Egypt. Poult. Sci. Vol. (39) (IV): (973-991) (2019) **(1911-1051)**

Egyptian Poultry Science Journal

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

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

EFFECT OF IN OVO INJECTION WITH RESVERATROL ON HATCHING TRAITS AND PHYSIOLOGICAL RESPONSE OF MANDARA CHICKS

Amina S. EL-Saadany; Osama M. Ali; A. A. EL Prollosy; Amal M. EL-Barbary; Ebtsam E. E. Iraqi and Hanaa M. Khalil

Dep. of Poult. Breed. Res. Anim. Prod. Res. Inst., Agric. Res. Center, Egypt

Corresponding author: Amina Shaaban El-Saadany Email: aelsaadany55@yahoo.com

Received: 17/11/2019 Accepted: 12 /12/2019

ABSTRACT: This study aimed to investigate the effect of *in ovo* injection with resveratrol (Trans.3, 4, 5-trihydroxystilbene) on hatching traits and physiological response of Mandara chicks. A total of 864 fertile eggs at ED14 were distributed randomly into four groups of 216 eggs each with three replicates. At the 14thday of incubation, fertile eggs were injected in the yolk sac as following groups: first, eggs were un-injected (intact control), second, eggs were injected with 100 µl /egg of sterile water (sham control), and third and fourth eggs were injected with 100 µl /egg of (25 and 50 µg) resveratrol (RV), respectively. Results show that hatchability %, hatched chicks' weight and chick quality significantly improved for resveratrol groups compared with intact or sham control groups. Moreover, group injected with 50µg/egg recorded the highest value of hatchability (94.68%). Additionally, in ovo resveratrol injection resulted in boosted plasma antioxidants enzymes (TAC and SOD) activities and significantly decreased in plasma lipid peroxidation (MDA) levels for hatched chicks. Also, RV injection increased plasma total protein, globulin, IgG and IgM and decreased cholesterol levels. In ovo resveratrol injection had a positive effect on hematological parameters (Hb, PCV, RBCs and WBCs) and triiodthyronine (T_3) of baby chicks. Relative intestinal weight, lengths of duodenum, jejunum and ileum and jejunum maltase enzyme value were significantly increased for chicks hatched from eggs injected with resveratrol. In conclusion, in ovo injection of resveratrol on 14th d of incubation has positive effect on hatchability%, chick quality, physiological, immunological, anti- oxidative status and intestinal development of hatched chicks. Yolk sac injection at E14 with 50 µg RV /egg could be recommended for improving chick's health.

Key words: Resveratrol, *in ovo*, antioxidant, hatching traits, physiological parameters.

INTRODUCTION

In commercial poultry rearing, *in ovo* feeding of nutrients is an important factor in maximizing profits. A number of nutrients have important physiological, nutritional and immunological functions related to bird's embryogenesis and growth rate. In ovo injection of these nutrients may help overcome any constraints imposed by inadequate egg nutrition (Selim et al., 2012). However, chick embryos through the incubation show an increased susceptibility to oxidative stress probably due to heightened metabolic rate and o2 consumption, as well as high levels of tissue polyunsaturated fatty acids and insufficient natural antioxidant reserves (Malheiros et al., 2012). Nowadays, *in ovo* feeding of antioxidants during incubation may enhance the antioxidant status of the chicken's embryo.

Resveratrol (Trans.3, 4, 5-trihydroxystilbene) is found in some fruits such as red grapes, grape products, raspberries, mulberries, grapevines (Vuong et al., 2014). In general, it is considered as a botanical polyphenolic compound and monomeric bioactive that are capable of scavenging free radicals of oxygen and lipids (Rubiolo et al., 2008), also protect DNA from oxidative damage (Yan et al., 2012). Additionally, resveratrol has highly lipophilic and hydrophilic properties therefor it is assumed to be more effective than certain other antioxidants like vitamin C and E (Murcia and Martinez 2001). The Trans form of powder resveratrol is stable under high air humidity up to 75% and temperature about 40 ^{o}C (Prokop et al., 2006).

However, Lopez-Velez et. al. (2003), reported that resveratrol is an effective eliminator of hydroxyl, superoxide and metal-induced free radicals and enhances the activities of antioxidant enzymes including SOD, GSH-px, and CAT. Previous studies indicated that supplemented dietary resveratrol reduced the MDA level and increased the activities of GSH-px, CAT and SOD in the serum (Sahin et al., 2010 and 2012).

Moreover, Zhang et al. (2014) found that adding resveratrol to chicks diet at levels of (200, 400 or 800 mg/kg) resulted in increasing of body weight gain, thymus weight, IgM, cell proliferation index, antibody titers against avian influenza viruses H5N than those fed control diet during the experimental period (1-40 day of age).

The positive effects and vital role of resveratrol in poultry could be attributed to its antioxidant and pharmacological effects and beneficial health impacts, such as anticancer, antiviral, antifungal, neuroprotective, and anti-inflammatory. Exploration of the modes of action of resveratrol such as pharmacological, nutritional and biological activities are crucial for successful farm animal and poultry management that may provide further understanding of the health and performance parameters in agriculture species (Alagawany et al.,2015). The embryonic stage of development and the timing of egg injection are the key areas that can be manipulated to maximize hatchability and chick quality following *in ovo* application.

One of the unique way is receiving nutrients to the incubating embryos through *in ovo* injection. Therefore, the current study aimed to evaluate the effect of in ovo yolk sac injection of resveratrol at ED14 on hatching traits and physiological response of Mandara chicks.

MATERIALS AND METHODS

The present study was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center.

Experimental Design:

A total of 1050 fresh hatching eggs with average weight of 53g from Mandara hens at 40 weeks old were collected. All eggs were incubated at 99.50F and 55% relative humidity (RH) in an automatic incubator. At the 14thday of incubation, the eggs were manually candled to remove infertile eggs and early dead embryos then 864 fertile eggs were distributed randomly into four groups of 216 eggs each with three replicates of 72 eggs each. Injection site on eggs were cleaned with 70% ethanol alcohol and punctured with a hard, thin stylus, and the resveratrol was injected into the yolk sac on 14-day-of incubation using insulin syringe. Immediately after the injection, the pinhole site was sealed with wax and eggs were returned to the incubator. At 18th day of incubation all eggs were transferred to the hatchery at 98.60F and 65% RH.

Trans-Resveratrol was purchased from Sigma-Aldrich (st. Louis, MO, USA). The four treatments were applied to the eggs as follow:

1-The 1st group, eggs were un-injected and served as (intact control).

2- The 2nd group, eggs were injected with 100 µl /egg of sterile water served as (sham control).

3- The 3rd group, eggs were injected with 100 µl /egg of (25µg) resveratrol.

