

# Effect of dietary stevia and ginger extracts on laying performance, fertility, hatchability, and serum biochemical parameters in laying Japanese quails exposed to heat stress

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**ABSTRACT.** The aim of this study was to determine the effect of various levels of supplemental stevia and ginger extracts (SGE) on laying performance, fertility, hatchability, and serum biochemical parameters in laying Japanese quails exposed to heat stress. SGE doses were added at the following levels: 0 (control), 0.5 (T1), 1.0 (T2), and 1.5% (T3) of the diet. In SGE-supplemented quails, feed intake ( $P < 0.0001$ ), egg production ( $P < 0.0001$ ), and egg weight ( $P < 0.002$ ) were significantly the highest in the treatment groups (days 1–90). Feed conversion ratio ( $P < 0.0001$ ) was lower in the treatment groups compared to the control group. The fertility index was higher in all experimental groups ( $P < 0.05$ ). The weights of hatching eggs were higher in groups T2 and T3 ( $P < 0.0001$ ). Egg weight in the laying period was higher in the 2nd month in control and group T1, and in the 2nd and 3rd month in groups T2 and T3 ( $P < 0.0001$ ;  $P < 0.05$ ). Hatched chick weights were higher in groups T2 and T3 ( $P < 0.0001$ ). Serum aspartate aminotransferase levels in quails of group T3 were significantly lower than in the other groups ( $P < 0.01$ ). Serum glucose and cholesterol levels were the lowest in groups T2 and T3 ( $P < 0.05$ ). Serum triiodothyronine levels in the treatment groups were significantly lower ( $P < 0.01$ ). In conclusion, these results suggested that dietary SGE improved laying performance, fertility, and hatchability, as well as mitigated the negative effects of heat stress on selected biochemical parameters in quails exposed to high environmental temperature.

## Introduction

Increasing environmental temperature along with climate change adversely affects immune system functions and reduces growth, development, and reproductive performance, as well as meat and egg quality in livestock, especially in poultry production. New poultry genotypes developed by commercial poultry companies have low heat tolerance and are exposed to life-threatening conditions due to

increasing environmental temperature (Nawaz et al., 2021). To reduce the effect of raising temperature in poultry houses, commercial enterprises have made large investments to control microclimate conditions using tunnel ventilation or air evacuation and sprinkler devices (Lara and Rostagno, 2013; Ranjan et al., 2019). However, these changes are not completely effective, and small-scale companies are more affected by heat stress. In addition to these technological advances, it is necessary to develop

feeding strategies and nutritional supplements (Jyotsnarani et al., 2022).

Phytogenic compounds are bioactive substances of plant origin that exert functional effects on animal health and productivity (Nair et al., 2019). Alkaloid, terpenoid, and phenolic compounds present in ginger (*Zingiber officinale*) and stevia (*Stevia rebaudiana*) have antioxidant, anti-inflammatory, antiparasitic, and antibacterial properties (Pirgozliev et al., 2021a; b; 2022). These features can effectively reduce energy loss and heat stress in animals, and improve nutrient absorption for growth and reproductive performance. They also show positive effects on fertility, hatchability, egg production, egg weight, and hatched chick weight (Windisch et al., 2008; Tchoffo et al., 2017; Rehman et al., 2019; Jiang et al., 2020). The objective of this study was to investigate the effects of different supplementary doses of stevia and ginger extracts (SGE) on laying performance, fertility, hatchability, and serum biochemical parameters in laying Japanese quails exposed to heat stress.

## Material and methods

### Animal management and treatments

All procedures used in the present study were approved by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Turkey (No.: 2022-7579450). The birds were supplied from the practice and research farm of the Faculty of Veterinary Medicine of Dicle University. A total of 120 female (70-day-old) Japanese quails (*Coturnix coturnix japonica*) with similar body weights ( $245.48 \pm 3.22$ ) were randomly allocated to 4 groups with 6 replicate cages ( $60 \times 120 \times 30$  cm), 5 quails per cage. A nipple drinker and a linear feeder were used in each cage compartment. The birds were adapted to the experimental conditions for a period of 10 days. After the adaptation period, the experimental study period lasted 90 days (day 10). Quails in all groups were reared at neutral temperature for 16 h ( $21 \pm 2$  °C the rest of the time) and long-term for 8 h ( $32 \pm 2$  °C from 10:00 to 18:00); the relative humidity was approximately 50–55%. Electric heaters with adjustable thermostats, air conditioners, and fans for air circulation were used to maintain the ambient temperature at the desired level. The photoperiod consisted of 16 h of light and 8 h of darkness.

