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INFLUENCE OF IN OVO INJECTION OF INORGANIC IRON AND ITS NANOPARTICLES FORM ON GROWTH, AND PHYSIOLOGICAL RESPONSE OF BROILER CHICKENS

[173]

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ABSTRACT

A total of 320 fertile eggs, were divided randomly into 4 treatments with four replicates and 20 eggs per each replicate. Treatments including: T1; control; without injection, T2; injected with 0.1ml saline solution; sham control, T3, injected with0.1 ml either containing 0.75 ppm inorganic or Nano – iron particles 75 ppm Fe-Nano inorganic, T4, injected with 75 ppm Fe inorganic On the 7th day of incubation, 0.1 ml solution was *In ovo* injected into the egg .Body weight and body weight gain were measured at biweekly intervals, at 42d of age, three hens per replicate were weighted, sacrificed for slaughtering weights of carcass, internal organs(heart, liver, and lymphoid organs i.e. (spleen, thymus and Bursa) were recorded. Blood samples were collected at slaughtering bird at 42d of age. There were significant increase in body weight and body weight gain in treatments injected with75 ppm Fe-Nano inorganic and 75 ppm Fe inorganic compared with control. There were no significantly differences among all experimental groups in dressed carcass, gizzard, and heart, liver. Abdominal fat decreased in groups injectedwith75 ppm both iron forms. Compared with the control group. No variation was observed in the weight of bursa and spleen, however thymus weight was significantly higher in both Fe inorganic and Fe nano inorganic injected groups than un-injected control group. High density lipoprotein concentration and cholesterol were significantly decreased by different treatment as compared with the control group. These results suggest that, 75 ppm Fe inorganic or75 ppm Fe-Nano injection at the 7th improved at the 7th day of incubation improved embryonic growth and development as well as decreasing post hatched chick's plasma cholesterol.

Keywords: Broiler chicken, *In ovo*, injection Iron nanoparticles, growth, blood constitutes.

INTRODUCTION

Iron (Fe) is essential trace element that playsan important role in manymetabolic processes in human and animal organism **(Dallman 1982)**. Majority of iron is present in the erythrocytes as hemoglobin (molecule that contains one hem group and one protein chain in each of its four units). Thus structure of hemoglobin will stabilize iron in the ferrous state and allow it to function as oxygen carrier from the lung where it is fully loaded with oxygen to the tissue **Lieu et al (2001)**. The **NRC (1994)** recommended 50-120 ppm of iron for poultry diet 2,000 ppm with tolerance limit of. Recently, nutritionists have been interested to altering the amount of certain menials in poultry products such as carcass and egg with recently increased consumer's interest in the nutritive value of foods. Iron content of the egg showed minimum variability with dietary change while some variation was possible in other trace minerals (**Naber, 1979).** Absorbability of minerals in monogastrics could be increased by providing them in the form of chelates **(Kratzer and Vohra, 1986; Paik, 2001).** Iron (Fe) is essential as a cofactor for the function of over 300 different enzymes (**Romanoff, 1967; Lozoff et al 2006)** and is an important structural cofactor for many proteins, including DNA synthesis and oxygen

transport **(Whitnall and Richardson, 2006; Scott et al 2008; Li and Zhao, 2009)**. Nanotechnology has the potential to impact many aspects of food and agricultural systems. Food security, disease treatment delivery methods, new tools for molecular and cellular biology, new materials for pathogen detection and protection of the environment are examples of the important links of nanotechnology to the science and engineering of agriculture and food systems. Iron nanoparticles were produced by co-precipitation from an aqueousFe3+/Fe2+ solution (ratio 3:2) using concentrated ammonium hydroxide in excess **(Reimers and Khalafalla 2011).** Iron nanoparticles and compounds may be considered a good alternative to existing treatments. Previous work has demonstrated the growth enhancement and wound healing effects of iron nanoparticles **(Gluschenko et al 2002; Sizova et al 2013),** and nano-Fe+3 has been used as a food additive (**Mohamad et al 2014).** Iron deficiency, or anemia, is thought to affect the health of more than 1 billion people worldwide **(World Health Organization, 2008).**and Agricultural animals also suffer from anemia.

