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# Effect of In-Ovo Injection of Ascorbic, Folic Acids and their Combination on Hachability and Subsequent Growth Performance of Broiler Chicks

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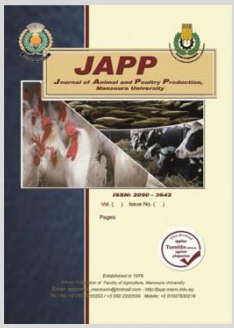


## ABSTRACT

A total number of 1260 fertile eggs were randomly divided into seven treatments to investigate effect of in ovo-injection of ascorbic, folic acids and their combination at 14th day of incubation on hatchability and subsequent growth performance of broiler chicks. The experimental design was as follow: 1- Eggs were without injection. 2- Eggs were injected with 0.1ml distiller water/ egg. 3- Eggs were injected with 0.1ml solution containing 6 µg ascorbic acid (AA) /egg. 4- Eggs were injected with 0.1ml solution containing 75 µg folic acid (FA) /egg. 5- Eggs were injected with 0.1ml solution containing 150 µg FA /egg. 6- Eggs were injected with 0.1ml solution containing 6 µg AA + 75µg FA/egg. 7- Eggs were injected with 0.1ml solution containing 6 µg AA+150µg FA /egg. Hatchability % was improved due to injection of $AA_6$/egg and with combination between $AA_6$ and $FA_{150}$/egg as compared to the control group. Also, in ovo injected with $AA_6$, $FA_{75}$, $FA_{150}$ and $AA_6$+$FA_{75}$ recorded the best value of live body weight compared to the control group at 28 days of age. In ovo injection of AA, FA and their combination significantly decreased triglycerides levels in serum blood. Also, the level of HDL significantly improved due to in-ovo injected with $AA_6$ and $FA_{75}$. Therefor, it could be mentioned that in-ovo injection of 6µg AA/egg and 6µg AA+150µg FA/egg in fertile eggs at 14th day of incubation can be applied to improve the hatchability and subsequent growth performance from 1 to 28 day of age.

***Keywords:*** Hatchability**,** Ascorbic acid, Folic acid, Productive performance

## INTRODUCTION

During embryonic development in ovo injection is a strategy used to adapt chicks to the new diet after hatch and to anticipate the chick's requirements, and there are an energy demanding activity thus this strategy for provision of high energy for chick's hatching, which is. In addition, there is a main growth of the gastrointestinal tract during the initial days of chick age, followed by skeletal and muscular development. There is not enough time for compensatory growth as a result of the short lifespan of broilers, (Kornasio *et al.*, 2011) thus, it is necessary to stimulate beneficial impacts on growth performance of the broiler chickens during the initial growth to chick. Also, in ovo injection of some nutrients such as vitamins and minerals may help overcome any constraint of inadequate egg nutrition (Selim *et al.*, 2012). The period between the hatch and grow-out phases is called transitional and residual yolk is the major source of nutrients (Henderson *et al.*, 2008). The growth rate of broiler's chick is very high during the initial growth (first two week) of post hatch (Oliveira *et al.*, 2015).

Folic acid is vitamin B-complex group (water-soluble) and it is l-glutamic acid, N-[4-[[(2-amino-1-4-dihydro-4-oxo-6-pteridinyl) methyl] amino] benzoyl] which present in liver and yeast (Nouri *et al.*, 2014). In respect of the function of folic acid, it is important in the synthesis of amino and nucleic acids and the metabolism of these compounds where it is acts as coenzyme in single-carbon transfer (Abd El-Azeem *et al.* 2014). In addition, the folic acid is a critical water soluble vitamin for reproduction to all animals, and its requirement for hatch the eggs are higher as comparing with for egg production (Vieira 2007). According to the NRC (1984) the folic acid requirement in case poultry for egg hatchability is comparatively higher than that for production. The amount of folic acid for broiler chicks was 0.55 mg/kg of diet from 1day to 42 days of age, while this requirement during the cycle of egg production is 0.25 mg/kg of diet (NRC 1984).

Ascorbic acid (AA), a water-soluble vitamin with multiple biochemical functions, has been shown to benefit avian embryonic development during incubation in a dose-dependent manner. Scientific publications stated that in ovo injection of ascorbic acid during the period of embryogenesis (incubation the embryo) may reduce the effects of stress due to overheating and, consequently, improve percentage of hatchability, embryo weight at different incubation days, reduction of embryo death during incubation and body weight after hatching (Zakaria and AL-Laif 1998; Zakaria *et al.* 1998). There is no recommended requirement established by NRC where the research studies have illustrated that under ordinary conditions, healthy animals do not respond to supplemental vitamin C.

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In addition, during embryonic development high metabolic rates can lead to production free radicals from reactive oxygen species (ROS) (Halliwell, 1994). Free radicals are extremely reactive; these reactions lead to peroxidation (oxidation in living system) of polyunsaturated fatty acids in plasma membrane of the cell and as a result loss of membrane functions. For example, deoxyribonucleic acid damage may directly induce inhibition of proteins such as enzymes synthesis and indirectly cause mutation or cells death (Vasudevan and Sreekumari, 2001).

Antioxidants play a main function in protecting cell membrane from the actions of reactive oxygen species (ROS) by reducing free radicals and regarding the process of lipid peroxidation which happen in living systems, antioxidants break these interactions, thus they may be prevent the damage induced due to (ROS) (Yu 1994). In ovo injection of some nutrients may help late-term embryos to overcome the constraints of limited nutrients in egg (Foye *et al.*, 2005). Antioxidant is considered determinant of chick viability during early post-hatch period (Surai, 2000).

