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Influence of *In-ovo* Feeding Vitamins E, B1, and B2 to Broiler Embryos on Hatchability, Chick Quality, and Blood Biochemical Parameters of Hatched Chicks

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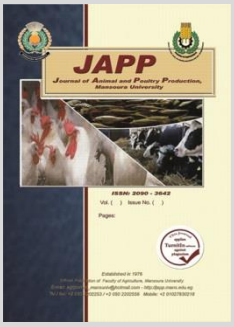

ABSTRACT

This study aimed to evaluate *in ovo* feeding of vitamins E, B1, and B2 on hatchability characteristics, and blood parameters of hatched chicks. Total of 1140 eggs (Cobb 500, 63.8 g/egg) were divided into five groups (228 in each). Experimental groups included negative control, positive control (0.2 ml sterile saline), eggs injected with Vit. E (1000 mg/100 µl sterile oil solvent), Vit. B1 (8.92 mg/100 µl sterile saline), or Vit. B2 (4.30 mg/100 µl sterile saline). Materials were injected into embryo on 17-day incubation. All eggs were subjected to the same incubation temperatures, relative humidity, and CO_2 concentration. After hatching, blood samples were collected from 6 hatched chicks in each group for analysis. Results showed no significant effect for vitamins on hatchability rates and hatched chick quality. Hatched chick weight was higher ($P<0.05$) with all treatments than in positive control. Vitamins E and B2 decreased ($P<0.05$) late embryonic mortality. Vit. B1 increased glucose and LDL. Vit. E decreased ($P<0.05$) HDL and triglycerides. Total protein was increased ($P<0.05$) by all vitamins injected. ALP activity was increased only by Vit. B1. Plasma T_3 concentration was increased by vitamins E and B1, but did not differ significantly from that in positive control and Vit. B2 groups. The highest corticosterone level was in the negative control, being lower with all materials injected, particularly with Vit. B1, reflecting positive impacts on eliminating hatching stress. Although hatchability rates were not affected by material injected, all vitamins had beneficial effects on hatched chick weight and health performance.

***Keywords*:** Egg injection, chicken, vitamins, hatching rate, blood analyses.

INTRODUCTION

In recent years, the technology of injecting embryos and *in ovo* vaccination has emerged in the field of poultry industry (Foye *et al*., 2006; Slawinska *et al*., 2019). The benefits of this technology are thus still growing and bringing about new advantages for the chicken business (Peebles, 2018). During hatching period, embryos of birds use specific sources of energy deposited in the egg by broiler breeder, such as protein, fats, vitamins and minerals (Ghobadi and Matin, 2015). Oxidation of egg yolk lipids is important for embryos to use these nutrients, therefore. Thus, lipids were metabolized by embryos and long-chain polyunsaturated fatty acids (PUFA) in embryonic tissues (Bautista-Ortega *et al*., 2009). The ability of embryo tissues to damage by free radicals or ROS (reactive oxygen species), and cellular death may be increased due to high PUFA content in cell membranes (Selim *et al*., 2012). In this respect, the internal organs of bird embryos provide antioxidant defenses to ensure a proper embryonic development in birds. In eggs, antioxidants agent was reported to reduce oxidation processes in embryos, affecting on hatchability rate (Surai *et al*., 2016). When chicks begin hatching stage, they perform internal pecking of the internal membranes of the shell, and at this stage there is a transition from breathing with the corioallantois membrane to pulmonary breathing. During and immediately after hatching, the rate of oxygen consumption increases and therefore the metabolic rate increases to cover the physical activities requirements and endothermic (Hohtola, 2002) In this context, the pulmonary respiratory process is considered to be essential for the broiler chick live, while this process elevates oxygen rates in the blood circulation and thus increase es the production of free radicals due to oxidation processes (Surai *et al*., 2016).

Nutrient utilization from the first day of incubation, as the albumen and yolk participate in providing nutrients and supplying them to the embryo. Providing appropriate nutrition to the developing embryo is of utmost importance. Feeding broiler breeder flocks is the only source of vitamins for the egg, but breeders' ignorance of diet formulation of broiler breeder or the use of poor-quality vitamins leads to deficiencies and death of the embryos during the middle or late incubation period ((Hossain *et al*., 1998). Increased rate absorption of residual nutrients in the yolk sac is one advantage of early feeding poultry embryos using *in ovo* injections. Improving productive performance in terms of increased body weight, better feed conversion ratio, high carcass features and meat quality, and good immunity in birds are all outcomes of scientific researches (Kermanshahi *et al*., 2015 and Tavaniello *et al*., 2020). Several studies have demonstrated benefits poultry embryos by vitamins, amino acids, carbohydrates, minerals, pro- and pre-biotics, as *in ovo* administration (Araújo *et al*., 2019). Egg feeding with antioxidants may enhance the condition of hatching, reflecting good quality chicks. Antioxidants in egg yolk control oxidation by reducing or deactivating free radicals before they act on chick organ tissues (Surai *et al*., 2016).

