

# Journal of Animal and Poultry Production

Journal homepage & Available online at: [www.jappmu.journals.ekb.eg](http://www.jappmu.journals.ekb.eg)

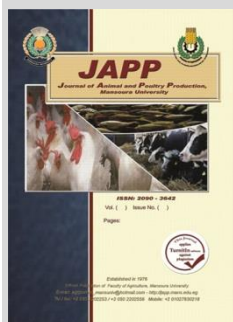
## Isin Ovo injection useful for Aged Broiler Breeders?

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### ABSTRACT

The aim of this study was to assess the effect of *in ovo* injection with saline, vitamins (D<sub>3</sub> and B<sub>12</sub>) and zinc and L-carnitine on the hatching rate and blood constituents in newly hatched chicks. A total of 1170 eggs from broiler breeders were divided into six treatment groups of eggs: a control group without injection which served as sham-operated or a negative control group and five groups were injected with saline, vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> or L-carnitine, respectively. All eggs were set at a temperature range between 24-26°C for 6 hours before exposure to the incubation temperature. Then the injection process occurred at the 18<sup>th</sup> day and 12 hours of the embryonic development by depositing the test materials into the air sac at the wide end of the eggs, then the hole was closed with wax. The highest value of fertile hatchability was recorded in eggs injected with vitamin D<sub>3</sub> and zinc, respectively, followed by vitamin B<sub>12</sub> and L-carnitine. The injected test materials (vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> and L-carnitine) had a positive effect on the percentage of late dead embryos compared to negative control group. All injected materials except zinc led to an increase in plasma levels of cholesterol and low-density lipoprotein but levels of glucose, triglyceride, high-density lipoprotein, total protein, albumin and globulin were not affected. In conclusion, *in ovo* injection of vitamins (D<sub>3</sub> and B<sub>12</sub>), zinc and L-carnitine may be suggested as an effective technique for increasing hatchability and profitability in aged broiler breeders.

**Keywords:** *In ovo* injection, Broiler breeder eggs, Fertile hatchability, Late dead embryos, vitamins (D<sub>3</sub>, B<sub>12</sub>), Zinc and L-carnitine.

### INTRODUCTION

The incubation period for chicken eggs persists 21 days, and represents about 37.5% of their life cycle. If we consider the period necessary to transfer the sound chicks from the hatchery when their hatchability reach 95%, along with the period required to transfer them to the farm, we can get a possibility of reducing the water and feed consumption of chicks for 2-3 days. This economic managerial procedure has prompted the application of the early feeding technique of the embryos through piercing the eggshell to allow injecting a nutrient or nutrients to pass through to the developing embryo (El-Sabrouet *et al.*, 2019).

Also, the hatch window is an important factor affecting the quality of the chicks, and in most cases the period required to transfer the chicks to the breeding farms ranges between 48 and 72 hours (Kadamat *et al.*, 2013). During this period, the only source of feeding for the continuation of the embryo life and its growth is the fats (lipids) and proteins found in the residual egg yolk (Sklanet *et al.*, 2000). During the fasting period (which may last 72 hours) the hatched chicks can rely on the components of the residual egg yolk to meet their nutrient requirements, but it may be insufficient for optimal growth rate. Therefore, the early feeding techniques were adopted, which had proved to be effective in supporting growth and improving the quality characteristics of the resultant chicks (Ferket, 2012). To enhance poultry growth and productivity, several methods were used. Such methods can be divided based on the target site of *in ovo* injection into five sites: the air cell, the embryo's body itself, the amniotic fluid, the allantoic membrane, and

the yolk sac (Saeed *et al.*, 2019). Therefore, the aim of this study was to investigate the effect of *in ovo* injection with saline, vitamins (D<sub>3</sub> and B<sub>12</sub>), zinc and L-carnitine on hatchability characteristics and some blood constituents of newly hatched chicks.

### MATERIALS AND METHODS

Injecting eggs with minerals; vitamins and amino acids was carried out in the commercial Matroh El-Watania incubator (it is a single-stage incubator equipped with a system of automatic turning for eggs), Matroh El-Watania Company for poultry, Matroh Governorate, Egypt. The laboratory analyses were performed at the Faculty of Agriculture; Mansoura University, Egypt.

