Effects of In-Ovo injection of Biotin on chick's embryonic development and physiological traits

Effects of In-Ovo injection of Biotin on chick's embryonic development and physiological traits

S. A. Alkubaisy1, A. A. Majid2, S. M. Abdulateef2*, F. A. Al-Bazy3, O. K. Attallah 2, O. M. Abdualmajeed2, Th. T. Mohammed2, F. M. Abdulateef4 and K.I. Mahmud5

[1]Desert studies centre, University of Anbar, Iraq
[2]Department of Animal Production, College of Agriculture, University of Anbar, Iraq
[3] Ministry of Agriculture, Agricultural Research Station
[4]Ministry of Agriculture, Directorate of Anbar Agriculture, Iraq
[5]College of Agriculture Engineering Sciences, Salahaddin University, Iraq

*Correspondence author's e-mail: ag.salwan.mahmood@uoanbar.edu.iq

Abstract. This study was conducted at the local hatchery in Ramadi, Anbar Iraq from 1st to 23th Dec. 2018, to 26 Feb 2019. The objectives of this study were to investigate the effect of injected eggs hatching in times and different concentrations of Biotin in growth and embryonic development, hatchability. Six hundred eggs of hatching types (Ross 308) and injected with different concentrations of biotin at age of 0 days (before placing in the hatchery) and 18 days of incubation. Eggs were divided into five groups (120 eggs for each) as follows: 1.T0: Control group placed in the hatchery without injection. 2.T1: Injected with a dose of 100 µg biotin at age of zero. 3. T2: Injected with dose 75 µg biotin at age zero. 4.T3: Injected with a dose of 100 µg biotin at age 18 days of incubation. 5.T4: Injected with a dose of 4175 µg biotin at age of 18 days of incubation. Statistical analysis was performed (CRD) (P=0.065) results show: Increase length of the embryo, diameter of vascular region and number of pairs of somites at 3 days of incubation for T2. Increase percentage of embryonic weight, decrease the percentage of albumin and the percentage of at 7 days of incubation for T1 and T2. Increase in percentage of embryonic weight and amniotic sac and liquid, decrease in the percentage of albumin and yolk, at 14 days of incubation for T2. Increase percentage of embryonic weight, decrease the percentage of yolk at the age of 17 days incubation for T2. Increase hatchability of total eggs and fertile eggs. Decrease embryonic mortality and different stages of embryonic development. Concluded that In-Ovo injection of the hatching eggs with biotin contributed to increase physiological traits and embryonic development.

1. Introduction

Supplement usage begins from the absolute first day of hatching, where both albumin and yolk of egg support creating embryo, avian embryos develop and grow from energy and nutrients stored in egg by hen. In this sense, breeder male contribution is not important. Follicular deposition of nutrients occurs over a wide range of time but becomes relevant during the week prior to ovulation. Amounts, but also forms, of nutrients deposited in egg, determine the success of embryo development and hatching of a healthy chick[1]. Vitamin B7 is a water-soluble vitamin, has an important role in transferring carbon dioxide in fat metabolism, carbohydrate, and protein by functioning as an enzyme cofactor. It is involved in multiple biochemical reactions including niacin metabolism, amino acid degradation, and formation of purine, which is an integral part of nucleic acids. It interacts with histone by the action of biosignal transferase. It may be prescribed as a supplement for diabetic patients due to its role in carbohydrate metabolism. Biotin is commonly found in vitamin B complex and many food sources,

such as milk, yeast, egg yolk, cereal, and mushroom[2]. Biotin is a coenzyme for 4 types of carbohydroxylase Acetyl-coenzyme A carboxylase (Acc), Pyruvate carboxylase (PC), Propionyl-coenzyme A carboxylase (PCC), and Methylcrotonyl CoA carboxylase (MCC)[3]. Robel [4] study the effect of addition Biotin to turkey Breeder and injecting turkey breeders' egg with Biotin then concluded that the injection of hatching egg aid to increasing hatchability and chick weight better than an addition of breeder diets. Being a close system, proper nutrient supply to developing embryo is of utmost importance. Maternal nutrition is the only source of vitamins for eggs but ignorance in breeder's diets or use of poor-quality vitamins results in deficiencies leading to embryonic mortality during the middle or late incubation period. Besides [5], deficiency of vitamin A results in reduced cellular immune responses. Water-soluble B-vitamins function as parts of coenzymes [6] and when present in a deficient concentration in hens diet result in high embryonic mortality. In-ovo feeding technique is used to supply nutrients directly into the developing embryo for improvement in post-hatch growth [7, 8]. The aim of this study to investigate the In-Ovo injection of Biotin on the embryonic development and physiological traits in a chick's embryo.

