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
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). 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, 14th and 30th 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 18th 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. At the 7th 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 (1ml/1liter). 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               | 5         | 5        |
| Ground Limestone     | 1.7       | 1.5      |
| Anti-coccidial       | 0.05      | 0.05     |
| 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 %            | 1         | 0.9      |
| Total phosphorus P%  | 0.55      | 0.51     |
| Sodium Na %          | 0.16      | 0.16     |

1Vitamin Provided per kilogram: vitamin A 400,000 IU; vitamin D 160,000 IU; 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.
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 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:** 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

| 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.** Not Significant

| **(P<0.01).** N.S. | **(P<0.01).** N.S. |
|---------------------|---------------------|

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 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 feed intake 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 had no significant effect on 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 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, 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 Control      | 156.92±3.14  | 418.69±13.11 | 837.24±21.22 | 1477.20±40.93| 2104.30±36.87|
| T2 Injection(N.S)| 147.48±2.14  | 403.16±5.78  | 823.02±14.72 | 1443.00±23.32| 2082.90±44.32|
| T3 Injection    | 145.86±1.92  | 387.76±10.97 | 793.36±13.71 | 1434.20±22.72| 2031.60±45.63|
| T4 Injection    | 148.41±5.60  | 404.53±11.88 | 818.53±20.19 | 1508.00±27.82| 2119.10±27.74|
| T5 Injection    | 148.42±2.88  | 405.74±9.69  | 855.53±18.01 | 1533.00±41.66| 2139.40±53.19|
| Spraying        | 148.34±2.48  | 418.53±3.48  | 836.85±12.99 | 1549.00±26.03| 2140.40±13.55|
| Dipping         | 150.04±4.65  | 419.56±13.29 | 870.37±28.70 | 1538.00±44.17| 2191.60±45.23|
| AD3E            | 153.06±3.45  | 413.22±12.42 | 843.90±17.09 | 1548.00±33.17| 2222.90±34.17|
| N.S             | 151.18±3.87  | 412.96±8.79  | 844.38±16.44 | 1552.10±36.22| 2208.60±28.75|
| 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|
| 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±12.24 | 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|

*(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. 1wk  | Wt. gain 2wk | Wt. gain 3wk | Wt. gain 4wk | 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 | 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  | 2097.45±53.18       |
| Injection       | AD3E      | 106.01±2.47  | 270.18±5.26  | 418.32±15.25 | 712.14±29.87  | 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        |
| 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        |
| N.S             | 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       |
| 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  | 409.08±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: Not Significant

***(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 1<sup>st</sup> wk. | F.I 2<sup>nd</sup> wk. | F.I 3<sup>rd</sup> wk. | F.I 4<sup>th</sup> wk. | F.I 5<sup>th</sup> 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 Control         | 131.70±2.48             | 462.21±11.60            | 586.10±15.95            | 896.56±37.02            | 1112.90±25.77           | 3189.49±63.79            |
| T2 Injection (N.S.)| 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 (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 Injection       | 120.36±3.71             | 482.81±10.53            | 616.88±11.50            | 916.00±24.19            | 1121.00±33.82           | 3257.05±70.25            |
| Spraying (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 Dipping (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            |
| 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            |
| 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±24.14            |
| 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            |

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 1\(^{st}\) wk. | FCR 2\(^{nd}\) wk. | FCR 3\(^{rd}\) wk. | FCR 4\(^{th}\) wk. | FCR 5\(^{th}\) 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 Control         | 1.12±0.01           | 1.77±0.03           | 1.40±0.04           | 1.47±0.05           | 1.72±0.09           | 1.54±0.02        |
| 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        |
| 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 = Not significant
*(P<0.05), ** (P<0.01)
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 Control    | 10.66 ± 3.99      | 348.34 ± 17.11        |
| T2 Control    | 8.00 ± 1.33       | 349.24 ± 17.17        |
| Injection(N.S)| 9.33 ± 3.99       | 349.58 ± 14.43        |
| T3 Injection  | 8.00 ± 2.49       | 339.16 ± 18.88        |
| T4 Injection  | 6.66 ± 2.10       | 358.71 ± 11.67        |
| T5 Injection  | 4.00 ± 2.66       | 377.88 ± 17.29        |
| T4 Spraying   | 2.66 ± 1.63       | 383.61 ± 13.23        |
| T5 Spraying   | 5.33 ± 1.33       | 411.37 ± 8.40         |
| T6 Dipping    | 5.33 ± 2.49       | 400.93 ± 10.40        |
| T7 Dipping    | 4.00 ± 2.66       | 395.35 ± 9.38         |

