



Effect of Egg Injection with Different Levels of Organic Selenium and Spirulina Algae Extract in the Blood Biochemistry of Broiler Chickens

Honar F. Taher*, Qana H. Al-Jabari and Mohammed S. Baha Aldeen

Animal Production Department, College of Agriculture, Kirkuk University, Kirkuk, Iraq.

Abstract

THE experiment was conducted for the period from (9/16/2023 to 11/18/2023), with the aim of studying the effect of injecting eggs with different levels of organic selenium and spirulina algae extract on the blood biochemistry of broiler chickens. The experiment of injection and hatching of chicks was conducted in the Lailani hatchery located in the Lailan area, which contains BROODTECH type hatcheries. The duration of the experiment was 21 days. (600) hybrid eggs (Ross 308) from a flock of 33-week-old hens imported from Turkey were used. The average egg weight was 58 g according to the certificate of origin. They were randomly distributed among (6) treatments with (100) eggs for each treatment and placed in BROODTECH incubator drawers until the age of (18 days), after which the eggs were injected and the drawers were transferred to the hatchery until the chicks emerged at the age of (21 days). The parameters used were as follows: T1 = no negative control injection T2 = injection of 0.5 ml of deionized distilled water per egg (positive control) T3 = injection of 0.5 ml/egg containing a concentration of 10 micrograms of organic selenium T4 = 0.5 ml/egg injection containing a concentration of 20 micrograms of organic selenium T5 = 0.5 ml/egg injection containing 1.5 mg of spirulina algae extract T6= 0.5 ml/egg injection containing 3 mg of spirulina algae extract. The study conducted that inject the egg with organic selenium and Spirulina were effected on the level of both calcium and phosphor, and decrease the MDA in the blood serum.

Keywords: Selenium, Spirulina, Injection, egg.

Introduction

Promoting the health and growth of broiler chickens using the same technology used to inoculate embryos into the egg can be done using a variety of nutrients to produce healthy, chickens [1]. Providing embryos with exogenous nutrients in eggs may improve hatchability and increase net weight and final body weight of broilers [2] through modification of embryo's gut morphology [3]. One-day-old chicks are considered the final product of the hatchery industry and provide an important starting point for broiler chicken production. High hatchability of marketable chicks within a narrow hatchability range is the main goal of the hatchery industry, because these chicks perform well, which explains their higher viability, growth rate, meat yield, and better feed conversion [4]. Spirulina Algae enhances immune function and increases growth because it is rich in nutrients such as vitamins, amino acids, gamma-linoleic acid, phycocyanin, tocopherol, chlorophyll, and carotenoids. Spirulina also

possesses as antitumor, antimicrobial, antiviral, and anti-activities [5]. Phycocyanin is a blue-coloured protein pigment abundantly available in Spirulina and has gained popularity due to its diverse applications in various industries. Phycocyanin is rich in natural properties that can be used as a nutraceutical, combining the food and pharmaceutical sectors. It has been explored in the pharmaceutical industry by combining it with medicines. In addition, phycocyanin has been used as a natural alternative to artificial food colourings, which add colour to food and enhance the nutritional value of food [6]. Selenium is mainly given to chickens through feeding chicks after hatching or injection into eggs. When nutrient fluid is injected into the egg, it allows chicken's embryos to consume nutrients before hatching [7].

Selenium used in traditional methods by inject the eggs by organic, such as selenium yeast, or inorganic. Chickens are naturally consumed before hatching; thus, injecting nutrients into the amniotic

*Corresponding authors: Honar F. Taher, E-mail: honarfataher70@gmail.com, Tel.: 07700950170

(Received 26 April 2024, accepted 10 July 2024)

DOI: 10.21608/EJVS.2024.285534.2040

©2025 National Information and Documentation Center (NIDOC)

fluid of the embryo's before it hatches will provide essential nutrients to the embryo's intestines. Several experiments conducted on selenium injection into eggs have reported that it can improve the expression of immune genes in avian cells enhance the immune response and antioxidants characters in chickens exposed to inflammatory bowel disease pathogens at hatching [8], and reduce oxidative damage. Incubation and the post-hatching period increase adipose tissue mass, cause hyperplasia of adipocytes during chicken embryonic development [9], increase the length of the villi in the small intestine and the width of the duodenal villi [10], and improve the weight of chicks, Hatching, final body weight gain, and feed conversion efficiency [11].