Among the well known antioxidants, AA and FA where, it is reported that FA has good effect on peroxidation in the living system by alleviate the superoxide dismutase, malondialdehyde and glutathione peroxidase (Goh and Koren 2008). The superoxide dismutase is a first-step defense that converts oxygen radicals ($O_2^-$) to hydrogen peroxide ($H_2O_2$) (Underwood and Suttle, 1999). Decreases in superoxide dismutase activity, may be resulted in an increase amounts of nucleic acid, protein and lipid damage, which can induce cellular damage (Kokoszka *et al.*, 2001).

Thus, the current study was designed to investigate thoroughly the hatchability of fertile eggs, subsequent effect growth performance as well as economic efficiency as a result from in-ovo injection of ascorbic, folic and their combination at 14$^{th}$ day of incubation.

## MATERIALS AND METHODS

**Artificial incubation and protocol of egg injection:**

This study was conducted in a commercial incubation company, Egypt. A total number of 1260 fertile eggs were obtained from Hubbard® breeder hens of 53 week of age were obtained from a commercial company and all eggs were incubated in automatic setter and incubator under stander conditions at 37.5 C and 65 % relative humidity in incubator. The average weight of the eggs was 68.98 g, with a range of 67.09 to 70.19 g/ egg. At day 14$^{th}$ of incubation, 1260 fertile eggs were randomly divided into seven treatments each of 180; in three replicates each of 60 fertile eggs. Eggs were allotted to a completely randomized experimental design with seven treatments where eggs were punctured in the large end to make a whole by hard and thin stylus; all the eggs were injected in air sac with 0.1ml/ egg by using insulin syringe (1ml) then sealed by non-toxic glue. Ascorbic acid (AA) and folic acid (FA) (Synth, 99% purity) were used in the current study as follow:

1- The control group, eggs were without injection.
2- The second group, eggs were injected with 0.1ml distiller water/ egg.
3- The third group, eggs were injected with 0.1ml solution 6 µg AA /egg.
4- The fourth group, eggs were injected with 0.1ml solution containing 75 µg FA /egg.
5- The fifth group, eggs were injected with 0.1ml solution containing 150 µg FA /egg.
6- The six group, eggs were injected with 0.1ml solution containing 6 µg AA + 75µg FA/egg.
7- The seven group, eggs were injected with 0.1ml solution containing 6 µg AA+150µg FA /egg.

**Birds, management and broiler's diets:**

A total number of 210 broiler chicks hatched from the experimental treatments were weighed, divided into seven treatments, three replicates for each(10 chicks each). The chicks were reared under similar environmental and managerial conditions. In addition, the chicks were fed on a standard starter diet contained 23% crude protein (CP) and 3060 Kcal/Kg diet from 1 to 11days of age then the grower diet contained 21%CP and 3180Kcal/kg diet from 12 to 24 days and were provided with feed and water *ad libitum*. The diet was formulated according to the requirement recommended by National Research Council (NRC)1994.Chemical compositions to the diets of broiler were shown in Table (1).

**Table 1. Composition and calculated analysis of the experimental diets (1-28 days of age).**

| Item | Starter | Grower |
|---|---|---|
| | 1 – 11 day | 12 – 24 day |
| Ingredients % | | |
| Corn | 50.7 | 58.0 |
| Soybean meal (44% cp) | 37.0 | 28.4 |
| Guillotine ( 60% cp) | 3.7 | 5.5 |
| Limestone | 1.9 | 1.8 |
| Soya oil | 4.0 | 3.9 |
| Mono calcium phosphate | 1.3 | 1.2 |
| L-Lysine | 0.3 | 0.2 |
| Vitamin – mineral premix | 0.3 | 0.3 |
| DL-methionine | 0.3 | 0.26 |
| Salt | 0.3 | 0.3 |
| Sodom bicarbonate | 0.1 | 0.1 |
| L-Threonine | 0.1 | 0.04 |
| Calculated analysis | | |
| Crude protein (%) | 23 | 21 |
| Metabolic energy (kcal/kg) | 3060 | 3180 |
| Crude fate (%) | 5.9 | 6.4 |
| Crude fiber (%) | 3.6 | 3.6 |
| Total lysine | 4.6 | 4.1 |
| Total methionine | 1.8 | 1.7 |
| Total Methionine + Cysteine | 3.6 | 3.2 |
| Total available phosphorus | 1.6 | 1.4 |
| Total calcium | 3.2 | 3.0 |

**Each 3 kg of the Vitamin – mineral premix manufactured by multi-vita Company, Egypt contains: Vitamin A 12 MIU, Vit. D 5 MIU, Vit E 80 g, Vit. K 4 g, Thiamin 4 g, Riboflavin 9 g, Pyridoxine 4 g, Niacin 60 g, Vit. B12 25 mg, Pantothenic acid 10 g,Folic acid 2 g, Biotin 150 mg, Choline chloride 500 g, Manganese 100g, Zinc 100 g, Iron 40 g, Copper 15 g, Iodine 1.25g, Selenium 0. 35 g, Cobalt 0.20 g. and carrier CaCO3 to 3000 g..**

**Experimental parameters measured:**

1-Hatchability traits: At hatch, all hatched chicks were individually weighted and the hatchability of fertile eggs was measured.
2-Growth performance: All chicks were weighed throughout the period at 1, 7, 14, 21 and 28 days of age. of age. Body weight gain , feed consumption and feed

conversion ratio were calculated through the periods 1–7, 8–14, 15–21 and 22-28 days of age.

