Effect of in Ovo injection with Nano- Selenium or Nano- Zinc on Post-Hatch Growth Performance and Physiological Traits of Broiler Chicks

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Abstract—The current study was aimed to investigate the effect of in ovo injection with Nano-selenium or Nano-zinc on post-hatch growth performance and physiological traits of broiler chicks under heat stress. Four hundred fertile broiler eggs from cobb500™ flock were randomly divided into four treatments (100 eggs each). First was normally without injection (control), second was injected with 15 ppm Nano-Selenium (SENPs) /egg, third treatment was injected by 15 ppm Nano-Zinc (ZnNPs)/egg and fourth treatment was injected with phosphate buffered saline (PBs) 15 ppm /egg. To study the post-hatch performance, A total number of 240 one day-old chicks were randomly distributed into 4 equal experimental treatments of 60 chicks each. Every treatment was sub-divided into three replicates (20 chicks/each), were at lasted 5 weeks.

Results obtained could be summarized as follows: Nano-selenium explained higher chick’s weight at hatch, chick’s weight to egg weight ratio and hatchability % than all other treatments. At first week of age, the body weight (BW) in the nano-selenium treatment increased than the untreated (control) treatment, although the gastrointestinal tract weight was 0.44 % and the intestine weight was 0.8 %, this is explained by an augmentation in the length of both the length of the small intestine and the gastrointestinal tract by 12 % at 7 day of age. The highest live body weight and body weight gain and the best-feed conversion ratio were recorded with Nano- selenium than all other treatments at 35 day of age.

In conclusion, under semi-arid conditions, usage the Nano-selenium are not harmful to the embryo (injected with 15 ppm) and can be used to improve the post-hatch performance of broiler under semi arid condition.

Keywords—Nano-selenium, Nano-zinc, performance, In-ovo injection, immunity.

I. INTRODUCTION

Feeding the embryos in fertilized eggs by In-ovo injection with some nutritious solutions is beneficial after hatching. The fertilized eggs will quickly growth the gastrointestinal system to improve digestion, metabolism and growth performance of chicks (Ohtsu et al., 2015). Who noted that injecting fertilized eggs into increased meat broiler production, and the trend is likely to continue in future with the advent of development in the field of genetics, nutrition, biotechnology, and developmental biology. In ovo nutrition could lead to improved digestive capacity, increased growth rate and feed efficiency, reduced post-hatch mortality and morbidity, improved immune response to enteric antigens, reduced incidence of developmental skeletal disorders, and increased muscle development and meat yield, Ferket (2011). However, further many advanced researches are required to explore further beneficial effects and safety of nano forms of minerals. In -ovo injection of minerals has also gained importance as the high-metabolism, fast-growing broiler embryos.

In tropical and semitropical regions, raising broiler out of their thermal comfort zone can cause economic loss in the poultry industry. It has been shown that, in poultry that exposed to elevated temperature showed a desperation on the immune responses, body weight and feed efficiency (Altan et al., 2000 and Ohtsu et al., 2015), while, plasma corticosterone and heterophil/lymphocyte ratio are improved (Quinteiro-Filho et al., 2012). Cells exposed to elevated temperature showed an inhibition of protein synthesis through alterations in the phosphorylation state of many components of the translational process. This clarified the rise the mortality and a rapid drop in the final body weight (Syafwan et al., 2011). Using fertilized egg injections can overcome such difficulties in under heat stress by in ovo feeding of minerals. Selenium is a trace mineral that is part of an antioxidant enzyme called glutathione peroxidase and is involved in the regulation of energy metabolism, thyroid hormone activation, immune response (Arthur et al., 2003 Ludwiczek et al., 2004; Lozoff et al., 2006 Whitnall and Richardson, 2006; Li and Zhao, 2009). Selenium is an essential micronutrient; plays
an important role in number of biological processes (essential component for the normal development of spermatozoa) and enhancing the activity of the glutathione peroxidase and seleno-enzymes which in-turn can help in protecting the body from the free radicals, which destroy the cells of the body causing autoimmune diseases. The toxicity of nano-selenium is 7 times lower than that of inorganic selenium and 3 times lower than that of organic selenium (Peng et al., 2007). Trace minerals are important nutritional components for imparting immunity and in ovo enrichment can be a way for improving the immune system of the birds.

