Egypt. Poult. Sci. Vol. (40) (I): (153-171) (2020) **(1912-1068)**

Egyptian Poultry Science Journal

http://www.epsj.journals.ekb.eg/

ISSN: 1110-5623 (Print) – 2090-0570 (Online)

EFFECTS OF OMEGA-3 ADMINISTERED IN-OVO DURING INCUBATION ON SOME PHYSIOLOGICAL PARAMETERS OF THE DEVELOPING CHICK EMBRYOS

Basuony H.A.[1], Elaroussi M.A.[1], and A.M.M. Badran[2]

[1] Poult. Res. Unit, Biolog. Applic. Dep., Nuc. Res. Center, Atom. Ener. Authority.

[2]Anim. Prod. Res. Inst., Agric. Res. Center, Ministry of Agric. Dokki, Giza

Corresponding Author: A.M.M. Badran[2] email aamel555@gmail.com

Received: 10/12/2019 Accepted: 29 /01/2020

ABSTRACT: Total of 400 hatching eggs with an average egg-weight of 60.5 ± 1.5g from Hy-Line Brown Strain acquired from layer breeder flock (46 weeks of age) were used in this study to investigate the impact of in-ovo injection of omega-3 fatty acids on hatchability and some physiological and histological parameters of the developing chick embryos and newly hatched chicks. At the 10th day of embryogenesis, fertile eggs were allocated into four equal groups. In the 1st group, embryonated eggs did not receive any treatment and served as the control group. Eggs of the 2nd, 3rd and 4th groups were injected into the yolk sac with 200µL sterile saline, 100 and 200µL of omega-3 fatty acids, respectively. Results revealed that in-ovo injection with low dose of omega-3 (100µl) resulted in a significant increase in the hatchability percent, embryonic and post-hatch body weight, serum total protein, globulin, thyroid hormones concentrations and total antioxidant capacity in the hepatic and brain tissues as compared with high dose of omega-3 (200µl), or control groups. While, significant increases in serum total lipids, cholesterol, triglycerides concentrations and alkaline phosphatase, alanine transaminase, and aspartate transaminase enzymes activities as well as harmful histological changes in hepatic tissues were observed in high dose of omega-3 (200µl). In conclusion, in-ovo injection with 100µl of omega-3 caused enhancement in hatchability, body weight, total antioxidant capacity of hepatic and brain tissues and some of the physiological aspects during embryonic development and at hatch.

Key Words: Chicken embryo, omega-3, antioxidant capacity, thyroid hormones, histological changes.

INTRODUCTION

Nutrient deficiencies (less supply of energy, vitamins or minerals in breeder diets) in eggs used for incubation can regularly cause malformed growth of embryos and noticeable decrease in hatchability (Uni *et al*., 2005). Egg yolk contains about 5-6 g of lipid, nearly 80% of it consumed by the developing embryos for structural membrane synthesis and energy production. Peroxidation of low quality lipid may predispose the increase of cellular membranes damage and metabolic disorders (Abdulwahid *et al*., 2017). Fat sources and poly unsaturated fatty acids (PUFA) deposition in embryonic tissues, such as brain and heart tissues, are essential for their supplementation with energy (Dewailly *et al*., 2001). Therefore, supplementary energy sources may support the late-termed embryos growth and improve the genetic potential for late embryos and early post-hatch growth via enhancing the ability of digestion and metabolism which led to improve the growth rate and accelerate access to marketing weight (Al-Daraji *et al*., 2010; Dos Santos *et al*., 2010; Selim *et al*., 2012). Natural fish oil is used as a source of omega-3 essential fatty acids (decosahexaenoic acid; DHA and eicosapentaenoic acid; EPA) that are important for normal metabolism (Uni & Ferket, 2004). Because poultry cannot synthesize omega-3 fatty acids they rely on the shorter-chain omega-3 fatty acid from feed sources, such as alpha-linolenic acid (ALA), in forming the long-chain omega-3 fatty acids. The essential fatty acids were given their name when researchers found that they are essential to normal growth in young animals (Van West & Maes, 2003). Recently, one of the new technologies introduced in poultry industry is in-ovo feeding. In this technique, liquid nutrients are injected into the embryos' air cell, amniotic fluid or yolk sac (Herfiana, 2007; Abd El-Moneim *el al*., 2019). This mechanism is useful for embryos development because protein and energy are first derived from the yolk of the egg (Vieira, 2007). The importance of such method appears more as it is believed that birds have access to feed only after 36-48 hours which may subsequently influence the body weight and muscle development (Noy &Uni, 2009). Recent twenty years, nutrition, management and genetic selection of poultry flocks has improved, however, in commercial hatcheries, hatchability of broiler eggs has not elevated (Schaal & Cherian, 2007). In-ovo technology may help to improve hatchability and health of the hatched chick's weight and growth performance through feeding metabolic modulators to the developing embryo (Bakyaraj *et al*., 2011; Selim *et al*., 2012).

Exogenous fatty acids and antioxidants also provided to the developing embryo during incubation period may enhance chicken embryos (Schaal, 2008; Perez *et al*., 2010). Therefore, the goal of the current study was to investigate the impact of in-ovo injection of natural fish oil as a source of omega-3 fatty acids, on hatchability and some physiological parameters of the developing embryos and hatching chicks.

MATERIALS AND METHODS

The present study was carried out in the Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority. All procedures were in accordance with the National Institutes of Health Guide for the care and Use of Laboratory Animals.

1-Eggs and Omega-3 sources:

Natural fish oil as a source of omega-3 fatty acids was obtained from Natural

Assets, (Omana Group, LLC. Garden Grove, CA92841, USA) and dissolved in liquid form (Gelatin capsule) containing 180 mg EPA (Eicosapentaenoic Acid), 120 mg DHA (Dococsahexaenoic Acid), gelatin, glycerine and purified water. Hatching eggs with an average egg-weight of 60.5 ± 1.5g from broiler breeder layers Hy-Line Brown Strain acquired from layer breeder ages (46 weeks of age) were a generous gift from Miser Poultry Company.

2-Experimental design:

Total of four hundred Hy-Line hatching eggs were individually weighed to the nearest 0.01 g using an electronic digital balance, and then incubated in a Victoria incubator (Guanzate Co, Italy) at 37.5°C and 60% relative humidity during the first 18th days of incubation period. At the 10th day of incubation (doi), eggs were candled to remove clear eggs (non-fertile), and to identify the air cell and yolk sac site. Handel egg injection method was applied for injection of natural fish oil as a source of omega-3 fatty acids after candled and only embryonated eggs were selected for injection. Thus three hundred and sixty fertile eggs were divided into four equal groups with three replicates for each group (30 fertile eggs for each replicate). In the first group, embryonated eggs do not receive any treatment (non injected group) and served as the control group. In the second group, each egg was injected with 200µL sterile phosphate buffer saline through a pinhole made at the narrow end of the egg with a 24G hypodermic needle (25 mm long) to reach the yolk sac according to Abd El-Moneim *et al*. (2019) and served as the sham control group. While, each egg of the third and fourth groups was injected into the yolk sac with 100 and 200µL of natural fish oil as a source of omega-3 fatty acids, respectively. Complete disinfection was done after every injection by alcohol 70% to prevent cross contamination between individual eggs. After the eggs were injected, the injection holes were sealed with sterile wax and return to the incubator to complete the incubation.