2. Materials and Methods

2.1 Animal Study:
The study was carried out according to the protocol approved by the University of Anbar, Ethics-Committee, Iraq. Fertile eggs from Ross (308) strain broiler breeder hens It was received from a commercial farm.

2.2 Experimental study:
In this study, 600 eggs had been Collected from Ross 308 Broiler breeders (53 weeks old). The egg had been divided into 5 groups, each one distributed to 120 eggs, and every one of this group sub-divided into 3 replicates, each replicate consist of 40 eggs. Eggs were injected using an automatic syringe that used for oil vaccination by using a needle of 25 millimeters [8].

2.3 In- Ovo:

The eggs were injected from the wide side by making more (13mlm) in the air sac than every egg injected by 0.1 of Biotin fluid (Latex CO. Ltd, Germany) as follows: Control group placed in the hatchery without injection. T1:Injected with a dose of 100 µg biotin at age of zero. T2:Injected with a dose of 75 µg biotin at age of zero. T3: Injected with a dose of 100 µg biotin at age of 18 days of incubation. T4: Injected with a dose of 75 µg biotin at age of 18 days of incubation. Egg candling was conducted to determine the Amnion sac for making the second injection (18 days of egg incubation), and the injection surface was sterilized by antiseptic (Dettol) before injection. The pores were closed by using Dye pedicures [5]. Eggs were incubated in (AFLO) mark setter by distributing the groups randomly. Prepare 400 mL of sterile water, is divided into four glass containers, Weighed quantities 100 & 75 mg of vitamin and amount of each dissolved in 100 ml of sterile water, Injection method of eggs at aged zero and 18 days of incubation, by the needle of size 25 mm, The needle is introduced from the petition of the eggshell after piercing through the air gap depth of 13 mm and the injection dose of 0.1 ml of the biotin prepared in both dates, and then underwent three tests embryonic, the mortality rate is calculated and the hatchability.

2.4 Embryonic test:

The first embryonic test conducted 3 days from incubation where we put the eggs horizontally and the shell is opened and the following traits are measured: Embryo length, vascular region, and pairs of somites. The second embryonic test conducted 7 days from incubation where we broke the eggshell, take out the contents of the egg to out, the following traits are measured: Embryo weight, Albumin, and shell. The third embryonic test conducted 14 days from incubation where we have broken the eggshell, take out the contents of the egg to out, the following traits are measured: Embryo weight, yolk, amniotic sac, and liquid and albumin. Fourth embryonic test conducted in 17 days from

incubation where we have broken the eggshell, take out the contents of the egg to out, the following traits are measured: Embryo weight and yolk.

2.5 Statistical Analysis:

This experiment was carried by using Complete Randomized Design (C.R.D). and the Data were analyzed by using the SAS program for statistical analysis [9]. The means for each treatment were compared by using Duncan's polynomial by using 0.05 and 0.01 significance levels to determine significant differences between the averages [10].