Zinc important in immune system of the broiler embryo, Kidd, et.al. (1992) and Kidd, (2003). Additional zinc in diet of broiler has improved enhanced antibody (Cardoso et. al. 2007). Zinc is function of cells mediating non-specific immunity such as neutrophils and natural killer cells (Shankar and Prasad, 1998). Therefore, the main objective of the present work was effect of ovo injection by nano form of selenium or zinc on post-hatch physiological and growth performance of broiler under semi-arid condition.

II. MATERIALS AND METHODS

First: Pre-hatch: Four hundred fertile broiler eggs from cobb500™ flock were used to investigate to effect of In-ovo injection by Nano forms of selenium or zinc on post-hatch physiological parameters and growth performance of broiler under semi-arid condition. The eggs were randomly divided into four treatments (100 eggs per treatment). First treatment was without injection and serves as control, second treatment was injected with 15 ppm Selenium (SE-NPs)/egg, third treatment was injected by 15 ppm Zinc (ZnNPs)/egg and fourth treatment was injected with phosphate buffered saline (PBS) 15 ppm /egg. The eggs were set in a hatchery in Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Nano form of selenium (SeNPs) was prepared by adopting the procedure of (Razi et al., 2011). Nano form of zinc (ZnNPs) was prepared by adopting the procedure of (Patric, et al., 2016). In ovo supplementation of nano selenium and zinc particle’s (SeNPs, ZnNPs) (15ppm/egg ) was carried out on 7th day of incubation, amniotic route was marked and a small pinpoint hole was made in the broad end of the egg to remove the egg shell by using Topaz Engraver as egg driller and in ovo supplementation was done according to the treatments through the amniotic route using a 24G hypodermic needle (25 mm long) and the pinpoint hole was sealed using wax (Bhanja, S.K., 2004). Eggs were candled on 7th and 17th day to remove infertile eggs. The hatchability percent was calculated using the following formulae:

\[ \text{Hatchability percent} = \frac{\text{Number of hatched chicks}}{\text{Number of eggs that were fed in ovo at 21 days}} \times 100. \]

While, Chick weight is to egg weight ratio= (Chick weight (g)/ Egg weight (g)) \times100.

Second: Post- hatch: to study the post-hatch performance was carried out at South Sinai Experimental Research Station (Ras-Suder City) which fits to the Desert Research Center. A total number of 240 one-day-old chicks were randomly distributed into 4 equal experimental treatments (60 chicks each) with three replicates (20 chicks each).

The experimental treatments were as follows:

- **T1**: Chicks produced from un-injected treatment as control
- **T2**: Chicks produced from the injection of nano-selenium (SeNPs).
- **T3**: Chicks produced from the injection of nano-zinc (ZnNPs).
- **T4**: Chicks produced from the injection with phosphate buffered saline (PBS).

All chicks were kept under similar managerial, hygienic and environmental conditions. The chicks were housed in cages from hatch up to 5 weeks of age. Average of indoor ambient temperature (35.70°C ±0.98) and relative humidity (24.2 RH (%) ±1.32) were recorded using electronic digital thermo-hygrometer. Feed was offered ad libitum that met NRC (1994) recommendations and fresh water was available all time. Weekly individual live body weight and feed intake were recorded before offering the feed. Feed conversion ratio = (g feed/g gain) were calculated. The chicks were examined against diseases and treated with antibiotics and vaccines to keep them healthy.

The end of the trial, 10 broiler from each treatment were taken randomly, blood samples were withdrawn from the wing vein into tube containing EDTA to examine immediately red blood cells count by means of hemocytometer and hemoglobin concentration according to Jaime method.

Blood samples were centrifuged at 3000 rpm for 20 minutes for the separation of serum and kept at (−20°C) until further analysis. Blood metabolites (total protein (TP), albumin (AL), total lipids (TL), Triglycerides (TG), liver enzymes (alanine transaminase (ALT), aspartic transaminase (AST)), plasma immunoglobulin IgG and IgM concentration, creatinine (Cr) and Triiodothyronine hormone (T3). While, globulin and albumin ratio (A/G ratio) were calculated. All measurements were determined calorimetrically by using kits (By BioSystems S.A. Costa Brava 30, Barcelona (Spain, Barcelona)). Thyroid hormone (Tri-iodothyronine) were measured by ELISA technique using IMMUNOSPEC kits supplied by
Statistical Analysis
Statistical analysis was carried out using General Linear Model (GLM) procedures by SAS (2010) using simple one-way analysis of variance according to the model: $Y_{ij} = \mu + T_i + e_{ij}$

Where: $Y_{ij}$ = any observation of $i$th chicks within $j$th treatment, $\mu$ = Overall mean, $T_i$ = Effect of $i$th treatment (i: 1- 4), $e_{ij}$ = Experimental error. Significant differences among treatment means were tested by Duncan’s multiple range tests, (Duncan, 1955).