3-Blood and organs samples:

At the 15th and 18th doi, nine blood samples per each group were collected from embryonic vitelline vein, while, at hatch nine blood samples per each group were collected by slaughtered. After coagulation, the blood was centrifuged at 4500 rpm for 15 minutes to obtain the serum. The serum samples were frozen at -20°C till biochemical analysis. At the 15th and 18th doi and at hatch, nine embryos and nine hatched chicks were individually weighed (g). The liver, brain and heart were collected, weighted and their percentages to live body weight were calculated. After removing and weighing the brain and liver they were quickly washed in ice cold saline and blotted individually on filter paper then homogenized separately in phosphate buffer (pH 7.4) and then kept at -20°C for total antioxidant capacity determination. Moreover, at the 15th and 18th days of embryonic development chorioallantoic fluid was collected to determine creatinine and uric acid concentrations.

At hatch, hatching percentage was calculated (after removing infertile and sampling eggs) as follows:

Hatching percentage = (No. of hatched eggs/No. of fertile eggs after sampling) × 100

Total embryonic mortality rate was calculated using the equation:

Embryonic mortality (%) = 100 - Hatching percentage

4- Biochemical analysis:

Serum total protein, albumin, cholesterol, triglycerides and total lipids concentrations as well as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) enzymes activities and creatinine and uric acid concentrations in the chorioallantoic fluid were determined using chemical reagent of commercial kits (Stanbio Company, USA) and measured on computerized spectrophotometer (model Spectronic 1201, Milton Roy, USA). Triiodothyronine (T_3) and thyroxine (T_4) levels were determined using commercial radioimmunoassay (RIA) kits (IZOTOP Co., INSTITUTE OF ISOTOPES Ltd., http://www.izotop.hu) and samples were counted on a Packard Gamma Counter (model 540501 RIA SAR, USA).

5-Histological Examination:

At the18th doi and at hatch, small pieces of the previously collected livers were quickly removed and fixed in 10% neutral buffered formalin solution for histological examinations. Following fixation, specimens were dehydrated in graded ethanol, embedded in wax, sectioned to 5 microns thickness. The sections were stained with Haematoxylin and Eosin (Banchraft *et al.*, 1996) and examined under Olympus light microscope.

6-Statistical analysis:

Data were statistically analyzed by one-way analysis of variance using the General Liner Model Procedure of the SAS software (SAS Institute, 2000). Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) at $p < 0.05$. The model applied was $Y_{ij} = \mu + Ti + e_{ij}$ where: Y_{ij} = any value from the overall population, μ = overall mean, T_i = the effect of the ith treatment (i=1, control; 2, sham control group; 3, 100 µL omega-3and 4, 200µL omega-3) and e_{ij} = the random error associated with the ijth individual.

RESULTS

1-Effect of in-ovo omega-3 injection on hatchability and embryonic mortality:

Hatchability percentage was significantly higher when the fertile eggs were injected with low dose of omega-3 (92.3%) as compared with the high dose of omega-3 (90.2%). The lowest hatchability percentage was recorded for the control (88.4%) and sham-injected control (88.5%) groups (Fig. 1). On the other hand, data obtained from Figure (1) shows that, mortality rate was significantly decreased in omega-3 injected groups compared with the untreated and sham control groups. The lowest mortality rate was occurred when the fertile eggs were injected with the low dose of omega-3 (7.7%) followed by the high dose of omega-3 (9.8%).

2-Effect of in-ovo omega-3 injection on body weight and some organs relative weight:

Embryonic and hatching body weights were significantly higher in the omega-3 treated groups as compared with the untreated or the sham control groups (Table 1). The highest body weights of embryos and hatchlings were recorded in the low dose of omega-3 injected group, while the lowest values were recorded for the control and sham control groups. On the other hand, injecting the fertile eggs with omega-3 doses had no significant effects on liver, heart and brain relative weights.

3-Effect of in-ovo omega-3 injection on serum proteins:

Results in Table (2) demonstrated that at the 15th, 18th doi and at hatch serum total protein and globulin concentrations were significantly higher in omega-3 injected

groups as compared with the control and the sham control groups. The highest concentrations of serum total protein and globulin were recorded for the low dose of omega-3 treated group at all studied ages. Contrarily, serum albumin concentration was significantly lower in the omega-3 treated groups. The lowest albumin values were recorded in the embryos and chicks injected with the high dose of omega-3.

4- Effect of in-ovo omega-3 injection on some serum enzymes activities:

Data in Table (3) demonstrates the effects of in-ovo omega-3 injection on some enzymes activities at the 15^{th}, 18^{th} days of incubation and at hatch. It could be observed that serum ASTALT and ALP activities were significantly higher in the omega-3 treated groups as compared with the control and sham control groups. Generally, the highest activities of the previous enzymes were recorded when the fertile eggs were injected with the high dose of omega-3 while the lowest values were recorded for control and sham control groups.

5-Effect of in-ovo omega-3 injection on serum lipids profile:

Table (4) shows the effect of in-ovo omega-3 injection on serum lipids profile. It could be observed that serum total lipids, cholesterol, and triglycerides concentrations were significantly lower at the 15^{th} and 18^{th} doi and at hatch when the fertile eggs were in-ovo injected with the low dose of omega-3 as compared with untreated-control or sham control groups.

6-Effect of in-ovo omega-3 injection on thyroid hormones concentrations:

Results presented in Table (5) shows the effect of in-ovo omega-3 injection on serum thyroid hormones concentrations during embryonic development periods and at hatch. T_3 and T_4 concentrations were significantly higher in the omega-3 injected groups comparing with the control and sham control groups. Furthermore, thyroid hormones levels were significantly higher ($P < 0.05$) in treated group with low doses of omega-3.

7-Effect of in-ovo omega-3 injection on allantoic uric acid and creatinine concentrations:

The effect of in-ovo omega-3 injection on allantoic fluid uric acid and creatinine concentrations are presented in Table (6). The results illustrated that at the 15^{th} and 18^{th} days of embryonic development, the highest concentrations of allantoic uric acid and creatinine were observed in the high dose of omega-3 injected group. While there were no significant differences in their concentrations between the low dose of omega-3 treatment, control and sham control groups.

8-Effect of in-ovo omega-3 injection on total antioxidant capacity in hepatic and brain tissues:

The results of Table (7) demonstrated that, at the 15^{th} and 18^{th} days of incubation and at hatch, total antioxidant capacity in the hepatic and brain tissues were significantly higher ($P \leq 0.05$) when the fertile eggs were injected with low dose of omega-3 as compared with the high dose and control groups. However, the lowest values ($P \leq 0.05$) of total antioxidant capacity in hepatic and brain tissues were recorded for the high dose of omega-3 injected group.