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Water-soluble B vitamins are constituents of coenzymes that, when deficient in hen diets, result in a high rate of embryonic death (Machlin, 1984). Thiamin ((Vit. B1) deficiency in eggs results in a high rate of embryo death just prior to hatching, and the chicks that hatch suffer from polyneuritis, it is a cofactor for numerous enzymes that promote decarboxylation and trns-skeletolation type processes (Charles, *et al.*, 1972). Riboflavin (Vit. B2) is a major source of flavin adenine dinucleotide (FAD) and flavin mononucleotide (riboflavin-5′-phosphate, FMN) are essential for energy metabolism. It also plays a crucial part in the respiratory chain and the oxidation of fatty acids and amino acids. Furthermore, it is a component of the flavin enzymes and coenzymes, which transfer electrons in oxidation and reduction reactions. Moreover, riboflavin is involved in the metabolism of folate, Vit. B6 (pyridoxine), and Vit. B12 (cobalamin), as well as the Krebs cycle (Massey, 2000). Therefore, riboflavin is crucial for the innate immunity of both plants and animals (Massey, 2000; Kidd, 2004). Particularly well-known is riboflavin's immunomodulatory function in avian immunity (Kidd, 2004). Vitamin B2 is considered essential for birds as a feed additive for commercial poultry flocks (National Research Council, 1994; Fefana, 2014). As a primary fat-soluble antioxidant, Vit. E halts lipid peroxidation chain reaction. In the brain, antioxidant system was found to be essential for the development of nutritional encephalomalacia, which is brought on by a deficiency of Vit. E in young chicks (Dror and Bartov 1982). Vitamin E supplementation in the feed of breeder of broiler chicks improve the hatchability rates of hatched eggs as well as the quality of the resulting chicks (Urso *et al*., 2015). Furthermore, Vit. E deficiency enhances humoral immune responses in chicks by acting as an antioxidant to lessen cellular free radical damage (Hossain *et al*., 1998).

The current study aimed to evaluate the effects of vitamins E, B1, and B2 *in ovo* feeding on hatchability characteristics, and blood biochemical parameters, enzyme activity, physico-meniral levels, and thyroid hormone profile in blood plasma of hatched chicks.

MATERIALS AND METHODS

Injecting eggs with vitamin E and vitamins B complex was carried out in single stage (Petersime Focus). Eggs were incubated in the commercial Dakahlia poultry hatchery, Qalubia Governorate, Obour, Egypt. The laboratory analyses were performed at laboratories of Faculty of Agriculture; Mansoura University; Egypt.

Egg injection and incubation:

A total of 1140 eggs with an average weight of 63.8 g were obtained from a commercial Dakahlia poultry broiler breeder flock (Cobb 500) at 68 weeks of age. Eggs received hatchery at temperature18°C stored for 4 days in troll's egg, were randomly divided into five groups (228 eggs in each), each included three replicates of 76 eggs.

In this experiment, eggs in a negative control group did not received any treatment, while those in a positive control groups were injected (0.2 ml) with sterile saline (0.9%NaCl). In other three groups, eggs were injected with Vit. E (1000 mg/100 µl sterile oil solvent), Vit. B1 (8.92 mg/100 µl sterile saline, hydrochloride thiamin 1000 mg equivalent 8.92 mg vitamin B1), or Vit. B2 (4.30 mg/100 µl sterile saline, riboflavin sodium phosphate equivalent 4.30 mg vitamin B2). Injections were performed on Day 17 of incubation.

Extreme care was taken into account when performing the injection process for all treated eggs. Different injection sites for egg were shown in figure 1. In our study the materials were injected into embryo (Fig. 1), under the supervision of specialists in the injection process with extensive experience, using 19G mm needle as standardized method (Bhanja *et al*., 2004). In this study, in order to avoid infections and contaminations of eggs, needles were changed constantly, and ethyl alcohol 70% was used, and it was deposited again in the incubator to complete the embryonic development process.

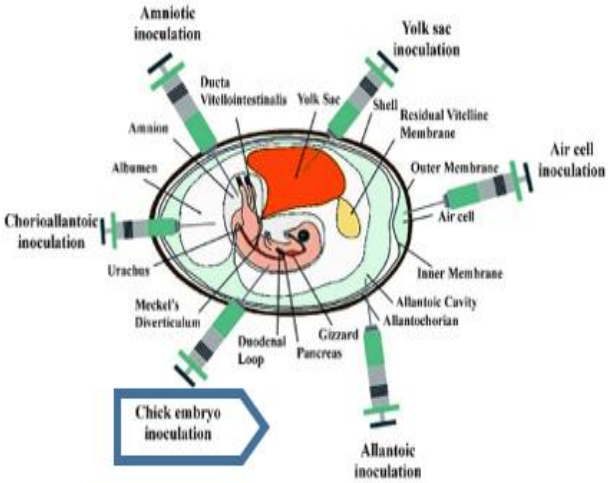

Fig. 1. Different injection sites for egg.