III. RESULTS AND DISCUSSION
Effect of SeNPs and ZnNPs on hatchability parameters:
Effects of In-ovo nutrition with Nano forms of selenium or zinc on hatchability parameters are summarized in Table 1. No significant variation (P>0.01) existed in the egg weight and hatchability percent between the treatments. While, hatchling weight (g) and ratio of chicks weight to egg weight % were significantly different (P<0.05) between treatments. It was noticed that the Nano- selenium recorded the highest value by 4.73, 4.31 and 4.73 %, for hatchling weight (g) and ratio of chicks weight to egg weight % and hatchability %, while, Nano-zinc recorded 2.28, 2.17 and 3.30 % for hatchling weight (g) and Ratio of chicks weight to egg weight % and hatchability % than control, respectively. Patric Joshua et al., (2016) recorded that In-ovo feeding of nano minerals (at 5 ppm level of zinc, copper and selenium) did not significantly influence (P>0.01) the hatchling weight, ratio of chicks weight to egg weight % and hatchability %.

Growth performance at 7 day of age.
At first week of age, (as shown in Tables 2, 3, 4, 5 and 6), the body weight (BW) in the nano-selenium treatment (T2) increased by 6.06 % than the untreated group, while, the gut weight was increased by 0.44 % and the intestine weight by 1.43 %. This is explained by an augmentation in the length of both the length of the small intestine and the gastro intestinal tract (Table 2). Thus, increased the absorption rate of the gastro intestinal tract, both mineral etc. In addition, this proves the increased of total protein, albumin and total fat by analysis of blood serum, (Table 3). It is seen from results that the chicks that got selenium Nano-particles suspension had different morphological blood indices as compared with those of the control. Which, the augmentation in the immunity of bird represented by the increase of white blood cells. White blood cells was amplified by 1.27 %, red blood cells by 1.53 % and hemoglobin by 68.1 than control. Therefore, this is explained by enhancement of hemoglobin concentration in the cell by 34.2 % and the diameter of the cell increased by 7 (Table 4). Therefore, the first week of age is that increased the protein produced because of growth hormone was directed to the production of immunity to the bird rather than to increase body weight. Immunoglobulin G (IgG), the most abundant type of antibody, is found in all body fluids and protects against bacterial and viral infections (Table 5). Immunoglobulin M (IgM), which is found mainly in the blood and lymph fluid, is the first antibody to be made by the body to fight a new infection. The immune system of the bird is partly developed at hatch. This correlates the present study as chicks receiving in ovo injection of Se had significantly lower expression of TNF-α gene (Zhang et al., 2012). Selenium compound affects the expression of TLRs by modulating the TLR signaling pathway. Expression of both TLR-2 and TLR4 gene was significantly increased in Se injected chick. Chicken embryo tissues are rich in long chain polyunsaturated fatty acids and as a result are very vulnerable to lipid peroxidation. Therefore, SeNPs is a crucial factor in maintaining appropriate antioxidant defense during embryonic development (Surai, 2002). Surai also considered as an immunological enhancement agent to enhance or recover immune functions of the organism (Ru-Duan et al., 1992). Se injected chicks by (Akshat et al., 2003) has shown a positive effect by increasing the expression. They sided that it can be concluded that in ovo feeding of either SeNPs at 14th day of embryonic age is beneficial for enhancing the immune response. Se has modulated the expression of adaptive or cellular immunity related genes in broiler.