9-Histological Examination:

Prominent histological changes in the liver tissue related to the in-ovo omega-3 injection as compared with the control at the 18^{th} doi and at hatch are illustrated in Figure 2: A, B and C and Figure 3: A, B and C, respectively. At the18^{th} doi, no histopathological alterations were observed and the normal histological

structure of the central vein and portal area with surrounding hepatocytes in the hepatic parenchyma were noticed in the control group (Fig. 2, A).When fertile eggs were injected with low dose of omega-3, normal histological structure of the central and portal veins with slight cytoplasmic vacuolization of hepatocytes were observed (Fig. 2, B). While, when fertile eggs were in-ovo injected with high dose of omega-3, liver of chicken embryo at the 18th doi showing dilatation of central and portal vein with few inflammatory cells infiltration in portal area and vacuolar degeneration in the hepatocytes (Fig. 2, C).

Liver tissues of control hatched chicks were showing normal histological structure of the central vein and portal area with surrounding hepatocytes in the hepatic parenchyma (Fig. 3, A).While, in-ovo injection with low dose of omega-3 showing few micro fatty change in hepatocytes all over the parenchyma (Fig. 3, B). Finally, few congestion in the central and portal vein were observed after hatch as a result of high dose of omega-3 injection (Fig. 3, C).

DISCUSSION

In our study, in-ovo injection with 100µL of omega-3 improved hatchability, embryonic and newly hatching weight and reduced mortality rates as compared with 200µL of omega-3 treated group and control groups. Recently, it has been shown that in-ovo administration of nutrients could be considered as an alternative method to improve hatchability (Amen, 2015). These results are compatible with the findings of previous reports (El-Sayed & Hashim, 2000; Uni & Ferket, 2003). Moreover, the obtained results are in agreement with Amen (2016) who reported that in-ovo omega-3 injection subsequently improved hatching weight and growth performance and immune response as well as reduces the risk of chronic diseases and mortality rates. It has also been reported that hatchability was improved with in-ovo feeding of omega-3 fatty acids which might ameliorate the production of energy during embryogenesis (Amen, 2016). The reserve of fatty acids leads to improve embryo's ability to hatch and to perform; therefore, supplying embryos with exogenous nutrients could increase final body weight of broilers (Al-Zuhairy & Alasadi, 2013). Fat sources rich with omega-3 increase growth by activating bile production which leads to increase post-hatch efficiency of diet digestion and absorption.

Findings of Abdulwahid *et al*. (2017) also agreed with ours, they reported that in-ovo injection with hatching eggs with Cod liver oil significantly increased hatchability and body weight as compared with the control. The authors attributed the increase in hatchability due to the higher level of omega-3 found in CLO which improve the energy production during embryogenesis. Bautista-Ortega *et al*. (2009) fed Cobb breeder hens with corn-soybean meal-based diet containing 3.5% sunflower oil (low n-3), 1.75% sunflower oil plus 1.75% fish oil (medium n-3), or 3.5% fish oil (high n-3). They found that hatchability for the low (1.8), medium (10.3), and high (13.3%) omega-3 supplementation was 89, 85, and 83%, respectively. Abdulwahid *et al*. (2017) also demonstrated that inoculation with CLO significantly increased mean body weight of chicks as compared with control. This improvement in growth rate could be attributed to the critical role of omega-3 on energy needed for the metabolic process without exhausting glycogen storage. Furthermore, omega-3 PUFAs have

important role in the cellular development as they involved in the structure of cellular membranes (Schaal, 2008). In addition, Salih (2009), Tako *et al*. (2004) and Lopes *et al*. (2006) reported that in-ovo inoculation of Omega-3 PUFAs enhanced the presence of more satellite cells in early post hatch development as well as the proliferation of myoblasts during embryonic development which in turn improve the muscular growth. The results obtained by Uni & Ferket (2004), Zhai *et al*. (2008), Dos Santos *et al*. (2010) and McGruder *et al*. (2011) demonstrated that in ovo inoculation with a variety of nutrients enhanced the energy status of late-term embryos, post hatch weights and growth. Furthermore, fat sources enriched with omega-3 increased could increase broiler growth due to activating bile secretion which leads to increase fat digestion and absorption (Bou *et al*., 2004; Safamerher *et al*., 2008).

In-ovo injection with omega-3 in the present study had no significant effect on relative liver, brain and heart weights during embryonic development or at hatch. Our obtained results are in agreement with Chashnidel *et al.* (2010) who found that male broiler fed fish oil up to 4.5% had no significant effects on weight of liver, gizzard, and heart. On the other hand, serum total protein and globulin concentrations were significantly higher with in-ovo low dose omega-3 injection, while, serum albumin concentration was significantly lowered. The increase in globulin and total protein concentrations may be due to the effect of source of omega-3 fatty acids, found in the natural fish oil, in elevating the immune response by stimulating lymphocytes proliferation. The present findings are in accordance with Abdulwahid *et al.* (2017) who noticed that injection of hatching eggs with (Cod Liver Oil) CLO increased total protein and globulin concentration and decreased albumin level significantly as compared with the control. Al-Mayah (2009) reported that dietary supplementation of 50 g of fish oil/kg diet accelerate the circulating immune-globulins M and G then increased serum total protein. Therefore, reduction in mortality rate in the current study with low dose of omega-3 group as compared with the high dose of omega-3 and control or sham control groups may be attributed to the increase in globulin concentrations.

In the current study, the elevation in hepatic enzyme activities in high dose of omega-3 treated group might indicate recent liver functions disturbance (Koreleski & Switakiewicz, 2006). Therefore, the constant increase in ALT, AST and ALP was indicative of hepatocellular dysfunction and destruction (Alparslan & Özdogan, 2006). Moreover, elevated AST activity is not exclusively specific for liver failure but also considered as a cardiac biomarker. The increase in ALT activity followed the administration of high dose of omega-3 might correlates with the number of damaged hepatocytes and evaluates the extent of hepatic damage (Ruxton *et al*., 2007). Furthermore, the increase in ALP activity with high dose of omega-3 administration was considered as an indication for hepatobiliary disease (Hunkar *et al*., 2002).

The significant reduction in total lipids, triglycerides and cholesterol concentrations at the15th and 18th days of embryogenesis and at hatch in 100µL omega-3 injected group may be due to that the low dose of omega-3 has hypolipidemic action (Hosseini, 2011). These results are accordance with Hosseini & Bahrami (2011) who found

that serum total lipids, cholesterol and triglycerides levels were significantly lowered with feeding low dose of fish oil (1%) as compared with the high dose of fish oil (4%) or control. Also, Sultan (2005) demonstrated that the effect of fish oil on the cholesterol concentration is primarily by decrease diverting lipids metabolism to phospholipids formation. Saleh (2009) also reported that fish oil supplementation in broiler diets decreased plasma levels of cholesterol and triglyceride in comparison to those of control group. Al-Mayah (2009) showed that the inclusion of low dose of fish oil in the broilers diet decreased plasma lipoprotein concentration and this may be due to the decline in triglycerides synthesis and secretion from the liver by increasing proximal beta oxidation, decreasing activity of synthetic enzymes and increasing the expression of hepatic receptor for LDL which caused by long chain of omega-3 such as EPA and DHA (Schumann *et al*., 2000). In addition, the reduction in serum cholesterol concentration in the present study might be due to suppressive hepatic synthetic enzymes HMG-CoA synthase (3-hydroxy-3-methylglutaryl–coenzyme A) which increase B-oxidation process and involved in cholesterol formation (Chashnidel *et al*., 2010). Omega-3 fatty acids can also reduce serum concentrations of triglycerides and cholesterol by increasing the removal of LDL from the liver or peripheral tissues, and increase bile excretion in the feces (Dewailly *et al*., 2001).

