Impact of Taurine in OVO Injection on Hatchability, Intestinal Development, and Biochemical Parameters of Newly Hatched Broiler Chicks

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Abstract

This experiment was conducted to study the effects of taurine in ovo injection on hatchability, chick quality, intestinal development, and biochemical parameters of broiler chick Ross 308, On 18th day of incubation, 1050 fertilized hatching eggs randomly distributed into seven treatments with 150 eggs for each treatment, T1 without injection (negative control), T2 control with injection of 0.2 ml normal saline /egg (carrier control), and T3–T7 (injected with 0.2 mL normal saline containing 50, 100, 150, 200, and 250 mg taurine per egg, respectively). Statistical analysis showed a significant effect of taurine injection on hatchability (P=0.000) and mortality (P=0.005), Treatments T3 and T4 recorded the highest hatchability and the lowest mortality. Additionally, treatment T3 showed significant improvement (P=0.000) in body weight, with a mean of 44.51 g, followed by treatment T4 with a mean weight of 43.03 g, compared to 40.06 and 40.75 g in T1 and T2, respectively. Similarly, chick length was significantly greater (P=0.000) in T3 (22.77 cm) and T4 (22.50 cm) compared to T1 (21.85 cm) and T2 (21.70 cm) .T3 and T4 led to a significant increase in total protein (P=0.001), globulin (P=0.006), and high - density lipoprotein (P=0.001) concentrations and a significant decrease in cholesterol (P=0.000), triglycerides(P=0.001), and liver enzyme concentrations, It can be concluded that In ovo injection with 50 mg/egg of taurine, improved hatchability, productivity, and physiological traits of broiler chicks.

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Introduction

The poultry industry is striving to meet the increasing demand for animal protein due to rapid population growth. Advances in genetic improvement, nutrition, and poultry management have led to the production of broilers with greater live weight, shorter lifespans, and higher feed conversion efficiency (Rajcic *et al.,* 2021; Al-Tememy *et al.,* 2023; AL-Tamimy *et al.,* 2024).

Many studies have proven that the early period of the broiler’s life affects the subsequent performance, as giving early feeding leads to an improvement in the bird’s ability to express their productive capacity, which reflects positively on the productive efficiency of birds. So, hatching can be a way to improve the broiler’s productive efficiency completely through injecting eggs with substances and nutrients, which is one of the methods of early feeding (Arain *et al.,* 2022).

In ovo injection of energy sources, essential amino acids, and mineral elements improves body weight and skeletal development, produces active chicks with high health and efficiency of food conversion, and improves the ability of birds to benefit from nutrients. It can be considered one of the methods to reduce the stress on hatched chicks, whether it is nutritional, health, or environmental (Ghane-Khoshkebijari *et al.,* 2024). This technique can also be used in conducting embryo insemination operations before hatching against viral diseases to provide early and effective protection for chicks and modulate cell- and antibody-mediated immune responses, leading to enhanced protection against pathogens (Das *et al.,* 2021; Ncho *et al.,* 2024).

Taurine, or β-Amino ethan sulfonic acid, is an amino acid derived from the metabolism of methionine and cysteine which is found in high concentrations in animal tissues. It has a fundamental role in protecting cell membranes and in the formation of bile salts as well as to its vital role in the safety of nerve cells and the retina (Alzawqari *et al.,* 2016). Taurine is essential for the development of photoreceptors and acts as a cell inhibitor against stress-related damage. Studies have indicated that dietary supplementation with taurine improves broiler performance by reducing fat accumulation in the liver and abdominal region, regulating plasma triglyceride levels, and enhancing thermal tolerance (Han *et al.,* 2020; Surai *et al.,* 2020), as well as taurine, in ovo injection reduces oxidative stress and improves organism homeostasis in chicken embryos (Lukasiewicz Mierzejewska *et al.,* 2024).

A comprehensive study on the effect of injecting broiler hatching eggs with taurine has not been conducted previously. Therefore, this experiment aims to investigate the impact of taurine in ovo injection on hatchability, chick quality, intestinal development, and biochemical parameters of newly hatched broiler chicks.

Materials and Methods

Ethics Approval

The scientific ethical committee of the Animal Production Department, College of Agriculture, University of Diyala, approved this study and gave the Ethical Number (4/2024). All applicable national and international guidelines for the care and use of animals were followed.

