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Productive and reproductive status, hematological traits, biochemical blood parameters, and immune response of Sinai chicken breeders as influenced by the dietary addition of bee venom during the second production cycle and summer season

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ABSTRACT

Bee venom (BV) is a product of bees and is produced by female worker bees. It contains several bioactive molecules, including peptides such as apamin, melittin, and adolapin, as well as enzymes such as phospholipase A2. These molecules have potential advantages for the treatment of central nervous system diseases and inflammation. Adding BV-derived material to animal diets has been shown to enhance productivity, provide health benefits, and act as a therapeutic agent. The present study tested the hypothesis that BV supplementation can ameliorate the stress induced by ambient temperature and advanced age in Sinai chicken breeders during the second production cycle (108-116 weeks of age). A total of 200 aged Sinai layer hens and 20 cocks were randomly allocated to four trial groups. Each trial group comprised 50 females and 5 males, further divided into 5 replicates, each with 10 females and 1 male. The first group received a control diet, while the other three groups (2, 3, and 4) received the control diet supplemented with 0.3, 0.4, and 0.5 g BV kg^{-1}, respectively (BV 0.3, BV 0.4, and BV 0.5). The present findings indicated that BV addition significantly ($P < 0.05$) improved the layers' final body weight (FBW), body weight gain (BWG), egg production (EP), egg weight (EW), egg mass (EM), and feed conversion ratio (FCR) at the 0.3, 0.4, and 0.5 g BV kg^{-1} levels compared to the control treatment. Moreover, serum total protein, total lipids, and total cholesterol, as well as immunoglobulin IgY and IgM, thyroid hormones (triiodothyronine and thyroxine), and redox status (total antioxidant capacity and malondialdehyde) were significantly ($P < 0.05$) improved in response to adding BV to layers' diets during the summer season compared to the control treatment. Despite these improvements, BV supplementation did not significantly ($P > 0.05$) affect red blood cell count, mean corpuscular volume, or lymphocyte, eosinophils, or monocyte counts at the 0.3, 0.4, and 0.5 g BV kg^{-1} levels compared to the control treatment. Moreover, significant improvements ($P < 0.05$) were detected in hatchability traits for the aged Sinai layer hens with only (BV 0.4 and BV 0.5) supplementation during the summer season. The number of unfertilized eggs decreased by 29.41% and 17.65% in the groups administered 0.4 and 0.5 g BV kg^{-1} of diet, respectively, compared to the control group. Simultaneously, the number of fertilized eggs increased by 7.54% and 4.52% in these respective groups, and the total number of chicks hatched rose by 27.14% and 17.14% in the 0.4 and 0.5 g BV kg^{-1} treatments, respectively, compared with the control. These outcomes validate the hypothesis that dietary BV could enhance productive, reproductive, and certain physiological parameters in aged Sinai layer hens.

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Introduction

The poultry sector has demonstrated resilience in the face of past difficulties and is expected to effectively address and adapt to current challenges (Korver, 2023; Attia et al., 2024). One main challenge is finding strategies to improve the performance of local aged hens during their second laying cycle. It is urgent to address this problem for several reasons (Youssef et al., 2014).

Firstly, the extended growth period for local chickens lasts from day one until they reach 23 weeks of age, the age of sexual maturity (Youssef et al., 2014). This extended rearing period results in high costs for producers, particularly given the rising poultry feed prices (Bist et al., 2024). Secondly, the high mortality rates during the growing phase and the rising costs of vaccines and medications further increase overall expenses (Mottet et al., 2017). Therefore, from a sustainability perspective, it could be more cost-effective to utilize aged chickens and enhance egg production efficiency during their second laying cycle (Wong et al., 2017).

However, aging in hens is related to hormonal changes, ovarian disruption, hepatic failure (Liu et al., 2013; May-Panloup et al., 2016; Huang et al., 2021a), and physiological changes, all of which contribute to a significant decrease in laying performance during the advanced laying stage (Dai et al., 2020). Among these, ovarian dysfunction is particularly critical, as it reduces the number of developing and pre-ovulatory follicles, resulting in lower serum oestrogen levels and reduced egg-laying rates (Hao et al., 2020; Yao et al., 2022).

There is strong evidence that ovarian function is related to the ovarian redox state, serum hormone levels, and the body's physiological state (Huang et al., 2021b). Similar to mammals, laying hens can develop ovarian dysfunction due to oxidative stress, which in turn reduces their reproductive performance and overall productivity (Wang et al., 2021). Heat-induced oxidative stress further exacerbates this condition, by inducing ovarian cell apoptosis, decreasing follicle numbers, and impairing egg production (Li et al., 2020).

According to Lee et al. (2020), aging is a standard and inevitable biological process that increasingly contributes to the accumulation of free radicals. When the body's endogenous antioxidant system fails to neutralize reactive oxygen species (ROS), oxidative stress and redox imbalance develop (Estevez, 2015). Furthermore, organ function declines with age, as evidenced by increased lipid deposition and reduced antioxidant capacity, both of which peak in early life and then decline over time (Gu et al., 2021).

To address these issues, bee venom (BV), a bioactive substance produced by the honeybee *Apis mellifera* and released during stinging, is used as a defensive secretion to protect the bee from potential threats (Abo-Zaid et al., 2023). BV comprises a diverse array of bioactive peptides, including phospholipase A2 (PLA2), apamin, and adolapin (Jang et al., 2003). These components confer several therapeutic effects, including antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, immunomodulatory, and cytoprotective effects (Baqer and Yaseen, 2018; Carpena et al., 2020; Hassan and Raghad, 2021). Adding BV-derived material to animal diets has been reported to boost productivity (Rabie et al., 2018), offer health benefits (Sturm et al., 2002), and act as a therapeutic agent (Han et al., 2010).

The laying hen sector faces a major challenge: a noticeable decline in egg production in the late stages of egg production (Dai et al., 2020). This problem is exacerbated by current global warming conditions (El-Kholy et al., 2024). It has been recommended that the optimal temperature-humidity index (THI) for the rearing of layer hens should not exceed 27.8 (Zulovich and DeShazer, 1990).

Global warming and aging have created a need for research tools to achieve sustainability. Despite BV's wide-ranging bioactivity, its impact on the functions of aged tissues and organs remains largely unexplored, especially under heat-stress conditions. It is hypothesized that the dietary supplementation of BV is expected to exert beneficial impacts on the laying hens. Therefore, the present work aimed to assess the potential of adding BV to layer diets to mitigate the adverse effects of age (108-116 weeks) and high ambient temperature on productive and reproductive performance, blood biochemistry, redox status, reproductive hormones, and some hatching traits.

Materials and methods

Birds, experimental design, and diet

All bird handling and care procedures adhered to the ethical standards established via the Institutional Animal Care and Use Committee of the Animal Production Research Institute, Agricultural Research Center, Egypt. The experiment followed the European Parliament and Council's September 22, 2010, directive 2010/63/EU guidelines on the protection of animals used in scientific experiments.

The trial conforms to the guidelines recognized by the Zagazig University Ethics Committee regarding the use of experimental animals (Approval No. ZU-IACUC/2/F/313/2023). The trial was performed at the El-Serw Poultry Research Station (Damietta Governorate), Agricultural Research Center, in cooperation with the Faculty of Agriculture, Damietta University, Damietta, Egypt.

A total of 200 adult Sinai layer hens and 20 cocks were randomly assigned to four experimental groups following a completely randomized design (CRD). Each trial group consisted of 50 females and 5 males, further subdivided into 5 replicates, each with 10 females and 1 male.

The trial lasted 8 weeks, between 108 and 116 weeks old. The birds were randomly assigned to four experimental groups. The first group received a control diet, while the other three groups (2, 3, and 4) received the control diet supplemented with 0.3, 0.4, and 0.5 g BV kg^{-1}, respectively (BV 0.3, BV 0.4, and BV 0.5). Purified BV from *A. mellifera*, in a lyophilized powder form, was sourced from the Egyptian company for the production of vaccines, sera, and drugs (VACSERA), Giza, Egypt.

The additives were mixed and added to the diets by hand for ten min to ensure uniform distribution in the feed. All birds were fed mash diets during the experimental period. The basal diet was formulated according to the National Research Council (NRC, 1994) recommendations to meet the nutrient requirements of breeding hens (Table 1). The AOAC (2011) methods were used to analyze the chemical composition of the basal diet.

