The Effect of Using Iron Nanoparticles or Iron Methionine by *In Ovo* Injection or As a Dietary Supplement on The Productive and Physiological Performance of Mamoura Chicken Strain

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Received: 01/12/2022 Accepted: 21/12/2022

Abstract

The aim of this experiment is to study the effect of *in ovo* injection with either Iron Nano Particles (INPs) or Iron Methionine (IM) and add them as nutritional supplements on productive and physiological performance of Maamaura chicken strains. Total number of 720 fertile eggs from Maamaura chicken strain was used in this study. Eggs were randomly divided in to 6 treatments as follows: T1 (control; without injection), T2 (injected 0.1 ml with concentrated saline 9.0%), T3 (injected 0.1 ml with concentrated 25 ppm of iron methionine, T4 (injected 0.1 ml concentrated with 25 ppm iron methionine nano particles/egg, T5 (added of 20 mg iron methionine per 1kg to feed chicks after hatch and T6 (added of 5 mg iron methionine nano-particles per 1kg to feed chick after hatch). Live body weight was taken at day old and every two weeks to the end of the experiment. Feed intake was determined every two weeks, hatchability, mortality percentages and chick weight at hatch were determined. Some blood parameters and carcass traits were determined at the end of experiment. These results revealed that treatments applied showed highly significant ($p>0.0001$) effect on live body weight and feed intake at all period of experiment, except during the period of iron addition for feed intake. T3 and T4, respectively significantly ($p>0.0001$) increased in body weight, hatchability percentage, body weight hatch and decreased embryo mortality. Treatments applied had highly significant ($p> 0.0001$) effect on all blood parameters and all carcass traits. Birds of T3 and T4 respectively significantly increased plasma total protein, albumin, cholesterol and triglycerides. Iron (Fe), HDL, LDL, and ALT while T3 and T4 respectively decreased significantly plasma AST. T3 and T4 respectively increased significantly in all absolute and relative weight of carcass traits except relative weight of liver and gizzard.

Key words: *In ovo* injection, Nano iron; Iron methionine; Growth performance; Physiological performance.

Introduction:

Iron is essential for animal and poultry so it supplemented in their diets. Iron is an integral part of many proteins and enzymes that maintain good health. 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) Conrad *et al*., (1999). It is also essential for the regulation of cell growth and differentiation, the

production of hemoglobin, myoglobin and the component of red blood cells that transports oxygen around the body. Iron (Fe) acts as a cofactor for the function of over 300 different enzymes (Lozoff *et al.*, 2006) and is an important structural cofactor for many proteins, including DNA synthesis and oxygen transport (Whitnall and Richardson, 2006; Scott and Chen, 2008; Li and Zhao, 2009). *In ovo* feeding can provide the embryo injection necessary to its optimum post-hatch growth. Despite the dependence of embryo growth on nutrients especially iron, data on the mineral content of the egg during incubation is limited. The injection of methionine was an important amino acid effective on performance. Iron sulfate nanoparticles alone and with methionine (Alimet) used to increase the growth during embryonic and post-hatch periods (Ali *et al*., 2020). Iron-deficiency anemia is as a public health problem (Stoltzfus, 2001). One of the strategies to overcome this problem is adding to food iron supplementary. Hemoglobin and myoglobin are important determinant agents of meat quality. Much of the organic iron in the body is found in the structure of hemoglobin, in muscles as myoglobin, and in liver, it is in the form of reserved ferritin and hemosiderin (Suttle, 2010). The use of iron nanoparticle (Fe) of dietary supplementation is important to improve the production and immunological performance of lay hens**.** In the poultry farms, birds expose to many stresses, such as vaccination, high stocking density, feed withdrawal, high and low ambient temperature, catching crating, and transportation. Stressful conditions whether acute or chronic, have various detrimental effects on the physiological features of birds, and such conditions can damage the production performance of poultry (Attia *et al.*, 2016, 2018). So, it uses iron nanoparticle to relieve the stress the birds are exposed lay hens. These particles have features, such as large surface area (increasing physical chemical and biological activities) and higher solubility and mobility (Toyooka, *et al*., 2009 and Diman *et al*., 2018). 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). The nanoparticle has a lower antagonism in the intestine that leads to improved absorption, reduced excretion to the environment and improved feed efficiency (Gopi *et al*., 2017). The bioavailability of nanoparticles can be increased as they have different physical and chemical properties than their original corresponding mineral (Raje *et al*., 2018). The aim of this study was to investigate the physiological and productive responses to the use of iron nanoparticles or iron methionine by *in ovo* injection or as a dietary supplement in Mamoura Chicken strain.

