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Effect of In-ovo Bee Venom Injection on Hatchability, Blood Traits, Immunity, and Antioxidant Status of Sinai chick's Strain at Hatch

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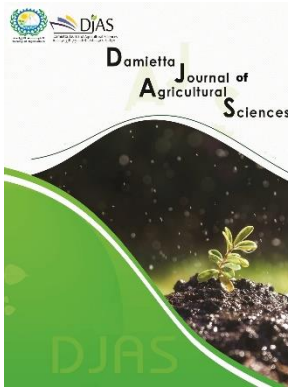

ABSTRACT

This study evaluated the effects of in-ovo bee venom (BV) on hatchability, blood traits, immunity, and antioxidant status of Sinai chickens. A total of 250 fertilized eggs were divided into five groups: non-injected control (CON), sterile distilled water-injected control (sham), and three BV-injected groups (10, 15, and 20 µg/egg). On day 18 of incubation, each egg was injected into the air sac. Results indicated that in-ovo injection of BV improved the hatchability of Sinai strain eggs, particularly at 15 and 20 µg/egg, and enhanced blood biochemistry by increasing glucose, total protein, albumin, and high-density lipoprotein "HDL" while reducing cholesterol, triglycerides, and total lipids. At the 15 µg, alanine transaminase (ALT) decreased while thyroxine (T4) concentration increased in compared to other experimental groups. Both 15 and 20 µg elevated triiodothyronine (T3) levels, enhanced immunity (while blood cells "WBC"; and Immunoglobulins "IgG, and IgM"), and improved antioxidant status by increasing TAC and reducing MDA level. In conclusion, in-ovo injection of 15 and 20 µg BV/egg improved immunity, antioxidant status, and hematological traits of Sinai chicks, which may enhance their post-hatch performance.*Keywords: Antioxidant, blood, CoQ10, Growing rabbits, productive.*

Keywords: *Antioxidant, bee venom; in-ovo; hatchability; hematology; Sinai chickens.*

INTRODUCTION

Efficient incubation and optimal embryonic development are important for poultry production (Foye et al., 2007). In-ovo nutrient administration and early post-hatch feeding have been reported to improve hatchability, chick quality, and performance (El-Kholy et al., 2021; Sarhan et al., 2023). The period from day 18 of incubation to four days post-hatch is critical for intestinal development and survival chicks (Iji et al., 2001; Uni and Ferket, 2004), as chicks shift from yolk reserves to external feed, accompanied by major metabolic and physiological changes (De Oliveira et al., 2008; Givisiez et al., 2020).

Several studies have investigated the use of bee venom (BV) and its main component, melittin (MLT), for in-ovo injection in poultry (Khalil et al., 2023; Khali et al., 2021). Globally, bee products such as BV, pollen, propolis, and royal jelly have been utilized for centuries due to their medicinal properties (Fratellone et al., 2016; Guha et al., 2021). Apitherapy, a branch of complementary medicine, employs honeybee products for therapeutic purposes (Trumbeckaite et al., 2015).

The BV also known as apitoxin (Abd El-Aziz et al., 2023), contains at least 18 pharmacologically active components, with melittin (≈50%) as the major peptide, along with phospholipase A2, apamin, adolapin, and hyaluronidase, in addition to amino acids, sugars, lipids, minerals, volatile compounds, and biogenic amines (Lee et al., 2009; Wehbe et al., 2019; Nowar, 2016; Zolfagharian et al., 2015; Abd El-Wahed et al., 2017; Hossen et al., 2017). These bioactive substances provide potent anti-inflammatory, antibacterial, analgesic, anticancer, and antiviral effects, in addition to stimulating immune responses (Guha et al., 2021; Wehbe et al., 2019).

In poultry, BV has been reported to improve performance and antioxidant capacity when administered either by injection or through drinking water (Han, 2010). Mechanistically, the bioactive components of BV can modulate immune responses by promoting dendritic cell maturation and activating host defense pathways against microbial pathogens (Perrin-Cocon et al., 2004; Ramoner et al., 2005; Banchereau & Steinman, 1998; Samuel, 2001).

Although numerous studies have investigated in-ovo injection techniques, the effects of in-ovo

injection with BV have not been sufficiently explored. Therefore, the present study was conducted to evaluate these effects. Based on the aforementioned properties of BV, this study aimed to investigate the effects of in-ovo injection of BV at three levels (10, 15, and 20 µg/egg) into Sinai strain hen eggs on day 18 of incubation. The evaluation focused on hatchability, immune response, antioxidant status, and selected blood biochemical parameters of the hatched chicks.

MATERIALS AND METHODS

Ethics statement

The experiment was conducted at Al-Serow Poultry Breeding Research Station, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Damietta Governorate, in cooperation with Faculty of Agriculture, Damietta University, Egypt, in compliance with Directive 2010/63/EU of the European Parliament and Council on the protection of animals and birds used for scientific purposes.