### Animal feeding and data collection

During the experiment, the quails were divided into the following groups: 0 (control), (T1), (T2), and (T3); the control group was fed a basal diet (Table 1)

without the addition of stevia (*Stevia rebaudiana* 20.000 mg/kg; Sigma-Aldrich, Istanbul, Turkey) and ginger (*Zingiber officinale* 5.000 mg/kg; Calbiochem, Istanbul, Turkey) extract mixture (SGE), while group T1 was supplemented with 0.5% SGE, T2 – 1% SGE and T3 – 1.5% SGE.

**Table 1.** Ingredient and nutrient composition of the basal diet

Ingredient	Amount, %
Maize	53.45
Soybean meal (48%)	29.26
Soy oil	4.88
Salt	0.30
DL-methionine	0.18
Limestone	9.94
Dicalcium phosphate	1.65
Vitamin and mineral premix <sup>1</sup>	0.34
Chemical analyses, % (dry matter basis)	
crude protein	18.93
crude fat	6.11
crude fibre	4.63
calcium	3.81
phosphorus	0.64
Calculated compositions <sup>2</sup>	
metabolisable energy, kcal/kg	2975
methionine	0.41
lysine	1.05

<sup>1</sup> vitamin and mineral premix provided per kilogram of diet: mg: retinyl acetate 1.8, cholecalciferol 0.025, dl-tocopheryl acetate 1.25, menadione sodium bisulphite 2.5, thiamine-hydrochloride 1.5, riboflavin 3, niacin 12.5, d-pantothenic acid 5, pyridoxine hydrochloride 2.5, vitamin B<sub>12</sub> 0.0075, folic acid 0.25, choline chloride 125, Mn (MnSO<sub>4</sub>·H<sub>2</sub>O) 50, Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O) 30, Zn (ZnO) 30, Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O) 5, Co (CoCl<sub>2</sub>·6H<sub>2</sub>O) 0.1, I as KI 0.4, Se (Na<sub>2</sub>SeO<sub>3</sub>) 0.15; <sup>2</sup>calculated according to tabular values listed for feed ingredients

Feed and fresh water were given *ad libitum*. Egg production and weight were calculated daily, while feed consumption and feed conversion rates were calculated weekly. In order to determine monthly fertility and hatchability percentages, 2 female and 1 male quail were caged 1 week before egg collection. During the last 5 days of each month, eggs were collected and weighed, and then placed in the hatcher. The eggs placed in the incubator were exposed to 37.56 °C and 65% RH for the first 14 days. Eggs were transferred to the hatchery unit in the last 3 days of incubation and 37.1 °C and 72% RH were applied until hatching; ventilation and temperature were adjusted automatically. Eggs were automatically turned once every two hours a day. Chicks were weighed on the same day and the weight of the chicks from individual groups was determined. Fertility, early and middle-late embryonic mortality, and hatchability parameters were calculated according to the formulas given below:

fertility % = (number of fertile eggs / total egg number) × 100,

early mortality = (number of dead embryos between days 1 and 9 of incubation / number of fertile eggs) × 100,

middle-late mortality = (number of dead embryos between days 10 and 21 of incubation / number of fertile eggs) × 100 (Reijrink et al., 2010),

hatchability % = number of hatched chicks / number of fertile eggs) × 100.

Twelve quails (2 quails from each cage) were killed by cervical dislocation to determine serum biochemical parameters. Blood samples were centrifuged at 4 °C at 3000 rpm for 10 min. Serum samples were stored at -20 °C until analysis.

### Laboratory analysis

Malondialdehyde (MDA) levels in the homogenate and serum were determined using the single heating method of Yoshioka et al. (1979). Serum aspartate aminotransferase (AST) (Archem, A2212, Istanbul, Turkey) activity and cholesterol, glucose, and creatine kinase (CK) (Archem, A2091, Istanbul, Turkey) levels were measured using commercially available kits and a biochemistry auto-analyzer (D280;

Sinnowa, Nanjing, China). Triiodothyronine (T<sub>3</sub>; ng/ml), and thyroxin (T<sub>4</sub>; ng/dl) were analysed by radioimmunoassay RIA according to Akiba et al. (1982).