Environmental Description of incubator and hatchery:

Tables 1 and 2 display the temperature (°F), wet bulb (°F), and percentage of carbon dioxide in the incubator and hatchery, respectively. In a single-stage incubator (Petersime Focus), the incubator temperature was changeable; from the first to the 19th day of incubation, and wet bulb (°F) and the percentage of CO2 were estimated. A lot of eggs must enter the incubator at once, resulting in the embryonic development of those eggs in a single phase. In order to provide the ideal conditions for embryonic development, a high degree of managerial control is advised. This development is determined by measuring the egg's temperature using the incubator's *ovo-scan* component during incubation period.

On Day 19 of incubation, all eggs were transferred to the hatchery at 98.5°F (85°F wet bulb). On Day 20 and 16 hours, the wet bulb temperature was gradually increased to 89°F, and at the end of the incubation period (Day 21), the wet bulb temperature gradually decreased to 85°F (Tables 2). With a variable system temperature of 100.1°F on the entry day and 99.9°F (*ovo-scan*) on day 15, the single stage (Petersime Focus) incubator gradually drops until the 19th day, when the hatch is transferred. At every stage of incubation, it regulated the temperature, humidity, ventilation, and CO_2 (Tables 1 and 2).

The hatching percentages were estimated by considering the number of hatched chicks relative to total number of eggs (commercial hatchability) or the number of fertile eggs (scientific Or fertile hatchability). and can be computed as follows:

Commercial hatchability = (Number of hatched chicks/number of total eggs) x100

Scientific or fertile hatchability = (Number of hatched chicks/ number of fertile eggs) x100

Table 1. Program of single-stage (petersime focus) incubator

Incubation time (Day: Hour)	*Ovo-scan* temperature (°F)	Incubator temperature (ºF)	Ventilation (%)	Wet bulb temperature (ºF)	CO_2 (%)	Cool
00 : 00	100.1	99.8	00	90	0.85	Water till day 16, followed by air flow ten seconds thereafter.
01 : 00	100.0	99.7	00	90	0.85	
02 : 00	100.0	99.6	00	90	0.85	
03 : 00	99.9	99.5	5-50	90	0.55	
07 : 00	99.8	99.5	10-60	90	0.45	
09 : 00	99.8	99.5	10-60	88	0.40	
10 : 00	99.9	99.7	10-60	88	0.40	
11 : 00	100.0	99.6	30-50	86	0.30	
11 : 18	99.9	99.5	30-50	86	0.30	
12 : 00	99.8	99.4	30-50	86	0.30	
13 : 00	99.9	99.3	40-65	84	0.30	
14 : 00	99.9	99.2	40-65	84	0.30	
15 : 00	99.9	99.0	80-100	84	0.30	
18 : 00	99.9	98.8	80-100	84	0.30	
18 : 24	99.9	97.7	80-100	84	0.30	

Table 2. Program of single-stage (petersium focus) hatchery

Incubation time (Day: Hour)	Temperature (ºF)	Wet bulb temperature (oF)	CO_2 (%)	Ventilation (%)	Cool
19 : 00	98.3	85	0.60	40 -100	Water till 12 hours post-the 20th day, followed by air flow ten seconds thereafter.
19 : 12	98.8	87	0.80	0 -100	
20 : 00	98.5	91	1.00	40 - 60	
20 : 10	98.3	90	0.90	10 -100	
20 : 12	98.3	89	0.75	25 - 60	
20 : 14	98.2	89	0.50	35 - 65	
20 : 16	98.0	89	0.50	40 - 70	
20 : 18	97.9	88	0.50	60 - 75	
20 : 20	97.5	88	0.50	70 - 95	
21 : 00	96.3	85	0.40	80 -100	

Embryonic mortality:

The embryonic mortality intervals during the incubation period were categorized based on when they occurred as shown in Table 3.

Table 3. Embryonic mortality categories.

Category	Embryonic mortality intervals (days)
Early embryonic mortality	0 – 7
Mid-embryonic mortality	8 – 14
Late embryonic mortality	15 – 21
Infertile eggs	11
Pipped eggs	19 - 21
Live pipped chicks	21
Dead pipped chicks	21

Blood parameters of hatched chicks:

After complete hatching process, blood samples were collected randomly from 6 hatched chicks in each group (three chicks for blood biochemical and three for blood physical characteristics). The blood sample was individually taken by slaughtering with a surgical scalpel, cutting the jugular vein, and then filtering the blood with a tube containing 0.1 ml heparin. Blood samples of the 1st three chicks were centrifuged at 3000 rpm per minute for 15 minutes to separate blood plasma for biochemical, enzyme, and hormone assays in blood plasma.

Using specific chemical kits (Spectrum Diagnostic Kits S.A.E., Egyptian Company of Biotechnology, 2022), plasma concentration of glucose, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, total protein, and albumin, as well as activity of plasma aspartate aminotransferase (AST), alanine aminotransferase(ALT) and alkaline phosphatase (ALP) were determined.

Calorimetrical methods and chemical kits (teco diagnostics, ANAHEM, CA, France) were used to measure Na, Cl, and K contents in blood plasma based on the Fundamentals of clinical chemistry method of Henry (1974) and Tietz (1974), respectively.