Thyroid hormone elevations during the incubation period have been shown to be required for embryonic development and hatch (McNabb, 2007). Moreover, exogenous nutrition remarkably influences thyroid hormone activities. These hormones are mainly implicated in the regulation of tissue metabolism and growth (Too *et al*., 2017). Therefore, a gradual increase in both T_3 and T_4 concentrations were observed by in-ovo injection of both doses of omega-3 groups as compared with the control groups. Safamerher *et al*. (2008) showed that the significant increase in both T_3 and T_4 concentrations with low doses of omega-3 groups may be due to increasing thyroid stimulating hormone as a result of increasing metabolic process than that in the highly dose of omega-3 groups and control group. Vanderpas (2006) concluded that increasing the basal metabolic rate is accompanied by elevated birds' appetite and subsequently their body weight. Therefore, the highly significant increasing in embryonic and newly hatched body weight in the low dose of omega-3 group might be attributed to the higher T_3 and T_4 concentrations.

The significant increase of both uric acid and creatinine levels in allantoic fluid by injecting the high dose of omega-3 may be attributed to damage in the development of the fetal excretory system. Our results are in agreement with Simopoulos (2006) who found that, high dose of omega-3 produces acute necrosis in proximal tubular epithelial cells and inhibit normal secretion of renal uric acid. Moreover, the elevation in allantoic levels of uric acid and creatinine may be due to nephrotoxic effects following the administration of omega-3 and the reduction in the glomerular filtration rate (Simopoulos, 2006).

Inoculation of exogenous n-3 fatty acids as antioxidants may enhance lipid and PUFAs profiles, and the antioxidant status of chicken embryos (Jameel, 2013). However, limited information is available concerning the impact of maternal omega-

3 enrichment on lipid peroxidation and antioxidant status. The UFAs found in CLO have major antioxidant properties because its valuable effects on cellular lipid peroxidation and antioxidant enzyme activities (Hunker *et al*., 2002). Also, they reported that the ability of CLO to reduce cholesterol and triglyceride concentrations which considered as a protective effect against lipid peroxidation. Our results demonstrated that, total antioxidant capacity in the hepatic and brain tissues were significantly higher in the low dose of omega-3 treated group as compared with the high dose of omega-3 and control groups. These results are in agreement with Bautista-Ortega *et al*. (2009) who found that thiobarbituric acid reactive substances were significantly increased in the liver tissues of chicks produced from hens fed 1.8% omega-3 than those hatched from hens fed 10.3 and 13.3% omega-3.

Biochemical data are consistent with the histological changes induced by high dose of omega-3 injection. The rise in serum ALP activity observed in the high dose of omega-3 treated group reflected a degree of obstruction of the biliary cells imposed by hepatocyte pressure (Edoardo *et al*., 2005). This is probably secondary to congestion of the central and portal vein in a dose dependent manner. The rise in ALT and AST serum activity justified the possibility that this congestion was accompanied with a degree of physical damage of hepatocytes (Del Campo *et al*., 2018). Data also demonstrated that this hepatocyte damage was aggravated by the inflammatory cells infiltration of the liver as evidence by the significant rise of both enzymes when compared between the high and the low omega-3 doses. This most likely was due to the extra harm imposed of liver cells by cytokine released from these inflammatory cells (Abbas *et al*., 2016).

CONCLUSION

The obtained results indicated that in-ovo injection with omega-3 improved hatchability, embryonic and newly hatched body weight and enhancement the antioxidant capacity. Implications, applications, recommendations and limitations of the study:

Both doses of omega-3 improved hatchability and embryonic and newly hatched body weight due to increased level of thyroid hormones. In-ovo administration of omega-3 at high dose had a negative effect on hepatic enzymes and lower total antioxidant capacity that might attributed to their deleterious impacts of liver cell structure and congestion in the central and portal vein. Therefore, our study recommended to using the low dose (100µL) of natural fish oil as a source of omega-3 fatty acid to enhancement hatchability, mean body weight and studied physiological aspect during embryonic development and at hatch.

ACKNOWLEDGEMENT

The authors express their thanks to Miser Poultry Company (MIPCO) for the generous gift of the fertilized eggs used in this study. The authors express also their thanks to Dr. K. A. Ahmed, Professor of Veterinary Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt for her help in the histological examination. The authors also express their gratitude to Dr. Abd-Moneim Eid Abd-Moneim Hassan, lecture of poultry physiology, Biological Application Department, Nuclear Research Center, Atomic Energy Authority, to his help in performing the statistical analysis and drafting the manuscript.

Table (1): Effect of in-ovo omega-3 injection on body and some relative organs weight

Age	Treatment	Body Weight (g)	Liver (%)	Brain (%)	Heart (%)
15th day of incubation	Control	11.36 ± 0.15[c]	1.23 ± 0.06	4.51 ± 0.12	0.894 ± 0.04
	Sham Control	11.25 ± 0.14[c]	1.22 ± 0.06	4.49 ± 0.11	0.891 ± 0.03
	100µL omega-3	14.47 ± 0.12[a]	1.27 ± 0.08	4.47 ± 0.09	0.875 ± 0.02
	200µL omega-3	12.92 ± 0.09[b]	1.29 ± 0.06	4.49 ± 0.08	0.851 ± 0.12
18th day of incubation	Control	30.42 ± 0.23[c]	2.29 ± 0.07	3.59 ± 0.11	0.671 ± 0.05
	Sham Control	30.22 ± 0.19[c]	2.26 ± 0.06	3.56 ± 0.10	0.666 ± 0.05
	100µL omega-3	34.81 ± 0.18[a]	2.27 ± 0.09	3.61 ± 0.12	0.640 ± 0.02
	200µL omega-3	32.92 ± 0.17[b]	2.24 ± 0.12	3.57 ± 0.09	0.622 ± 0.02
At hatch	Control	35.36 ± 1.06[c]	2.75 ± 0.06	2.38 ± 1.98	0.755 ± 0.07
	Sham Control	35.22 ± 1.11[c]	2.71 ± 0.05	2.32 ± 1.93	0.751 ± 0.08
	100µL omega-3	39.11 ± 1.21[a]	2.78 ± 0.08	2.41 ± 1.82	0.724 ± 0.09
	200µL omega-3	37.02 ± 1.31[b]	2.70 ± 0.08	2.37 ± 1.88	0.766 ± 0.09

*Values are expressed as means ± standard error of the mean.

