http://dx.doi.org/10.22161/ijeab/3.2.6 *ISSN: 2456-1878*

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

Amal M. Hassan

Cairo, Egypt. B.O.P. 11753Matariya Street, 1 Mathaf El-matria & Poultry Production Division, Desert Research Center (DRC).

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 5weeks.*

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 (SENPs)/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:

Hatchability percent (%) = (Number of hatched chicks / number of eggs that were fed in ovo at 21 days) × 100.While, Chick weight is to egg weight ratio= (Chick weight (g)/ Egg weight (g)) ×100.

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 (35.70C ±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-iodothronine) were measured by ELISA technique using IMMUNOSPEC kits supplied by

(Immunospec Corporation, 7018 Owensmounth Ave. Suite 103 Canoga Park, CA 91303, USA).

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, μ = 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, hatching 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 hatching weight (g) and ratio of chicks weight to egg weight % and hatchability %, while, Nano-zinc recorded 2.28, 2.17 and 3.30 % for hatching 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 hatching 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 14^{th} 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.

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 and1.63%), HGB by about (18.14 and 3.49%), MCHC by (4.59 and 2.14%), HCT by about (13.41 and 4.99 %), RDWCV by about (4.00 and 2.00%) and 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 .016 %, 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. Zhanga *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 selenium - dependent enzyme biosynthesis but with much lower acute toxicity (Zhanga, *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.

REFERENCES

[1] Akshat G., S.K. Bhanja, M. Mehra and V. Pande. 2017. In ovo supplementation of selenium or iron enhanced the expression of immune related genes in broiler chickens. https://www.researchgate.net [accessed Nov 04 2017].
[2] Altan, O.; Altan, A.; Oguz, I.; Pabuccuolu, A.; and Konyaliouglu, S., 2000. Effects of heat stress on growth, some blood variables and lipid oxidation in broilers exposed to high temperature on broiler exposed to high temperature at an early age. Br. Poult. Sci., 41:489-493.
[3] Arthur, J.R., R.C. McKenzie, G.J. Beckett, 2003. Se in the immune system. J. Nutrition. 133, 1457-1459.
[4] ARTHUR, J.R., R.C. MCKENZIE, G.J. BECKETT, 2003. Se in the immune system. J. Nutrition. 133, 1457-1459.
[5] Bhanja, S.K., Mandala, A.B. and Johri, T.S., 2004. Standardization of injection sites, needle length, embryonic age and concentration of amino acids for in ovo injected in broiler breeder eggs. Indian J. Poult. Sci., 39: 105-111
[6] Cardoso, A., Albuquerque, R. and Tessari, E., 2007. Humoral immunological response in broilers vaccinated against Newcastle disease and supplemented with dietary zinc and vitamin E. Rev. Bras. Cien. Avic. 8(2): 2501-2509.
[7] Duncan, D.B., 1955: Multiple range and multiple F tests. Biometrics. 11, 1-42.
[8] Ferket, P.R., 2011. In ovo feeding and the promise of perinatal nutrition. In: Proceedings of Alltech International Nutrition Symposium, Lexington, Kentucky, United States of America.
[9] Kidd, M.T., 2003. A treatise on chicken dam nutrition that impacts progeny. World Poult. Sci. J., 59: 475-494.
[10] Kidd, M.T., Anthony, N.B. and Lee, S.R., 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci., 71: 1201-1206.
[11] LI, M., and C. ZHAO, 2009: Study on tibetan chicken embryonic adaptability to chronic hypoxia by revealing differential gene expression in heart tissue. Sci. China C. Life Sci. 52, 284-295.

[12] Lozoff, B., N. Kaci rot and T. Walter, 2006. Iron deficiency in infancy: Applying a physiologic framework for prediction. Am. J. Clin. Nutr. 84, 1412-1421.
[13] Ludwiczek, S., I. Theurl, E. Artner- Dworzak, M. Chorney and G. Weiss, 2004. Duodenal HFE expression and hepcidin levels determine body iron homeostasis: Modulation by genetic diversity and dietary iron availability. J. Mol. Med., 82: 373-382.
[14] NRC (National Research Council), 1994. Selenium in nutrition. Rev. ed. Subcommittee on Selenium. National Academy Press. Washington, DC.
[15] Patric Joshua1 P., C. Valli and V. Balakrishnan, 2016. Effect of in ovo supplementation of Nano forms of zinc, copper, and selenium on post-hatch performance of broiler chicken. Veterinary World, Vol.9/March-2016/11.
[16] Peng, D., Zhang, J., Liu, Q. and Taylor, E.W, 2007. "Size effect of elemental selenium nanoparticles (Nano-Se) at supranutritional levels on selenium accumulation and glutathione S-transferase activity", Journal of Inorganic Biochemistry, Vol. 101(10), pp. 1457-1463.
[17] Quinteiro-Filho, WM, Gomes AV and Pinheiro, M. L., 2012. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with Salmonella enteritis's. Avian Pathol 41:421–427.
[18] Razi, K.M., Maamoury, R.S. and Banihashemi, S., 2011. Preparation of nano selenium particles by water solution phase method from industrial dust. Int. J. Nano Dimens., 1(4): 261-267.
[19] Ru-Duan, W., W. Chang-Sen and F. Zuo-Hua, L. Yi, 1992. Investigation on the effect of selenium on T lymphocyte proliferation and its mechanisms. J. Tongji Med. University. 12, 33-38.
[20] SAS, (2010). SAS proprietary Software, Release 9. 1. Cary, NC. SAS Institute, Inc.
[21] Shankar, A.H. and Prasad, A.S., 1998. Zinc and immune function: The biological basis of altered resistance to infection. Am. J. Clin. Nutr. 68: 447-463.
[22] Surai, P.F., 2002. Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. World's Poult. Sci. 58, 431-450.
[23] Syafwan S, Kwakkel R. P. and M.W.A. Verstegen, 2011. Heat stress and feeding strategies in meat-type chickens. Worlds Poult. Sci. J 67:653–674.
[24] Whitnall, M. and D.R. Richardson, 2006. Iron: A new target for pharmacological intervention in neurodegenerative diseases. Seminars Pediatric Neurol. 13, 186-197.
[25] Zhang, Z. W., Q. H. Wang, J. L. Zhang, S. LI, X. L. Wang and S. W. XU., 2012. Effects of oxidative stress on immunosuppression induced by selenium deficiency in chickens. Biol. Trace Elem. Res. 149, 352-361.
[26] Zhang, Z. W., Q. H. Wang, J. L. Zhang, S. Li, X. L. Wang, S. W. Xu. 2012. Effects of oxidative stress on immunosuppression induced by selenium deficiency in chickens. Biol. Trace Elem. Res. 149, 352-361.