#### Egg Injection and Incubation:

A total of 1170 eggs with an average weight of 67 to 70g were obtained from a commercial Matroh El-Watania broiler breeder flock (Cobb-500) at 67 weeks of age. In this study, eggs were injected with five test solutions of saline (0.9% NaCl); vitamin D<sub>3</sub>; Zinc; vitamin B<sub>12</sub> or L-carnitine vs. sham-operated eggs (a negative control group). Injections were made at the 18<sup>th</sup> day and 12 hours of incubation. The test materials were prepared as follows: 1.5mg/100µl sterile saline and L-carnitine (8mg/100µl sterile saline), vitamin D<sub>3</sub> (100,000IU/100µl sterile saline), zinc gluconate (72.9mg/100µl sterile saline), B<sub>12</sub> (1000mg/ 100µl sterile saline). Extreme care was taken into account when performing the injection process for all treated eggs, as the test materials were injected into the air sac at a depth of 0.28 mm in the wide end of the egg under the supervision of specialists

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DOI: 10.21608/jappmu.2023.220100.1080

in the injection process with extensive experience. The injection process was organized as follows: 195 eggs were sham-operated and served as a negative control (NC) group, 195 eggs were injected with sterile saline (0.9%) and served as a positive control (PC) group, 195 eggs were injected with vitamin D<sub>3</sub> and served as treatment three, 195 eggs were injected with zinc gluconate and served as treatment four, 195 eggs were injected with vitamin B<sub>12</sub> and served as treatment five, 195 eggs were injected with L-carnitine and served as treatment six. In this study, in order to avoid infections and contaminations among eggs of the injected groups, insulin syringes were changed constantly, and ethyl alcohol 70% was used to prevent the spread of infection among eggs, and it was deposited again in the hatchery to complete the embryonic development process.

**Environmental Description of Incubator and Hatchery:**

The characterization of temperature, relative humidity (RH), and carbon dioxide values in both incubator and hatchery are shown in Tables 1 and 2, respectively. Incubator temperature was variable in single-stage incubator (EMKA); relative humidity (RH) and percent of CO<sub>2</sub> were estimated from day one to 18 days of incubation. To our knowledge we must be aware that a large amount of eggs enter the incubator simultaneously and thus their embryonic development occur in one phase. So that a high level of managerial control is recommended to supply the optimal conditions of embryonic development and this development is inferred by sensing the heat of the egg from *ovo scan* compartment of the incubator.

The hatching percentage was estimated by considering the number of hatched chicks relative to the number of fertile eggs. It is also known as scientific or fertile hatchability (FH), and can be computed as follows:

$$\text{Fertile Hatchability} = \frac{\text{no of hatched chicks}}{\text{no of fertile eggs}}$$

**Table 1. Program of single-stage incubator.**

Age	Eggshell	Incubator	Vent	RH	CO <sub>2</sub>	Cool
D: H	Temp. (°F)	Temp. (°F)	%	(%)	%	
00:00	100.4	100.4	00	90	0.25	
01:00	100.4	100.3	00	90	0.25	
02:00	100.3	100.2	00	90	0.25	
03:00	100	100	10-15	90	0.35	
07:00	100	99.9	10-15	90	0.60	Water till
09:00	100	99.8	10-15	88	0.60	day
10:00	99.9	99.7	10-15	88	0.45	16, followed
11:00	99.9	99.6	30-50	86	0.45	by air flow
11:18	99.8	99.5	30-50	86	0.45	ten seconds
12:00	99.7	99.4	30-50	86	0.45	thereafter.
13:00	99.7	99.3	40-65	84	0.45	
14:00	99.7	99.2	40-65	84	0.45	
15:00	99.7	99.0	40-65	84	0.45	
18:00	99.7	98.8	60-85	84	0.30	

D: Day. H: Hours. RH: Relative humidity.