[a, b, c] Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

Table (2): Effect of in-ovo omega-3 injection on some serum proteins concentrations

Age	Treatment	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
15th day of incubation	Control	3.33 ± 0.03^{c}	1.66 ± 0.09^{a}	1.67 ± 0.09^{c}
	Sham Control	3.31 ± 0.05^{c}	1.62 ± 0.08^{a}	1.69 ± 0.07^{c}
	100µL omega-3	3.93 ± 0.09^{a}	1.35 ± 0.06^{b}	2.58 ± 0.06^{a}
	200µL omega-3	3.67 ± 0.03^{b}	1.22 ± 0.14^{c}	2.45 ± 0.14^{b}
18th day of incubation	Control	4.39 ± 0.12^{c}	2.36 ± 0.03^{a}	2.03 ± 0.03^{c}
	Sham Control	4.36 ± 0.11^{c}	2.31 ± 0.03^{a}	2.05 ± 0.03^{c}
	100µL omega-3	4.97 ± 0.03^{a}	2.24 ± 0.12^{b}	2.73 ± 0.12^{a}
	200µL omega-3	4.62 ± 0.04^{b}	2.09 ± 0.29^{c}	2.53 ± 0.29^{b}
At hatch	Control	6.14 ± 0.12^{c}	2.34 ± 0.02^{a}	3.80 ± 0.02^{c}
	Sham Control	6.11 ± 0.12^{c}	2.30 ± 0.02^{a}	3.81 ± 0.02^{c}
	100µL omega-3	6.70 ± 0.05^{a}	3.06 ± 0.05^{b}	3.64 ± 0.05^{a}
	200µL omega-3	6.38 ± 0.03^{b}	2.86 ± 0.06^{c}	3.52 ± 0.06^{b}

*Values are expressed as means ± standard error of the mean.
$^{a, b, c}$ Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

Table (3): Effect of in-ovo omega-3 injection on some serum enzymes activities

Age	Treatment	ALT (U/L)	AST (U/L)	ALP (IU/L)
15th day of incubation	Control	18.1 ± 0.5^{c}	29.4 ± 0.4^{c}	118.00 ± 1.16^{c}
	Sham Control	18.4 ± 0.6^{c}	29.9 ± 0.5^{c}	119.06 ± 1.11^{c}
	100µL omega-3	19.6 ± 0.9^{b}	32.6 ± 0.7^{b}	122.00 ± 1.00^{b}
	200µL omega-3	21.4 ± 0.11^{a}	36.6 ± 0.9^{a}	129.66 ± 0.66^{a}
18th day of incubation	Control	21.2 ± 0.8^{c}	62.9 ± 0.11^{c}	127.00 ± 1.00^{c}
	Sham Control	21.6 ± 0.11^{c}	63.2 ± 0.08^{c}	128.09 ± 1.00^{c}
	100µL omega-3	23.1 ± 0.9^{b}	66.6 ± 0.9^{b}	133.33 ± 0.66^{b}
	200µL omega-3	24.9 ± 0.12^{a}	71.9 ± 0.9^{a}	163.33 ± 5.71^{a}
At hatch	Control	26.6 ± 0.7^{c}	83.7 ± 0.9^{c}	139.80 ± 0.60^{c}
	Sham Control	27.1 ± 0.6^{c}	84.2 ± 0.9^{c}	141.02 ± 0.77^{c}
	100µL omega-3	29.2 ± 0.9^{b}	89.6 ± 0.12^{b}	146.00 ± 0.96^{b}
	200µL omega-3	32.1 ± 0.13^{a}	96.6 ± 0.11^{a}	154.60 ± 1.29^{a}

*Values are expressed as means ± standard error of the mean.
$^{a, b, c}$ Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

Table (4): Effect of in-ovo omega-3 injection on serum lipids profiles

Age	Treatment	Total lipids (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
15th day of incubation	Control	167.66 ± 3.18^{b}	123.66 ± 3.72^{b}	24.00 ± 2.51^{b}
	Sham Control	169.12 ± 3.01^{b}	126.09 ± 3.15^{b}	27.12 ± 3.23^{b}
	100µL omega-3	145.66 ± 2.26^{c}	109.33 ± 5.71^{c}	17.33 ± 4.09^{c}
	200µL omega-3	224.00 ± 4.35^{a}	146.66 ± 0.34^{a}	46.00 ± 4.05^{a}
18th day of incubation	Control	242.33 ± 3.8^{b}	168.66 ± 5.8^{b}	31.66 ± 4.6^{b}
	Sham Control	247.01 ± 3.4^{b}	171.61 ± 3.9^{b}	33.03 ± 5.1^{b}
	100µL omega-3	232.00 ± 2.64^{c}	153.33 ± 7.23^{c}	40.33 ± 2.40^{c}
	200µL omega-3	284.33 ± 0.87^{a}	188.00 ± 0.58^{a}	59.30 ± 2.84^{a}
At hatch	Control	312.60 ± 3.57^{b}	229.04 ± 5.44^{b}	55.20 ± 1.97^{b}
	Sham Control	315.04 ± 3.12^{b}	232.11 ± 4.88^{b}	59.78 ± 1.64^{b}
	100µL omega-3	299.80 ± 3.89^{c}	209.80 ± 7.24^{c}	48.40 ± 1.68^{c}
	200µL omega-3	346.20 ± 3.39^{a}	240.60 ± 6.84^{a}	67.40 ± 1.56^{a}

*Values are expressed as means ± standard error of the mean.

$^{a, b, c}$ Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

Table (5): Effects of in-ovo omega-3 injection on thyroid hormones concentrations

Age	Treatment	T_3 (ng/dL)	T_4 (µg/dL)
15th day of incubation	Control	1.64 ± 0.5^{c}	0.23 ± 0.03^{c}
	Sham Control	1.59 ± 0.4^{c}	0.21 ± 0.04^{c}
	100µL omega-3	2.02 ± 0.08^{a}	0.44 ± 0.11^{a}
	200µL omega-3	1.85 ± 0.08^{b}	0.32 ± 0.08^{b}
18th day of incubation	Control	1.94 ± 0.11^{c}	0.58 ± 0.18^{c}
	Sham Control	1.91 ± 0.09^{c}	0.54 ± 0.13^{c}
	100µL omega-3	2.74 ± 0.11^{a}	0.96 ± 0.12^{a}
	200µL omega-3	2.22 ± 0.09^{b}	0.72 ± 0.10^{b}
At hatch	Control	2.26 ± 0.10^{c}	1.43 ± 0.06^{c}
	Sham Control	2.21 ± 0.09^{c}	1.37 ± 0.05^{c}
	100µL omega-3	2.98 ± 0.11^{a}	1.98 ± 0.14^{a}
	200µL omega-3	2.43 ± 0.08^{b}	1.76 ± 0.07^{b}

*Values are expressed as means ± standard error of the mean.