In blood plasma of chicks, thyroid hormones, including level of triiodothyronine (T_3) and tetraiodothyronine (T_4) was assayed, using specific kits (Diagnostic examination, Equipar, Italy) after the methods of Sterling (1975) and Liewendahi (1990), respectively. According to the method of Sainio *et al*. (1988), level of corticosterone in blood plasma was also measured, using chemical kits (Diagnostic products corporation, DPC, Los Angeles, USA)

In the whole blood samples of the 2nd three slaughtered chicks, blood pH value and sedimentation rate were determined. Blood sedimentation rate was measured following the procedure described by Brown (1980), whereas blood pH value was measured by a pH-meter (CG 728 SCHOTT)

Statistical analysis:

Our data were subjected to statistical analysis by SAS, (2006) computer program using one-way ANOVA. When the *F* statistic was significant ($p<0.05$), a mean separation was performed using the least significant difference by Tukey test.

RESULTS AND DISCUSSION

Hatchability characteristics:

Table 4 displays the results of impacts of vitamin E, B1, and B2 *in ovo* injection on characteristics of hatching, chick quality, and hatched chick weight. The present results showed no significant effect of the materials injected into the embryos at the 17th day of incubation on hatchability rates and hatched chick quality. However, hatched chick weight was affected significantly ($P<0.001$) by *in ovo* feeding, being significantly ($P<0.05$) higher in positive control and all treatment groups. On the other hand, chick weight was significantly ($P<0.05$) higher in all treatment groups than in positive control one. Generally, *in ovo* feeding with Vit. B2 tended to show the highest chick weight.

Table 4. Effect of *in ovo* administration with vitamins E, B1, and B2 on hatchability characteristics.

Treatment	Fertility rate	Hatchability rate		Chick type			Chick weight (g)
		Commercial	Scientific	A	B	C	
Negative control	89.04	70.18	78.81	64.53	11.33	2.95	40.80^{c}
Positive control	88.60	74.12	83.66	73.26	8.41	1.98	41.88^{b}
Vitamin E	89.04	73.25	82.26	70.44	8.37	3.44	42.52^{a}
Vitamin B1 (Thiamin)	89.04	71.92	80.78	71.42	4.92	4.43	42.49^{a}
Vitamin B2 (Riboflavin)	89.91	72.81	80.97	73.17	4.87	2.92	42.60^{a}
SEM	2.06	2.96	2.74	3.20	1.85	1.22	0.062
P-value	0.994	0.905	0.784	0.287	0.07	0.72	<.0001

$^{a-c}$: Means in the same column having different superscripts are significantly different (P≤0.05).

The significant increase of chick weight in positive than in negative control group can be assumed that the injection of sterile saline may help in regulation of the acid-base balance in the hatched chick's body. Unchanged hatchability rates with different *in ovo* treatments are in agreement with the findings of Bhanja *et al.* (2007), who showed that vitamin injections *in ovo* had no detrimental effect on the hatchability percentage. Also, Trzeciak *et al.* (2014) concluded that riboflavin supplied *in ovo* did not affect hatchability rate. In our study, the average weight of the chicks increased as a result of injections of vitamin E, B1, and B2 as compared to the negative or positive control groups. Similar results were reported by Araújo *et al.* (2019), who found that treatments of 49.5 or 60.4 IU of Vit. E resulted in significant (P<0.05) increase in newborn chick weights as compared to control group.

The highest chick weight observed by Vit. B2 might be due to embryo's responses to Vit. B2 *in ovo* feeding as it is involved in metabolism of nutrients (Robel and Christensen, 1987). One potential benefit of vitamin B1 injections in chicks is through conversion thiamin to thiamine pyrophosphate in presence of ATP, which functions as a cofactor for several enzymes like pyruvate dehydrogenase and ex-ketoglutarate dehydrogenase and involved in conversion of glucose to energy, CO, and H2 and O2 (Buckle, 1965). Exogenous Vit. E injection at the critical time of fatty acid oxidation, around days 14-17th of incubation, may help in increasing lipid utilization for energy production and lowering the production of free radicals that seriously damage cellular membranes (Cherian and Sim, 1997 and Surai, 2000). Vitamin E improved antioxidant status of the eggs (Surai, 2000) and had an ability to protect eggs against oxidation (Puthpongsiriporn *et al*., 2001) which could be the cause of a higher body weight because injected eggs produced more energy and prevented the effects of hydro peroxides, which promote embryonic growth (Schaal, 2008). In contrast to the present results, some authors did not found any improvement in newborn chicks from eggs with *in ovo* Vit. E supplementation after 17.5 days of embryonic development (Bhanja *et al*., 2012; Salary *et al*., 2014; Rajkumar *et al*., 2015). The confliction in the success of results experiments with injecting eggs with nutritional supplements depends on various factors, including; materials injected, diluent type, time of injection, and the location of injection.

