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EFFECT OF DIETARY SUPPLEMENTATION AND INJECTING HATCHING EGGS WITH PYRIDOXINE ON SOME HATCHABILITY AND INCUBATION CHARACTERS AND SOME PHYSIOLOGICAL TRAITS OF CHICKS.

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ABSTRACT

A total number of 198 Silver Montazah strain birds (180 hens and 18 cocks) 24-wks-old were used in this experiment up to 36 wks. of age was divided into two experimental groups (90 hens and 9 cocks per each group). The birds of the 1st group were individually weighed and randomly divided into 3 equal treatments (30 hens and 3 cocks in each) with three replicates (10 hens and 1 cock in each) with almost similar initial average body weight. Replicates were randomly housed in floor pens (280 cm long x 220 cm wide). The 1st group of birds (90 hens and 9 cocks) was divided to 3 treatment groups, the 1st treatment group was fed the basal diet without supplementation and served as control (T1), the 2nd and 3rd treatment groups were fed the basal diet supplemented with 8.25 and 11.00 mg pyridoxine/kg diet (T2 and T3), respectively. At the end of the experiment (36 wks. of age), a total number of 300 hatching eggs were collected from experimental groups (100 eggs in each treatment). At the same time (36 wks. of age), the second 300 hatching eggs were collected from the same breeder flock fed the basal diet without supplementation and incubated. At the 18th day of incubation, 300 eggs were divided into three treatment groups (100 eggs in each). The 4th treatment group was injection with 0.2 ml saline solution (T4). The 5th and 6th treatment groups were injected into amniotic sac through the air sac with 300 and 400µg pyridoxine, respectively.

***The results obtained could be summarized as follow**: Results showed significant (P≤0.05) differences among treated groups in*

embryonic mortality, hatchability percentage, chick weight and chick quality at hatch. The best values of embryonic mortality, hatchability percentage and chick weight at hatch were recorded by pyridoxine injection at different doses compared with either un-supplemented or un-injected pyridoxine treatments which recorded the worst values. The best (P≤0.05) values of percentage of grade A chicks were recorded by pyridoxine injection at different doses treatments when compared with other treated groups. The highest (P≤0.05) values of plasma total protein, globulin, calcium, phosphorus , T3 and T4 concentrations and albumin/globulin ratio of chicks at hatch day were recorded by pyridoxine injection, especially at the high level (400 µg) when compared with either supplemented or un-injected pyridoxine groups.

***In conclusion**, in - ovo injection of pyridoxine at level 400µg to laying eggs at 18 days of incubation may be a practical and beneficial procedure for improving the hatchability traits and the early post hatch physiological responses as indicated with better blood biochemical and metabolic hormones expression*.

Key words: Pyridoxine injection, diet, hatching eggs, incubation characters, physiological traits, Silver Montazah strain.

INTRODUCTION

Pyridoxine (vitamin B6) plays an important role in the synthesis and degradation of aspartate aminotransferase in the chicken embryo (Sharma and Gehring, 1987). Pyridoxine has an important role in amino acids, carbohydrates, and fatty acids metabolism and also plays a major role in the energy-producing citric acid cycle (McDowell, 1989). Deficiency of pyridoxine leads to early embryonic death and decreased IgM and IgG response to antibody challenge (Blalock *et al*., 1984) as well as, depressed appetite, poor growth and characteristic nervous symptoms in chicks (Scott *et al*., 1976). Pyridoxine concentration in egg yolk remains stable in response to incremental levels in turkey breeder diets whereas it increases in albumen (Robel, 1992).

Robel (2002) reported that dietary supplemental pyridoxine had limited influence on increasing the transfer of vitamin B6 in turkey eggs, while hatchability was improved by pyridoxine egg injection. Eggs injection of pyridoxine is vital for increasing hatchability even though the

hen's diet was fully supplemented with Pyridoxine add are finance. Elsayed *et al*. (2010) reported that injection of quail eggs with 120 µg/egg pyridoxine at the 7th day of incubation period improved hatchability percentage, chick weight at hatch, growth performance and carcass traits. Ibrahim *et al*. (2012) found that hatchability was positively affected by pyridoxine injection of fertile ostrich eggs (10 mg) at the 7th day of incubation compared to non-injected group. Pyridoxine is water-soluble vitamins (Bender, 1999), any deficiency results in embryonic growth retardation that leads to its death and eventually results in poor hatchability.

Therefore, the present study was conducted to estimate hatchability percentage, embryonic mortality, chick weight at hatch and some blood biochemical traits of montazah strain chickens as affected by dietary supplementation and injecting hatching eggs with pyridoxine.

MATERIALS AND METHODS

This experiment was carried out at Inshas Poultry Breeding Station, Animal Production Research Institute, Agricultural Research Center.

