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Effect of dietary electrolyte balance and arginine to lysine ratio on hematological, antioxidant and immunological traits in dual-purpose breeding hens under cyclic heat stress condition

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ABSTRACT

A total of 245 hens and 35 cocks (32 weeks age) were assigned to seven treatment groups (five replicates with seven hens and one cock) to investigate the effect of dietary electrolyte balance (DEB) and arginine to lysine ratio (Arg/Lys) on birds' physiological and biochemical traits under cyclic heat stress (CHS) condition. Birds were housed in an environmentally controlled facility having four sectors. The first group (positive control, PC) was kept under thermoneutral conditions and fed diet with DEB of 180 mEq and Arg/Lys of 1.25, whereas the other six treatments were kept in the second sector under CHS and fed diet with DEB and Arg/Lys equal to: 180 mEq and 1.25 (negative control, NC); 250 mEq and 1.25; 320 mEq and 1.25; 180 mEq and 1.37; 250 mEq and 1.37; 320 mEq and 1.37, respectively. Hens on NC group had significantly decreased red blood cells (RBCs), white blood cells (WBCs) and its fractions. The groups fed different DEB and Arg/Lys in diet significantly enhanced the blood parameters and plasma lipid profile compared NC group. Hens under CHS fed on 250 and 320 DEB with 1.37 Arg/Lys recorded the lowest concentration of low-density lipoprotein (LDL) compared with the other groups. Triiodothyronine (T_3) activity was not differed among groups, while T4 activity in layer exposed to CHS (NC group) recorded the highest activity compared to PC. From findings, it can be concluded that laying hens fed a diet having DEB 250 mEq with 1.37 Arg/Lys could be successfully applied to counteract the adverse effect of CHS and to improve blood hematological and biochemical traits, antioxidants, and immunity response.

1. Introduction

High relative humidity and ambient temperature raise body temperature and respiration rate, which in turn reduce blood CO_2 levels and alter the acid-base balance, leading to respiratory alkalosis (Macari et al., 1994), causing detrimental effects on productive traits, feed efficiency, and survival rate of poultry (Borges et al., 2003a).

Environment, nutrition, and metabolism play a key-role in the acid-base balance of chickens (Altan et al., 2003; Yalcin et al., 2004). Because of electrolyte balance and concentration are essential to bird's survival, they are strictly controlled in both its extracellular and intracellular fluids (Borges et al., 2003a). According to Mushtaq et al. (2013), electrolytes are chemical substances that break down into positive (cation) and negative (anion) molecules in solution and have a built-in capacity to transmit electric current. The principal ions that are essential for maintaining the osmotic pressure and acid-base equilibrium of bodily fluids are Na^+, K^+, and Cl^- (Borges et al., 2003b). The equation DEB = $Na^+ + K^+ - Cl^-$ (McDonald et al., 2011) is used to determine the dietary electrolyte balance (DEB), which is defined as the ratio and dietary amounts of Na^+, K^+, and Cl^- (Mongin, 1980). These minerals must be

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consistently administered to all classes of poultry in order to reach the desired production levels since they are essential for tissue protein synthesis, cellular acid-base balance maintenance, and cellular homeostasis (Shahsavari et al., 2012; Mushtaq et al., 2013). It is possible that the bird requirements for amino acids shift during hyperthermia due to the gradual changes in protein pools and amino acid metabolism that occur during heat stress (HS) (Temim et al., 2000a; 2000b).

The amino acid L-arginine (L-Arg) can be used in the biosynthesis of several substances, including protein, nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine, and dimethyl arginine (Brugaletta et al., 2023). As a result, it aids in a variety of crucial biochemical and physiological processes in chickens (Khalaji and Wideman, 2010). Additionally, Arg is an essential amino acids that has vital functions including immunological effects (T-cell proliferation and nitric oxide substrate), urea cycle, antistress agent, and acid/base (Attia et al., 2006, 2011; Vance and Adrian, 2017). In order to mitigate the harmful effects of heat stress and avoid the harmful consequences of an excess of free radicals produced during normal metabolism (Atakisi et al., 2009), L-Arg needs to be introduced to diets (Attia et al., 2011). The Arg is a strong inducer of growth hormone secreted by the anterior pituitary gland in animals and insulin secreted by pancreatic cells (Flynn et al., 2002).

Therefore, the present study was conducted to investigate the effect of DEB and Arg/Lys ratio on some physiological and biochemical traits in dual-purpose breeding hens exposed to cyclic heat stress.