Experiment Design

This experiment was conducted at the Al-Safa hatchery in Diyala Governorate and the Department of Animal Production Laboratory at the College of Agriculture, University of Diyala during 15/11/2023 to 31/12/2023 to study the effect of taurine in ovo injection on hatchability, chick quality, intestinal development, and biochemical parameters of broiler newly hatched chicks. On the 18th day of incubation, eggs were candled to remove unfertilized eggs and the eggs with dead embryos and 1050 fertilized hatching eggs from broiler breeder flocks (Ross 308) from the same flock 34- week-old, with an average weight of 62.00±2 g which is stored for two days. On the 18th day of incubation, the eggs were randomly distributed into seven treatments, with three replicates per treatment (50 eggs per replicate) as follows:

1. T1: No injection (negative control).
2. T2: Injection of 0.2 mL normal saline per egg (carrier control).
3. T3: Injection of 0.2 mL normal saline containing 50 mg taurine per egg.
4. T4: Injection of 0.2 mL normal saline containing 100 mg taurine per egg.
5. T5: Injection of 0.2 mL normal saline containing 150 mg taurine per egg.
6. T6: Injection of 0.2 mL normal saline containing 200 mg taurine per egg.
7. T7: Injection of 0.2 mL normal saline containing 250 mg taurine per egg.

From 1 to 18 days, eggs were incubated in an incubator (Petersime, Belgium) at 99.8 °F and 87-87.5% relative humidity. On the 18th day of incubation, the eggs were injected with amniotic fluid through a hole in the shell using an automated syringe (Socorex Swiss) and a 22-gauge needle after disinfection with a 70% ethanol solution. The hole was then covered with Parafilm® tape. All experimental solutions were prepared on the day of injection by using taurine produced by the Turkish company Exvitamin with normal saline and prepared by stirring using a magnetic stirrer type, and before the in ovo injection procedure, all solutions were warmed to 37°C, and the eggs were transferred in hatching baskets to the hatcher at 98.9 °F and 90% relative humidity.

Data collection of hatchability, chick quality, intestinal development, and biochemical parameters

After hatching, hatchability and mortality were measured for each replicate group of chicks in each treatment group according to Desha *et al.* (2015), while body weight and chick length were measured for ten chicks from each replicate randomly selected according to Willemsen *et al.* (2008). Then, three chicks from each replicate were slaughtered to measure the weight and length of the small intestine and the weight of the remaining yolk. Blood samples were collected from the jugular vein of nine randomly selected chicks per treatment using tubes containing a separating gel, and serum blood samples were analyzed by an automatic biochemical analyzer (Apple, Co., Japan) with kits produced by (Spinrect Co. Espain) during 15/12/2023 to 31/12/2023. The analyzed blood serum traits included Glucose (mg/dl) according to Coles (1986), uric acid (mg/dl) according to Henry *et al.* (1982), protein profile (mg/dl) according to Wotton (1964), lipid profile (mg/dl) according to Franey and Elias (1986) and liver enzymes according to Reitman and Franke (1957).

Statistical analysis

The data were analyzed by the SPSS program (IBM, 2019) version 26 according to a complete randomized design (CRD), and the Duncan multi-range test (Duncan, 1955) at a significance level of 5%.

Results and Discussion

The results in Table 1 showed a highly significant effect of taurine injection treatments on hatchability (P=0.000) and mortality rates (P=0.005). Treatment T7 recorded the lowest hatchability rate (72.00%) and the highest mortality rate (28.00%), while treatments T3 and T4 recorded the highest hatchability rate and the lowest mortality rates. Additionally, treatment T3 showed significant superiority (P=0.000) over the other treatments in body weight, with a mean of 44.51 g, followed by treatment T4 with a mean weight of 43.03 g, which is compared to

