematological parameters; the haemoglobin concentration increased while heterophil and H/L ratio decreased.

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