EFFECT OF DIFFERENT ADMINISTRATION METHODS OF VITAMIN AD3E TO HATCHING EGGS AND SUPPLEMENT IT WITH DRINKING WATER ON HATCHABILITY TRAITS AND POST HATCH PERFORMANCE AND BIOCHEMICAL PARAMETER OF BROILER

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ABSTRACT

This study was conducted at the Badi Hatchery and poultry house / department of animal production / College of Agriculture engineering science/ University of Duhok. The aim of study was to investigate the effect of different administration methods of vitamin AD3E to hatching eggs on hatchability and post hatch productive performance and some physiological parameter of broiler. A total of one thousand and two hundred fifty (1250) hatched eggs were distributed into five groups each groups 250 eggs. The experimental treatments were as the follow: T1 (Control), T2 (sham control)(injected with 0.1 ml normal saline), T3 (injected with 0.1 ml of vitamin AD3E dissolved in 5 ml of vitamin in 1 ml of water) T4 (Spraying vitamin AD3E 1 ml /L water), T5 (Dipping vitamin AD3E 1 ml /L water). During the rearing period, each treatment divided into 2 group of replications, (5 replication) for each group that is from one treatment exactly. First 5 five replications (R1-R5) for each treatment given different doses of vitamin at different rearing age via drinking water and other 5 five replications (R6-R10) were drink normal water throughout the rearing period which is at least 5 weeks. Weekly Live body weight, body weight gain, feed consumption, feed conversion ratio, mortality percentage, Production index were recorded, (Total protein, glucose and cholesterol), blood serum titer for testing the immunity of chicken against diseases Newcastle (ND) and Gumboro, infectious bronchitis (IB). The overall data shows the following results: - In hatching stage there were significant affect in chick weight and chick to egg ratio and in rearing stage live body weight, body weight gain, feed conversion ratio, production index, serum glucose, Newcastle Gambaro and infectious bronchitis disease.

Key word: AD3E. injection. spraying. dipping. broiler. hatching eggs. Vitamin

مجلة العلوم الزراعية العراقية -2021 :52 (2):322-335 **حسن وطيب**

تاثير طرئق مختلفة لأعطاء فيتامين AD3E لبيض التفقيس و عن طريق ماء الشرب على صفات التفقيس والاداء الانتاجي و بعص صفات البايوكيمائية والمناعية لفروج اللحم

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المستخلص

أجريت هذه الدراسة في مفقس (بادي) وحقول دواجن قسم الإنتاج الحيواني – كلية هندسة علوم الزراعة – جامعة دهو استهدفت الدراسة لمعرفة تأثير طرق اعطاء فيتامينAD3Eلبيض تفقيس امهات فروج اللحم و عن طريق ماء الشرب على كفاءة التفقيس ,الأداء الانتاجى, وبعض الصفات الفيسيولوجية والمناعية لأفراخ الفاقسة لفروج اللحم. وزعت 1250 بيضة على 5 مجموعات 250 بيضة لكل مجموعةوكانت معاملات التجربة كالاتيT1معاملة السيطرة,T2حقن البيض 0.1مل محلول ملحي عاودي/ بيضة,T3حقن البيض بمحلول فيتامين0.1AD3E مل/ بيضة5(مل فيتامين لكل لتر من الماء عند عمر18يوم من الحضن) وعند عمر7يوم من الحضنT4رش البيض في محلول (AD3E) مل /لتر, T5غطس البيض في محلول فيتامن (AD3E) مل /لتر . خلال فترة التربية الافراخ الفاقسة من كل معاملة وزعت على مجموعتين 5 مككرات لكل مجموعة من (R1–R5)لكل مجموعة تم اعطائهم تراكييز مختلفة من فيتامين من خلال ماء الشرب ومككرات الاخرى من كل المعامله) (R6–R10تم اعطائهم ماء صافيطيلة فترة التربية 35 يوم.خلال التجربة تم قياس الوزن الحي الاسبوعي, الزيادة الوزنية الاسبوعية, استهلاك العلف, كفاءة تحويل الغذائي ,نسبة الهلاكات, دليل الانتاجي, مستوى الكلوكوز,كوليسترول و البروتين الكلي في مصل الدم, و مستوى المناعة ضد مرض نيوكاسل, كمبورو والتهاب القصبات. وكانت النتائج كالاتي : فى المرحلة الفقس كان هناك تاثير المعنوى على وزن الافراخ عند الفقس ونسبة الفرخة الى البيضة مقارنة مع السيطرة وفى المرحلة التربية الوزن الحي, الزيادة الوزنية , كفاءة تحويل الغذائي , دليل الانتاجي, مستوى الكلوكوز, مستوى المناعة ضد مرض نيوكاسل , كمبورو و التهاب القصبات.

الكلمات المفتاحية : حقن . رش . غطس . لفروج اللحم.بيض التفقيس .فيتامين

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INTRODUCTION

Hatching performance and chick weight may be influenced by the process of egg formation, which is affected by various factors such as egg weight (EW), egg quality, yolk nutrients yolk antioxidant concentrations and variations in embryonic development (37).The rapid growing of modern breeds makes suffering less supply of energy, less supply of some vitamins and minerals which led to decrease in hatchability and vitality of newly hatched chicks (41). Many researchers were administrated that possible increasing hatchability, vitality with enhance growing with early nutrition of chicken embryos through exogenous antioxidant and nutrient solution by in ovo technology (31**)**. A number of nutrients have an important physiological, nutritional and immunological functions related to the bird's embryogenesis and growth rates. In ovo injection of these nutrients may help overcome any constraints imposed by inadequate egg nutrition (34). Moreover, in ovo injection is one of the unique ways of entering the nutrients of the fetus during the incubation period is through the injection process (27) injections are egg-like nutrients such as Carbohydrates, proteins, amino acids, vitamins and vaccines can promote embryo growth, improve energy production and promote early intestinal development by increasing the development of intestinal function and improving the of immunity (19).Vitamin E is a biological antioxidant that could contribute to improved growth, physiological, and immunological performance in broiler chickens because of its ability to neutralize free radicals and reduce lipid peroxidation in both the plasma and skeletal muscle (20;35). Oxidative stress has been regarded as one of the major factors negatively affecting the performance of birds in the poultry industry (40). Vitamin A considers one of the necessary vitamins during normal embryo development stages and plays an important role in maintaining the differentiation and cellular specialization in the organism. (32).Vitamin D3 is one of the fat soluble vitamin, which is important for the mussel growth the embryo, where the target organs include the intestine, kidney, bone, ovary, testis and pituitary gland (15). Vitamin D3 increases the proliferation and differentiation of muscle cells through its role in DNA synthesis and support the growth of the muscles of the emptying fetus and an increase in its length and maturity (10). Spraying of hatching eggs is one of the methods for administration of nutrients for the eggs, (28) found that spraying Japanese quails eggs with different level of glucose had effect on the hatching traits. The hatchability percentages of Muscovy duck eggs were improved by dipping eggs into ascorbic acid solution when compared to the control on the 0, 14^{th} and 30^{th} days of incubation (21).