Embryonic mortality:

Results in Table 5 revealed no discernible variations were found in the percentage of cull eggs, early- and mid-embryonic mortality rates, percentage of live and dead pipped embryos, abnormal embryo percentage, and contamination rates after a statistical analysis of the unhatched egg data. However, vitamins E and B2 used in the injections significantly (P<0.05) decreased the rates of late embryonic death as compared to Vit. B1 and both positive and negative control groups.

Table 5. Outcomes residual analysis conducted on unhatched eggs and embryonic mortality as affected by *in ovo* administration with vitamins E, B1, and B2.

Treatment	Culled egg (%)	Mortality rate			Pipped (%)		Abnormal	Contamination
		Early	Mid	Late	Live	Dead		
Negative control	21.18	2.96	0.00	7.39^{a}	4.93	2.96	1.48	1.46
Positive control	16.34	2.48	0.99	4.95^{ab}	1.49	3.96	1.49	0.98
Vitamin E	17.73	5.42	0.99	1.97^{b}	3.94	2.46	1.97	0.98
Vitamin B1	19.21	4.43	1.48	3.94^{ab}	2.46	3.94	1.97	0.99
Vitamin B2	19.03	5.37	0.98	1.95^{b}	4.39	1.46	0.98	3.90
SEM	2.71	1.40	0.66	1.38	1.28	1.19	0.87	0.78
P-value	0.698	0.436	0.612	0.028	0.292	0.532	0.924	0.975

$^{a-b}$: Means in the same column having different superscripts are significantly different (P≤0.05).

In our study, occurrence of late embryonic rate was detected at 15-21 d interval and the injection time was on Day 17 of the incubation period, so the present results (Table 5) may indicate beneficial impacts of vitamins E and B2 *in ovo* feeding on embryonic mortality. According to Ebrahimi *et al.* (2012), the injection materials particularly the vitamin E and riboflavin had a positive impact on reducing the incidence of late embryonic mortality. This improvement may be attributed to that the injected materials (Vit. E and Vit. B2) reduced the concentrations of oxidative stress, free radicals, and reactive oxygen metabolites which cause oxidation of lipids induced cellular membranes damage during the stage of rapid embryonic development in chicken. In chicken embryos, antioxidant defense mechanisms, including SOD (superoxide dismutase), GPx (glutathione peroxidase), Vitamins E and C, and alipoic acid prevent damage to major organs and systems (El-Senousey *et al*., 2018) and *in ovo* feeding was found to be the best way for fortifying the embryonic antioxidant defense (Araújo *et al*., 2019; El-Senousey *et al*., 2018). On the other hand, Trzeciak *et al.* (2014) showed no effect of riboflavin *in ovo* supplementation on chicken embryo mortality.

Blood parameters of hatched chicks:

Glucose and lipid profiles:

Table 6 shows glucose concentration and lipid profile in plasma of hatched chicks. Results revealed that when thiamin (B1) was injected during the late incubation period, it was discovered that the newly hatched chicks' plasma glucose

concentration significantly (P<0.05) increased as compared to other treatment or control groups. In poultry, until the initiation of feed intake, level of blood glucose is important at a late embryonic stage of hatching and immediately post-hatching. Towards the termination of the hatching period, embryos draw on reservoir energy to cover the increased glucose requirements during incubation period (John *et al*., 1988; Christensen *et al*., 2001). Based on our results, Vit. B1 may have a role in glucose synthesize from protein and fat (Elwyn and Bursztein, 1993 a, b, c). Because oxygen is scarce in the final quarter of hatching period, Vit. B1 may increase glucose level by stimulating the gluconeogenesis or glycolysis of stored glycogen (Bjønnes *et al*., 1987; John *et al*., 1987). A tendency of increase in plasma glucose level by Vit. B2 in comparing with both positive and negative controls may be attributed to that Vit. B2 acts as a cofactor for multiple enzymes, it can help break down dietary carbohydrates. This may explain why vitamin B2-injected birds had higher serum glucose (Kitakoshi *et al*., 2007).

Table 6. Effect of *in ovo* administration with vitamins E, B1, and B2 on glucose level and lipid profile in plasma of hatched chicks.

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)
Negative control	196.17^{b}	171.37^{a}	37.46^{b}	133.91^{a}	34.73^{a}
Positive control	208.00^{ab}	149.57^{b}	30.26^{c}	119.31^{b}	33.11^{ab}
Vitamin E	221.18^{ab}	140.03^{b}	36.53^{b}	103.50^{c}	30.99^{c}
Vitamin B1	234.00^{a}	161.57^{a}	54.62^{a}	106.95^{c}	31.60^{bc}
Vitamin B2	222.50^{ab}	148.59^{b}	32.33^{c}	116.26^{bc}	31.70^{bc}
P-value	0.04	<.0001	<.0001	<.0001	<.0001
SEM	8.48	2.61	1.46	3.51	0.49

$^{a-c}$: Means in the same column having different superscripts are significantly different (P≤0.05).

LDL= Low density lipoprotein; HDL= High density lipoprotein.