Eggs and incubation

Sinai chickens (a local strain) are a mongrel breed originating from the Sinai desert, derived from crosses between old local strains and exotic types (Eltanany et al., 2011). A total of 250 fertile eggs were collected from Sinai breeder hens at 116 weeks of age. The eggs were transferred to an automatic incubator (Econoom, Holland, model 1965) set at 37.5 °C and 65–70% relative humidity. The eggs were automatically turned 24 times per day at an angle of ±45°, and after injection, they were moved to the hatcher in covered trays for the last three days of incubation under 37.5 °C and 70 ± 3% relative humidity. Throughout the incubation period, all eggs were maintained according to standard routine procedures. Before incubation, the eggs were randomly divided into five experimental groups (50 eggs per group). The first group was not injected and served as the negative control (CON). The second group was injected with 0.2 ml of sterile distilled water and served as the positive control (sham). The third, fourth, and fifth groups were injected with 10, 15, and 20 µg of BV per egg, respectively. All eggs were candled on day 10 of incubation to check for fertility and embryonic development.

The in-ovo injection solution

Three in-ovo injection solutions were prepared containing 10, 15, and 20 µg BV/egg. Bee venom (BV; freeze-dried, 95%) was purified from Apis mellifera in lyophilized powder form and obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Egypt). The BV powder was dissolved in sterile distilled water, prepared immediately before injection, and gently warmed reach the incubation temperature.

The in-ovo injection procedure

All eggs, including those of the control groups, were taken out of the incubator for nearly 20 min to equalize the conditions during the injection process. A mini grinder (Model CT13428, CROWN, China) was used to make a proper hole on the broad side of the eggshell. Using a 21-gauge needle at 432 h (18 d of incubation), eggs from all injection groups were injected from the top of the large end of the egg with in-ovo injection solutions (0.2 ml/egg) into the air sac, following the procedures described by Uni & Ferket (2003) and Uni et al. (2005). After injection, the site was disinfected with 70% ethanol and sealed with sterile paraffin wax. In parallel, eggs of the control group were taken out of the incubator and kept under the same environmental conditions as the injected eggs.

Measurements

Hatchability traits

The number of hatched chicks on days 21th and 22nd of incubation was compared with the number of fertile eggs to determine hatchability for each replicate/treatment (North, 1984). The hatchability percentage based on the total eggs set was determined as (number of hatched chicks / total eggs set × 100). The numbers of pipped eggs and embryonic mortality were also recorded.

Blood hematology and biochemical constituents

Three chicks were randomly selected from each treatment group and slaughtered for blood sample collection via the jugular vein to assess hematological and biochemical parameters. Blood samples were collected in heparinized tubes to hematological parameters, while other samples in non-heparinized tubes and it centrifuged (3500 rpm) for 20 minutes to separate blood serum to biochemical analysis. Serum samples were carefully transferred into Eppendorf tubes and stored at −20 °C in a deep freezer. Hematological parameters were evaluated according to the recommendations of Schalm et al. (1975).

Blood samples were used to measure hematological parameters, including white blood cells (WBCs), lymphocytes (L), heterophils (H), red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet count (PLT). Erythrocyte and leukocyte counts were analyzed using the hemocytometer method.

Biochemical analysis were included: serum total protein (TP, g/dl) and albumin (ALB, g/dl) using a commercial kit, following the method of Doumas and Maume (1977). Globulin (GLU, g/dl) levels were calculated by subtracting albumin values from total protein. The ALB/GLU ratio was calculated. Serum samples were also analyzed for aspartate aminotransferase (AST, U/L) and alanine

aminotransferase (ALT, U/L) using commercial kits from Linear Chemicals (Barcelona, Spain), according to Friedman and Young (2005). Furthermore, serum levels of total cholesterol (TC, mg/dl), total lipids (TL, mg/dl), triglycerides (TG, mg/dl), high-density lipoprotein (HDL, mg/dl), and low-density lipoprotein (LDL, mg/dl) were determined as described by Vogel (1997). Creatinine (mg/dl) was measured according to Fabiny and Ertingshausen (1971), and urea (mg/dl) was determined following Patton and Crouch (1977). Serum immunoglobulins IgG and IgM were assessed using the method of Akiba et al. (1982).

Serum hormone analysis

Using commercially available enzyme-linked immunosorbent assay (ELISA) kits, serum endocrine hormone concentrations, such as triiodothyronine (T3, ng/ml) and thyroxine (T4, ng/ml), were measured in accordance with the manufacturer's instructions. All measurements were made in duplicate to include accuracy. An ELISA kit (Autobio Diagnostics, Co., China, E-1002) with a sensitivity of 0.4 µg/dL, intra-assay precision of >3.58%, and inter-assay precision of >9.64% was used to measure the levels of serum T4. Commercial ELISA kits (Autobio Diagnostics, Co., China, E-1001) with an assay sensitivity of 0.2 ng/mL, an intra-assay precision of >3.94%, and an inter-assay precision of >7.83% were used to measure the levels of serum T3.