MATERIALS AND METHODS

The experiment was conducted at the (Badi) hatchery that located near to the Badi village at Duhok governorate, all the rearing and sampling work of this study were at the poultry farm of Animal Production Department, College of Agriculture, University of Duhok, Kurdistan region, Iraq. A total of one thousand two hundred and fifty 1250 forming the broiler breeders flock their origin was from Portugal (Rose 308: broiler breeders age, 32 weeks, first cycle of percentage, average eggs weight 57 to 58 g . The trial was started on January 7, 2019 for the period 10 weeks. This investigation was approved by the animal production department scientific committee of college of agriculture, University of Duhok.

Eggs incubation and flock rearing

A total of one thousand two hundred and fifty 1250 eggs distributed into 5 groupsof 250 eggs and set in a force draft incubator at temperature were Eggs incubated at 37.5℃and 54–55 % relative humidity environment, ventilated by a big fan and water tube cooling system. First group (T1) acted as a control (un-injected) and second group(T2) injected with distilled water or normal saline (0.1ml /egg), forth group (T3) was injected by the vitamin AD3E (0.1 ml / eggs) and after dissolved 5 ml vitamin to 1 litter of sterile water on 18^{th} day of incubation by using a 27 gauge 0.40mm × 25 mm needle this needle was teeth disposable endodontic and periodontal irrigation tips from the company (Dia Dent) and with Socorex® syringe that is very good for injection due to not make embryo be injured and coming blood if touch body of

chicks inside eggs . At7th day of incubation (T4) was acted as a spraying with AD3E solution (1ml/ 1liter)and fifth group (T5) was acted as a dipping with AD3E solution (1m/1litter).All unhatched eggs were broken to insure proper hatching result; after that the hatched chicks were transported separately to the farm, using special individuals transport cages for each treatment group. On the day of hatch the chicken were weighting and hatching percentage and mortality was recorded.

Serum biochemical and immunity

Blood samples were collected (5 ml) at the day 35 of the study by venipuncture of the neck vein, then, the blood samples will keep on ice and transfer to the laboratory blood tubes were kept in the refrigerator for 48 hours and then placed in centrifuge to 1500 revolution for 10 min to separate the serum . The serums was separate and stored at -20 °C till the measurements of the (Glucose and Cholesterol and Total protein) by auto analyzer (COBAS INTEGRA 400 plus). And immune parameters Antibiotics titration against Newcastle disease (ND), IBD and infectious bronchitis (IB) diseases were performed using ELISA by specific kits of OVATEC® Plus, SERELISA® Rabies (Synbiotic,USA),and BIA-CK® 121 Biochek , Netherland.

Table 1. Composition and nutritional levels of experimental diets

Feed stuff	Starter %	Grower %
Corn	47.73	51.47
Soya bean meal	31.5	24.6
Wheat	11	16
L-Methionine	0.2	0.17
L-Lysine HCl	0.14	0.19
Threonine	0.08	0.09
Enzyme	0.05	0.05
Anti-toxin	0.15	0.15
Soya oil	2.1	0.5
Mono calcium phosphate	0.3	0.22
Premix [1]	5	5
Ground Limestone	1.7	1.5
Anti-coccidial	0.05	0.05
Calculated chemical analyses		
Metabolic energy kcal/ kg	3072	3122
Crud protein %	22.5	20
Fat%	4.5	4
C/P ratio	136.5	156
Crud fiber	2.67	2.62
Calcium Ca %	1	0.9
Total phosphorus P%	0.55	0.51
Sodium Na %	0.16	0.16

[1]**Vitamin Provided per kilogram: vitamin A 400.000 I.U. ; vitamin D 160.000 I.U; vitamin E 1200 mg : vitamin B1 120 mg : vitamin B2 280 mg : vitamin B6 160 mg : vitamin B12 1400 Mcg : Biotin 4 mg; Folic acid, 40 mg; Niacin 1600 mg; Vitamin K3 100 mg : choline chloride 12000 mg : choline 10411.2 mg : calcium –D-Pantothenate 600 mg .Trace element Provided per kilogram of diet: Fe, 2000 mg; Cu, 400 mg; Mn , 3200 mg; Zn, 2400 mg; iodine, 40 mg : Se, 10 mg. Antioxidant added per kilogram: B.H.T (E321) 67 mg: Propyl Galate (E310) 5.60 mg: Citric Acid (E330) 10 mg.**

Statistical analyses

The statistical analysis of data was carried out using (GLM) General Linear Models within (SAS Institute, 2002) program and Duncan's multiple range test (Duncan, 1955) was used to test the differences between the sub classes of each factor.

RESULTS AND DISCUSSION

Hatchability traits: The effect of using different ways of AD3E administration for hatching eggs in hatchability traits presented in (Table 2). Treatments had no significant effect on the hatchability percentage, embryonic mortality compared to control. Chick weight and Chick weight to egg weight ratio was significantly higher in control compared to treated eggs. Results were in line with finding of (9; 17) who reported that injection broiler hatching eggs at 14 day of incubation with Vitamin had no significant effect on the hatchability %, embryonic mortality compared to non-injected eggs as a control. While, our results were in contrast with finding of (25) who found in a trail injected quail hatching eggs with vitamin significantly ($P<0.05$) increased hatchability percentage and chick weight at hatching and significantly reduced total embryonic mortality compared to control.