Growth performance at 35 day of age.
Effects of In -ovo injection of broiler eggs with nano forms of selenium and zinc on growth performance of broiler during the experimental period (0-5 weeks of age) are shown in table (8). Final weight (gm) and weight gain (gm) values during the whole experimental period increased significantly (P<0.01) with the SeNPs and ZnNPs. The FI of the T2 was significantly decreased compared to other treatments. It is clear that SeNPs decreased feed intake by 9.89 % than that of the controls. Results of feed conversion ratio (FCR) (gm feed/gm gain) revealed a significant difference (P<0.01) among the experimental treatments. It was observed in this study, that SeNPs recorded the best FCR than other treatments and this may be due to the increase in feed intake and reduction of daily weight gain. It is worth to note that SeNPs improved growth performance of broiler chickens compared with other treatments and control. Ferket, P.R. (2011) recorded that In ovo feeding could lead to improved digestive capacity, increased growth rate and feed efficiency.

Blood analysis.

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The results demonstrate that the effect of In-ovo injection of broiler eggs with SeNPs and ZnNPs showed increased (P<0.01) RBC counts by about (24.39 and 1.63%), HGB by about (18.14 and 3.49%), MCHC by (4.59 and 2.14%), HCT by about (13.41 and 4.99%), RDWSD by about (9.38 and 6.26%) , while it decreased (P<0.01) MCV by about by 8.78 and 9.46% , MCH by about 4.78 and 6.83% as compared to control, respectively (Table 10). The treatment of SeNPs showed a significant increase in body weight compared to the untreated treatment by about 0.28%, this is supported by increased gastrointestinal length and weight by about 0.54 % and 0.16 %, respectively and stomach weight by 0.69 %. Therefore, there was an increase in absorption and utilization of nutrients. Injected with nanoparticles at the rate of 0.16 %, but these few were not significant and white blood cells the lymphoma increased significantly by 0.022%, while red blood cells increased by 0.55%, increased platelets by 0.27% and increased cell diameter by 0.27%. Table (10) showed the effect of different treatment on the blood parameters of broiler at 35 day old. These results found that these results are supported by increasing the body weight by increasing the growth hormone and increase the total protein in blood and albumin, although it is an insignificant increase, followed by an increase in the immune proteins. The form of nano elemental SeNPs depends on the presence of protein and it would be interesting to investigate the relationship between protein and Se0 atoms. We found that Se0 atoms adhered easily to protein and that the coexisting system of protein and selenium could directly scavenge ROS (OH•, O−2 and H2O2). However, Se0 itself, after being separated from protein by centrifugation, did not show this distinctive property. Zhang et al., (2012) reported that present the first report of the preparation of a novel selenium form, nano red elemental selenium, with biological activity and effects similar to those of sodium selenite on sodium dependent enzyme biosynthesis but with much lower acute toxicity (Zhang et al., 2012).

It can stabilization that In-ovo injection with Nano particles at level 10 nm Selenium in 1 ml at 7 days increases body weight and the immune efficiency of the bird. Akshat et al., (2017) found that selenium supplemented chicks had higher cellular immune gene expression in vivo response to mitogen PHA-P was also higher (P<0.01) in ZnNPs or SeNPs supplemented chicks. In ovo supplementation of ZnNPs and SeNPs did not improve the post-hatch growth, but increased the growth related gene expression. SeNPs and ZnNPs enhanced cell-mediated immune genes expression in broiler (Akshat et al., 2017).

Nano-particles can evade conventional physiological ways of nutrient distribution and transport across tissue and cell membranes, as well as protect compounds against destruction prior to reaching their targets. In ovo administration of nanoparticles, may be seen as a new method of nano-nutrition, providing embryos with an additional quantity of nutrients. The research clearly shows that nano minerals are not harmful to the embryo and can be used to improve the post-hatch performance of broiler. However, further many advanced researches are required to explore further beneficial effects and safety of nano forms of minerals.

In conclusion, under semi-arid conditions, can be used the nano form of selenium or zinc are not harmful to the embryo (injected with 15 ppm) and can be used to improve the post-hatch performance of broiler.