Redox Status

Total antioxidant capacity (TAC, mM/L) and serum malondialdehyde (MDA, nmol/mL) levels were measured using a UV4802 spectrophotometer (Unico Co., Dayton, USA) (Botsoglou et al., 1994). Every redox index was calculated in accordance with the guidelines provided by the corresponding assay kit manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis

This study analyzed by using a MIXED approach for repeated measurements (SAS, 2012, release 9.2, Cary, NC, USA) to evaluate redox status indicators, biochemical characteristics, and blood hematology as dependent variables. Meteorological, physiological, and fertility and hatchability data were analyzed using a general linear model approach (one-way analysis of variance; SAS, 2012). Differences between treatment groups were determined using Duncan's new multiple range post-hoc test, and $p < 0.05$ was deemed significant. All data were expressed as least square means ± pooled standard error of the mean (SEM). The Shapiro-Wilk and Levene tests were used to assess variance homogeneity and normality. For statistical significance, a p-value of < 0.05 was chosen.

RESULTS:

Hatchability Performance

With respect to hatchability on days 20 and 21, the results revealed highly significant differences ($P \leq 0.001$) among treatments in the total hatchability percentages of Sinai strain eggs in compared to the control group (Table 1). In-ovo injection with the lower BV dose resulted in a significantly ($P \leq 0.0001$) lower number of pipped and dead chicks in compared to the corresponding values in the control and higher-dose groups. Additionally, hatchability traits showed a significant ($P \leq 0.0001$) higher linear and quadratic response.

Biochemical Parameters of Chicks at Hatch:

The results of glucose, total protein (TP), albumin (ALB), globulin (GLU), and lipid profile parameters in the blood of chicks at hatch are presented in Table 2. Blood glucose levels differed significantly ($p < 0.05$) among the treatment groups, with the highest values observed in the in-ovo injection groups treated with 15 and 20 µg of BV in compared to the control and other experimental groups. Significant increases ($p \leq 0.01$) in TP concentrations were recorded in the in-ovo groups injected with 15 and 20 µg BV, relative to the control, sham, and 10 µg BV groups. In contrast, GLU concentrations were significantly ($p < 0.05$) higher in the control and sham groups, while the lowest value was observed in the 15 µg BV in-ovo injection group. Furthermore, ALB levels and the ALB / GLU ratio were significantly ($p < 0.05$) higher in the group injected in-ovo with 15 µg BV in compared to other experimental groups. Both TChol and TL concentrations were significantly ($p \leq 0.01$) reduced in the 15 and 20 µg BV in-ovo injection groups. Triglyceride concentrations were also significantly ($p \leq 0.01$) decreased in the 15 µg BV in-ovo injection group. Conversely, LDL-C and HDL-C levels showed no significant differences among in-ovo experimental groups. In the linear response analysis, glucose, ALB, GLU, TC, and TL exhibited highly significant effects ($P < 0.001$). A significant ($P < 0.05$) linear response was also detected for TP, GLU, and the ALB/GLU ratio. Moreover, the ALB/GLU ratio demonstrated a highly significant quadratic response ($P = 0.0031$). In addition, ALB, GLU, and TG showed significant ($P < 0.05$) quadratic responses.

Hematological Parameters

The hematological parameters of chicks at hatch are presented in Table 3. The parameters (RBC, HCT, MCV, MCH and PLT) showed no significant differences ($p > 0.05$) among all experimental groups. In contrast, HGB and MCHC recorded significant ($p < 0.05$) higher values in the groups injected with 15 and 20 µg BV. Additionally, HGB and MCHC showed a highly significant linear effect ($P < 0.0001$).

Liver and Kidney Function Parameters in Chicks at Hatch

The results of liver and kidney function parameters in the blood of chicks at hatch are presented in Table 4. The AST values showed no significant differences among the BV treatment groups (P > 0.1809). The ALT was significantly (p < 0.0001) lower in the in-ovo BV-injected groups in compared to control and sham groups. The 15 µg BV dose showed the significant lowest ALT level in compared to other experimental groups. Creatinine and urea-N were showed no significant differences (P > 0.05) among all experimental groups. Additionally, ALT showed highly significant linear (P < 0.001) and quadratic (P < 0.0001) responses.

Table 1. Effect of in-ovo bee venom (BV) injection on hatchability traits of Sinai chick's strain at hatch.