2. Materials and methods

The current study was conducted at the Animal Production Research Institute, ARC, Ministry of Agriculture's El-Sabahia Poultry Research Station in the Alexandria Governorate in collaboration with Damanhour University's Faculty of Agriculture, Egypt. The Mandara local strain, which is a hybrid of ♂ Alex and ♀ Dokki-4 (Abd-EI-Gawad and Elham, 1981), was used in this study.

A total of 245 hens and 35 cocks were kept in 35-floor pens equipped with wheat straw split into seven treatment groups during the 12-week trial, which ran from 32 to 44 weeks of age. Five replicates of each treatment were used, each replicate consisting of one cock and seven hens each. The hens were kept in a light-proof, climate-controlled building. As a positive control (PC), the first treatment was maintained at the ideal temperature of 22–24 °C and relative humidity (RH) of 45–55%. It was fed a baseline meal with dietary electrolyte balance (DEB) of 180 mEq and the Arg/Lys ratio of 1.25. In contrast, the other treatments were maintained for three consecutive days a week from 11:00 a.m. to 15.00 p.m. under cyclic heat stress (CHS) conditions (38 °C and 55–65% RH). As a negative control (NC), the first HS group was given the baseline diet along with dietary electrolyte balance (DEB) 180 mEq and Arg/Lys 1.25. The five groups under HS fed with DEB 250 mEq and Arg/Lys 1.25; basal diet with DEB of 320 mEq and Arg/Lys 1.25; basal diet with DEB 180 mEq and Arg/Lys 1.37; basal diet with DEB 250 mEq and Arg/Lys 1.37; and basal diet with DEB 320 mEq and Arg/Lys 1.37 (Table 1).

The DEB was calculated according to The mEq/Kg used as the unit for expressing electrolytes is a measure of the electrolyte's power/potential of activity (Kahn, 2005).

DEB = Na+ + K + - Cl - mEq/kg DM of diet. The Na, K and Cl were determined using a Varian ICP-optical emission spectrometer 720-ES (LabMakelaar Benelux B.V., Netherlands). The arginine and lysine were determined after grounding the samples through 1-mm mesh screen. The samples were hydrolyzed for 24 h with 6NHCl at 110 C for the determination using High-Speed Amino Acid Analyzer (Hitachi LA8080 AminoSAAYA) as outlined (AOAC, 2007).

Water and feed were available at all times. In accordance with standard veterinary care procedures, vaccinations and treatment programs were carried out. The photoperiod that the birds were subjected to consisted of 16 light and 8 dark cycles. The detail on temperature degree (Tdb°C) and relative humidity (RH, %) during the experimental periods (before and after heat stress) is reported in Table 2.

A 5 ml blood samples were collected from wing vein of five hens of 44 weeks old (at the end of experimental period), in two blood tubes with and without heparin, respectively. Blood samples were centrifuged at 3000 rpm × 20 min, to separate the plasma and serum which were kept at −20 °C until analysis. While, blood samples for determination

Table 1
Ingredient and chemical composition of the experimental diets.

Ingredients, g/kg	Treatments					
	D1	D2	D3	D4	D5	D6
Yellow corn	630	630	628	630	630	628
Soybean meal 44% CP	260	260	260	260	260	260
Limestone	82.47	77.55	76.33	81.59	76.67	75.3
$CaHPO_4$	19.4	19.4	17.2	19.4	19.4	17.5
NaCl	3.90	1.95	1.7	3.80	1.95	1.50
Vit.-Min. Premix[a]	3.00	3.00	3.00	3.00	3.00	3.00
DL-Methionine	1.00	1.00	0.90	1.00	1.00	0.92
$NaHCO_3$	–	1.90	2.00	–	1.90	2.00
KCO_3	0.23	3.00	8.47	0.23	3.00	8.50
KCl	–	2.20	2.40	–	2.10	2.30
Arginine	–	–	–	0.98	0.98	0.98
Calculated composition						
CP, g/kg	165.4	165.4	165.2	165.4	165.4	165.2
ME, kcal/kg	2706	2706	2699	2706	2706	2699
Ca, g/kg	32.7	31.1	30.2	32.4	30.8	29.9
Available P, g/kg	4.90	4.90	4.50	4.90	4.90	4.50
Arginine g/kg	10.5	10.5	10.5	11.5	11.5	11.5
Lysine g/kg	8.4	8.4	8.4	8.4	8.4	8.4
Arginine/Lysine	1.25	1.25	1.25	1.37	1.37	1.37
Methionine, g/kg	3.5	3.5	3.5	3.5	3.5	3.5
TSAA, g/kg	6.3	6.3	6.2	6.3	6.3	6.2
Na, g/kg	1.70	1.70	1.70	1.65	1.70	1.60
K, g/kg	7.20	9.70	12.40	7.20	9.60	12.40
Cl, g/kg	2.80	2.60	2.60	2.70	2.60	2.40
DEB (mEq)[b]	180	250	320	180	250	320
ER[c]	3.6	4.3	5.4	3.6	4.3	5.4
Ether extract, g/kg	26.4	26.4	26.4	26.9	26.4	26.4
Crude fibre, g/kg	28.2	28.2	28.2	28.2	28.2	28.2
Determined analysis, g/kg						
Crude protein	161.3	164.3	165.1	163.3	162.7	163.1
Lysine	10.32	10.41	10.47	11.38	11.51	11.47
Arginine	8.46	8.45	8.51	8.47	8.49	8.53
Arginine/Lysine	1.22	1.23	1.23	1.34	1.36	1.34
Ether extract	25.0	24.9	26.0	25.8	24.5	26.0
Crude fibre	29.0	28.0	27.8	27.4	26.8	28.9
Ash	57.8	52.3	88.5	62.6	58.1	67.0
Na, g/kg	1.89	1.91	1.88	1.66	1.71	1.59
K, g/kg	7.40	9.80	12.35	7.30	9.70	12.35
Cl, g/kg	2.75	2.56	2.57	2.73	2.58	2.38