Table 2. Effect of different administration methods of vitamin AD3E on Hatchings traits of broiler breeders hatching eggs (Mean ± Standard Error)

Treatments	Hatchability from Fertilized eggs (%)	Total embryonic Mortality (%)	Chick weight (gm.)	Chick weight to eggs weight ratio (%)
T1 control	91.24±1.71	8.75±1.71	40.69±0.33a	70.38±0.65a
T2 Injection (Normal S.)	88.50±2.51	11.49±2.51	39.73±0.19b	69.59±0.34a
T3 Injection (AD3E)	85.56±1.68	14.43±1.68	39.58±0.28b	69.04±0.48ab
T4 Spraying (AD3E)	88.69±2.51	11.30±2.51	38.38±0.30c	66.31±0.42c
T5 Dipping (AD3E)	87.96±1.53	12.03±1.53	38.79±0.13c	67.76±0.19b
Over all mean	88.39±0.91	11.60±0.91	39.43±0.19	68.62±0.34
	N.S	N.S	**	**

**** (P<0.01).N.S: Not Significant**

Live body weight: The impact of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the weekly live body weight presented in (Table 3). Result shows that interaction and using AD3E in drinking water had no significant on the weekly body weight compared to control. Concerning the administration way of AD3E on hatching eggs shown that at 3rd week of age chicks formed from eggs sprayed with AD3E significantly (P<0.05) had higher weight compared to those injected with normal saline as a positive control being, 857.14 g and 805.94 g ,respectively. At 4th week of age body weight of chicken hatched from eggs sprayed, injected, and dipped with AD3E was significantly higher compared to control. At 35 days of age the live body weight of chicken produced from eggs sprayed and dipped with AD3E had significantly (P<0.05) higher body weight compared to both negative and positive controls and best weight recorded for spraying group. Result were in agreements with finding of (6:2) reported that chicks produced from eggs of Ross 308 injected with different level of vitamin with inactivated Newcastle disease vaccine had significantly (P<0.05) better live body weight and weight gain compared to chicks produced from eggs injected with sterile PBS. Furthermore (24) who added vitamin E in Ross 308 broilers diet recorded no significant effect on the live body weight of chicken at 42 days of age. While, Results were in contrast of the finding of. reported that chicks hatched from broiler breeder eggs injected with vitamin E (50µl) with Newcastle vaccine significantly (P<0.05) improved live body weight and weight gain at different age compared chicks produced from eggs injected with sterile PBS as control. While results were in centenary with finding of (5) who used different doses of Vitamin AD3E in broiler drinking water recorded that all treatments lead to significantly(P<0.05) improvement in live body weight compared to the control. And the cause that chicken formed from egg treated with AD3E had more weight may be due to that vitamin A has important role in the function of the thyroid gland (42). Vitamin A work on the increase activity of the pituitary gland to release thyroid stimulating hormone (TSH) in the body to increase secretion of thyroid hormone (thyroxine) that enrolled in the increase metabolic rate in the body tissue to increase absorption of monosaccharide and increase protein metabolism that lead to increase body weight (11). Also vitamin E kept the gastro intestinal cell healthy led to increase activity of nutrient absorption led to positively effect on live body weight (30). Furthermore, (1) mentioned that vitamin A improve growth and development of the epithelial cell of the digestive system make the villus healthier and increase growth of small intestine percent that increase digestion absorption led to increase body weight..

Live body weight gain: The effect of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the weekly live body weight gain presented in (Table 4). Result shows that interaction and using AD3E in drinking water had no significant on the weekly and total weight gain compared to control. Concerning the administration way of AD3E on hatching eggs shown that at 4th week of age chicks formed from eggs injected, sprayed and dipped with AD3E significantly (P<0.05) had higher weight compared to those injected with normal saline and control, being 694.8, 685.8 and 686.83, respectively. While, total live body weight gain (1-35) days of age chicken produced from eggs sprayed and dipped with AD3E got significantly (P<0.01) more weight

gain compared to both negative and positive control (injection with normal saline). The results was in contrast with results obtained by other researchers (6;2) who found that chicks produced of the broiler hatching eggs injected with vitamin had significantly (P<0.05) increase weight gain compared to chicks produced from eggs injected with normal saline.

Feed intake: The impact of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the weekly and total feed intake presented in (Table 5). Results shown that interaction, using different dose of AD3E in drinking water and different way of administration of AD3E for hatching eggs had no significant effect on the weekly and total feed intake compared to control. Result were in agreement with the findings of (33) who found that injection of broiler breeder eggs with different levels of Vitamin E had no significant effect on the feed intake of chicken at 42 days of age compared to un injected eggs. Furthermore, (24) who added vitamin E in Ross 308 broilers had no significant effect on the feed intake of chicken at 42 days of age. While, results were in contrast with finding of (3) reported that chicks formed from broiler breeder eggs injected with vitamin with Newcastle vaccine significantly (P<0.05) improved feed intake from 1st to 4th week of age compared to chicks produced from eggs injected with sterile PBS as control.

FEed conversion ratio

The impact of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the weekly and total feed conversion ratio presented in (Table 6). Results shown that interaction and administration of AD3E in drinking water had no significant effect on the weekly and total feed conversion ratio, except at first week of age using AD3E in drinking water significantly(P<0.05) increase feed conversion ratio value or lower feed conversion ratio compared to no AD3E in drinking water, being 1.16 and 1.11 respectively. Regarding to the method of administration of AD3E for hatching eggs had significant (P<0.05) effect on the feed conversion ratio at 14 and total feed conversion ratio 1-35 days of age, Also had significant (P<0.01) effect on the feed conversion ratio at 28 day of age. Best result at total FCR (1-35) was for chicken hatched from eggs sprayed and dipped with AD3E compared to control (injected with normal saline), being 1.50, 1.50 and 1.56, respectively. Results were in line with finding of (9; 22) who investigated there was no significant effect on feed conversion ratio of chicks produced of broiler breeder eggs injected with vitamin compared to un injected eggs as a control. However, results were in contrast with finding of (5) who used different doses of vitamin AD3E in broiler drinking water stated that all doses lead to significantly(P<0.05) improvement in feed conversion ratio compared to the control.

Mortality and production index

The impact of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the total mortality and production index presented in (Table 7). Results shown that interaction, using different dose of AD3E in drinking water and different way of administration of AD3E for hatching eggs had no significant effect on mortality but the mortality percent in treatment numerically lower than negative and positive control. Also Results shown that interaction, using different dose of AD3E in drinking water had no significant effect on Production index except chicks produced from eggs sprayed and dipped in AD3E had significantly (P<0.01) higher production index compared to negative and positive control, being 397.49, 398.14, 348.79 and 344.37, respectively. Our results were in agreement with the finding of (4) who reported that adding different doses of vitamin AD3E in drinking water had no significant effect on the mortality percentage of chicks at 42 days of age. Furthermore, (24) who found that adding vitamin in Ross 308 broilers diet had no significant effect on the mortality percentage of chicken at 42 days of age. Furthermore, while, our result were in contrast with the finding of (23) who reported that using AD3E in broiler drinking water significantly (P<0.05) improved production compared to control.