Based on the inclusion levels (0.3, 0.4, and 0.5 g BV kg^{-1} feed), the estimated concentrations of the two main active components (melittin, and PLA2) were calculated according to their average proportions in BV reported in the literature (approximately 40–50% melittin and 10–12% PLA2 of dry BV weight (Rady et al., 2017).

The calculated amounts of melittin and PLA2 incorporated into the experimental diets are presented in Table 2. These estimates provided an approximate indication of bioactive compound levels, as no direct laboratory quantification of BV components in the diets was performed.

Housing

Birds were housed in separate floor pens (dimensions: 260 × 210 cm) with litter flooring. Surrounding ambient temperature (°C) and relative humidity (RH) were documented daily to compute the temperature-humidity index (THI). The wet-bulb temperature (Twb) was calculated following the methodology of Stull (2011), and THI was derived using the formula developed by Zulovich and DeShazer (1990):

$$THI = 0.6 \times Tdb + 0.4 \times Twb,$$

Where: Tdb = dry-bulb temperature (°C).

THI values were categorized into four heat stress levels: no heat stress (<27.80°C), moderate (27.80–28.80°C), severe (28.90–29.90°C), and very severe (>30.00°C).

Table 1
Composition and chemical analysis of the basal diets fed during 108-116 weeks of age.

Ingredients	%
Yellow corn	59.70
Soybean meal (44%CP)	24.02
Wheat bran	5.40
Corn oil	1.00
Di-Calcium phosphate	1.45
Limestone	7.77
Vitamin-mineral premix[1]	0.30
Sodium chloride	0.30
DL-Methionine	0.06
Total	100
Calculated[2] -nutritional analysis	
ME, kcal/kg[2]	2700
Crude protein, %[3]	16.0
Crude fiber, %[3]	3.72
Calcium, %[2]	3.30
Available phosphorus, %[2]	0.40
Lysine, %[2]	0.90
Methionine, %[2]	0.35
Methionine + cysteine, %[2]	0.62
Sodium, %[2]	0.14
Crude ash, %[3]	13.72
Dry matter, %[3]	91.17
Analyzed nutrients[3] (%)	
Crude protein	16.0
Crude fiber	3.88
Calcium	3.13
Crude ash	13.72
Dry matter	91.71

[1] Each 3 kg of vitamins and minerals. Mixture contains vitamin A, 10000,000 IU; vitamin D3, 2000,000 IU; vitamin E, 10,000 mg; vitamin K_3, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 5000 mg; vitamin B6, 1500 mg; vitamin B12, 10 mg; pantothenic acid, 10,000 mg; niacin, 30,000 mg; folic acid, 1000 mg; biotin, 50 mg; choline, 250,000 mg; manganese, 60,000 mg; zinc, 50,000 mg; copper, 10,000 mg; iron, 30,000 mg; iodine, 1000 mg; selenium, 100 mg; and cobalt, 100 mg. [2]Calculated according to NRC (1994), [3]Analyzed according to AOAC (2011).

Table 2
Estimated concentrations of major active components [melittin and phospholipase A2 (PLA2)] in the experimental diets supplemented with bee venom (BV).

Bee venom supplementation	Bee venom active components (mg kg^{-1} feed)
(BV 0.3) = 0.3 g kg^{-1} feed	Melittin: 0.24 mg kg^{-1}; PLA2: 0.06 mg kg^{-1}
(BV 0.4) = 0.4 g kg^{-1} feed	Melittin: 0.32 mg kg^{-1}; PLA2: 0.08 mg kg^{-1}
(BV 0.5) = 0.5 g kg^{-1} feed	Melittin: 0.40 mg kg^{-1}; PLA2: 0.10 mg kg^{-1}

The estimated concentrations of melittin and PLA2 were calculated based on the proportion of bee venom added to the feed and previously reported average contents of these bioactive components in *Apis mellifera* venom.

Effect of dietary supplementation with BV on productivity measurements

Average live body weight and feed intake (FI) were monitored (g) for each replicate throughout the trial. The body weight gain (BWG) was computed by deducting the initial body weight (IBW) from the final body weight (FBW). Egg production data, including daily egg count and weight, were documented to compute egg production (EP) rate, egg weight (EW), and egg mass (EM). The feed conversion ratio (FCR) was computed as FI/EM.

Effect of dietary supplementation with BV on blood hematology and biochemical constituents

At the end of the trial (116 weeks old), 28 blood samples (7 hens per group) were gathered from the wing vein in heparinized tubes for hematology tests, including hemoglobin (HGB) content, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet count (PLT), red blood cells (RBC), white blood cells (WBC), white blood cells' differentiation types, and heterophiles/lymphocytes ratio (H/L).

The selection was performed randomly across all replicates within each treatment to ensure representative sampling, rather than selecting a fixed number of birds per replicate. Accordingly, one or two hens were randomly chosen from different replicates, ensuring that all replicates were proportionally represented and minimizing potential sampling bias. RBC and WBC counts were performed using a Neubauer improved hemocytometer (Paul Marienfeld GmbH and Co. KG, Lauda-Königshofen, Germany) after appropriate dilution with Natt-Herricks solution (Irizarry-Rovira, 2004).

Other blood samples were collected from 7 females per treatment group (28 samples) in plain tubes (without anticoagulant), left to clot for 30 min, then centrifuged at 3,000 × *g* for 15 min using a T32c centrifuge (Janetzki, Wallhausen, Germany).

The obtained serum was transferred into Eppendorf tubes and preserved at −20°C for biochemical analyses. Total protein (TP) and albumin (ALB) concentrations were analyzed (g dL^{1}) using standard commercial kits, in accordance with Doumas and Maume (1977), while globulin (GLO) was determined by deducing ALB from TP.

Lipid profile parameters (mg dL^{-1}), including total cholesterol (TC), triglycerides (TG), total lipids (TL), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were determined as described by Vogel (1997).

Enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed (U L^{-1}) using commercial kits from Linear Chemicals (Barcelona, Spain) according to the protocols outlined by Friedman and Young (2001). Kidney function indicators, creatinine and urea, were estimated (mg dL^{-1}) using methods by Fabiny and Ertingshausen (1971) and Patton and Crouch (1977), respectively.

Effect of dietary supplementation with BV on serum hormone analysis

Serum thyroid hormone concentrations, triiodothyronine (T3) and thyroxine (T4) were measured using ELISA kits according to the manufacturer's instructions. ELISA kits (E-1001, Autobio Diagnostics, Zhengzhou, China) with an assay sensitivity of 0.2 ng mL^{-1}, an intra-assay precision of >3.94%, and an inter-assay precision of >7.83% were used to measure serum T3 levels.

ELISA kits (E-1002, Autobio Diagnostics) with a sensitivity of 0.40 ng dL^{-1}, intra-assay precision of >3.580%, and inter-assay precision of >9.640% were used to measure serum T4 levels.

Effect of dietary supplementation with BV on the levels of immunoglobulins

Serum levels of immunoglobulin Y (IgY) and immunoglobulin M (IgM) were measured (mg dL^{-1}) using a sandwich ELISA kit (MyBioSource, San Diego, USA), adapted from the work of Bianchi et al. (1995). All procedures followed the manufacturer's instructions.

Effect of dietary supplementation with BV on redox status

Blood serum levels of total antioxidant capacity (TAC, mmol L^{-1}) and serum malondialdehyde (MDA, μmol mL^{-1}) were measured using a UV-Vis spectrophotometer (Biochrom Libra S22, Biochrom Ltd., Cambridge, UK) in accordance with Botsoglou et al. (1994).

Each redox index was calculated according to the guidelines provided by the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Effect of dietary supplementation with BV on hatching traits

At 116 weeks of age, hatching traits were evaluated in the control

and BV-treated groups. For analysis, a total of 200 eggs (50 per treatment) were manually collected over five consecutive days and stored at ambient temperature (11-15°C) and RH (75-85%). Eggs were transported to setter trays (50 eggs per tray), fumigated with formaldehyde for 15 min, and then moved to the incubator. Incubation was conducted in PTO incubators (Fathallah Company, Alexandria, Egypt) under standard conditions of 37.8°C and 65–70% RH, with automatic 90° turning every 1 h.

Candling was performed on day 10 individually to assess embryo viability using a hand lamp. Dead embryos and infertile eggs were discarded, while fertilized eggs containing viable embryos were retained. Fertility was calculated using the formula of van Wageningen et al. (2004).