3-Slauter test: at the end of study, three chicks per treatment were randomly selected and slaughtered; data of carcass traits (including eviscerated carcass, giblets, edible parts and abdominal fat) and pancreas were calculated as a percentage of live weight. And the blood samples were collected to estimate hematological and biochemical traits as follow:

**Biochemical analysis of blood:**

At time of slaughter, samples of blood from each chick were collected without anticoagulant. The tubes which contained blood clot were centrifuged at 3500 rpm for 20 minutes to separate the serum that used for determination of serum total protein, total cholesterol, triglycerides, HDL , LDL cholesterol, AST and ALT enzymes according to the methods by (Peters, 1968); (Ellefson and Caraway, 1976); (Bucolo and David, 1973); (Siedel, 1983) and (Reitman and Frankel, 1957) respectively. These biochemical measurements were determined calorimetrically by using commercial kits.

**Statistical analysis:**

Statistically, data were analyzed using General Linear Models Procedure of the SPSS (2008), differences between treatments were subjected to Duncan´ s Multiple Range – test (Duncan, 1955).

The following model was used to study the effect of treatments on the parameters investigated as follows:

$$Yij = \mu + Ti + eij$$

**where:**

**Yij = an observation, μ = overall mean, Ti = effect of treatment (i=1, 2, 3, 4, 5, 6,7) and eij = Random error.**

## RESULTS AND DISCUSSION

Effect of in ovo injected with ascorbic acid (AA), folic acid (FA) and their combination into air sac at day 14 of incubation on % hatchability of fertile egg and chick body weight (BW) at hatch are shown in table (2). According to the results, significant alternatives were detected due to in ovo injection in Hubbard® broiler breeder eggs by AA, FA and their combination. The results showed that 6 µg AA/egg or 150 µg FA + 6 µg AA / egg resulted in a higher hatchability values than control fertile eggs by about 9.97 and 3.71 % respectively. Regarding BW at hatch, no significant differences were detected among chicks at hatch due to injection by different levels of AA, FA and their combination.

**Table 2. Effect of In-ovo injection of folic acid, ascorbic acid and their combination at 14th day of incubation on hatchability of fertile eggs.**

| Trails | Control | Sterile Distilled Water Contained A.A , F.A And A.A + F.A | | | | | | SEM | Sig |
|---|---|---|---|---|---|---|---|---|---|
| | | Distiller water | A.A | F.A 75 | F.A 150 | F.A 75 + A.A | F.A 150 + A.A | | |
| Fer. % | 60.33 | 59.67 | 61.67 | 60.00 | 59.33 | 58.00 | 62.33 | 0.833 | N.S |
| E.W | 68.18 | 68.78 | 69.12 | 69.38 | 69.26 | 69.17 | 68.97 | 0.16 | N.S |
| N.C.H | 46.00$^{ab}$ | 43.67$^{ab}$ | 51.67$^{a}$ | 44.33$^{ab}$ | 45.67$^{ab}$ | 37.33$^{b}$ | 49.33$^{a}$ | 1.30 | * |
| Chick.W | 46.92 | 48.25 | 48.51 | 47.89 | 47.89 | 48.71 | 48.25 | 0.27 | N.S |
| H.of F.E | 76.20$^{ab}$ | 73.53$^{ab}$ | 83.80$^{a}$ | 73.90$^{ab}$ | 77.13$^{ab}$ | 67.13$^{b}$ | 79.03$^{a}$ | 1.76 | * |

**a,b: means in the same row bearing different superscripts are significantly different ( $p \leq 0.05$ ).**

According to the current results, no significant differences were detected on hatchability rate of fertile eggs due to in ovo injected eggs with AA, FA and their combination. However, the hatchability rate was improved due to injected with 6 µg AA/egg and with combination between 6 µg AA and 150 µg FA/egg by about 9.97 and 3.71% respectively. These results agreement with published results by (Zakaria and AL-Latif 1998; Zakaria *et al.* 1998) who demonstrated that vitamin C injection into chicken eggs have a favorable impact on percentage of hatchability rate, embryo weight during incubation, as well as on the reduction of embryo death during incubation and on chick body weight after hatching. Also, a favorable influence on hatchability due to injected with 3 mg/egg of vitamin C into broiler breeder eggs on day 13 of incubation was also reported by Ipek *et al.* (2004). On the other hand, Nowaczewski *et al.*, (2012) illustrated that injection of AA in chicken eggs failed to influence hatchability but, in case of duck eggs in ovo injection of different doses of AA (4 and 8 mg/egg) on the 20th day of incubation increased hatchability rate by decreasing proportions of dead and un-hatched embryos. Nouri *et al.* (2017) mentioned that in ovo feeding with FA (40, 80 and 120 µg/egg did not effect on hatchability percentage of broiler's eggs.

This improvement in hatchability rate might be explained according to some scientific basics during embryonic development where during this period high metabolic rates can cause chain reaction leads to thousands of events which resulted in production of reactive oxygen species (ROS) (Halliwell, 1994). Free radicals are extremely reactive where they react with a normal compound, other free radicals are generated. Peroxidation of polyunsaturated fatty acids leads to loss of plasma membrane functions. Moreover, deoxyribonucleic acid damage may cause inhibition of proteins directly such as enzymes and indirectly cause mutation or cell death and as a result carcinogenesis (Vasudevan and Sreekumari, 2001).