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Table 1: Effect of in ovo injection by broiler eggs with Nano form (Mean ±SE) on egg weight, hatch weight of chicks, their ratio and hatchability percent.

| Items | Egg weight (g) | Hatch weight of chicks (g) | Rat of chicks weight to egg weight | Hatchability % |
|-------|----------------|----------------------------|----------------------------------|----------------|
| T1    | 60.73±0.80     | 47.32±0.80                 | 77.92±0.78                       | 92.01±4.11     |
| T2    | 60.90±0.92     | 49.56±0.75                 | 81.28±0.90                       | 96.36±3.08     |
| T3    | 60.81±0.78     | 48.40±0.94                 | 79.61ab±1.02                     | 95.05±2.15     |
| T4    | 60.8±0.79      | 47.43±0.77                 | 78.01ab±0.83                     | 94.21±3.57     |

a,b: Means within a column with different superscripts are significantly different (P<0.01).

Sig. = Significance, * (P<0.01), ns = not significant.

Table 2: Effect of In-ovo injection with broiler eggs with Nano form (Mean ±SE) on carcasses treaties of chicks at 7 day of age.

| Items | BW (g)  | GW (%)  | SIW (%) | DTW (%) | SIL (cm) |
|-------|---------|---------|---------|---------|----------|
| T1    | 462.00±1.05 | 3.96±4.02 | 6.56±3.01 | 11.32±3.50 | 44.50±2.40 |
| T2    | 490.00±1.21 | 5.74±4.11 | 7.50±2.71 | 17.34±3.11 | 50.57±2.50 |
| T3    | 448.67±1.89 | 4.91±4.89 | 7.37±2.90 | 13.69±3.72 | 49.10±2.87 |
| T4    | 480.8±1.11  | 5.33±4.56 | 7.50±2.88 | 16.67±2.85 | 48.00±2.32 |

a,b: Means within a column with different superscripts are significantly different (P<0.01).

Sig. = Significance, * (P<0.01), ns = not significant.

BW= body weight; GW= gut weight; SIW= small intestine weight; DTW= digestive tract weight and SIL= small intestine length.
Table 3: Effect of In-ovo injection with broiler eggs with Nano form (Mean ±SE) on white blood cell definition treaties of chicks at 7 day of age

| Items | WBC (10⁹/l) | LY % | MO% | BA% |
|-------|-------------|------|-----|-----|
| T1    | 53.30b ± 5.9 | 52.79b ± 0.01 | 0.56e ± 0.10 | 0.01±0.01 |
| T2    | 68.11b ± 5.9 | 67.28c ± 0.01 | 0.76c ± 0.10 | 0.01±0.01 |
| T3    | 42.41b ± 0.01 | 42.32b ± 0.01 | 0.16b ± 0.10 | 0.01±0.01 |
| T4    | 43.63b ± 0.01 | 43.42b ± 0.01 | 0.17b ± 0.10 | 0.01±0.01 |

a,b: Means within a column with different superscripts are significantly different (P< 0.01).

Table 4: Effect of In-ovo injection with broiler eggs with Nano form (Mean ±SE) on hematological parameters of chicks at 7 day of age

| Items | RBC(x10⁶) | HGB(g/dl) | HCT (%) | MCV µm (fl) | MCH(pg) | MCHC (%) | PLT |
|-------|-----------|-----------|----------|-------------|----------|-----------|-----|
| T1    | 1.23±0.19 | 6.00±0.28 | 18.42±1.90 | 148±4.30 | 48.92±0.81 | 32.7±1.01 | 119±5.8 |
| T2    | 1.53±0.20 | 7.1±0.30  | 20.89±1.99 | 135±3.85 | 46.58±0.98 | 34.2±2.33 | 84±5.9 |
| T3    | 1.2±0.25  | 5.4±0.29  | 16.34±0.22 | 135±3.05 | 45.58±0.75 | 33.5±1.88 | 74±6.4 |
| T4    | 1.29±0.22 | 5.8±0.27  | 16.42±0.99 | 126±3.99 | 45.43±0.90 | 35.7±0.97 | 71±6.2 |

Table 5: Effect of In-ovo injection with broiler eggs with Nano form (Mean ±SE) on serum analysis of chicks at 7 day of age

| Items | Cr (mg/dL) | AST(g/dL) | ALT(g/dL) | TL (mg/dL) | TC (mg/dL) | TG (mg/dL) |
|-------|------------|-----------|-----------|------------|------------|------------|
| T1    | 0.90±0.09  | 184±1.50  | 20.80±1.46 | 455±20.32  | 159±0.04   | 277±5.34   |
| T2    | 0.88±0.09  | 199±1.44  | 18.40±1.01 | 567±20.32  | 137±0.02   | 169±5.34   |
| T3    | 0.89±0.09  | 207±1.46  | 24.40±1.08 | 532±20.32  | 157±0.22   | 230±5.34   |
| T4    | 1.20±0.09  | 198±1.48  | 23.60±1.44 | 555±20.32  | 176±0.03   | 256±5.34   |