Results in Table 6 showed significant effects on plasma lipid profile in all treatment and positive control groups. Plasma cholesterol concentration was significantly (P<0.05) decreased by vitamins E and B2 and even in positive control group. Concentration of plasma LDL was significantly (P<0.05) increased by Vit. B1 and significantly (P<0.05) decreased by Vit. B2, as did positive control group. HDL level in plasma of chicks was significantly (P<0.05) decreased by all treatments, being significantly (P<0.05) lower in vitamins E, and B1 than in positive control groups. However, *in ovo* feeding with vitamins E, B1, and B2 during embryonic development resulted in a significant (P<0.05) decrease in plasma triglycerides concentrations in comparing with the negative control groups, being significantly (P<0.05) the lowest in Vit. E. These results indicated pronounced effects of Vit. B1 on increasing LDL level and decreasing HDL level in plasma of chicks, and decreasing plasma HDL and triglycerides levels by Vit. E.

Overdosing on vitamin E supplements lowers triglyceride and plasma cholesterol levels (Bell, 1971; Clegg *et al*., 1976), which prevents atherosclerosis in chickens (Donaldson, 1982; Smith and Kummerow, 1989). Aa a dietary supplementation, vitamin E (325 ppm) reduced the levels of triglycerides and cholesterol up until 49 days of age in broilers (Franchini *et al*., 1988). Additionally, turkeys fed vitamin E on day 42 showed a decrease in cholesterol (Franchini *et al*., 1990). Supplementing both dietary vitamins E and A led to a significant decrease in serum triglyceride (Sahin *et al*., 2001).

Total proteins and their fractions:

According to the results in Table 7, total protein concentration was significantly (P<0.05) higher in plasma of newly hatched chicks that received injections of vitamins E, B1, and B2 as compared to positive and negative control groups. Plasma albumin concentration was affected significantly (P<0.05) by treatments, but means of all treatments did not differ significantly from that of positive and negative control groups. However, plasma globulin concentration was not affected significantly by *in ovo* treatments.

Increases in the overall plasma protein concentrations in the embryos injected with Vit. E may be due to that VE fortified the chicks' tissues' resistance to protein oxidation. As per Willemsen *et al*. (2008), the initiation of pulmonary respiration of embryos was observed with the moment the chicks begin pecking the internal membrane of egg shell, leading to an increase in oxygen concentration in the tissues. Consequently, high oxygen availability during the pre-hatching stage facilitates the formation of free radicals, which intensify the oxidative process of the embryo tissues' membranes and Vit. E shields the tissues of embryos, particularly from lipid oxidation (Surai *et al*., 2016).

In comparable with the obtained results, Vit. E supplementation raised plasma protein concentration in heat-stressed layer birds (Ajakaiye *et al*., 2010). Also, supplementing both dietary vitamins E and A led to a significant increase in protein concentrations (Sahin *et al*., 2001). However, broilers fed vitamin E (100 and 200 mg/kg) have been shown to have significant increases in total proteins and albumin; however, globulin levels did not differ significantly (Desoky, 2018). Increasing plasma total proteins by Vit. B2 may indicate the role of Vit. B2 as a cofactor for multiple enzymes to help breakdown dietary proteins and increasing serum protein levels in Vit. B2-injected birds Kitakoshi *et al*. (2007).

Table 7. Effect of *in ovo* administration with vitamins E, B1, and B2 on in protein profile plasma of hatched chicks.

Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Negative control	6.01^{b}	2.88^{ab}	3.13
Positive control	5.93^{b}	2.67^{b}	3.26
Vitamin E	6.27^{a}	2.83^{ab}	3.44
Vitamin B1	6.44^{a}	3.39^{a}	3.05
Vitamin B2	6.39^{a}	3.31^{ab}	3.08
SEM	0.05	0.16	0.16
P-value	<.0001	0.0208	0.4549

$^{a-b}$: Means in the same column having different superscripts are significantly different (P≤0.05).

Enzyme activity:

Concerning the enzyme activity in plasma of hatched chicks (Table 8), the effect of *in ovo* treatment was significant on activity of AST (P<0.01) and ALP (P<0.05) and not significant on ALT activity in plasma of chicks. Activity of AST was significantly (P<0.05) lowered only in positive control group by sterile water injection, however, ALP activity was significantly (P<0.05) increased only by Vit. B1.

In accordance with our results, Arslan *et al*. (2001) reported no effect of Vit. E treatment on the activity of AST or ALT in broilers at the fifth and seventh week of life.

Franchini *et al*. (1990) found that AST activity in turkey have increased in response to dietary Vit. E levels at early age, and have decreased in older birds (140 days old). However, Desoky (2018) found that the broiler group fed 200 mg/kg of Vit. E had a significant decrease in AST and ALT activities.

Table 8. Effect of *in ovo* administration with vitamins E, B1, and B2 on in enzyme activity in plasma of hatched chicks.

Treatment	AST (U/l)	ALT (U/l)	ALP (U/l)
Negative control	28.13[a]	33.11	63.98[b]
Positive control	25.71[b]	37.71	63.30[b]
Vitamin E	27.36[ab]	34.94	70.51[ab]
Vitamin B1	28.24[a]	79.41	75.97[a]
Vitamin B2	27.62[ab]	31.78	65.64[ab]
SEM	0.47	22.76	2.76
P-value	0.006	0.541	0.017

[ab]: Means in the same column having different superscripts are significantly different (P≤0.05).

