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Effect of *IN-OVO* injection with Nano Iron - Particles on Physiological Responses and Performance of Broiler Chickens under Saini Conditions

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Abstract— *A total of 600 fertile eggs, in a completely randomized design were used to investigate the effects of Iron nano-particles IN-OVO injection on productive performance, immune status and physiological responses in broiler chickens. The eggs were divided into 6 groups that assigned as: T1 (control; without injection), T2 (injected with 0.1 ml saline 9.0%; sham control), T3; (injected with 0.1 ml of 20 ppm Fe-NPs organic, T4 (injected with 0.1 ml of 20 ppm Fe-Nano inorganic), T5 (injected with 0.1 ml of 20 ppm Fe organic) and T6 (injected with 0.1 ml of 20 ppm Fe-inorganic). At 7th day of incubation, the corresponding doses were in- ovo injected in 0.1 ml solution into the air sac.*

The results showed that: Hatchability was highly significant (P< 0.01) in T1, 0.1 ml of 20 ppm Fe-NPs, 0.1 ml of 20-ppm Fe-NPs-Alimet chelate, 0.1 ml of 20 ppm Fe-Aliment chelate and 0.1 ml of 20-ppm Fe-Aliment chelate. The egg weight was higher (P< 0.01) in T2. There was an increase (P< 0.01) in chick weight in controls, other Fe-NPs organic or Fe-NPs-inorganic and Fe organic in comparison with other treatments. In addition, chick body weight to egg weight ratio in controls, Fe-Nano organic and FeNPs- inorganic was higher (P < 0.01) than in the other groups. T3 has shown the highest (P< 0.01) relative weight compared to the other treatments. Serum Fe content and liver function were (P< 0.01) higher in by using Fe-NPs, Fe-NPs alimet inorganic and Fe-organic than other treatments. The treatments of Fe-NPs- organic and Fe-Aliment chelate, chickens' blood hemoglobin increased significantly compared with the other treatments. These results suggest that Fe-NPs, Fe-NPs-Alimet chelate and Fe-Alimet chelate improved embryonic growth and development.

Keywords— Broiler chicken, hatchability, in- ovo, iron nano-particles, immunity.

I. INTRODUCTION

Minerals play a vital role for maintaining homeostatic conditions in living organisms. Nanotechnology (the use of nano-particles of diameters between 1 and 100 nm) is nowadays applied in science, engineering, and agriculture (Scott and Chen, 2002 and Oberdorster and Donaldson, 2007). Nano-particles activities depend on their physical and chemical characteristics. Nanoparticles can show unique biological behavior, yet, the main mechanism of their action is still unknown (Shimizu, *et al.,* 2009). These particles have features, such as large surface area (increasing physical, chemical, and biological activities) and higher solubility and mobility (Diman*et al.,* 2018 and Toyooka, *et al.,* 2009). High surface to volume ratio allows the functionalizing of nanoparticles with different ligands, coatings and other useful tools for lots of biomedical applications. High surface to volume ratio allows the functionalizing of nanoparticles with different ligands, coatings and other useful tools for lots of biomedical applications. Thus, it allows the functionalizing of nanoparticles with different ligands, coatings and other useful tools for lots of biomedical applications. However, the new physical and chemical properties of novel engineered nanoparticles make them extremely attractive for use in applications like medical sciences (Park, *et al*., 2010). Nano-particles have many novel properties compared with the bulk materials. Thus, inorganic nano-particle elements are widely used to enhance the productive performance of livestock, Ma *et al.,* (2006).

Embryonic development relays upon the availability of the required nutrients within the egg. Nutrient management *in-ovo* may provide an alternative method for poultry industry to increase hatchling weight. Chicks are affected by the nutrients in yolk remaining in the peritoneal cavity post hatching (Romanoff, 1960). Thus, a continually and precisely regulated supply of trace elements derived from stores within the egg is essential to ensure avian embryonic survival.