Table 6: Effect of In-ovo injection with broiler eggs with Nano form (Mean ±SE) on serum analysis of chicks at 7 day of age

| Items | TP (g/dL) | Al (g/dL) | Gl (g/dL) | A/G % |
|-------|-----------|-----------|-----------|-------|
| T1    | 2.40±0.48 | 1.11±0.11 | 1.31±0.14 | 0.84±0.08 |
| T2    | 3.85±0.55 | 1.49±0.11 | 2.36±0.14 | 0.63±0.08 |
| T3    | 3.56±0.61 | 1.92±0.11 | 1.64±0.14 | 0.85±0.08 |
| T4    | 2.78±0.50 | 1.68±0.11 | 1.10±0.14 | 0.65±0.08 |

Table 7: Effect of In-ovo injection with broiler eggs with Nano form (Mean ±SE) on serum analysis of chicks at 7 day of age

| Items | Tg (g/dL) | Al (g/dL) | Gl (g/dL) | A/G % |
|-------|-----------|-----------|-----------|-------|
| T1    | 2.40±0.48 | 1.11±0.11 | 1.31±0.14 | 0.84±0.08 |
| T2    | 3.85±0.55 | 1.49±0.11 | 2.36±0.14 | 0.63±0.08 |
| T3    | 3.56±0.61 | 1.92±0.11 | 1.64±0.14 | 0.85±0.08 |
| T4    | 2.78±0.50 | 1.68±0.11 | 1.10±0.14 | 0.65±0.08 |
Table 8: The effect of in ovo injection of broiler on final weight, weight gain, feed intake and feed efficiency ratio at 35 day of age

| Items | Chick Weight (g) | Final weight (g) | Weight gain (g period) | Feed intake (g period) | Feed conversion ratio |
|-------|------------------|------------------|------------------------|-----------------------|----------------------|
| T1    | 47.41±0.08       | 1499.23±22.82    | 1451.82±21.89          | 2822.75±28.22         | 1.94±0.09            |
| T2    | 49.57±0.10       | 1890.43±24.55    | 1840.86±23.08          | 2543.45±30.01         | 1.38±0.17            |
| T3    | 46.40±0.13       | 1765.27±26.78    | 1718.87±25.66          | 2886.80±32.05         | 1.68±0.24            |
| T4    | 47.43±0.18       | 1540.90±25.91    | 1493.47±26.14          | 2676.80±27.08         | 1.79±0.11            |

Sig.: a,b: Means within a column with different superscripts are significantly different (P<0.01).

Sig.: = Significance, * (P< 0.01), ns = not significant

Table 9: The effect of In-ovo injection of broiler on carcass traits at 35 day of age

| Items | LBW | HBW | CBW | DTW | DTL | DTW |
|-------|-----|-----|-----|-----|-----|-----|
| T1    | 1514.00±23.22 | 1459.80±22.22 | 1324.14±23.63 | 190.80±5.5 | 4.08±0.8 | 190.80±20.5 |
| T2    | 1940.00±23.22 | 1881.33±22.22 | 1798.10±23.63 | 212.67±5.5 | 6.29±0.8 | 221.67±20.5 |
| T3    | 1811.67±23.22 | 1770.33±22.22 | 1622.61±23.63 | 188.67±5.5 | 5.66±0.8 | 188.67±20.5 |
| T4    | 1725.67±23.22 | 1668.67±22.22 | 1349.53±23.63 | 200.67±5.5 | 4.80±0.8 | 200.67±20.5 |

Sig.: a,b: Means within a column with different superscripts are significantly different (P<0.01).

Sig.: = Significance, * (P< 0.01), ns = not significant.