[a] Provided per kg of diet: vitamin A, 12,000 IU, vitamin E, 10 IU, menadione, 3 mg, Vit. D_3, 2200 ICU, riboflavin, 10 mg, Ca pantothenate, 10 mg, nicotinic acid, 20 mg, choline chloride, 500 mg, vitamin B_{12}, 10 μg, vitamin B_6, 1.5 mg, vitamin B_1, 2.2 mg, folic acid, 1 mg, biotin, 50 μg. Trace minerals (mg/kg of diet): Mn, 55, Zn, 50, Fe, 30, Cu, 10, Se, 0.10, Anti oxidant, 3 mg.
[b] Dietary electrolyte balance (DEP) = $Na^+ + K^+ - Cl^-$).
[c] Electrolyte ratio (RE) = $[(K^+ + Cl^-)/Na^+]$.

Table 2
The temperature degree (Tdb°C) and relative humidity (RH, %) during the experimental periods.

Period	Before Heat Stress			During Heat Stress		
	Tdb	RH	THI	Tdb	RH	THI
January	19.67	48.83	61.37	37.17	35.67	89.73
February	22.00	50.25	65.77	37.42	41.58	91.92
March	23.00	48.75	67.56	38.08	40.83	92.89
Mean ± SE	21.6 ± 1.7	49.3 ± 0.8	64.9 ± 3.2	37.6 ± 0.5	39.4 ± 3.2	91.5 ± 1.62

THI = temperature-humidity index. Mild (72–79 THI), Moderate (80–89 THI), Severe (90 THI or greater). Tdb = dry bulb temperature. RH = relative humidity. Calculated according to Berman (2016).

the hematological constituents were taken randomly from each treatment group before the end of the heat stress by 2 h. All the measurements on blood samples were made using commercial diagnostic kits (Egypt Spectrum diagnostic) as previously reported by Attia et al. (2017, 2020). The blood hematological characteristics [haemoglobin (Hgb) and PCV, white blood cells (WBCs) and fractions] were measured as suggested by Drubkin (1964) and Hawkey and Dennett (1989), whereas creatinine and urea levels according to Bartles et al. (1972). The value of pH was determined using digital electric by pH meter immediately after collection of samples.

The indoor temperature (°C) and RH % were recorded through the experimental period (January to March). The estimation of the temperature-humidity index (THI) was calculated according to Berman (2016). Weather Safety Index zone was assessed (LCI, 1970; Thom, 1959; Gross and Siegel, 1983).

2.1. Statistical analysis

One way ANOVA was used for statistical analysis and data normality testing (SAS Inistitute, 2009). Using Tukey's test, variables with significant differences were compared. The replication served as the experiment unit.

3. Results and discussion

The results in Table 3 showed that hens exposed to HS on NC group had a significant decrease in RBCs count, PCV, Hgb, MCV, MCH, and MCH, while blood pH was increased by 7.1% compared to PC group. Moreover, hens fed different DEB and Arg/Lys improvement considerably RBCs count, PCV, Hgb, MCV, MCH, and MCHC values compared to NC birds. However, panting under CHS may be the cause of the increase in pH values (alkalosis). This increases in pH value needs further verification. Thus, the reduced hydrogen ion concentration led to an increase in plasma pH, often known as alkalosis (Richards, 1970). Additionally, when a bird pants to remove excess heat by evaporation cooling, the blood's bicarbonate to carbon dioxide ratio increases, but in turn causes a shift in blood pH (a condition known as respiratory alkalosis) (Franco-Jimenez and Beck, 2007). It's possible that the rise in blood pH will result in less ionized calcium in the blood. According to Wang et al. (1989), panting causes the lungs to exhale more carbon dioxide, which reduces the partial pressure of carbon dioxide and, as a result, blood bicarbonate.

