Research Article

Effect of in Ovo Injection of Spirulina Algae Extract on Hatchability Traits and Biochemical Parameters of Iraqi Local Ducks

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Abstract | This experiment was conducted to study the effect of *in ovo* injection of spirulina algae extract on hatchability traits and biochemical parameters of Iraqi local ducks. Three hundred sixty hatching eggs from Iraqi local ducks were used; the eggs were divided into four treatments at random, with three replicates of each treatment and 30 eggs per replicate. The treatments were arranged as follows: a negative control (without injection, NC), a positive control (injected with sterile distilled water alone, PC), and spirulina (SP) at 3 and 6 mg/egg, respectively. The results showed a significant effect of spirulina injection on hatchability rate and embryonic mortality rate. The highest value of hatchability percentage was achieved by injecting eggs with spirulina at levels of 3 and 6 mg compared to the negative and positive control treatments (P≤0.05). Injecting eggs with 3 and 6 mg of spirulina resulted in the lowest rate of embryonic mortality compared to the negative and positive control treatments (P≤0.05). In ovo spirulina injection had no significant effect on chick hatch weight (P>0.05). Hatched chicks from spirulina-treated eggs showed significantly higher serum total protein and albumin concentrations than those from control treatments (P≤0.05). No significant differences were observed in serum globulin or glucose levels among treatments (P>0.05). In contrast, serum cholesterol and triglyceride concentrations were significantly reduced in chicks hatched from eggs injected with spirulina at both levels (P≤0.05). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not significantly affected by spirulina injection (P>0.05). In conclusion, in ovo injection of spirulina at 3 and 6 mg/egg can be effectively used to improve hatchability performance and enhance the physiological condition of Iraqi local duck chicks.

Keywords | Ovo injection, spirulina, hatchability, biochemical parameters, Iraqi local ducks

Received | September 22, 2025; **Accepted** | December 14, 2025; **Published** | May 06, 2026
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Citation | Sultan ATM, Awad NM, Jassim AK (2026). Effect of in Ovo injection of spirulina algae extract on hatchability traits and biochemical parameters of Iraqi local ducks. J. Anim. Health Prod. 14(2): 775-779.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.2.775.779
ISSN (Online) | 2308-2801

INTRODUCTION

Injecting nutrients into eggs prior to hatching is a common practice nowadays to enhance the health and growth of poultry and produce healthy chicks (Al-Jabari *et al*., 2024). Providing exogenous nutrition to embryos in ovo may improve the rate of hatching, raising the weight of the chicks and the final body weight (Rufino *et al*., 2019). There are many substances such as vitamins, carbohydrates, some mineral elements, and supplements used in the feeding process to inject hatching eggs (Aljumaily and Taha, 2019).

A premium natural feed addition for the nutrition of animals and poultry is spirulina, sometimes known as blue-green algae. People and animals have used the blue-green algae (*Spirulina platensis*) as a food source for hundreds of years due to its high carotenoid content and exceptional nutritional value. Spirulina contains all of the essential amino acids and has a comparatively high protein content,

ranging from 55 to 65% (Jamil *et al*., 2015). Spirulina is expected to have a phosphorus availability of 41% and an energy content of 2.50-3.29 kcal/g, Vitamins (thiamin, riboflavin, pyridoxine, and vitamin B), amino acids, gamma linoleic acid, phycocyanin, tocopherol, chlorophyll, beta-carotene, carotenoids, and minerals, particularly iron, are also abundant in it (Abd El-Hady and El-Ghalid, 2018). There have been reports of the health advantages of spirulina for ailments like diabetes and arthritis. Additionally, it has been demonstrated to have antiviral and immunostimulatory properties (Khan *et al*., 2020). Therefore, the present study aimed to evaluate the effects of in ovo injection of Spirulina on hatchability, fertility, embryonic mortality (%), subsequent growth performance, and the physiological status of hatched ducklings.