### Statistical analysis

All performance and serum biochemical parameters were analysed using orthogonal polynomial contrast in the analysis of variance, and multiple comparisons were performed using the Duncan-HSD multiple range test. All analyses were determined using SPSS for Windows (IBM, SPSS Version 22.0). The results were considered significant at  $P < 0.05$ . Reproductive parameters (fecundity, fertility, hatchability, etc.) were calculated using the chi-square test, while egg and chick weights were analysed using one-way ANOVA implemented in SPSS. Variation between groups was detected using Tukey's multiple comparisons. Data are presented as mean and standard deviation or as a percentage (Chi-square). The level of statistical significance was assumed at  $P < 0.05$ .

## Results

### Laying performance

Feed intake of quails fed SGE on days 31–60, 61–90, and 1–90 ( $P < 0.01$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively), egg production on days 1–30, 31–60, 61–90 and 1–90 ( $P < 0.001$ , for all), egg weight on days 31–60 and 1–90 ( $P < 0.05$ , and  $P < 0.01$ , respectively) were significantly higher in the treatment groups than in the control group.

**Table 2.** Effect of stevia and ginger extract (SGE) supplementation on laying performance in quails

Variables	SGE, %				SEM	Statistical significance, $P > F_{ij}$		
	Control	T1	T2	T3		S	L	Q
Feed intake, g								
Days 1–30	27.46	27.56	27.71	27.61	0.466	0.985	0.772	0.837
31–60	24.80 <sup>b</sup>	26.59 <sup>a</sup>	27.44 <sup>a</sup>	26.29 <sup>a</sup>	0.388	0.001	0.008	0.001
61–90	22.57 <sup>c</sup>	23.76 <sup>bc</sup>	24.64 <sup>b</sup>	26.73 <sup>a</sup>	0.396	0.0001	0.0001	0.288
1–90	24.93 <sup>c</sup>	25.97 <sup>b</sup>	26.59 <sup>a</sup>	26.87 <sup>a</sup>	0.215	0.0001	0.0001	0.118
Egg production, %								
Days 1–30	72.04 <sup>b</sup>	72.78 <sup>b</sup>	71.48 <sup>b</sup>	76.85 <sup>a</sup>	0.599	0.0001	0.0001	0.001
31–60	67.96 <sup>c</sup>	74.07 <sup>b</sup>	73.89 <sup>b</sup>	78.15 <sup>a</sup>	0.555	0.0001	0.0001	0.110
61–90	54.26 <sup>d</sup>	63.89 <sup>b</sup>	58.70 <sup>c</sup>	69.26 <sup>a</sup>	0.703	0.0001	0.0001	0.516
1–90	64.75 <sup>d</sup>	70.25 <sup>b</sup>	68.02 <sup>c</sup>	74.75 <sup>a</sup>	0.623	0.0001	0.0001	0.333
Egg weight, g								
1–30	11.89	12.23	12.44	12.47	0.179	0.107	0.022	0.391
31–60	11.94 <sup>b</sup>	12.36 <sup>ab</sup>	12.64 <sup>a</sup>	12.41 <sup>a</sup>	0.140	0.016	0.014	0.034
61–90	11.90	12.00	12.19	12.27	0.154	0.325	0.071	0.953
1–90	11.90 <sup>b</sup>	12.20 <sup>a</sup>	12.44 <sup>a</sup>	12.40 <sup>a</sup>	0.084	0.002	0.0001	0.079
Feed conversion ratio, (feed intake, g / (egg weight, g × egg production, %))								
1–30	3.21 <sup>a</sup>	3.10 <sup>b</sup>	3.12 <sup>b</sup>	2.88 <sup>c</sup>	0.025	0.0001	0.0001	0.016
31–60	3.06 <sup>a</sup>	2.90 <sup>b</sup>	2.94 <sup>b</sup>	2.71 <sup>c</sup>	0.019	0.0001	0.0001	0.086
61–90	3.50 <sup>a</sup>	3.10 <sup>b</sup>	3.44 <sup>a</sup>	3.15 <sup>b</sup>	0.021	0.0001	0.0001	0.019
1–90	3.26 <sup>a</sup>	3.03 <sup>c</sup>	3.17 <sup>b</sup>	2.91 <sup>d</sup>	0.022	0.0001	0.0001	0.430