4- The 4th group, eggs were injected with 100 µl/egg of (50 µg) resveratrol.

Treatment solutions were warmed to 30 0C before injection.

At day 21 of incubation, chicks' weight (g) were recorded. Hatchability was calculated as the percentage of hatched chicks from fertile eggs. Eggs that failed to hatch were inspected by naked eye to estimate embryonic mortality during intervals (early) 1-7, (mid) 8-14 and (late) 15-21 days of incubation. Chick quality assessment was done using some of Tona scoring system (Tona et al., 2003). Addition to this method, activity, down and appearance, navel area were scored as a physical parameters. The quality score for a chicks was defined as the sum of the scores assigned to each quality parameter. Chick length was measured from the tip of the beak to the end of the middle toe with the chicks' dorsal surface extended over a ruler (Wolanski et al., 2007).

At the hatch, six chicks from each treatment were selected randomly, weighed and slaughtered. Then, blood samples were collected to determine biochemical constituents of blood using commercial kits. A portion of the anticoagulant was used to measure the white blood cells count (WBCs), red blood cells count (RBCs), hemoglobin (Hb) and packed cell volume (PCV). Plasma and serum were obtained from the blood samples by centrifugation for 15 min. at 3000 rpm and were stored at -20 C until the time of analysis. Plasma total protein, albumin, cholesterol, urea, Total antioxidant capacity (TAC), Malondialdehyde (MDA) and Superoxide dismutase (SOD) activities were calorimetrically determined using commercial Kits. Plasma immunoglobulin, IgG and IgM were determined using the method of Leslie and Frank (1989). Serum triiodothyronine (T3) concentration was measured by using commercial ELISA kit.

The small intestine of slaughtered chicks were removed, weighed and their segments (duodenum, jejunum and ileum) were separated and measured by

centimeter to obtain their lengths. Weight of small intestine was expressed as a percentage of life body weight. Jejunum part was immediately frozen in physiological saline for assayed maltase activity (nmol/min/mg protein) at Animal Health Research Institute according to the method of Leon et al. (2014). Intestinal aerobic and anaerobic microflora counts were measured. Aerobic plate count (APC), total coliform count and total anaerobic count were carried out according to American Public Health Association (A.P.H.A, 1985).

Data were statistically analyzed according to SAS program (SAS, 2004) using GLM Procedure. All the data were subjected to one way analysis of variance model. Mean differences were tested by Duncan's multiple range (Duncan, 1955).

RESULTS AND DISCUSSION

Hatching traits:

Table 1 represents the effect of in ovo injection with Resveratrol (RV) on the 14th day of incubation on embryonic mortality %, hatchability of fertile eggs % and hatched chick body weight (g). There were no significant differences among the experimental treatments for embryonic mortality % in early (1-7days) and mid (8-14days) stages of incubation. While, the embryonic mortality % at late stage of incubation (15-20days) was significantly ($p \leq 0.0001$) decreased for RV injected groups compared to both control groups. Results also indicated that the group injected with 50 µg RV /egg significantly lowered embryonic mortality % at late stage of incubation comparing with intact control group. Similar results were obtained by El-kholy et al. (2019) who revealed that injected quail eggs with vitamin C and B before incubation resulted in decreased the late embryonic mortality % compared to the positive control group. With respect to the hatchability of fertile eggs %, the results showed that hatchability rates were significantly ($p \leq 0.0001$) improved for in ovo injection of Resveratrol (25 and 50 µg/egg) by 4.39 and 7.54 %, respectively compared with intact control group. Moreover, the injected group with 50µg/egg recorded highest hatchability of fertile eggs (93.89%) compared to the other experimental groups.

According to, Baxter (2008) the biological efficient of resveratrol for the prevention of free radicals or lipid peroxidation was 95% compared to the traditional antioxidants such as vitamin E and C which their biological efficient were about 65 and 37%, respectively. The present results concerning the improvement of hatchability % of fertile eggs injected with resveratrol on 14th day of incubation (critical time of fatty acid oxidation) may be due to reduce the production of free radicals that cause a serious damage to the cellular membranes (Surai,2000), and increase lipid utilization for energy production to improve hatchability (Schaal,2008). Therefore, higher hatchability percentage after injection of RV may be due to the improvement of the antioxidant status of the eggs and could help the embryo to overcome oxidative stress at hatch time. Our findings were supported by Hajati et al. (2014) who found that in ovo injection with 4.5 mg grape seeds extract (GSE) /egg on 18th day of incubation significantly increased the hatchability in broiler breeder hens. Also the improvement of hatchability by 6.54 % compared with control group was reported by Kalantar et al. (2019) who injected broiler chickens eggs with Coenzyme Q10 before incubation. Similarly, *in ovo* administration of L-Arginine at the rate of (100 µg/µl/egg) on the 14th day of

incubation resulted in improvement of survival and hatching rates (Subramaniyan et al. 2019).

Regarding the hatched chick weight, the result showed that chicks' weight at hatch was significantly higher ($p \leq 0.001$) in resveratrol injected eggs compared to sham or intact control groups. Our results are consistent with those previously reported by Nazem et al. (2017) who found that in ovo injection of Met-Cys caused improvement in chicks' weight at hatch compared to the non-injected groups. Similar results were confirmed by Elwan et al. (2019).

Our results revealed that *in ovo* injection of resveratrol on 14th day of incubation resulted in increasing the chicks weight at hatch, and this increasing may be attributed to the improved antioxidant status of embryos .However, the alleviation of the hatch-related oxidative stress may lead to a higher hatch weight and post-hatch performance through protection of skeletal muscle stem cells from oxidative damages (Choi et al. 2016).

Data of chicks' quality as affected by in ovo resveratrol injection on 14th day of incubation are summarized in Table 2. It can be observed from this data, RV had a significant ($p \leq 0.0001$) effect on chicks' activity (%) and chicks' length (cm) at hatch. Weak activity % for hatched chicks was significantly increased in control groups than in other treatment groups. Injected of 50 µg RV/egg recorded higher good activity % of hatched chicks followed by those injected with 25 µg RV/egg compared to intact or sham control groups. The same trend of improvement was observed in hatched chick length where the enhancement of chick length were 6.9 and 11.6 % respectively, compared to control group. Moreover, there were no significant differences among all treatment groups with respect to downs and appearance% (clean and dry – wet) and navel (completely closed and clean or it's not completely closed and not discolored) for hatched chicks.