Table 3. Effect of different administration methods of vitamin AD3E on weekly live body weight of broiler chickens in 35 day of age (Mean ± Standard Error):

Treatment		Wt. 7 days	Wt. 14 days	Wt. 21 days	Wt. 28 days	Wt. 35 days
Over all mean		150.62±1.07	407.71±3.87	832.52±6.36	1510.89±11.19	2140.74±13.18
T1	No AD3E	156.92±3.14	418.69±13.11	837.24±21.22	1477.20±40.93	2104.30±36.87
Control	AD3E	147.48±2.14	403.16±5.78	823.02±14.72	1443.00±23.32	2082.90±44.32
T2	No AD3E	145.86±1.92	387.76±10.97	793.36±13.71	1434.20±22.72	2031.60±45.63
Injection(N.S)	AD3E	148.41±5.60	404.53±11.88	818.53±20.19	1508.00±27.82	2119.10±27.74
T3	No AD3E	148.42±2.88	405.74±9.69	855.53±18.01	1533.00±41.66	2139.40±53.19
Injection	AD3E	148.34±2.48	418.53±3.48	836.85±12.99	1549.00±26.03	2140.40±13.55
T4	No AD3E	154.04±4.65	419.56±13.29	870.37±28.70	1538.00±44.17	2191.60±45.23
Spraying	AD3E	153.06±3.45	413.22±12.42	843.90±17.09	1548.00±33.17	2222.90±34.17
T5	No AD3E	151.18±3.87	412.96±8.79	844.38±16.44	1552.10±36.22	2208.60±28.75
Dipping	AD3E	152.52±1.74	392.98±24.25	802.05±23.62	1526.40±25.71	2166.60±17.34
		N.S	N.S	N.S	N.S	N.S
All treatment without AD3E		151.28±1.61	408.94±5.19	840.17±9.83	1506.90±17.93	2135.10±21.76
All treatment with AD3E		149.96±1.46	406.48±5.84	824.87±7.98	1514.88±13.72	2146.38±15.27
		N.S	N.S	N.S	N.S	N.S
T1 control		152.20±2.38	410.92±7.23	830.13±12.40ab	1460.10±22.89b	2093.60±27.41b
T2 Injection (Normal S.)		147.14±2.82	396.14±8.12	805.94±12.24b	1471.10±20.93ab	2075.35±28.48b
T3 Injection (AD3E)		148.38±1.79	412.14±5.29	846.19±10.92ab	1541.00±23.31a	2139.90±25.87ab
T4 Spraying (AD3E)		153.55±2.73	416.39±8.64	857.14±16.35a	1543.00±26.06a	2207.25±27.23a
T5 Dipping (AD3E)		151.85±2.01	402.97±12.59	823.22±15.29ab	1539.25±21.37a	2187.60±17.30a
		N.S	N.S	*	*	**

*(P<0.05), ** (P<0.01), N.S =Not significant

Table 4. Effect of different administration methods of vitamin AD3E on weekly weight gain of broiler chickens in 35 day of age (Mean ± Standard Error):

Treatment		Wt. 1 wk	Wt. gain 2 wk	Wt. gain 3 wk	Wt. gain 4 wk	Wt. gain 5wk	Total wt. gain 1-35
Over all mean		109.79±7.23	257.08±9.18	424.81±7.13	666.88±10.01	641.33±11.25	2099.91±4.44
T1	No AD3E	117.58±3.12	261.77±10.74	418.54±12.55	612.76±43.66	654.30±36.94	2064.97±36.90
Control	AD3E	107.81±2.10	255.68±5.33	419.86±10.28	619.97±3.39	639.90±29.12	2043.23±44.34
T2	No AD3E	104.53±1.96	241.89±10.22	405.60±6.01	611.64±19.99	626.60±53.14	1990.27±45.66
Injection(N.S)	AD3E	106.74±5.62	256.12±9.38	414.00±11.31	689.46±19.48	611.10±29.89	2077.43±24.77
T3	No AD3E	106.42±2.88	257.32±8.39	449.78±12.20	677.46±28.82	606.40±29.10	2097.40±53.18
Injection	AD3E	106.01±2.47	270.18±5.26	418.32±15.25	712.14±29.87	591.40±20.09	2098.07±13.56
T4	No AD3E	114.04±4.62	265.52±8.88	450.81±15.44	667.62±16.24	653.60±13.90	2151.60±45.18
Spraying	AD3E	112.73±3.42	260.16±9.47	430.68±6.55	704.09±20.00	674.90±21.12	2182.57±34.14
T5	No AD3E	110.52±3.84	261.77±6.19	431.42±11.10	707.71±23.72	656.50±20.99	2167.93±28.77
Dipping	AD3E	111.52±1.76	240.46±22.87	409.06±21.90	665.94±40.57	698.60±45.06	2125.60±17.35
		N.S	N.S	N.S	N.S	N.S	N.S
All treatment without AD3E		110.62±1.70	257.65±4.05	431.23±6.03	655.44±13.83	639.48±14.36	2094.43±21.79
All treatment with AD3E		108.96±1.48	256.52±5.39	418.38±5.92	678.32±12.74	643.18±14.80	2105.38±15.27
		N.S	N.S	N.S	N.S	N.S	N.S
T1 control		112.70±2.41	258.72±5.74	419.20±7.65	616.36±21.56b	647.10±22.30	2054.10±27.43b
T2 Injection (N.S)		105.64±2.83	249.00±6.95	409.80±6.20	650.55±18.47ab	618.85±28.97	2033.85±28.47b
T3 Injection (AD3E)		106.22±1.79	263.75±5.13	434.05±10.59	694.80±20.40a	598.90±16.86	2097.73±25.87ab
T4 Spraying (AD3E)		113.38±2.72	262.84±6.18	440.74±8.59	685.86±13.58a	664.25±12.44	2167.08±27.19a
T5 Dipping (AD3E)		111.02±2.00	251.12±11.72	420.24±12.16	686.83±23.22a	677.55±24.46	2146.77±17.34a
		N.S	N.S	N.S	*	N.S	**

**(P<0.01). * (P<0.05), N.S: Not Significant

Table 5. Effect of different administration methods of vitamin AD3E on weekly feed intake of broiler chickens in 35 day of age (Mean ± Standard Error)