40.06 and 40.75 g in treatments T1 and T2, respectively. Moreover, treatments T3 and T4 also showed a significant superiority (P=0.000) over other treatments in chick length, with lengths of 22.77 and 22.50 cm, respectively, which is compared to 21.85 and 21.50 cm in treatments T1 and T2, respectively. Our results are consistent with Vidyarthi *et al.* (2010); Belal *et al.* (2018), and Han *et al.* (2020). They found the addition of taurine to the diet led to a significant improvement in live body weight and weight gain, as Orhan *et al.* (2020) noted improved coefficient digestion of nutrients in quail as a result of the addition of taurine to the diet, and according to Hafeez *et a.* (2021), taurine led to improve body weight, weight gain, and feed conversion ratio under heat stress conditions due to its role in improving the health of birds and increasing the efficiency of the metabolism, Orhan *et al.* (2020) noted improved coefficient digestion of nutrients in quail as a result of the addition of taurine to the diet, which is positively reflected on the productive traits as in the results of our experiment. Taurine also leads to an increase in the length of the small intestine and its weight compared to the control treatment, thereby increasing its development and activity and stimulating the absorption of nutrients from the yolk sac (Table 2) which enhances the growth and development of the chicks in the last period of hatching because feeding the embryos in the amniotic fluid during this period will increase the absorption of nutrients by enterocytes, stimulate the secretion of digestive enzymes, promote the absorption of nutrients from the yolk sac, and support the development of vital organs of the fetus which is represented by the digestive system especially the small intestine (Salahi, 2015). In addition to the role of taurine in increasing the mass and volume of skeletal muscles due to the increased formation of muscle proteins, which ultimately leads to an increase in body mass and weight (Huang *et al.,* 2014).

Table 1. Impact of Taurine in OVO Injection on productive traits of chicks (mean ± SE)

Treatments	Hatchability (%)	Mortality (%)	Chick weight (g)	Chick length (cm)
T1	88.89±2.22 a	11.11±2.22 b	40.06±0.35 c	21.85±0.10 b
T2	90.00±1.92 a	10.00±1.92 b	40.75±0.41 c	21.70± 0.09 b
T3	96.67±1.92 a	3.33±1.92 b	44.51± 0.39 a	22.77± 0.11 a
T4	96.67±1.92 a	3.33±1.92 b	43.03±0.43 b	22.50±0.10 a
T5	90.00±1.92 a	10.00±1.92 b	40.91±0.40 c	21.76±0.12 b
T6	88.00±2.30 a	12.00±2.30 b	40.43±0.41 c	21.82±0.33 b
T7	72.00±8.00 b	28.00±8.00 a	40.29±0.34 c	21.80±0.15 b
P-value	0.000	0.005	0.000	0.000

- T1 Negative control treatment without injection, T2 carrier control treatment with injection of 0.2 mL/egg normal saline, T3, T4, T5, T6 and T7 Hatching egg Injection of 0.2 mL normal saline containing 50, 100, 150, and 250 mg taurine per egg respectively.
- Different letters within the same column indicate significant differences according to Duncan's multiple range test 5%.

Table 2 shows the effect of in ovo injecting with different levels of taurine on some anatomical traits of hatched chicks, the finding table showed a significant effect of taurine injection on small intestine weight (P=0.019) and length (P=0.014). T3 and T4 treatments recorded the highest small intestine weight (1.64 and 1.63 g) and length (50.16 and 49.00 cm length) compared with the T1 and T2 treatments. As for the weight of the remaining yolk (P=0.044), control treatment T1 recorded the highest weight of the remaining yolk, followed by treatments T2 and T5, while injection treatments T3 and T4 recorded the lowest weight of the remaining yolk, which recorded 4.18 and 4.15 g, respectively. The reason for the increase in small intestine weight and length is due to the role of taurine in enhancing the proliferation of intestinal cells, improving the intestinal mucosa, reducing apoptosis in the intestines, and stimulating the proliferation of epithelial cells in the small intestine, which reflects positively on the functional and structural traits of the small intestines, Taurine also increases the activity and effectiveness of the lipase enzyme and bile salts, which cause an increased digestion of fats and benefit from them (Zeng *et al.,* 2012).

Table 2. Impact of Taurine in OVO Injection on some anatomical traits of chicks (mean ± SE)

Treatments	Remaining yolk weight (g)	Small intestine weight (g)	Small intestine length (cm)
T1	4.83±0.02 a	1.17 ± 0.04 b	42.33±1.45 b
T2	4.70±0.01 bc	1.16±0.03 b	43.00±0.57 b
T3	4.18±0,05 c	1.64±0.06 a	50.16±0.91 a
T4	4.15±0.09 c	1.63±0.19 a	49.00±0.57 a
T5	4.75±0.20 b	1.20±0.13 b	43.40±1.13 b
T6	4.62±0.33 bc	1.24±0.09 b	42.66±1.45 b
T7	4.65±0.13 bc	1.23±0.11 b	43.33±3.38 b
P-value	0.044	0.019	0.014

- T1 Negative control treatment without injection, T2 carrier control treatment with injection of 0.2 mL/egg normal saline, T3, T4, T5, T6 and T7 Hatching egg Injection of 0.2 mL normal saline containing 50, 100, 150, and 250 mg taurine per egg, respectively.
- Different letters within the same column indicate significant differences according to Duncan's multiple range test 5%.