Regarding ascorbic acid, it is well known for its antioxidant properties as it assists the body in contesting toxicity, viral and bacterial infections. Ascorbic acid is an important structural component of tendons, bones, blood vessels and muscles; also it is required for collagen synthesis. Vitamin C enhances iron adsorption and regenerates other antioxidants such as vitamin E (Brody, 1994). Also, folic acid may play an important role as an antioxidant in vivo, both by preventing the negative effects of free radicals, as well as by inhibiting lipid peroxidation (Merola *et al.*, 2013). The antioxidant potency of FA was also reported by (Gliszczyńska-Świgło 2006). The nutritional importance of FA lies in its vital role in one-carbon metabolism; these include amino acid synthesis such as synthesis of serine and glycine (Bailey, 2007), the latter is critical for synthesis and repair DNA, for all cell replication, including normal foetal development (Stover, 2010). In

addition, data obtained by Bekhet *et al*., (2013) suggest that supplementation with vitamin c and folic acid during incubation may prevent defects in heart of chick embryo by environmental xenobiotic. The advantageous impact of ascorbic acid injected into duck eggs is attributed to its modifying effect on adrenal gland metabolism and inhibiting the synthesis of 21-hydroxylase and 11-beta hydroxylase, i.e. enzymes which take active part in production of corticosterone (Tullet 1990).

The results concerning live body weight (BW) from 7 to 28 day of age as influenced by in-ovo injection are shown in Table 3. The results illustrated that all in-ovo injection treatments during embryogenesis led to a significant ($p \leq 0.05$) higher BW at 7 day of age compared to the control group. Also, live BW of birds from in-ovo injected with 6 µg AA, 150 µg FA and 6 µg AA + 75 µg FA was significantly increased as compared to the control treatment at 14 day of age. In addition, at 21 day of age, all in-ovo injection at 14$^{th}$ of incubation except for in-ovo injected with distiller water and 6 µg AA/ egg resulted in a significant increase in live BW at 21 day of age compared to the control group. No significant alternations in live BW at 28 day of age were detected due to in-ovo injection of AA, FA and their combination at 14$^{th}$ day of incubation; however, the results revealed that in-ovo injection treatments with $AA_6$, $FA_{75}$, $FA_{150}$ and $AA_6$+$FA_{75}$ recorded the best value of live BW compared to the control group by about 51.46 to 69.48 g/ bird.

**Table 3. Effect of in-ovo injection with folic acid, ascorbic acid and their combination at the 14$^{Th}$ day of incubation on live body weight.**

| Trails | Control | Sterile Distilled Water Contained A.A , F.A And A.A + F.A | | | | | | SEM | Sig |
|---|---|---|---|---|---|---|---|---|---|
| | | Distiller water | A.A | F.A 75 | F.A 150 | F.A 75 + A.A | F.A 150 + A.A | | |
| 0 day | 46.92 | 48.25 | 48.51 | 47.89 | 47.90 | 48.71 | 48.26 | 0.27 | NS |
| 7 day | 193.32$^{b}$ | 215.36$^{a}$ | 220.00$^{a}$ | 215.55$^{a}$ | 213.36$^{a}$ | 223.14$^{a}$ | 211.14$^{a}$ | 2.53 | * |
| 14 day | 498.42$^{b}$ | 522.11$^{ab}$ | 536.76$^{a}$ | 523.05$^{ab}$ | 529.61$^{a}$ | 529.39$^{a}$ | 523.96$^{ab}$ | 3.64 | * |
| 21 day | 877.36$^{b}$ | 900.60$^{b}$ | 905.59$^{b}$ | 1018.27$^{a}$ | 1017.37$^{a}$ | 1024.36$^{a}$ | 1029.23$^{a}$ | 16.61 | * |
| 28 day | 1148.37 | 1131.02 | 1199.83 | 1212.63 | 1217.85 | 1214.80 | 1160.30 | 14.19 | NS |

**a,b: means in the same row bearing different superscripts are significantly different ( $p \leq 0.05$ ).**

In respect of body weight gain (BWG) at different stages of study as shown in Table 4, it is clearly showed that at 7 days of age, chicks from eggs in-ovo injected with AA, FA and their combination had the highest significant values of BWG compared to the control treatment, while all in-ovo injection treatments did not actually differ from control group in the value of BWG at 14 days after hatch. Body weight gain values at 21 days almost showed the same trend observed at 7 days with a slight variation where , the highest values of BWG were recorded due to in-ovo injection of 75 µg FA/egg , 150 µg FA , AA + FA75 and AA + FA 150 as compared to the control treatment. However, body weight gain of chicks hatched from eggs injected with $AA_6$ significantly increased as compared to those hatched from eggs injected with the tow levels of combination between AA and FA at 28 day of age. Dealing with the collective data showed that no significant differences in average BWG could be detected among treatments. However, all in- ovo treatments with exception injected with 0.2 ml distiller water/egg ted to clearly improve in BWG ranged from 49.87 to 67.64 g/ bird as compared to the control group.

**Table 4. Effect of in-ovo injection with folic acid, ascorbic acid and their combination at the 14$^{Th}$ day of incubation on body weight gain.**

| Trails | Control | Sterile Distilled Water Contained A.A , F.A And A.A + F.A | | | | | | SEM | Sig |
|---|---|---|---|---|---|---|---|---|---|
| | | Distiller water | A.A | F.A 75 | F.A 150 | F.A 75 + A.A | F.A 150 + A.A | | |
| 7 day | 146.40$^{b}$ | 167.11$^{a}$ | 171.49$^{a}$ | 167.65$^{a}$ | 165.46$^{a}$ | 174.42$^{a}$ | 162.88$^{a}$ | 2.47 | * |
| 14 day | 305.10 | 306.75 | 316.76 | 307.50 | 316.24 | 306.25 | 312.81 | 2.87 | NS |
| 21 day | 378.93$^{b}$ | 378.48$^{b}$ | 368.83$^{b}$ | 495.22$^{a}$ | 487.76$^{a}$ | 494.97$^{a}$ | 505.27$^{a}$ | 15.37 | * |
| 28 day | 271.01$^{a}$ | 230.42$^{ab}$ | 294.23$^{a}$ | 194.36$^{ab}$ | 200.47$^{ab}$ | 190.43$^{ab}$ | 131.06$^{b}$ | 15.81 | * |