Experimental design:

This experiment aimed to study the effect of dietary supplementation and injecting hatching eggs with pyridoxine (vit B6) on hatchability, embryonic mortality, Chick quality at hatching , hatch weight and some blood plasma traits of chicks at hatching day.

A total number of 198 Silver Montazah strain birds (180 hens and 18 cocks) 24-wks-old were used in this experiment up to 36 weeks of age. Birds were divided into two experimental groups (90 hens and 9 cocks per each group). The birds of the 1st group were individually weighed and randomly divided into 3 equal treatments (30 hens and 3 cocks of each) with three replicates (10 hens and 1 cock each) with almost similar initial average body weight. Replicates were randomly housed in floor pens (280 cm long x 220 cm wide). The first treatment of that group was fed the basal diet without extra supplementation and served as control (T1), the second and third treatments were fed the basal diet supplemented with extra 8.25 and 11.00 mg pyridoxine/kg diet (T2 and T3), respectively. At the end of the experiment (36 wks of age), a total number of 300 fertile eggs were collected from the experimental treatments (100 eggs in each) and prepared for incubation.

The 2nd group of birds were individually weighed, randomly divided into 3 treatments and were fed the basal diet without extra supplementation of pyridoxine. 300 eggs were collected from that group (100 eggs per each treatment) and prepared for incubation . At the 18th day of incubation each egg of all treatments of the 2nd group were injected into amniotic sac through the air sac (blunt end of the egg) as follow: the 1st treatment was injection with 0.2 ml saline solution (T4), while the T5 and T6 were injected with 300 and 400µg pyridoxine, respectively. Eggs were injected through the air sac with a blunt-tip injector needle [18.4 mm length and 1.27 mm bore width (outside diameter)] to target the amnion. The needle provided an injection depth of approximately 2.49 cm from the top of the large end of the egg. The injected area was disinfected with an ethyl alcohol and the pinhole site was sealed with sterile paraffin wax immediately after injection. The injected eggs were transferred to the hatcher after the injection. All eggs were collected from the same breeder flock and weighed on a balance with 0.1 g precision (48 ± 1 g) and were incubated at 37.8 °C and 63% RH. At the 7th and 14th days of incubation, the eggs were candled, and the infertile ones or those containing early dead embryos were recorded then removed.

Management and feeding:

All birds were kept under the same managerial house, hygienic and environmental conditions. Birds were kept in an open house with light cycle regimen of 16 h light: 8 h darkness, throughout the experimental period (24-36 wks of age). Feed and water were provided for *ad libitum* consumption. Birds were fed layer diets according to **NRC (1994)**. The composition and calculated analysis of the basal diet are shown in Table 1.

Measurements:-

Embryonic mortality was divided into three categories: early embryonic mortality during 0 - 9 days of incubation, middle embryonic mortality during 10 - 17 days of incubation and late embryonic mortality during 18 - 21 days of incubation. Stages of embryonic mortality were determined according to Reijrink *et al.*, (2009). Hatchability was calculated as a percentage of fertile eggs. All chicks were weighed at hatch and graded into first 'A' and second 'B' grade chicks. A chick was classified as a grade A when the chick was clean, dry, free of deformities or lesions and bright eyes as well as navel was completely closed and clean (Tona *et al.*, 2004). The other chicks were classified as grade B. The first and the second grade of chicks were calculated as a percentage of total hatched chicks.

Table 1: The composition and calculated analyses of the experimental basal diet.

Ingredients	**Percentage (%)**
Yellow corn	61.57
Soya bean 44%	17.00
Wheat bran	6.70
Corn gluten 60%	4.50
Di Ca P	1.39
Lime stone	8.16
Salt	0.37
Premix*	0.30
L Methionine	0.01
Total	**100.00**
Calculated analyses **	
Protein (%)	16.5
Metabolizable energy (kg cal/kg)	2699
Crude fiber (%)	3.468
Ether extract (%)	2.964
Calcium (%)	3.399
Available Phosphorous (%)	0.397
Total Phosphorous (%)	0.610
Sodium (%)	0.164
Arginine (%)	1.28
Lysine (%)	0.730
Methionine (%)	0.335
Methionine & cysteine (%)	0.619

*__Premix added to the 1 kg of diet including__: Vit. A 10000 I.U; Vit. D_3 2000 I.U; Vit. E 15 mg; Vit. K_3 1 mg; Vit. B_1 1mg; Vit. B_2 5 mg; Vit. B_{12} 10 µg; Vit B_6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; Folic acid 1mg; Biotin 50 µg; Choline 300 mg; Zinc 50mg; Copper 4mg; Iodine 0.3 mg; Iron 30mg; Selenium 0.1mg; Manganese 60mg; Cobalt 0.1mg and carrier $CaCo_3$ up to 1kg.