3. Results and Discussion

3.1 Embryonic test:

The results in table (1) refers to that treated eggs with Biotin led to a significant increasing (P<0.05) in Embryo Length, Vascular length and Pairs of Somites for treatment (T2) at 3 days from incubation in table (2) the results refer to significant increasing (P<0.05) in Embryo weight, and significant decreasing (P<0.05) in Albumin and Shell weight for (T2) treatment compared with other treatments in 7 days of incubation. In table (3) the results refer to a significant increasing (P<0.05) in Embryo weight for (T2) treatment compared with (T0), significant increasing (P<0.05) in Amniotic Sac and Liquid for (T2) treatment compared with other treatments, significant decreasing in yolk and Albumin weight for (T2) treatment compared with other treatments. in table (4) the results refer to significant increasing (P<0.05) in Embryo Weight at the age of 7 days from incubation for (T2) compared with (T0) and significant decreasing (P<0.05) in Yolk weight at the age of 7 days from incubation for (T2) compared with other treatments. The significant increase in embryonic Characters for Biotin treatments may be as a result of a Biotin role in embryonic development. Manthy et al., [11] reported that Biotin plays a major role of histone in DNA synthesis in cell proliferation. The Biotin play a major role of growing head and trunk of the embryo separately at the age 7-10 day, where Taniguchi and Watanabae [12] showed that the embryo of chicken develops more with a large amount of Biotin during this period, and chick shows a high Biotin concentration in the liver at age 9-11days of incubation, these high levels may be led to high increasing in coenzyme ACC, PC, PCC and MCC. Acc is a regulatory enzyme for fatty acid metabolism synthesis [13]. PC catalyzed the reaction of pyruvic acid to oxalacetic acid, involved in gluconeogenesis [14]. The increase in PC within embryonic growth may be associated with glucose metabolism. Because the role of Biotin in the Embryos differs among development periods and organs, this suggested the involvement of Biotin in the formation of each tissue and organs.

3.2 Hatchability:

In figure (1) the results refer to a significant increasing (P<0.05) in hatchability of total egg and hatchability of fertilized egg for (T2) and (T4) compared with other treatments. The significant

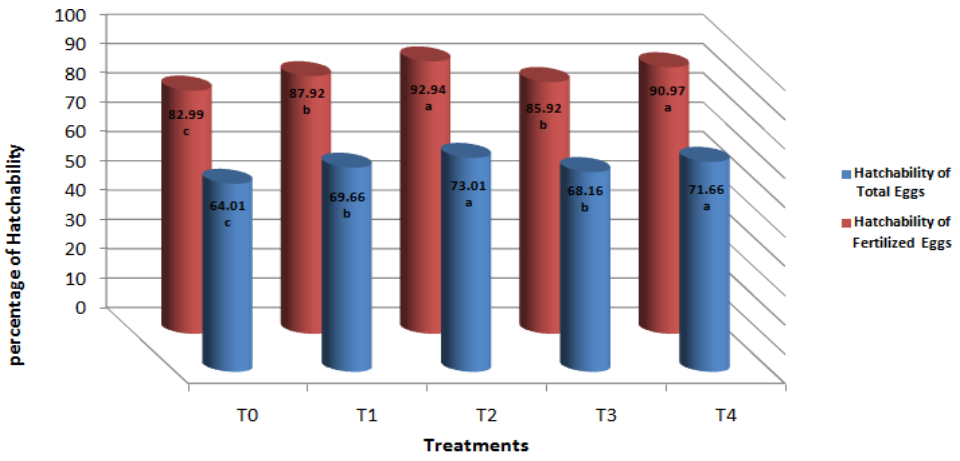

Fig. 1. The Effect of injection of Biotin on hatchability

increasing in hatchability for Biotin treatments may be a cause of the association between the liver Bioton concentration and the development and severity of Fatty liver and kidney syndrome (FLKS)[12]. incremental dietary biotin levels did not the amount of biotin deposited in the egg, biotin has been shown to be necessary for normal embryonic development and hatchability [15]. On the other hand, Robel and Christensen [16]; [17]. obtained improved hatchability in turkey eggs injected with biotin at 25 d of incubation. Dietary biotin supplementation may elicit a greater response in older breeders [18]; [19].

Table 1. Effect of injection of hatching eggs in different concentrations of Biotin in embryonic growth at the age 3 days from incubation (%As a percentage relative to egg weight at examination)

Treatments	Embryo Length (Mm)	Vascular Region (Mm)	Pairs of Somites
T0	9.00 ± 0.50b	10.57±0.38b	38.20±1.08 b
T1	9.90± 0.64ab	10.50±0.42b	39.20±0.30a
T2	11.16± 0.68a	12.23± 0.45a	40.73±0.50a
T3	11.56± 0.68a	12.43± 0.45a	40.73±0.50a
T4	11.26± 0.68a	12.23± 0.45a	41.73±0.50a
Significant	*	*	*

T0 = control treatment (no injection), T1 = 100µg injection Biotin/egg age zero, T2 = 75µg Biotin Injection / zero-old egg

* Different lowercase letters within a column indicate significant differences at the level of probability (P <0.05) Average ± standard error