Treatment		F.I 1st wk.	F.I 2nd wk.	F.I 3rd wk.	F.I 4th wk.	F.I 5th wk.	Total Feed intake 35 day
Over all mean		124.94±1.22	466.72±3.93	599.33±5.76	908.86±7.42	1111.52±7.69	3211.39±18.47
T1	No AD3E	131.70±2.48	462.21±11.60	586.10±15.95	896.56±37.02	1112.90±25.77	3189.49±63.79
Control	AD3E	122.68±1.77	452.56±14.70	586.98±9.81	899.36±17.05	1083.60±11.83	3145.19±36.91
T2	No AD3E	117.86±2.77	481.09±6.75	569.97±17.95	869.60±18.71	1083.60±33.74	3122.13±62.82
Injection(N.S)	AD3E	122.70±4.16	476.72±13.06	592.62±28.76	718.08±17.34	1121.90±19.13	3232.03±51.18
T3	No AD3E	120.36±3.71	482.81±10.53	616.88±11.50	916.00±24.19	1121.00±33.82	3257.05±70.25
Injection	AD3E	12.20±5.49	460.88±14.97	587.80±14.31	910.04±15.38	1086.70±24.04	3174.62±37.46
T4	No AD3E	124.48±4.64	469.00±19.77	419.86±22.04	927.32±20.56	1153.96±15.70	3294.63±63.31
Spraying	AD3E	130.34±4.00	448.92±7.71	670.81±15.90	931.32±23.72	1113.90±24.88	3232.30±59.15
T5	No AD3E	122.32±2.91	471.01±12.86	619.25±19.40	932.12±15.32	1108.20±29.16	3252.91±51.66
Dipping	AD3E	127.81±3.36	462.02±8.93	606.00±21.69	888.28±38.20	1129.40±19.32	3213.52±82.70
		N.S	N.S	N.S	N.S	N.S	N.S
All treatment without AD3E		123.34±1.68	473.22±5.54	602.41±8.36	908.32±10.98	1115.93±12.50	3223.24±28.43
All treatment with AD3E		126.54±1.74	460.22±5.37	596.24±8.06	909.41±10.20	1107.10±9.14	3199.53±23.94
		N.S	N.S	N.S	N.S	N.S	N.S
T1 control		127.19±2.08	457.38±8.97	586.54±8.83	897.96±19.22	1098.25±14.23	3167.37±35.52
T2 Injection (Normal S.)		120.28±2.49	478.90±6.96	581.30±16.42	893.84±14.49	1102.75±19.36	3177.08±42.36
T3 Injection (AD3E)		124.78±3.45	471.84±9.37	602.34±9.92	913.02±13.55	1103.85±20.38	3215.84±39.96
T4 Spraying (AD3E)		127.41±3.05	458.96±10.55	613.84±12.96	929.32±14.81	1133.93±15.39	3263.46±42.14
T5 Dipping (AD3E)		125.06±2.29	466.52±7.53	612.62±13.90	910.20±20.73	1118.80±16.86	3233.21±46.43
Treatment		N.S	N.S	N.S	N.S	N.S	N.S

N.S= Not significant

Table 6. Effect of different administration methods of vitamin AD3E on weekly feed intake of broiler chickens in 35 day of age (Mean ± Standard Error)

Treatment		FCR 1st wk.	FCR 2nd wk.	FCR 3rd wk.	FCR 4th wk.	FCR 5th wk.	Total FCR (1-35)
Over all mean		1.14±0.009	1.81±0.021	1.41±0.01	1.37±0.01	1.75±0.02	1.53±0.006
T1	No AD3E	1.12±0.01	1.77±0.03	1.40±0.04	1.47±0.05	1.72±0.09	1.54±0.02
Control	AD3E	1.13±0.01	1.76±0.04	1.40±0.04	1.45±0.02	1.70±0.06	1.54±0.01
T2	No AD3E	1.12±0.02	2.00±0.08	1.40±0.04	1.42±0.03	1.76±0.1	1.57±0.03
Injection(N.S)	AD3E	1.15±0.03	1.86±0.07	1.42±0.04	1.33±0.04	1.85±0.07	1.55±0.008
T3	No AD3E	1.13±0.02	1.87±0.02	1.37±0.02	1.35±0.03	1.86±0.08	1.55±0.01
Injection	AD3E	1.22±0.05	1.70±0.05	1.41±0.06	1.28±0.04	1.84±0.03	1.51±0.009
T4	No AD3E	1.09±0.03	1.76±0.06	1.37±0.02	1.38±0.01	1.76±0.05	1.53±0.01
Spraying	AD3E	1.15±0.03	1.73±0.08	1.41±0.02	1.32±0.03	1.65±0.06	1.48±0.01
T5	No AD3E	1.10±0.01	1.80±0.06	1.43±0.02	1.32±0.03	1.69±0.07	1.50±0.008
Dipping	AD3E	1.14±0.02	1.83±0.05	1.49±0.09	1.34±0.04	1.64±0.11	1.51±0.02
		N.S	N.S	N.S	N.S	N.S	N.S
All treatment without AD3E		1.11±0.009b	1.84±0.02	1.39±0.01	1.39±0.01	1.76±0.03	1.54±0.009
All treatment with AD3E		1.16±0.01a	1.78±0.03	1.43±0.02	1.34±0.02	1.74±0.03	1.52±0.008
		*	N.S	N.S	N.S	N.S	N.S
T1 control		1.13±0.01	1.77±0.02b	1.40±0.03	1.46±0.02a	1.71±0.05	1.54±0.01ab
T2 Injection (Normal S.)		1.14±0.01	1.93±0.05a	1.41±0.03	1.38±0.03b	1.80±0.06	1.56±0.01a
T3 Injection (AD3E)		1.17±0.03	1.79±0.04b	1.39±0.03	1.32±0.02b	1.85±0.04	1.53±0.01ab
T4 Spraying (AD3E)		1.12±0.02	1.75±0.05b	1.39±0.01	1.35±0.02b	1.71±0.04	1.50±0.01b
T5 Dipping (AD3E)		1.12±0.01	1.82±0.04ab	1.46±0.04	1.33±0.02b	1.67±0.06	1.50±0.01b
		N.S	*	N.S	**	N.S	*

*(P<0.05), ** (P<0.01), N.S =Not significant

Table 7. Effect of different administration methods of vitamin AD3E on percentage of Mortality and Production index (PI) of broiler chickens in 35 day of age (Mean ± Standard Error)