**a,b: means in the same row bearing different superscripts are significantly different ( $p \leq 0.05$ ).**

Results obtained in Table (5) clearly observed that the subsequent effect of the experimental in-ovo injection of AA, FA and AA + FA on feed intake /bird/day was significantly among treatment at 7, 14 and 28 day of age. It is clear from the results that birds from eggs injected with distilled water recorded significantly higher feed intake than those from eggs injected with $FA_{75}$ + $AA_6$ at 7 day. The same treatment (injected with distilled water) tend to a significant increase in feed intake /bird /period compared to control and other treatments with exception AA6 and $AA_6$ + $FA_{150}$ at 14 day of age. On the other hand, no significant differences were detected in feed intake/bird/period due to using any of the in-ovo injection treatments at 21 of age. However, feed intake was significantly decreased due to in-ovo injection at 14$^{th}$of incubation with $AA_6$a scompared to other injection treatments except for distiller water and control group at 28 days of age. It could be generally concluded that irrespective of the fluctuation observed in feed intake, feed intake/ bird during the whole period of study did not affected by in-ovo injection at 14$^{th}$ of incubation, however it was insignificant increased as a result of all in-ovo injection treatments with exception feed intake of chicks from eggs injected with $AA_6$ as compared to the control group.

**Table 5. Effect of in-ovo injection with folic acid, ascorbic acid and their combination at the 14Th day of incubation on feed intake.**

| Trails | Control | Sterile Distilled Water Contained A.A , F.A and A.A + FA | | | | | | SEM | Sig |
|---|---|---|---|---|---|---|---|---|---|
| | | Distiller water | A.A | F.A 75 | F.A 150 | F.A 75 + A.A | F.A 150 + A.A | | |
| 7 day | 197.29$^{ab}$ | 217.28$^{a}$ | 202.53$^{ab}$ | 205.13$^{ab}$ | 212.35$^{ab}$ | 193.87$^{b}$ | 199.86$^{ab}$ | 2.77 | * |
| 14 day | 362.49$^{b}$ | 414.49$^{a}$ | 387.43$^{ab}$ | 356.11$^{b}$ | 377.81$^{b}$ | 365.53$^{b}$ | 385.63$^{ab}$ | 5.34 | * |
| 21 day | 631.50 | 593.19 | 612.83 | 626.94 | 608.20 | 618.86 | 613.63 | 8.34 | NS |
| 28 day | 682.74$^{bc}$ | 681.36$^{bc}$ | 641.38$^{c}$ | 736.94$^{ab}$ | 734.43$^{ab}$ | 771.91$^{a}$ | 738.01$^{ab}$ | 10.85 | * |

**a,b,c: means in the same row bearing different superscripts are significantly different ( p ≤ 0.05 ).**

Data of feed conversion ratio (FRC) of chicks hatched from eggs injected with AA, FA and their combination are showed in Table 6. The results observed that FRC of chicks was significantly improved due to in-ovo injected with AA+$FA_{75}$ / egg compared to the control group during the first week. Also all in-ovo injection treatments resulted in insignificant improves in FRC except for in-ovo injected with AA 6 compared to control during the third week of age. But, chicks from eggs injected with distiller water had significantly low FRC than the control and other treatments at 14 day of age. Significant differences were observed in FRC as a result of subsequent effect to in-ovo injection of AA, FA or their combination at 28 day of age where, the combination between AA and FA either low or high level resulted in a significant decrease in FCR as compared to the control group, therefore,. In addition, no significant influence due to in–ovo injected with AA, FA and their combination on collective data of FCR could be detected as compared to the control group, but among treatment was observe significant differences, as chicks from eggs injected with AA6 confirmed the significant superiority in respect of FCR compare to combination between AA and FA, thus these results suggest that the effect between AA and FA is antagonistic effect.

**Table 6. Effect of in-ovo injection with folic acid, ascorbic acid and their combination at the 14Th day of incubation on feed conversion ratio.**

| Trails | Control | Sterile Distilled Water Contained F A.A , F.A And AA + FA | | | | | | SEM | Sig |
|---|---|---|---|---|---|---|---|---|---|
| | | Distiller water | AA | FA 75 | FA 150 | AA+ FA 75 | AA+ FA 150 | | |
| 7 day | 1.35$^{a}$ | 1.30$^{ab}$ | 1.18$^{ab}$ | 1.22$^{ab}$ | 1.28$^{ab}$ | 1.11$^{b}$ | 1.23$^{ab}$ | 0.02 | * |
| 14 day | 1.18$^{b}$ | 1.35$^{a}$ | 1.22$^{b}$ | 1.15$^{b}$ | 1.19$^{b}$ | 1.19$^{b}$ | 1.23$^{b}$ | 0.01 | * |
| 21 day | 1.66$^{ab}$ | 1.61$^{ab}$ | 1.70$^{a}$ | 1.26$^{ab}$ | 1.24$^{ab}$ | 1.25$^{ab}$ | 1.21$^{b}$ | 0.06 | * |
| 28 day | 2.67$^{b}$ | 3.08$^{ab}$ | 2.25$^{b}$ | 3.92$^{ab}$ | 3.68$^{ab}$ | 4.29$^{ab}$ | 8.94$^{a}$ | 0.74 | * |