Therefore, the current study aims to evaluate egg injections with Spirulina algae and organic selenium and study their effect on the biochemical blood characteristics of broilers chicken.

Material and Methods

The experiment was conducted in a period from 9/16/2023 to 11/18/2023 in order to study the effect of inoculating eggs with different levels of organic selenium and spirulina algae extract on the hatchability characteristics, productive performance, and blood biochemistry of broiler chickens. The experiment of injection and hatching of chicks was conducted in the Lailani hatchery located in the Lailan area, Kirkuk, Iraq, which contains BROODTECH type hatcheries. The duration of the experiment was 21 days. Six hundred hybrid eggs (Ross 308) that collected from 33-week-old hens flock were imported from Turkey. The average egg weight was 58 grams according to the certificate of origin. They were randomly distributed among (6) treatments with (100) eggs for each treatment and placed in BROODTECH incubator drawers until the age of (18 days), after which the eggs were injected and the drawers were transferred to the hatchery until the chicks emerged at the age of (21 days). The parameters used were as follows:

T1 = Negative control (Not inoculated)

T2 = Positive control (Inoculated with 0.5 ml of deionized distilled water per egg

T3 = injection of 0.5 ml/egg containing a concentration of 10 µg of organic selenium

T4 = 0.5 ml/egg injection containing a concentration of 20 µg of organic selenium

T5 = 0.5 ml/egg injection containing 1.5 mg of spirulina algae extract

T6= 0.5 ml/egg injection containing 3 mg of spirulina algae extract.

The spirulina algae that was injected into the egg was prepared by dissolving 0.15 g of spirulina algae in 100 ml of distilled water. The second

concentration was by dissolving 0.3 gm of spirulina algae in 100 ml of distilled water. The selenium substance that was injected into the egg was prepared by dissolving 0.04 micrograms of selenium in 100 ml of distilled water. The second concentration was by dissolving 0.08 micrograms of selenium in 100 ml of distilled water. When the embryo's reached 18 days of old, small hole was made above the air chamber to inject selenium, and the spirulina. Using a diabetic needle (0.5 gauge and 30G needle size), 0.5 ml of spirulina solution was injected into the air chamber, the injection site on the shell was closed with nail polish, and the eggs were returned to the hatchery. The hatched chicks were raised during the period (42) days in the poultry field of the Department of Animal Production, College of Agriculture - University of Kirkuk, Kirkuk, Iraq. The average temperature was (35) °C and the relative humidity was (20) % in the poultry field. The chicks were distributed randomly, with twenty chicks per replicate. The incubation temperature was adjusted to 35°C using electric heaters. Electric lighting was used to stimulate the chicks to eat and drink. At the end of experiment, the chickens were slaughter, blood was taken from each replicate, in an amount of (3 cm³), and placed in a special clotting tube. After leaving it for 3 hours to ensure the process of separating the serum, a centrifuge was used at 5000 rpm for 10 minutes. After the separation process, the samples were preserved by freezing until biochemical tests were performed (total protein, total cholesterol, glucose, Globulin, Aspartate Transaminase (AST), Alanine Transaminase (ALT), calcium, and phosphorus), where they were measured using a kit (ACCENT-200) from the company (CORMAY), of Polish origin. In addition to the antioxidant parameters represented by malondialdehyde (MDA) and glutathione (GSH) were measured using Chicken malondialdehyde ELISA Kit and Chicken Glutathione ELISA Kit, respectively, manufactured by (Sunlong Biotech Co., Ltd). The data were subjected to Statistical analysis by applying one-way ANOVA using SAS software. Differences between treatments were tested using Duncan's multiple comparison test [12], at a significance level of $P \leq 0.05$.