Items	Treatments[1] (*TRTS*)						*p*-values[3]		
			B V (µg/egg)						
	CON	Sham	10	15	20	SEM[2]	*T*	*L*	*Q*.
Number eggs	5 0.00	5 0.00	5 0.00	5 0.00	5 0.00	0 .000	-	-	-
No. chicks produced	2 8.00[b]	4 0.20[a]	4 1.40[a]	4 1.60[a]	4 3.00[a]	1 .277	< .0001	< .0001	< .0001
No. Un hatched produced	1 1.80[a]	6 .40[b]	4 .00[bc]	4 .20[bc]	3 .20[c]	0 .822	< .0001	< .0001	< .0001
Hatchability total eggs%	5 6.00[b]	8 0.40[a]	8 2.80[a]	8 3.20[a]	8 6.00[a]	2 .555	< .0001	< .0001	< .0001
Pipped egg	1 1.80[a]	7 .00[b]	3 .20[c]	4 .60[bc]	3 .20[c]	0 .892	< .0001	< .0001	< .0001
Mortality	3 0.02[a]	1 3.86[b]	8 .81[b]	9 .39[b]	6 .94[b]	2 .257	< .0001	< .0001	< .0001

[a,b] Means within a row with different superscripts are significantly different (p <0.05).
[1]CON= control (not injected); Sham= injected with sterile distilled water; BV = bee venom.
[2]SEM= Standard error of means. [3]T= treatments; L, linear response; Q., and quadratic response

Table 2. Effect of in-ovo bee venom (BV) injection on biochemical parameters of Sinai chick's strain at hatch.

Items[1]	Treatments[2] (*TRTS*)						*p*-values[4]		
			B V(µg/egg)						
	CON	Sham	10	15	20	SEM[3]	*T*	*L*	*Q*.
glucose (mg/dL)	103.39[b]	107.95[b]	107.27[b]	126.39[a]	124.92[a]	1.768	<.0001	<.0001	0.1681
TP (g/dL)	2.23[b]	2.17[b]	2.17[b]	2.45[a]	2.35[ab]	0.0674	0.0217	0.0418	0.8040
ALB(g/dL)	1.68[c]	1.61[c]	1.75[c]	2.12[a]	1.93[b]	0.059	<.0001	0.0002	0.0280
GLU(g/dL)	0.55[a]	0.56[a]	0.42[ab]	0.33[b]	0.43[ab]	0.047	0.0001	0.0230	0.0123
ALB/GLU ratio	3.42[b]	3.22[b]	4.38[b]	6.71[a]	4.60[b]	0.475	0.0082	0.0094	0.0031
TC (mg/dL)	242.86[a]	238.14[a]	224.86[ab]	192.71[b]	199.29[b]	11.062	0.0082	0.0059	0.3184
TL (mg/dL)	280.57[a]	276.43[a]	275.29[a]	254.29[b]	258.57[b]	4.587	0.0006	0.0003	0.3034
TG (mg/dL)	138.29[ab]	135.43[ab]	132.00[ab]	114.57[b]	152.29[a]	8.139	0.0450	0.5421	0.0207
LDL (mg/dL)	99.54	98.97	100.67	93.70	94.09	3.292	0.4353	0.1258	0.9117
HDL (mg/dL)	68.14	67.43	68.43	73.00	72.29	2.117	0.2313	0.1005	0.8241

[a,b] Means within a row with different superscripts are significantly different (p <0.05).
[1]TP= total protein; ALB= albumin; GLU= globulin; ALB/GLU= albumin/globulin ratio; TC= total cholesterol; TL- total lipids; TG= triglyceride; LDL-C= low density lipoprotein cholesterol; HDL= high density lipoprotein cholesterol.
[2]CON= control (not injected); Sham= injected with sterile distilled water; BV = bee venom.
[3]SEM= Standard error of means.
[4]*T*= treatments; *L*, linear response; *Q*., and quadratic response.

Table 3. Effect of in-ovo bee venom (BV) injection on hematological parameters of Sinai chick's strain at hatch.

Items[1]	Treatments[2] (*TRTS*)					SEM[3]	*p*-values[4]		
			BV(µg/egg)						
	CON	sham	10	15	20		*T*	*L*	*Q.*
RBC($\times10^6$/mm^3)	2.43	2.156	2.17	2.51	2.32	0.168	0.4967	0.9889	0.8465
HGB (g/dl)	8.06^b	7.89^b	7.91^b	11.06^a	10.87^a	0.359	<.0001	<.0001	0.9693
HCT (%)	24.29	23.87	24.09	25.79	24.46	1.115	0.7664	0.6804	0.6355
MCV µm	84.33	84.33	82.77	83.69	83.99	0.760	0.5833	0.9728	0.2167
MCH (pg)	3.84	3.70	3.65	4.58	4.79	0.403	0.1914	0.0705	0.5681
MCHC (g/dl)	33.38^b	33.26^b	33.24^b	43.82^a	44.81^a	2.208	0.0003	0.0003	0.8128
PLT (10^3/µl)	11.24	10.98	10.99	11.82	11.24	0.620	0.8456	0.7692	0.7890

[a,b, c] Means within a row with different superscripts are significantly different (p <0.05).