**a,b: means in the same row bearing different superscripts are significantly different ( p ≤ 0.05 ).**

Regarding subsequent effect on FI, BW and FCR, in ovo injected with vitamin C decreased average daily FI at 28 day of age. However, there were insignificant improve in FCR due to in ovo injected with AA. This is in agreement with the results by Zakaria (2001) who recorded that body weight of male broiler chickens was improved due to in ovo injected with 3 mg ascorbic acid at the 15th day of incubation. Also, in agreement with results in present study, Hajati *et al.*, (2014) found that in ovo feeding of ascorbic acid improved average daily weight gain of chickens comparing with penetrated control. According to the results by Nouri *et al.*, (2017) FI increased in birds hatched in ovo feed with FA (40, 80 and 120 µg/egg) and BWG improved in birds in ovo injected with 120 µg/egg on 21 days of age after hatch which our observations was similar to previous study. These results may be due to the beneficial impact of the FA for cellular functions, protein biosynthesis and optimizing the balance between amino acids either essential or nonessential (Abd El-Azeem *et al.*, 2014). Furthermore, folates donate one-carbon units in the process of DNA -biosynthesis with implications for the regulation of gene expression, transcription, chromatine structure, genomic repair and genomic stability (Stanger2002).

As shown in Table 7, it could be observed that the lowest value of ALT was those of in-ovo injected with AA6 and AA6 + FA75 treated chicks being significantly lower than control birds, while the highest record was attained by in-ovo injection of FA75 alone.

**Table 7. Effect of in-ovo injection with folic acid, ascorbic acid and their combination at14Th day of incubation on serum biochemical traits.**

| Trails | Control | Sterile Distilled Water Contained A.A , FA And AA + FA | | | | | | SEM | Sig |
|---|---|---|---|---|---|---|---|---|---|
| | | Distiller water | AA | FA 75 | FA 150 | AA+ F.A 75 | AA+ FA 150 | | |
| ALT (U/L) | 43.00$^{ab}$ | 31.50$^{cd}$ | 31.67$^{cd}$ | 50.00$^{a}$ | 43.50$^{ab}$ | 29.50$^{d}$ | 39.00$^{bc}$ | 1.79 | * |
| AST (U/L) | 67.00$^{a}$ | 52.00$^{bc}$ | 55.00$^{b}$ | 63.00$^{a}$ | 50.50$^{cd}$ | 63.00$^{a}$ | 47.00$^{d}$ | 1.62 | * |
| T.Protein (gm/dl) | 4.36$^{a}$ | 3.72$^{a}$ | 4.04$^{a}$ | 4.06$^{a}$ | 3.55$^{a}$ | 4.29$^{a}$ | 2.52$^{b}$ | 0.15 | * |
| Albumin (gm/dl) | 2.13$^{a}$ | 1.34$^{cd}$ | 1.22$^{de}$ | 1.66$^{b}$ | 1.51$^{bc}$ | 2.06$^{a}$ | 0.96$^{e}$ | 0.93 | * |
| Creatinine (mg/dl) | 0.61$^{d}$ | 2.19$^{ab}$ | 1.21$^{c}$ | 2.48$^{a}$ | 1.03$^{c}$ | 1.22$^{c}$ | 2.15$^{b}$ | 0.15 | * |
| Trig. (mg/dl) | 80.00$^{a}$ | 61.75$^{c}$ | 62.30$^{c}$ | 47.50$^{d}$ | 44.35$^{d}$ | 50.40$^{d}$ | 72.10$^{b}$ | 2.83 | * |
| Choles. (mg/dl) | 170.00$^{ab}$ | 170.67$^{ab}$ | 187.00$^{a}$ | 171.50$^{ab}$ | 170.50$^{ab}$ | 154.50$^{b}$ | 179.00$^{a}$ | 2.87 | * |
| HDL (mg/dl) | 59.00$^{bc}$ | 59.00$^{bc}$ | 66.00$^{a}$ | 65.50$^{a}$ | 53.50$^{c}$ | 56.50$^{c}$ | 63.00$^{ab}$ | 1.10 | * |

**a,b,c,d: means in the same row bearing different superscripts are significantly different ( p ≤ 0.05 ).**

On the other hand, the chicks hatched from in-ovo eggs injected with all treatments with exception FA75 and AA6 + FA75 were significantly lower in respect AST than those hatched from control eggs. While, all in-ovo injection treatments did not show any significant effect on total protein with exception birds from in-ovo injected with AA6 + FA150 where they had significantly lower total protein compared to control and other groups, also the same treatment (AA6 + FA150)resulted in a significant decrease in albumin compared to the control and most treatments.

Regarding kidney function the results obtained showed that blood serum kreatinine of chicks from in-ovo injected with FA75 was significant higher than control and other in-ovo injected with 0.2 ml distiller water/egg. On the other hand, the results in the current study evident that in-ovo injected with different levels of FA and combination between FA and AA significantly decreased serum triglycerides compared to the control group. But, serum cholesterol was insignificant reduced to in-ovo injected with AA6 + FA75 compared to control group. Also, in this respect in-ovo injected with AA6 and FA75 resulted in a significant increase in serum HDL (mg/dl) when compared to control group, while no significant differences were observed among the other treatments and control group in serum HDL.

As seen, in ovo injection of AA, FA and their combination significantly decreased triglycerides (TG) levels in serum blood, also in ovo injected with AA6+FA150 increased cholesterol levels. While the level of HDL significantly improved due to in ovo injected with AA6 and FA75. These results may be explaining the improvement which is observed in hatchability and subsequent effect in productive performance where TG levels might an indicator for lipolysis (Owoyele *et al.*, 2005). In addition, HDL (good cholesterol) removes cellular cholesterol and transferring it to the liver for excretion (Pinto *et al.*, 2005). The results are in agreement with those reported by Nouri *et al.* (2017), who reported that that in ovo injection of FA decreased cholesterol and triglyceride but who found that HDL is decreased in broilers. Whitehead *et al.*, (1995) reported that administration of FA to the broilers diet (2 mg/kg) did not effect on red blood cell phosphoribosyl pyrophosphate concentrations.