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Iron (Fe) is essential for a variety of physiological processes in livestock (e.g. DNA synthesis, oxygen transport, etc.) as illustrated by Lozoff*et al.*, (2006);Whitnall and Richardson, (2006) and Li and Zhao, (2009). NRC (1994) recommended 50-120 ppm daily intake of iron for poultry. Iron in the form of nano-particles has been reported to be less toxic than inorganic iron salts (Nikonov*et al.*, 2012). Additionally, they have prolonged effects on biological activities (Kovalenko and Folmanis, 2006). Iron nano-particles are more stable in air and have the ability to be degraded or metabolized in vivo, making them excellent candidates for a large number of applications (Bronstein *et al*., 2007).

Iron oxide nanoparticles (IONPs) are frequently used in biomedical applications, yet their toxic potential is still a major concern. While most studies of biosafety focus on cellular responses after exposure to nanomaterials, little is reported to analyze reactions on the surface of nanoparticles as a source of cytotoxicity. Results showed that IONPs had a concentration-dependent cytotoxicity on human glioma U251 cells, and they could enhance H_2O_2-induced cell damage dramatically. However, many studies have been conducted to evaluate the potential toxicity of iron oxide nanoparticles, Das, *et al.*, (2007).

The goal of present study was to investigate the effects of *in- ovo* injection of iron, iron nanoparticle and iron chelates nanoparticles methionine during broiler embryonic devolvement on productive performance, physiological and immunological responses and the absorption of iron.

II. MATERIALS AND METHODS

Experimental Design and Management

A total of 600 fertile broiler eggs obtained from cobb500™ parent stock were randomly divided into six equal groups. Eggs were individually weighed with an average of 60.83± 0.80g. Eggs were set in the hatchery and injection site was disinfected with ethyl alcohol, sealed with wax after injection then transferred to hatching baskets. The eggs were divided into 6 groups that assigned as: T1 (control; without injection), T2 (injected with 0.1 ml saline 9.0%; sham control), T3; (injected with 0.1 ml of 20 ppm Fe-NPs organic, T4 (injected with 0.1 ml of 20 ppm Fe-Nano inorganic), T5 (injected with 0.1 ml of 20 ppm Fe organic) and T6 (injected with 0.1 ml of 20 ppm Fe-inorganic). At 7th day of incubation, the corresponding doses were *in-ovo* injected in 0.1 ml solution into the air sac. Iron oxide nano-particles were prepared according to Reimers and Khalafalla (2011), suspended in Kno DMEM cell culture medium and dispersed by an ultrasonic bath. The injection was performed at day 7 of incubation into the air sac. Eggs were candled on 7th day of hatchery and 17th day to remove infertile eggs. Aliment according to HMTBA, Novus International, Inc., Charles, MO, USA. Iron Aliment Chelate according to Predieri*et al*. (2005), Fe-Nano Alimet Chelate Based on Marinescu *et al.* (2006).

Post- hatch, a total number of 360 one-day-old chicks were randomly distributed into six equal| (n = 60 / treatment) groups with three replicates (20 chicks/ each) according the corresponding treatments.

Experimental 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 (AT, ^{o}C) and Relative Humidity (RH, %) were recorded using electronic digital thermo-hygrometer. Average of AT and RH was 35.7 ±0.98^{o} C and 24.2 ±1.32 %, respectively. Feed was offered *ad libitum* according to NRC (1994) recommendations. Fresh water was made available all the daytime. Live body weight and feed intake were recorded weekly before offering feed. At the end of the trial, five broiler chicks from each group were picked randomly for blood sampling.

Blood samples (n= 30) were randomly withdrawn from 5 chicks immediately before slaughtering of chicks (at day 35) from the (brachial) wing vein into tubes containing EDTA as anticoagulant and centrifuged at 3000 rpm for 20 minutes for the separation of plasma and kept at (−20°C) until further analysis.

Experimental traits:

1. Hatchability percentage and ratio of chick weight to egg weight.
2. Weekly body weight, body weight gain, feed consumption and feed conversion ratio.
3. Hematological parameters: Red blood cells count, and hemoglobin concentration were measured immediately after blood collection.