Material and Methods:

Experimental design:

A total number of 720 eggs from Maamaura strain were divided randomly into 6 groups (120 eggs/group) and every group was divided in to three replicates, (40 eggs/replicate). All eggs were incubated at 37.6 ˚ C and relative humidity of 55 to 60% during the first 18 days of incubation, then temperature was decreased to 36.2˚ C and 65-70% relative humidity in the setter until hatching at the seventh day of hatching eggs were divided as follows:

1- The first group (T1) eggs without any injection and considered as control group.

2- The second group (T2) eggs were (injected with 0.1 ml saline 9.0%).

3- The third group (T3) eggs were (injected with 0.1 ml with concentrated 25 ppm of nano iron methionine.

4- The fourth group (T4) eggs were (injected, 0.1 ml concentrated with 25 ppm iron methionine nano particles/egg**.**

5- The fifth group (T5) hatches chicks were fed dietary supplementation with 20 mg iron methionine per 1kg to feed chicks from non-injected spawning eggs.

6- The sixth group (T6) hatches chicks were fed dietary supplementation with the addition of 5 mg iron methionine nano-particles per 1kg to feed chick from injected spawning eggs**.**

A total number of 480 one-day hatched chicks were distributed into six equal treatments in three replicates each according to previously mentioned treatments. Chicks were kept under similar environmental conditions. Chicks received a commercial starter diet (1- 21 days) containing 22% of crude protein (CP) and 3100 kcal/kg, while, grower diet until 8 weeks containing 20% CP and 3000 kcal/kg diet.

Experimental parameters:

Growth performance:

Performance of Maamaura strain was evaluated by recording body weight, daily feed intake during the experiment period. Individual body weight (BW) of birds was recorded at hatch, 0, 14, 28, 42 and 56 days of age in each treatment group. Traits of feed intake (FI) in each treatment was recorded per two weeks and then calculated during the whole experiment period (56 days).

Blood collection for biochemical studies:

At the 56^{th} day of the experiment (8 weeks), 10 ml of blood samples were collected from three birds per treatment group through neck slitting into sterile disposable hypodermic syringes. About 5 ml of blood samples were transferred immediately into plastic tubes containing anti-coagulant Heparin. Blood samples were centrifuged at 2500 rpm for (15- min). Plasma samples were stored in deep freezer at approximately 20 ^{o}C until the time of chemical analysis. The chemical analysis of blood samples was carried out by colorimeter method using commercial kits for determination of plasma total protein, albumin, globulin, cholesterol, triglyceride, HDL, LDL, liver enzymes (ALT and AST) and iron (Fe).

Plasma total protein (g/dl) was determined according to the method described by Grant *et al*., (1987), the determination of plasma albumin was carried out according to the method of Doumas *et al*., (1971), and globulin was calculated by subtraction of plasma albumin from total plasma protein. Plasma cholesterol (Stein *et al.*, 1986), triglycerides (Fossati and Prencipe, 1982), and HDL (Lopez-Virella, 1977) and transferrin were determined by commercial Kits (Spectrum Diagnostic Kits, Spec. Corp. Egypt), while LDL was determined according to the formula of Friedewald *et al.*, (1972), Iron concentration (Fe), Alanine transaminase (ALT) and Aspartate transaminase (AST) was determined according to the formula of Kaneko *et al*., (2008).