Since the chickens may deposit Ca into the eggshell using ionized calcium, it is vital (Odom et al., 1986). When the chickens are subjected to high ambient temperatures, this can lead to a decline in the thickness and quality of the eggshells (Samara et al., 1996). Furthermore, the present findings showed that CHS significantly decreased the proportion of WBCs and lymphocytes as compared to the PC group, but heterophil and the heterophil/lymphocyte ratio (H/L ratio) significantly increased (Table 4). In contrast to the NC treatment, hens given various amounts of DEB and Arg/Lys, however, showed a reduction in the detrimental effects of CHS on the percentages of WBCs, lymphocytes, and heterophils. The negative effect of CHS may cause animal health alternation due to alternation in cell meditated and antibody production due to the increase in corticosterone levels. Might imply an impact on systematic inflammation response (Xu et al., 2018). Simultaneously, the PC group matched the H/L seen for the layer's groups fed distinct DEB and Arg/Lys quantitatively. This may intensify the negative impact of HS, as seen by the experimental groups, H/L ratios becoming more equal than those of the NC group. One useful measure of stress in poultry blood is the H/L ratio. Mashaly et al. (2004) found that hens exposed to CHS had a considerably greater H/L ratio than chickens kept in thermoneutral circumstances. These results were consistent with their findings. According to Heller et al. (1979) and Gross and Siegel (1983), heat stress increased the H/L ratio, a stress indicator, and decreased the amount of circulating white blood cells/unit. Total WBC was shown to be decreased by heat exposure by Nathan et al. (1976). According to these results, leukocyte number and activity may be decreased by heat stress exposure. The HS reduced the synthesis of antibody, in agreement to Zulkifli et al. (2000). According to Sapolsky et al. (1987) and Ogle et al. (1997), there's a chance that this decline is the consequence of corticotropin-releasing factor (CRF) being activated by stress/inflammatory cytokine surges. After four weeks of heat exposure, Mashaly et al. (2004) found that the total WBCs of the birds exposed to CHS were lower than control birds and cyclic group. These findings could imply that heat stress exposure reduces leukocyte production and activity. According to the findings of Attia and Hassan (2017), CHS markedly reduced the packed cell volume (PCV) while dramatically increasing Hgb. However, Youssef et al. (2015) demonstrated that adding L-Arg to laying hens' diets at levels beyond those advised by the NRC (1994) did not have a detrimental effect on the birds' performance because there were no changes in blood parameters. Moreover, Jaiswal et al. (2017), Attia and Hassan (2017), and Aswathi et al. (2019) demonstrated similar outcomes. Al-Hassani (2011) showed no appreciable differences between the layer fed 0.0, 0.4, 0.7, and 0.9% Arg on RBCs and Hgb, PCV, MCV, MCH, and MCH. The RBC results are in line with his findings in a similar manner.

Moreover, Youssef et al. (2015) showed that physiological characteristics of laying hens, such as biochemical blood parameters, were unchanged when 0.728% of L-Arg was added to diet compared to the quantities recommended by the NRC (1994). When comparing the NC group to the PC group, the exposure of hens to HS resulted in a substantial rise in total lipid and cholesterol, LDL, Trg, and Malondialdehyde (MDA) activity by 11.2, 3.3, 2.7, 2.7, and 33.3%, respectively. The NC group under HS and the other experimental groups showed the same outcomes. Nonetheless, there was no discernible difference in HDL concentration across the regimens. When compared to the other experimental groups, the hens under HS and fed on 250 and 320 mEq DEB with 1.37 Arg/Lys produced the lowest LDL concentration. While CAT activity showed the opposite tendency, the NC group had the

Table 3
Effect of dietary electrolyte balance (DEB, mEq) and Arginine to Lysine ratio on hematological parameters of laying hens under heat stress.