The fertility percentage was estimated as: total fertile eggs/total incubated eggs × 100. Hatchability was calculated on days 21 and 22 of incubation. Hatchability was calculated according to Fayeye et al. (2005) as a percentage of total incubated eggs (number of hatched chicks/number of incubated eggs × 100) and as a percentage of fertile eggs (number of hatched chicks/number of fertile eggs × 100).

Statistical analysis

Data were statistically analyzed using SAS software version 9.2 (SAS, 2012). The MIXED model procedure for repeated measures was used to evaluate variables including BW, BWG, FI, EM, and blood biochemical indices. The GLM procedure (ANOVA) was applied for meteorological and reproductive traits.

Duncan's multiple range test was employed for a post-hoc test when the P value ≤ 0.05. Data normality and variance homogeneity were confirmed via the Shapiro–Wilk and Levene's tests.

Results

Meteorological data

Throughout the study, the average ambient temperature, RH, and THI were 32.47 ± 0.31°C, 71.58 ± 0.58%, and 30.72 ± 0.30, respectively, indicating that the birds were under very severe heat stress.

Effect of dietary supplementation with BV on laying performance traits

The impact of BV addition on layers' productive performance during the hot summer season is presented in Table 3. The IBW of all treatment groups was similar ($P = 0.763$). Also, FI was similar across the experimental groups ($P = 0.789$). Interestingly, the addition of BV at all concentrations used to birds' diets significantly improved FBW ($P < 0.0001$), BWG ($P < 0.0001$), EP ($P < 0.0001$), EW ($P < 0.0001$), EM ($P < 0.0001$), and FCR ($P < 0.0001$) (Table 3).

It can be noticed that BWG, EW, EM, and FCR were affected quadratically ($P < 0.0001$, $P = 0.0013$, $P = 0.0195$, and $P = 0.0169$, respectively) by the treatment (Table 3 and Fig. 1). Increasing the level of BV increased EP of laying hens ($P = 0.0001$) linearly (Table 3).

Dose–response curves of BWG, EW, EM, and FCR for different levels of dietary BV addition are presented in Fig. 1A, B, C, and D, respectively. The response equations showed diminishing increases in BWG, EM, and FCR with increasing BV level. However, these quadratic equations detected maximum BV levels of 0.41, 0.40, and 0.40 (g kg^{-1} diet) for the above-mentioned characters, respectively, to produce the maximum performance of (BWG) 338 g and (EM) 29.77, as well as the minimum FCR of 3.44. Meanwhile, EW showed a cubic response with a maximum response to 0.44 (g kg^{-1} diet) to obtain the maximum EW of 54 g (Fig. 1).

Effect of dietary supplementation with BV on blood hematological traits

Impacts of the dietary addition of BV on the blood hematology of Sinai chickens during the hot summer season are illustrated in Table 4 and Fig. 2. The dietary treatments with only (BV 0.4 and BV 0.5) supplementation significantly affected HCT ($P < 0.0001$). Dietary treatments at BV levels of 0.3, 0.4, and 0.5 g kg^{-1} significantly ($P < 0.0001$) affected HGB levels (Table 4). HGB was quadratically ($P = 0.0011$) and cubically ($P = 0.1042$) affected by BV addition (Table 4).

Whilst HCT was quadratically ($P = 0.0778$) and cubically ($P = 0.0138$) affected by BV addition (Table 4). Fig. 2 showed dose–response curves for HGB (A) and HCT (B) at different levels of dietary BV addition. The response equations illustrated that HGB responded up to 0.40 (g kg^{-1} diet). This quadratic response predicted a maximum HGB of 10.0 (g dL^{-1}). Whereas the response equation for HCT was cubic and responded at 0.42 (g kg^{-1} diet) to give the maximum (HCT) of 26.37% (Fig. 2).

Increasing levels of BV increased MCH ($P = 0.0015$) linearly (Table 4). The highest MCH values ($P < 0.0001$) were observed in the (BV 0.3) and (BV 0.4) groups. Increasing levels of BV increased PLT ($P = 0.0063$). Despite these improvements, BV supplementation did not significantly ($P > 0.05$) affect MCV, RBC, lymphocyte, eosinophils, and monocytes count (Table 4). On the other hand, basophils, heterophils, and the H/L ratio were decreased with dietary BV supplementation.

Effect of dietary supplementation with BV on blood proteins, lipid profile, and liver and kidney function parameters

The effects of dietary BV supplementation on blood proteins, lipid profile, and liver and kidney function parameters in heat-stressed layers are shown in Table 5 and Fig. 3. Dietary supplementation at BV levels of 0.4, and 0.5 g kg^{-1} significantly affected ALB, GLO, ALB/GLO ratio, TC, TL, TG, and ALT significantly ($P < 0.0001$, $P = 0.0006$, $P = 0.0006$, $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively) (Table 5).

ALB and GLO were cubically changed ($P = 0.0407$ and $P = 0.0556$, respectively) among the experimental groups (Table 5). Ascending

Table 3
Effect of dietary addition of bee venom (BV) on productive parameters of heat-stressed Sinai laying hens during the second production cycle.

Characteristics	Treatments				SEM	*P* values			
	CON.	(BV 0.3)	(BV 0.4)	(BV 0.5)		TRTS	Lin.	Quad.	Cub.
IBW (g)	1540 *a*	1552 *a*	1526 *a*	1534 *a*	16.462	0.7636	0.5763	0.8577	0.3833
FBW (g)	1737 *c*	1854.8 *b*	1890.6 *a*	1863 *b*	8.278	<0.0001	0.0003	0.0023	0.8385
BWG (g)	197 *c*	302.8 *b*	364.6 *a*	329 *b*	8.884	<0.0001	<0.0001	<0.0001	0.4273
EP (%)	41.25 *c*	48.39 *b*	54.32 *a*	55.07 *a*	1.464	<0.0001	0.0001	0.1071	0.4823
EW (g)	50.30 *c*	51.88 *b*	53.82 *a*	53.19 *a*	0.285	<0.0001	<0.0001	0.0013	0.0352
EM	20.75 *c*	25.11 *b*	29.77 *a*	29.31 *a*	0.777	<0.0001	<0.0001	0.0195	0.3240
FI (g day^{-1})	101.87 *a*	102.04 *a*	101.84 *a*	102.30 *a*	0.357	0.789	0.6823	0.7983	0.7020
FCR	4.91 *a*	4.07 *b*	3.44 *c*	3.51 *c*	0.103	<0.0001	<0.0001	0.0169	0.4032

CON. = control; (BV 0.3) = 0.3 g BV kg^{-1} diet; (BV 0.4) = 0.4 g BV kg^{-1}; (BV 0.5) = 0.5 g BV kg^{-1} diet; SEM = standard error of means; TRTS, treatments; Lin. = linear response; Quad. = quadratic response; Cub. = cubic response; IBW = initial body weight; FBW = final body weight; BWG = body weight gain; EP = egg production; EW = egg weight; EM = egg mass; FI = feed intake; FCR = feed conversion ratio. The data represent the means of five independent replicates, and values with the same letter within a row are not significantly different ($P > 0.05$) according to Duncan's multiple-range test.