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^{b}±0.80	77.92^{b}± 0.78	92.01± 4.11
T2	60.90±0.92	49.56^{a}± 0.75	81.28^{a}±0.90	96.36±3.08
T3	60.81±0.78	48.40^{ab}±0.94	79.61^{ab}±1.02	95.05±2.15
T4	60.8±0.79	47.43^{ab}±0.77	78.01^{ab}±0.83	94.21±3.57
Sig.	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.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	480.83±1.11	5.33±4.56	7.50±2.88	16.67±2.85	48.00±2.32
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.
BW= body weight; GW= gut weight, SIW= small intestine weight, DTW= digestive tract weight and SIL= small intestine length.

http://dx.doi.org/10.22161/ijeab/3.2.6 *ISSN: 2456-1878*

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 (10^9/l)	LY %	MO%	BA%
T1	53.30^{ab} ± 5.9	52.79^{ab} ±6.01	0.56^{ab} ±0.10	0.01±0.01
T2	68.11^{a} ±5.9	67.28^{a} ±6.01	0.76^{a} ±0.10	0.01±0.01
T3	42.41^{b} ±5.9	42.32^{b} ±6.01	0.16^{b} ±0.10	0.01±0.01
T4	43.63^{b} ±5.9	43.42^{b} ±6.01	0.17^{b} ±0.10	0.01±0.01
Sig.	*	*	*	ns

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), Lymphocytes (LY), Basophils (BA), Monocytes (MO), Eosinophils (EOS)

Table.5: 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($x10^6$)	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
Sig.	ns	ns	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.
RBC= read blood cell; HG= hemoglobin; HCT= hematocrit; MCV= mean curricular volume; MCH= Mean corpuscular hemoglobin, pg; MCHC= Mean corpuscular hemoglobin concentration; PLT= plaited cell.

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	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
Sig.	ns	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.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	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
Sig.	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.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^{ab}±0.08	1499.23^{b}±22.82	1451.82^{b} ±21.89	2822.75^{a}±28.22	1.94^{a}± 0.09
T2	49.57^{a}±0.10	1890.43^{a}±24.55	1840.86^{a}±23.08	2543.45^{b}±30.01	1.38^{b}±0.17
T3	46.40^{b}±0.13	1765.27^{a}±26.78	1718.87^{a}±25.66	2886.80^{a}±32.05	1.68^{ab}±0.24
T4	47.43^{ab}±0.18	1540.90^{b}±25.91	1493.47^{b}±26.14	2676.80^{b}±27.08	1.79^{ab}±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	221.67±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	ns	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.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.	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. 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	T1	T2	T3	T4
Hb (g/l)	11.57±2.51	10.27±2.51	4.00±2.51	10.77±2.51
RBCS (10^{6}/l)	2.78±0.98	2.94±0.98	9.70±0.98	3.00±0.98
HCT %	35.50±2.6	35.30±2.6	2.44±2.6	35.13±2.6
MCV µm (fl)	127.53±20.7	121.67±20.7	31.67±20.7	88.67±20.7
MCH (pg)	44.13±4.5	38.87±4.5	119.67±4.5	22.37±4.5
MCHC %	32.30±5.9	30.37±5.9	38.57±5.9	30.20±5.9
PLT	36.00±5.7	37.00±5.7	40.53±5.7	37.33±5.7
Sig	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. Hemoglobin (Hb), Red blood cells (RBC), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV).

http://dx.doi.org/10.22161/ijeab/3.2.6 *ISSN: 2456-1878*

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	17.87±5.45	353.33±40.80	143.33±5.98	159.67±35.50
T4	0.62±0.07	173.67±20.4	13.63±5.45	342.00±40.80	143.67±5.98	215.67±35.50
Sig.	ns	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: Table 2: 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^{c}±0.85	$1.76a^{b}$±0.85	1.98^{a}±0.85	$1.58b^{c}$±0.85	ns
IGG(nmol/ L)	3.96^{d}±0.31	2.98^{e}±0.31	5.43^{b}±0.31	6.75^{a}±0.31	*
IGM(nmol/ L)	5.18^{b}±0.24	5.93^{a}±0.24	3.32^{c}±0.24	4.43^{c}±0.24	*
CK (mg/dl)	176.20^{a} ±20.51	162.00^{b}±20.51	166.00^{b}±20.51	174.03^{a}±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.