MATERIALS AND METHODS

The study was conducted at the poultry farm, Faculty of Agriculture, University of Basrah, Iraq, from December 16, 2024, to February 10, 2025. Three hundred sixty hatching eggs for Iraqi local ducks were used; the eggs were divided into four treatments at random, with three replicates of each treatment and 30 eggs per replicate. The treatments arranged as follows: Negative control (without injection, NC), while the other groups were injected with sterile distilled water alone as a positive control (positive control, PC), spirulina (SP) with 3 and 6 mg/egg, respectively.

Following the sterilization of the puncture site with 70% ethyl alcohol, the injection was performed in the egg's air chamber, and the eggshell was punctured using a drill bit with a diameter of 1.00 mm. The solution was administered via a 1 ml medical syringe (Gauge 26). Following the injection, paraffin wax was used to close the hole. The incubator was set at 37.8°C and 60% relative humidity for the first 25 days of incubation, then 37.5°C and 65% for the final three days.

Study parameters

After hatching, the hatchability and mortality rates of the chicks were measured for each replicate of each treatment according to Desha *et al*. (2015), the body weight of ten randomly selected chicks from each replicate was measured according to Willemsen *et al*. (2008). Subsequently, three randomly selected chicks from each replicate per treatment were slaughtered, and blood samples were collected using EDTA-free tubes. The blood samples were then centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum samples were then stored at refrigerated temperatures for subsequent biochemical analyses, including total protein, Albumin, glucose, cholesterol, and triglycerides. These analyses were estimated using ready-made kits purchased from the commercial company (Biolabo SAS, France). Serum globulin was measured by subtracting albumin concentrations from total protein. The activity of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was measured according to the method described by Tietz (1999).

Statistical analysis

All data were analyzed using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA). Differences among treatment means were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test. Statistical significance was declared at $p \leq 0.05$.

RESULTS AND DISCUSSION

The impacts of the egg injection with spirulina algae extract on hatchability, mortality rates, and hatched chick weight are revealed and presented in Table 1. A significant difference ($P \leq 0.05$) in hatchability and embryonic mortality (%) of fertile eggs was observed among the different experimental treatments. The highest hatchability value was achieved by eggs injected with 3 and 6 mg SP/egg, compared to the positive (PC) and negative (NC) control groups. mbryonic mortality (%) was significantly reduced ($p \leq 0.05$) in eggs injected with 3 and 6 mg of Spirulina (SP) per egg compared with the positive (PC) and negative control (NC) groups. In contrast, no significant differences were observed among the experimental treatments in hatched chick weight. The results agreed with the findings of Aljumaily and Taha (2019), who found that injecting eggs with spirulina at concentrations of 1% and 2% led to a significant increase in the hatchability of fertilized eggs compared to the control treatment. No significant differences were observed among the study treatments in the weight of the hatched chicks. This finding was confirmed by Ismail *et al*. (2019), who demonstrated that injecting hatching eggs with spirulina at a concentration of 2.5 mg/egg resulted in a significant increase in hatchability of fertilized eggs. Additionally, the injection resulted in a reduction in the embryonic mortality rate compared to the control treatment. The hatchability rate is considered one of the important economic traits, as it depends on several factors, including males and females. Females produce eggs after fertilization by male sperm, in addition to being supplied with the necessary nutritional elements for growth and development (Selim *et al*., 2018). The hatching period, especially the last three days of incubation, is a critical period for the embryo's life, during which vital organs develop, along with physiological and metabolic changes. These include increased secretion of adrenal hormones to meet its needs for non-carbohydrate substances, particularly fats, which represent the main source of energy during the hatching period and the following 72 hours (Ibrahim and Abdel-Daim, 2015).

Table 1: The effect of injecting eggs with spirulina algae extracts on hatchability, mortality rates, and hatched ducklings weight (Mean± SE).