$^{a, b, c}$ Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

Table (6): Effect of in-ovo omega-3 injection on allantoic uric acid and creatinine concentrations

Age	Treatment	Uric acid (mg/dL)	Creatinine (mg/dL)
15th day of incubation	Control	1.68 ± 0.08[b]	0.62 ± 0.03[b]
	Sham Control	1.64 ± 0.07[b]	0.60 ± 0.04[b]
	100µL omega-3	1.66 ± 0.06[b]	0.63 ± 0.03[b]
	200µL omega-3	2.05 ± 0.10[a]	0.78 ± 0.08[a]
18th day of incubation	Control	2.19 ± 0.08[b]	0.72 ± 0.03[b]
	Sham Control	2.14 ± 0.11[b]	0.70 ± 0.04[b]
	100µL omega-3	2.14 ± 0.14[b]	0.68 ± 0.03[b]
	200µL omega-3	2.40 ± 0.14[a]	0.89 ± 0.05[a]

*Values are expressed as means ± standard error of the mean.
[a, b, c] Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

Table (7): Effect of in-ovo omega-3 injection on total antioxidant capacity in hepatic and brain tissues

Age	Treatment	Hepatic Tissue (µmol/g)	Brain Tissue (µmol/g)
15th day of incubation	Control	1.47 ± 0.02[b]	1.39 ± 0.02[b]
	Sham Control	1.42 ± 0.01[b]	1.33 ± 0.05[b]
	100µL omega-3	1.79 ± 0.01[a]	1.80 ± 0.08[a]
	200µL omega-3	1.27 ± 0.10[c]	1.28 ± 0.03[c]
18th day of incubation	Control	1.89 ± 0.02[b]	1.43 ± 0.01[b]
	Sham Control	1.82 ± 0.01[b]	1.38 ± 0.03[b]
	100µL omega-3	2.25 ± 0.18[a]	1.63 ± 0.17[a]
	200µL omega-3	1.62 ± 0.02[c]	1.30 ± 0.09[c]
At hatch	Control	2.57 ± 0.09[b]	1.69 ± 0.04[b]
	Sham Control	2.51 ± 0.07[b]	1.63 ± 0.05[b]
	100µL omega-3	2.23 ± 0.09[a]	1.98 ± 0.06[a]
	200µL omega-3	1.86 ± 0.02[c]	1.44 ± 0.05[c]

*Values are expressed as means ± standard error of the mean.
[a, b, c] Means with different superscripts, within column for each age differ significantly ($P \leq 0.05$).

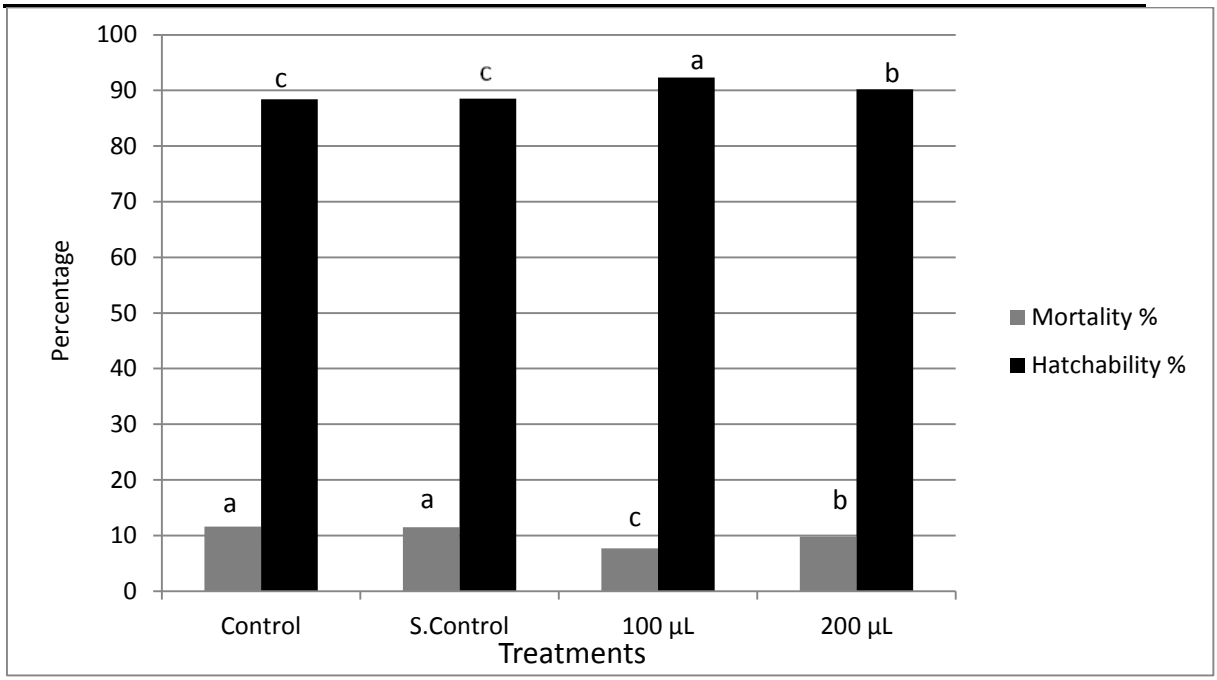

Fig (1): Effects of in-ovo omega-3 injection on hatchability and embryonic mortality.

Fig.(2): Liver of chicken embryo at the 18th day of embryogenesis (**A**) control group showing normal histological structure of the central vein and portal area with surrounding hepatocyts in the hepatic parenchyma H&Ex16, (B) group treated with 100µL of omega-3 in the 18th day of incubation showing normal histological structure of the central and portal veins with slight cytoplasmic vacuolization of hepatocytes H&Ex16, (C) group treated with 200µL of omega-3 in the 18th day of incubation showing dillatation of central and portal vein with few inflammatory cells infiltration in portal area and vacuolar degeneration in the hepatocytes H&Ex16.

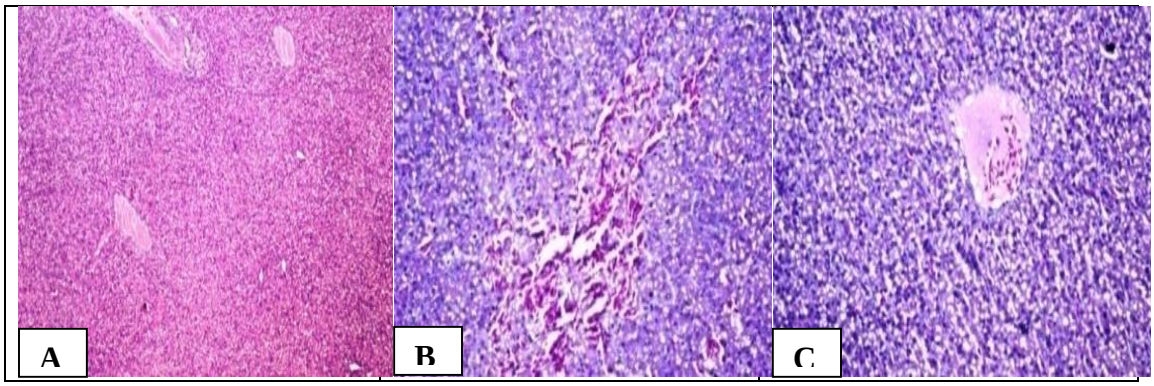

Fig.(3): Liver of newly hatched chicks (**A**) control group showing normal histological structure of the central vein and portal area with surrounding hepatocytis in the hepatic parenchyma. H&Ex16, (B) group treated with 100µL of omega-3 showing few microfatty change in hepatocytes all over the parenchyma. H&Ex40, (**C**) group treated with 200µL of omega-3 showing few congestion in the central and portal vein. H&Ex40.