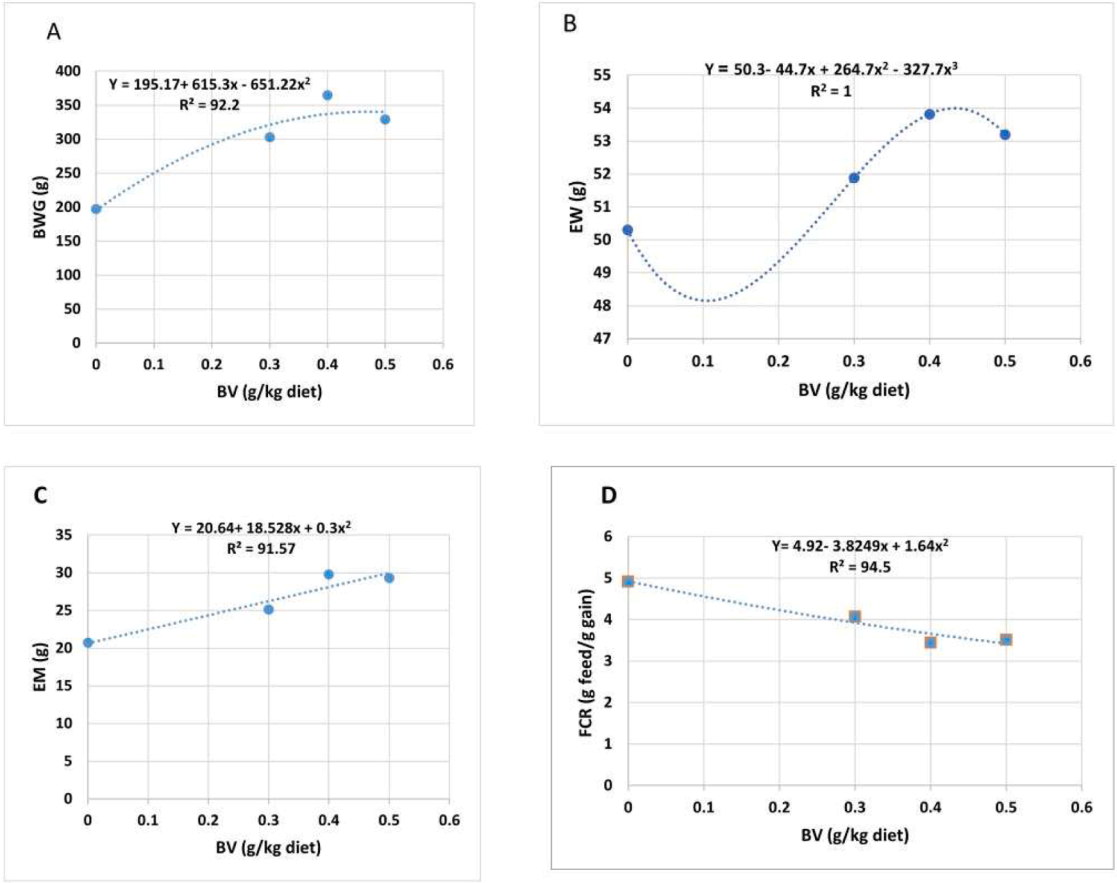

Fig. 1. Dose–response curve of body weight gain (BWG) (A), egg weight (EW) (B), egg mass (EM) (C), and feed conversion ratio (FCR) (D), for different levels of dietary bee venom (BV) addition. X and Y are the dependent (BV levels) and the independent variables of the regression equation, respectively.

Table 4
Effect of dietary addition of bee venom (BV) on blood haematological indices of heat-stressed Sinai laying hens during the second production cycle.

Characteristics	Treatments				SEM	*P* values			
	CON.	(BV 0.3)	(BV 0.4)	(BV 0.5)		TRTS	Lin.	Quad.	Cub.
HGB (g dL^{-1})	8.09 *d*	9.62 *b*	11.15 *a*	8.68 *c*	0.134	<0.0001	0.1819	0.0011	0.1042
HCT (%)	24.29 *b*	24.16 *b*	26.50 *a*	22.71 *c*	0.301	<0.0001	0.5946	0.0778	0.0138
MCV (μm)	84.33 *a*	84.12 *a*	83.68 *a*	83.51 *a*	0.390	0.4264	0.3890	0.9788	0.8812
MCH (pg)	3.45 *b*	4.48 *a*	4.66 *a*	3.60 *b*	0.1535	<0.0001	0.0015	0.4634	0.7530
RBC (× 10^6/mm^3)	2.43 *a*	2.15 *a*	2.40 *a*	2.41 *a*	0.110	0.267	0.8901	0.4372	0.3015
PLT (10^3/μL)	11.24 *ab*	10.65 *b*	11.96 *a*	12.00 *a*	0.544	0.0063	0.1529	0.5681	0.2040
WBC (10^3/mm^3)	22.57 *a*	21.58 *b*	23.21 *a*	21.29 *b*	0.3159	0.0008	0.4089	0.4547	0.0578
Lymphocytes (%)	43.50 *a*	42.73 *a*	42.75 *a*	43.44 *a*	0.313	0.1676	0.9115	0.0278	0.9275
Eosinophils (%)	10.10 *a*	10.33 *a*	9.81 *a*	10.47 *a*	0.265	0.3354	0.6323	0.4235	0.1156
Basophils (%)	0.63 *a*	0.57 *ab*	0.52 *b*	0.53 *b*	0.0253	0.0314	0.1074	0.4655	0.7974
Monocytes (%)	13.57 *a*	13.43 *a*	14.00 *a*	14.14 *a*	0.2474	0.1584	0.3687	0.8001	0.6510
Heterophils (%)	33.94 *a*	32.94 *a*	32.92 *a*	31.42 *b*	0.3967	0.0016	0.1258	0.8161	0.6128
H/L ratio	0.781 *a*	0.772 *a*	0.771 *a*	0.723 *b*	0.0129	0.0192	0.1586	0.4758	0.6630

CON. = control; (BV 0.3) = 0.3 g BV kg^{-1} diet; (BV 0.4) = 0.4 g BV kg^{-1}; (BV 0.5) = 0.5 g BV kg^{-1} diet; SEM = standard error of means; TRTS, treatments; Lin. = linear response; Quad. = quadratic response; Cub. = cubic response; HGB = hemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; RBC = red blood cells; PLT = platelet count; WBC = white blood cells; H/L = heterophiles/lymphocytes ratio. The data represent the means of seven independent replicates, and values with the same letter within a row are not significantly different ($P > 0.05$) according to Duncan's multiple-range test.

levels of BV increased ALB and GLO linearly ($P = 0.0027$ and $P = 0.0137$, respectively) (Table 5). The highest blood serum levels of ALB were detected in the (BV 0.4) group, and the highest blood serum levels of the ALB/GLO ratio were also detected in the (BV 0.4) group (Table 5).

The dietary treatments with (BV 0.3, BV 0.4, and BV 0.5 g kg^{-1}) supplementation significantly reduced TC and TL (Table 5). The dietary treatments with only BV supplementation at 0.4 g kg^{-1} significantly reduced LDL levels (Table 5).

TC and TL were affected quadratically ($P = 0.0138$ and $P = 0.0072$, respectively), and TG and ALT were affected cubically ($P = 0.0145$ and $P = 0.0097$, respectively) by dietary treatments with BV (Table 5). Ascending levels of BV decreased TL and ALT ($P = 0.0503$ and $P < 0.0001$, respectively) linearly (Table 5). On the other hand, dietary BV supplementation did not affect AST levels (Table 5).

The lowest TL values ($P < 0.05$) were observed in the (BV 0.3) and (BV 0.4) groups, whilst the lowest ALT values ($P < 0.05$) were observed

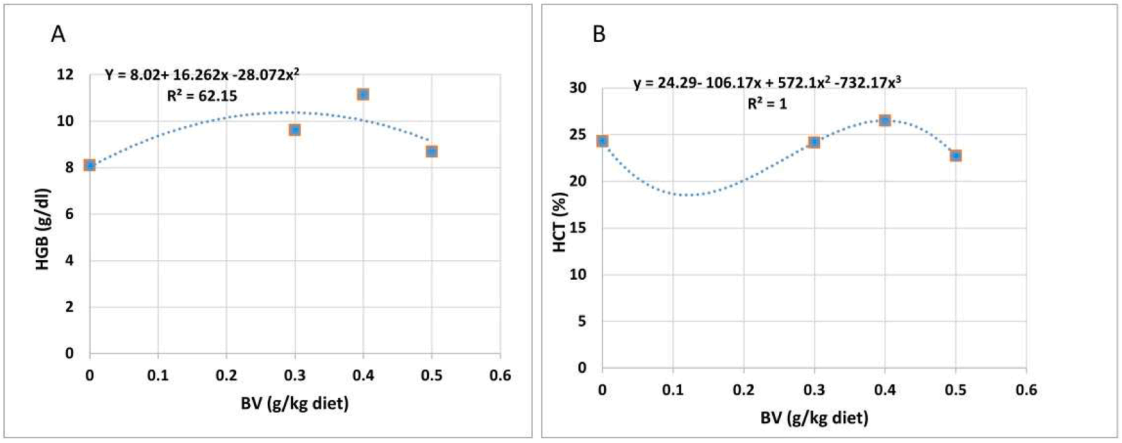

Fig. 2. Dose–response curve of hemoglobin (HGB) (A), and haematocrit (HCT) (B) for different levels of dietary bee venom (BV) addition. X and Y are the dependent (BV levels) and the independent variables of the regression equation, respectively.