DEB + (Arg/Lys)		RBCs, $\times 10^6$/mm^3	PCV, %	Hgb, g/dl	MCV, μm^3/RBCs	MCH, pg/dl	MCHC, %	pH
180 + 1.25 (PC)		2.93^{a}	31.9^{ab}	13.8^{a}	118^{ab}	47.1^{bc}	41.2^{a}	7.71^{b}
Heat stress	180 + 1.25 (NC)	2.40^{b}	28.3^{c}	11.3^{c}	108^{c}	44.1 ^{cd}	39.7^{b}	8.26^{a}
	250 + 1.25	2.46^{b}	30.7^{b}	12.9^{b}	125^{a}	52.5^{a}	42.0^{a}	7.73^{c}
	320 + 1.25	2.93^{a}	33.0^{a}	12.6^{bc}	116^{b}	47.9 ^{b}	40.2 ^{ab}	7.63^{c}
	180 + 1.37	2.65^{ab}	31.6^{ab}	12.7^{b}	113^{bc}	47.9^{b}	40.2^{ab}	7.74^{b}
	250 + 1.37	2.63^{ab}	30.9^{b}	12.6^{b}	118^{ab}	47.8^{b}	40.7 ^{ab}	7.88^{b}
	320 + 1.37	2.56^{b}	30.7^{b}	12.2^{b}	121^{ab}	47.8^{b}	40.8^{ab}	7.86^{b}
SEM		0.300	0.152	0.078	0.942	0.339	0.580	0.43
P Value		0.0001	0.0006	0.0001	0.0004	0.0001	0.0004	0.0001

$^{a\text{-}c}$ Means having different superscripts in the same column are significantly different ($P < 0.05$). SEM: standard error of means, RBC: Red blood cells, PCV: Packed-cell volume, Hgb: haemoglobin, MCV: Mean corpuscular volume. MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration. DEB = dietary electrolyte balance. Arg/Lys = arginine to lysine ratio. PC: positive control; NC: negative control.

Table 4
Effect of dietary electrolyte balance (DEB, mEq) and Arginine to Lysine ratio on white blood cells and differential leukocyte of laying hens under cyclic heat stress.

DEB + (Arg/Lys)		WBCs, × 10^3/mm^3	Lymphocyte, %	Monocyte, %	Heterophil, %	Eosinophils, %	H/L ratio
180 + 1.25 (PC)		64.0^{a}	78.8^{c}	3.80	14.8 ^{b}	2.70	19.0 ^{b}
Heat stress	180 + 1.25 (NC)	53.3^{d}	64.8 ^{d}	4.10	28.8 ^{a}	2.80	45.1 ^{a}
	250 + 1.25	58.1^{c}	83.4 ^{ab}	4.10	10.4 ^{bc}	2.00	12.5 ^{b}
	320 + 1.25	58.3^{bc}	80.4 ^{bc}	5.80	11.2 ^{bc}	2.90	14.0 ^{b}
	180 + 1.37	61.3^{ab}	85.2 ^{a}	4.20	9.00 ^{c}	2.80	10.7 ^{b}
	250 + 1.37	58.4^{bc}	80.0^{bc}	5.80	10.8 ^{bc}	3.50	13.6 ^{b}
	320 + 1.37	63.0^{a}	80.8 ^{bc}	6.10	10.3 ^{bc}	2.80	12.8 ^{b}
SEM		0.324	5.210	0.650	1.110	0.263	1.211
P-value		<0.0001	<0.0001	0.0508	<0.0001	0.571	<0.0001

[a-c] Means having different superscripts in the same column are significantly different (P < 0.05). SEM: standard error of means. WBCs: White blood cell, H/L ratio: heterophil/lymphocyte, DEB = dietary electrolyte balance. Arg/Lys = arginine to lysine ratio. PC: positive control; NC: negative control.

greatest Trg concentration and MDA activity when compared to the other groups, which were statistically similar (Table 5). These findings concur with those of Aswathi et al. (2019), Jaiswal et al. (2017), and Attia and Hassan (2017). In comparison to a NC group, these authors showed that dietary DEB substantially reduced total plasma lipid, total cholesterol, LDL, Trg, and MDA. Similarly, Fouad et al. (2013) demonstrated that chickens given L-Arg up to 1% for three weeks had decreased levels of total cholesterol and triglycerides in their plasma. Yang et al. (2016) found that hens supplemented with 8.5 mg/kg L-Arg exhibited reduced levels of total blood cholesterol and triglycerides in comparison to hens given 0 mg/kg L-Arg ($p < 0.05$).

Furthermore, Arian broiler chicks given Arg 139 and 154% beyond guidelines had the greatest blood HDL levels compared to those fed Arg 100 and 124%, according to Pirsaraei et al., (2018). Furthermore, 0.25% L-Arg supplementation may reduce plasma total cholesterol levels via inhibiting the liver's generation of 3-hydroxy l-3-methyl glutaryl-Co A reductase, according to a 2013 theory by Fouad et al. This may reduce the plasma Trg and belly fat yield through the upregulation of the expression of the genes associated to fatty acid oxidation, CPT1 and 3HADH, in the broiler heart while downregulating the FAS expression of gene in liver. Nitric oxide, an Arg metabolite, has been demonstrated to inhibit the production of corticosterone and ACTH (Tsai et al., 2002; Heinzen and Pollack, 2003). When the hypothalamus becomes active, the adrenal gland cortex responds by preventing the release of corticosterone and the metabolite nitric oxide on ACTH (Puvadolpirod and Thaxton, 2000a; 2000b). Unganbayar et al. (2005) have demonstrated that MDA levels, a soluble lipid breakdown product, are a useful indication of the extent of lipid peroxidation. Moreover, Jiang et al. (2013) demonstrated that Arg supplementation decreased liver and layer serum MDA levels.