The improvement in hatched chick quality (activity and length) by *in ovo* resveratrol injection could be attributed to resveratrol is absorbed at faster rates into the circulatory system as compared to the other antioxidants found within grab seeds extract (Goldberg et al. 2003). However, antioxidants concentrations in the tissues transferred from yolk sac are responsible for normal embryonic/chick development (Surai, 1999) so antioxidant protection is an important mechanism on chick development at hatching time (Surai, 2002). These results are in agreement with findings of Araujo et al. (2018) who consisted that in ovo VE injection on 17.5th day of incubation resulted in improved the chick' oxidative status, which led to enhancement of incubation results and chick quality. In addition to Babacanoglu et al. (2018) concluded that in ovo injection of α tocopherol caused positive effects on chick quality and length.

Hematological parameters:

Results displayed in Table 3 showed the effect of in ovo injection of RV on some blood hematological parameters. It was found that there were a significant increase ($p \leq 0.0001$) in each of red blood cells (RBCs), white blood cells (WBCs) counts, hemoglobin (Hb) concentration and packed cell volume (PCV) percent in Mandara chicks hatched from eggs injected with resveratrol compared with either intact or sham control groups. With regard to the *in ovo* resveratrol injection levels, the best improvement of hematological parameters was recorded

for chicks hatched from eggs injected with 50 µg RV/egg. The previous of blood picture parameters are consider a good indicators for the physiological status of animals (Lassen and Swardson, 1995).

These results are in line with those obtained by Tedesc et al. (2000) who stated that resveratrol had a positive effect on hematological parameters due to its ability to maintain the membrane integrity of erythrocyte. The improvement of hematological traits in this study is supported by (Ognik et al. 2016) who found the addition of resveratrol to the diet caused an increase in blood hemoglobin concentration of turkey hens. This finding also agrees with the result obtained by (Atmac et al., 2014) in rats where resveratrol reduced hemolysis induced by fluoride treatment. However, reactive oxygen species (ROS) produced during stress is known to play essential role in tissue damages and exert adverse effect on RBCs counts (Gumulu et al. 2002). Whereas, resveratrol a potent antioxidant agent maintained the membrane integrity of red blood cells (Ememe et al., 2016).

Hence, we may suppose that the increase in the count of WBCs was a result of stimulation of the immune system by the use of resveratrol. Similar result was confirmed by Ognik et al. (2016) who indicated that use of resveratrol at the amount of 200g/tone as a feed additive in turkey hens can stimulate aspects of specific and non-specific immunity by increasing the activity of lysozyme and the percentage of phagocytic cells.

Antioxidants activities and lipid peroxidation:

Significant differences ($p \leq 0.0001$) were observed in plasma total antioxidants capacity (TAC), superoxide dismutase (SOD) and malondialdehyde (MDA) activities of hatched chicks by *in ovo* resveratrol injection Table (Figure 1). Whereas, plasma TAC and SOD levels were significantly higher in the in ovo resveratrol injected groups compared with intact or sham control groups. As indicated, in ovo RV injection resulted in boosted plasma TAC values by 39.2 and 54.4 % for 25 and 50 µg injected groups respectively, compared with intact control. The same trend was shown in the plasma SOD activity, which showed a significant ($p \leq 0.001$) increase by increasing resveratrol level. On the contrary, in ovo RV injection caused a significant decrease of plasma MDA concentration compared with two control groups. The improvement of antioxidant status for baby chicks in the current study related to the antioxidant activity of resveratrol which may due to the ability of hydroxyl group in resveratrol to inhibit of the production of reactive oxygen species (ROS) and malondialdehyde (MDA). Thus, the reduced in levels of lipid peroxides may have been associated with increase antioxidant enzymes activity. This result is confirmed by Hao et al. (2011) who reported that resveratrol supplementation (5, 22.5 and 45 mg/kg diet) could decrease MDA concentrations of mice liver with high fat diets and the level of SOD was increased with resveratrol groups. Moreover, Lopez-Velez et al. (2003) noted that resveratrol is an effective eliminator of superoxide, hydroxyl and metal-induced free radicals and enhances the activities of antioxidant enzymes. Similarly, previous studies indicated that dietary supplementation of resveratrol reduced the MDA level and increased the activities of SOD, TAC, CAT and GSH-px in the serum of laying hens and quails(Sahin et al. 2010, 2012).

Blood biochemical parameters:

The levels of plasma total protein and its fractions (albumin and globulin), for Mandara hatched chicks as affected by in ovo resveratrol injection are illustrated in Table 4. Results showed that in ovo injection with resveratrol significantly ($p \leq 0.0001$) increased plasma total protein and globulin in comparison to both control groups. While, no significant differences were observed among experimental treatments in plasma albumin level. Consistent with our results, Sridhar et al. (2014) reported that broilers fed diet supplemented with resveratrol (0.5 and 1.0 %) had higher plasma total protein than control group. They suggested that the increasing of total proteins level may due to indicate hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and production of liver cells. In the same line, Taha et al. (2019) found that injection with 0.5ml royal jelly/egg significantly increased plasma total protein and globulin in hatched chicks.

Data of Table 4 indicate that plasma cholesterol level was significantly ($p \leq 0.001$) decreased in chicks hatched from eggs injected with resveratrol compared with the two control groups. The lowest cholesterol value was recorded for in ovo injected with 50 µg RV/egg group by 14% compared with control group. This result is in agreement with those reported by Feng et al. (2017) who observed that dietary resveratrol supplementation of at 0.5, 1.0, 2.0 and 4.0 g/kg layer diet decreased cholesterol status. Also, Abu Hafsa and Ibrahim (2017) found that broilers fed a diet supplemented with grape seeds at (10, 20 and 40 g/kg diet) had lower levels of plasma total lipids and cholesterol compared with the control birds. The hypocholesterolemic effect by resveratrol in the present study may be due to the antioxidizing properties phenolic compounds in resveratrol which may prevent of the lipid peroxidation and regulate cholesterol synthesis. In the same respect, El-Kholy (2013) concluded that in ovo injection of Japanese quail eggs with 100 mg/egg vitamin B_6 had decreased plasma cholesterol. Also, El-Kholy et al. (2019) recommended that the plasma cholesterol was decreased for chicks hatched from eggs injected with vitamin C, B_6 and B_{12}.

Table 4 shows triiodothyronine (T_3) concentrations as affected by in ovo injection of resveratrol. Plasma T_3 level was significantly ($p \leq 0.0001$) increased in chicks hatched from eggs injected with resveratrol compared with those hatched from both control groups. This increasing was in a level-dependent manner. The same finding was found by Elwan et al. (2019) when they injected broiler eggs by Met-Cys after heat stress at d 17.5 of incubation. Similarly, El-Kholy et al. (2019) concluded that plasma T_3 and T_4 were increased in chicks hatched from eggs injected with vitamin C, B_6 and B_{12}. The improvement in thyroid hormone (T_3) in current study may be attributed to the effect of resveratrol for stimulating the energy metabolism. According to, Lu et al. (2007) levels of thyroid hormones were positively correlated with chick embryonic weight and they suggested that thyroid hormones could appear to be critically important for maintaining the normal growth and development during chick embryogenesis.