Treatment		Total Mortality %	Production index (PI)
Over all mean		6.40 ± 0.78	371.42 ± 5.35
T1	No AD3E	10.66 ± 3.99	348.34 ± 17.11
Control	AD3E	8.00 ± 1.33	349.24 ± 17.17
T2	No AD3E	9.33 ± 3.99	349.58 ± 14.43
Injection(N.S)	AD3E	8.00 ± 2.49	339.16 ± 18.88
T3	No AD3E	6.66 ± 2.10	358.71 ± 11.67
Injection	AD3E	4.00 ± 2.66	377.88 ± 17.29
T4	No AD3E	2.66 ± 1.63	383.61 ± 13.23
Spraying	AD3E	5.33 ± 1.33	411.37 ± 8.40
T5	No AD3E	5.33 ± 2.49	400.93 ± 10.40
Dipping	AD3E	4.00 ± 2.66	395.35 ± 9.38
		N.S	N.S
All treatment without AD3E		6.93 ± 1.24	368.23 ± 6.96
All treatment with AD3E		5.86 ± 0.96	374.60 ± 8.23
		N.S	N.S
T1 control		9.33 ± 1.77	348.79 ± 11.43b
T2 Injection (Normal S.)		8.66 ± 2.00	344.37 ± 11.33b
T3 Injection (AD3E)		5.33 ± 1.66	368.29 ± 10.34ab
T4 Spraying (AD3E)		4.00 ± 1.08	397.49 ± 8.71a
T5 Dipping (AD3E)		4.66 ± 1.73	398.14 ± 6.66a
		N.S	**

**(P<0.01). * (P<0.05), N.S: Not Significant

Serum biochemical parameter

The influence of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the serum glucose, cholesterol and total protein shown in (Table 8) . Results shown that interaction, administration of AD3E and method of using with hatching eggs had no significant effect on the serum content of cholesterol, glucose and total protein except chicks hatched from egg injected and sprayed with AD3E had significantly(P<0.05) lower serum glucose content compared to control being, 229.10, 229.80 and 248.95, respectively. Results were in line with findings of by (7) who added different level of vitamin in broiler diet stated that no significant difference found among treatments on the serum cholesterol and total protein at 35 and 49 days of age compared to control. While results were in contrast with result of (22) who injected broiler breeder eggs with vitamin recorded that treatments significantly (P<0.05) increased serum glucose content compared to control (2) reported that chicks produced from hatching eggs of Ross 308 injected with different level of vitamin had significantly (P<0.05) higher serum total protein and globulin with lower serum albumin compared to chicks produced from eggs injected with sterile PBS.

Table 8. Effect of different administration methods of vitamin AD3E on serum biochemical profiles of broiler chickens in 35 day of age (Mean ± Standard Error)

Treatment		Glucose(gm./L)	Cholesterol(gm./L)	Total protein(mg/L)
Over all mean		235.99±2.54	120.82±2.07	2.87±0.03
T1	No AD3E	241.70±9.63	114.40±1.63	2.82±0.05
Control	AD3E	256.20±8.39	117.50±9.73	2.87±0.12
T2	No AD3E	232.60±6.48	122.80±4.35	2.78±0.10
Injection(N.S)	AD3E	233.60±6.64	114.40±8.83	2.77±0.06
T3	No AD3E	229.50±4.98	127.00±3.40	2.99±0.07
Injection	AD3E	228.70±11.94	120.70±3.90	2.85±0.23
T4	No AD3E	228.80±6.0 b	129.40±7.43	3.09±0.05
Spraying	AD3E	224.80±7.16	128.30±4.96	2.96±0.04
T5	No AD3E	238.00±8.13	122.40±9.58	2.84±0.09
Dipping	AD3E	246.00±4.10	121.30±7.13	2.77±0.06
		N.S	N.S	N.S
All treatment without AD3E		234.12±3.12	121.20±2.79	2.90±0.04
All treatment with AD3E		237.86±4.05	120.44±3.11	2.84±0.05
		N.S	N.S	N.S
T1 control		248.95±6.49 a	115.95±4.68	2.84±0.06
T2 Injection (Normal S.)		233.10±4.38ab	113.60±4.65	2.77±0.05
T3 Injection (AD3E)		229.10±6.10 b	123.85±2.65	2.92±0.11
T4 Spraying (AD3E)		226.80±4.45 b	128.85±4.21	3.03±0.03
T5 Dipping (AD3E)		242.00±4.49 ab	121.85±5.63	2.81±0.05
		*	N.S	N.S

*** (P<0.05), N.S: Not Significant**

Immunity parameters

The impact of using different ways of AD3E administration for hatching eggs, using different doses in drinking water and their interaction on the immunity parameters presented in (Table 9). Results show that interaction had significant (P<0.01) effect on the total antibody titer against Newcastle disease and had significant (P<0.05) effect on the total antibody titer against Gambaro. Higher titer was recorded for chicken produced from eggs injected with AD3E and administrated AD3E in drinking water. While the lower titer recorded for chicken produced from control hatching eggs and without using AD3E in drinking water. Infectious bronchitis titer did not significantly affected by interaction. Administration of AD3E in chicken drinking water significantly (P<0.01) improved total antibody titer against Newcastle, Gambaro, and infectious bronchitis compared to without using AD3E in drinking water being , 5350.28, 1576.46, 522.48 and 3761.32, 1330.58, 498.02, respectively. Concerning the method of administration of AD3E for hatching eggs. Results show that chicken produced from eggs from eggs injected, sprayed and dipped in AD3E had significantly (P<0.01) higher antibody titer against Newcastle disease and Gambaro compared to both controls. Regarding to titer against infectious bronchitis chicken produced from eggs injected with AD3E had significantly higher titer (522.80) compared to chicken produced from eggs injected with normal saline (492.45) and control eggs (504.35). Results were in agreement with the finding of (3 ; 2) who reported that chicks produced from hatching eggs of Ross 308 injected with different level of vitamin E with inactivated Newcastle disease vaccine had significantly (P<0.05) higher antibody titer against Newcastle disease at 21 and 35 days of age compared to chicks produced from eggs injected with sterile PBS. Also, Furthermore, (29) who found that using different level of AD3E in drinking water of Ross chicks significantly (P<0.05) increased antibody titer against Newcastle disease compared to control. While the results were in contrast with finding of , (22) who investigated that there was no significant effect on antibody titer against Newcastle disease of chicks produced of broiler breeder eggs injected with compared to un injected eggs as a control. And this may be due to that deficiency of vitamin E significantly decrease immunity response (18;26) and excess (26) have been shown to depress immune responses in chicks. Most research suggests that vitamin A deficiency is associated with reduced cellular immune responses whereas vitamin A excess impairs antibody responses. Vitamin A deficiency has been shown to directly impact T-cell functions that are vital for a bird to mount an immune response to an infection (36; 14).Furthermore, Vitamin E is primarily known for its role as an

antioxidant in reducing cellular free radical damage, but its deficiency causes a number of reduced immune responses (13). (16) Evaluated immune responses in male broilers fed diets varying in DL-*α*-tocopherolacetatefrom 0 to 87 mg/kg of diet. Thymic and splenic T cell populations were altered indicating that more helper T cells (CD4) were present with increased dietary vitamin E and thus improved responsiveness to immunologic stimuli. Improved disease resistance as mediated by dietary vitamin E has been noted with bacteria (38;39), coccidiosis (12), and viruses (17). Additionally, Vitamin D is critical for proper bone development in poultry. Research has elucidated negative effects on broiler cellular immunity as affected by vitamin D deficiency. For example, (8) fed female broilers a diet devoid of vitamin D or a diet containing 800 IU/kg of cholecalciferol Broilers fed diets devoid of vitamin D had depressed cellular immunity as measured by cutaneous basophil hypersensitivity response to phytohemagglutinin- P, depressed thymus weight, and depressed macrophage function. However, although SRBC is a Tdependent antigen, differences in primary or secondary responses did not occur (8).