When compared to the PC and other experimental groups, the plasma Ca and P contents of the birds in the NC group were considerably lower. The other experimental groups were statistically equivalent, but the plasma Ca concentration in the NC group was considerably lower. The decrease in plasma Ca and P in the NC group may be due to lower Ca and P intake and/or absorption (Attia et al., 2006, 2011), and plasma T3 and corticosterone hormones (Jingjing et al., 2015).

When compared to the other experimental groups, the group fed on 320 DEB with 137 Arg/Lys had the greatest P content (Table 6). Furthermore, compared to the NC group, the groups given DEB exhibited a significantly higher Ca concentration and a steadily increasing P concentration, according to the data presented here. However, the group given 250 mEq DEB with 1.37 Arg/Lys had the greatest Ca concentration. 320 mEq DEB with 1.37 Arg/Lys was the group with the greatest P concentration compared to the other groups. However, there was no discernible difference in the Ca/P ratio between any of the groups. Minerals are required for many biological activities, including the production of tissue proteins, preservation of the cell's acid-base balance, expression of cellular homeostasis, gene regulation, detoxification systems, and structural metabolism of bone (Borges et al., 2003b; Shahsavari et al., 2012). Our findings concur with those of Aswathi et al. (2019), who found that heat-stressed birds had lower serum Ca and P levels than a control group.

Additionally, Attia et al. (2018) discovered that while plasma P and the Ca/P ratio were constant across treatments, chickens raised in hot conditions and fed a diet devoid of supplements had lower plasma Ca concentrations. On the other hand, Aml and Abd-Elaal (2020) discovered that serum Ca and P concentrations decreased on the first and third days of HS exposure before rising to normal on the fifth day of HS exposure. Furthermore, it was shown by Odom et al. (1986) that duodenal epithelial cells absorb and use less calcium when exposed to high ambient temperatures. The laying hens' reproductive system may not be receiving enough nutrients due to the poor blood supply brought on by the high temperature. This would limit the quantity of nutrients that reach the reproductive tract and permit healthy egg formation (Ezekwe, 2011). Furthermore, Belay and Teeter (1996) found that broilers grown at high-cycling ambient temperatures had lower retention rates for minerals. Furthermore, Chiericato et al. (2005) discovered

Table 5
Effect of dietary electrolyte balance (DEB, mEq) and Arginine to Lysine ratio on lipid profile constituents, catalase and malondialdehyde of laying hens under cyclic heat stress.

DEB + (Arg/Lys)		T lipid g/dl	Chol, mg/dl	HDL mg/dl	LDL, mg/dl	HDL/LDL	Trig, mg/dl	CAT mg/dl	TAC, mg/dl	MDA, mg/dl
180 + 1.25 (PC)		4.66 ^{b}	210^{b}	44.2	94.8 ^{ab}	0.46 ^{bc}	183^{b}	39.5^{a}	411	0.168^{b}
Heat stress	180 + 1.25 (NC)	5.18 ^{a}	217^{a}	42.6	97.3 ^{a}	0.44 ^{d}	187^{a}	32.3^{c}	405	0.224^{a}
	250 + 1.25	4.67 ^{b}	206^{c}	46.0	92.0 ^{bc}	0.50 ^{abc}	180^{bc}	38.8^{a}	419	0.167^{b}
	320 + 1.25	4.36 ^{b}	204^{c}	44.6	90.6 ^{cd}	0.480^{bc}	181^{bc}	38.1^{ab}	420	0.140^{b}
	180 + 1.37	4.38 ^{b}	212^{b}	46.6	91.3 ^{bc}	0.51 ^{ab}	181^{bc}	37.8^{b}	412	0.167^{b}
	250 + 1.37	4.54 ^{b}	210^{b}	45.4	89.6 ^{d}	0.48 ^{bcd}	180^{bc}	38.2^{b}	410	0.163^{b}
	320 + 1.37	4.54 ^{b}	210^{b}	47.4	87.7 ^{d}	0.54 ^{a}	181^{bc}	37.8^{b}	415	0.157^{b}
SEM		0.045	0.403	1.201	0.324	0.0127	0.878	0.117	14.28	0.003
P Value		0.0001	0.0001	0.0845	0.0001	0.0023	0.0001	0.0001	0.4291	0.0005

[a-c] Means having different superscripts in the same column are significantly different (P < 0.05). SEM: standard error of means, P value: probability level. T lipid = Total lipid; Chol = Cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Trig = Triglycerides; CAT: Catalase, MDA: Malondialdehyde. DEB = dietary electrolyte balance. Arg/Lys = arginine to lysine ratio. PC: positive control; NC: negative control.