As seen in Table 4, there was a significant ($p \leq 0.01$) decrease in plasma urea concentration in chicks hatched from eggs

injected with resveratrol in comparison to both control groups. While, no significant differences were observed between intact and sham control groups. Whereas, plasma urea concentration was decreased by 14.8 and 22.2 % for 25 and 50 µg RV injected groups respectively, compared with intact control value. Our result was in agreement with those obtained by Zhang et al. (2019) who reported that dietary supplementation of laying hens with resveratrol (400 and 800 mg/kg diet) resulted in significant reduction in serum urea levels. The protective effect of resveratrol on kidney function in our study may be attributed to its properties as potential polyphenolic compound that considered as an important monomeric bioactive compound that exhibits a strong antioxidant capacity to scavenge free radicals of oxygen and lipids (Rubiolo et al. 2008). Thereby in kidney cells, resveratrol was found to exert its protective action through up regulation of NO (Giovannini et al. 2001).

The effect of in ovo injection of resveratrol on plasma immunoglobulin (IgG and IgM) in Mandara hatched chicks are presented in Table 4. Plasma IgG and IgM were significantly ($p \leq 0.01$) increased by increasing resveratrol level. Whereas, plasma concentration of IgG were increased by 8.5 and 13.1 % for the two RV treatments (25 and 50 µg/egg), respectively compared with intact control value. The same trend was shown in plasma IgM concentration. These findings are confirmed with Zhang et al. (2014) found that chicks fed diets supplemented with resveratrol (200, 400 and 800 mg/kg diet) achieved the highest values of IgG and antibody titers against avian influenza viruses than those fed control diet. Likewise, Zhu et al. (2019) suggested that in ovo 3mg vitamin C injection at 15th day of incubation increased IgM at hatch also, IgG and IgM concentrations at 21 day of age. The improvement in immunity system by in ovo injection of resveratrol in present study may be due to antioxidant properties of resveratrol that protects immunological tissue from destruction. According to Malaguarnera (2019) reported that resveratrol regulates the immunity by interfering with immune cell regulation, proinflammatory cytokines' synthesis, and gene expression. This cytokines stimulate B lymphocytes activities which would be able to produce immunoglobulin (Freitas et al. 2011).

Intestinal segments and maltase activity:

Data in Table 5 summarized the differences in intestinal segments for hatched chicks. There were significantly increased in relative intestinal weight, length of duodenum, jejunum and ileum of chicks hatched from eggs injected with resveratrol compared to the intact or sham control groups. Likewise, data of Figure 2 illustrate that there was a significant ($p \leq .0001$) increase in jejunum maltase enzyme value of hatched chicks as a result of in ovo injection with resveratrol compared to control groups. The highest improvement in intestinal development was recorded for the chicks hatched from egg injected with 50 µg RV /egg. According to, Pinchasove and Noy (1993) reported that physiological maturation of the gastrointestinal tract occurs mainly through increased production of intestinal enzymes such as maltase enzyme. Therefore, our results reflected on nutrients absorption improvement and in turn increase chicks' performance. These results confirmed the hypothesis that in ovo injection sulfur amino acids improves the surface area and crypt depth of the villus as indicator of the intestinal

developmental and functional status in broiler chicks (Elwan et al., 2019).

Microbiological study:

In ovo injection of resveratrol on 14th days of incubation caused appreciable reduction in the intestinal total aerobic and anaerobic counts and the count of total coliform of hatched chicks compared with control groups (Table 6). Perumalla and Hettiarachchy (2011) stated that phenolic compounds of grape have inhibitory effect on bacteria. In agreement to our results, Hajati et al., (2014) reported that *in ovo* injection of 4.5, 6 mg grape seeds extract/egg or 3 mg vitamin C/egg resulted in decreased ileal population of Coliforms and E. Coli in broiler chicks. Perumalla and Hettiarachchy (2011) summarized that the outer cell membrane or cytoplasmic of a bacterium is essentially composed of a phospholipids bilayer and proteins and is the major site of interaction with antimicrobial compounds. Damage to this vital membrane can result in death of the bacteria. The function of hydroxyl group and conjugated double bonds in the reactive group of natural plant extracts may be involved in their binding to the cell wall components. Also, major phenolic constituents may alter the cell morphology through influencing the osmotic pressure of the cell, thus disrupting the cytoplasmic membrane and causing leakage of the cell constituents (Sivarooban et al., 2008)

CONCLUSION

Based on the current study, it is concluded that *in ovo* injection of resveratrol on 14th d of incubation has positive effect on hatchability%, chick quality, physiological, immunological, and microbiological, anti-oxidative status and intestinal development of hatched chicks. Yolk sac injection at E14 with 50 µg RV /egg could be recommended for improving chick's health.

Table (1): Effect of *in ovo* injection with Resveratrol (RV) on embryonic mortality, hatchability of fertile eggs and chick weight at hatch.

Treatments	Embryonic mortality%			Hatchability of fertile eggs %	Chick weight (g)
	early (1-7 days)	mid (8-14 days)	late (15-20days)		
Intact control	2.87	1.35	8.47 [a]	87.31 [c]	37.42 [b]
Sham control	2.96	1.24	8.99 [a]	86.81 [c]	36.02[b]
25 µg RV/egg	2.71	1.14	5.01[b]	91.14 [b]	39.98[a]
50 µg RV/egg	2.84	1.19	2.08[c]	93.89 [a]	40.33 [a]
SEM	0.011	0.015	0.130	1.04	0.451
P-value	0.660	0.713	0.000	0.000	0.001

a, b, c, Means in the same column with different superscripts, differ significantly (p≤0.05).
SEM=Standard error mean.

Table (2): Effect of *in ovo* injection with Resveratrol (RV) on chick quality.

Treatments	Activity %		Downs and appearance %		Navel %		Chick length (cm)
	Weak	Good	Clean and dry	Wet	Completely closed and clean	Not completely closed and not discolored	
Intact control	11.20 [b]	88.80 [C]	96.00	4.00	98.48	1.52	15.98 [c]
Sham control	12.80 [a]	87.20 [d]	96.20	3.80	98.80	1.20	15.86 [c]
25 µgRV/egg	9.07 [c]	90.93 [b]	96.60	3.40	98.78	1.22	17.08 [b]
50 µgRV/egg	5.13 [d]	94.87 [a]	96.80	3.20	98.80	1.20	17.84 [a]
SEM	0.389	0.389	0.211	0.211	0.120	0.120	0.108
P-value	0.000	0.000	0.540	0.540	0.749	0.749	0.000

a, b, c, d, Means in the same column with different superscripts, differ significantly (p≤0.05).
SEM=Standard error mean.