**Table 2. Program of single-stage hatchery.**

Age	Temp	RH	CO <sub>2</sub>	Vent	Cool
D: H	(°F)	(%)	%		
18:12	98.5	85	0.6	25-50	
19:12	98.2	87	0.8	25-65	
20:00	98.0	91	1.0	25-90	
20:10	97.8	90	0.9	35-100	Water till 14 hours post-
20:12	97.5	89	0.75	35-100	the 20 <sup>th</sup> day, followed by
20:14	97.0	89	0.50	35-100	air flow ten seconds
20:16	96.8	89	0.50	50-100	thereafter.
20:18	96.5	88	0.50	50-100	
20:20	96.0	88	0.50	70-100	
21:00	95.8	85	0.40	70-100	

**Embryonic Mortality:**

The stages of mortality were divided according to the timing of their occurrence during the incubation period as it was given in Table 3.

**Plasma Blood Parameters of Hatched Chicks:**

After complete hatching, random sample of 9 chicks was chosen from each treatment to blood sampling in heparinized test tubes, and the blood samples were immediately centrifuged at 3000 rpm for 15 minutes to separate the plasma. Plasma concentrations of glucose (Glu), cholesterol (Cho), triglyceride (Tri), low-density lipoprotein (LDL) high-density lipoprotein (HDL), total protein (TP) and albumin (Alb), as well as activity of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by commercial kits (Spectrum Diagnostic Kits S.A.E., Egyptian Company of Biotechnology, 2022). Plasma globulin (Glo) concentration was also calculated.

**Table 3. Embryonic mortality categories.**

Category	Time of occurrence from incubation period (days)
Early Dead Embryos	0 – 7
Mid Dead Embryos	8 – 14
Late Dead Embryos	15 – 21
Infertile eggs	11
Pipped eggs	19-21
Live pipped chicks	21
Dead pipped chicks	21

**Statistical Analysis:**

The statistical analysis of results was performed by using one-way analysis of variance of the GLM procedure of the Statistical Analysis System (SAS, 2006). Significant differences between means of different estimated variables were identified by Duncan's new multiple range test at P<0.05 (Duncan, 1955). The following statistical model was used:

$$Y_{ij} = \mu + E_i + e_{ij} \text{ Where:}$$

Y<sub>ij</sub> = observed trait; μ = the overall mean; E<sub>i</sub> = Effect of injected material; and e<sub>ij</sub> = experimental random error.

**RESULTS AND DISCUSSION**

**Reproductive Performance:**

Data summarized in Table (4) showed some characteristics of hatchability in aged broiler breeders as influenced by *in ovo* injection with saline, vitamin D<sub>3</sub>, Zinc, vitamin B<sub>12</sub> and L-carnitine. The highest value of fertile hatchability (FH) was achieved by the groups injected with vitamin D<sub>3</sub>, Zinc and vitamin B<sub>12</sub>, respectively. The group of eggs treated with L-carnitine also achieved better mean of FH but was not significantly different from As was expected, fertility rate (%) was not significantly affected by the injection of test materials. Likewise, the hatch weight of chicks was not affected by the injected materials. Our results are in agreement with those of Stevens *et al.* (1984), who found that vitamin D<sub>3</sub> deficiency led to a decrease in the hatching rate and an increase in the late embryonic mortality. A similar trend of response was also observed by Bello *et al.* (2013).

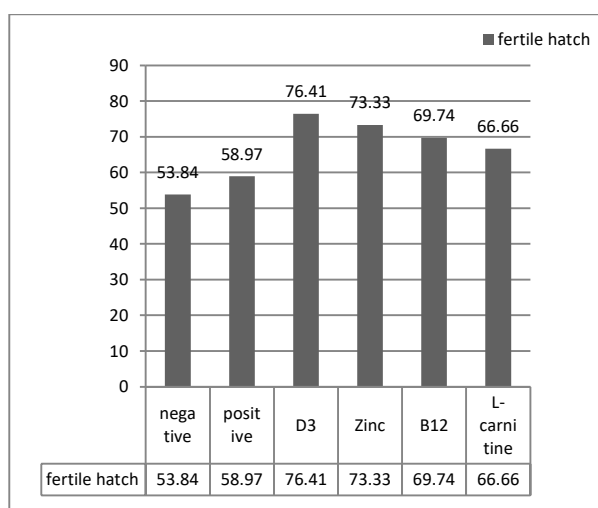
In an early report, Narbaitz *et al.* (1987) also attributed the effectiveness of vitamin D<sub>3</sub> in improving the hatching rate to its role in the growth and development of the poultry embryo and its regulation of calcium metabolism which enhances the vitality of the embryo in the period between the internal pipping of the shell to the completion of the hatching process. Recently, El-Fiky *et al.* (2022) reported a positive