Control – 0% SGE in the diet, T1 – 0.5% of SGE in the diet, T2 – 1% SGE in the diet, T3 – 1.5% SGE in the diet; SEM – standard error of the mean;  $F_{ij}$  – statistical contrast: S – SGE supplementation effect (quail supplemented with SGE vs quail not supplemented with SGE), L – linear effect of increasing dietary SGE levels, Q – quadratic effect of increasing dietary SGE levels; <sup>a-d</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$

In contrast, feed conversion ratio on days 1–30, 31–60, 61–90 and 1–90 ( $P < 0.001$ , for all) was lower in all treatment groups compared to the control group (Table 2).

### Serum biochemical parameters

The effect of SGE on serum biochemical parameters in quails exposed to heat stress is given in Table 3. Serum MDA levels decreased in all treatment groups compared to the control group ( $P < 0.001$ ). Serum AST levels in quails from group T3 were significantly lower than in the other groups ( $P < 0.01$ ). Serum glucose and cholesterol levels were the lowest in groups T2 and T3 ( $P < 0.05$ , for both). However, there was no difference between the groups in serum CK levels. Serum  $T_3$  levels in the treatment groups were significantly lower compared to the control group ( $P < 0.01$ ). However, there was no change in serum  $T_4$  levels.

### Fertility and hatchability percentages, and egg and chick weights

Fertility rates were higher in the experimental groups ( $P < 0.05$ ), however, there was no statistical difference between the groups in hatchability, early embryonic mortality (EEM), and middle-late embryonic mortality (MLEM) ( $P > 0.05$ ) (Table 4). An increase was observed in hatching egg weights in groups T2 and T3 ( $P < 0.0001$ ). Egg weights during the laying period were higher in the 2nd month in group T1 and control, while in the 2nd and 3rd months in groups T2 and T3 ( $P < 0.0001$ ;  $P < 0.05$ ) (Table 5). Hatched chick weight (one-day) were higher in groups T2 and T3. The weight of the hatched chicks of the eggs collected in the laying period in the 2nd month were higher in the control group, while in months 2 and 3 in group T2 ( $P < 0.0001$ ) (Table 6).

**Table 3.** Effect of dietary stevia and ginger extract (SGE) supplementation on serum MDA, AST, CK, glucose, cholesterol,  $T_3$  and  $T_4$  levels in heat-stressed quails

Variables	SGE, %				SEM	Statistical significance, $P > F_{ij}$		
	Control	T1	T2	T3		S	L	Q
MDA, nmol/ml	10.8 <sup>a</sup>	6.2 <sup>b</sup>	5.0 <sup>b</sup>	6.1 <sup>b</sup>	0.559	0.0001	0.004	0.0001
AST, U/l	330 <sup>a</sup>	321 <sup>a</sup>	282 <sup>ab</sup>	233 <sup>b</sup>	20.7	0.014	0.002	0.362
CK, U/l	3066	2998	2969	2800	230.5	0.226	0.235	0.099
Glucose, mg/dl	306 <sup>a</sup>	290 <sup>ab</sup>	270 <sup>bc</sup>	255 <sup>c</sup>	9.7	0.008	0.001	0.938
Cholesterol, mg/dl	242 <sup>a</sup>	220 <sup>ab</sup>	198 <sup>bc</sup>	187 <sup>c</sup>	9.9	0.004	0.0001	0.583
$T_3$ , ng/ml	3.75 <sup>a</sup>	2.63 <sup>b</sup>	2.58 <sup>b</sup>	2.15 <sup>b</sup>	0.282	0.005	0.001	0.253
$T_4$ , ng/dl	0.410	0.404	0.409	0.403	0.005	0.136	0.296	0.021

Control – 0% SGE in the diet, T1 – 0.5% of SGE in the diet, T2 – 1% SGE in the diet, T3 – 1.5% SGE in the diet; MDA – malondialdehyde, CK – creatine kinase, AST – aspartate aminotransferase,  $T_3$  – triiodothyronine,  $T_4$  – thyroxine; SEM – standard error of the mean;  $F_{ij}$  – statistical contrast: S – SGE supplementation effect (quail supplemented with SGE vs quail not supplemented with SGE), L – linear effect of increasing dietary SGE levels, Q – quadratic effect of increasing dietary SGE levels; <sup>abc</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$