[1]RBC= red blood cells; HGB= hemoglobin; HCT= haematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; PLT= Platelet count; MCHC= Mean corpuscular hemoglobin concentration.

[2]CON= control (not injected); Sham= injected with sterile distilled water; BV = bee venom.

[3]SEM= Standard error of means.

[4]*T*= treatments; *L*, linear response; *Q*., and quadratic response.

Table 4. Effect of in-ovo bee venom (BV) injection on liver and kidney function parameters of Sinai chick's strain at hatch.

Items[1]	Treatments[2] (*TRTS*)					SEM[3]	*p*-values[4]		
			B V (µg/egg)				*T*	*L*	*Q.*
	CON	sham	10	15	20				
AST (mg/dL)	188.92	188.23	186.37	182.21	183.97	2.185	0.1809	0.0685	0.3435
ALT (mg/dL)	24.79^a	24.63^a	16.62^{bc}	12.96^c	18.72^b	1.267	<.0001	0.0003	<.0001
Creatinine (mg/dL)	0.24	0.20	0.20	0.24	0.21	0.028	0.7492	0.6396	0.9204
Urea-N (mg/dL)	3.55	3.51	3.63	3.44	3.22	0.353	0.9364	0.4666	0.6750

[a,b] Means within a row with different superscripts are significantly different (p <0.05).

[1]AST= aspartate transaminase; ALT= alanine transaminase.

[2]CON= control (not injected); Sham= injected with sterile distilled water; BV = bee venom.

[3]SEM= Standard error of means.

[4]*T*= treatments; *L*, linear response; *Q*., and quadratic response.

Thyroid Hormone Levels in Chicks at Hatch

Data on T3 and T4 concentrations, showing significant effects in Sinai chicks at hatch, are presented in Table 5. The results showed that in-ovo injection with higher BV doses (15 and 20 µg) resulted in a significant ($p < 0.0001$) increase in T3 concentrations in compared to the 10 µg BV dose, sham, and control groups. Also, Table 5 shows insignificant differences between 15 and 20 BV groups for T3 concentrations. Additionally, T4 concentrations were significantly ($p < 0.0001$) higher in the group injected in-ovo with the 15 µg BV dose in compared to the other experimental groups (Table 5). Furthermore, both T3 and T4 exhibited significant linear responses ($P < 0.0001$), while T4 also showed a significant quadratic response ($P < 0.0001$).

Immune Response Indicators (Cellular and Humoral)

The results of the cellular immune parameters (WBC and leukogram) and humoral immune parameters (IgG and IgM) in the blood of chicks at hatch are presented in Table 2. No significant ($p > 0.05$) differences were observed in all leukocyte indices (WBC, eosinophils, basophils, monocytes, heterophils, and the H/L ratio) across all experimental groups. IgG and IgM levels also showed significant ($p < 0.0001$) differences among the treatments, with the highest values observed in the in-ovo injection group that received the 15-µg bee venom dose. Additionally, IgG and IgM showed significant linear and quadratic responses ($P < 0.0001$).

Antioxidant Status Indicators

Table 7 shows the effect of in-ovo injection of BV on the antioxidant status. TAC levels increased significantly ($p < 0.0001$) in BV-treated groups compared to the control and sham, while MDA levels decreased. The 15 and 20 µg BV/egg doses showed the greatest improvement in antioxidant status.

Additionally, TAC and MDA showed significant linear and quadratic responses (P < 0.0001).

Table 5. White blood cells differential values of growing NZW rabbits as affected by different feeding program and CoQ10 administration.

Items[1]	Treatments[2] (*TRTS*)					SEM[3]	*p*-values[4]		
	CON	Sham	BV(µg/egg) 10	15	20		*T*	*L*	*Q.*
T3, ng/ml	1.84[c]	1.88[bc]	2.00[b]	2.48[a]	2.43[a]	0.055	<.0001	<.0001	0.0820
T4, ng/ml	9.49[c]	9.71[c]	11.84[b]	12.93[a]	11.89[b]	0.314	<.0001	<.0001	<.0001

[a,b, c] Means within a row with different superscripts are significantly different (p <0.05).
[1]T3= triiodothyronine; T4= thyroxine.
[2]CON= control (non injected); Sham= injected with sterile distilled water; BV = bee venom.
[3]SEM= Standard error of means.
[4]T= treatments; L, linear response; Q., and quadratic response.

Table 6. Plasma total protein and its fractions in growing NZW rabbits as affected by different feeding program and CoQ10 administration.