REFERENCES

Abbas MW, Shamshad T, Ashraf MA, Javaid R, 2016. Jaundice: a basic review. International Journal of Research in Medical Sciences 4: 1313-1319.

Abd El-Moneim EA, El-Wardany I, Abu-Taleb AM, Wakwak MM, Ebeid TA, Saleh AA, 2019. Assessment of in ovo administration of Bifidobacterium bifidum and Bifidobacterium longum on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. Probiotics and Antimicrobial Proteins: In Press.

Abdulwahid MT, AlZuhary MAA, Kadhum MJ, 2017. Effect of hatching eggs injection with inactivated Newcastle disease vaccine and two doses of cod liver oil on some productive traits and immune response of broilers. Advances in Animal and Veterinary Sciences 5: 100-106.

Al-Daraji HJ, Al-Mashadani HA, Al-Hyani WK, Mirza HA, Al-Hassani AS, 2010. Effect of dietary supplementation with different oils on productive and reproductive performance of quail, Journal of Poultry Science 9: 429-435.

Al-Mayah AA, 2009. Effect of Fish oil on humeral immunity of broiler chicks. Basrah Journal of Veterinary Research 8: 23-34.

Alparslan G, Özdogan M, 2006. The effects of diet containing fish oil on some blood parameters and the performance values of broilers and cost efficiency. International Journal of Poultry Science 5: 415-419.

Al-Zuhairy MA, Alasadi YJ, 2013. Effect of in-ovo injection with Newcastle disease vaccine, multivitamins AD3E, and omega-3 on performance and immune response of broiler. International Journal of Sdv Biological Research 3: 208-211.

Amen MHM, 2015. In-ovo injection of omega-3 fatty acids improve hatchability and chick performance, Published in 2nd scientific conference at Gramian University in May 7th. Garmain-Iraq.

Amen MHM, 2016. In ovo feeding of omega-3 fatty acids improved production traits, hematological parameters and immune response in broiler. Research Opinions in Animal and Veterinary Sciences 6: 1-6.

Bakyaraj S, Subrat KB, Samir M, Banabihari D, 2011. Modulation of post-hatch growth and immunity through in ovo supplemented nutrients in broiler chickens. Journal of the Science of Food and Agriculture 92: 313–320.

Banchraft JD, Stevens A, Turner DR, 1996. Theory and practice of histological techniques. Forth ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

Bautista-Ortega J, Goeger DE, Cherian G, 2009. Egg yolk omega-6 and omega-3 fatty acids modify tissue lipid components, antioxidant status, and ex vivo eicosanoid production in chick cardiac tissue. Poultry Science 88: 1167–1175.

Bou R, Guardiola F, Tres A, Barroeta AC, Codony R, 2004. Effect of dietary fish oil, alpha-tocopherl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. Poultry Science 83: 282–292.

Chashnidel Y, Moravej H, Towhidi A, Asadi F, Zeinodini S, 2010. Influence of different levels of n-3 supplemented (fish oil) diet on performance, carcass quality and fat status in broilers. African Journal of Biotechnology, 9: 687-691.

Del Campo JA, Gallego P, Grande L, 2018. Role of inflammatory response in liver diseases: Therapeutic Strategies. World Journal of Hepatology 27(1): 1-7.VIEWS

Dewailly E, Blanchet C, Lemieux S, Sauve L, Gingras S, Ayotte P, Holub BJ, 2001. N-3 fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik. American Journal of Clinical Nutrition 74: 464-473.

Dos Santos TT, Corzo A, McDaniel CD, Filho TRA, Araujo LF, 2010. Influence of in ovo inoculation with various nutrients and egg size on broiler performance. Journal of Applied Poultry Research 19: 1-12.

Duncan DB, 1955. Multiple range and multiple F-test. Biometrics 11: 1.

Edoardo GG, Testa R, Savarino V, 2005. Liver enzyme alteration: a guide for clinicians. Canadian Medical Association or its licensors 1: 172.

El-Sayed EM, Hashim ME, 2000. Effect of Nigella sativa on the immune response to Eimeria vaccination in chicken. Egyptian Journal of Agricultural Research 78: 231- 239.

Herfiana IM, 2007. The effect of Glutamine, Dextrin and Its Combination Through In Ovo Feeding on Immune Response, Blood Profiles and The Carcass Composition of MaleBroiler Chicken. Msc thesis. Sekolah Pascasarjana, Institute pertanin, Bogor.

Hosseini MN, 2011. Effect of fish oil on performance and blood indices in broiler chicks immunized with sheep erythrocytes. Advances in Environmental Biology 5: 1166-1171.

Hosseini MN, Bahrami Y, 2011. Influence of dietary fish oil supplementation on humoral immune

response and some selected biochemical parameters of broiler chickens. Journal of Agricultural and Biological Science 28: 67–77.

Hunkar T, Aktan F, Ceylan A, Karasu C, 2002. Effects of cod liver oil on tissue antioxidant pathways in normal and streptozotocin-diabetic rats. Cell Biochemistry and Function 20: 297–302.

Jameel YJ, 2013. The effect of in-ovo injection with Newcastle disease vaccine, multivitamins AD3E and omega-3 oil on subsequence productive performance and some physiological parameters of broiler chicks. Ph.D. Dissertation in science of poultry hygiene. College of Veterinary Medicine. University of Baghdad. Iraq.

Koreleski J, Switakiewicz S, 2006. The influence of dietary fish oil and vitamin E on the fatty acid profile and oxidative stability of frozen stored chicken breast meat. Journal of Animal and Feed Science 15: 631-640.

Lopes KLAM, Pedroso AA, Leandro NSM, Stringhini JH, Barbosa CE, 2006. Glutamine in ovo inoculation effect at the starter performance of broilers. **Brazillian Journal of Avian Science 8: 103.**

McGruder BM, Zhai W, Keralapurath MM, Bennett LW, Gerard PD, Peebles ED, 2011. Effects of in ovo injection of electrolyte solutions on broiler embryogenesis and pos-thatch performance. Poultry Sciences 90: 1058-1066.

McNabb FM, 2007. The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. Critical Reviews in Toxicology 37: 163–193.

Noy Y, Uni Z, 2009. Early Nutritional Strategies 147- 152.