**Table 4.** Effects of stevia and ginger extract (SGE) supplementation and laying period on fertility and hatchability rates

Variables	SGE, %				P-value Chi-square
	Control	T1	T2	T3	
Fertility, n	257/300	272/300	276/300	273/300	$P = 0.0477$
%	85.7 <sup>b</sup>	90.7 <sup>ab</sup>	92.0 <sup>a</sup>	91.0 <sup>a</sup>	$\chi^2 = 7.920$
Hatchability, n	237/257	255/272	259/276	256/273	$P = 0.7863$
%	92.2	93.8	93.8	93.8	$\chi^2 = 0.786$
EEM, n	4/257	5/272	6/276	5/273	$P = 0.9654$
%	1.5	1.8	2.1	1.8	$\chi^2 = 0.2709$
MLEM, n	16/257	12/272	11/276	12/273	$P = 0.6272$
%	6.2	4.4	4.0	4.4	$\chi^2 = 1.7438$

Control – 0% SGE in the diet, T1 – 0.5% of SGE in the diet, T2 – 1% SGE in the diet, T3 – 1.5% SGE in diet; EEM – early embryonic mortality, MLEM – middle and late embryonic mortality; fertility, % = (number of fertile eggs / total eggs set)  $\times$  100; the Pearson Chi-square test or exact Chi-square test were used to analyse the data ( $P < 0.05$ ); hatchability, % = number of hatched chicks / number of fertile eggs  $\times$  100; early mortality = (number of dead embryos between day 1 and 9 of incubation / number of fertile eggs)  $\times$  100; middle mortality = (number of dead embryos between day 10 and 17 of incubation / number of fertile eggs)  $\times$  100; late mortality = (number of dead embryos between day 18 and 21 of incubation / number of fertile eggs)  $\times$  100 (Rejzink et al., 2010); the Pearson Chi-square test was used to analyse the data ( $P < 0.05$ ); <sup>abc</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$

**Table 5.** Effect of stevia and ginger extract (SGE) supplementation and laying period on hatching egg weight

Variables	SGE, %				P-value
	Control	T1	T2	T3	
1st month	12.20 ± 0.58 <sup>cb</sup>	12.35 ± 0.59 <sup>cb</sup>	12.51 ± 0.65 <sup>abB</sup>	12.58 ± 0.58 <sup>aA</sup>	0.0001
2nd month	12.64 ± 0.60 <sup>bA</sup>	12.56 ± 0.51 <sup>bA</sup>	12.91 ± 0.58 <sup>aA</sup>	12.69 ± 0.57 <sup>bA</sup>	0.0001
3rd month	12.07 ± 0.61 <sup>cb</sup>	12.20 ± 0.67 <sup>cb</sup>	12.73 ± 0.46 <sup>aA</sup>	12.49 ± 0.58 <sup>bB</sup>	0.0001
P-value*	0.0001	0.0002	0.0001	0.0469	
Total	12.30 ± 0.64 <sup>b</sup>	12.37 ± 0.61 <sup>b</sup>	12.71 ± 0.59 <sup>a</sup>	12.59 ± 0.58 <sup>a</sup>	0.0001

Control – 0% SGE in the diet, T1 – 0.5% of SGE in the diet, T2 – 1% SGE in the diet, T3 – 1.5% SGE in the diet; \* one-way analysis of variance (ANOVA) and Tukey's post hoc test were used to analyse the data ( $P < 0.05$ ); <sup>abc</sup> – groups with different letters in the same row are significantly different; <sup>AB</sup> – groups with different letters in the same column are significantly; data are presented as means and standard deviations