Table 9. Effect of different administration methods of vitamin AD3E in antibody titers against Newcastle Disease, Gamboro and IB of broiler chickens in 35 day of age

Treatment		Newcastle Disease	Gamboro	IB
Over all mean		4555.80±169.24	1453.52±20.58	510.25±3.29
T1	No AD3E	2465.20±116.78e	1262.82±10.05e	495.30±7.27
Control	AD3E	4348.40±64.68d	1540.00±7.91bcd	513.40±2.68
T2	No AD3E	2679.40±158.62e	1280.30±18.05e	480.30±11.26
Injection(N.S)	AD3E	4772.00±61.09cc	1529.20±15.44cd	504.60±2.93
T3	No AD3E	4409.60±156.16cd	1475.10±17.03d	513.70±5.06
Injection	AD3E	6157.20±77.30aa	1613.30±21.08aa	531.90±6.25
T4	No AD3E	4630.00±177.24cd	1311.00±15.58e	501.70±7.95
Spraying	AD3E	5906.20±186.56ab	1593.00±21.25abc	534.60±8.99
T5	No AD3E	4622.40±57.76cd	1323.70±55.83e	499.10±15.07
Dipping	AD3E	5567.60±79.41b	1606.80±13.74ab	527.90±3.26
		**	*	N.S
All treatment without AD3E		3761.32±207.33b	1330.58±19.31b	498.02±4.62b
All treatment with AD3E		5350.28±146.21a	1576.46±9.85a	522.48±3.23a
		**	**	**
T1 control		3406.80±320.11c	1401.40±46.59c	504.35±4.74bc
T2 Injection (Normal S.)		3725.70±357.85b	1404.75±42.96c	492.45±6.82c
T3 Injection (AD3E)		5283.40±302.62a	1544.20±26.33a	522.80±4.85aa
T4 Spraying (AD3E)		5268.10±244.86a	1452.00±48.61b	518.15±7.88ab
T5 Dipping (AD3E)		5095.00±164.19a	1465.25±54.41b	513.50±8.71ab
		**	**	**

****(P<0.01). * (P<0.05), N.S: Not Significant**

CONCLUSION: 1- Hatchability traits: Hatchability percentage, embryonic mortality, Chick weight and Chick weight to egg weight ratio was reduced in AD3E administration methods

2-Parameter performance: During the rearing period, Live body weight, body weight gain and production index was increased, feed conversion ratio and mortality was improved, immune response was better in AD3E administration methods. The feed intake, Carcass cut percentage and biochemical parameter did not effected with AD3E administration

REFERENCES

1- Abdalla, A. A., A. M. Nema and N. Y. Elnagdy. 2009. Effect of Vitamin A Supplementation the Performances and Immune Response of Banarah Chicken. Egypt. Poultry Science, (29) (I): (221-239).

2- Abdulwahid, M. T. 2015. Effect of injection hatching eggs with Newcastle disease vaccine and different doses of vitamin E on some productive traits and immune response of broilers.The Iraqi Journal of Veterinary Medicine, 39(2): 98-107

3- Abdulwahid, M. T. and M. A. Al-Zuhairy. 2013. Effect of injection the broiler hatching eggs with vitamin E and cod liver oil on their productive traits and immune response to Newcastle disease vaccine. The Iraqi journal of Veterinary Medicine, 37(2): 199 -205

4- Al-Hamed A. M. Y. 2013. Study of effects Broiler density on some production characteristics by supplement by addition of different levels of group of Vitamin AD3E. Tikrit Journal for Agricultural Sciences. 13 (2): 84-91

5- Al-Taleb S.S. 2003. The effect of different levels of ad_3e vitamins alone or with added vitamin c on the productive performance of broiler chicks.Pakistan Journal of Biological Sciences, 6: 485-487

6- Al-Zuhairy M. A., and Y. J. Jameel.2014. Effect of ND vaccine, multivitamin AD3E, and omega-3 on performance and immune response of broilers. Mirror of Research in Veterinary Sciences and animals. 3 (1), 42-50

7- Arslan, M., Z. Mukaddes, M. Erdal, T. Ulker, and E. Elif. 2001. The Effects of Vitamin E on Some Blood Parameters in Broilers. Turk. J. Vet. Anim. Sci. 25:711-716

8- Aslam, S. M., J. D. Garlich and M. A. Qureshi 1998. Vitamin D deficiency alters the immune response of broiler chicks. Poult. Sci. 77:842–849

9- Bahanja S., K. Mandal, A. B. Agarwal, S. K., S. Majumdar, and A.Bhattacharyya.2012. Effect of in ovo injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens.16th European symposium poultry nutrition.

10-Capiati, D.A., M.T. Téllez-Iñón, and R.L.Boland, . 1999. Participation of protein kinase C α in 1, 25-dihydroxy-vitamin D3 regulation of chick myoblast proliferation and differentiation. Molecular and Cellular Endocrinology. Volume 153, Issues 1–2, 20 July 1999, Pages 39–45

11-Cheryl, F.N., L. E. David, P. H. Karen and M.N. Kendall..1984. Hypothyroidism: An early sign of vitamin A deficiency in chickens. British Journal of Nutrition, 114: 1733-1736

12-Colnago, G. L., L.S. Jensen. and P.L. Long, .. 1984.Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens. Poult. Sci. 63:1136–1143

13- Cook M. E. 1991. Nutrition and immune response to the domestic fowl. Crit. Rev. Poult. Biol. 3:167–189

14-Dalloul, R. A., H. S. Lillehoj, T. A. Shellem and J. A. Doerr.2002. Effect of vitamin A deficiency on host intestinal immune response to Eimeriaacervulina in broiler chickens. Poult. Sci. 81:1509–1515