effect of vitamin D<sub>3</sub> on the hatching rate of poultry eggs. In addition, Hamza *et al.* (2022) indicated that the effect of zinc on the hatching rate of poultry egg depends primarily on the source of zinc. In this regard, Sogunle *et al.* (2018) observed that when the eggs were injected with zinc at a level of 80 µg per egg, the hatching rate increased in the treated eggs. Also, Uni and Ferket (2004) pointed out that the effect of vitamin B<sub>12</sub> in improving the hatching ratio appears when the injection process occurs on the eighteenth day of incubation. They attributed to the fact that at this age the embryo has completed its development and is able to be exposed to a change in temperature in the injection environment, which is less than the temperature of the hatching machine. This viewpoint was supported by Teymouriet *al.* (2020) as the eggs were injected with vitamin B<sub>12</sub> on the thirteenth and fifteenth days at a concentration of 20-40 µg, and it did not exert a beneficial effect on the hatching rate. Furthermore, Momeneh and Torki (2018) mentioned that the percentage of hatching in eggs treated with vitamin B<sub>12</sub> improved to 70.83%, compared to that of the control group (58.33%) when the injection process occurred on the eighteenth day of incubation and this is in harmony with the results of Lillie *et al.* (1949), who noticed an improvement in the hatching rate in eggs treated with vitamin B<sub>12</sub>.

**Table 4. Effects of *in ovo* injection of saline, vitamins, zinc and L-carnitine on hatchability characteristics in aged broiler breeders.**

Treatments	No. of Eggs.	Infertility %	Fertility %	FH %	Chick weight at hatch (g)
NC	195	17.95	82.05	53.84 <sup>c</sup>	41.58
PC	195	17.44	82.56	58.97 <sup>bc</sup>	45.76
Vitamin D <sub>3</sub>	195	15.38	84.62	76.41 <sup>a</sup>	42.91
Zinc	195	16.42	83.58	73.33 <sup>a</sup>	42.68
Vitamin B <sub>12</sub>	195	16.93	83.07	69.74 <sup>ab</sup>	42.21
L-carnitine	195	17.44	82.56	66.66 <sup>abc</sup>	42.84
SEM	-	2.69	2.69	3.34	1.43
Significance	-	NS	NS	**	NS

Means with different superscripts in the same column differ significantly at P≤0.05. NS: Not significant, \*\*: Significant at P≤0.05, NC: Negative control, PC: Positive control, SEM: Standard error of the means.



**Figure 1. Effects of the test materials on fertile hatchability compared with the negative control**

On the other hand, Dooley *et al.* (2011) detected a limited significant effect of *in ovo* L-carnitine injection on the hatching rate of poultry eggs. While Zahiet *al.* (2008) stated that when L-carnitine injection was performed at the age of

18 days of the embryonic development, there was no positive effect on the hatching rate, which confirms that the absence of a beneficial effect to injecting L-carnitine into the hatching eggs is not due to the timing of the injection process, but to the effect of the L-carnitine itself. A similar trend of response was also observed by Keralapurathet *al.* (2010).