Demortiere et al (2011) concluded that *In ovo* feeding of either Se or Fe at 14th day of embryonic age is beneficial for enhancing the immune response. Fe was found modulating the expression of humoral or cellular immunity related genes

Zhai et al (2015) indication that *In ovo* injected into the egg yolk sac with25, 75, 125 ppm Fe-Nano improved embryonic growth and higher chick body weightcompared with the other treatments.

Saki et al (2014) suggest that 25 ppm iron nanoparticles (Fe-Nano), 100 ppm iron nanoparticles Aliment chelate (Fe-Nano-Aliment chelate) and 150 ppm Fe-Aliment chelate as injection contributed to embryonic growth development. Thus there for present study was designed to evaluate the effect of in ovo injection of broiler eggs with different levels and forms of iron on growth performance and some blood parameters of hatched chicks.

Therefore, the objective of this study was carried out to determine the possible effect(s) of *in ovo* injection of broiler eggs with different levels and forms of iron on growth performance, blood parameters and slaughter traits of post hatch broiler chicks

MATERIALS AND METHODS

The present study was carried out in the Faculty of Agriculture, Ain Shams University, from October, November to December (2016). The laboratory work was done at Poultry Breeding Department, Animal Production Research Institute (APRI), Ministry of Agriculture, Dokki, Giza, Egypt.

Experimental design

In ovo Injection of eggs

A total of 320 eggs from Acres broiler breeder flock (Cobb500) were used for this study. All eggs were individually numbered and weighed prior to the beginning of the incubation. Average egg weight was approximately 55 g. Eggs were divided randomly into four treatment groups, 80 eggs each. The 1st group was kept as a control group without injection, the 2nd group injected with 0.75 ml saline solution (sham), The 3rdgroup was injected with 75 ppm Fe Nano inorganic. The 4th group was injected with75 ppm Fe inorganic. Fertile eggs were incubated at 37.5°C and a relative humidity of 55 to 60% during d 1-18 and at 36°C with relative humidity of 60-65% during d 19-21.On d 6 of incubation, unfertilized eggs or those with early embryonic mortality were discarded. At 7d of incubation the eggs from group two to four were injected into the air sac with 0.1 ml the previous of solutions. Immediately after the injection, the hole was sealed with sterile tape as mention before. Two sources of Iron, Fe Oxide and Fe-Nano, were used at dose (75ppm) Fe-Nano. All hatched chicks were used for a grow-out study.

Preparation of Iron nanoparticles Solutions

Iron nanoparticles were produced from an aqueous Fe^{3+}/Fe^{2+} solution using ammonium hydroxide solution. 1 mL $FeCl_3.6H_2O$ (1 M) was mixed with 1 mL $FeCl_2$ (0.5 M) were mixed in 50 ml distilled water. Thereafter, 3 g of glutamine were added at once under vigorous stirring. Finally, 100 µL of ammonium hydroxide were added rapidly. The mixture was kept under stirring for about 30 min. Iron nanoparticles have particle size ranged from 17-26 nm.

Birds and housing

To study the post-hatch performance 80 chicks from each treatment were weighed in their same respective groups and were reared for a period of 6 weeks. The experimental birds were housed in -tiered, well-ventilated battery cages provided with artificial lighting. The standard management practices were adopted, and they were uniform for all the treatment groups. All the chicks in the various treatments were fed *ad libitum* quantity of a common experimental ration. The chicks were fed with broiler starter ration from 0 to 10 days and broiler grower ration from 11 d to 6weeks. Clean drinking water was provided *ad libitum.* The ingredient and nutrient composition of the experimental diets are presented in **Table (1)**.