Results and discussion

Table (1) shows the effect of injecting hatching eggs with different concentrations of organic selenium and spirulina algae extract on liver enzymes. There were no significant differences between the treatments in AST and ALT. [13] in his study in which he injected the eggs of ISA brown chickens with different concentrations of selenium, concluded that there was no significant difference in the liver enzymes AST and ALT between the treatments. [14] concluded, no significant differences in the concentration of liver enzymes represented by

AST and ALT following egg inoculation with different selenium concentrations.

Table (2) shows the effect of injecting hatching eggs with different concentrations of organic selenium and spirulina algae extract on biochemical blood parameters. It was found that there were no significant differences between the treatments in glucose concentration, and there were also no significant differences between the treatments in the concentration of total cholesterol. As for the concentration of total albumin, the first and second treatments outperformed the rest of the treatments by 14.20 and 14.66, respectively, and there were no significant differences between treatments at the concentration of total globulins. The current study did not agree with the study conducted by [14] who inoculate embryo chicken eggs with different concentrations of spirulina algae, where he observed significant differences in the levels of albumin, globin, and cholesterol. While [13] injected the eggs of ISA brown chickens with different concentrations of selenium and found that there was no significant difference in albumin, triglycerides, and total proteins between the treatments.

Previous study [13] concluded, that the injected the hatching eggs with different concentrations of selenium didn't effect significantly on the blood parameters represented by the concentration of glucose, albumin, and globulin.

The effect of injecting hatching eggs with different concentrations of organic selenium and spirulina algae extract on the minerals such as calcium and phosphorus is represented in Table (3).. It was found that in calcium metal, the first, second, and third treatments outperformed the rest of the treatments, 14.30, 13.96, and 14.04, respectively. As for phosphorus metal, the first treatment outperformed the rest of the treatments by 6.94.

Table (4) shows the effect of injecting hatching eggs with different concentrations of organic

selenium and spirulina algae extract on immune parameters. It was found that in MDA, the third treatment outperformed the rest of the treatments by 0.242, but in glutathione, there were no significant differences between the treatments. Previous researcher [13] found, in his study, that inject selenium with different concentrations into hatching eggs, didn't effect significantly on the concentration of MDA and glutathione.

Conclusion

The study conducted that inject the egg with organic selenium and Spirulina were effected on the level of both calcium, and phosphorus, and decrease the MDA in the blood serum. Moreover, the selenium and Spirulina did not effect on each of AST, ALT, total protein, Glucose, total cholesterol, globulin, and GSH.

Acknowledgment

The authors are very grateful to the College of Agriculture, Kirkuk university, Kirkuk, Iraq especially for the farm staff for providing most of the requirements for supporting this research.

Funding statement

The research mentioned above was funded by the researchers themselves and without any external funding

Authors contributions

All named authors have made an active contribution to the conception and design and analysis and interpretation of the data and the drafting of the paper and All have critically reviewed its content and have approved the final version submitted for publication.

Conflicts of interest

The authors declared no competing interests

TABLE 1. Effect of injecting hatching eggs with different concentrations of organic selenium and spirulina algae extract on liver enzymes (mean $\pm$ standard error)

Treatment	AST	ALT
1	755.16 $\pm$ 68.26 ^a	13.12 $\pm$ 1.44 ^a
2	621.56 $\pm$ 37.87 ^a	13.92 $\pm$ 1.08 ^a
3	645.22 $\pm$ 108.79 ^a	13.66 $\pm$ 1.06 ^a
4	632.26 $\pm$ 99.62 ^a	13.54 $\pm$ 0.54 ^a
5	631.72 $\pm$ 54.45 ^a	12.48 $\pm$ 0.20 ^a
6	727.58 $\pm$ 124.10 ^a	18.16 $\pm$ 3.75 ^a
Sig	N.S.	N.S.

T1=negative control treatment (no injection), T2=positive control treatment (injection of 0.5 ml of deionized pure water), T3=injection of 0.5 ml/egg (containing a concentration of 10 μ g organic selenium), T4=injection of 0.5 ml/egg (contains a concentration of 20 micrograms of organic selenium), T5 = injection 0.5 ml/egg (contains 1.5 mg of spirulina algae extract), T6 = injection 0.5 ml/egg (contains 3 mg of spirulina algae extract). Means not having a common letter within each row differ significantly (P<0.05).