Carcass characteristics:

At 56 days of age of three birds were randomly selected from each treatment individually weighed and were slaughtered after they have been fasted for 12 hens. Measurements were live body weight, carcass weight. The weights of the heart, liver, gizzard, spleen and bursa of fabricius and their percentages of carcass weight.

Statistical analysis:

Data collected were analyzed by one way analysis using SAS program (SAS, 2006).

Statistical model: Yij = m + ai + eij.

Yij = observation.

m = general mean.

ai = effect of *in ovo* injection nano iron methionine particles and dietary supplementation.

eij = experimental error**.**

In order to determine significant differences between all possible mean comparisons Duncan`s multiple range test (Duncan, 1955) was applied. Statistical significance was accepted at a probability level of 0.05.

Results and Discussion:

Productive performance:

Live body weight:

Results presented in Table (1) show that highly significant (p>0.0001) effect of *in-ovo* injection of different iron methionine sources and dietary supplementation of powder on live body weight at different ages. Birds produced from T3 and T4 which injected with 0.1 ml of nano iron methionine and methionine iron had respectively significantly higher live body weight at all estimated periods. The results obtained are in agreement with those reported by Ali Saki *et al.,* (2020) who stated that *in ovo* feeding can provide the embryo injection necessary to its optimum post-hatch growth. Despite the dependence of embryo growth on nutrients especially iron, data on the mineral content of the egg during incubation is limited. The injection of methionine was an important amino acid effective on performance. Iron sulfate nanoparticles alone and with methionine (Alimet) was used to increase the growth during embryonic and post-hatch periods**.** This result may be returned to due to circulatory activity and increasing secretion of digestive enzyme, the metabolic rate is increased, and therefore the conversion rate is increased, resulting in an increase in live body weight at different age. Zhai *et al*., (2015) reported that *in-ovo* injection of Fe-nano (25-125 ppm) improved chick performance traits. Also, iron nanoparticles and compounds may be considered a good alternative to existing treatments. Mohammed (2018) suggested that 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-Alimet chelate and Fe-Alimet chelate improved embryonic growth and development.

Feed intake:

Results presented in Table (2) show that highly significant (p>0.0001) effect of *in-ovo* injection of different iron methionine sources and dietary supplementation of powder (P< 0.0001) on feed intake at different ages. The birds at T3 and T4 which injected with 0.1 ml of either nano iron methionine or methionine iron significantly increased feed intake than the other fife groups at different ages**.** These results may be leads to increase of secretion many enzyme systems including catalase, peroxidase, and phenylalanine hydroxylase**.** The results agree with Amal (2018), who found that, significantly (P<0.05) increased feed intake when *in ovo* injection by nano forms of FeNano particles. Diman *et al.,* (2018) reported that, these particles have features, such as large surface area (increasing physical, chemical, and biological activities) and higher solubility and mobility. On other vie Ghada and Abdalla, (2019) injected 0.1 ml/egg of iron solution containing 75 ppm of different iron sources (nano organic and organic) and concluded that, injection with 0.1 ml of nano iron methionine improve feed intake in Maamaura local strain**.**

Some blood parameters:

Results presented in Tables (3 and 4) show highly significant (P<0.0001) effect of *in ovo* injection of different iron methionine sources and dietary supplementation on some blood parameter (total protein, albumen, cholesterol, triglycerides and iron) at different ages. T3 and T4 injected with 0.1 ml of either nano iron methionine or methionine iron respectively significantly (P<0.0001) increased some blood