Table 1. Ingredients and the chemical Composition of the experimental diet

Composition (per 100 kg)	Starter (1-10 day)	Grower (11-42 day)
Yellow corn	52.28	63.19
Soybean meal (44% CP)	34.00	22.5
Corn gluten (60% CP)	6.00	6.30
Soy bean oil	3.00	4.00
Di-calcium phosphate	1.84	1.59
Limestone	1.43	1.10
L-Lysine HCl	0.32	0.28
Dl-Methionine	0.26	0.17
Sodium chloride	0.24	0.24
Sodium bicarbonate	0.23	0.23
Vitamins Premix *	0.10	0.10
Minerals Premix**	0.30	0.30
Total	100.00	100.00
Calculated analysis**		
Crude protein	23.17	21.25
Metabolizableenergy (Kcal/kg)	3100	3110
Ether extract	5.63	5.08
Crude fiber	3.80	3.45
Calcium	1.04	0.90
Av. Phosphorus	0.50	0.45
Lysine	1.44	1.24
Methionine	0.68	0.60
Methionine+cysteine	1.06	0.95
Sodium	0.15	0.16

*Supplied per kg of diet: Vit. A, 11000 IU; Vit. D3, 5000 IU; Vit. E, 50 mg; Vit K3, 3 mg; Vit. B1, 2 mg; Vit. B2 6 mg; B6 3 mg; B12, 14 mcg; Nicotinic acid 60mg; Folic acid 1.75 mg, Pantothenic acid 13mg; and Biotin 120 mcg

**Supplied per kg of diet: Choline 600 mg; Copper 16 mg; Iron 40 mg; Manganese. 120 mg; Zinc 100 mg and Iodine 1.25 mg

Physiological traits

1. Blood parameters

Blood samples were collected from 3 chicks per each replicate at 42 d of age to evaluate the blood biochemical constituents. For each sample, 5 ml blood was collected at slaughtering in heparinized tubes. The tubes were centrifuges at 4000 rpm for 15 minutes and clear plasma was separated, and then stored in a deep freezer at -20^{o}C until the time of biochemical analysis. Cholesterol, HDL, LDL triglycerides, total protein, and albumin were determined by available commercial kits (Biodiagnostic.com. Egypt).

2. Slaughter traits

At 42d of age, three hens per replicate were weighted, sacrificed for of carcass traits, internal organs (heart, liver and lymphoid organs (spleen and Bursa) weights to the nearest 0.1 gm. The relative weights of these organs were calculated in relation to live body weight.

Statistical analysis

Results were analyzed for all variables using the general linear models procedure (GLM) to establish differences between means using SAS software (version 9.1). Means showing significant differences were compared using Duncan's multiple range test. Statistical significance was based on $P< 0.05$.

RESULTS AND DISCUSSION

Body weight and weight gain

Results presented in **Table 2, 3** showed that groups injected with 75 ppm of inorganic iron and Nano inorganic iron particle improved body weight and body weight gain compared with control. These results agree with **Nikonov, et al (2011**) they reported that supplementation of diets for broiler breeders hens with Fe improved of broiler chicks Performance. **Shinde et al (2011)** found that organic sources of Fe supplementation also improved performance.

Table 2. The effect of *In ovo* injection of iron on body weight of broiler chickens at different ages, 1to 6 week of age

Week / Treatments	Body weight (g)					
	1st WK	2nd WK	3rd WK	4th WK	5th WK	6th WK
CONTROL	88.33^{b} ±1.66	208.33^{c} ±3.33	439.00^{c} ±3.78	875.33^{d} ±20.53	1426.67^{c} ±26.66	1918.7 ±42.15
SHAM	98.33^{b} ±1.66	297.00^{b} ±2.88	520.67^{bc} ±11.56	943.33^{c} ±22.78	1593.33^{b} ±52.06	1976.0 ±31.78
NANO IN ORGANIC Iron	103.33^{a} ±3.33	325.00^{ab} ±5.00	534.00^{ab} ±9.45	1083.32^{b} ±3.00	1596.67^{b} ±21.85	2143.3 ±145.78
IN ORGANIC Iron	103.33^{a} ±3.33	336.67ab ±18.55	576.67^{a} ±29.20	1133.67^{a} ±3.38	1786.67^{a} ±128.10	2266.7 ±10.13
Probability	0.0002	0.0001	0.0009	0.0001	0.0068	0.3709

Means within a column with different superscripts are significantly different (P≤0.05).