## CONCLUSION

According to the current results, it could be mentioned that in-ovo injection of 6µg AA/egg and 6µg AA+150µg FA/egg in broiler eggs at 14$^{th}$ day of incubation can be applied to improve the hatchability of fertile eggs and subsequent growth performance during the stage from 1 to 28 days of age.

## REFERENCES

Abd El-Azeem N.A.; Abdo M.S.; Madkour, M. El-Wardany. I. (2014). Physiological and histological responses of broiler chicks to in ovo injection with folic acid or l-carnitine during embryogenesis. Glob Vet 13(4):544–551

Bailey, L. B. (2007). Folic acid. In J. Zempleni, R. B. Rucker, D. B. McCormick & J. W. Suttie (Eds.), *Handbook of Vitamins*, (4th edition, pp. 385–413), Boca Raton, Forida, USA: CRC Press (Taylor & Francis Group, LLC. http: //www. Foodnetbase .com /books /5534 /4022_C012.pdf (accessed November 15, 2012).

Bekhet, M. G.; Al-Kahtani, M. A. and Abdel-Moneim, A. M. (2013). The protective effects of vitamin C and folic acid against methylmercury teratogenicity in chick embryo. Jornal of cell and animal biology Vol.7 pp.77-84.

Brody, T. (1994). Nutritional Biochemistry. (Pp. 450–459). San Diego, CA: Academic Press.

Bucolo, G. and David, H (1973). Quantitative determination of serum triglycerides by the use of the enzyme .Clin. Chem., 19:475.

Campbell, T.W. (1995). Avian Hematology and Cytology, Iowa State University Press, Ames, Iowa, USA. pp: 3-19.

Duncan, D.B. (1955). Multiple ranges and multiple f-test, Biometries 11: 1-42.

Ellefson, R. D. and Caraway, W.T. (1976). Fundamental of clinical chemistry .Ed Tietz NW, p 506.

Foye, O.T.; Uni, Z. and Ferket, P.R. (2006). Effect of in ovo feeding egg white protein, beta-hydroxy-betamethylbutyrate, and carbohydrates on glycogen status and neonatal growth of turkeys. Poultry Science, 85: 1185-1192.

Gliszczyńska-Świgło, A. (2006). Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. *Food Chemistry*, 96, 131–136.

Gross, W.B. and Siegel, P.B. (1986). Effects of initial and second periods of fasting on heterophil/lymphocyte ratios and body weight. Av. Dis., 30:345 346.

Halliwell, B. (1994). Free radicals and antioxidants: A personal view. Nutr. Rev. 52 (8): 253-265.

Henderson S.N.; Vicente, J.L.; Pixley, C.M.; Hargis, B.M. and Tellez, G. (2008). Effect of an early nutritional supplement on broiler performance. Int J Poult Sci 7:211–214

Ipek, A.; Sahan, U. and Ylmaz, B (2004). The effect of *in ovo* ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. Arch. Geflügelk. 68: 132-135.

Kornasio, R.; Halevy, O.; Kedar, O. and UNI, Z. (2011). Effect of in ovo feeding and its interaction with timing of first feed on glycogen reserves, muscle growth, and body weight. Poultry Science 90: 1467-1477.

Merola, N.; Alonso, F. J. G.; Ros, G. and Cáston, M. J. P. (2013). Antioxidant activity comparison between [6S]-5-methyltetrahydrofolic acid calcium salt and the related racemate form. *Food Chemistry*, 136, 984–988.

Nouri1, S.; Jamshid G.; Ghalehkandi, S.; Hassanpour and Shahryar, H. A. (2014). The effect of *in ovo* injection of grape seed extract and vitamin C on hatchability, antioxidant activity, yolk sac weight, performance and ileal micro flora of broiler chickens. Res. Opin. Anim. Vet. Sci., 2014, 4(12): 633-638

Nowaczewski, S.; Kontecka. H. and Krystianiak, S. (2012). Effect of *In Ovo* Injection of Vitamin C During Incubation on Hatchability of Chickens and Ducks. Foliabiologica (Kraków),vol.60,No1-2.

Oliveira, T,F,B,; Bertechini A.G.; Bricka, R.M.; Kim, E.J.; Gerard, P.D. and Peebles, E.D. (2015). Effects of in ovo injection of organic zinc, manganese, and copper on the hatchability and bone parameters of broiler hatchlings. Poult Sci 94:2488–2494

Owoyele, B.V.; Yakubu, M.T.; Alonge. F.; Olatunji, L.A. and Soladoye, A.O. (2005). Effects of folic acid intake on serum lipid profiles of apparently healthy young adult male Nigerians. Afr J Biomed Res 8:139–142

Peters, T. (1968). Determination of total protein in serum. Clinical Chemistry, 14:1147.

Pinto, X,; Vilaseca, M.A.; Balcells, S.; Artuch, R.; Corbella, E.; Meco, J.F.; Vila, R,; Pujol, R. and Grinberg, D. (2005). A folate-rich diet is as effective as folic acid from supplements in decreasing plasma homocysteine concentrations. Int J Med Sci 2:58–63. Foye OT (2005) The biochemical and molecular effects of amnionic nutrient administration, "in ovo feeding" on intestinal development and function and carbohydrate metabolism in turkey embryos and poults. Ph.D

Reitman, S. and S. Frankel (1957). Coloric determination of GOT or GPT activity. Am. J. Clin. Path., 28-56.

Ritchie, B. W.; Harrison, J. G. and Harrison, R. L. (1994). Avian Medicine. Winger's Publishing Inc, Florida, USA, pp. 176-198.