TABLE 2. The effect of injecting hatching eggs with different concentrations of organic selenium and spirulina extract on blood biochemical parameters (mean $\pm$ standard error)

Treatment	Total protein	GLU-G	T-CHOL	Glo	ALB
1	3.60 $\pm$ 0.26 ^a	271.60 $\pm$ 14.61 ^a	112.00 $\pm$ 9.14 ^a	22.40 $\pm$ 0.60 ^a	14.20 $\pm$ 0.36 ^a
2	3.86 $\pm$ 0.19 ^a	265.00 $\pm$ 8.84 ^a	119.20 $\pm$ 10.75 ^a	22.40 $\pm$ 0.81 ^a	14.66 $\pm$ 0.60 ^a
3	3.78 $\pm$ 0.27 ^a	265.60 $\pm$ 5.27 ^a	122.40 $\pm$ 5.05 ^a	21.40 $\pm$ 0.75 ^a	13.90 $\pm$ 0.72 ^{ab}
4	4.30 $\pm$ 0.23 ^a	262.00 $\pm$ 6.01 ^a	119.00 $\pm$ 9.30 ^a	21.60 $\pm$ 1.29 ^a	12.54 $\pm$ 0.35 ^b
5	4.02 $\pm$ 0.24 ^a	259.40 $\pm$ 7.99 ^a	121.40 $\pm$ 7.20 ^a	22.40 $\pm$ 1.21 ^a	13.90 $\pm$ 0.45 ^{ab}
6	3.36 $\pm$ 0.34 ^a	255.20 $\pm$ 3.12 ^a	114.00 $\pm$ 4.87 ^a	23.40 $\pm$ 0.98 ^a	13.96 $\pm$ 0.27 ^{ab}
Sig	N.S.	N.S.	N.S.	N.S.	*

T1=negative control treatment (no injection), T2=positive control treatment (injection of 0.5 ml of deionized pure water), T3=injection of 0.5 ml/egg (containing a concentration of 10 μ g organic selenium), T4=injection of 0.5 ml/egg (contains a concentration of 20 micrograms of organic selenium), T5 = injection 0.5 ml/egg (contains 1.5 mg of spirulina algae extract), T6 = injection 0.5 ml/egg (contains 3 mg of spirulina algae extract). Means not having a common letter within each row differ significantly ($P < 0.05$).

TABLE 3. The effect of injecting hatching eggs with different concentrations of organic selenium and spirulina algae extract on the minerals calcium and phosphorus (mean $\pm$ standard error)

Treatment	Ca	PHOS
1	14.30 $\pm$ 0.50 ^a	6.94 $\pm$ 0.15 ^a
2	13.96 $\pm$ 0.18 ^a	6.01 $\pm$ 0.20 ^b
3	14.04 $\pm$ 0.26 ^a	5.67 $\pm$ 0.20 ^{bc}
4	13.52 $\pm$ 0.35 ^{ab}	5.64 $\pm$ 0.14 ^{bc}
5	12.46 $\pm$ 0.75 ^{ab}	5.46 $\pm$ 0.29 ^{bc}
6	11.88 $\pm$ 1.20 ^b	5.15 $\pm$ 0.41 ^c
Sig	*	**

T1=negative control treatment (no injection), T2=positive control treatment (injection of 0.5 ml of deionized pure water), T3=injection of 0.5 ml/egg (containing a concentration of 10 μ g organic selenium), T4=injection of 0.5 ml/egg (contains a concentration of 20 micrograms of organic selenium), T5 = injection 0.5 ml/egg (contains 1.5 mg of spirulina algae extract), T6 = injection 0.5 ml/egg (contains 3 mg of spirulina algae extract). Means not having a common letter within each row differ significantly ($P < 0.05$).