Table 2. Effect of injection hatching eggs in different concentrations of Biotin in embryonic growth at the age 7 days from incubation (%As a percentage relative to egg weight at examination)

Treatments	Embryo Weight	Albumin	Shell
T0	1.10±0.04c	25.57±0.71a	11.67±0.33ab
T1	2.03± 0.07b	16.75±0.59b	11.31±0.35a
T2	2.98±0.04a	16.76±0.64b	10.14±0.22b
T3	2.18±0.04a	16.26±0.64b	10.20±0.22b
T4	2.98±0.04a	16.76±0.64b	10.04±0.22b
Significant	*	*	*

T0 = control treatment (no injection), T1 = 100µg injection Biotin/ egg age zero, T2 = 75µg Biotin Injection/ zero-old egg

* Different lowercase letters within a column indicate significant differences at the level of probability (P <0.05) Average ± standard error

Table 3. Effect of injection hatching eggs in different concentrations of Biotin in embryonic growth at the age of 7 days from incubation (%As a percentage relative to egg weight at examination)

Treatments	Embryo Weight	Yolk	Amniotic Sac And Liquid,	Albumin
T0	19.19±0.26 b	33.36±0. 2a	33.35±0.45 b	6.9 ± 0.23a
T1	19.32±0.25a b	33.53±0. 2a	33.21±0.14 b	6.1±0.14a
T2	30.3±0.22 a	21.27±0. 2b	33.3±0.19 a	5.33±0.21b
T3	29.32±0.25a b	23.53±0. 2a	33.11±0.14 b	5.1±0.14a
T4	27.3±0.22a	21.17±0. 2b	33.5±0.19 a	5.03±0.21b
Significant	*	*		*

T0 = control treatment (no injection), T1= 100µg injection Biotin/egg age zero, T2 = 75µg Biotin Injection/ zero-old egg

* Different lowercase letters within a column indicate significant differences at the level of probability (P <0.05) Average ± standard error

Table 4. Effect of injection hatching eggs in different concentrations of Biotin in embryonic growth at the age 17 days from incubation (%As a percentage relative to egg weight at examination)

Treatments	Embryo Weight	Yolk
T0	33.3±0.62b	31.21±0.51 a
T1	33.9±0.59ab	31.32±0.23a
T2	39.10±0.60a	19.10±0.20 b
T3	33.9±0.59ab	31.32±0.23 a
T4	39.10±0.60a	19.10±0.20b
Significant	*	*

T0 = control treatment (no injection), T1 = 100µg injection Biotin/egg age zero, T2 = 75µg Biotin Injection/ zero-old egg

* Different lowercase letters within a column indicate significant differences at the level of probability (P <0.05) Average ± standard error

4. Conclusion

Injected eggs concentration of 75 µg biotin/ egg in 0 and 18 days. Led to give the best result to improve embryonic growth and reduce mortality. Consequently, increase hatchability and improve the quality of chicks, so the increased weight of the chicks hatched.

5. References

[1] Vieira, S. L. 2007. Chicken embryo utilization of egg micronutrients. *Brazilian Journal of Poultry Science*, **9**(1):01- 08. doi.org/10.1590/S1516-635X2007000100001

[2] Calsamiglia, S. & Rodríguez, M. 2012. Optimum vitamin nutrition in dairy cattle. In: Barroeta, A. C., Cepero Briz, R., Lopez Bote, C., Baucells, M. D., Davin, R., Rey, I. A., Blanco Pérez, A., Gonzalez, G., Rodriguez, M., Calsamiglia, S., Hernandez, J. M., Sanz, J., Casals, R., Isabel, B., Soto-Salanova, M. F. & Weber, G. Optimum vitamin nutrition in the production of quality animal foods. DSM Nutritional Products Limited, 355- 357pp. http://www.optimumvitaminnutrition.com