Table 3 shows the effect of in ovo injecting with different levels of taurine on protein profile and uric acid concentration in blood serum and shows a highly significant effect of taurine injection treatments on total protein (P=0.001) and globulin (P=0.006) concentration serum, and T3 and T4 treatments recorded the highest concentration, while control treatments T1 and T2 recorded the lowest concentration of total protein and globulin. Table 3 also shows that there is no significant effect of in ovo injecting with different levels of taurine on albumin (P=0.061) and uric acid (P=0.484) concentration. Taurine may contribute to elevated blood protein levels by supporting liver function and enhancing protein synthesis, as the liver is primarily responsible for producing plasma proteins such as globulins, taurine's antioxidant and anti-inflammatory properties help protect hepatic cells and optimize their metabolic activity (Table 5) Additionally, taurine influences amino acid transport and cellular hydration, which can facilitate protein production and stability in the bloodstream. These combined effects may lead to a measurable increase in serum protein concentrations (Lee *et al.,* 2004; Lukasiewicz Mierzejewska *et al.,* 2024).

Table 3. Impact of Taurine in OVO Injection on protein profile and uric acid concentration (mean ± SE)

Treatments	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Uric acid (mg/dl)
T1	2.33±0.008 c	1.00±0.11	1.33±0.20 b	4.40±0.60
T2	2.20±0.001c	0.85±0.02	1.35±0.02 b	3.95±0.02

T3	2.70±0.15 a	0.80±0.05	1.90±0.15a	3.70±0.15
T4	2.70±0.10 a	0.90±0.20	1.80±0.17 a	4.00±0.56
T5	2.45±0.02 b	1.30±0.05	1.15±0.08 b	4.25±0.14
T6	2.06±0.06 c	0.93±0.03	1.13±0.03 b	3.83±0.17
T7	2.36±0.06 b	0.96±0.08	1.40±0.15 b	4.73±0.41
P-value	0.001	N.S	0.006	N.S

- T1 Negative control treatment without injection, T2 carrier control treatment with injection of 0.2 mL/egg normal saline, T3, T4, T5, T6 and T7 Hatching egg Injection of 0.2 mL normal saline containing 50, 100, 150, and 250 mg taurine per egg, respectively.
- Different letters within the same column indicate significant differences according to Duncan's multiple range test 5%.
- N.S: meaning non-significant effect.

The finding in Table 4 shows there was no significant effect (P=0.610) of taurine injection treatments on low-density cholesterol concentration. As for cholesterol and triglyceride concentration, the results show a significant decrease (P=0.000) in T3, T4, and T5 treatments compared with control treatments (T1 and T2). Regarding high-density cholesterol concentration, T3 and T4 treatments recorded the highest concentration and reached 33.66 and 34.29 mg/dL, respectively, compared with 26.33 mg/dl in the control treatment T1.

Table 4. Impact of Taurine in OVO Injection on lipid profile concentration (mean ± SE)

Treatments	Cholesterol (mg/dl)	Triglyceride (mg/dl)	High-density cholesterol (mg/dl)	Low-density cholesterol (mg/dl)
T1	351.33±4.70 a	55.33±0.88 a	26.33±0.66 c	69.00±5.03
T2	353.66±1.20 a	50.66±0.66 a	29.00±0.57 b	73.00 ± 1.15
T3	292.66±5.36 b	40.00±0.57 b	33.66±1.33 a	73.33±2.40
T4	302.00±3.21 b	39.66±0.33 b	34.29±1.20 a	68.33±1.20
T5	303.33±0.66 b	40.00±0.57 b	29.50±1.44 b	74.50±2.02
T6	352.33±5.23 a	55.66±1.20 a	32.00±1.15 ab	71.33±2.90
T7	335.00±18.23 a	56.00±5.50 a	32.66±1.20 ab	71.33±0.88
P-value	0.000	0.000	0.001	N.S

- T1 Negative control treatment without injection, T2 carrier control treatment with injection of 0.2 mL/egg normal saline, T3, T4, T5, T6 and T7 Hatching egg Injection of 0.2 mL normal saline containing 50, 100, 150, and 250 mg taurine per egg, respectively.
- Different letters within the same column indicate significant differences according to Duncan's multiple range test at 5%.
- N.S: meaning non-significant effect.