Table 5
Effect of dietary addition of bee venom (BV) on blood protein fractions, lipid profile, and liver and kidney function parameters of heat-stressed Sinai laying hens during the second production cycle.

Characteristics	Treatments				SEM	*P* values			Cub.
	CON.	(BV 0.3)	(BV 0.4)	(BV 0.5)		TRTS	Lin.	Quad.	
Blood protein									
TP (g dL^{-1})	2.21 *c*	2.27 *b*	2.42 *a*	2.36 *a*	0.0197	<0.0001	0.0961	0.4908	0.3662
ALB (g dL^{-1})	1.67 *c*	1.71 *c*	2.06 *a*	1.95 *b*	0.0359	<0.0001	0.0027	0.3519	0.0407
GLO (g dL^{-1})	0.58 *a*	0.56 *a*	0.36 *b*	0.42 *b*	0.0378	0.0006	0.0137	0.6574	0.0556
ALB/GLO ratio	2.98 *b*	3.07 *b*	6.05 *a*	5.15 *a*	0.5304	0.0006	0.0086	0.3618	0.0634
Lipid profile									
TC (mg dL^{-1})	242.86 *a*	226.14 *b*	192.71 *c*	226.00 *b*	3.0756	<0.0001	0.0684	0.0138	0.0799
TL (mg dL^{-1})	280.86 *a*	265.00 *b*	253.71 *c*	268.57 *b*	2.95	<0.0001	0.0503	0.0072	0.3650
TG (mg dL^{-1})	138.57 *a*	135.14 *a*	115.86 *c*	127.14 *b*	2.531	<0.0001	0.0566	0.9777	0.0145
LDL (mg dL^{-1})	99.67 *a*	98.78 *a*	90.36 *b*	97.76 *a*	2.0548	0.0146	0.3064	0.1839	0.0973
HDL (mg dL^{-1})	68.43 *a*	70.29 *a*	71.14 *a*	71.00 *a*	2.390	0.8445	0.4305	0.6794	1.0000
Glucose (mg dL^{-1})	103.39 *c*	114.60 *b*	129.07 *a*	112.98 *b*	2.696	<0.0001	0.0015	<0.0001	0.0098
Liver functions									
AST (mg dL^{-1})	189.18 *a*	184.58 *a*	183.79 *a*	184.89 *a*	2.06	0.2714	0.2049	0.2366	0.8561
ALT (mg dL^{-1})	25.08 *a*	23.60 *a*	14.15 *b*	16.12 *b*	1.545	<0.0001	<0.0001	0.2752	0.0097
AST/ALT ratio	7.76 *b*	8.11 *b*	13.52 *a*	11.64 *a*	0.714	<0.0001	0.1834	0.0241	0.0639
Kidney functions									
Creatinine (mg dL^{-1})	0.26 *a*	0.18 *b*	0.22 *ab*	0.21 *ab*	0.0149	0.0159	0.4319	0.5762	0.6034
Urea (mg dL^{-1})	3.56 *a*	3.59 *a*	3.87 *a*	2.99 *b*	0.1913	0.0249	0.3434	0.1856	0.3519

CON. = control; (BV 0.3) = 0.3 g BV kg^{-1} diet; (BV 0.4) = 0.4 g BV kg^{-1}; (BV 0.5) = 0.5 g BV kg^{-1} diet; SEM = standard error of means; TRTS, treatments; Lin. = linear response; Quad. = quadratic response; Cub. = cubic response; TP = total protein; ALB = albumin; GLO = globulin; ALB/GLO = albumin/globulin ratio; TC = total cholesterol; TL = total lipids; TG = triglyceride; LDL = low density lipoprotein; HDL = high density lipoprotein; AST = aspartate aminotransferase; ALT = alanine aminotransferase. The data represent the means of seven independent replicates, and values with the same letter within a row are not significantly different ($P > 0.05$) according to Duncan's multiple-range test.

in the (BV 0.4) and (BV 0.5) groups (Table 5). The highest AST/ALT ratio was observed in the (BV 0.4) group (Table 5). On the other hand, dietary BV supplementation with BV did not affect HDL, creatinine, or urea (Table 5).

Fig. 3 illustrates the dose–response curve of ALB (A), TC (B), TL (C), TG (D), ALT (E), and GLO (F) for different levels of dietary BV addition. The results revealed that both TC and TL decreased quadratically, reaching maximum values of 0.45 and 0.37 (g kg^{-1} diet), and yielding minimum values of 214.72 (mg dL^{-1}) and 260.30 (mg dL^{-1}) for TC and TL, respectively (Fig. 3).

On the other hand, the response equations for the remaining traits were cubic and demonstrated that ALB, TG, ALT and GLO responded up to 0.44, 0.43, 0.45 and 0.41 (g kg^{-1} diet) to predict maximum ALB and GLO of 2.15 (g dL^{-1}) and 129.16 (mg dL^{-1}) and minimum TG and ALT of 115.03 (mg dL^{-1}) and 13.11 (mg dL^{-1}), respectively (Fig. 3).

Effect of dietary supplementation with BV on thyroid hormones, immunoglobulins, and redox status

Effects of the dietary addition of BV on thyroid hormones, immunoglobulins, and redox status of heat-stressed layers are shown in Table 6 and Fig. 4. The dietary BV administration at BV levels of 0.4 and 0.5 g kg^{-1} significantly increased blood levels of T3 and T4 ($P = 0.0054$ and $P < 0.0001$, respectively). Dietary treatment with BV affected T3 and T4 quadratically ($P = 0.0408$ and $P = 0.0694$, respectively) (Table 6).

The dietary BV administration significantly increased serum IgY and IgM levels (Table 6). Ascending levels of BV increased IgY and IgM linearly ($P < 0.0001$) (Table 6). The highest IgY and IgM values were observed in the BV 0.4 and BV 0.5 groups. Notably, there were no significant differences between the two BV levels (0.4 and 0.5 g kg^{-1} diet) for T3, T4, and IgM (Table 6).

Regarding redox status, adding BV to the layers' diets at 0.4 and 0.5 g kg^{-1} significantly increased blood TAC ($P < 0.0001$) and decreased blood