Parameters	Experimental treatments				Sig.
	Control		SP, mg		
	NC	PC	3	6	
Hatchability (%)	64.16±1.48^{b}	65.02±2.24^{b}	73.05±1.04^{a}	76.75±1.92^{a}	*
Mortality (%)	35.83±1.48^{a}	34.98 ±2.24^{a}	26.94±1.04^{b}	23.25±1.92^{b}	*
Hatched chick weight(g)	31.33±0.66	31.00±0.57	33.33±0.33	33.66±0.66	N.S

Means bearing different superscripts in a row differ significantly (P≤0.05). NC: negative control; PC: positive control; SP: spirulina extract. * Significantly different (P≤0.05); N.S: Non-significant.

Table 2: The effect of injecting eggs with spirulina algae extracts on total protein, albumin, globulin and glucose level of hatched ducklings (Mean± SE).

Parameters	Experimental treatments				Sig.
	Control		SP, mg		
	NC	PC	3	6	
Total Protein (mg/dL)	2.46±0.03^{b}	2.44±0.02^{b}	2.61±0.02^{a}	2.64±0.04^{a}	*
Albumin (mg/dL)	1.43±0.02^{b}	1.42±0.02^{b}	1.56±0.01^{a}	1.58±0.02^{a}	*
Globulin (mg/dL)	1.02±0.01	1.01±0.02	1.04±0.04	1.06±0.02	N. S
Glucose (mg/dL)	256.33±2.60	252.66±1.85	257.33±2.66	260.00±2.64	N. S

Means bearing different superscripts in a row differ significantly (P≤0.05). NC: negative control; PC: positive control; SP: spirulina extract. * Significantly different (P≤0.05); N.S: Non-significant.

This process increases the likelihood of embryos being exposed to oxidative stress resulting from the hatching process and metabolic changes (Abdel-Daim *et al.*, 2015). Liquid spirulina extract can be used to inhibit oxidation due to its content of nutrients, vitamins, and antioxidants (Miranda *et al.*, 1998). It has been observed that liquid spirulina extract contains high levels of fat-soluble vitamins, such as vitamins A, E, and C. Vitamin A helps in the reconstitution of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), in addition to its role in improving metabolic processes and cellular differentiation of the embryo during its growth (Sporn *et al.*, 1994). As for vitamin E, it acts as an antioxidant to prevent protein damage, leading to improved digestion and utilization of nutrients (Khaligh *et al.*, 2017). It also plays a role in maintaining nitric oxide and protecting it from the harmful effects of free radicals, which helps stimulate cellular metabolism (Lorenzoni and Ruiz-Feria, 2006).

Table 2 shows the effects of in ovo injection of spirulina on serum biochemical parameters of hatched domestic ducklings. A significant increase (P≤0.05) in the concentrations of total protein and albumin is observed in injected eggs with 3 and 6 mg SP/egg in comparison with the positive (PC) and negative (NC) control groups. However, no significant differences (P≤0.05) were observed among the study treatments in the concentrations of globulin and glucose. Our results were consistent with the findings of Ismail *et al.* (2019), who indicated that injecting hatching eggs with spirulina at levels of 2.5 and 5.0 mg/egg led to a significant increase in the concentrations of total protein and albumin in the serum of chickens. Our results were consistent with the findings of Omar *et al.* (2022), who reported that adding spirulina to broiler diets at levels (0.25, 0.5, 0.75, 1 g/kg) led to a significant increase in total protein and albumin concentrations, while the supplementation had no significant effect on blood serum glucose concentrations. This was confirmed by Zahir *et al.* (2019), who found that dietary supplements containing spirulina at concentrations of 0.5%, 1%, and 1.5% had no significant effect on blood serum glucose levels in broilers. This was confirmed by Taher *et al.* (2024), who found that injecting eggs with spirulina at concentrations of 1.5 and 3 mg/egg did not significantly affect the concentrations of glucose in the blood serum. The significant increase in the total protein concentrations observed in the treatments where spirulina was injected into the eggs may be attributed to spirulina's content of proteins, essential amino acids, minerals, vitamins, phospholipids, and antioxidants (Jamil, et.al., 2015).