Table (3): Effect of in ovo injection with Resveratrol (RV) on hematological parameters of Mandara hatched chicks.

Treatments	RBCs (10^6 /mm^3)	Hb (g/dl)	PCV (%)	WBCs (10^3 /mm^3)
Intact control	2.67 [c]	11.44 [c]	35.89 [c]	4.22[c]
Sham control	2.64 [c]	11.16 [d]	35.64 [c]	4.18[c]
25 µg RV/egg	2.92 [b]	12.12 [b]	38.58 [b]	4.86 [b]
50 µg RV/egg	3.06 [a]	12.68 [a]	40.40 [a]	5.40 [a]
SEM	0.026	0.099	0.310	0.084
P-value	0.000	0.000	0.000	0.000

a, b, c, d, Means in the same column with different superscripts, differ significantly ($p \leq 0.05$). SEM=Standard error mean. RBC=red blood cells; Hb=hemoglobin; PCV=packed cell volume; WBC=white blood cells.

Table (4): Effect of in ovo injection with Resveratrol (RV) on some blood constituents of Mandara hatched chicks.

Items	Intact control	Sham control	25 µg RV/egg	50 µg RV/egg	SEM	p-value
Total protein (g/dl)	5.92 [c]	5.66 [d]	6.22 [b]	6.58 [a]	.069	.000
Albumin(g/dl)	3.72	3.66	3.66	3.90	.029	.076
Globulin(g/dl)	2.20 [b]	2.00 [c]	2.56 [a]	2.68 [a]	.051	.000
IgG (mg/dl)	176.00 [c]	174.40 [c]	191.00 [b]	199.00 [a]	1.72	.012
IgM (mg/dl)	275.20 [c]	273.40 [c]	296.20 [b]	313.00 [a]	2.65	.010
Cholesterol(mg/dl)	166.00 [a]	167.20 [a]	151.40 [b]	142.80 [c]	1.71	.004
Urea (mg/dl)	27.00 [a]	27.12 [a]	23.00 [b]	21.20 [c]	.439	.002
T_3 (ng/ml)	1.63 [c]	1.65 [c]	1.80 [b]	1.96 [a]	.022	.000

a, b, c, d, Means in the same row with different superscripts, differ significantly ($p \leq 0.05$). SEM=Standard error mean. T_3 =Triiodothyronine .

Table (5): Effect of *in ovo* injection with Resveratrol (RV) on intestinal segments of hatched chicks.

Treatments	Relative intestine weight	Duodenum length (cm)	Jejunum length (cm)	Ileum length (cm)
Intact control	2.45 ^{c}	3.89 ^{c}	10.28 ^{c}	7.69 ^{c}
Sham control	2.51 ^{c}	3.77 ^{c}	10.34 ^{c}	7.58 ^{c}
25 µg RV/egg	2.68 ^{b}	4.34 ^{b}	11.13 ^{b}	8.27 ^{b}
50µg RV/egg	2.87 ^{a}	4.61 ^{a}	11.34 ^{a}	8.77 ^{a}
SEM	0.04	0.01	0.13	0.07
P-value	0.000	0.001	0.005	0.000

a, b, c, , Means in the same column with different superscripts, differ significantly ($p \leq 0.05$). SEM=Standard error mean.

Table (6): Effect of *in ovo* injection with Resveratrol (RV) on counts of aerobic, anaerobic and total coliform bacteria in intestine of hatched chicks.

Treatments	Aerobic plate count	Total coliform Count	Total anaerobic count
Intact control	$11x10^{5}$	$15x10^{4}$	$6x10^{2}$
Sham control	$6x10^{5}$	$14x10^{4}$	$5x10^{1}$
25 µg RV/egg	$5x10^{5}$	$12x10^{4}$	$2x10^{1}$
50 µg RV/egg	$2x10^{5}$	$9x10^{4}$	-ve