**Selected Parameters of Hatchability in Aged Broiler Breeders**

The effects of *in ovo* injection with saline, vitamin D<sub>3</sub>, Zinc, vitamin B<sub>12</sub> and L-carnitine on selected parameters of hatchability and stages of embryonic mortality are presented in Table 5. The results showed that no significant alternations in live pipped and mid dead embryos due to *in ovo* injection with saline, vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> and L-carnitine, and contamination could be detected from captured eggs classification. However, capturing eggs from the NC group displayed significantly (P≤0.01) higher mean (28.20%) of culled eggs than those injected with vitamin D<sub>3</sub> (8.20%), vitamin B<sub>12</sub> (13.33%), L-carnitine (15.89%) and zinc (10.25%). The mean of culled eggs in the PC group (23.58%) was slightly lower than that of the NC group but significantly higher (P≤0.01) than those injected with vitamin D<sub>3</sub> or zinc, and insignificantly different from those injected with vitamin B<sub>12</sub> or L-carnitine. Based on data obtained from captured eggs classification, the rate of pipped embryos (%) was significantly lower (P≤0.01) in groups of eggs injected with vitamin D<sub>3</sub> or zinc compared with the NC group but comparable to those of the PC and other treated groups. Our results indicated also that the groups of eggs injected with vitamin D<sub>3</sub>, zinc or vitamin B<sub>12</sub> recorded significantly lower percentages of dead pipped embryos compared with the NC group but were insignificantly different from those of the PC and other treated groups. Also, early- and late-dead embryos significantly decreased (P≤0.01) by using *in ovo* injection of the test materials as compared to the NC group.

In this respect, Han *et al.* (2016) observed a beneficial linear effect to supplemental dietary vitamin D<sub>3</sub> on the mortality rate of broiler chicks from one to three weeks of age. In line with our results, Wang *et al.* (2016) found a positive effect of vitamin D<sub>3</sub> on the mortality rate of broiler chicks fed Ca- and P-deficient diets during the first three weeks of life. In accordance with the current findings, embryonic mortality increased when hatching eggs were low in their contents of vitamin D<sub>3</sub> (Sunde *et al.*, 1978; Steven *et al.*, 1984; Elaroussiet *al.*, 1993). On the other hand, El-Fiky *et al.* (2022) attributed the absence of a significant effect of vitamin D<sub>3</sub> on the late mortality rate to the error resulting from the manual injection method. It is notable that the significant effect of vitamin B<sub>12</sub> in reducing the rate of late mortality is due to its effect on various physiological processes in which cell division occurred (Momeneh and Torki, 2018). They clarified that such processes involve synthesis of tissues producing red blood cells, which in turn help in the transfer of oxygen from the lungs to the rest of the body tissues, especially during this critical stage of the embryonic development because the embryo transits from water respiration for aerobic respiration, resulting in enhancement of the embryonic viability and reducing the rate of late mortality. Sogunle *et al.* (2018) attributed the role of zinc in reducing the late mortality to its role in reducing cases of dead-in-shell embryos during the last stage of hatching, via its direct effect in enhancing the blood and cellular immunity of the embryo. The studies conducted

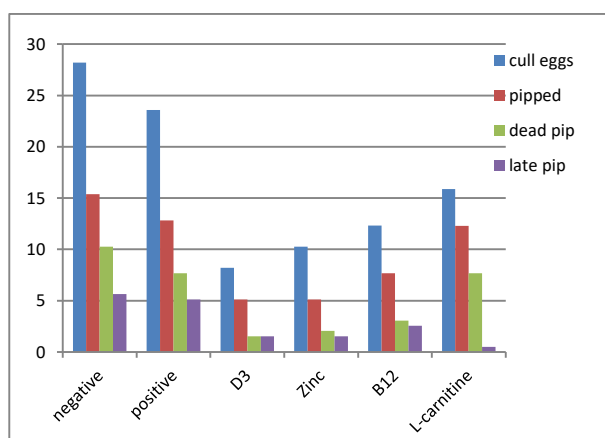
by Zahiet *al.* (2008) and Shafeyet *al.* (2010) separately indicated that neither the in ovo injected dose of L-carnitine nor the timing of its injection during the embryonic

development had a positive effect on the rate of late embryonic mortality.