The results in Table 3 showed a significant effect (P≤0.05) of spirulina injection treatments on cholesterol, triglyceride, ALT and AST concentrations in hatched ducklings. In ovo injection with 3 and 6 mg SP/egg resulted in the lowest cholesterol and triglyceride concentrations as compared with the control groups. As for the enzymes ALT and AST, there was no significance between the treatments.

Table 3: The effect of injecting eggs with spirulina algae extracts on cholesterol, triglyceride, AST and ALT concentrations of hatched ducklings (Mean± SE).

Parameters	Experimental treatments				Sig.
	Control		SP, mg		
	NC	**PC**	3	**6**	
Cholesterol (mg/dl)	317.66 ±4.63[a]	318.33 ±3.17[a]	301.6 ±2.40[b]	293.66 ±3.17[b]	*
Triglyceride (mg/dl)	68.33 ±2.96[a]	69.00 ±2.51[a]	53.00 ±3.05[b]	46.33 ±3.33[b]	*
AST (U/L)	254.66±3.38	257.33±2.18	251.00±4.04	249.66±2.60	N.S
ALT (U/L)	14.66±1.66	15.66±2.02	13.33±1.76	11.00±1.73	N.S

Means bearing different superscripts in a row differ significantly (P≤0.05). NC: negative control; PC: positive control; SP: spirulina extract; AST: aspartate aminotransferase; ALT: alanine aminotransferase; * Significantly different (P≤0.05); N.S: Non-significant.

These results are consistent with those obtained by Mobarez *et al*. (2018), who reported that adding spirulina to the diet of laying hens led to a significant decrease in serum cholesterol and triglyceride concentrations. These results were consistent with the findings of Aljumaly and Taha (2019), who found that injecting hatching eggs with 50 microliters containing 1 and 2% spirulina did not significantly affect the concentrations of AST and ALT in the serum of hatched quail birds. Park *et al*. (2015) observed a reduction in blood cholesterol levels in treatments fed spirulina supplements compared to the control treatment. Abd El-Hady and El-Ghalid (2018) indicated that adding spirulina resulted in a significant decrease in serum cholesterol and triglyceride concentrations in broiler chickens. Serum cholesterol and triglyceride levels significantly decreased when spirulina was added to broiler diets at levels of 0.2 and 0.3 g/kg (Mariey *et al*., 2012). This was confirmed by Selim *et al*. (2018), who found that adding spirulina to laying hen diets at levels of 2 and 3 kg/ton led to a decrease in serum cholesterol concentrations. The decrease in serum cholesterol and triglyceride levels observed in the third and fourth treatments may be attributed to the presence of microalgae, particularly spirulina, which contains docosahexaenoic acid (DHA). DHA has a beneficial effect in reducing the risk of cardiovascular diseases, including lowering blood triglyceride levels (Holub, 2009). Chen *et al*. (2011) indicated that docosahexaenoic acid (DHA) from microalgae can inhibit the activity of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), thereby reducing cholesterol synthesis and consequently lowering plasma cholesterol levels. The HMG-CoA enzyme is a rate-limiting enzyme in cholesterol synthesis, and its inhibition can lead to decreased plasma cholesterol levels.

CONCLUSION

From obtained results, we concluded that in ovo injection of Iraqi local duck eggs with Spirulina could be used to improve hatching traits and subsequent growth performance as well as physiological status of hatched chicks.

ACKNOWLEDGEMENT

Many thanks to The Research team who Worked with me.

NOVELTY STATEMENT

This manuscript presents interesting findings showing that in Ovo Spirulina algae extract can improve hatchability and biochemical parameters in Iraqi local ducks.

AUTHOR'S CONTRIBUTION

All the research team for this work have equal roles.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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