The plasma of hatched chicks receiving Vit. B1 injection showed an increase in the activity of ALP. In spite of this, there were no appreciable variations observed between Vit. B1 and each of Vit. E and Vit. B2 injection treatments. Also, there was no discernible difference between the two control treatments' positive and negative outcomes when Vit. E and Vit. B2 injections were used. In contrast to our results, some studies have shown that birds' activity of ALP rise in response to high dosages of Vit. E supplements. According to Arslan *et al*. (2001), broilers treated with 100, 200, and 300 ppm of Vit. E demonstrated a statistically significant reduction in ALP levels by the seventh week. Similarly, turkeys supplemented with 30, 90, 180, or 360 ppm of Vit. E/kg of ration showed similar plasma ALP activity; however, as the bird grew older, its plasma ALP levels increased (Franchini *et al*., 1990).

Physico-mineral parameters:

Red blood cell sedimentation rate, blood pH value, and concentration of plasma Na and K was significantly affected by treatment (P<0.05), while there was no significant effect of treatment on plasma Cl concentration in plasma of chicks (Table 9). The group that received Vit. E injections had significantly (P<0.05) the lowest sedimentation rate, being significantly (P<0.05) lower than negative control group only. The negative control treatment had the maximum 24-hour erythrocyte sedimentation rate. Regarding the pH values, it was discovered that vitamins E and B2 injections caused the pH levels to drop and settle around the normal range (7.42) as compared to positive and negative control groups and even Vit B1 treatment.

Table 9. Effect of *in ovo* administration with vitamins E, B1, and B2 on sedimentation rate, pH value, acid base balance in blood of hatched chicks.

Treatment	Sedimentation rate (mm/24 h)	pH value	Na (mEq/l)	K (mEq/l)	Cl (mmol/l)
Negative control	112.66[a]	7.59[a]	115.47[b]	1.78[b]	105.56
Positive control	105.67[ab]	7.46[ab]	124.76[ab]	1.87[ab]	103.03
Vitamin E	96.17[b]	7.42[b]	130.22[a]	2.27[a]	101.20
Vitamin B1	108.33[ab]	7.44[ab]	131.46[a]	2.07[ab]	102.80
Vitamin B2	108.67[ab]	7.42[b]	128.42[ab]	1.94[ab]	103.95
SEM	3.03	0.04	3.29	0.10	1.53
P-value	0.010	0.023	0.015	0.036	0.38

[ab]: Means in the same column having different superscripts are significantly different (P≤0.05).

Increases in non-respiratory HCO_3 (Tazawa, 1986) reduces the acidity shift brought on by the buildup of CO_2. In blood, hemoglobin (Hb) acts as a non-carbonate buffer, is partially to blame for the pH change that is decreased during the latter half of the incubation period. The buffer value increases from 9-10 to 15-18 days of incubation, reflecting the developmental increase in hematocrit value and Hb concentration (Erasmus *et al*., 1970/1971; Tazawa and Piiper, 1984). According to other research (Tazawa *et al*., 1983; Andrewartha *et al*., 2011), there is no rise in the buffer value during development (Burggren *et al*., 2012). Depending on the gas mixture and the age of the chicken embryos, there are differences in the responses in acid-base balance after a single day of exposure to modified environmental gas mixtures (Burggren *et al*., 2012). An increase in blood parameters might have enhanced the circulation of oxygen and nutrients, thereby raising the respiratory rate of chicks. It is possible that circulating VE affected the hematopoietic process by causing the synthesis of more red blood cells loaded with hemoglobin (Araújo *et al*., 2019).

Contents of plasma Na and K were significantly (P<0.05) increased only by Vit. E, content of Na was significantly (P<0.05) increased by Vit. B2; however, plasma Cl content was not affected by *in ovo* feeding treatments (Table 9). However, these rates of increases showed insignificant differences with the positive control group. Therefore, the detected increase may be attributed to the dose of sterile saline for positive control and vitamins B1 and B2 and oil as a solvent for Vit. E.

Hormonal profile:

Data in Table 10 reflected significant effect of *in ovo* feeding on plasma T_3 and corticosterone concentrations; however, there were no changes in plasma T_4 levels. The average plasma T_3 concentrations were increased significantly (P<0.05) in by Vitamins E and B1 as compared to negative control, but did not differ significantly from that in positive control group or injected with Vit. B2. On the other hand, different vitamins injected significantly (P<00.05) reduced plasma corticosterone concentration of chicks as compared to negative control, but only Vit. B1 was significantly (P<0.05) lower than positive control treatment.

Table 10. Effect of *in ovo* administration with vitamins E, B1, and B2 on hormonal profiles of Triiodothyronine (T_3), thyroxin (T_4), and corticosterone in plasma of hatched chicks.