N.S: Not Significant

**(P<0.01), * (P<0.05)

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                  |                 |                     |                     |
| T1 Control                     | 248.95±6.49     | 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±6.10b    | 123.85±2.65         | 2.92±0.11           |
| T4 Spraying (AD3E)             | 226.80±4.45b    | 128.85±4.21         | 3.03±0.03           |
| T5 Dipping (AD3E)              | 242.00±4.49ab   | 121.85±5.63         | 2.81±0.05           |
| All treatment without AD3E    |                 |                     |                     |
| No AD3E                        |                 |                     |                     |
| Injection (N.S)                |                 |                     |                     |
| T1                             | 241.70±9.63     | 114.40±1.63         | 2.82±0.05           |
| T2                             | 232.60±6.64     | 114.40±8.83         | 2.77±0.06           |
| T3                             | 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           |
| Spraying (AD3E)                | 224.80±7.16     | 128.30±4.96         | 2.96±0.04           |
| T5                             | 233.60±6.64     | 117.50±3.03         | 2.99±0.07           |
| Dipping (AD3E)                 | 246.00±4.10     | 121.30±7.13         | 2.77±0.06           |
| All treatment with AD3E        |                 |                     |                     |
| No AD3E                        |                 |                     |                     |
| Injection (N.S)                |                 |                     |                     |
| T1                             | 234.12±3.12     | 121.20±2.79         | 2.90±0.04           |
| T2 Injection (N.S.)            |                 |                     |                     |
| T3 Injection (AD3E)            |                 |                     |                     |
| T4 Spraying (AD3E)             |                 |                     |                     |
| T5 Dipping (AD3E)              |                 |                     |                     |

*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 ; 29) 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      |                   |         |        |
| T1                 |                   |         |        |
| Control            | AD3E              |         |        |
| T2                 | No AD3E           |         |        |
| Injection (N.S)    | AD3E              |         |        |
| T3                 | No AD3E           |         |        |
| Injection          | AD3E              |         |        |
| T4                 | No AD3E           |         |        |
| Spraying           | AD3E              |         |        |
| T5                 | No AD3E           |         |        |
| Dipping            | AD3E              |         |        |

**All treatment without AD3E**

|                    | Newcastle Disease | Gamboro | IB     |
|--------------------|-------------------|---------|--------|
| T1                 |                   |         |        |
| Control            | AD3E              |         |        |
| T2                 | No AD3E           |         |        |
| Injection (Normal S.) | AD3E              |         |        |
| T3                 | No AD3E           |         |        |
| Injection          | AD3E              |         |        |
| T4                 | No AD3E           |         |        |
| Spraying           | AD3E              |         |        |
| T5                 | No AD3E           |         |        |
| Dipping            | AD3E              |         |        |

**All treatment with AD3E**

|                    | Newcastle Disease | Gamboro | IB     |
|--------------------|-------------------|---------|--------|
| T1                 |                   |         |        |
| Control            | AD3E              |         |        |
| T2                 | No AD3E           |         |        |
| Injection (Normal S.) | AD3E              |         |        |
| T3                 | No AD3E           |         |        |
| Injection          | AD3E              |         |        |
| T4                 | No AD3E           |         |        |
| Spraying           | AD3E              |         |        |
| T5                 | No AD3E           |         |        |
| Dipping            | AD3E              |         |        |

***(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

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**Table Data**

- **Newcastle Disease**
  - T1: 4555.80±169.24
  - Control: 2465.20±116.78
  - T2: 2679.40±158.62
  - Injection (N.S): 4772.00±61.09
  - T3: 4409.60±158.56
  - Injection: 6157.20±77.30
  - T4: 4630.00±177.24
  - Spraying: 5906.20±186.56
  - T5: 4622.40±57.76
  - Dipping: 5567.60±79.41

- **Gamboro**
  - T1: 1453.52±20.58
  - Control: 1262.82±10.05
  - T2: 1540.00±7.91
  - Injection (N.S): 1200.30±18.05
  - T3: 1475.10±17.03
  - Injection: 1613.30±21.08
  - T4: 1311.00±15.86
  - Spraying: 1593.00±21.25
  - T5: 1323.70±55.83
  - Dipping: 1606.80±13.74

- **IB**
  - T1: 513.50±8.71
  - Control: 518.15±7.88
  - T2: 522.80±4.85
  - Injection (Normal S.): 522.48±3.23
  - T3: 504.35±6.82
  - Injection (AD3E): 492.45±6.82
  - T4: 522.80±4.85
  - Spraying (AD3E): 513.70±7.27
  - T5: 504.60±2.93
  - Dipping (AD3E): 510.25±3.29

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**Newcastle Disease Traits**

| Parameter       | T1 | Control | T2 | Injection (N.S) | T3 | Injection | T4 | Spraying | T5 | Dipping |
|-----------------|----|---------|----|----------------|----|-----------|----|----------|----|---------|
| Hatchability    | 5095.00±164.19 | 513.50±8.71 | 518.15±7.88 | 522.80±4.85 | 504.35±6.82 | 522.48±3.23 | 504.60±2.93 | 513.70±7.27 | 504.60±2.93 | 510.25±3.29 |

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**Gamboro Traits**

| Parameter       | T1 | Control | T2 | Injection (N.S) | T3 | Injection | T4 | Spraying | T5 | Dipping |
|-----------------|----|---------|----|----------------|----|-----------|----|----------|----|---------|
| Hatchability    | 513.50±8.71 | 518.15±7.88 | 522.80±4.85 | 522.48±3.23 | 504.35±6.82 | 522.45±6.82 | 522.80±4.85 | 513.70±7.27 | 504.60±2.93 | 510.25±3.29 |

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**IB Traits**

| Parameter       | T1 | Control | T2 | Injection (N.S) | T3 | Injection | T4 | Spraying | T5 | Dipping |
|-----------------|----|---------|----|----------------|----|-----------|----|----------|----|---------|
| Hatchability    | 513.50±8.71 | 518.15±7.88 | 522.80±4.85 | 522.48±3.23 | 504.35±6.82 | 522.45±6.82 | 522.80±4.85 | 513.70±7.27 | 504.60±2.93 | 510.25±3.29 |
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