**According to Feed composition tables for Animal & Poultry feedstuffs used in Egypt (2001).

At hatching day, 12 chicks were randomly selected from each treatment and slaughtered. Blood samples were collected from each four birds in heparinized test tube. Samples were centrifuged at 3000 rpm for 20 minutes. The separated plasma was stored in a deep freezer at -20°C until assayed for triiodothyronine (T_3) and thyroxin (T_4) hormones, total protein, albumen, globulin, A/G ratio, total cholesterol, calcium, phosphorus,

aspartate amino transaminase (AST) and alanine transaminase (ALT) according to the manufacture recommendations of commercial kits.

Statistical analysis:

Data were subjected to one-way analysis of variance using general linear models (GLM) procedure of SAS (2001). Means were separated by using Duncan's multiple range test (Duncan, 1955). The percentage values were transferred to percentage angle using arcsine equation before subjected to statistical analysis, and then actual means are presented.

The following model in each experiment was used:

$$Y_{ij} = \mu + T_i + e_{ij}.$$

Where, Y_{ij} = observation for each dependent variable; μ = Overall means; T_i = Treatment effects (i = 1, 2... and 6); e_{ij} = Random error.

RESULTS AND DISCUSSION

Embryonic mortality, hatchability and chick weight and quality at hatching day:

The effects of dietary supplementation and injecting hatching eggs with pyridoxine on embryonic mortality (EM), hatchability (H) percentages and chick weight (ChW) and quality (ChQ) at hatching day are presented in Table 2. Results showed significant ($P \leq 0.05$) differences among the treated groups of EM, H, ChW and ChQ. However, the greatest H and ChW values were recorded by pyridoxine injection at different doses treatments compared with either un-supplemented or un-injected pyridoxine treatments which recorded the worst values of EM, H and CWH.

Moreover, the highest ($P \leq 0.05$) percentage of grade A chicks were recorded by pyridoxine injection at different doses compared with the other treatment groups. The lowest ($P \leq 0.05$) percentage of grade B chicks was recorded by pyridoxine injection at the highest dose treatments compared with the other treated groups. In this respect, Pyridoxine (vitamin B6) is a water soluble vitamin (Bender, 1999), this is important from a nutritional standpoint, because the water soluble vitamins are not stored to any extent in the body. Therefore, a constant supply must be provided in the maternal diet to be deposited in adequate quantities in the eggs for future offspring consumption (Stevens, 1991). Any deficiency could result in an embryonic growth retardation that leads to death and eventually poor hatchability. This is due to the important functions of pyridoxine that it involved in the metabolism of protein, lipids, phospholipids, fatty acid, cholesterol and carbohydrates (Squires and Naber, 1993). Also, it is required to several enzymes, particularly those

Table (2): Effect of dietary and injecting hatching eggs with pyridoxine on embryonic mortality, hatchability, hatch weight and chick quality of Silver Montazah laying hens.

Treatment groups	Hatchability of fertile eggs (%)	Embryonic mortality (%)				Chick weight at hatch (g)	Chick quality	
		Early	Intermediate	Late	Total		Grade A	Grade B
T1	82.99^{bc}	6.67	4.33	6.01	17.01^{ab}	35.75^{bc}	93.72^{c}	6.28^{a}
T2	85.00^{abc}	5.67	4.66	4.67	15.00^{abc}	37.67^{ab}	94.00^{bc}	6.00^{ab}
T3	85.45^{ab}	6.33	4.22	4.00	14.55^{bc}	37.66^{ab}	94.59^{bc}	5.41^{ab}
T4	80.08^{c}	7.67	5.25	7.00	19.92^{a}	35.06^{c}	93.59^{c}	6.41^{a}
T5	88.88^{a}	5.67	3.00	2.45	11.12^{c}	38.40^{a}	95.92^{a}	4.08^{bc}
T6	88.96^{a}	5.00	4.33	1.71	11.04^{c}	38.08^{a}	96.13^{a}	3.87^{c}
MES	**1.12**	**0.50**	**0.36**	**0.50**	**1.572**	**0.57**	**0.31**	**0.31**

$^{a, b, c}$ …Means within a column with different superscripts are significantly differ (P≤0.05). T1:Control without supplementation T2: Pyridoxine (8.25 mg/kg diet) T3: Pyridoxine (11.00 mg/kg diet) T4: Injection with 0.2 ml saline solution: T5: Injection with 0.2 ml saline solution + 300µg pyridoxine : T6: Injection with 0.2 ml saline solution + 400 µg pyridoxine.