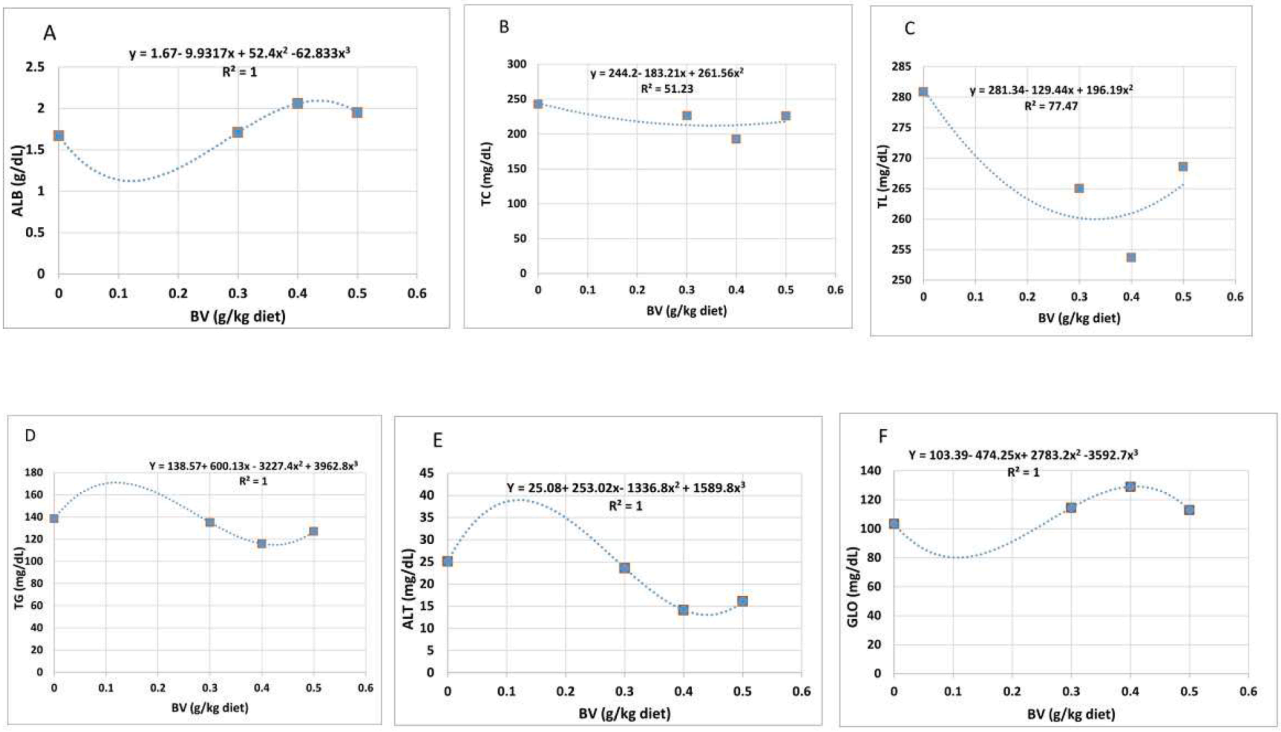

Fig. 3. Dose–response curve of albumin (ALB) (A), total cholesterol (TC) (B), total lipids (TL) (C), triglyceride (TG) (D), alanine aminotransferase (ALT) (E), and globulin (GLO) (F) for different levels of dietary bee venom (BV) addition. X and Y are the dependent (BV levels) and the independent variables of the regression.

Table 6
Effect of dietary addition of bee venom (BV) on thyroid hormones, immunoglobulins, and redox status of heat-stressed Sinai laying hens during the second production cycle.

Characteristics	Treatments				SEM	*P* values			
	CON.	(BV 0.3)	(BV 0.4)	(BV 0.5)		TRTS	Lin.	Quad.	Cub.
Thyroid hormones									
T3 (ng dL^{-1})	1.84 *b*	2.13 *ab*	2.41 *a*	2.23 *a*	0.1029	0.0054	0.0054	0.0408	0.7206
T4 (ng dL^{-1})	9.65 *c*	10.55 *b*	12.70 *a*	12.12 *a*	0.295	<0.0001	<0.0001	0.0694	0.0325
Immunoglobulins									
IgY (mg dL^{-1})	176.15 *d*	226.85 *c*	299.36 *a*	271.77 *b*	8.575	<0.0001	<0.0001	0.0705	0.0650
IgM (ng dL^{-1})	274.19 *c*	285.97 *b*	308.4 *a*	299.99 *a*	3.58	<0.0001	<0.0001	0.0768	0.1139
Redox status									
TAC (mmol L^{-1})	1.65 *c*	2.24 *bc*	3.39 *a*	2.79 *ab*	0.2139	<0.0001	0.0020	0.0425	0.0882
MDA (μmol L^{-1})	28.98 *a*	26.13 *b*	22.21 *c*	25.77 *b*	0.718	<0.0001	0.0110	0.0076	0.0952

CON. = control; (BV 0.3) = 0.3 g BV kg^{-1} diet; (BV 0.4) = 0.4 g BV kg^{-1}; (BV 0.5) = 0.5 g BV kg^{-1} diet; SEM = standard error of means; TRTS, treatments; Lin. = linear response; Quad. = quadratic response; Cub. = cubic response; T3 = triiodothyronine; T4 = thyroxine; IgY = immunoglobulin Y; IgM = immunoglobulin M; TAC = total antioxidant capacity; MDA = malondialdehyde. The data represent the means of seven independent replicates, and values with the same letter within a row are not significantly different ($P > 0.05$) according to Duncan's multiple-range test.

MDA ($P < 0.0001$) compared with the untreated group (Table 6). TAC and MDA were also affected quadratically by BV administration ($P = 0.0425$ and $P = 0.0076$, respectively (Table 6).

Concerning dose–response curve of T3, T4, TAC, and MDA for different levels of dietary BV addition (Fig. 4A, B, C and D) the results showed that each of T3 and TAC were increased quadratically and responded up to 0.45 and 0.40 (g kg^{-1} diet) to give the maximum T3 and TAC of 2.28 (ng dL^{-1}) and 2.84 (mmol L^{-1}), respectively (Fig. 4). Whereas the response equations for the remaining traits were cubic and recorded that T4 and MDA responded up to 0.44 and 0.42, (g kg^{-1} diet) to predict maximum T4 of 12.92 (ng dL^{-1}) and minimum MDA of 22.15 (μmol L^{-1}), respectively (Fig. 4).

Effect of dietary supplementation with BV on hatching traits

The data on incubation performance and hatching traits are presented in Table 7. Significant improvements ($P < 0.05$) were detected in hatchability traits for the aged Sinai layer hens with only (BV 0.4 and BV 0.5) supplementation during the summer season (Table 7). Data presented in Table 7 revealed statistically significant effects of BV (BV 0.4 and BV 0.5) supplementation on the number of fertilized or unfertilized eggs, the number of chicks hatched, the fertility percentage, and the percentage of pipped eggs (Table 7).

The proportion of unfertilized eggs declined by 29.41% and 17.65% in the groups receiving 0.4 and 0.5 g BV kg^{-1} of diet, respectively, relative to the control (Table 7). Concurrently, fertilized egg counts rose by 7.54% and 4.52% in these same groups (Table 7). Furthermore, the number of chicks hatched increased by 27.14% and 17.14% in the BV 0.4 and BV 0.5 treatments, respectively, compared to the control (Table 7).

Interestingly, the percentage of pipped eggs decreased by 39.0% and 25.4% in the (BV 0.4) and (BV 0.5) groups, respectively, suggesting a

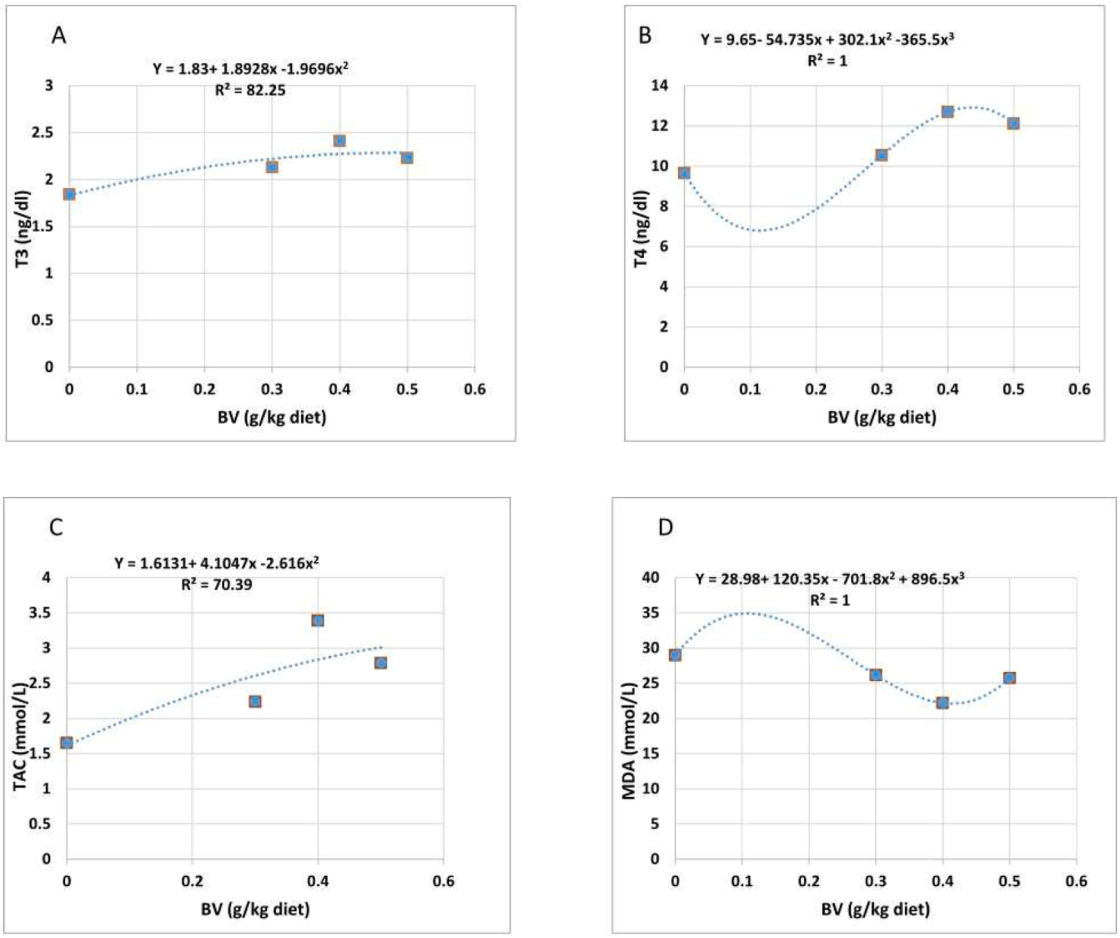

Fig. 4. Dose–response curve of triiodothyronine (T3) (A), thyroxine (T4) (B), total antioxidant capacity (TAC) (C), and malondialdehyde (MDA) (D) for different levels of dietary bee venom (BV) addition. X and Y are the dependent (BV levels) and the independent variables of the regression equation, respectively.