Table 3. The effect of *In ovo* injection on Body weight gain of broiler chicks

Week / Treatments	Body weight gain (g)				
	1-2 Week	2-3 Week	3-4 Week	4-5 Week	5-6 Week
CONTROL	120.00^{b} ±1.66	230.67^{c} ±3.33	436.33^{c} ±3.78	551.34^{b} ±20.53	492.03^{c} ±26.66
SHAME	198.67^{b} ±1.52	223.67^{b} ±1.85	422.66^{b} ±10.47	650.00^{a} ±22.27	382.67^{b} ±50.96
NANO IN ORGANIC	209.00^{a} ±3.71	209.33^{b} ±5.66	549.32^{b} ±9.95	513.35^{b} ±3.66	546.63^{b} ±22.12
IN ORGANIC	233.34^{a} ±3.38	240.67^{a} ±18.65	557.67^{a} ±29.68	653.67^{a} ±3.52	480.03^{a} ±128.45
Probability	0.0001	0.0001	0.0008	0.0001	0.0063

Means within column with different superscripts are significantly different (P≤0.05).

Sizova (2015) reported that the use of Iron nanoparticles increased the live weight of the chickens. also,

Saki (2014) found that Fe Nano particles increase production of broiler chicks and improve embryonic growth and development.

Carcass criteria

The results presented in **(Table 4)** indicated that the effect of treatments on dressed carcass, abdominal fat and relative weights of some edible organs such as gizzard, liver, heart. There were no significantly differences among all experimental groups in dressed carcass, gizzard, and heart, liver. Contrary, abdominal fat decreased in groups injected 75 ppm of inorganic iron and Nano inorganic iron particle compared with other groups. No variation was observed in the weight of bursa and spleen, however thymus weight was significantly higher in both Fe inorganic and Fe nano inorganic injected groups than un-injected control group. These results are agreement with those obtained by **Goel et al (2013**) found that *In ovo* feeding of iron may influence the embryonic development, while iron can play an important role in posthatch growth also.

Table 4. The effect of *In ovo* injection on organs weight (g) of broiler chickens at 6 week of age

Item / Treatment	Carcass weight (g)	gizzard weight (g)	Heart (g)	Liver (g)	fat weight (g)	Thymus weight (g)	Bursa (g)	Spleen (g)
CONTROL	1625.0^{b} ±122.20	30.677 ±2.83	8.92 ±1.31	38.89^{b} ±2.45	30.96^{a} ±0.95	2.49^{c} ±0.26	3.32 ±0.54	2.08 ±0.75
SHAME	2045.0^{a} ±55.67	29.743 ±0.91	13.36 ±1.62	69.92^{a} ±9.07	26.54^{a} ±2.25	3.91^{b} ±0.26	3.25 ±0.59	3.28 ±0.64
NANO IN ORGANIC	1820.0^{ab} ±118.14	28.233 ±1.34	12.89 ±0.92	51.38^{ab} ±7.90	19.63^{b} ±1.33	4.58^{ab} ±0.29	2.66 ±0.30	2.30 ±0.13
IN ORGANIC	2050.0^{a} ±63.50	28.163 ±1.40	12.56 ±1.50	51.70^{ab} ±1.47	17.16^{b} ±2.65	5.35^{a} ±0.76	2.68 ±0.91	2.49 ±0.53
Probability	0.0526	0.0628	0.2590	0.1319	.0001	0.0095	0.6504	0.3685

Means within a column with different superscripts are significantly different (P≤0.05).

Blood plasma Constituents

Data of blood plasma constituents throughout the experiment period are shown **(Table 5)** plasma total protein concentration, albumin and globulin were not significantly affected by treatments.