involved in red blood cell formation transamination, decarboxylation, desulfuration and amine oxidation of amino acids, the coenzymes are in the form of pyridoxal phosphate and pyridoxamine phosphate, and they play essential role in muscle phosphorylase activity, amino acid interaction and transport in nucleic acid synthesis via the production of active formaldehyde and in the actions of steroid hormones. The deficiency of vitamin B6 reduces the oxidation of linolenate (Pregnolato *et al*., 1994). Okada *et al*. (1998) confirmed that the function of pyridoxine in amino acid metabolism is reflected in an increased requirement when high levels of protein are fed. Pyridoxine deficient chicks show depressed appetite, poor growth and feed consumption, hyper excitability, weakness, microcytic hypochromic anemia, convulsion, and death perosis, toe deviations and also produces characteristic nervous symptoms which play an important role in jerky, movements of the legs when walking and often undergo extreme spasmodic convulsions that usually terminate in death (Yang and Jenq, 1998). Moreover, pyridoxine deficiency lead to early embryonic death (Landauer, 1967) and decreased IgM and IgG response to antibody challenge (Blalock *et al*., 1984). So that, in - ovo pyridoxine was vital for increasing hatchability even though the hen's diet was sufficiently supplemented with pyridoxine (Robel, 2002). Robel and Christensen (1991) reported that the injection of turkey eggs with 600 µg/egg

pyridoxine (B6) at the 25 days of incubation resulted in approximately 4.6% higher hatchability than the control (non injected). Also, York *et al*., (2004) and Bhanja *et al*., (2007) reported that the injection of chicken eggs with 100 µg/egg pyridoxine at 14 day of incubation period resulted in apparently higher hatchability (81.5%) than in non-injected control (80%). These results were confirmed by Elaroussi *et al*., (2003) and El- sayed *et al*., (2010) in quail and Ibrahim *et al*., (2012) and Amer, (2012) in ostrich.

Pyridoxine has an important role in amino acid, carbohydrate, and fatty acid metabolism and also plays a major role in the energy producing citric acid cycle (McDowell, 1989). Robel (1983) assayed turkey eggs for pyridoxine and observed significant decrease in their pyridoxine concentrations associated with maternal age. This aging feature coupled with the biological variable in the deposition of the vitamin in hen's eggs means that it is conceivable that some contain insufficient pyridoxine for embryo survival. It was considered that the transport of vitamin from the egg to the chick was responsible for the failure in embryo development. This reasoning reinforces that the practice of injection of vitamins, as well as other nutrients, may become a routine in poultry production (Vieira 2007).

Biochemical traits:

a-*Plasma proteins:*

Data of blood plasma proteins of Silver Montazah chicks at hatching day as affected by pyridoxine dietary supplementation and injecting eggs with pyridoxine are presented in Table 3. It is evidently shown that all studied plasma proteins were significantly ($P \leq 0.05$) affected due to treatments effect except of plasma albumin concentration. It is evidently shown that injection of pyridoxine at the highest level (400 µg) had significantly ($P \leq 0.05$) increased of plasma total protein and globulin concentration compared to un-supplemented and un-injected treatments. There were non- significant ($P \geq 0.05$) differences duo to treatments on plasma albumin concentration. However, the highest ($P \leq 0.05$) ratio of albumin/globulin ratio was recorded by eggs injected with the highest level of pyridoxine (400 µg) and pyridoxine supplemented at different levels compared to the other treatments. The lowest albumin/globulin ratio indicates more disease resistance and immune response (Lee *et al*., 2003). These results are, in agreement to some extent with the results of El-Sayed *et al*. (2010) who found that serum total protein, albumin and globulin were significantly higher ($P \leq 0.01$) in the groups of quail eggs injected with 120 µg pyridoxine than the other groups injected with 40 and 80 µg pyridoxine. In addition, there were non-significant differences in albumin concentration

Table (3): Effect of dietary and injecting hatching eggs with pyridoxine on blood plasma proteins of Silver Montazah chicks at hatch day.

Treatment groups	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A/G ratio
T1	3.780^{bc}	2.007	1.773^{ab}	1.137^{ab}
T2	4.227^{ab}	2.013	2.214^{ab}	0.970^{b}
T3	4.253^{ab}	2.007	2.246^{ab}	0.933^{b}
T4	3.347^{c}	1.847	1.500^{b}	1.287^{a}
T5	4.290^{ab}	2.090	2.200^{ab}	1.007^{ab}
T6	4.683^{a}	2.120	2.563^{a}	0.857^{b}
MES	**0.219**	**0.105**	**0.281**	**0.169**

[a, b, c] …Means within a column with different superscripts are significantly differ (P≤0.05). T1:Control without supplementation T2: Pyridoxine (8.25 mg/kg diet) T3: Pyridoxine (11.00 mg/kg diet) T4: Injection with 0.2 ml saline solution: T5: Injection with 0.2 ml saline solution + 300µg pyridoxine : T6: Injection with 0.2 ml saline solution + 400 µg pyridoxine.

between the treatments of quail received 120 µg and 80 µg pyridoxine. Also, Roussel *et al*. (1988) studied the effects of pyridoxine deficiency on the blood traits of neonatal chicken and found that total serum protein concentration was significantly decreased than normal, while serum albumin was declined from one-third to one-fifth of the control values. Moreover, serum protein level was higher (P≤0.01) in vitamin B6 injected birds compared with control ones, (Goel *et al*., 2013).