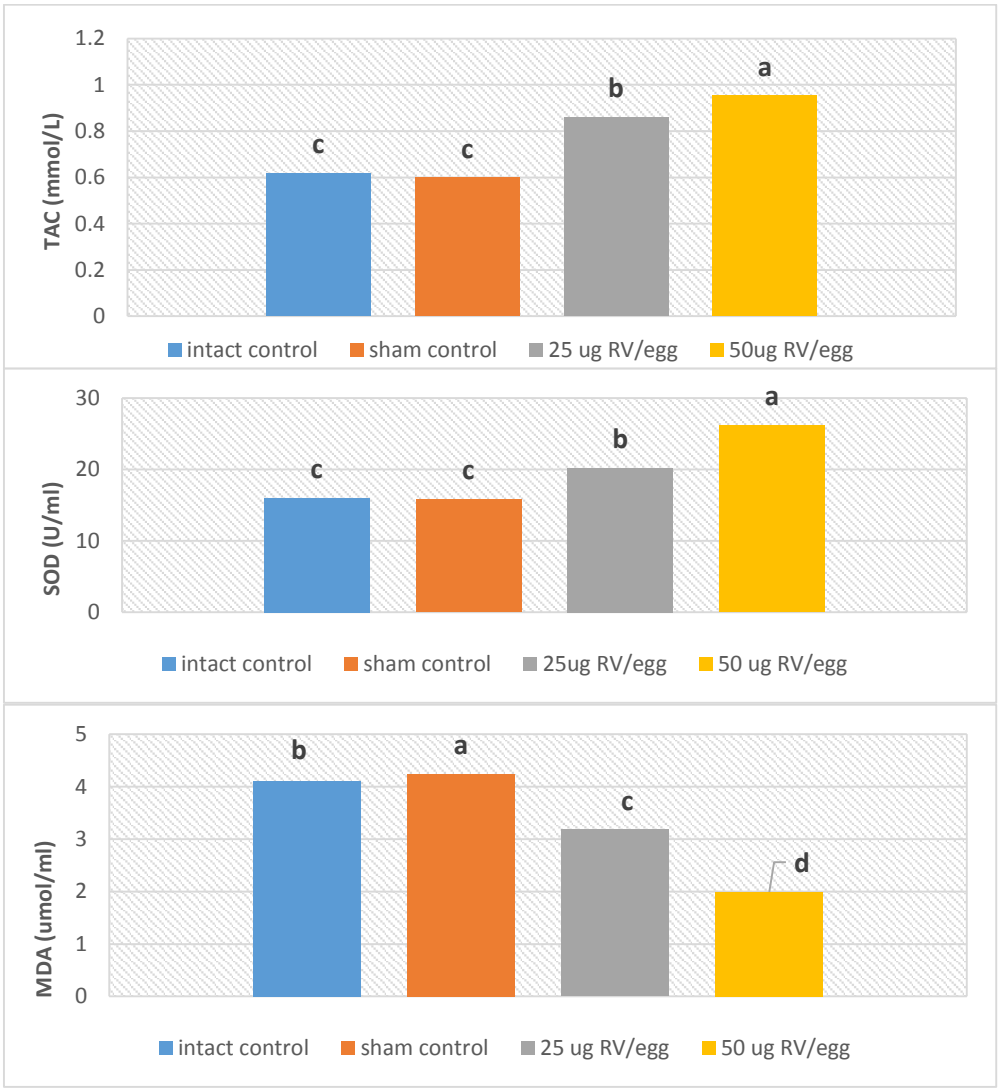
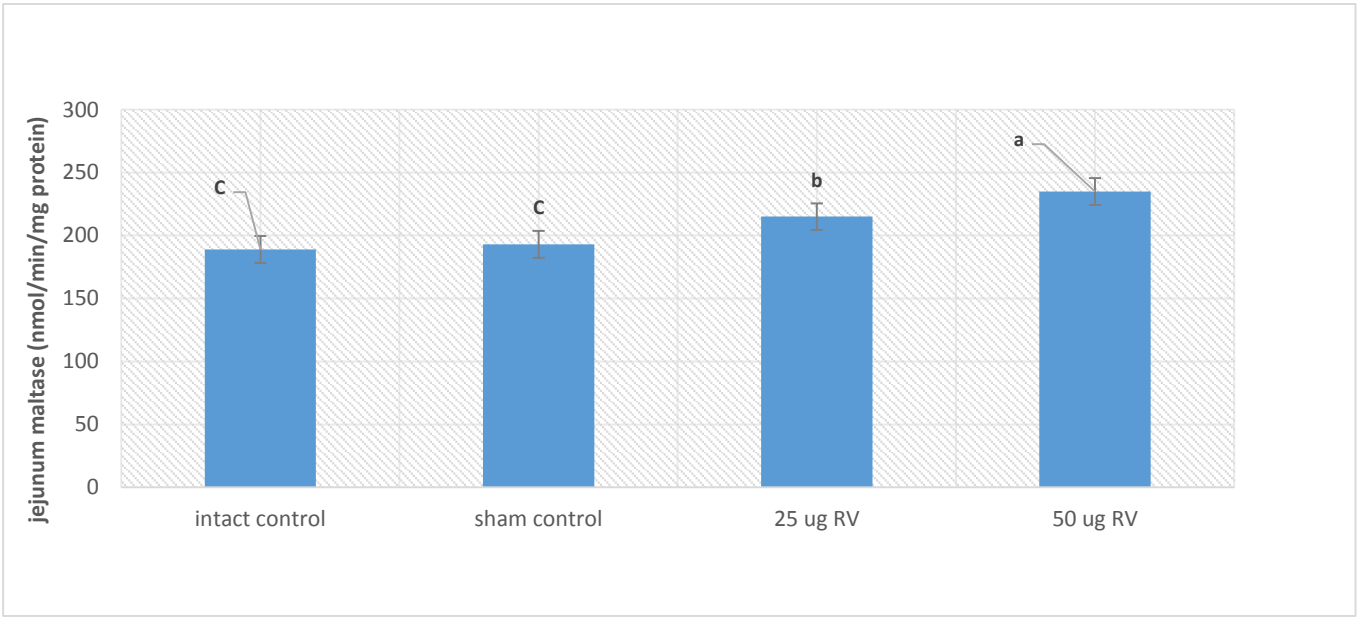


Fig (1): Effect of *in ovo* injection with resveratrol (RV) on total antioxidants capacity (TAC), superoxide dismutase (SOD) and malondialdehyde (MDA) of Mandara hatched chicks.

Fig. (2): Effect of *in ovo* injection with Resveratrol (RV) on jejunum maltase activity (nmol/min/mg protein) of hatched chicks.



REFERENCES

Abu Hafsa, S.H. and Ibrahim, S. A. 2017. Effect of dietary polyphenol-rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. J. Anim. Physiol. Nutr. 1-8.

A. P. H. A. American Public Health Association, 1985. Standard Methods for the Examination of Water and Wastewater, 16th Ed. APHA, Washington, D.C.

Alagawany, M. M.; Farag, M. R.;Dhama, K.; Abd-EL-Hack, M.; Tiwari, R.and Alam, G. M. 2015. Mechanisms and beneficial applications of resveratrol as feed additive in animal and poultry nutrition: A Review. Science Alert, 213-221.

Araujo, I. C. S.; Café, M. B.; Noleto, R. A.; Martins, J. M. S.; Ulhoa, C. J.; Guareshi, G. C.; Reis, M. M. and Leandro, N. S. M. 2018. Effect of vitamin E in ovo feeding to broiler embryos on hatchability, chick quality, oxidative state and performance. Poult. Sci. 98(9): 1-10.

Atmac, N.; Yilduim, E.; Guner, B.; Kabakci, R. and Bilmen, F. S. 2014. Effect of resveratrol on hematological biochemical alteration in rats exposed to fluorid. Bio. Med. Res. Int. Article ID 698628, 5 pages.

Babacanoglu, E.; Karagecili, M.R.Aand Karadas, F. 2018. Effects of egg weight and in ovo injection of α-tocopherol on chick development, hatching performance, and lipid soluble antioxidant concentration in quail chick tissues. Archiv Fuer Tierzucht, 61(2): 179.

Baxter, R. A., 2008. Anti-aging properties of resveratrol: Review and report of a potent new antioxidant skin care formulation. J. Cosmet.Dermatol., 7: 2-7.

Choi, M. H.; Ow, J. R.; Yang, N. D. and Taneja, R. 2016. Oxidative stress mediated skeletal muscle degeneration: molecules, mechanisms, and therapies. Oxidative medicine and cellular longevity. Article ID 6842568, 13 pages, https://doi.org/10.1155/2016/6842568.

Duncan, D. B., 1955. Multiple Rang and Multiple F Test Biometrics. 11: 42.

EL-Kholy, M. S. 2013. Physiological study on Japanese quail as affected by some nutritional treatments. PhD Thesis, Fac. Agric., Zagazig Univ.., Egypt.

EL-Kholy, M. S.; Zenat, A. I.; EL-Mekkawy, M. M.and ALagawany. M. 2019. Influence of in ovo administration of some water-soluble vitamins on hatchability traits, growth, carcass traits and blood chemistry of Japanese quails .Ann. Anim. Sci., 19: 97-111.

Elwan,H. A. M.; ELnesr, S.S.; Xu, Q.; Xie, C.; Don, X. and Zou, X. 2019. Effect of in ovo Methionine-Cysteine injection on embryonic development, antioxidant status, IGF-1and TLR4 Gene expression and iejunum histomorphmetry in newly hatched broiler chicks exposed to heat strees during incubation. Animals (Basel),9(1):25.