Items[1]	Treatments[2] (*TRTS*)					SEM[3]	*p*-values[4]		
	CON	sham	B V(µg/egg) 10	15	20		*T*	*L*	*Q.*
WBC(10^3/mm^3)	22.39	21.92	21.05	22.92	21.96	0.685	0.4279	0.8430	0.8968
Lymp. (L, %)	43.49	43.16	43.08	43.02	42.42	0.323	0.2368	0.0353	0.7829
Eosiophils, %	10.09	9.89	10.55	10.08	9.98	0.276	0.5050	0.5128	0.3360
Basophil, %	0.57	0.57	0.56	0.51	0.56	0.039	0.8182	0.6893	0.4141
Monosytes, %	13.29	13.29	13.96	13.96	13.96	0.558	0.8661	0.5203	0.6312
Hetero. (H, %)	33.94	33.19	31.95	32.54	33.18	1.006	0.7027	0.7311	0.2353
H/L	0.78	0.87	0.74	0.86	0.78	0.036	0.7789	0.8563	0.2513
IgG mg/dl	176.09[d]	174.73[d]	250.55[c]	305.06[a]	292.71[b]	3.705	<.0001	<.0001	<.0001
IgM mg/dl	274.19[d]	273.23[d]	295.50[c]	309.74[a]	301.47[b]	1.387	<.0001	<.0001	<.0001

[a,b, c] Means within a row with different superscripts are significantly different (p <0.05).
WBC= white blood cells; Lymp.= lymphocytes; Hetero.= heterophiles; H/L= heterophiles/ lymphocytes ratio ; IgG= immunoglobin G; IgM= immunoglobin M.
[2]CON= control (not injected); Sham= injected with sterile distilled water; BV = bee venom.
[3]SEM= Standard error of means.
[4]*T*= treatments; *L*, linear response; *Q*., and quadratic response.

Table 7. Effect of in-ovo bee venom (BV) injection on antioxidant status of Sinai chick's strain at hatch.

Item's	Treatments[2] (*TRTS*)					SEM[3]	*p*-values[4]		
	CON	Sham	B V (µg/egg) 10	15	20		*T*	*L*	*Q.*
TAC, mM/L	1.65[c]	1.60[c]	2.28[b]	3.45[a]	3.25[a]	0.197	<.0001	<.0001	0.0563
MDA, nmol/mL	2.98[ab]	2.52[a]	2.46[b]	1.79[c]	2.28[c]	0.579	<.0001	<.0001	0.1029

[a,b, c] Means within a row with different superscripts are significantly different (p <0.05).
[1]TAC= total antioxidant capacity; MDA= malondialdehyde.
[2]CON= control (not injected); Sham= injected with sterile distilled water; BV = bee venom.
[3]SEM= Standard error of means.
[4]*T*= treatments; *L*, linear response; *Q*., and quadratic response.

DISCUSSION

The present study demonstrated that in-ovo injection of BV had a positive effect on hatchability percentages. These findings are consistent with previous studies, which reported that in-ovo injection of different nutrients can improve the nutritional status of embryos and, consequently, enhance hatchability percentages in broiler breeder eggs (Al-Shamery & AlShuhaib, 2015; Edwards et al., 2016). The improvement in hatchability observed in the current study may be attributed to the enriched nutritive value of BV, which contains essential minerals, sugars and amino acids that support embryonic growth and hatchability. Similarly, Hassan et al. (2021) reported that in-ovo injection of BV into Alexandria breeder hens' eggs at day 18 of incubation significantly increased hatchability percentages. However, Khalil et al. (2023) indicated that a higher dose of melittin, the main bioactive component of BV (approximately 50%), at 15 µg/egg in-ovo injection at day 18 of incubation, had a negative effect on hatchability percentages.

The present results revealed that the highest glucose values were recorded in the in-ovo injection groups treated with 15 and 20 µg of bee venom, in compared to the control and other groups. These findings agree with Khali et al. (2021), who reported significantly higher glucose levels in the 20 µg BV group than in the 10 µg BV and control groups. In the same pattern Khalil et al. (2023) observed that significantly higher glucose values in chicks from the 10 and 15 µg MLT/egg groups compared to the control and 5 µg groups. Collectively, these results suggest that in-ovo injection of BV enhances blood glucose levels at hatch, particularly at higher doses. The increase in glucose is consistent with its role as the main energy source for hatching activities, derived from glycogen in the liver, yolk sac membrane, and muscle (Christensen et al., 2001; Yadgary & Uni, 2012; van de Ven et al., 2013). This may explain the reduced of pipped and mortality rates observed in the BV-treated groups in current study.

Regarding the protein profile in current study, significant increases in TP concentrations were observed in the in-ovo groups injected with 15 and 20 µg BV. GLU concentrations were higher in the control and sham groups, with the lowest value recorded in the 15 µg BV group. Moreover, ALB levels and the ALB/GLU ratio were significantly elevated in the 15 µg BV group. These findings differ from those of Khalil et al. (2023), who reported no significant changes in TP and GLU concentrations following in-ovo melittin injection in Alexandria chicks. They also differ from the results of Naglaa et al. (2019), Han et al. (2010), and El-Banna et al. (2023), who found that BV supplementation in the drinking water of broiler chickens had no significant effect on serum protein profile parameters. In contrast, our results are consistent with several studies that demonstrated the positive effect of BV on protein metabolism. Hassan and Raghad (2021) reported that BV increased TP, ALB, and GLU levels in the blood.