Perez TI, Zuidhof MJ, Renema RA, Curtis JM, Ren Y, Betti M, 2010. Effects of vitamin E and organic selenium on oxidative stability of ω-3 enriched dark chicken meat during cooking. Journal of Food Science 75: 25-34.

Ruxton CHS, Reed SC, Simpson MJA, Millington KJ, 2007. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. Journal of Human Nutrition and Dietetics 20: 275–285.

Safamerher A, Aghaei N, Mehannavaz Y, 2008. The influence of different levels of dietary fish oil on the performance, carcass traits and blood parameters of broilers chickens. Research Journal of Biological Sciences 3: 1202-1207.

Salih GE, 2009. Effects of inclusion of local disposed roasted fish meal on the performance and carcass charateristics of broiler. Egyptian of Poultry Science 29: 735-745.

SAS Institute, 2000. SAS OnLine Doc.Version 8.SAS Institute Inc., Cary, NC.

Schaal TP, 2008. The effects of in-ovo feeding of fatty acids and antioxidants on broiler chicken hatchability and chick tissue lipids. University Honors College, Oregon State University.

Schaal T, Cherian G, 2007.A survey of the hatchability of broiler and Turkey eggs in the United States from 1985 through 2005. Poultry Science 86: 598-600.

Schumann BE, Squires EJ, Leeson S, 2000. Effect of dietary flaxseed, flax oil and n-3 fatty acid supplement on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. British

Journal of Poultry Science 41: 465–472.

Selim SA, Gaafer KM, El-ballal SS, 2012. Influence of in ovo administration with vitamin E and Ascorbic acid on the performance of Muscovy ducks. Emirates Journal of Food and Agriculture 24(3): 264-271.

Simopoulos AP, 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomedicine and Pharmacotherapy 60: 502.

Sultan SIA, 2005. Effect of dietary fish oil on production traits and lipid composition of laying hens. International Journal of Poultry Science 4: 586-588.

Tako E, Ferket PR, Uni Z, 2004. Effects of in ovo feeding of carbohydrates and β-hydroxy-β-methylbutyrate on the development of chicken intestine. Poultry Science 83: 2023-2028

Too HC, Shibata ., Yayota M, Darras VM, Iwasawa ., 2017. Expression of thyroid hormone regulator genes in the yolk sac membrane of the developing chicken embryo. Journal of Reproduction and Development 63: 463–72.

Uni Z, Ferket PR, 2003. Enhancement of development of oviparous species by in-ovo feeding. North Carolinas State Univ. and Yissum Research Development Company Assignees. US Pat., vol. 6(592): 878.

Uni Z, Ferket PR, 2004. Methods for early nutrition and their potential. World's Poultry Science Journal 60: 101–111

Uni Z, Ferket PR, Tako E, Kedar O, 2005. In-ovo feeding improves energy status of late-term chicken embryos. Poultry Science 84: 764-770.

Vanderpas J, 2006. Nutritional epidemiology and thyroid hormone metabolism. Annual Review of Nutrition 26: 293–322.

Van West D, Maes M, 2003. Polyunsaturated fatty acids in depression. Acta Neuropsych, 15: 15-21.

Vierira SL, 2007. Chicken embryo utilization of egg micronutrients. Brazilian Journal of Poultry Science 9: 1-8.

Zhai W, Neuman S, Latour MA, Hester PY, 2008. The effect of in ovo injection of L-carnitine on hatchability of White Leghorns. Poultry Science 87:569–572.

Chicken embryo, omega-3, antioxidant capacity, thyroid hormones, histological changes.

الملخص العربي

تأثير الحقن بالاوميجا -3 للبيض خلال التحضين على بعض القياسات الفسيولوجية خلال تطورالنمو الجنينى للدجاج

حمدى عبد الرحمن بسيونى[1]–محمود على العروسى[1] - امل محمد محمد بدران[2]

1- وحدة بحوث الدواجن – قسم التطبيقات البيولوجية- مركز البحوث النووية- هيئة الطاقة الذرية- مصر
بحوث تربية الدواجن - مركز البحوث الزراعيه– الدقي - مصر 2- معهد بحوث الانتاج الحيوانى – قسم

تم اجراء التجربة بوحدة بحوث الدواجن بقسم التطبيقات البيولوجية بمركز البحوث النووية – هيئة الطاقة الذرية.استخدم في هذة الدراسة عدد 400 بيضة تفريخ (متوسط وزن ما بين 60.5 ± 1.5جم) من سلالة دجاج الهايلاين من قطيع امهات عمره 46 اسبوع و ذلك لدراسة تأثير حقن بيض التفريخ بمستويات مختلفة من الاوميجا-3 علي نمو الاجنه و نسب الفقس و وزن الكتاكيت الفاقسة و بعض التغيرات الفسيولوجية و الهيستولوجية خلال مراحل التطور الجنيني و عند الفقس. في اليوم العاشر من التفريخ تم اجراء الفحص الضوئي و استبعاد البيض غير المخصب. تم تقسييم البيض المخصب (و عدده 360 بيضة مخصبة) عشوائيا الي اربعة مجموعات متساوية و تم تقسيم كل مجموعة الي ثلاث مكررات كل منها 30 بيضة مخصبة حيث تم ترقيم البيض و وزن البيض كل على حدة. لم يتم حقن المجموعة الاولي و اعتبرت مجموعة المقارنة. بينما تم حقن المجموعة الثانية في كيس الصفار بمائتين ميكروليتر محلول ملحي معقم و حقن المجموعة الثالثة و الرابعة في كيس الصفار بمائة و مائتين ميكروليتر من الاوميجا-3 علي التوالي.

و قد اظهرت النتائج ان الحقن بالمستوي المنخفض من الاوميجا-3 (100 ميكروليتر) ادى الي ارتفاع معنوي لكل من نسبة الفقس و وزن الاجنة و وزن الكتاكيت عند الفقس وكذلك محتوي سيرم الدم من كل من البروتين الكلي و الجلوبيولين و مستويات هرمونات الدرقية والقدرة الكلية المضادة للأكسدة في أنسجة الكبد والمخ مقارنة بالمستوي الاعلي من الاوميجا-3 (200 ميكروليتر) و مجموعة المقارنة. بينما تسبب الحقن بمستويات مرتفعة من الاوميجا-3 في الارتفاع المعنوي لكل من نسبة النفوق الجنيني و محتوي سيرم الدم من كل من الليبيدات الكلية و الكوليستيرول و الدهون الثلاثية بالاضافة الي انزيمات الكبد. كما اظهرت نتائج التركيب النسيجى للكبد تلازم الحقن بالمستوى المرتفع من الاوميجا -3 (200 ميكروليتر) و ما تبعه من ارتفاع ليبيدات الدم و انزيمات الكبد مع الاثار الضارة علي خلايا الكبد.

و مما تقدم فان الدراسة توصي بحقن يض التفريخ للدجاج بالاوميجا – 3 بمستوي 100 ميكروليتر و ذلك لتحسين نسب الفقس و وزن الكتاكيت الفاقسة و ارتباط ذلك بالتحسن في بعض الصفات الفسيولوجية في الدم.