b-Plasma cholesterol, calcium and phosphorus:

Data of plasma cholesterol, calcium and phosphorus concentrations of chicks at hatching day as affected by dietary supplementation pyridoxine to laying hens and injecting eggs with pyridoxine are presented in Table 4. It is evidently shown that the treatments had a significant (P≤0.05) effect on plasma calcium and phosphorus concentrations of chicks at hatching day. However, eggs injected with the highest level of pyridoxine (400µg) recorded significantly (P≤0.05) increased of plasma calcium concentration of chicks at hatching day compared with un-injected treatments. Moreover, injecting eggs with pyridoxine at different levels recorded significantly (P≤0.05) increased of plasma phosphorus concentration compared with other treated groups except those supplemented pyridoxine at the highest level (11.00 mg/kg) to laying hens diet.

Table (4): Effect of dietary and injecting hatching eggs with pyridoxine on blood plasma cholesterol, calcium and phosphorus of Silver Montazah chicks at hatch day.

Treatment groups	Cholesterol (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
T1	289.29	8.937^{ab}	5.423^{c}
T2	284.33	9.137^{ab}	5.667^{bc}
T3	295.76	9.187^{ab}	5.977^{ab}
T4	297.15	8.777^{b}	5.367^{c}
T5	296.22	9.223^{ab}	6.093^{a}
T6	283.00	9.530^{a}	6.247^{a}
MES	**8.680**	**0.182**	**0.129**

[a, b, c] …Means within a column with different superscripts are significantly differ (P≤0.05). T1:Control without supplementation T2: Pyridoxine (8.25 mg/kg diet) T3: Pyridoxine (11.00 mg/kg diet) T4: Injection with 0.2 ml saline solution: T5: Injection with 0.2 ml saline solution + 300µg pyridoxine : T6: Injection with 0.2 ml saline solution + 400 µg pyridoxine.

On the other hand, there was insignificant (P≥0.05) differences duo to treatments effect on plasma cholesterol concentration of chicks at hatching day. These findings are consistent with the findings of El-Sayed *et al*. (2010) who showed that serum cholesterol concentration was significantly elevated by saline and pyridoxine injections with non-significant differences between the experimental groups. The same authors found that cholesterol concentration was positively correlated with increasing pyridoxine. Also, Frances *et al*. (1979) found that serum cholesterol levels of vitamin B6 deficient hens were lower than those of hens received an adequate diet. On the other hand, serum cholesterol level was not significantly changed due to vitamin B6 injected (Goel *et al*., 2013). The current results were supported by Siegel *et al*. (2006) who reported that serum calcium concentration were non-significantly differ by pyridoxine injection. Further, El-Sayed *et al*. (2010) found that serum calcium concentration were non-significantly affected by pyridoxine injection. The same authors added that phosphorus level was negatively affected by both saline and pyridoxine injections, therefore, control group had significant higher serum level of phosphorus than other groups. These results effectively support the current findings of hatching traits and coincided with the earlier suggestion of Christensen and Eden (1985) who showed that the improvement in hatchability percentages of turkey eggs was associated with higher embryonic calcium and

phosphorus. Moreover, this increment had important role in stimulating muscular activity and contraction (Christensen and Biellier, 1982).

C-*Thyroid activity and liver functions*:

Results in Table 5 showed significant differences ($P \leq 0.05$) in concentrations of plasma thyroid hormones (T_3 and T_4), as an indication of thyroid activity of chicks at hatch day due to dietary supplementation of pyridoxine to laying hens and injecting eggs with pyridoxine. The highest ($P \leq 0.05$) values of plasma T_3 and T_4 hormones concentrations were recorded by eggs injected with the highest level of pyridoxine (400µg) compared with un-injected treatments. On the other hand, there were non-significant differences in hepatic enzymes activities {aspartate amino transaminase (AST) and alanine amino transaminase (ALT) enzymes} of chicks at hatching day duo to treatments groups effect. In this respect, El-Sayed *et al*. (2010) found that serum triiodothyronine (T_3) concentration was significantly increased in the group of quails received 120 µg pyridoxine than the other groups (40 and 80 µg pyridoxine), while non-significant differences were found between the groups of quails received 120 µg pyridoxine and control one. Also, Virden *et al*. (2003 and 2004) showed that serum T_3 concentration was significantly elevated in Hubbard hens received high levels of pyridoxine (100 µg) in comparison with the low pyridoxine levels (40 and 60 µg).

Table (5): Effect of dietary and injecting hatching eggs with pyridoxine on liver function and thyroid activity of Silver Montazah chicks at hatch day.