[3] Taniguchi, A. &Watanabe T. 2007. A study on the utilization of the Biotin in the Embryos in domestic fowl. *Japanese Journal of Poultry Science*, **44**(1): 9-16.
[4] Robel, E. J. 2002. Assessment of dietary and egg injected d- biotin, pyridoxine and folic acid on turkey hatchability: folic acid and poult weight. *World's Poultry Science Journal*, **58**(3): 305-315. doi: https://doi.org/10.1079/WPS20020024
[5] Abdulateef, S. M. 2017. The effect of maternal care (Acoustic stimulus) on embryonic development and hatching muscle of broiler chickens. *Iraqi Journal of Agricultural Sciences*, **48**(5): 1263-1274. Doi: https://doi.org/10.36103/ijas.v48i5.336
[6] Mock, D. M. 2007. Biotin. In: Zempleni, J., Rucker, R. B., McCormick, D. B. & Suttie, J. W. Handbook of Vitamins. Chapter 11, 4th ed., CRC Press, Taylor & Francis Group, Boca Raton, London, New York, 361- 377pp. doi:10.1201/9781420005806
[7] Ohta, Y., Tsushima, N., Koide, K., Kidd, M. T. & Ishibashi, T. 1999. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poultry Science*, **78**(11): 1493- 1498. doi: 10.1093/ps/78.11.1493
[8] Bhanja, S. K., Mandal, A. B. & Johri, T. S. 2004. Standardization of injection site, needle length, embryonic age and concentration of amino acids for in ovo injection in broiler breeder eggs. *Indian Journal of Poultry Science*, **39**: 105–111.
[9] SAS. 2012. Statistical analysis system. User's Guide. Statistical. Version 9th ed. SAS. Inst. Inc. Cary N. C., USA.
[10] Duncan, D. 1955. Multiple Range and Multiple F Tests. *Biometrics*, **11**(1): 1-42. https://doi.org/10.2307/3001478. https://www.jstor.org/stable/3001478.
[11] Manthey, K. C., Griffin, J. B. & Zempleni, J. 2002. Biotin supply affects expression of Biotin transporters. Biotinylation of carboxylases and metabolism of interlukin-2 in Jurkat cells. *The Journal of Nutrition*, **132**(5):887-892. doi: 10.1093/jn/132.5.887
[12] Taniguchi, A. & Watanabe, T. 2007. Roles of biotin in growing ovarian follicles and embryonic development in domestic fowl. *Journal of Nutritional Science and Vitaminology*, **53** (6): 457- 463. doi: 10.3177/jnsv.53.457
[13] Sweetman, L. 2000. Pantothenic acid and biotin. In Biochemical and physiological aspects of human nutrition. Philadelphia, PA: WB Saunders Company. 520-529pp. https://www.elsevier.com/books/biochemical-physiological-and-molecular-aspects-of-human-nutrition/stipanuk/978-1-4377-0959-9
[14] Dukshinamurti, K. & Chauhan, J. 1989. Biotin. *Vitamins & Hormones*, **45:** 337- 384. doi:10.1016/S0083-6729(08)60398-2
[15] Whitehead, C. C., Pearson, R. A. & Herron, K. M. 1985. Biotin requirements of broiler breeders fed diets of different protein content and effect of insufficient biotin on the viability of progeny. *British Poultry Science*, **26**(1):73- 82. doi:10.1080/00071668508416789
[16] Robel, E. J. & Christensen, V. L. 1987. Increasing hatchability of turkey eggs with biotin egg injection. *Poultry Science*, **66**(1): 1429–1430. doi:10.3382/ps.0661429
[17] Saed, Z. J. M., Abdulateef, S. M., Mohammed, Th.T. & Al-Khalani, F. M. H. 2018. Effect of dried tomato pomace as alternative to vitamin C supplemented diets in hematological indices and oxidative stability of egg yolk of laying hens in high-ambient temperature. *Biochemical and Cellular Archives*, **18**(2): 1647-1652
[18] Chen, F., Noll, S. L. & Waibel, P. E. 1994. Dietary biotin and turkey breeder performance. *Poultry Science*, **73**(5): 682-686. doi:10.3382/ps.0730682
[19] Al-Enzy, A. F. M., Saed, Z. J. M., Naser, A. S., Mohammed, Th. T., Abdulateef, S. M., Al-Khalani, F. M. H. & Abdulateef, F. M. 2020. The role of adding sodium chloride in broiler chicks diets to improve production performance and antioxidant status during heat stress. *Annals of Tropical Medicine and Public Health*, **23**(16): 231- 612. Doi: 10.36295/ASRO.2020.231612