parameters plasma total protein, albumen, cholesterol, triglyceride and iron) than the other treatments applied. The results obtained with those of Ghada *et al.,* (2019) reported that, plasma total protein was significantly increased in all the control groups compared to the different forms of iron in nano particle or in the organic or inorganic forms injected groups. The previous result indicates that *in ovo* injection has no harmful effect on different plasma protein parameters, this means that iron injection has a positive impact on protein synthesis. It suggested that plasma proteins profile of a given bird is a reflection of the metabolic activities related to protein synthesis and/or degradation, this support the findings of many authors who observed that iron has the ability to bind protein and enhanced DNA synthesis which in turn affect plasma protein level at different growth periods (Yair and Uni, 2011). These results agree with Mohammed, (2018) who suggest that 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-Alimet chelate and Fe-Alimet chelate improved Serum Fe content and liver function significantly and also, Amal, (2018) who found that, the iron injection significantly ($P<0.01$) enhanced different blood parameters. On other vie, effects of *in-ovo* injection on broiler eggs on plasma iron definitions in chicks on 35 days of age, recently, showed major variations between treatments which plasma iron were increased in the treatment by *in-ovo* injection iron- nano particle while it was decreased and lowest value in other treatment with no significant difference. And showed highly significant ($p>0.0001$) effect of *in-ovo* injection of different iron methionine sources and dietary supplementation of powder on high density lipoprotein (HDL), lower density lipoprotein (LDL) and liver enzyme Aspartate transaminase **(**AST) and Alanine transaminase (ALT) at different ages. T3 and T4 injected with 0.1 ml of either nano iron methionine or methionine iron respectively increased significantly HDL, LDL and plasma ALT when compared with different treatments applied, while, T2 and T6 decreased significantly plasma AST (263.0 and 258.0 mg/dl, respectively). The results agree with those of Mohammed, (2018) which suggest that 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-Alimet chelate and Fe-Alimet chelate improved serum liver function were significant.

Carcass characteristics:

Results presented in Tables (5 and 6) show that highly significant ($P<0.0001$) effect of *in ovo* injection of different iron methionine sources on carcass absolute and relative weight of giblet (liver, gizzard and heart). T3 and T4 injected with 0.1 ml of either nano iron methionine or methionine iron respectively significantly increased ($p>0.0001$) carcass weight and absolutely and relatively weights of giblets than the other treatments. The results obtained agree with El-Said1 and Gogary, (2019), who reported that, carcass parameters could be detected that inclusion of *in ovo* injection of different iron sources and supplementations of folic acid. In the broiler diet did not have significant effect on gizzard and heart. However, the experimental group's *in ovo* injection of different iron sources achieved significantly higher LBW and the proportions of carcass and liver or immunity organs (spleen and thymus). Also, the results obtained disagree with those of Azza, *et al.,* (2018), whom reported 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 significant differences among all experimental groups in dressed carcass, gizzard, heart and liver. Also, showed that significantly ($p<0.0001$) effect of *in ovo* injection dietary methionine iron supplementation on absolute and relative weights of bursa and spleen at different ages. The birds of (T3) and (T4) injected with 0.1 ml of either nano iron methionine or methionine iron had

significantly (P< .0001) greater obtained and relative weights of bursa and spleen than those of the other treatments applied. These results are agreement with those obtained by Goel *et al.,* (2013), who found that *in ovo* feeding of iron may influence the embryonic development, while iron can play an important role in post hatch growth. Akshat *et al.,* (2012) reported that the trace minerals are important nutritional components for imparting immunity and *in ovo* injected of iron during incubation into the yolk sac/amnion of the broiler embryos can be a way for improving the immune system of the birds. Ghada and Abdalla, (2019) noticed that injected with dose of each iron solution is 0.1ml/egg containing 75 ppm of different iron sources (nano organic and organic) increased of the relative weights of spleen, bursa and thymus, and stimulated some histological change in the immune related organs which may result in improvement of chick immunity. Inversely, Azza, *et al*., (2018), reported that, 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.

Conclusion:

It can be concluded that *in ovo* injection technique can contribute to improving the productive and physiological characteristics of chickens, especially local breeds. Also, the use of iron nanoparticles as one of the nutrients through this path of this technology.