Ememe, M. U.; Edeh, R.E.1; Abdullahi, U. S1; Sackey, A.K.B1. and Ayo, J. O. 2016. Changes in hematological parameters and erythrocyte osmotic fragility in lame and aged horses administered with resveratrol supplement. Afr. J. Biomed. Res. 19(1): 1-7**.**

Feng, Z. H.; Gong, J. G.; Zhao, G. X.; Lin, X.; Liu, Y.C. and Ma, K. W.

2017. Effects of dietary supplementation of resveratrol on performance, egg quality, yolk cholesterol and antioxidant enzyme activity of laying hens.Br Poult. Sci. 5: 544-549.

Freitas, J. A.; Vanat, N.; Pinheiro, J. W.; Balarin, M. R. S.; Sforcin, J. M. and Venacio, E. J. 2011. The effect of propolis on antibody production by laying hens. Poult. Sci., 90(6): 1227-1233.

Giovannini, L.; Migliori, M.; Iongoni, B.; Dipak Das; Bertelli, A.; Panichi, V; Filippi, C. and Bertelli, A. 2001. Resveratrol a polyphenol found in wine, reduces ischemia reperfusion injury in rat kidney. Journal of Cardiovascular Pharmacology, 37(3):262-270.

Goldberg, DM.; Yan, J.and Soleas, GL. 2003. Absorption of three wine related polyphenols in three different matrices by healthy subject. Clin Biochem, 36: 79-87.

Gumulu, S.; Bilmen, S.; Sarikciog, L.; Sahin, E.; Yargicog, P. and Aysel,A. 2002. Influences of different stress models on the antioxidant status and lipid peroxidation in rat erythrocyte. Free Rad Res. 36: 1277-1282.

Hajati,H.; Hassanabadi, A.; Golian, A.; Moghaddam, H. N. and Nassiri, M. R. 2014. The effect of in vivo injection of grape seed extract and vitamin Con hatchability, antioxidant activity, yolk sac weigh, performance and ileal micro flora of broiler chickens. Res. Opin. Anim. Vet. Sci., 4(12): 633-638.

Hao, R.Y.; Li, Y. X.; Yu, F.; Zhao, Y. and Zheng, X. N. 2011. Effects of resveratrol on lipid peroxidation in mice with high fat and high cholesterol diet. J. China Med. Univ., 40:17-25.

Kalantar, M.; Hosseini, S. M.; Hosseini, M. R.; Kalantar, M. H.; Farmanullah, and Yang, L. G .2019. Effect of in ovo injection of Coenzyme Q10 on hatchability, subsequent performance, and immunity of broiler chickens. BioMED Research International, 1-8.

Lassen, E.D. and Swardson, C. J.1995. Hematology and hemostasis in the horse: normal functions and common abnormalities. Vet. Clin. North Am. Equine Pract. 11: 351-389.

Leon, M.; Garrido, G.; Csstaneda, M. and Rueda, E. 2014. Early feeding to modify digestive enzyme activity in broiler chickens. Rev. MVZ Cordoba 19(3): 4316-4327.

Lopez-Velez, M.; Martinez-Martinez, F.and del Valle-Ribes, C, 2003. The study of phenolic compounds as natural antioxidants in wine. Crit. Rev. Food Sci. Nutr., 43: 233- 244.

Leslie, H.; and Frank, C. H., 1989. Practical Immunology. Third ed., p: 23.

Lu, J. W.; McMurtry, J. P. and Coon, C. N. 2007. Developmental changes of plasma insulin, glucagon, insulin-like growth factors, thyroid hormones, and glucose concentration in chick embryos and hatched chicks. Poult. Sci. 86: 673-683.

Malaguarnera, L. 2019. Influence of resveratrol on the immune response. Nutrients, 11(5):
946-968.

Malheiros, R. D.; Ferket, P. R.and Goncalves, F. M., 2012. Oxidative stress protection of
embryos by in ovo supplementation. Salvador- Bahia- Brazil:World's Poultry Congress.

Murcia, M. A. and Martinez-Tome, M. 2001. Antioxidan activity of resveratrol compared with common food

additives.J. Food Prot., 64: 379- 384.

Nazem, M. N.; Sayed, M. S.; Reza, K. and Hamideh, M. 2017.Histomorphometric analysis of the small intestine of broiler chick embryos injected in ovo with methionine. Anim. Prod. Sci., 59(1): 133-139.

Ognik, K.; Cholewinska, E. and Czech,A. 2016. The effect of adding Hesperdin, Diosmin, Quercetin and Resveratrol extracts to feed for turkey hens on selected immunological and biochemical blood indices. Ann. Anim. Sci., 16 (4): 1101–1114.

Perumalla, A.V.S. and Hettiarachchy, N.S. 2011. Green tea and grap seed extracts- Potential applications in food safety and quality. Food Research International, 44: 827-839.

Pinchasove, Y. and Noy, Y. 1993. Comparison of post hatch holding time and subsequent early performance of broiler chicks and turkey poults. Br. Poult. Sci. 34: 111-120.

Prokop, J.; Abrman, P.; Seligson, A. L. and Sovak, M. 2006. Resveratrol and its glycon piceid are stable polyphenols. J. Med. Food, 9: 11-14.

Rubiolo, J. A.; Mithieux, G. and Vega, F. V. 2008. Resveratrol protects primary rat hepatocytes against oxidative stress damage: Activation of the Nrf2 transcription factor and augmented activities of antioxidant enzymes. Eur. J. Pharmacol., 591: 66-72.

Sahin, K.; Orhan, C.;Akdemir, F.; Tuzcu, M.; Iben, C. and Sahin, N. 2012. Resveratrol protects quail hepatocytes against heat stress: Modulation of the Nrf2 transcription factor and heat shock proteins. J. Anim. Physiol. Anim. Nutr., 96: 66-74.

Sahin, K.; Akdemir, F.; Orhan, C. Tuzcu, M.; Hayirli, A. and Sahin, N. 2010. Effects of dietary resveratrol supplementation on egg production and antioxidant status. Poult. Sci., 89: 1190- 1198.

SAS, 2004. SAS/STAT User's Guide Version 9.1.SAS Inc., Cary, NC.

Schaal TP.2008. The effect of in ovo feeding of fatty acids and antioxidants on broiler chicken hatchability and chick tissue lipid [dissertation]. Corvallis: Oregon State University, University Honors College.

Selim, Sh. A.; Gaafar, K. M.; and EL-ballal, S. 2012. Influence of in ovo administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. Emir. J. Food Agric., 24: 264-271.