Treatment groups	Liver function		Thyroid activity	
	AST	**ALT**	**T3**	**T4**
T1	48.94	20.05	1.780^{ab}	8.783^{ab}
T2	49.02	20.62	1.820^{ab}	8.837^{ab}
T3	50.20	20.42	1.830^{ab}	8.953^{ab}
T4	48.46	19.93	1.657^{b}	8.320^{b}
T5	51.17	20.61	1.847^{ab}	8.953^{ab}
T6	49.59	20.99	1.913^{a}	9.060^{a}
MES	**1.826**	**0.639**	**0.058**	**0.20**

$^{a, b, c}$ …Means within a column with different superscripts are significantly differ ($P \leq 0.05$). T1:Control without supplementation T2: Pyridoxine (8.25 mg/kg diet) T3: Pyridoxine (11.00 mg/kg diet) T4: Injection with 0.2 ml saline solution: T5: Injection with 0.2 ml saline solution + 300µg pyridoxine : T6: Injection with 0.2 ml saline solution + 400 µg pyridoxine.

The present results are consistent with previous results De Oliveira (2007) reported an increase in the thyroxin hormones during the late stage of embryonic development, when the chick embryo needs more oxygen and energy to survive and hatch after being switched to lung respiration. Furthermore, the embryonic ability to consume the liver and muscular glycogen as a source of energy during the hatching days depends mainly on thyroid hormone levels.

The present results indicated that supplementing with the pyridoxine to laying hens or injecting eggs with pyridoxine had no deleterious effect on liver functions and may protect the hepatocytes of chicks from being destroyed and that is meant a better liver function associated with pyridoxine supplementation or injecting eggs with pyridoxine. The current results were supported by Siegel *et al*., (2006) who reported that serum AST was significantly ($P \leq 0.01$) changed by pyridoxine injection while serum ALT concentration were non-significantly differed. El-Sayed, *et al*. (2010) found that serum AST level was significantly increased in the groups of quails received 40 µg pyridoxine. Moreover, non-significant differences were observed between both groups of quails received 80 µg and 120 µg pyridoxine or between control and saline groups. The later authors added that serum ALT concentration was non-significantly affected by pyridoxine injection.

In conclusion, *in-ovo* injection of pyridoxine, especially at 400µg at 18 days of incubation is a practical and beneficial procedure for improving the hatchability traits and the early post-hatch physiological responses as indicated with better blood traits and metabolic hormones expression.

REFERENCES

Amer N. S. I. (2012). Studies on improving ostrich egg hatchability and its relation with some factors affecting embryonic development during artificial incubation. Ph.D. Thesis, Faculty of Agriculture, Cairo, Al -Azhar University.

Bender D.A. (1999). Non-nutritional uses of vitamin B6. Br. J. Nutr.,81(1):7-20.

Bhanja S.K., Mandal A.B., Agarwal S.K., Majundar S., and Bhattacharyya A. (2007). Effect of in ovo injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens. World Poult. Sci. Association, Proceeding on Poultry Nutrition, Strasbourg, France, pp 143-146.

Christensen V.L., and Biellier H.V. (1982). Physiology of turkey embryos during pipping and hatching. IV. Thyroid function in embryos from selected hens. Poult. Sci. 61:2482- 2488.

Christensen V.L., and Edens F.W. (1985). Magnesium calcium, and phosphorus content of shells from hatching and non-hatching turkey eggs. Poult. Sci. 64: 1020 1027.

De Oliveira J.E. (2007). Effects of in *ovo* feeding on Turkey embryos development, energy status, intestinal maturation, gene expression and post-hatch development. A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy Nutrition Raleigh, North Carolina.

Duncan D.B. (1955). *Multiple range and multiple F-tests. Biometrics,11:1-42*.

Elaroussi M.A., Abu-Taleb A.M., and Elbarkouky E. (2003). Manipulating embryonic growth by in ovo nutrient administration to Japanese quail eggs. J. Egypt. German Society of Zoolgy, Vertebrate Anatomy & Embryology, 40B:31-48.

El-sayed M.A., Wakwak M.M., and Mahrose Kh. M. (2010). Effect of pyridoxine injection in Japanese quail eggs on hatchability, performance and some of physiological parameters. Isotope & Rad. Res., 472(1): 109-123.

Frances G. and Scott L., (1979). Influence of Vitamin B-6 upon Reproduction and upon Plasma and Egg Cholesterol in Chickens. The Journal of Nutrition, Volume 109, Issue 6, 1, 1010–1017**.**

Goel A., Bhanja S. K., Pande V., Mehra M. and Manddal A. (2013). Effects of *in ovo* administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens. Indian Journal of Animal Sciences **83** (9): 916–921.

Ibrahim N.S., Wakwak M.M., and Khalifa H.H. (2012). Effect of in ovo injection of some nutrients and vitamins upon improving hatchability and hatching performance of ostrich embryos. Egypt. Poult.Sci. (32): 981-994.

Landauer W. (1967). The hatchability of chicken eggs as influenced by environment and hereditary. Storrs Agricultural Experimental Station, Monogr1University of Connecticut, Storrs.