**Table 5. Effects of *in ovo* injection of saline, vitamins, zinc and L-carnitine on selected parameters of hatchability in aged broiler breeders.**

Treatments	Culled eggs (%)	Pipped Embryos (%)	LivePipped (%)	DeadPipped (%)	EarlyDead (%)	Mid Dead (%)	Late Dead (%)	Cont. %
NC	28.20 <sup>a</sup>	15.38 <sup>a</sup>	5.13	10.25 <sup>a</sup>	4.10 <sup>a</sup>	1.53	5.64 <sup>a</sup>	1.53
PC	23.58 <sup>ab</sup>	12.82 <sup>ab</sup>	5.13	7.69 <sup>ab</sup>	1.53 <sup>ab</sup>	2.56	5.12 <sup>ab</sup>	1.53
Vitamin D <sub>3</sub>	8.20 <sup>c</sup>	5.12 <sup>b</sup>	3.58	1.54 <sup>b</sup>	1.53 <sup>ab</sup>	0.00	1.53 <sup>ab</sup>	0.00
Zinc	10.25 <sup>c</sup>	5.12 <sup>b</sup>	3.07	2.05 <sup>b</sup>	1.01 <sup>b</sup>	1.02	1.53 <sup>ab</sup>	1.02
Vitamin B <sub>12</sub>	13.33 <sup>bc</sup>	7.69 <sup>ab</sup>	4.62	3.07 <sup>b</sup>	2.05 <sup>ab</sup>	0.00	2.56 <sup>ab</sup>	1.02
L-carnitine	15.89 <sup>bc</sup>	12.30 <sup>ab</sup>	4.61	7.69 <sup>ab</sup>	2.05 <sup>ab</sup>	0.00	0.51 <sup>b</sup>	1.02
SEM	2.62	2.10	1.46	1.60	0.97	0.65	1.18	0.72
Significance	**	**	NS	**	**	NS	**	N.S

Means with different superscripts in the same column differ significantly at P≤0.05. NS: Not significant, \*\*: Significant at P≤0.05, NC: Negative control, PC: Positive control, SEM: Standard error of the means.



**Figure 2. Effects of test materials on selected parameters of hatchability in aged broiler breeders.**

**Blood Plasma Lipid Profile Parameters of Cobb-500 Broiler Chicks:**

Data on blood plasma components (Glu, Cho, Tri, HDL and LDL) of day-old broiler chicks as affected by injecting eggs with saline, vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> and L-carnitine are shown in Table 6. The obtained data showed a lack of significant effect of the injected test materials on the plasma concentrations of Glu, Tri and HDL of broiler chicks. The plasma levels of Cho were significantly higher (P≤0.05) in chicks hatched from eggs injected with vitamins (D<sub>3</sub> and B<sub>12</sub>), L-carnitine and saline (PC group) than those hatched from eggs injected with zinc or the NC group, with no significant differences between them. Also, there were significant increases (P≤0.01) in plasma concentrations of LDL in chicks hatched from eggs injected with vitamins (D<sub>3</sub> and B<sub>12</sub>), L-carnitine and saline compared with those hatched from eggs injected with zinc or the NC group. No clear reason could be suggested for such observed increase in plasma levels of LDL of chicks of these treated groups in comparison to those injected with Zn or the NC group.

**Blood Plasma Protein Profile and Liver Function of Cobb-500 Broiler Chicks:**

Data on blood plasma protein fractions and liver function enzymes (TP, Alb, Glo, AST and ALT) of day-old broiler chicks as affected by injecting eggs with saline, vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> and L-carnitine are given in Table 7. The injected test materials did not significantly affect

the plasma concentrations of TP, Alb and Glo of broiler chicks. As presented in Table 7, the injected test materials produced slight erratic differences in plasma activity of AST and ALT of broiler chicks, with no clear-cut trend. Interestingly, broilers hatched from eggs injected with L-carnitine exhibited significantly higher (P≤0.05) plasma AST activity than the PC group but comparable to other treated groups and the NC group. Also, broilers hatched from eggs treated with L-carnitine displayed significantly higher (P≤0.05) plasma ALT activity than those of chicks hatched from eggs treated with vitamin D<sub>3</sub> and zinc but comparable to the NC group and other treated groups.