Selim, S.A.; Gaafar, K.M. and El-ballal, S.S. (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

Siedel, J. (1983). Test combination of cholesterol. Clin. Chem. 29: 1075.

Stanger O. (2002). Physiology of folic acid in health and disease. Curr Drug Metab 3:211–223.

Stover, P. J. (2010). Folate biochemical pathways and their regulation. In L. B. Bailey (Ed.), *Folate in Health and Disease*, (2nd edition, pp. 49–74). Boca Raton, Forida, USA: CRC Press (Taylor & Francis Group, LLC).

Surai, P.F. (2000). Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. British Poultry Science, 41: 235-243.

Tullett, S. C. (1990). Science and the art of incubation. Poult. Sci. 69: 1-15.

Vasudevan, D.M. and Sreekumari, S (2001). Free radicals and antioxidants. In "Textbook of biochemistry for medical students". pp 212- 215. 3rd edition. Jaypee Brothers Medical Publishers(P) Ltd, New Delhi.

Vieira, S.L. (2007). Micronutrients in eggs for the chicken embryo. Br J Poult Sci 9(1):1–8

Zakaria, A. H. and AL-Latif, A. A. (1998). Effect of ascorbic acid treatment during egg incubation and after hatch on embryonic development, hatch time, and body weight changes of posthatch incubation time of broiler chickens. Arch. Geflugelk 62: 176-182.

Zakaria, A. H.; AL-Latifa, A. and AL-Anezi, M. A. (1998). Effect of ascorbic acid on embryonic development, hatch time and growth of extended delayed placement of broiler chickens. Arch. Geflugelk. 62: 11-15.

## تأثير حقن البيض بفيتامين ج وحامض الفوليك والخليط بينهما علي نسبة الفقس والأداء الإنتاجي اللاحق لكتاكيت اللحم

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استخدم في هذا البحث عدد 1260 بيضة مخصبة تم تقسيمها عشوائيا الي 7 معاملات تجريبية (180 بيضة / معاملة و3 مكررات لكل منها) بهدف دراسة تأثير حقن البيض المخصب بفيتامين ج , حامض الفوليك والخليط بينهما في اليوم 14 من التفريخ علي نسبة الفقس وأداء الأنتاج اللاحق في الفترة من 1 الي 28 يوم من العمر بعد الفقس. تم توزيع البيض المخصب في تصميم تام الشوائية علي 7 معاملات كما يلي: 1- مجموعة المقارنة : بيض بدون حقن كمجموعة مقارنة. 2- المجموعة الثانية: بيض تم حقنه بـ 0.1 مل ماء مقطر / بيضة. 3- المجمعهة الثالثة: بيض تم حقنة بـ 0.1 مل ماء مقطر يحتوي علي 6 ميكروجرام فيتامين ج. 4- المجموعة الرابعة: بيض تم حقنة بـ 0.1 مل ماء مقطر يحتوي علي 75 ميكروجرام حامض الفوليك. 5- المجموعة الخامسة: بيض تم حقنة بـ 0.1 مل ماء مقطر يحتوي علي 150 ميكروجرام حامض الفوليك. 6- المجموعة السادسة: بيض تم حقنة بـ 0.1 مل ماء مقطر يحتوي علي 6ميكروجرام فيتامين ج+75 ميكروجرام حامض الفوليك. 7- المجموعة السابعة: بيض تم حقنة بـ 0.1 مل ماء مقطر يحتوي علي 6ميكروجرام فيتامين ج+150 ميكروجرام حامض الفوليك. اوضحت النتائج ان نسبة فقس البيض المخصب تحسنت نتيجة حقن البيض المخصب بـ 6 ميكروجرام فيتامين ج / بيضة وكذلك نتيجة الحقن بحقن البيض بمستوي 6 ميكروجرام فيتامين ج + 150 ميكروجرام حامض الفوليك / بيضة وبلغ التحسن 9.97 و 3.71 % علي التوالي مقارنة بمجموعة المقارنة. وفيما يتعلق بالأداء الأنتاجي بعد الفقس , اوضحت النتائج ان الكتاكيت الناتجة من البيض الذي تم حقنه بمستوي 6ميكروجرام فيتامين ج و75 ميكروجرام حامض الفوليك و150 ميكروجرام حامض الفوليك و6ميكروجرام فيتامين ج+75 ميكروجرام حامض الفوليك سجلت افضل قيمة لوزن الجسم الحي مقارنة نمجموعة المقارنة وذلك بحوالي من 51.46 الي 69.48 جرام / طائر. ذاد استهلاك العلف بدرجة عير معنوية للكتاكيت الناتجة من كل معاملات الحقن فيما عدا تلك الناتجة من البيض المحقون بمستوي 6 ميكروجرام فيتامين ج/ بيضة. لم يتضح وجود فروق معنوية في معدل التحويل الغذائي. أدي حقن البيض بكل من حامض الأسكوربيك والفوليك والخليط بينهما الي انخفاض معنوي في مستوي الجليسريدات الثلاثية في سيرم الدم. أيضا تحسن مستوي الكولستيرول عالي الكثافة نتيجة الحقن بكل من 6 ميكروجرام فيتامين ج و 75 ميكروجرام حامض الفوليك / بيضة. لذلك فإن النتائج توضح امكابية حقن البيض المخصب بمستوي 6 ميكروجرام فيتامين ج و150 ميكروجرام حامض الفوليك / بيضة في اليوم الرابع عشر من التحضين لحسين نسبة فقس البيض المخصب والأداء الأنتاجي اللاحق خلال الفيرة من 1 الي 28 يوم من العمر بعد الفقس.