Sridhar, M.; Suganthi, R.U. and Thammiaha, V. 2014. Effect of dietary resveratrol in ameliorating aflatoxin B1-induced changes in broiler birds. J. Anim. Physiol. Anim. Nutr.,(In press). 10.1111/jpn. 12260.

Sivarooban, T.; Hettiarachchy, N.S. and Johnson, M.G. 2008. Transmission electron microscopy study of Listeria monocytogenes treated with nisin in combination with either grape seed or green tea extract. Journal of Food Protection, 71: 2105-2109.

Subramaniyan, S. A.; Kang, D. R.; Park, J.R.; Siddiqui, S. H.; Ravichandiran, P.; Sam Na, C. and Shim, K. S. 2019. Effect of in ovo injection og L-Arginine in different chicken embryonic development stages on posy-hatch growth performance, immune response and muscle development proteins. Doi: 10.20944/preprints 201905, 013, V1.

Surai, P.F. 1999. Tissue specific changes in the activities of antioxidant enzymes

during the development of the chicken embryo. British Poultry Science, 40: 397-405.

Surai, P.F. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. Br Poult Sci, 41: 235-243.

Surai, P.F. 2002. Natural antioxidants in avian nutrition and reproduction. NottinghamUniversitypress,Nottingham,UK.

Taha, A. E.; AbdAllah, O.A.; Attia, K.M.; AbdEl-Karim, R.E.; AbdEl-Hack, M.E.; El-Edel, M. A. and Saadeldin, I. M.2019. Does in ovo injection of two chicken strains with royal jelly impact hatchability, post-hatch growth performance and hematological and immunological parameters in hatched chicks? Animals, 9,486; doi: 10.3390/ani9080486.

Tedesco, I.; Russo, M.; Russo, P.; Iacomino, G.; Russo, G.L.; Carraturo,A.; Faruolo, C.; Moio, L. and Palumbo, R. 2000. Antioxidant effect of red wine polyphenols on red blood cells. J. Nut. Biochem, 1: 114-119.

Tona, K.; Bamelis, F.; De Ketelaere,B.; Bruggeman, V.; Moraes,V. M.; Buyse, J.; Onagbesan, O. and Decuypere, E. 2003. Effect of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poult. Sci.82:736-64

Vuong, T. V.; Franco, C. and Zhangi, W. 2014. Tteatment strategies for high resveratrol induction in Vitis vinifera L. cell suspension culture. Biotechnol. Rep., 1: 15-21.

Wolanski, N. J.; Renema, R. A.; Robinson, F.E.;Carney, I. V. L. and Fancher, B. 2007. Relationships among egg characteristics, chick measurements and early growth traits in Ten Broiler breeder. strains. Poult. Sci. 86: 1784-1792.

Yan, Y.; Yang, J. Y. ; Mou, Y. H.; Wang, L. H.; Zhou, Y. N. and Wu, C. F. 2012. Differences in the activities of resveratrol and ascorbic acid in protection of ethanol-induced oxidative DNA damage in human peripheral lymphocytes. Food Chem. Toxicol., 50: 168-174.

Zhang, C. Y.; Tian, Y. D.; Yan, F. B.; Kang, X. T.; Han, R. L.; Sun, G. R. and R. 2014. Modulation of growth and immunity by dietary supplementation with resveratrol in young chickens receiving conventional vaccinations. Am. J.Vet. Res., 75: 752-759.

Zhang, C.; Kang, X.; Zhang, T. and Huang, J. 2019. Positive effect of resveratrol on egg-laying ability, egg quality, and antioxidant activity in hens. J. Appl. Poult. Res. 0:1-7.

Zhu, Y. F.; Li, S. Z.; Sun, Q. Z. and Yang, J. 2019. Effect of in ovo feeding of vitamin C on antioxidation and immune function of broiler chickens. Animal Doi:https://doi.org/10.1017/S1751731 1180035

Resveratrol, *in ovo*, antioxidant, hatching traits, physiological parameters.

الملخص العربى

تاثير حقن بيض التفريخ بالريسفيراترول على صفات الفقس و الاستجابة الفسيولوجية لكتاكيت المندرة

امينة شعبان السعدنى، اسامة محمود على، على عبد الهادى البرلسى، امال محمود البربرى، ابتسام السيد ابراهيم عراقى، هناء محمد خليل

قسم بحوث تربية الدواجن، معهد بحوث الانتاج الحيوانى،مركز البحوث الزراعية،وزارة الزراعة

اجريت هذه التجربة لدراسة تاثيرحقن بيض التفريخ بالمركب الفينولى (ريسفيراترول) وذلك عن طريق استخدام 864 بيض مخصب لسلالة المندرة. تم تقسيم البيض الى 4 معاملات بكل منها 216 بيضة (72 بيضة/مكررة). فى اليوم 14 من التحضين تم حقن البيض المخصب فى كيس الصفار كالاتى: المجموعة الاولى (كنترول سليم) بدون حقن والمجموعة الثانية (كنترول زائف) حقنت 100 ميكروليتر ماء معقم والمجموعة الثالثة والرابعة حقنت 100 ميكروليتر ريسفيراترول بتركيز 25 و50 ميكروجرام/بيضة على التوالى. اظهرت النتائج تحسن معنوى فى نسبة الفقس ووزن وجودة الكتاكيت عند الفقس فى المجاميع المحقونة بالريسفيراترول مقارنة بالكنترول. ارتفع معنويا كل من انزيمات المضادة للاكسدة والبروتينات الكلية والجلوبيولين وجلوبيولينات المناعة فى بلازما الدم عند الفقس للمجاميع المحقونة بالريسفيراترول مقارنة بالكنترول وكانت اعلاها فى المعاملة المحقونة 50 ميكروجرام/بيضة. ولوحظ انخفاض فى كوليستيرول الدم والمحتوى الميكروبى للامعاء فى المجاميع المحقونة بالريسفيراترول . واوضحت النتائج زيادة معنوية فى نسبة الهيموجلوبين والهيماتوكريت وعدد كرات الدم الحمراء والبيضاء فى كتاكيت المندرة عند الفقس فى المجاميع المعاملة مقارنة بالكنترول. وجدت زيادة معنوية فى الوزن النسبى للامعاء واطوال كل من الاثنى عشر والصائم واللفائفى ونشاط انزيم المالتيز فى الصائم فى الكتاكيت الفاقسة من بيض محقون بالريسفيراترول مقارنة بالكنترول. ونستخلص من هذه الدراسة ان حقن بيض التفريخ بالريسفيراترول عند اليوم14 من التحضين ادى الى تحسين نسبة الفقس وجودة الكتاكيت وايضا تحسن الحالة الفسيولوجية والمناعية للكتلكيت الناتجة وقد سجلت المعاملة 50 ميكروجرام/بيضة افضل