**Table 6.** Effect of stevia and ginger extract (SGE) supplementation and laying period on chick weight

Variables	SGE, %				P-value
	Control	T1	T2	T3	
1st month	8.16 ± 0.47 <sup>cb</sup>	8.31 ± 0.55 <sup>bc</sup>	8.40 ± 0.56 <sup>abB</sup>	8.55 ± 0.73 <sup>a</sup>	0.0001
2nd month	8.40 ± 0.60 <sup>bA</sup>	8.40 ± 0.55 <sup>b</sup>	8.89 ± 0.80 <sup>aA</sup>	8.71 ± 0.78 <sup>a</sup>	0.0001
3rd month	8.02 ± 0.55 <sup>bB</sup>	8.22 ± 0.58 <sup>b</sup>	8.6 ± 0.92 <sup>aA</sup>	8.63 ± 0.72 <sup>a</sup>	0.0001
P-value*	0.0001	0.1219	0.0002	0.3331	
Total	8.20 ± 0.56 <sup>b</sup>	8.31 ± 0.56 <sup>b</sup>	8.66 ± 0.79 <sup>a</sup>	8.63 ± 0.75 <sup>a</sup>	0.0001

Control – 0% SGE in the diet, T1 – 0.5% of SGE in the diet, T2 – 1% SGE in the diet, T3 – 1.5% SGE in the diet; \* one-way analysis of variance (ANOVA) and Tukey's post hoc test were used to analyse the data ( $P < 0.05$ ); <sup>abc</sup> – groups with different letters in the same row are significantly different; <sup>AB</sup> – groups with different letters in the same column are significantly; data are presented as means and standard deviations

## Discussion

### Laying performance

It is commonly known that yield performance decreases in birds exposed to heat stress. This is due to a decrease in feed consumption and a negative effect on the digestive system, which in turn adversely affects the intestinal microflora and gastrointestinal peptides, thereby reducing nutrient absorption from the intestine (Liu et al., 2019; Wang et al., 2021). Heat stress was applied in the present study and resulted in an increase in feed consumption and a decrease in feed conversion ratio in all treatment groups compared to the control group. Similar studies in broilers and pigs reported that dietary stevia supplementation increased feed consumption and improved feed conversion ratio (Atteh et al., 2008; Xiong et al., 2022). Likewise, researchers showed that the addition of ginger to the diet had significant positive effects on feed consumption and feed conversion ratio (Asghar et al., 2021; Nemati et al., 2021). Since ginger contains digestive enzymes and many active ingredients that can stimulate digestion, it is able to increase feed consumption and improve feed conversion ratio (Mohamed et al., 2012). Contrary to our results, Pirgozliev et al. (2022) added 2% stevia to laying hens' feeds and did not find significant differences; egg production did not change compared to control, differences were recorded only between 1% and 2% inclusion groups.

Egg production in the present study was higher in all experimental groups compared to the control group, with the highest levels detected in group T3. In previous studies, the authors found that the addition of stevia and ginger did not have any negative effects on egg production, and even stated that there was a numerical increase in the experimental groups (Tchoffo et al., 2019; Pirgozliev et al., 2022). The increase in egg weight in the experimental groups may have been caused by terpenic and phenolic compounds contained in ginger extract. Due to the antimicrobial properties of terpenes and the antioxidant properties of phenolic compounds, it may have contributed to the production of quality eggs by supporting cells and organs involved in egg production (Tchoffo et al., 2017).

### Serum biochemical parameters

Thermal stress is one of the main reasons of oxidative damage. Exposure to heat stress raises the concentration of free radicals and causes various diseases by damaging cell phospholipid membranes (El-Deep et al., 2016). Stevia and ginger extracts exhibit high levels of antioxidant activity, i.e. the elimination of free radical electrons and superoxides (Thomas and Glade, 2010; Zhao et al., 2011). MDA is an indicator of oxidative stress and is the end product of lipid peroxidation (Rehman et al., 2018). In our study, the addition of SGE to the diet statistically decreased serum MDA levels in

the treatment groups. Researchers reported that the supplementation of stevia (Tchoffo et al., 2019) and ginger (Wen et al., 2019) to the diet reduced serum MDA levels. In the present study, serum AST, glucose, and cholesterol levels were found to be lower in the experimental groups. Serum AST level is utilised as one of the important indicators of liver health status. This enzyme is synthesized in the liver, and when liver damage occurs, its blood levels increase due to release from damaged hepatic cells. The results of Malekizadeh et al. (2012) and Tchoffo et al. (2019) were consistent with our findings, as they reported that the addition of ginger to the ration reduced serum AST levels in laying and broiler chickens. Low serum AST level in the experimental groups could be due to the liver damage-alleviating effect of antioxidant compounds such as 6-gingerol present in ginger (Joshi et al., 2017). An increase in temperature affects the birds and increases their blood glucose level, which is one of the important stress indicators. Our results concerning blood glucose concentrations were similar to many previous studies, which reported that the addition of stevia (Khalifah et al., 2021) and ginger (Rehman et al., 2019) to the diet reduced blood glucose levels. This decrease in blood glucose could be due to stevia's ability to lower glucose concentration without inducing hypoglycaemia (Chen et al., 2005).