The results of our study in Table 4 demonstrated that in ovo injecting of taurine significantly decreased cholesterol and triglyceride concentrations while increasing high-density cholesterol in the serum of broilers. This may be due to bile-conjugated taurine promoting lipolysis and fatty acid formation, which then leads to improved lipid metabolism, many studies associated with taurine and cholesterol 7 α-hydroxylase enzyme activity, which catalyzes the conversion of cholesterol to bile acids, and the formation of bile acids from cholesterol which is one of the important pathways in regulating cholesterol in body tissues and fluids (Alzawqari *et al.,* 2016). According to Zeng *et al.* (2012), taurine leads to an increase lipoprotein lipase activity on the vascular endothelial surface that degrades circulating triglycerides in the bloodstream to very low-density lipoproteins and chylomicrons as well as increased hepatic lipase activity that degrades hydrolyzes triglycerides and phospholipids. Also, taurine could enhance triglyceride hydrolysis in plasma and high-density cholesterol maturation, and then reduce the content of triglyceride and cholesterol, also Nandhini *et al.* (2002) reported that taurine leads to an increase in gene expression of the carnitinepalmitoyltransferase 1 enzyme and hormone-sensitive lipase hydroxylase enzyme activity, thus enhancing the metabolism of fats. As shown in Table 5, hepatic enzyme activity significantly decreased in T3, T4, and T5 treatments compared with the control treatment. Aspartate transaminase concentration reached 219.00, 215.50, 210.66, and 215.33 U/L in T1, T2, T6, and T7 treatments and decreased significantly (P=0.003) to 169.00, 184.66, and 181.33 U/L in T3, T4, and T5 treatments, respectively, while Alanine transaminase concentrations reached 6.00 and 8.00 U/L in T1 and T2 treatments, respectively, and significantly decreased (P=0.002) to 4.00 U/L in T3 and 3.66 U/L in T4 and T5 treatments, as well as T3, T4 and T5 treatments recorded the lowest concentration of the enzyme alkaline phosphatase compared with T1 and T2 . The reason for the low concentration of liver enzymes in taurine injection coefficients is attributed to its role in enhancing the antioxidant status and reducing oxidative stress by enhancing glutathione and glutathione peroxidase activities. Many studies have demonstrated that taurine significantly inhibits free radicals and reduces lipid peroxidation (Xu *et al.,* 2019; Han *et al.,* 2020; Xu *et al.,* 2023).

Table 5. Impact of Taurine in OVO Injection on hepatic enzymes activity (mean ± SE)

Treatments	Aspartate transaminase (U/L)	Alanine transaminase (U/L)	Alkaline phosphatase (U/L)
T1	219.00±15.50 a	6.00±0.57 b	25.66±0.33 a
T2	215.50±8.94 a	8.00±0.57 a	32.50±0.22 a
T3	169.00±9.29 b	4.00±0.57 c	20.00±0.57 b
T4	184.66±4.48 b	3.66±0.33 d	20.33±0.66 b
T5	181.33±5.36 b	3.66±0.33 d	20.00±0.57 b
T6	210.66±6.22 a	6.33±1.20 ab	25.33±0.66 a
T7	215.33±2.84 a	5.00±0.57 bcd	24.33±1.25 a
P-value	0.003	0.002	0.000

- T1 Negative control treatment without injection, T2 carrier control treatment with injection of 0.2 mL/egg normal saline, T3, T4, T5, T6, and T7 Hatching egg Injection of 0.2 mL normal saline containing 50, 100, 150, and 250 mg taurine per egg respectively.
- Different letters within the same column indicate significant differences according to Duncan's multiple range test 5%.

Conclusions

In ovo injection with 50 or 100 mg/egg of taurine significantly improves hatchability, post-hatch chick quality, and early physiological development in Ross 308 broilers and enhances body weight and chick length at hatch, as well as improved biochemical indicators, including elevated total protein, globulin, and high-density cholesterol levels, and decreased cholesterol, triglycerides, and hepatic enzyme activity. Based on these findings, we recommend injecting broiler eggs with 50 mg/egg of taurine.

Conflict of interest

The authors of this manuscript have no conflicts of interest to report. They have all seen and agreed on the content of the paper and there is no financial interest to declare, as well as the authors certify that this submission is original work and it is not under review in other publications.

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