The present results also showed that TC and TL levels decreased significantly in the 15 and 20 µg BV groups, while triglycerides were lowest in the 15 µg BV group. These findings are consistent with the hypolipidemic effects of bee venom reported in previous studies (Zahran et al, 2021). Rabie et al. (2018) demonstrated that broiler chickens supplemented with BV (2 mg/L in drinking water) showed significantly lower plasma cholesterol concentrations compared to the control group. Similarly, Lee et al. (2010) found that BV administration at physiologically optimal levels reduced total cholesterol and triglyceride contents in mice with atherosclerotic lesions. Furthermore, Rabie et al. (2018) reported that increasing the supplementation level of BV from 0.5 to 2 mg/L drinking water significantly ($P \leq 0.01$) decreased triglyceride concentrations.

The present study demonstrated that Hb and MCHC were significantly increased in chicks injected with 15 and 20 µg of BV. These results are consistent with El-Hanoun et al. (2020), who reported that injecting rabbits with different doses of bee venom significantly elevated RBCs and Hb values, with the highest responses observed in the treated groups. Likewise, Khali et al. (2023) indicated that chicks injected with melittin (10 mg/egg) exhibited significantly higher hemoglobin concentrations. On the other hand, Khali et al. (2023) found no significant differences in RBCs and MCH values among treatments, while the control chicks showed higher MCV and MCHC values compared to the treated groups. From a physiological perspective, the elevation of hemoglobin and MCHC in the present study suggests that BV may stimulate erythropoietic activity and enhance the oxygen-carrying capacity of blood. Bee venom is known to contain biologically active components, such as melittin and apamin (Wehbe et al., 2019), which can improve blood circulation, modulate immune responses (El-Sabrout et al., 2025), and possibly stimulate hematopoietic stem cells. The increase in hemoglobin concentration could therefore improve oxygen transport to tissues, supporting embryonic growth and metabolic activity. Conversely, as noted by Isaac et al. (2013), a reduction in RBCs compromises the transport of oxygen and the return of carbon dioxide to the lungs, which could negatively impact tissue metabolism.

In the present study, in-ovo injection of bee venom (BV) resulted in an increase in AST, creatinine,

and urea levels, while decreasing ALT, with the lowest ALT observed at 15 µg. These results contrast with those of Gurafi (2004), Elkomy et al. (2021), and El-Hanoun et al. (2020), who reported significant decreases in urea, creatinine, AST, and ALT in BV-treated rabbits in compared to the control group.

The present results showed that the T3 levels were significantly increased in the 15 and 20 mg BV/egg groups, while the 15 mg BV group showed the highest T4 level among all treatments. However, Khalil et al. (2021) and Hassan et al. (2021) reported that BV in-ovo at dose of 10 and 20 µg/egg significantly decreased both serum triiodothyronine and thyroxine concentrations in Alexandria chicken at hatching day. The thyroid gland is an endocrine organ that secretes the hormones triiodothyronine and thyroxine. Its function can be influenced by several environmental and physiological conditions' factors (Dawson et al., 1992). Normal thyroid hormone levels are essential for the growth, development, and maintenance of physiological functions. They play a key role in regulating both antibody- and cell-mediated immune responses (Cremaschi et al., 2000; Klecha et al., 2000) as well as the basal metabolic rate of mammals and birds (Hulbert & Else, 2004). Among thyroid hormones, T3 is considered the primary physiological regulator of oxygen consumption and daily activity, particularly in young chickens (Bobek et al., 1977). Compared to T4, T3 is metabolically more active (Klandorf et al., 1981).

The present results demonstrated that in-ovo injection with BV did not induce significant changes in leukocyte indices. This is consistent with the findings of Khalil et al. (2023), who reported that in-ovo injection of melittin on day 18 of incubation in Alexandria chicks produced no significant differences in WBCs, lymphocytes, monocytes, eosinophils, or H/L ratio among the studied treatments. Similarly, Naglaa et al. (2019) reported that supplementation of BV in drinking water at concentrations of 1 and 2 mL/L had no effect on lymphocytes, basophils, or eosinophils, while it increased heterophil and monocyte counts. In addition, the elevation of immunoglobulins IgG and IgM, particularly at the 15 µg dose, highlights the potential of moderate BV supplementation to enhance humoral immunity. These findings agree with previous reports on the immunomodulatory properties of BV bioactive compounds such as melittin and apamin (El-Sabrout et al., 2025), which have been shown to improve poultry health and resistance to infections (El-Banna et al., 2023; Bava et al., 2023 and Han et al., 2010). Supporting this, Khali et al. (2023) reported that in-ovo injection of 10 µg bee venom extract (melittin) on day 18 of incubation enhanced post-hatch immunological indicators, including immunoglobulins, T cells, and B cells. Similarly, supplementation of BV through drinking water at levels up to 2 mg/L was found to improve broiler immunity (El-Banna et al., 2023). In another trial, Ali and Mohanny (2014) demonstrated that direct injection of 0.5 mg bee venom per chick enhanced immune responses. Comparable effects have also been observed in rabbits, where bee venom administration significantly increased immunoglobulin A (IgA) and immunoglobulin M (IgM) levels compared with control groups (El-Hanoun et al., 2020). Collectively, these outcomes highlight bee venom as a promising natural alternative to antimicrobial growth promoters, supporting poultry immunity and reducing reliance on routine antibiotic use (Abd El-Aziz et al., 2023).