**Table 6. Blood plasma lipid profile parameters of day-old broilers as affected by injecting eggs with saline, vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> and L-carnitine.**

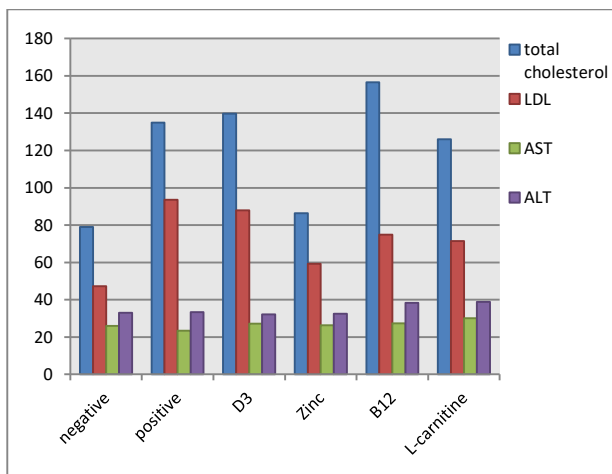
Treatments	Glu mg/dl	Cho mg/dl	Tri mg/dl	LDL mg/dl	HDL mg/dl
NC	230.60	79.00 <sup>b</sup>	65.20	47.62 <sup>d</sup>	23.80
PC	240.60	135.00 <sup>a</sup>	66.80	93.52 <sup>a</sup>	27.00
Vitamin D <sub>3</sub>	248.60	139.80 <sup>a</sup>	74.40	87.96 <sup>ab</sup>	24.40
Zinc	244.00	86.40 <sup>b</sup>	68.80	59.20 <sup>cd</sup>	38.00
Vitamin B <sub>12</sub>	249.60	156.60 <sup>a</sup>	68.60	74.80 <sup>bc</sup>	32.60
L-carnitine	236.80	126.00 <sup>a</sup>	71.20	71.40 <sup>bc</sup>	35.40
SEM	5.84	7.91	4.53	3.82	3.28
Significance	NS	*	NS	**	NS

Means with different superscripts in the same column differ significantly at P≤0.05. NS: Not significant, \*: Significant at P≤0.05, \*\*: Significant at P≤0.05, NC: Negative control, PC: Positive control, and SEM: Standard error of the means.

**Table 7. Blood plasma protein profile and liver function enzymes of day-old broilers as affected by injecting eggs with saline, vitamin D<sub>3</sub>, zinc, vitamin B<sub>12</sub> and L-carnitine.**

Treatments	TP mg/dl	Alb mg/dl	Glo mg/dl	AST U/l	ALT U/l
NC	3.12	1.18	1.84	25.96 <sup>ab</sup>	33.07 <sup>abc</sup>
PC	3.08	1.16	1.98	23.39 <sup>b</sup>	33.26 <sup>abc</sup>
Vitamin D <sub>3</sub>	3.56	1.28	2.28	27.20 <sup>ab</sup>	32.19 <sup>c</sup>
Zinc	3.54	1.38	2.16	26.36 <sup>ab</sup>	32.52 <sup>bc</sup>
Vitamin B <sub>12</sub>	3.44	1.28	2.16	27.40 <sup>ab</sup>	38.40 <sup>ab</sup>
L-carnitine	3.60	1.30	2.30	30.00 <sup>a</sup>	38.80 <sup>a</sup>
SEM	0.1	0.09	0.137	1.05	1.35
Significance	NS	NS	NS	*	*

Means with different superscripts in the same column differ significantly at P≤0.05. NS: Not significant, \*: Significant at P≤0.05, NC: Negative control, PC: Positive control, and SEM: Standard error of the means.