4. Blood metabolites: Total protein (TP), albumin (AL), total lipids (TL), Triglycerides (Tg), cholesterol, iron, TIBC and ferritin, liver enzymes (alanine transaminase (ALT), aspartic transaminase (AST)), plasma immunoglobulin IgG and IgM concentration, creatinine (Cr) and globulin and albumin ratio (A/G ratio) were calculated. Blood metabolites were determined calorimetrically by using commercial kits (Bio Systems S.A. Costa Brava 30, Barcelona. Spain, Barcelona).
5. Blood hormones: Triiodothyronine (T3) hormoneswas measured by ELISA technique using IMMUNOSPEC kits supplied by (Immunospec Corporation, 7018 Owensmounth Ave. Suite 103 Canoga Park, CA 91303, USA).

Statistical analysis was carried out using General Linear Model (GLM) procedures by SAS (2010) using simple one-way analysis of variance. Significant differences among treatment groups were tested using Duncan's multiple range tests, Duncan, (1955)**.**

III. RESULTS AND DISCUSSION

Effect of ovo injection by Fe-Nano, Fe-Nano-Alimet chelate, Fe-Aliment chelate and Fe-Aliment chelate on hatchability traits

Table (1) shows the egg performance when injected with different forms of supplementary Iron. . There was a significant difference (P<0.01) between control and sham control with respect to hatchability percent. There seem to be a need for NaCl solution because of a deficiency of this mineral in the egg, which might explain the positive effect of saline injection. Sodium chloride is a mineral salt and it seemed to close a gap in the requirements of egg growth to this mineral. It might also have a positive effect with respect to buffering the medium inside the egg, which led to facilitating the growth performance, livelihood of the embryo and therefore the hatchability percent improved as a result. It seems that these explanations are logic since there was no significant difference between sham control (saline solution injection) and injection of different forms of Iron either as in nano particle form or not and the form of being organic or inorganic. The different forms of Iron in nano particle or in the organic or inorganic forms showed the same significant difference as the saline solution injection did. The same explanations might, therefore, apply. The check weight/egg weight ratio of control and sham control were not significantly different (74.5 and 74.8 for control and sham control, respectively). The injection of different forms of Iron positively enhanced this ratio. The ratios were 85.2, 4.6 and 84.4 for T3, T4 and T5, respectively).The inorganic form of Iron (T6) was similar to both controls. Saki *et al.* (2014) found no significant effects on hatchability percent among the groups fed 50 and150 ppm Fe-Aliment chelate relative to control one. This may be explained by the deficiencies or excesses of individual trace elements that can cause impaired growth, abnormal development, thus, affecting all of the major organ systems and in extreme cases, death of the embryo (Richards and Steele, 1987). Appropriate amounts of each trace element are required to support embryonic growth and development, Richards, (1989). In mammals, Fe link to amino acids increased the transfer of Fe across the placenta and into the embryo, Ashamead and Graff, (1982)**.**

The form of nano Fe in any form depends on the presence of protein and it would be interesting to investigate the relationship between protein and Fe atoms. Foye, *et al*., (2006) found that Fe atoms adhered easily to protein and that the co-existing system of protein and iron could directly scavenge ROS (OH•, O•− 2 and H2O2). 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.

Growth performance at 7 day of age:

Effects of *in-ovo* injection of nano forms of Fe-Nano particles (either organic or inorganic) on average weight gain and feed efficiency ratio of broiler during the first week of age are shown in table (2). Body weight (gm) values during the first week gradually increased significantly (P<0.01). The control group showed the lowest body weight over the period of first seven day period (90.55 gm). Sham control showed higher significant body weight (120.5 gm) over this period compared to regular control. It was lower than the treatment of the injection of nano-Iron in either form (132.4 and 123.9 gm for T3 and T4, respectively). The injection of regular Fe salt in both forms (organic and inorganic) showed lower (105.99 and 118.9 gm for organic and inorganic forms of regular Fe injection, respectively) body weight than both controls. Therefore, the percent increments of T2, T3 and T4 were 33.68, 46.26 and 36.83%, compared to T1, respectively. Therefore, the weight gains of T2, T3 and T6 were significantly (P<0.01) higher compared to other treatments. They increased by 65.69, 65.69 and 58.01 % than T1. With regard to feed intake, T2, T3, T4 increased by 59.03, 37.9, 6.19 %, respectively, than that of the T1 control. Results of feed conversion ratio (gm feed/gm gain) revealed a highly significant difference (P<0.01) among the experimental treatments (97.75, 155.45, 134.8, 103.8, 112.75, and 155.75 for T1, T2, T3, T4, T5 and T6,

respectively). It is observed that T3, T4, T2 and T5 recorded the best FCR and this may be due to the increase in feed intake and reduction of daily weight gain. This explained was introduced byFoye, *et al*., (2006) who noted that, *in-ovo* injection could lead to improved digestive capacity, increased growth rate and feed efficiency. Uni.*et al.,* 2005 and Foye, *et al*., (2006) reported that the breast weight percentage was not significantly different among all treatments.