Table 7
Effect of dietary addition of bee venom (BV) on hatching traits of heat-stressed Sinai laying hens during the second production cycle.

Characteristics	Treatments				SEM	*P* values			
	CON.	(BV 0.3)	(BV 0.4)	(BV 0.5)		TRTS	Lin.	Quad.	Cub.
Number of unfertilized eggs	10.20 *a*	9.20 *ab*	7.20 *c*	8.40 *b*	0.346	0.0001	0.1038	0.2683	0.3421
Number of fertilized eggs	39.80 *c*	40.80 *bc*	42.80 *a*	41.60 *b*	0.346	0.0001	0.1038	0.2683	0.3421
Number of produced chicks	28.00 *c*	30.20 *c*	35.60 *a*	32.80 *b*	0.758	<0.0001	0.0527	0.2053	0.1970
Number of unhatched eggs	11.80 *a*	10.80 *a*	7.20 *b*	8.80 *b*	0.5385	0.0001	0.0596	0.3212	0.2563
Fertility (%)	79.60 *c*	81.60 *bc*	85.60 *a*	83.20 *b*	0.6928	0.0001	0.1038	0.2683	0.3421
Hatchability of total set eggs (%)	56.00 *c*	60.40 *c*	71.20 *a*	65.60 *b*	1.517	<0.0001	0.0527	0.2053	0.1970
Hatchability of fertilized eggs (%)	70.30 *b*	73.50 *b*	83.17 *a*	78.85 *a*	1.460	0.0001	0.0441	0.3018	0.2599
Percentage of pipped eggs	11.80 *a*	10.60 *a*	7.20 *b*	8.80 *b*	0.539	0.0001	0.0596	0.3212	0.2563

CON. = control; (BV 0.3) = 0.3 g BV kg^{-1} diet; (BV 0.4) = 0.4 g BV kg^{-1}; (BV 0.5) = 0.5 g BV kg^{-1} diet; SEM = standard error of means; TRTS, treatments; Lin. = linear response; Quad. = quadratic response; Cub. = cubic response. The data represent the means of five independent replicates, and values with the same letter within a row are not significantly different ($P > 0.05$) according to Duncan's multiple-range test.

potential improvement in hatchability (Table 7). Additionally, the hatchability percentage of fertilized eggs was linearly affected by BV addition ($P = 0.0441$), with the highest value in the group treated with 0.4 g BV kg^{-1} diet (Table 7).

Discussion

The THI in the current study was 30.72, indicating very severe heat stress, as reflected in decreased performance metrics. Heat stress poses a substantial management challenge, jeopardizing antioxidant integrity, as evidenced by increased oxidative stress and lipid peroxidation, and reduced serum antioxidant levels (El-Ratel et al., 2023; El-Kholy et al., 2024).

Recently, a variety of bee products have been incorporated into the diets of broilers and rabbits to increase their antioxidant capacity (Abd El-Aziz et al., 2023; El-Banna et al., 2023). Concrete data are scarce concerning the impact of BV on laying hens' growth; nevertheless, the results presented here align with the findings observed in broiler chickens.

The addition of BV to birds' diets in the current study significantly improved FBW, BWG, EP, EW, EM, and FCR compared with the control group. These outcomes were confirmed by Han et al. (2010) and Kim et al. (2018), who showed that treating drinking water with BV resulted in a statistically significant ($P < 0.05$) increase in BWG. The increased

EW observed in BV-treated birds, as shown in Table 3, is consistent with these results.

These findings could be attributed to BV being considered one of the most efficient natural supplements, owing to its unique composition, which includes a variety of beneficial enzymes and peptides, as noted by Kim et al. (2010). In contrast, Ali and Mohanny (2014) pointed out that BV reduced ($P < 0.05$) broilers' BW and BWG throughout the interval of 0-3 weeks of age. The differences between those results and the current results could be related to the differences in the birds' strain or age.

Overall, in the current study, supplementing layers' diets with BV increased EP and EM. Such an effect was previously confirmed by Boka et al. (2014). There has been no evidence of beneficial effects of natural antioxidants in feeds on EM, egg number, or FCR. Recently, El-Kholy et al. (2024) found that laying hens fed natural antioxidants had a positive effect on EP and EM during heat stress.

The increased egg production parameters and improved FCR in the present study could be related to the antibacterial effect of BV, which may improve reproductive performance and increase laying hen production during the second production cycle or under heat stress. This could explain the rise in BWG in the current study. The findings suggest that dietary BV supplementation positively influenced performance traits in laying hens, possibly due to its biologically active components. Nevertheless, further studies involving extended laying periods and larger sample sizes are required to validate these effects across the entire laying cycle.

Blood hematologic, and biochemical parameters are crucial markers for assessing an animal's clinical and nutritional health (Olafedehan et al., 2012; El-Kholy et al., 2024). These profiles provide vital data for examining environmental factors, pathology, avian immunology, and other stressors that impact bird health (Afolabi et al., 2011; El-Kholy et al., 2022).

The current data indicate that dietary treatments at BV levels of 0.3, 0.4, and 0.5 kg^{-1} of diet significantly affected HGB levels (Table 4). The current results are consistent with those of Rabie et al. (2018), who reported that HGB content was significantly elevated in BV-treated chicks compared with the control group. On the other hand, dietary treatments with sole supplementation of BV 0.4 and BV 0.5 significantly influenced HCT levels. The maximum MCH values were recorded in the (BV 0.3) and (BV 0.4) groups (Table 4).

Han et al. (2010) and El-Banna et al. (2023) reported that BV treatments had no statistically significant effect on most hematological parameters in broiler chickens. Still, HTC increased significantly with BV supplementation from 0.5 to 2 mg L^{-1} in drinking water. These findings are comparable to those reported by Mohammed and Hassan (2019), who found higher HGB levels ($P < 0.05$) and an insignificantly higher HCT in BV-treated arthritic rats. It has been demonstrated that BV treatment can improve blood circulation in coronary, peripheral, and microvascular blood vessels, and that it plays a role in erythropoiesis (Son et al., 2007).

Notwithstanding these enhancements, BV supplementation in the current study did not significantly ($P > 0.05$) influence RBC count, MCV, or the counts of lymphocytes, eosinophils, or monocytes (Table 4). These findings suggested that BV treatments did not have any harmful or adverse consequences on the blood hematology of Sinai layers. These data were confirmed by Meligi et al. (2020), who found no significant differences in RBCs, MCV, MCH, or WBC count between BV-treated and untreated rats. Similarly, Hussein et al. (2001) noted insignificant increases in red blood cell indices, including MCV and MCH.

According to data on liver enzymes and blood metabolites, the BV dietary supplement improved most of the criteria evaluated in this study (Table 5). ALB, GLO, the ALB/GLO ratio, TC, TL, TG, ALT, and AST/ALT ratio were all significantly impacted by the dietary treatments that contained solely (BV 0.4 and BV 0.5) supplementation. ALB and GLO rose linearly as BV levels rose (Table 5). The highest serum levels of ALB and ALB/GLO ratio were seen in the (BV 0.4) group (Table 5).

The current results, however, contradict those of Han et al. (2010), who found that adding BV to broiler drinking water had no significant effect on TP or GLO. Similarly, the present results are supported by the work of Mousavi et al. (2012), who reported that BV treatment restored TG and TC levels to normal in diabetic rats. Conversely, these findings diverged from those of Han et al. (2010) and Ali and Mohanny (2014), who reported that the inclusion of BV in broilers' drinking water did not significantly impact TC or TL.