**Figure 3. Effects of test materials on plasma levels of Cho, LDL, AST and ALT) of day-old broilers..**

Our results are consistent with those obtained by Kim and Kang (2022), who found that *in ovo* Zn injection and dietary zinc supplementation did not significantly affect blood serum levels of TP, Alb or activity of AST and ALT in broiler chicks. In this regard, Biria *et al.* (2020) evaluated the effect of *in ovo* injection with Zn (zinc oxide nanoparticles) on blood serum parameters in broiler chicks, and found that the response depends of the applied dose of zinc (50, 75 or 100 ppm). They observed that in serum Tri concentration increased in broiler chicks (10 days of age) linearly with increasing the injected level of zinc while levels of Cho, LDL and HDL of 10-day-old broiler chicks hatched from eggs treated with 50 ppm zinc nanoparticles were lower than that of that control group; but were significantly higher than their control counterparts when the level of zinc administration reach 75 or 100 ppm. While Teymouriet *al.* (2020) found a significant effect of vitamin B<sub>12</sub> injections on the levels of glucose and total protein in the blood of chicks on days 1 and 21 post hatch. Wang *et al.* (2013) pointed out to the potential of L-carnitine in reducing the level of triglycerides in the blood of broilers it leads to a significant increase in the total protein and globulin. On the other hand, Arslan *et al.* (2003) concluded that the effect of carnitine on cholesterol and blood glucose was not significant, and this is supported by the findings of Parsaeimehret *al.* (2014), who found no significant effect of L-carnitine on blood concentrations of glucose, TP, Alb and Glo, Tri, Cho, LDL and HDL of broiler chicks. El-Fiky *et al.* (2022) detected a significant effect of vitamin D<sub>3</sub> injections at a rate of 100 µl on both ALT and AST. They also noticed that the effect of vitamin D<sub>3</sub> on blood components is based on the existence of an overlapping relationship between the level of vitamin D<sub>3</sub> transmitted from breeder to the embryo through blood plasma and the level of *in ovo* injection of vitamin D<sub>3</sub> which increases its effectiveness and compensates for the percentage consumed by the chick from the yolk sac.

### CONCLUSION

The current study indicated that injecting eggs produced from aged older broiler breeders with vitamins (D<sub>3</sub>, B<sub>12</sub>) and Zinc can improve the hatchability characteristics and reduce the embryonic late dead ratio. When compared to the control, *in ovo* injection of vitamin D<sub>3</sub> and zinc enhanced plasma liver enzymes and lipid profile.

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## هل حقن ببيض أمهات كتاكيت اللحم المسنة مفيداً ؟

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### الملخص

تهدف هذه الدراسة لتقييم تأثير حقن البيض بالمحلول الملحي، فيتامين (د3)، وفيتامين (ب12)، وكذلك كل من الزنك والكارنتين علي معدل الفقس وبعض مكونات الدم في الكتاكيت حديثة الفقس. تم استخدام عدد 1170 بيضة من قطيع أمهات كبيرة في العمر، وتم تقسيم البيض الي 6 مجاميع تجريبية هي: مجموعة التحكم (لم يتم حقنها) وتم اعتبارها كمجموعة سلبية وخمس مجموعات تم حقنها بمحلول ملحي وفيتامين د3 والزنك وفيتامين ب12 والكارنتين علي التوالي. تم وضع البيض في درجة حرارة 24-26 درجة مئوية لمدة 6 ساعات قبل عملية التفريخ. تم اجراء عملية الحقن في اليوم الثامن عشر و12 ساعة من التطور الجنيني عن طريق وضع مواد الاختبار في كيس الهواء في الطرف العريض للبيض ثم تم اغلاق الحفرة بالشمع، سجلت أعلى قيمة فقس للبيض المخصب عند المعاملة بفيتامين د3 والزنك علي التوالي يليهما فيتامين ب12 والكارنتين، كان لمواد الاختبار المحقونة (فيتامين د3، الزنك، فيتامين ب12 والكارنتين) تأثير ايجابي علي نسبة التفوق الجنيني المتأخر مقارنة بمجموعة السيطرة السلبية، أدت جميع المواد المحقونة باستثناء الزنك الي زيادة مستويات الكوليستيرول في البلازما والبروتين الدهني منخفض الكثافة ولكن لم تتأثر مستويات الجلوكوز والدهون الثلاثية والبروتين الدهني عالي الكثافة والبروتين الكلي والألومين والجلوبيولين، في الختام يمكن اقتراح حقن البيض بفيتاميني د3 وب12 والزنك والكارنتين بطريقة فعالة لزيادة قابلية الفقس والربحية لمربي الدجاج اللاحم المتقدم في العمر.