Growth performance at 35 day of age:

Effects of *in-ovo* injection by nano forms of Fe-Nano particles on average weight gain and feed efficiency ratio of broiler during the experimental period (0-5 weeks of age) are shown in table (3). The weight gain (gm) of the T2, T3, T4, T5 and T6 (2101.94, 2118.94, 2124.6, 2049.67 and 2003.47 gm, respectively) significantly (P<0.05) increased than T1 (1855.23 gm). They increased by 13.29, 14.2, 10.27 and 10.48% than T1. It is clear that T2, T3, T4 were increased feed intake by 21.43, 15.03, 2.45 and 3.38%, respectively, than that of the T1 treatment. Results of feed conversion ratio (FCR) (gm feed/gm gain) revealed a significant difference (P<0.01) among the experimental treatments. It was monitored in this study, that T2, T3, T4, T5 and T6 recorded the best FCR; these results match up the increase in feed intake and reduction of daily weight gain.

Blood analysis.

The effects of *in-ovo* injection of broiler eggs on plasma iron definitions in chicks on 35day of age are shown in Table (4). The results indicate that the effect of *in-ovo* injection of broiler eggs with nano forms of Fe-Nano, Fe-NPs-Alimet chelate, Fe-Aliment chelate and Fe-Aliment chelate recorded significant increased (P<0.01) the values of WBC's, HGB, MCHC and HCT, while it was insignificant in RBC's and MCV , MCH, RDWCV and RDWSD compared to control treatment (Table 3).

On the other view, it was found through the results in table 4 that the iron injection significantly (P<0.01) enhanced different blood parameters for T2 and T1 compared to other treatment (T3, T4, T5 and T6). Which the reduction value were 25.72, 40.7, 57.76, 32.63 and 30.5% compared to T1, respectively. There were significant (P<0.01) decrease in T2, T3, T4, T5 and T6 in TIBC. This decrease were by 47.8, 97.89. 84.6, 3.86%, respectively. The same trend was observed in feretin, where there were significant deferent between T2, T3, T4 and T5 compere to T1 (by 49.45, 49.12, 33.42 and 37.87%, respectively). This data was synchronized with the data showed of hematological parameters in table 3, especially in RBC's, Hb and HTC.

The treatments 25 ppm Fe-NPs, 100 ppm Fe-NPs-Alimet chelate and 150 ppm Fe-Alimet chelate have shown higher Fe content in serum and liver compared with those in other treatments. Seo*et al*. (2008) concluded that iron content of broiler meat could be effectively enriched by supplementation of 200 ppm of Fe as Fe-Aliment chelate for 5 weeks. The results was demonstrated iron concentrations in the liver and kidney (Bertechini, *et al.,* 2012) and chickens for fattening (Shinde, *et al.,* 2011).The greatest mean increase was +22% and +31.9% for broiler muscle and liver, respectively. In addition, hemoglobin in two treatments of 100-ppm Fe-NPs- Alimet chelate and150-ppmFe-Alimet chelate significantly increased compared with other treatments.

The results of Warner *et.al.* (2006) indicated that the absolute amount of iron per liver increased steadily up to hatching time. Their results showed that the highest liver weight was observed in treatment having 25 ppm of Fe-NPs. The treatments 25 ppm Fe-Nano, 100 ppm Fe-NPs-Alimet chelate and 150 ppm Fe-Alimet chelate have shown higher Fe content in serum and liver compared with those in other treatments.