Table 10: The effect of in ovo injection of broiler on white blood cell defection at 35 day of age

| Items | WBCS | LY% | MO% | BAS% |
|-------|------|-----|-----|------|
| T1    | 144.77±20.4 | 60.00±5.6 | 31.67±6.7 | 5.00±1.2 |
| T2    | 142.33±20.4 | 61.33±5.6 | 29.67±6.7 | 5.33±1.2 |
| T3    | 104.23±20.4 | 52.00±5.6 | 38.00±6.7 | 6.00±1.2 |
| T4    | 118.00±20.4 | 53.67±5.6 | 36.67±6.7 | 6.00±1.2 |

Sig.: a,b: Means within a column with different superscripts are significantly different (P<0.01).

Sig.: = Significance, * (P< 0.01), ns = not significant. White blood cells (WBC), 10^9/l, Eosinophils (EOS), Monocytes (MO), Basophils (BAS), Lymphocytes (LYM),

Table 11: The effect of In-ovo injection of broiler on hematological parameters at 35 days of age.

| Items | Hb (g/l) | RBCS (10^6/l) | HCT % | MCV µm (fl) | MCH (pg) | MCHC % | PLT |
|-------|----------|--------------|-------|-------------|----------|--------|-----|
| T1    | 11.57±2.51 | 2.78±0.98 | 35.50±2.6 | 127.53±20.7 | 44.13±4.5 | 32.30±5.9 | 36.00±5.7 |
| T2    | 10.27±2.51 | 2.94±0.98 | 35.30±2.6 | 121.67±20.7 | 38.87±4.5 | 30.37±5.9 | 37.00±5.7 |
| T3    | 4.00±2.51  | 9.70±0.98 | 2.44±2.6  | 31.67±20.7  | 119.67±4.5 | 31.67±20.7 | 40.53±5.7 |
| T4    | 10.77±2.51 | 6.70±0.98 | 3.00±2.6  | 22.37±4.5   | 29.67±4.5  | 2.44±2.6  | 37.33±5.7 |

Sig.: a,b: Means within a column with different superscripts are significantly different (P<0.01).

Sig.: = Significance, * (P< 0.01), ns = not significant. Hemoglobin (Hb), Red blood cells (RBC), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV).
Table 12: Effect of In-ovo injection with broiler eggs with Nano form (Mean± SE) on plasma fractions (g/dl) of broiler chicks at 35 day of age.

| Items | Cr(mg/dl) | ALT(mg/dL) | AST(mg/dL) | TL(mg/dL) | TC(mg/dL) | TG (mg/dL) |
|-------|-----------|------------|------------|-----------|-----------|------------|
| T1    | 0.51±0.08 | 164.60±20.4| 25.34±5.45 | 409.20±40.80 | 139.80±5.98 | 166.80±35.50 |
| T2    | 0.73±0.09 | 194.00±20.4| 10.73±5.45 | 431.67±40.80 | 134.67±5.98 | 234.33±35.50 |
| T3    | 0.56±0.11 | 159.00±20.4| 10.73±5.45 | 431.67±40.80 | 143.33±5.98 | 159.67±35.50 |
| T4    | 0.62±0.07 | 173.67±20.4| 13.63±5.45 | 432.00±40.80 | 143.67±5.98 | 215.67±35.50 |

Sig.  ns  Ns  ns  ns  ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.

Table 13: The effect of In-ovo injection of broiler on blood parameters of broiler chicks at 35 day of age.

| Items        | T1       | T2       | T3       | T4       | Sig. |
|--------------|----------|----------|----------|----------|------|
| TP (g/dL)    | 2.82±0.48| 2.95±0.48| 2.48±0.48| 2.78±0.48| ns   |
| AL (g/dL)    | 1.37±0.11| 1.45±0.11| 1.33±0.11| 1.44±0.11| ns   |
| G (g/dL)     | 1.46±0.14| 1.51±0.14| 1.15±0.14| 1.34±0.14| ns   |
| A/G ratio    | 1.12±0.08| 1.14±0.08| 1.21±0.08| 1.08±0.08| ns   |
| T3(nmol/ L)  | 1.38±0.85| 1.76a±0.85| 1.98a±0.85| 1.58b±0.85| ns   |
| IGG(nmol/ L) | 3.96±0.31| 2.98±0.31| 5.43±0.31| 6.75±0.31| *    |
| IGM(nmol/ L) | 5.18±0.24| 5.93±0.24| 3.32±0.24| 4.43±0.24| *    |
| CK (mg/dl)   | 176.20±20.51| 162.00±20.51| 166.00±20.51| 174.03±20.51| *    |

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.