Table 6
Effect of dietary electrolyte balance (DEB, mEq) and Arginine to Lysine ratio on blood calcium and phosphors concentrations and reproductive and thyroid hormones activity of laying hens under cyclic heat stress.

DEB + (Arg/Lys)		Ca, mg/dl	P, mg/dl	Ca/P	E_2, ng/ml	P_4, pg/ml,	T_3, μg/dl	T_4, μg/dl	T_3/T_4
180 + 1.25 (PC)		61.5[a]	4.97 [ab]	12.40 [a]	133[a]	7.2	0.229	11.7 [d]	0.019 [a]
Heat stress	180 + 1.25 (NC)	49.0[c]	4.24 [c]	10.87 [b]	114 [c]	7.6	0.224	17.0 [a]	0.014 [c]
	250 + 1.25	57.0 [b]	4.67 [b]	12.23 [ab]	124 [b]	7.4	0.226	15.6 [abc]	0.014 [c]
	320 + 1.25	55.3 [b]	4.70 [b]	11.78 [ab]	124 [b]	7.4	0.224	15.9 [ab]	0.014 [c]
	180 + 1.37	56.3[b]	4.67 [b]	12.07 [ab]	126 [b]	7.5	0.225	15.0 [bc]	0.015 [b]
	250 + 1.37	57.7 [b]	4.90 [ab]	11.79 [ab]	124 [b]	7.6	0.224	14.7 [c]	0.015 [b]
	320 + 1.37	57.0 [b]	5.23 [a]	10.90 [b]	124 [b]	7.5	0.223	14.7 [c]	0.015 [b]
SEM		0.256	0.036	0.139	0.265	0.042	0.002	0.097	0.001
P Value		0.0001	0.0001	0.0001	0.0001	0.095	0.2758	0.0001	0.0001

[a-c] Means having different superscripts in the same column are significantly different ($P < 0.05$). SEM: standard error of means, P value: probability level. P_4: Progesterone, E_2: Estrogen, T3: Triiodothyronine, T_4: Thyroxine, Ca: Calcium, P: Phosphorus, DEB = dietary electrolyte balance. Arg/Lys = arginine to lysine ratio. PC: positive control; NC: negative control.

that for Hy-Line Brown laying hens fed on isoproteic and isocaloric diets containing 170 mEq/kg and 25 mEq/kg, respectively, Ca, P, Na, K, Mg, and Cl had comparable effects. However, Ghorbani and Fayazi (2009) found that feeding Hy-Line hens exposed to HS with dietary NaHCO3 supplementation had no effect on plasma Na or Ca. The same authors proposed that electrolyte balance might potentially affect the optimal Arg/Lys ratio for birds under HS. According to Balnave and Brake (2001), birds under HS that received NaHCO3 supplements saw a drop in plasma Lys. Additionally, they saw a rise in plasma Arg/Lys of 1.36 Arg/Lys. The scientists came to the conclusion that, in electrolyte balances, amino acid digestibility might not depend on NaHCO3. Moreover, Brake et al. (1998) found that when additional NaCl was supplied to the meal, a lower Arg/Lys was needed, suggesting a connection between dietary electrolytes and the Arg/Lys necessary to optimize birds' performance under HS. When compared to the PC group and the other experimental groups, the layer on the NC group showed the lowest estrogen (E_2) activity, while the progesterone (P_4) activity did not vary between the groups (Table 6). These findings are in line to Rozenboim et al. (2007), who assessed that White Leghorn laying hens exposed to high ambient temperatures for two days (12 h at a peak temperature of 42 3 °C) had lower plasma P_4 and testosterone levels. Attia et al. (2016) found that chickens grown under cyclic HS and fed diet without supplements had reduced blood levels of E_2 and P_4. Native Thai chickens kept at a low ambient temperature (27 °C) generated significantly higher plasma oestradiol than those exposed to temperatures of 31 °C at week 4 and 35 °C at week 7 according to Sartsoongnoen et al. (2018). However, by week 11, there was a substantial difference in plasma E_2 levels between the hens maintained at 35 °C and the control group. Furthermore, with extended exposure to ambient temperature of 27 °C, the levels of circulating E_2 rose.