Effects of *in-ovo* injection on broiler eggs on plasma iron definitions in chicks on 35 day of age are shown in Table (5) .The data showed major variations in TP for T1, T2, T3 and T5 compared to T4 and T6, where, they were increased by (11.8, 12.57 and 2.96 %) related to T1, while the lowest value was for T6 (by 1.97 %) and no significant difference. The same trend was observed in A, G and A/G ratio. Since the albumen is synthesized mainly in liver, that liver function was enhanced by the injection of Iron in its different forms, and that the albumin is a main source for amino acid formation, the protein synthesis increased leading to more formation of muscles, which in turn leads to increased final body weight. This is clearly manifested in the results obtained in this study (Table 3).

IV. CONCLUSION

These results suggest that under semi-arid conditions, the *in-ovo* injection of 20-ppm iron nanoparticles (Fe-NPs), 20-ppm iron nanoparticles Alimet chelate (Fe-NPs-Alimet chelate) and 20-ppm Fe-Alimet chelate as injection contributed to embryonic growth development. Iron nanoparticles and Alimet chelate form, as the active in gradient of feed additives, premixes, and compound feed, due to the high surface activity and penetration into cell can actively influence the intracellular metabolism by stimulating various processes.

The nano form of Fe are not harmful to the embryo (injected with 20 ppm) and can be used to improve the post-hatch performance of broiler.

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Table.1: Mean ± SE of egg weight, checks hatching weight, ratio between egg and checks weights and hatchability percent as affected byiIn ovoinjection

Items	Egg weight (g)	Hatch weight of chicks (g)	Ratio between chicks weight to egg weight %	Hatchability %
T1	60.83^{a}±0.80	45.32^{b} ± 0.80	74.52^{b}± 0.78	92.01 ^{b} ± 4.11
T2	60.81 ^{a} ±0.79	48.56^{ab} ± 0.75	74.81^{b}±0.90	96.36 ^{a} ±3.08
T3	60.91 ^{a} ±0.78	51.90^{a}±0.94	85.22^{a}±1.02	95.05 ^{a} ±2.15
T4	60.81 ^{a} ±0.79	51.43^{a} ± 0.77	84.58^{a}±0.83	94.21 ^{a} ±3.57
T5	60.80 ^{a} ±0.91	51.33^{a} ± 0.84	84.42^{a}±0.90	95.15 ^{a} ±2.5
T6	60.82 ^{a} ±0.91	47.43^{b} ± 0.77	77.88^{ab}±0.90	94.92^{a}±0.90
Sig.	n. s	*	*	*

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

Table.2: Effect of ovov injection on broiler eggs on final weight, weight gain, feed intake and feed efficiency ratio at 7 day of age

Items	Chick Weight (g)	Body weight (g)	Weight gain (g/period)	Feed intake (g/period)	Feed conversion ratio
T1	45.32^{c}±0.80	90.55^{c}±22.82	45.23^{b}±21.89	97.75^{c}±28.22	2.15^{c}± 0.09
T2	48.56^{ab}±0.75	120.50^{a}±24.55	74.94^{a}±23.08	155.45^{a}±30.01	2.08^{b}±0.17
T3	51.90^{a}±0.94	132.44^{a}±26.78	74.94^{a}±25.66	134.80^{a}±32.05	1.80^{a}±0.24
T4	51.43^{a}±0.77	123.90^{ab}±25.91	54.67^{b}±26.14	103.8^{a}±27.08	1.89^{a}±0.11
T5	51.33^{a}±0.84	105.99^{b}±25.91	54.67^{b}±24.14	112.75a^{b}±29.23	2.05^{b}±0.17
T6	47.43^{b}±0.7	118.90^{b}±25.91	71.47^{b}±25.14	155.75^{ab}±27.25	2.18^{bc}±0.17
Sig.	*	*	*	*	*

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

Table.3: The effect of ovo injection of broiler on final weight, weight gain, feed intake and feed efficiency ratio at 35 day of age.