Lee H.G., Seong C.S., Kim Y.C., Davis R.L., Han K.A. (2003). Octopamine receptor OAMB is required for ovulation in Drosophila melanogaster. Dev. Biol. 264(1): 179--190.

McDowell L.R. (1989). Vitamins in animal nutrition: comparative aspects to Human Nutrition. Academic Press, San Diego, CA. p:155.

NRC (1994). National Research Council. National Requirements of Poultry. 9th Rev. Edn., National Academy Press, Washington, DC. USA.

Okada M., Shibuya M., Akazawa T., Muya H., and Murakami Y. (1998). Dietary protein as a factor affecting vitamin B6 requirement. J. Nutr. Sci. Vitaminol (Tokyo).44(1):37-45.

Pregnolato P., Maranesi M., Marchetti M., Barzanti V., Bergami R., Tolomelli B. (1994). Interaction among dietary vitamin B6, proteins and lipids: effects on liver lipids in rats. Int. J. Vitam. Nutr. Res. 64(4):263-269.

Reijrink I.A.M., Meijerhof R., Kemp B., Graat E.A.M. and van den Brand H. (2009). Influence of prestorage incubation on embryonic development, hatchability, and chick quality. Poultry Science 88, 2649–2660.

Robel E.J. (1983). "The effect of age of breeder hen on the levels of vitamins and minerals in Turkey eggs", Poult. Sci. 62:1751-1756.

Robel E.J. (1992). Effect of dietary supplemental pyridoxine levels on the hatchability of turkey eggs. Poult. Sci. 71(10):1733-1738.

Robel E.J. (2002). Assessment of dietary and egg injected d-biotin, pyridoxine and folic acid on turkey hatchability: folic acid and poultry weight. World's Poult. Sci. J. 58:305-315.

Robel E.J., and Christensen V.L. (1991). Increasing hatchability of turkey eggs by injecting eggs with pyridoxine. Bri. Poult. Sci. 32(3):509–513.

Roussel P.H., Lamblin G., Lhermitte M., Houdret M., Lafitte J.J., Perini J.M., Klein A. and Scharfman A., Biochimie (1988). The complexity of mucins. *Biochimie.* 70(11):1471–1482.

SAS institute (2001). *SAS Users Guide Statistics*. Version 10th, 16-Edition, SAS Inst., Cary, NC.

Scott M.L., Nesheim M.G., and Young R. (1976). *Nutrition Of The Chicken* 2nd edition, Published by M. L. Scott & Associates, Ithaca, New York.

Sharma C.P., and Gehring H. (1987). Effect of vitamin B6 on the synthesis and degradation of aspartate aminotransferase in chicken embryo fibroblasts. *J. Biol. Chem.* , 262:16503-16508.

Siegel P.B., Blair M., Gross W.B., Meldrum B., Larsen C., Boa Amponsem K. and Emmerson D.A., (2006). Poult Performance as Influenced by Age of Dam, Genetic Line, and Dietary Vitamin E .*Poultry Science*, 85, 939-942.

Squires M.W., and Naber E.C. (1993). Vitamin profiles of egg as indicators of nutritional. Status in laying hen: riboflavin study. *Poult. Sci.* 72: 483-499.

Stevens L. (1991). Egg white proteins. Comp. Biochem. *Physiol. B.* , 100 (1):1-9.

Tona K., Onagbesan O., De Ketelaere B., Decuypere E. and Bruggeman V. (2004). Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and post hatch growth to forty-two days. Journal of Applied Poultry Research 13, 10–18.

Vieira S. L. (2007). Chicken embryo utilization of egg micronutrients. Brazilian Journal of Poultry Science, 9(1), 1–8.

Virden W.S., Yeatman J.B., Barber S.J., Willeford K.O., Ward T.L., Fakler T.M., Wideman R.F. and Kidd M.T., (2004) Immune system and cardiac functions of progeny chicks from dams fed diets differing in zinc and manganese levels and source. Poultry Sci., 83, 344-351.

Virden W.S., Yeatman J.B., Barber S.J., Zumwalt C.D., Ward T.L., Johnson A.B. and Kidd M.T.,(2003). Hen Mineral Nutrition Impacts Progeny Livability. J. Appl. Poult. Res. 12:411–416.

Yang C.P., and Jenq S.L. (1998). pyridoxine deficiency and requirement in mule ducklings. J. Chin. Agric. Chem. Soc. 27: 450-459.

York M.A., Gul M., Hayirli A., and Karaoglu M. (2004). Laying performance and egg quality of hens supplemented with sodium bicarbonate during the late laying period. Inter. J. Poult. Sci.. 3(4): 272-278.

تأثير كل من التغذية وحقن بيض التفريخ بالبيرودوكسين على بعض صفات الفقس والتفريخ وبعض الصفات الفسيولوجية فى الدجاج.