TABLE 4. The effect of injecting hatching eggs with different concentrations of organic selenium and spirulina algae extract on immunological parameters (mean $\pm$ standard error)

Treatment	MDA	GSH
1	0.256 $\pm$ 0.004 ^{ab}	0.239 $\pm$ 0.018 ^a
2	0.266 $\pm$ 0.019 ^{ab}	0.278 $\pm$ 0.028 ^a
3	0.242 $\pm$ 0.006 ^a	0.230 $\pm$ 0.012 ^a
4	0.269 $\pm$ 0.012 ^{ab}	0.288 $\pm$ 0.027 ^a
5	0.293 $\pm$ 0.017 ^b	0.282 $\pm$ 0.017 ^a
6	0.294 $\pm$ 0.022 ^b	0.289 $\pm$ 0.012 ^a
Sig	**	N.S.

T1=negative control treatment (no injection), T2=positive control treatment (injection of 0.5 ml of deionized pure water), T3=injection of 0.5 ml/egg (containing a concentration of 10 μ g organic selenium), T4=injection of 0.5 ml/egg (contains a concentration of 20 micrograms of organic selenium), T5 = injection 0.5 ml/egg (contains 1.5 mg of spirulina algae extract), T6 = injection 0.5 ml/egg (contains 3 mg of spirulina algae extract). Means not having a common letter within each row differ significantly ($P < 0.05$).

References

- Mustafa, N. A., Ameen, Q. A., Shaker, A. S. and Muhamad, H. A. Effect In-Ovo Injection (MethioGrow) Liquid Methionine in Broiler Breeder Eggs On Hatching Characters and Growth Performance. *Journal of Kirkuk University for Agricultural Sciences*, **12**(4),14-22 (2021)
- Amin, Q. A., Khidhir, Z. K., Mustafa, N. A. and Sami, A. Impact of Utilizing Liquid Methionine in Drinking Water for Broiler Chicks' on Some Meat Traits. *Mesopotamia Journal of Agriculture*, **47**(1),1-15(2019)
- Rufino, J., Cruz, F., Costa, V., Silva, A., Melo, L. and Sami, A. Impact of Utilizing Liquid Methionine in Dri Bezerra, N. Effect of In Ovo Feeding of L-Glutamine to Chick Embryos. *Brazilian Journal of Poultry Science*, **21**(4), eRBCA-2018-0852(2019).
- Al-Khayyat, M. and Sami, A. Impact of Utilizing Liquid Methionine in Dri Shanon, A. Comparison the Effect of Adding Local Anemone coronaria L. Flower Powder and Vitamin C in the diet on the Productive Performance of Laying Hens Exposed to Heat Stress in Summer. *Kirkuk University Journal For Agricultural Sciences*, **15**(1), 138-150(2024).
- Farag, M. R., Abd El-Aziz, R. M., Ali, H. A. and Ahmed, S. A. Evaluating the Ameliorative Efficacy of Spirulina Platensis on Spermatogenesis and Steroidogenesis in Cadmium-Intoxicated Rats. *Environ. Sci. Pollut. R.*, **23**, 2454–2466 (2016).
- Rashid, S. A., Ameen, Q. A., Mustafa, N. A., Sadq, S. M. and Sami, A. Impact of Utilizing Liquid Methionine in Dri Shaker, A. S. Influence of Liquid Methionine (methiogrow) in Drinking Water on Broiler Chicks Performance, Drinking Water, and Carcass Traits. *Tikrit Journal for Agricultural Sciences*, **21**(4), 9-16(2022).
- Goel, A., Bhanja, S.K., Mehra, M., Mandal, A. and Pande, V. In ovo trace element supplementation enhances expression of growth genes in embryo and immune genes in post-hatch broiler chickens. *J. Sci. Food Agric.*, **96**(8), 2737-2745 (2016).
- Lee, S.H., Lillehoj, H.S., Jang, S.I., Jeong, M.S., Xu, S.Z., Kim, J.B., Park, H.J., Kim, H.R., Lillehoj, E.P. and Bravo, D.M. Effects of in ovo injection with selenium on immune and antioxidant responses during experimental necrotic enteritis in broiler chickens. *Poult. Sci.*, **93**(5),1113-1121. (2014)
- Hassan, A.A., El-Mokhtar, N.M. and El-Hamaky, A.M. Evaluation of the efficacy of ozone fumigation and zinc oxide nanoparticles in control of aflatoxins contamination in cattle feeds. *Anim. Health Res. J.*, **5**, 165–180 (2017).
- Chandiranathan, T., Pasupathi, K., Valli, C. and Omprakash, A.V. Effect of in ovo supplementation of amino acids and nanoselenium on small intestine histomorphometry. *Indian Vet. J.*, **94**(11), 59-61 (2017) .
- Hassan, A.M. Effect of in ovo injection with nano-selenium or nano-zinc on post-hatch growth performance and physiological traits of broiler chicks. *Int. J. Environ. Agric. Bioetec.*, **3**(2), 350-357 (2018) .
- Duncan, D. B. Multiple Range and Multiple Test. *Biometrics*, **11**, 1-42 (1955).
- Kwassi,T., Pitala, W., Ngueda, D., Sergers, L. and Fafiolu, A. Effect of in ovo Injection of Selenium in Isa Brown Fertile Eggs on Hatching Process, Chicks Quality and Post Hatch Growth. *International Journal of Poultry Science*, **20**, 152-157 (2021).
- Ismail, F., Sherif, Kh, Rizk, Y. and Hassan, M. Effect of Spirulina and Canthaxanthin Injection into Hatching Eggs on Hatchability Traits and Subsequent Growth Performance of Chicks. *Journal of Animal and Poultry Production*, **10**, 197-202 (2019).