Items	Chick Weight (g)	Body weight (g)	Weight gain (g/period)	Feed intake (g/period)	Feed conversion ratio
T1	45.32^{c}±0.80	1900.5^{b}±22.82	1855.23^{b}±21.89	3691.75^{c}±28.22	1.99^{a}±0.09
T2	48.56^{ab} ±0.75	2150.50^{a}±24.55	2101.94^{a}±23.08	2900.45^{a}±30.01	1.38^{b}±0.17
T3	51.90^{a}± 0.94	2170.44^{a}±26.78	2118.94^{a}±25.66	3136.80^{a}±32.05	1.48^{b}±0.24
T4	51.43^{a}±0.77	2175.90^{a}±25.91	2124.67^{a}±26.14	3059.8^{a}±27.08	1.44^{b}±0.11
T5	51.33^{a}±0.84	2100.99^{ab}±25.91	2049.67^{ab}±24.14	3566.75^{ab}±29.23	1.74^{ab}± 0.09
T6	47.43^{b}±0.70	2050.90^{ab}±25.91	2003.47^{ab}±25.14	3766.75^{ab}±27.25	1.88^{ab}±0.17
Sig.	*	*	*	*	*

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

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Table.4: Effect of ovo injection on broiler eggs on plasma iron definitions in chicks on 35day of age.

TR	T1	T2	T3	T4	T5	T6
WBCS (10^9/l)	108.70^{b}±8.45	144.77^{a}±9.55	142.33^{a}±8.24	104.23^{b}±7.99	121.27^{ab}±7.85	113.33^{b}±10.00
L1%	60.00^{ab}±2.20	60.00^{ab}±2.30	61.33^{a}±2.40	52.00^{c}±2.00	56.67^{abc}±2.33	59.67^{ab}±2.22
N1%	31.67^{abc}±1.96	31.67^{abc}±2.01	29.67^{c}±1.92	38.00^{a}±2.12	35.33^{abc}±1.89	30.33^{bc}±1.89
M1%	5.00^{a} ±0.45	5.00^{a}±0.51	5.33^{a}±0.53	6.00^{a}±0.49	5.00^{a}±0.60	6.00^{a}±0.44
E1%	3.33^{a}±0.50	3.33^{a}±0.61	3.67^{a}±0.61	4.00^{a}±0.55	3.00^{a}±0.70	4.00^{a}±0.50
HB (g/l)	10.60^{a}±0.65	11.57^{a}±2.30	10.27^{a}±1.85	10.70^{a}±1.45	10.37^{a}±2.61	10.60^{a}±1.35
RBCS ($10^{/6}$µl)	3.12^{a}±0.38	2.78^{a}±0.21	2.94^{a}±0.29	2.44^{a}±0.19	2.56^{a}±0.20	3.09^{a}±0.33
HCT %	34.80^{a}±4.16	35.50^{a}±5.56	35.30^{a}±3.96	31.67^{a}±4.47	31.30^{a}±6.12	34.27^{a}±6.32
MCV (µm, fl)	90.63^{b}±8.52	127.53^{a}±9.18	121.67^{a}±12.25	119.67^{a}±10.25	122.00^{a}±9.98	82.33^{b}±14.59
MCH (pg)	35.67^{ab}±5.52	44.13^{a}±6.72	38.87^{a}±4.56	38.57^{a}±4.95	39.57^{a}±4.04	29.63b^{c}±6.07
MCHC (µm, fl)	30.90^{ab}±6.42	32.30^{ab}±4.12	30.37^{ab}±4.25	29.67^{b}±5.21	32.53^{ab}±5.51	33.63^{a}±4.95
RDW_CV	15.23^{a}±0.91	12.17^{b}±0.74	15.50^{a}±0.81	14.97^{a}±0.81	15.50^{a}±0.84	15.50^{a}±0.75
RDW_SD	33.37^{a}±4.01	45.97^{a}±3.85	37.93^{a}±4.13	40.53^{a}±3.95	34.70^{a}±4.21	37.93^{a}±4.00

a, b, c Means within the same row with no common superscript differ significantly.
** P≤ 0.01, NS= non-significant

Table.5: Effect of ovo injection on broiler eggs on plasma iron definitions in chicks on 35 day of age.