Triiodothyronine (T_3) activity was constant across all experimental groups; however, the NC group's (HS-exposed layer) T_4 activity showed the highest activity when compared to PC, and PC T3/T4 ratio was higher than other treatments (Table 6). Our findings align with those of Aml and Abd-Elaal (2020), who discovered that 40-week-old Hy-line chickens' T_3 and T_4 concentrations were considerably reduced when they were subjected to heat stress conditions (40 ± 1 °C for five days, 4 h each day) as opposed to a control group that was kept at 25 ± 1 °C. Conversely, Bowen and Washburn (1985) reported that elevated room temperature lowers chicken thyroid function. Furthermore, circulating concentrations of T_3 and T_4 are known to drop in response to high temperatures (Hillman et al., 1985). However, Attia et al. (2016) assessed that the least amount of T_3 and T_4 was detected in heat-stressed hens fed a diet without supplements. When compared to PC treatment, hens in the NC group showed considerably higher values of both phagocytic activity (PA) and index (PI) (Table 7). Furthermore, all experimental groups under HS (except from NC) had statistically equivalent PI values. There were no statistically significant changes in the IgG and IgM readings across the treatments. In addition, the NC group's IgA levels dropped considerably in comparison to the PC group. In contrast to those on PC, birds fed 320 mEq DEB and 1.37 Arg/Lys showed a considerable drop in IgA. Our data are in agreement to Atakisi et al. (2009), reporting that supplementing Japanese quails' diets with dietary L-Arg and Zn raised total antioxidant capacity (TAC) and lowered MDA levels relative to a control diet. Supplementing diets with L-Arg (5 mg/kg) and Zn (60 mg/kg) may decrease oxidative stress and boost antioxidant capacity. Moreover, Duan et al. (2015) discovered that digestible dietary Arg had a substantial impact on the levels of TAC in broiler breeder serum, which may shield tissues from the damaging effects of lipid oxidation products.

Table 7
Effect of dietary electrolyte balance (DEB, mEq) and Arginine to Lysine ratio on immune parameters of laying hens under cyclic heat stress.

DEB + (Arg/Lys)		PA, %	PI, %	IgG, mg/ml	IgM, mg/ml	IgA, mg/ml
180 + 1.25 (PC)		16.4 [d]	1.53 [b]	9.78	2.33	0.833[a]
Heat stress	180 + 1.25 (NC)	20.6 [a]	1.33 [a]	9.80	2.36	0.663 [c]
	250 + 1.25	18.4 [abc]	1.70 [b]	9.72	2.42	0.823[a]
	320 + 1.25	16.8 [cd]	1.77 [b]	9.73	2.38	0.823[a]
	180 + 1.37	16.6 [cd]	1.67[b]	9.71	2.38	0.837[a]
	250 + 1.37	19.3 [abc]	1.70 [b]	9.79	2.36	0.813[a]
	320 + 1.37	20.6 [a]	1.63 [b]	9.82	2.40	0.753[b]
SEM		0.215	0.838	3.590	0.0345	0.0236
P-value		0.0001	0.0215	0.0663	0.2910	0.0177

[a-c] Means having different superscripts in the same column are significantly different ($P < 0.05$). SEM: standard error of means. TAC = Total antioxidant capacity. PA: Phagocytic activity. PI: Phagocytic index. IgG: Gamma immunoglobulin. IgM: Mu immunoglobulin. IgA: Alpha immunoglobulin. DEB = dietary electrolyte balance. Arg/Lys = arginine to lysine ratio. PC: positive control; NC: negative control.

4. Conclusion

From findings, it is possible to effectively counteract the negative effects of heat stress by feeding breeder laying hens a diet with a DEB of 250 mEq and an Arg/Lys ratio of 1.37. This will also improve the blood hematological and biochemical traits, antioxidant status, and immunity response of the birds. These conclusions can be drawn from the obtained data.

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CRediT authorship contribution statement

Youssef A. Attia: Conceptualization, Data curation. **Ahmed A. Abdallah:** Formal analysis, Investigation. **Fulvia Bovera:** Methodology, Writing – original draft, Writing – review & editing. **Abd El-Hamid E. Abd El-Hamid:** Investigation. **Asmaa Sh El-Naggar:** Formal analysis, Investigation. **Rashed A. Alhotan:** Validation, Visualization. **Vincenzo Tufarelli:** Methodology, Supervision, Writing – original draft, Writing – review & editing. **Reda M. Zaki:** Methodology, Validation, Visualization.

Declaration of competing interest

This is to confirm that "The authors declare no conflict of interest".

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