Results illustrated in **(Table 4)** shows that no significant differences in serum low density lipoprotein at six week of age. On the other hand values of cholesterol and high density lipoprotein concentration were significantly decreased by different experimental diets as compared with the control group.

Table 5. The effect of *In ovo* injection on some Blood plasma Constituent of broiler chickens at 6 week of age

Parameter / Treatment	Total protein (mg/dl	Albumin (mg/dl)	Globulin (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
CONTROL	194.06 ±0.50	116.60 ±0.04	77.46 ±5.46	98.35^{b} ±8.77	95.70^{ab} ±8.67	50.27^{e} ±0.34
SHAME	184.73 ±0.44	116.73 ±0.03	68.00 ± 6.33	115.84^{a} ±1.87	113.24^{a} ±2.47	44.13^{f} ±0.63
NANO IN ORGANIC	208.30 ±1.27	112.69 ±3.96	95.61 ±2.38	82.22^{c} ±12.57	98.66^{ab} ±12.02	58.01^{b} ±0.23
IN ORGANIC	193.89 ±0.75	116.70 ±0.01	77.19 ±9.22	78.70^{c} ±3.58	85.94^{ab} ±3.42	54.93^{c} ±0.31
Probability	0.3690	0.4777	0.2349	0.03683	0.2669	0.0001

Means within a column with different superscripts are significantly different (P≤0.05).

CONCLUSION

The results of the present study indicate that Injection with Fe inorganic and Fe nano inorganic can improve body weight and body weight gain. Moreover, it reduces the total lipids and cholesterol in serum of chickens and produces more healthy food for human consumption.

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تأثير حقن البيض بالحديد الغيرعضوي واشكال جزيئات الحديد النانومترية على النمو والاستجابة الفسيولوجية فى دجاج التسمين

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الموجـــــــز

استخدم فى هذه الدراسة عدد 320 بيضة مخصبة تم تقسيمها الى اربعه مجاميع بكل مجموعة 80 بيضةفي اربع مكررات بكل منها 20 طائر وكانت المجموعات على النحو التالى:

المعاملة الاولى (T_1) : للمقارنه (بدون حقن)، المعاملة الثانية (T_2) : الحقن بمحلول ملحى ،المعاملة الثالثة (T_3) :الحقن ب اكسيد الحديد بتركيز 75 ppm، المعاملة الرابعة (T_4): الحقن ب الصوره النانومتريه لاكسيد الحديد بنفس التركيز.

وتم تسجيل وزن الجسم اسبوعيا ومع نهاية الأسبوع السادس تم أخذ 48 طائر عشوائياً (3 طيور من كل مكررة) و وزنت ثم ذبحت لاخذ وزن الأعضاء الداخلية مثل القلب –الطحال–الكبد– غدة البرسا –الغدة الثيموثية كما اخذ عينات الدم لتقدير بعض مكونات الدم مثل مستوى البروتين الكلى فى بلازما الدم، الألبيومين، الجلوبيولين ومستوى الليبيدات والكوليستيرول .

أوضحت نتائج هذه الدراسة أن هناك فروقاً معنوية فى وزن الجسم والزيادة فى الوزن وكانت أعلاها هى المعاملات التى تم حقنها بإوكسيد الحديد او جزيئات الحديد النانومترية، بينما لا يوجد اختلافات معنوية فى اوزان القلب و الكبد والطحال و البرسا ولكن هناك اختلافات معنوية فى وزن الغدة الثيموثية ومستوى الكوليستيرول في البلازما.

توصى هذه الدراسة بإستخدام الحديد بصورته النانومتريه لدجاج التسمين لتحسين الآداء الانتاجى والفسيولوجى والذى يمكن أن ينعكس على زيادة وزن الجسم وقلة استهلاك العليقة وإنتاج لحم منخفض الكوليسترول والدهون الضارة بصحة الانسان .

الكلمات الدالة: دجاج التسمين، الحقن بجزئيات النانوحديد ’النمو’ مكونات الدم

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