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أجريت هذه الدراسة فى محطة بحوث تربية الدواجن بأنشاص– معهد بحوث الانتاج الحيوانى وذلك بهدف دراسة تأثير كل من تغذية وحقن البيض المخصب للدجاج بالبيريدوكسين (فيتامين ب ٦) فى تحسين نسبة الفقس وتقليل النفوق الجنينى وزيادة وزن الكتاكيت الفاقسة وبعض مكونات الدم فيها. واستخدم فى هذه الدراسة عدد ٦٠٠ بيضة مخصبة من سلالة المنتزه الفضى بواقع ٣٠٠ بيضة لكل من تغذية وحقن البريدوكسين.

تم جمع عدد٣٠٠ بيضة مخصبة من الأمهات عند عمر ٣٦ أسبوع وتم تقسيمهم الى ٣ معاملات متساوية (١٠٠ بيضة/المعاملة) تبعا لتغذية الأمهات على البريدوكسين كالتالى:

-**المعاملة الأولى**:تم جمع البيض فيها من أمهات غذيت على العليقة الأساسية دون إضافات، أما **المعاملتين الثانية والثالثة** تم جمع البيض فيها من أمهات غذيت على العليقة الأساسية مضاف اليها البريدوكسين بمعدل ٨.٢٥ ، ١١.٠٠ مجم/كجم عليقة على الترتيب فى الفترة من ٢٤-٣٦ أسبوع من العمر. في نفس الوقت تم جمع عدد ٣٠٠ بيضة مخصبة أخرى من نفس القطيع ، ونفس العمر من أمهات غذيت على العليقة الأساسية دون إضافات، ووضعهم في ماكينة التفريخ. فى اليوم الثامن عشر من التفريخ تم تقسيم البيض الى ٣ معاملات تبعا لحقن البيض بالبريدوكسين بواقع (١٠٠ بيضة/معاملة) كالتالى:

- **المعاملة الرابعة**: تم فيها أخذ البيض المخصب و حقنه بمقدار ٠.٢ مل محلول ملحى فقط ، المعاملتين الخامسة والسادسة: تم فيها حقن البيض بمقدار ٠.٢ مل محلول ملحى يحتوى على ٣٠٠،٤٠٠ ميكروجرام بيرودوكسين على الترتيب.

وتتلخص أهم النتائج المتحصل عليها فيما يلى:

- وجد أن هناك اختلافات معنوية نتيجة المعاملات على كل من نسب النفوق الجنينى، الفقس وفرز الكتاكيت الفاقسة وكذلك وزن الكتاكيت الفاقسة.
- وجد أن أفضل القيم معنويا في نسب الفقس والنفوق الجنيني ووزن الكتاكيت الفاقسة قد تم تسجيلها في المجموعتين الخامسة والسادسة والتي تم حقن البيض فيهما بالبريدوكسين بالمستويات المختلفة (٣٠٠، ٤٠٠ميكروجرام) على الترتيب مقارنة بالمجموعتين الأولى والرابعة والتي لم يتغذى فيها الأمهات (المجموعة الأولى) وكذلك التي حقن البيض فيها بالمحلول الملحي فقط (المعاملة الرابعة). والتى سجلت أسوأ النتائج فى تلك الصفات. كما وجد أن أفضل القيم معنويا بالنسبة لجودة الكتاكيت الفاقسة من الفئة A قد تم تسجيلها أيضا في المعاملتين الخامسة والسادسة مقارنة بالمعاملات الأخرى، بينما أفضل القيم معنويا بالنسبة لجودة الكتاكيت الفاقسة من الفئة B قد تم تسجيلها فى المعاملة السادسة والتى تم حقن البيض فيها بالمستوى الأعلى من البريدوكسين (٤٠٠ميكروجرام) مقارنة بالمعاملات الأخرى
- وجد أيضا أن أفضل القيم معنويا في تركيزات البروتينات الكلية، الجلوبيولينن، الكالسيوم، الفسفور، هرمونات الغدة الدرقية (T_4 , T_3) ونسبة الألبيومين إلى الجلوبيولين في بلازما دم الكتاكيت الفاقسة قد تم تسجيلها في المعاملة التي تم حقن بيض التفريخ فيها بالمستوى الأعلى من البريدوكسين (٤٠٠ميكروجرام) مقارنة بالمعاملات التي غذيت فيها الأمهات بالبريدوكسين والتي تم حقن البيض فيها بالمحلول الملحي فقط.

التوصية : يتضح من هذه النتائج أن حقن بيض تفريخ الدجاج بالبريدوكسين خاصة بمعدل ٤٠٠ ميكروجرام فى اليوم الثامن عشر من التفريخ أدى الى تحسين نسب التفريخ وتقليل النفوق الجنينى وزيادة وزن الكتاكيت الفاقسة من خلال تحفيز مكونات الدم البيوكيميائية ونشاط الغدة الدرقية خلال المرحلة الأخيرة من التفريخ.