15-De Boland, A. R. and A. W. Norman. 1990. Influx of extracellular calcium mediates 1,25dihydroxyvitamin D3-dependent transc-altachia (the rapid stimulation of duodenal Ca+2 tansport). Endocrinology, 127: 2475 – 2480

16-Erf, G. F., W.G. Bottje, T.K.., Bersi, M.D.., Headrick, M. D. and C.A. Fritts. A. 1998. Effects of dietary vitamin E on the immune system in broilers: Altered proportions of CD4 T cells in the thymus and spleen. Poult. Sci. 77:529–537

17-Franchini A., S. Bertuzzi, C. Tosarelli and G. Manfreda.1995. Vitamin E in viral inactivated vaccines. Poult. Sci. 74:666–671

18- Friedman, A. and D. Sklan. 1989a. Impaired T lymphocyte immune response in vitamin A depleted rats and chicks. Br. J. Nutr. 62:439–449

19-Gaafar, K.M., S.A. Selim, and S.S. El-ballal.2013. Effect of in ovo administration with two levels of amino acids mixture on the performance of Muscovy ducks. Emir. J. Food. Agric., 25: 58–65

20-Gao, J., H, Lin,. X.J., Wang, Z.G., Song, and H.C., Jiao, 2010. E, Vitamin supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. Poult. Sci. 89, 318 327

21-Ghonim, A. I. A., Awad, A. L., El- Shhat, A. M., Fatouh, M. H. A. and Ali, Kh. A. A. 2008. Effect of dipping and spraying hatching eggs of Muscovy duck by ascorbic acid solutions during incubation period on hatchability traits. Egypt. J. Poult. Sci., 28: 283–298

22-Goel, A., S.K. bhanja, V. Pande, M. Mehra and A.B. Mandal. 2013. Effects of *in ovo*administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens. Indian Journal of Animal Sciences, 83: 916-21

23-Kamalzadeh, A., N. Ila and O. Heydarnejad, 2009. Effects of Emulsified vitamins on broiler performance. World Journal of Zoology 4 (1): 42-46

24-Karadas, F., S. Erdoğan, D. Kor, G. Oto and M. Uluman. 2016. The effects of different types of antioxidants (se, vitamin e and carotenoids) in broiler diets on the growth performance, skin pigmentation and liver and plasma antioxidant concentrations. Brazilian Journal of Poultry Science. 18 (1): 101-116.

25-Kussy M. J., M. K. Salah and F. Sh. Tarki. 2016. Effect of in ovo injection of various

vitamins on hatchability and embryonic mortality during embryogenesis stages of Japanese quails Coturnix japonica. Journal of Kerbala for Agricultural Sciences, Volume 3, Issue 1, Pages 132-145

26-Lessard, M., D. Hutchings and N. A. Cave. 1997. Cell-mediated and humoral immune responses in broiler chickens maintained on diets containing different levels of vitamin A. Poult. Sci. 76:1368–1378

27-Macalintal L. M. 2012. In ovo selenium (SE) injection of incubating chicken eggs: effects on embryo viability, tissue SE concentration, lipid peroxidation, immune response and post hatch development. Theses and Dissertations-- Animal and Food Sciences, University of Kentucky UK knowledge

28-Merkhan M. M. 2013. Effect of spraying Japanese quail hatching eggs with different levels of Glucose on hatchability traits. Int.J. Appl. Poult. Res. 2(1): 12-14

29-Naimi, D., V. Reza, and S.S. Heshmat. 2014. Effect of different levels of vitamin AD3E on performance, response of humoral immune and percentage of tibia ash broilers. Advances in Environmental Biology, 8(9): 303-306

30-Puthpongsiriporn, U., S. E. Scheideler, J. L. Shell and M. M. Beck.2001. Effect of vitamin E and C supplementation on performance in vitro lymphocyte proliferation and antioxidant status of laying hens during heat stress. Poultry Science, 80(8):1190-1200

31-Romao, J. M., T. G. Vasconcelos de Moraes, R. P. Ramos Salles, W. M. Cardose and C. C. Buxade. 2011. Effect of in ovo vaccination procedures on Japanese quail embryos (*Coturnix japonica*) and incubation performance. J. Anim. Brasileira, 12(4):584-592

32-Ross, T.K., V.E. Moss, J.M.., Prahl, J.M., and H.F. Deluca, 1992. A nuclear protein essential for binding of rat 1, 25 dihydroxyvitamin D3 receptor to its response elements. Proc. Natl. Acad. Sci. U S A 1992; 89:256–260

33- Salary J., S. Fatemeh, K. Majid and R. H. M. Hamid.2014. Inovo injection of vitamin E on post-hatch immunological parameters and broiler chicken performance. Asian Pacific Journal of Tropical Biomedicine 4(S2):S616-S619

34-Selim, S.A., K.M. Gaafar and S.S. El-ballal. 2012.Influence of in-ovo administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. Emir. J. Food. Agric.,24 (3): 264-271

35-Selim, N.A., F.S.Youssef, A.F.., Abdel-Salam, and S.A. Nada, 2013b. Evaluations of some natural antioxidant sources in broiler diets: 1-Effect on growth, physiological and immunological performance of broiler chicks. Int. J. Poult. Sci. 12, 561–571

36-Sklan, D., D. Melamed and A. Friedman. 1994. The effect of varying levels of dietary vitamin A on immune response of the chick. Poult. Sci. 73: 843-847

37-Surai, P. F., V. I. Fisinin and F. Karadas. 2016. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium, Animal Nutrition, 2(1), 1–11

38-Tengerdy, R. P. and C. F. Nockels. 1975. Vitamin E or vitamin A protects chickens against E. coli infection. Poult. Sci. 54:1292–1296

39-Tengerdy, R. P. and C. J. Brown. 1977. Effect of vitamin E and A on humoral immunity and phagocytosis in *E. coli* infected chicks. Poult. Sci. 56:957–963

40-Xiao, R., R.F. Power, D., Mallonee, C., Crowdus, . K.M., Brennan, , T. Ao, J.L., Pierce, and K.A. Dawson., 2011.A comparative transcriptomic study of vitamin E and an algae based antioxidant as antioxidative agents: Investigation of replacing vitamin E with the algae-based antioxidant in broiler diets. Poult. Sci. 90, 136–146

41-Yegani, M. and D. R. Korver. 2008. Factor affecting intestinal health in poultry. Poult. Sci., 87: 2052–20633

42- Zimmermann, M. B., P. L. Jooste, N. S. Mabapa, S. Schoeman, R. Biebinger, L. F. Mushaphi and X. Mbhenyane. 2007. Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate1-3. American Journal Clinical of Nutrition, 86: 1040-1044.