In the current study, the BV-treated birds exhibited reduced blood ALT (Table 5), indicating enhanced hepatoprotective efficacy. Increasing levels of BV resulted in a reduction in ALT (Table 5), with the lowest ALT values ($P < 0.05$) recorded in the (BV 0.4) and (BV 0.5) groups (Table 5). In contrast, Ali and Mohanny (2014) observed no significant effect on ALT activity after adding BV to broiler drinking water. According to Hwang et al. (2015), the antioxidant properties of BV, particularly its capacity to scavenge free radicals, may protect liver function, as indicated by reduced ALT activity in the current study. On the other hand, dietary BV supplementation did not significantly affect AST levels (Table 5). In conclusion, BV, as a dietary addition, has beneficial effects on liver function (ALT) in heat-stressed layers.

In addition, treatment with BV reduced oxidative stress, as evidenced by higher TAC and lower MDA levels (Table 6), thereby exerting a hepatic protective effect. Kim et al. (2019) found that dietary BV improved TAC and altered fatty acid metabolism in broilers. Furthermore Kim et al. (2010), demonstrated the strong hepatoprotective properties of BV, which operate by inhibiting the production of proinflammatory cytokines, including TNF-α, and by reducing elevated serum transaminase levels. Exposure to stressors, such as thermal stress and aging, is known to increase ROS production, leading to lipid peroxidation and disruption of cellular membrane integrity (Rehman et al., 2018).

In BV-treated birds, the MDA level in the blood was less than that of the untreated control group (Table 6). These findings are comparable to those reported by El Senosi et al. (2018) and Abo-Zaid et al. (2023), who observed a marked reduction in blood MDA content and substantial increases in blood TAC content in albino rats following BV therapy. Furthermore, Hegazi (2012) reported that BV acts as a powerful antioxidant, effectively reducing ROS and MDA levels. Han et al. (2010) indicated that the antioxidant properties of BV samples were associated with their ability to suppress lipid peroxidation and enhance TAC activity.

BV appears to mitigate oxidative effects, as evidenced by improved liver enzyme levels (ALT) and reduced MDA levels in treated hens, particularly at the 0.40 g kg^{-1} dose. These findings indicate stabilization of the blood's redox state. The TAC of BV has been validated through investigations in diverse disease models and health conditions (Hwang et al., 2015; Hanafi et al., 2018). The pharmacological properties of BV include protective effects against oxidative stress, microbial infections, inflammation, and tumors (Carpena et al., 2020; Hassan et al., 2021). Additionally, BV may improve oxidative balance by upregulating antioxidant defense systems, including increased activity of enzymes such as SOD and GSH-Px, thereby reducing lipid peroxidation and cellular damage under stress conditions (Im et al., 2016; Bava et al., 2023).

Moreover, the presence of compounds such as apamin and PLA2 in BV suggests potential immunomodulatory benefits, which could make BV effective in managing immune-related disorders (Hossen et al., 2017). The current study suggested that BV, with its bioactive components, could be effective for stressed-layer hens due to heat stress, aging, or the second reproductive cycle. Emerging evidence suggests that BV could influence hormonal regulation, potentially affecting reproductive and stress-related hormones, which may, in turn, affect productive and reproductive performance (Abd El-Aziz et al., 2023; Elkomy et al., 2023).

Thyroid hormones, particularly T3 and T4, are essential for the acclimatization process in laying hens and are adversely affected by high ambient temperatures (Attia et al., 2016). To prevent hyperthermia, a lower metabolic rate may result from heat-stress-induced decreases in serum T4 and T3 levels (El-Kholy et al., 2022, 2024). The current

investigation demonstrated that dietary BV treatment significantly increased blood concentrations of T3 and T4 (Table 6). Significantly, no substantial variations were seen between the two BV levels (0.4 and 0.5 g kg^{-1} diet) for T3 and T4 (Table 6).

Several studies have shown that chickens exposed to higher ambient temperature have lower serum T3 and T4 levels (Anjum et al., 2014, 2016). Conversely, Sechman (2013) observed that acute heat stress did not significantly affect blood levels of thyroid hormones. The reduction in thyroid hormones (T3 and T4) under elevated ambient temperatures appears to be associated with a downregulation of metabolic activity, likely serving as a physiological adaptation to minimize heat production and protect the body from hyperthermia. The reduction in T3 could be due to decreased T4-to-T3 conversion or decreased T3 synthesis, as suggested by Ozbey and Ozcelik (2004).

In the present study, dietary BV treatment at all concentrations significantly increased serum IgY and IgM levels compared with the untreated group (Table 6). The peak levels of IgY and IgM were identified in the BV 0.4 and BV 0.5 groups. Significantly, no discernible variation was observed between the two BV levels (0.4 and 0.5 g kg^{-1} diet) for IgM (Table 6). These observations align with those of Lee et al. (2020) and Abo-Zaid et al. (2023). Additionally, Ali and Mohanny (2014) reported that BV can enhance the immune response in broiler chickens without adverse effects. BV contains a complex mixture of bioactive compounds, including peptides such as melittin and apamin, as well as enzymes like phospholipase A_2, which are known to influence immune function by enhancing macrophage activity, stimulating cytokine production, and modulating both innate and adaptive immunity (Hossen et al., 2017; El Basuini et al., 2024). Eze et al. (2016) provided additional support for this conclusion, noting that BV, a complex mixture of peptides, has a major positive impact on the immune systems of living organisms. This effect may be linked to superoxide production, as reported by Han et al. (2010).

The effects of dietary BV supplementation on broiler breeders' fertility and hatchability attributes have not been adequately studied. Because of this discrepancy, the current results were compared with those of other studies examining the effects of various bee-derived compounds on layer chickens. In the present study, enhancements in hatchability characteristics were observed in older Sinai layer hens supplemented with BV at 0.4 and 0.5 kg^{-1} of diet during the summer season. Table 7 demonstrated statistically significant effects of BV (BV 0.4 and BV 0.5) supplementation on the quantity of fertilized and unfertilized eggs, the number of chicks hatched, the fertility percentage, and the percentage of pipped eggs.

The percentage of unfertilized eggs decreased by 29.41% and 17.65% in the groups administered 0.4 and 0.5 g BV kg^{-1} of diet, respectively, compared with the control (Table 7). Simultaneously, the number of fertilized eggs increased by 7.54% and 4.52% in these respective groups. Additionally, the number of chicks hatched increased by 27.14% and 17.14% in the BV 0.4 and BV 0.5 treatments, respectively, compared with the control. The percentage of pipped eggs also decreased by 39.0% and 25.4% in the (BV 0.4) and (BV 0.5) groups, indicating a possible enhancement in hatchability (Table 7). The dietary BV-related enhancements in hatching characteristics seen in this study contradict the findings of Rizk et al. (2023), who demonstrated that the inclusion of bee pollen in the diets of 60-week-old Siani laying hens led to insignificant increases in hatchability, both as a percentage of total set eggs and fertile eggs.

The BV content of *A. mellifera* enzymes (hyaluronidase, PLA2, α-glucosidase, and acid phosphatase), especially hyaluronidase, which causes dilation and increased blood vessel permeability resulting in increased blood circulation (Kim et al., 2019), may be related to the enhancement and improvement of production performance and immunity in treated-BV heat-stressed and aged hens.

The practical application and economic feasibility of BV in commercial poultry systems remain important considerations warranting further investigation. In addition, BV availability is comparatively low compared with conventional feed additives, which may lead to supply fluctuations and price instability. Therefore, detailed cost–benefit analyses and field-scale trials are important for evaluating whether improvements in production, health, and product quality can offset costs and support the scalability of BV application in modern poultry production systems.

Conclusion

In conclusion, dietary supplementation of BV during the second production cycle in Sinai chicken breeders under summer conditions had beneficial effects on productive and reproductive performance. Birds receiving BV exhibited improved egg production, fertility, and hatchability, along with enhanced hematological profiles and favorable biochemical parameters, indicating better physiological and metabolic status. Additionally, BV supplementation strengthened the immune response and helped alleviate the negative impacts of heat stress. Overall, these findings suggest that BV can serve as a promising natural feed additive to enhance the performance and health status of chicken breeders. Further research at various concentrations (above 0.5 g kg^{-1}) and studies on the optimal dosage will be required to provide more insight.

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Disclosures

There were no conflict of interest.

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