TR	T1	T2	T3	T4	T5	T6
Iron(µg/L)	360.33^{a}± 37.98	267.67^{ab}± 38.10	213.67^{b}± 38.2	163.00^{b}± 37.99	180.33^{b} ±38.22	147.33^{b}± 38.15
TIBC(µg/L)	158.00^{b}± 25.21	276.33^{a}± 26.50	312.67^{a}± 26.19	291.67^{a}± 25.22	276.00^{a} ±26.19	343.67^{a}± 25.21
Ferritin (µg/L)	51.17^{ab}± 9.77	76.47^{a}± 10.02	76.30^{a}±9.99	68.27^{ab}± 9.89	47.53^{ab} ±9.94	40.20^{b}± 10.10

a, b, c Means within the same row with no common superscript differ significantly.
** P≤ 0.01, NS= non-significant

Table.6: Effect of ovo injection on broiler eggs on blood analysis at 35 day of age

TR	T1	T2	T3	T4	T5	T6
TP (g/dL)	2.73^{ab}±0.34	3.05^{a}±0.40	2.67^{ab}±0.39	2.40^{b}±0.37	2.96^{ab}±0.35	2.99^{b}±0.39
Alb (g/dL)	1.33^{ab}±0.18	1.70^{a}±0.22	1.50^{ab}±0.21	1.35^{b}±0.20	1.60^{ab}±0.19	1.57^{ab}±0.18
Gl (g/dL)	1.40^{a}±0.24	1.35^{a}±0.21	1.17^{b}±0.20	1.05^{b}±0.25	1.36^{a}±0.23	1.42^{a}±0.22
A/g	1.05^{ab}±1.01	0.79^{ab}±1.10	0.78^{ab}±0.98	0.78^{b}±0.99	0.85^{ab}±1.99	0.91^{a}±1.00
ALT (g/dL)	103.67^{a}±4.33	135.67^{a}±3.90	94.67^{b}±2.22	84.33^{b}±2.22	83.33^{b}±2.22	75.67^{c}±4.97
AST (g/dL)	13.27^{a}±2.28	14.17^{a}±1.80	12.53^{a}±2.10	13.50^{a}±1.90	15.40^{a}±2.08	14.40^{a}±1.98
Urea (g/dL)	12.33^{a}±1.57	15.67^{a}±1.55	13.00^{a}±1.45	13.33^{a}±1.77	14.33^{a}±1.65	16.33^{a}±1.57

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Uric Acid (mg/dL)	4.36^{b}±0.79	4.30^{b}±0.87	6.17^{a}±0.77	6.24^{a}±1.00	4.56^{b}±0.95	4.44^{b}±0.99
Cr (mg/dL)	0.52^{b}±0.06	0.5^{b}±0.05	0.66^{ab}±0.07	0.81^{a}±0.08	0.59^{b}±0.05	0.57^{b}±0.04
Ch (mg/dL)	148.33^{b}±5.98	129.67^{c}±5.29	178.00^{a}±6.19	162.67^{a}±6.49	163.00^{a}±5.99	156.67^{b}±6.29
Tg(mg/dL)	267.67^{b}±13.38	191.67^{c}±14.38	323.33^{a}±15.18	299.67^{b}±13.28	317.00^{a}±12.38	166.67^{c}±14.38
HDL (mg/dL)	60.01^{abc}±3.78	68.01^{a}±3.48	44.67^{c}±3.89	63.67^{ab}±4.00	49.33^{bc}±4.08	56.5^{bc}±3.98
LDL (mg/dL)	46.66^{a}±4.98	49.33^{a}±5.58	34.01^{b}±4.78	42.01^{ab}±5.01	33.33^{b}±4.58	44.55±4.68
T L (mg/dL)	229.01^{d}±18.83	315.33^{bc}±19.93	453.67^{a}±18.93	368.33^{b}±20.03	294.01^{c}±19.03	295^{c}±20.20
A P (U/dL)	27.03^{a}±0.99	25.82^{ab}±1.00	22.89^{ab}±0.99	18.84^{b}±0.89	25.17^{ab}±0.98	24.9^{ab}±0.99
T3 (nmol/L)	1.38^{a}±0.18	1.44^{a}±0.19	1.46^{a}±0.17	1.23^{ab}±0.18	1.07^{b}±0.17	1.25^{ab}±0.19

a, b, c Means within the same row with no common superscript differ significantly.

** P≤ 0.01, NS= non-significant