Table 1. Effect of treatments on body weight (g) at different ages.

Treatment	Ages after hatching				
	(1 day)	(14 day)	(28 day)	(42 day)	(56 day)
T1	37.3^{b}±0.5	135.5^{e}±0.2	315.5^{d}±1.9	492.2^{b}±6.1	715.5^{e}±1.8
T2	38.9^{a}±0.5	135.5^{e}±0.2	315.5^{d}±1.9	478.7^{bc}±6.1	699.7^{f}±1.8
T3	40.1^{a}±0.5	154.3^{a}±0.2	372.3^{a}±1.9	550.4^{a}±6.1	821.4^{a}±1.8
T4	39.1^{a}±0.5	145.3^{b}±0.2	352.3^{b}±1.9	475.7^{bc}±6.1	791.5^{b}±1.8
T5	36.9^{b}±0.5	143.3^{c}±0.2	342.3^{c}±1.9	460.4^{c}±6.1	775.5^{c}±1.8
T6	36.1^{b}±0.5	141.5^{d}±0.2	315.4^{d}±1.9	458.7^{c}±6.1	757.2^{d}±1.8
Pr.	0.0005	0.0001	0.0001	0.0001	0.0001

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

Table 2. Effect of treatments on feed intake (g/birds) at different ages.

Treatment	Ages after hatching			
	(1 day)	(14 day)	(42 day)	(56 day)
T1	2^{a} ±0.6	20.3^{e} ±0.9	31.4^{d} ±0.1	67.3^{de} ±0.3
T2	2^{a} ±0.6	20.5^{e} ±0.9	31.4^{d} ±0.1	66.7^{e} ±0.3
T3	2^{a} ±0.6	23.5^{a} ±0.9	35.3^{a} ±0.1	71.5^{a} ±0.3
T4	2^{a} ±0.6	22.2^{b} ±0.9	33.4^{b} ±0.1	69.4^{b} ±0.3
T5	2^{a} ±0.6	21.3^{c} ±0.9	32.6^{c} ±0.1	68.4^{c} ±0.3
T6	2^{a} ±0.6	20.7^{d} ±0.9	31.7^{d} ±0.1	68.3^{cd} ±0.3
Pr.	1.0000	0.0001	0.0001	0.0001

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

Table 3. Effect of treatments on plasma total protein, albumin, ALT, AST and Iron (Fe) at 56 days of Age.

Treatment	Plasma blood parameter (mg/dl)				
	T. Protein	Albumen	ALT	AST	Iron (Fe)
T1	2.4^{e}±0.06	1.2^{d}±0.03	3.6^{b}±0.3	179.3^{b}±13.4	34.9^{f}±0.33
T2	2.8^{d}±0.06	1.3^{d}±0.03	3.7^{b}±0.3	263.0^{a}±13.4	91.9^{e}±0.33
T3	4.6^{a}±0.06	1.9^{a}±0.03	5.7^{a}±0.3	173.0^{b}±13.4	116.4^{a}±0.33
T4	3.4^{b}±0.06	1.6^{a}±0.03	4.3^{b}±0.3	256.0^{a}±13.4	110.6^{b}±0.33
T5	3.1^{c}±0.06	1.5^{b}±0.03	3.7^{b}±0.3	233.0^{a}±13.4	105.9^{c}±0.33
T6	2.9^{d}±0.06	1.4$^{c\pm}$0.03	3.3^{b}±0.3	258.0^{a}±13.4	102.8^{d}±0.33
Pr.	0.0001	0.0001	0.004	0.0008	0.0001

a, b: Means ± SEM within a column with different superscripts are significantly different (P<0.01). AST= Aspartate transaminase, ALT = Alanine transaminase.

Table 4. Effect of treatments on plasma lipid profile at 56 days of Age.