Treatment	T_3 (g/ml)	T_4 (ng/ml)	T_3/T_4 ratio	Corticosterone (ng/ml)
Negative control	1.92[b]	12.79	0.15	17.07[a]
Positive control	3.34[ab]	12.18	0.27	14.58[ab]
Vitamin E	3.80[a]	11.58	0.32	12.76[bc]
Vitamin B1	3.91[a]	12.13	0.32	11.15[c]
Vitamin B2	3.21[ab]	13.95	0.23	12.44[bc]
SEM	0.35	0.80	-	0.78
P-value	0.0037	0.3114	-	0.0001

[a and b]: significant group differences in the same column at P≤0.05.

A dramatic rise in plasma T_3 concentration that happens during the hatching period and appears without pronounced change in T_4 concentration is connected to the change in the respiratory system of the chick embryo from chorioallantoic to pulmonary. It is linked to an increase in the activity of D1 deiodinase, which converts T_4 to T_3, and a decrease in the activity of D3 deiodinase (Van der Geyten *et*

al., 2002 and McNabb, 2006). Based on these findings, it can be inferred that the alterations in T_3 concentrating after all vitamins E and B1 administration are caused by its impact on the activity of D1- and D3-deiodinases since nearly all circulating T_3 in avian species is of peripheral origin (McNabb, 2006). This theory is supported by the fact that the T_3/T_4 ratio increased by vitamins E and B1 treated embryos, as compared to those in positive control and Vit. B2, indicating that vitamins E and B1 may directly boosts D3 deiodinase activity and increase D1 deiodinase activity during the last stages of embryogenesis.

It is well known that the stress raised the amount of corticosterone in the plasma. As a result of having to perform both internal and external pecking and transition from breathing through the corioallantois membrane to pulmonary breathing, the chick experienced stress during the hatching process. The highest plasma corticosterone level after hatching of the chicks was recorded in the negative control treatment, being lower with all materials injected, particularly in the Vit B1 group, reflecting positive impacts on eliminating hatching stress.

CONCLUSION

Considering the above results, the injection of all vitamins had a beneficial effect on hatched chick performance including increased hatching weight, while Vit. E and B2 reduced mortality rate at late stage of hatching with improving most blood plasma biochemicals, increasing T_3 level, and decreasing corticosterone levels, reflecting positive impacts on eliminating hatching stress.

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تأثير تغذية أجنة دجاج التسمين داخل البيض بفيتامينات E, B1, B2 على نسبة الفقس وجودة الكتاكيت والمؤشرات البيوكيميائية للكتاكيت الفاقسة

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الملخص

تهدف هذه الدراسة إلى تقييم تغذية البيض بالفيتامينات E وB1 وB2 على خصائص الفقس ومؤشرات الدم للكتاكيت الفاقسة. تم تقسيم 1140 بيضة (Cobb 500 ، 63.8 جم/بيضة) إلى خمس مجموعات (228 في كل مجموعة). شملت المجموعات التجريبية الكنترول السلبي والكنترول الإيجابي (0.2 مل من المحلول الملحي المعقم) والبيض المحقون بفيتامين E (1000مجم/100 ميكرولتر من مذيب الزيت المعقم) ، فيتامين B1 (892 مجم/100 ميكرولتر من محلول ملحي معقم) ، أو فيتامين B2 (4.30 مجم/100 ميكرولتر من محلول ملحي معقم). تم حقن المواد في الجنين في الحضانة في اليوم 17 من فترة الحضانة. تم تعريض جميع البيض لنفس درجات حرارة التحضين والرطوبة النسبية وتركيز ثاني أكسيد الكربون. بعد الفقس ، تم جمع عينات الدم من 6 كتاكيت فاقسة في كل مجموعة لتحليلها. أظهرت النتائج عدم وجود تأثير معنوي للفيتامينات على معدلات الفقس وجودة الكتاكيت المفقسة. كان وزن الكتكوت المفقس أعلى ($P<0.05$) في جميع المعاملات منه في مجموعة الكنترول الإيجابية. خفض فيتامين E و B2 ($P<0.05$) معدل نافق الأجنة المتأخرة. زاد الجلوكوز و LDL ($P<0.05$) بفيتامين B1. خفض فيتامين E ($P<0.05$) كل من HDL والدهون الثلاثية. زاد البروتين الكلي ($P<0.05$) بجميع الفيتامينات المحقونة. تم زيادة نشاط ALP فقط عن طريق فيتامين B1. تم زيادة تركيز T_3 في البلازما بواسطة فيتامين E وB1 ، لكنه لم يختلف معنوياً عن الكنترول الإيجابي و مجموعات فيتامين B2. كان أعلى مستوى للكورتيكوستيرون بعد الفقس لمعاملة الكنترول السلبي ، والأقل مع جميع المواد المحقونة ، وخاصة في مجموعة فيتامين B1 ، مما يعكس تأثيرات إيجابية في القضاء على إجهاد الفقس. على الرغم من أن معدلات الفقس لم تتأثر بالمواد المحقونة، إلا أن جميع الفيتامينات كان لها آثار مفيدة على وزن الكتاكيت المفقسة والأداء الصحي.