Total cholesterol levels were lower in the experimental groups compared to the control group. Asghar et al. (2021) reported that ginger supplementation in various doses reduced blood cholesterol levels. Similarly, other study found that the addition of 2% ginger to broiler diets reduced blood cholesterol levels (Rehman and Haq, 2014). The hypolipidemic effect of ginger has also been previously described. The mechanism of this phenomenon may be based on the presence of hydroxyl methyl glutaryl coenzyme A reductase (HMG-COA) in ginger (Malekizadeh et al., 2012). In addition, ginger was also shown to lower blood cholesterol levels by stimulating glycogenic enzymes (Zhang and Tan, 2000).

Thyroid hormones ( $T_3$  and  $T_4$ ) play an important role in stress factor control. The thyroid attempts to balance nutrient metabolism by regulating thermoregulation during heat stress.  $T_3$  and  $T_4$  levels are essential for maintaining body temperature through energy metabolism in homeothermic animals such as chickens (Mancini et al., 2016). Lower serum  $T_3$  levels in the experimental groups compared to the control group could be due to the higher activation of body's defence mechanisms against the adverse

effects of temperature stress (Beckford et al., 2020). Similarly, Atteh et al. (2008) reported that the addition of stevia leaves and stevioside decreased serum  $T_3$  levels. The latter author explained the differential effects of stevia supplementation on blood  $T_3$  and triglyceride levels, and fat accumulation, could be due to different mechanisms of action of pure stevioside compared to the thyroid axis.

### **Fertility and hatchability percentages, egg and chick weights**

The results of the current study showed that the fertility rates were higher in groups T2 and T3 compared to the control group. Tchoffo et al. (2017) found that the addition of different doses of ginger essential oil to the diet in quails increased their fertility rates. Jiang et al. (2020) stated that the addition of stevioside (stevia leaf extract) to the ration of laying hens numerically increased the fertility and hatchability rates in the treatment groups, but did not result in differences between these groups. The results of Jiang et al. (2020) were consistent with our findings. Tchoffo et al. (2017) reported that the addition of different ginger doses to the diet exerted a significant effect on hatchability. In the present study, the positive effect of SGE addition in the form of increased number of fertile eggs and hatchability was due to the antioxidant, anti-inflammatory, and antibacterial properties of the compounds (alkaloid and terpenoids) contained in stevia and ginger (Tchoffo et al., 2017; Jiang et al., 2019).

We also recorded a significant increase in hatching egg weight and hatched chick weight, which were higher in groups T2 and T3 than in the control group. Safavipour et al. (2022) reported that herbal feed additives added to the ration at different doses increased egg weight. The findings of Tchoffo et al. (2017) were consistent with our study, as the addition of ginger increased the weight of both eggs and hatched chicks. The latter authors believed that phenolic compounds contained in ginger increased the mass of reproductive organs and function, as well as improved ovarian activity. In addition, the increase in egg weight could be due to the estrogenic effect of stevia, enhancing ovarian follicle function and improving steroidogenesis (Jiang et al., 2020).

### **Conclusions**

Economic losses caused by heat stress are quite high in livestock farming, especially in poultry. These losses are more evident in feed efficiency, egg weight and yield, fertility, and hatchability in laying

birds. The results of the study showed that dietary SGE supplementation increased the rate of egg production and feed intake, as well as improved feed conversion ratio. On the other hand, SGE extract addition decreased the blood levels of MDA, AST, glucose, cholesterol, and T<sub>3</sub>. SGE supplementation also significantly increased the fertility rate, had a positive effect on hatchability, as well as increased the weight of hatching eggs and hatched chicks.

## Conflict of interest

The Authors declare that there is no conflict of interest.

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