The present results demonstrated that TAC levels were significantly increased, whereas MDA levels were significantly decreased in the groups treated with 15 and 20 µg doses of in-ovo BV injection in Sinai strain eggs. This suggests that BV enhances the antioxidant defense system of the developing embryos, thereby reducing lipid peroxidation and oxidative damage. The antioxidant activity of BV observed in this study is consistent with earlier reports. Kim et al. (2019) found that dietary supplementation of bee venom in broiler chickens improved antioxidant status, while Mohamed et al. (2019) demonstrated a reduction in MDA concentrations in rats. Furthermore, El-Hanoun et al. (2020) reported that injecting rabbits with different doses of BV resulted in a significant increase in antioxidant indices (TAC) and a corresponding decrease in MDA levels. In poultry production, oxidative stress represents a major challenge as it can impair immunity, reduce growth performance, and compromise productivity (Mohamed et al., 2025). Bee venom has been reported to possess strong antioxidant properties that mitigate oxidative stress and improve poultry health (Abd El-Aziz et al., 2023; Bava et al., 2023). Therefore, incorporating BV, either through in-ovo injection or dietary supplementation, may serve as a promising strategy to enhance flock resilience, promote overall health, and improve productivity in commercial poultry systems (Qin et al., 2023; Abd El-Aziz et al., 2023).

Conclusively, according to the current study's results, it's recommended that in-ovo injection of bee venom at doses of 15 and 20 µg per egg, could be enhanced hatchability, hematological and biochemical traits, immune response, and antioxidant status in Sinai chickens, leading to improved post-hatch performance of chicks.

CONFLICTS OF INTEREST:

The authors of this work declare that they have no known conflicts of interest.

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Upon reasonable request, the data supporting the study's conclusions can be provided by the corresponding author.

AUTHORS CONTRIBUTION

S.H.M.H., Y.S.R., M.M.E. & K.H.M.E developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript.

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الملخص العربى

تأثير حقن سم النحل داخل البيضة على معدل الفقس، صفات الدم، المناعة، وحالة مضادات الأكسدة لسلالة دجاج سينا عند الفقس

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أُجريت هذه الدراسة لتقييم تأثير حقن سم النحل (BV) داخل البيضة على نسبة الفقس، وصفات الدم، والمناعة، وحالة مضادات الأكسدة في دجاج سلالة سيناء. تم تقسيم عدد 250 بيضة إلى خمس مجموعات: مجموعة كنترول لم يتم حقنها (CON)، ومجموعة كنترول تم حقنها بماء مقطر (sham)، وثلاث مجموعات محقونة بسم النحل (BV) بجرعات (10، 15، و20 ميكروجرام/بيضة). في اليوم الثامن عشر من الحضانة، تم حقن السم في الغرفة الهوائية بالطرف العريض للبيضة. أظهرت النتائج أن الحقن داخل البيضة بسم النحل أدى إلى تحسن في نسبة الفقس في بيض سلالة سيناء، وخاصةً عند مستوى 15 و 20 ميكروجرام، كما حسن مؤشرات الدم الكيميائية من خلال زيادة نسبة الجلوكوز، والبروتين الكلي، والألبيومين، والدهون عالية الكثافة، مع خفض الكوليسترول، والدهون الثلاثية، والدهون الكلية. كما لوحظ أنه عند مستوى حقن 15 ميكروجرام، انخفض مستوى إنزيم ALT وارتفع مستوى هرمون الثيروكسين، بينما أدى كلٌّ من مستويَّ حقن 15 و20 ميكروجرام إلى رفع مستوى هرمون التراي أيونوثيرونين، وتحفيز المناعة (خلايا الدم البيضاء، IgG، IgM)، وتحسين حالة مضادات الأكسدة من خلال رفع مستوى TAC وخفض مستوى MDA. عموما أظهرت النتائج النهائية لهذه الدراسة أن حقن 15 و20 ميكروجرام من BV / بيضة أدى إلى تحسين المناعة وحالة مضادات الأكسدة، وخصائص الدم لدى كتاكيت سيناء، مما قد يُحسّن أداؤها بعد الفقس.