Treatment	Plasma blood parameter (mg/ dl)			
	T. Cholesterol	HDL	LDL	Triglycerides
T1	3.6^{b}±0.3	62.0^{d}±0.38	21.2^{e}±0.1	179.3^{b}±13.4
T2	3.7^{b}±0.3	63.7^{c}±0.38	23.4^{d}±0.1	263.0^{a}±13.4
T3	5.7^{a}±0.3	67.3^{a}±0.38	28.5^{a}±0.1	173.0^{b}±13.4
T4	4.3^{b}±0.3	65.3^{b}±0.38	26.5^{b}±0.1	256.0^{a}±13.4
T5	3.7^{b}±0.3	63.7^{c}±0.38	24.3^{c}±0.1	233.0^{a}±13.4
T6	3.3^{b}±0.3	62.7^{cd}±0.38	23.4^{d}±0.1	258.0^{a}±13.4
Pr.	0.004	0.0001	0.0001	0.0008

a, b: Means ± SEM within a column with different superscripts are significantly different (P<0.01). HDL= high density lipoprotein, LDL= lower density lipoprotein,

Table 5. Effect of treatments on some carcass traits at 56 days of Age.

Treatment	Body (g)	Carcass. (g)	Liver. (g)	Liver. (%)	Gizzard (g)	Gizzard (%)
T1	745^{d} ± 4.4	434.67^{c}±14.0	22.8^{e}±0.12	3.1^{c}±0.03	22.5^{f}±0.14	3.02^{d}±0.03
T2	735^{d} ± 4.4	469.00^{cd}±14.0	22.8^{e}±0.12	3.1^{c}±0.03	22.0^{f}±0.14	2.99^{d}±0.03
T3	880^{a} ± 4.4	578.67^{a}±14.0	28.4^{a}±0.12	3.2^{b}±0.03	28.5^{a}±0.14	3.24^{c}±0.03
T4	805^{b} ± 4.4	515.00^{b}±14.0	27.4^{b}±0.12	3.4^{a}±0.03	27.5^{b}±0.14	3.4^{a}±0.03
T5	775^{c} ± 4.4	485.00^{cb}±14.0	26.3^{c}±0.12	3.3^{a}±0.03	26.4^{c}±0.14	3.4^{a}±0.03
T6	761^{c} ± 4.4	466.67^{cd}±14.0	25.4^{d}±0.12	3.3^{a}±0.03	25.4^{d}±0.14	3.3^{b}±0.03
Pr.	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001

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

Table 6. Effect of treatments on some carcass traits at 56 days of Age.

Treatment	Heart.		Bursa		Spleen	
	(g)	(%)	(g)	(%)	(g)	(%)
T1	3.86^{e}±0.04	0.52^{d}±0.01	2.2^{d}±.0.06	0.29^{c}±0.009	2.3^{cb}±0.06	0.31^{b}±0.3
T2	3.80^{e}±0.04	0.51^{d}±0.01	2.3^{cd}±0.06	0.31^{bc}±0.009	2.3^{cb}±0.06	0.31^{b}±0.3
T3	5.7^{a}±0.04	0.65^{a}±0.01	3.3^{a}±0.06	0.38^{a}±0.009	3.0^{a}±0.06	0.35^{a}±0.3
T4	4.8^{b}±0.04	0.59^{b}±0.01	2.7^{b}±0.06	0.34^{b}±0.009	2.5^{b}±0.06	0.31^{b}±0.3
T5	4.5^{c}±0.04	0.58^{bc}±0.01	2.5^{c}±0.06	0.32^{bc}±0.009	$2.4c^{b}$±0.06	0.31^{b}±0.3
T6	4.2^{d}±0.04	0.55^{c}±0.01	2.3^{cd}±0.06	0.30^{c}±0.009	2.2^{c}±0.06	0.29^{b}±0.3
Pr.	0.0001	0.0001	0.0001	0.0008	0.0001	0.02

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

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