تأثير حقن البيض بمستويات مختلفة من السيلينيوم العضوي ومستخلص طحالب السبيرولينا في الصفات الكيموحيوية لدم فروج اللحم

هونر فهمي طاهر ، قانع حسين الجباري و محمد صباح بهاء الدين

قسم الانتاج الحيواني، كلية الزراعة، جامعة كركوك، كركوك، العراق.

الملخص

أجريت التجربة للمدة من (2023/9/16 إلى 2023/11/18) بهدف دراسة تأثير حقن البيض بمستويات مختلفة من السيلينيوم العضوي ومستخلص طحالب الإسبيرولينا في الكيموحيوية لدم للدجاج الفروج. أجريت تجربة حقن وتققيس الافراخ في مقياس ليلاني الواقع في منطقة ليلان والذي يحتوي على مقياسات من نوع BROODTECH. وكانت مدة التجربة 21 يوما. تم استخدام (600) بيضة هجين (روس 308) من قطيع أمهات بعمر 33 أسبوع مستوردة من تركيا. متوسط وزن البيضة 58 غم حسب شهادة المنشأ. تم توزيعها عشوائيا على (6) معاملات بواقع (100) بيضة لكل معاملة ووضعت في أدراج حاضنة BROODTECH حتى عمر (18 يوما) وبعد ذلك تم حقن البيض ونقل الأدرج إلى الفقس حتى خروج الافراخ عند عمر (21 يوم). كانت المعاملات المستخدمة كما يلي: T1 = لا يوجد حقن سيطرة سالبة = T2 حقن 0.5 مل من الماء المقطر منزوع الأيونات لكل بيضة (سيطرة موجبة) = T3 حقن 0.5 مل / بيضة تحتوي على تركيز 10 ميكروغرام من السيلينيوم العضوي T4 = 0.5 مل / بيض تحتوي على تركيز 20 ميكروغرام من السيلينيوم العضوي T5 = 0.5 مل / بيض تحتوي على 1.5 ملغم من مستخلص طحالب السبيرولينا T6 = 0.5 مل / بيضة تحتوي على 3 ملغم من مستخلص طحالب السبيرولينا. وأظهرت الدراسة أن حقن البيضة بالسيلينيوم العضوي والسبيرولينا أثر على مستوى كل من الكالسيوم والفسفور ، وانخفاض مستوى MDA في مصل الدم.

الكلمات الدالة: سيلينيوم، سبيرولينا، حقن، بيضة.